CMFRI

Winter School on

Recent Advances in Mussel and Edible Oyster Farming & Marine Pearl Production

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Technical Notes

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1 Production trends in Indian marine Fisheries and need for Mariculture

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India is one among the top ten fish producing countries in the world contributing over 3% (6 million tonnes (mt)) of the world marine fish production. The fisheries sector in India contributes nearly Rs.22, 000 crores to the total national income and form about 1.4% of total national gross domestic product (GDP) and 4.5% of agricultural GDP. The sector provides employment and income to over 10 million fishers and fish farmers. The marine fisheries sector in the country contributes about 50% of the total fish production.

Among the countries bordering the Indian Ocean, India, endowed with a coastline of 8129 km, 2.02 million km^2 of EEZ and 0.5 million km^2 of continental shelf has a catchable annual marine fishery potential of 3.93 million tonnes occupies a unique position. Besides, there are vast brackish water spread areas all along the coastline, which offer ideal sites for seafarming and coastal mariculture. Among the Asian countries, India ranks second in culture and third in capture fisheries production, and is one of the leading nations in seafood export earning annually over Rs.6500 crores (forming about 29% of agri. exports). Marine fisheries sector occupies a very important place in the socio-economic development of the country. The sector has been recognized as a powerful instrument to generate income and employment as it stimulates growth of a number of subsidiary industries and is source of cheap and nutritious food besides being a foreign exchange earner. At the same time it is an instrument of livelihood for a large section of economically backward coastal population of the country.

The fisheries research during the last five decades together with the technological advancements in the harvest and post-harvest areas have accelerated the process of transformation of a traditional, subsistence oriented marine fisheries into a market driven multicrore industrial sector. With the result the marine fish production has made great leaps through successive stages, first with a change from natural to synthetic fibers in gear fabrication and a concurrent introduction of mechanised trawlers in fifties, second with the introduction of mass harvesting gear, the purse seine along the southwest coast in 70s and immediately followed by the introduction of motorisation (outboard engine) of country crafts and the subsequent proliferation of innovative gears like ring seine in late eighties, and introduction of multiday fishing in 90s and the yield reached around 2.7 million t during the year 1997. This production remains almost static since 1997, probably waiting for another technological breakthrough in the harvesting sector.

The availability and distribution pattern of marine fishery resources in India are typical of tropical waters. The fishery resource is constituted by a large variety of species coexisting in the same ground. There are nearly 1570 species of finfishes and about 1000 species of shellfishes known from our seas. The multispecies

fishery comprises of over 200 commercially important finfish and shellfish species (Table 1). The important varieties belonging to the pelagic groups such as the sardines, anchovies, mackerel, carangids, Bombay duck, ribbonfishes, seerfishes, tunas; demersal finfish groups such as the sharks, rays, croakers (sciaenids) perches, silverbellies, lizardfishes, catfish; crustaceans such as the penaeid and non-penaeid shrimps, crabs and lobsters; and cephalopods *viz.*, squids and cuttlefishes are common. The abundance of these stocks varies from region to region and from season to season with large pelagics like tunas being more abundant around Island Territories and small pelagics like sardines and mackerel supporting a fishery of considerable magnitude along the southwest and southeast coasts (Fig.1). The Bombay duck and non-penaeid shrimps form a good fishery along the northwest coast, while perches are dominant in the southwest and southeast coasts, especially in the Gulf of Mannar, Palk Bay and Wadge Bank. Among this, species/groups contributing to more than one lakh tonnes a year are oil sardine, mackerel, Bombay duck, ribbonfishes, carangids, perches, croakers, shrimps and cephalopods.

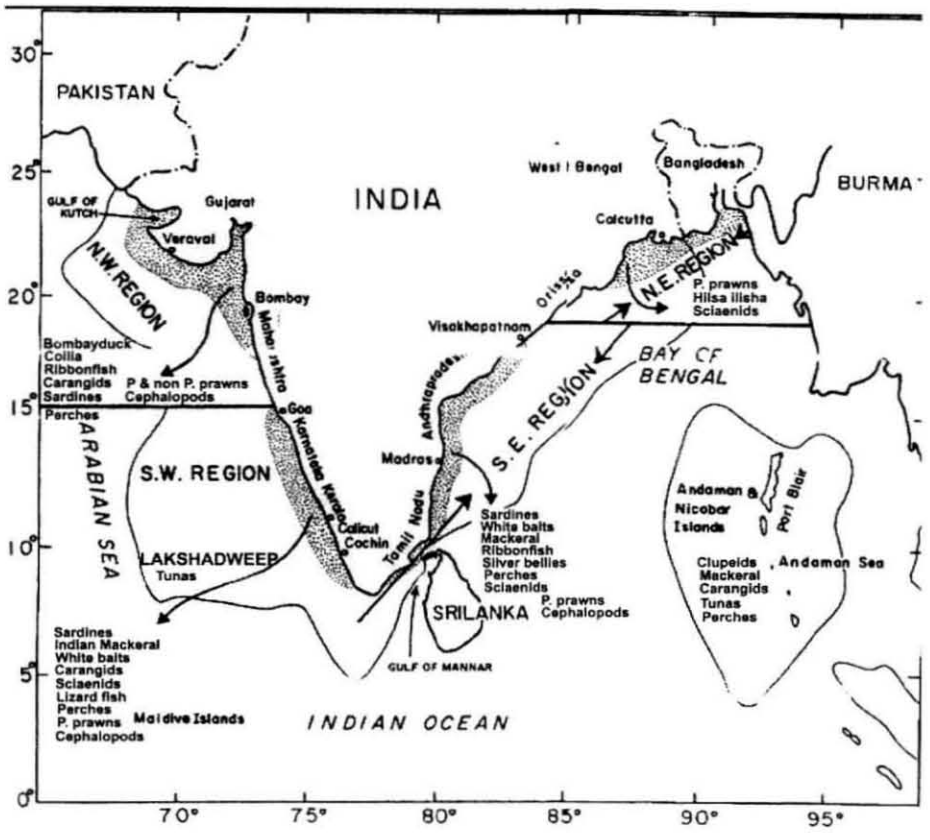

Fig.1. EEZ of India and geographical distribution of major fishery resources

Table 1. Catch trends and potential yield estimates of different groups

Group	Average catch (t)		Group Contribution (%)	Potential yield* (t)
	1985-89	1999-2003		
Elasmobranchs	54027	62799	2.46	71408
Oil sardine	141831	319419	12.53	294869
Other sardines	76541	101130	3.97	101490
Anchovies	68630	115598	4.53	141817
Other clupeoids	132626	43987	1.73	78932
Bombay duck	93185	105601	4.14	116227
Ribbonfishes	78384	172102	6.75	193670
Carangids	111040	120608	4.73	238148
Indian mackerel	123832	128430	5.04	295040
Seerfishes	35171	48905	1.92	61719
Coastal tunas	34185	50337	1.97	65472
Barracudas	-	17125	0.67	20849
Catfishes	50630	53711	2.11	51255
Eels	6317	9637	0.38	9081
Croakers	102934	141933	5.57	273027
Perches	90083	189093	7.42	226793
Flatfishes	29612	45482	1.78	47304
Silverbellies	60766	53849	2.11	67247
Pomfrets	37356	38378	1.51	46088
Penaeid shrimps	143073	196464	7.70	194192
Non-penaeid shrimps	48057	142929	5.61	138711
Stomatopods	-	43663	1.71	120351
Lobster	-	1938	0.08	3874
Cephalopods	39799	107415	4.21	101259
Others	40034	239327	9.39	975594
Total	1598113	2549860		3934417

Source: Modified CMFRI, 1997a *Anon, 2000

The annual catchable potential yield in the Indian EEZ is validated by a Committee as 3.93 mt consisting of 2.02 mt of demersal, 1.67 mt of pelagic and 0.24 mt of oceanic resources (Anon, 2000). This Working Group for the first time estimated the potential yield of as many as 68 species/groups of fishes occurring in the EEZ. The present annual average production of about 2.55 mt forms 64.8% of the revalidated fishery potential.

The coastal fisheries exploit a large number of species using different crafts and gears mostly in the depth range of 0 to 50 m. In recent years, however, the depth of operation has been extended upto about 120 m in some regions. Being a multigear fishery (gillnets, drift nets, hooks & line, pole & line, troll line, bag nets, ringseines, purseseines, trawls, etc.), fishing practices vary between different regions, depending on the nature of the fishing grounds and the distribution of the fisheries resources. The marine fish production in the country progressively

increased from 0.58 mt in 1950 to 2.73 mt in 1997 showing an average annual growth rate of 6.4% over a period of 4 decades (Fig.2). The annual growth rate during the different decades commencing from 1950, declined from 6.5% during 1950-60 to 2.3% during 1960-70; increased to 4.3% during 1970-80 and to 4.8% during 1980-90 but declined to 4.0% during 1990-96. This fall in the growth rate is reflected in the annual catch attaining the optimum level in the inshore fishing grounds extending upto a depth of 50 m. As could be seen from Figure 2 the marine fish production has reached a plateau since 1991, which is because the fishing effort is mainly concentrated in the 0-100 m depth zone. Over these years the trawling effort has increased considerably leading to excess pressure in the coastal waters.

The annual average landing during 1999-2003 was 2.55 mt against an annual catchable potential yield of 3.93 mt principally constituted by oil sardine (12.5%), penaeid prawns (7.7%), perches (7.4%), ribbonfishes (6.7%), non-penaeid prawns (5.6%), croakers (5.6 %), mackerel (5.1%), carangids (4.7%), anchovies (4.5%), cephalopods (4.2%), and Bombay duck (4.1%) (Table 1).

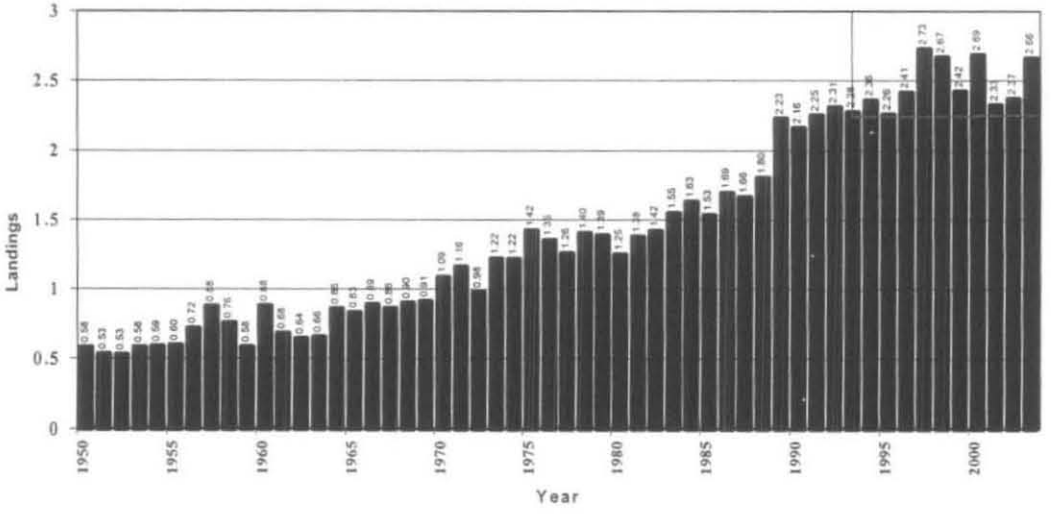

Fig. 2. All India marine fish landing (in mt.) during 1950-2003.

The mechanised sector accounted for 67.9%, motorised sector 25.0% and artisanal sector 7.1% of the total production. The sector-wise landings in different regions during 2003 are given in Figure 3. Comparative output of the marine fishing sector of different coastal states in 1985 and 2000 are given in Table 2. Catch trend during 2003 (Fig.4) indicate that the northwest coast contributed 34% to the total marine fish production followed by southwest coast (33%), southeast coast (23%) and the remaining (10%) by northeast coast (CMFRI, 2003).

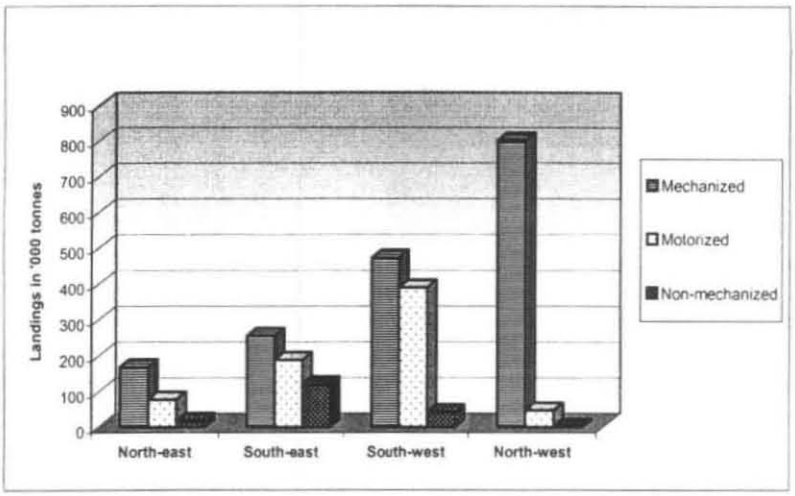

Fig.3.Sector-wise landings in different regions during 2003

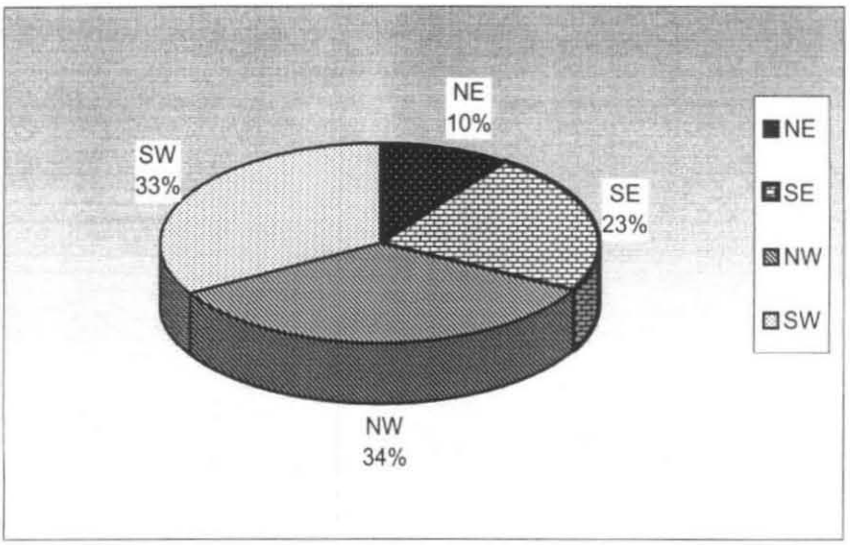

Fig.4. Regionwise fish landings in India during 2003

The increase or decrease in the annual marine fish production of the country by and large depends on the success or failure of oil sardine, mackerel, Bombay duck and shrimp fisheries (Pillai, 2003). The wide fluctuations in the annual yield of oil sardine and mackerel are well known and are generally due to factors such as spawning success, recruitment strength and environmental factors. However, in the case of the shrimp fishery, particularly the penaeid prawns, which are much, sought after by the export trade, the landings have been fluctuating from year to year with no definite trend. In most of the years, the margin of fluctuation has been varying from 10 to 15%. Further, the data on production, CPUE and other parameters of the coastal shrimp fishery at centres such as Sassoon Dock, Karwar, Mangalore, Calicut, Cochin, Neendakara, Mandapam and Chennai have indicated that further increase of effort may not yield increased production as the exploitation has reached the optimum level.

Table 2. Comparative output (in tonnes) of the primary marine fishing industry of different coastal States/Union Territories of India in 1985 and 2000

States/Union Territories	1985		2000	
	Output	Rank	Output	Rank
Andhra Pradesh	1,26,848	6	1,66,482	6
Gujarat	2,88,500	3	6,70,951	1
Goa	39,927	8	61,460	9
Karnataka	2,00,828	5	1,65,653	7
Kerala	2.95,339	2	5,75,500	2
Maharashtra	3,88,088	1	3,97,901	3
Orissa	49,205	7	1,25,935	8
Tamilnadu	2,57,000	4	3,93,000	4
West Bengal	39,350	9	1,71,500	5
Andamans	6,304	11	28,147	11
Lakshadweep	4,676	12	13,600	13
Pondicherry	19,913	10	38,620	10
Daman and Diu			15,946	12

Source: Korakandy, 1994 and Sudarsan, 2000

Although the achievements were tremendous, slowly but gradually, this common property was stressed and led to over harvest of at least a few easily vulnerable and target species and degradation of fish habitats perhaps to the extent of denudation by the unbridled human greed.

Status of exploitation of dominant species-stocks along the Indian coast in the 0-50m depth zone is given in Table 3. It is evident from the table that exploitation of many of the species at different regions have reached optimum level and in the case of certain prime species, the exploitation rate has even crossed the maximum sustainable level. The substantial increase in the effort over the last 4 decades resulted in the decrease in per capita area per active fisherman and per boat in the inshore fishing grounds, and also in the catch per unit effort. It also gave rise to conflicts among different categories of fishermen, particularly between the artisanal and mechanised sectors. Ultimately the sustainability of many resources in the coastal areas has been jeopardized by the incessant fishing pressure coupled with the impacts of pollution, and other anthropogenic causes. Such a critical situation warrants effective management of the exploited stocks in the coastal waters for sustaining the current production and to augment it further by focusing attention on the deep sea and oceanic sector.

Table 3. Status of exploitation of different species-stocks along the Indian coast in the 0-50 m depth zone

Species	State of Exploitation		
	Full	Over	Under
Sardinella longiceps	All along	-	-
S. gibbosa	SW coast	-	West coast
Hilsa ilisha	NE coast	-	-
Encrassicolina devisi	-	-	All along
Stolephorus waitei	-	-	-
Rastrelliger kanagurta	All along	-	-
Scomberomorus commerson	-	SE&SW coast	-
Euthynnus affinis	All along	-	-
Thunnus tonggol	All along	-	-
A. rochei	-	-	All along
Katsuwonus pelamis	-	-	All along
Megalaspis cordyla	-	-	SW coast
Decapterus russelli	-	-	All along
Selaroides leptolepis	SE coast	-	-
Atropus atropus	NW coast	-	-
Alepes kalla	SW coast	-	-
Atule mate	-	-	SW coast
Caranx carangus	SE coast	-	-
Parastromateus argenteus	-	West coast	-
Formio niger	-	SW coast	-
Trichiurus lepturus	-	East coast	West coast
Harpadon nehereus	NW coat	-	-
Nemipterus japonicus	All along	-	-
Nemipterus mesoprion	All along	-	-
Leiognathus bindus	East coast	-	-
L. dussumieri	Tamil Nadu	-	-
L. jonesi	Tamil Nadu	-	-
Secutor insidiator	East coast	-	-
Tachysurus tenuispinis	-	West coast	-
T. thalassinus	-	W&NE coast	-
Otolithus cuvieri	NW coast	-	-
Johnius macrorhynus	NW coast	-	-
J. vogleri	NW coast	-	-
J. sina	SW coast	-	-
J. carutta	SE coast	-	-
Penaeus monodon	East coast	-	-
P. indicus	-	East coast	-
P. semisulcatus	-	SE coast	-
Metapenaeus monoceros	All along	-	-
M. dobsoni	All along	-	-
Acetes indicus	NW coast	-	-
Panilurus polyphagus	-	NW coast	-

Loligo duvauceli	All along	-	-
Sepia aculeata	East coast	-	West coast
S. pharaonis	East coast	-	West coast

Source: Murty & Rao, 1996

There is increasing awareness in recent years among researchers, policy planners and management experts that any additional increase in fish production has to be obtained from offshore, deep sea and oceanic waters beyond the harvesting range of coastal fishing fleet. The estimated potential yield from deeper areas in the EEZ beyond 50 m depth is 1.69 mt. This includes several conventional and non-conventional resources. Oceanic resources consist of tunas (*Thunnus albacares, T.obesus, Katsuwonus pelamis*), billfishes, myctophids (*Benthosema* spp., *Myctophum* spp. and *Diaphus* spp.) and oceanic squids (*Symplectoteuthis oualaniensis*, *Onychoteuthis banskii*, *Thysanoteuthis rhombus*). But there is no directed fishery for these species, except marginal exploitation by chartered vessels, which operated under the deep sea fishing schemes in the nineties but were later suspended. Longline surveys conducted by Fishery Survey of India (FSI) have revealed abundant resources of skipjack (*K.pelamis*) and yellowfin *(T.albacares*) tunas and pelagic sharks in our waters (Somavansi, 2001). For exploitation and management of tuna resources of the coastal areas and the high seas, separate strategies should be evolved (Pillai *et al.,* 2002).

Among the multigears, gillnets, drift nets and bag nets of varied mesh sizes are widely employed by traditional fishermen along both the coasts while ring seines, purse seines and mechanized gillnets are confined to the southwest coast. Bottom trawlers upto 13 m OAL are operated along the entire coast, while the second-generation large trawlers 13-17m are operated from selected harbours along both the east and west coasts.

The growth of the fleets shows that the artisanal fleet (including the motorized) increased by about 110% from the 1960s to the 1990s and the mechanized fleet by about 570% during the same period (CMFRI, 1997) and has resulted in an over capacity of fleet operating in the inshore waters.

Currently 2251 traditional landing centres, 33 minor and six major fishing harbours serve as base for 208 thousand traditional nonmotorised crafts, 55,000 small scale beach landing motorised crafts, 51,500 mechanised crafts (mainly bottom trawlers, drift gillnetters and purse seiners) and 180 deep sea fishing vessels of 25 m OAL (Anon, 2001). The development of harbours and landing jetties, motorization of artisanal crafts and rapid expansion of mechanized fishing have contributed towards a significant increase in fish production, employment generation and revenue earnings. It has also resulted in declining per capita area for the boats (Table 4) and given rise to serious conflicts between artisanal and mechanised sectors in the inshore waters where CPUE for most of the fisheries and especially the shrimp are showing a declining trend. The pattern of marine fish landings in India during the past fifty years clearly reveals that the contribution by the artisanal sector to the total production was significant only up to 1960s while presently, the contribution by the mechanized and motorized sector accounts for 93% of the marine fish catch (CMFRI, 2003). Under these circumstances adoption

of sustainable fishing practices, diversified multi-gear and resource specific fishing and complementary mariculture practices are being advocated.

Table 4. Change in per capita area in ha/boat (non-mechanised + mechanised) in the shore areas (0-50 m) and offshore shelf areas (50-200 m) during successive periods

State	1961-62		1973-77		1980		1990	
	Inshore	Offshore	Inshore	Offshore	Inshore	Offshore	Inshore	Offshore
Gujarat	1453	2214	1095	1669	862	1314	499	760
Maharashtra	257	852	251	833	205	680	108	359
Goa	3030	7070	229	534	87	204	94	220
Karnataka	114	244	109	233	89	190	51	109
Kerala	59	123	57	118	44	92	40	84
Tamilnadu	78	55	74	53	52	36	53	38
Pondicherry	-	-	82	55	77	51	25	17
Andhra Pradesh	84	69	64	53	46	38	31	25
Orissa	528	599	317	359	147	166	96	109
West Bengal	1503	626	599	249	234	97	192	80
Lakshadweep	-	-	-	-	-	-	-	347
Andamans	-	-	-	-	-	-	-	3043

Source: *CMFRI Vision 2020*

Ornamental fish and fisheries

Besides the marine fishery resources for human consumption, there are certain resources of commercial value. Marine aquarium fish trade is gaining increasing popularity the world over with an estimated value of 4.5 billion US$ (Srivastava, 1994). The Gulf of Mannar, Palk Bay, Gulf of Kutch, southwest coast and the Lakshadweep and Andaman group of islands are known to be rich in ornamental fishes (Murty *et al.*, 1989, Murty 2002). The wrasses, damselfish, surgeons, butterflyfish, moorish idol, squirrelfish, triggerfish, rabbitfish, parrotfish, angels, goatfish and pufferfish are the major aquarium fishes represented by nearly 180 species. Most of these fishes are abundant and offer scope for live fish export and development of home aquariculture in the country. The results of the survey and assessment of marine ornamental fishes of Lakshadweep (nine islands) implemented by the Central Marine Fisheries Research Institute indicate an annual potential yield of 25 million fish consisting mainly of wrasses (38.0%), damsel fishes (32.7%), goat fish (8.4%), parrot fish (7.4%), squirrel fish (4.9%), surgeon fish (4.8%), butterfly fish (2.1%), trigger fish (0.8%) and others. Their exploitation, utilization and trade should be exercised with caution without trampling the habitat or other co-habitants of ecological value.

Key issues in marine fisheries sector

- Multi-gear, multi-species, Open Access Fisheries
- Increased and excessive fishing pressure in the coastal areas up to about 50 m depth zone
- Optimal exploitation of resources in the inshore waters
- Indiscriminate exploitation of juveniles of many commercially important species by reducing the mesh size
- Damage to the benthos and benthic ecosystem by continuous sweeping of the same ground by shrimp trawlers
- Decrease in area available in the sea per active fisherman and boat for conducting fishing operations
- Conflicts among different categories of fishermen particularly between the artisanal and mechanised groups of fishermen
- Conflicts between those engaged in coastal artisanal fishing and coastal aquaculture
- Ecosystem degradation
- Lack of proper fishery management system (Participatory Fisheries Management)
- Lack of National Marine Fisheries Policy
- Need for popularisation of Code of Conduct for Responsible Fisheries
- Absence of informed management regime

The annual growth rate of marine fisheries production increased from 4.3% during 1970s to 4.8% during 1980s and declined to 4.0% during 1990s(CMFRI, 1997a) and lowering down in growth rate is reflected in the annual catch attaining the optimum levels in the inshore fishing grounds up to a depth of 50m. The substantial increase in fishing effort since the 1970s has resulted in the decrease in per capita area per active fishermen and per boat in the inshore fishing grounds and also in the CPUE, which, in turn has given rise to intra/inter sector conflicts among difference categories of fishermen, especially artisanal and mechanized sectors (Sathiadas, 1996). Such a critical situation warrants effective management of the exploited stocks in the coastal waters for sustaining the current production and to augment it further by focusing attention on the offshore sector and on seafarming and coastal mariculture. There is also urgent need to formulate national and state level regulations/policies in marine capture and culture fisheries in conformity with the objectives of the FAO Code of Conduct for Responsible Fisheries and other relevant global conventions and regulations, within the ambit of the prevailing socio-political and economic objectives.

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2 Molluscan Mariculture – Global status

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Aquaculture has been in existence for centuries as a traditional practice in Europe and Asian countries mainly for edible oysters, freshwater fin fishes and brackish water shrimps. Farming of molluscs is one of the earliest form of mariculture especially, farming of oysters in the western hemisphere from Roman times. Since eighties scientific shrimp farming commenced in the country with traditional, extensive and semi-intensive farming techniques. Experimental farming of Mussels, pearl oyster and pearl production and clams were initiated in the early seventies in the country by national research laboratories. Since marine fish production is stagnating at an optimum level with further increase in production not envisaged, there is increase in awareness in the country to produce more seafood through scientific aquaculture.

Global Scenario

Global aquaculture production has shown a continuous and steady increase over the years, increasing from 3.9% of the total fish production by weight in 1970 to 29.8%in 2002. Of the total world fisheries production of 132.9 million metric tonnes in 2002, aquaculture contributed to 39.7 million tonnes. Of the total world aquaculture production in 2002, molluscs, mainly consisting of oysters, mussels, scallops, clams and cockles contributed 11.27 million mt, *ie* 28.3%. Among mulluscs, oysters dominated contributing to 36.67%, followed by clams and cockles (29.15%) and mussels (12.2%). Asian countries contributed 87.98% of the total aquaculture production .The percentage of molluscs in the total world aquaculture production was 28.3%.

Scientific pearl culture was initiated by Japan and till recently the international market for cultured pearls were controlled by them. However in recent years several other nations have started utilizing their pearl oyster resources and Japan lost its monopoly in the production and trade of cultured pearls. In 1998, the world pearl exports, valued at 396 million US dollars were controlled by French Polynesia (28%), Australia (20%), Japan (14%) and Indonesia (14%).

The world production of edible oysters in 2002 was 4317380 t and the important contributions were by China (83.9 %), Japan (5.1 %) and Republic of Korea (3.9 %).

The world production of mussels by aquaculture was 1444734 t dominated by China (67.1%), Italy (9.3 %) and France (7.3%).

The total clam production by aquaculture was 3430820 tonnes forming 30.3% of the total molluscan production by aquaculture and the leading countries were China, (92.5%), Malaysia (2.2%) and Italy (1.2%).

The total production of scallops by aquaculture was 1226568 tonnes, of China contributed 76.2%, and followed by Japan contributing 22.1%.

Cultured gastropods contributed 2816 tonnes and the major contributors were Taiwan province of China (82.56%) and South Africa (7.81%).

Cephalopods, mostly experimental culture, contributed 14 tonnes, mostly by Spain.

2002- World Aquaculture Production (FAO)

Group	Production (Mt)	Pecentage
Fish	1201060	5.72
Crustaceans	246525	1.17
Molluscs	11770659	56.09
Sea weeds	151387	0.72
Others	7616891	36.29
Total	**20986522**	

2002- World Aquaculture Production- Molluscs (FAO)

Group	Production (Mt)	Pecentage
Abalones, Winkles & Conchs	2816	0.02
Oysters	4317380	36.68
Mussels	1444734	12.27
Scallops, Pectens	1226568	10.42
Clams, Cockles, Ark shells	3430820	29.15
Squids, Cuttlefishes, Octopuses	14	0.0001
Miscellaneous marine mollusks	1348327	11.45
Total	**11770659**	

Methods of Bivalve culture

Bivalves, including mussels, oysters and clams contribute to the bulk of production of molluscs. The different farming techniques used for bivalve mariculture are

a) **On bottom culture**: It involves the transfer of young seed mussels from areas of great abundance were growth is poor owing to over crowding, to areas of good growth and fattening. This practice is followed in Holland, Denmark and Germany

b) **Pole culture**: Rows of poles interwoven with branches are used. Conducted in the intertidal mudflats along the Atlantic coast, mostly in France

c) **Raft culture**: Both floating and anchored rafts are used from which rens or cages holding oysters are hung. Eg: culture of mussels in Spain, Southern France, Yugoslavia and Italy.

d) **Long line culture:** Shallow waters of 10-15 m depth. It is able to withstand severe monsoon along the southwest coast of India.

Major Molluscan Species Cultured

Family Arcidae	
Anadara broughtonii	Japan (Experimental culture)
Anadara granosa	Tropical Pacific, Thailand, Malaysia, Korea
Anadara granosa bisinensis	Korea
Anadara inflata	Banten (West Java)
Anadara subcrenata	Japan
Arca granosa	Pacific islands, Japan
Family Ostreidae	
Crassostrea angulata (Potuguese oyster)	France, Tunisia
Crassostrea commercialis (Sydney rock oyster)	Australia, Hawaii
Crassostrea cucullata (Indian rock oyster)	Japan, Oyster, New Zealand
Crassostrea gigas (Pacific oyster)	Japan, Korea, N.America, Tasmania, New Zealand
Crassostrea glomerata (Rock oyster)	New Zealand
Crassostrea rhizophorae	Cuba, Jamaica, Puerto Rico
Crassostrea rivularis	Japan
Crassostrea virginica (Atlantic oyster)	USA
Ostrea edulis (European flat oyster)	Spain, France, Tunisia, Greece, Scotland, Ireland
Ostrea iredalie	Philippines
Ostrea lurida (Olympia oyster)	Puget Sound (USA)
Pycnodonta numisma	Thailand
Family Mactridae	
Mactra sulcataria	Japan
Family Mercenaridae	
Mercenaria mercenaria	Gulf of St Lawrence to Gulf of Mexico, USA
Family Veneridae	
Meretrix lamarckii	Japan
Meretrix lusoria	Japan, Korea
Meretrix meretrix	Japan, Korea
Protothaca staminea	N. America
Saxodomus giganteus (Butter clam)	USA
Tapes decussatus (Meditteranean clam)	Meditteranean
Tapes semidecussata (Japanese little neck clam)	Pacific coast of Asia (Japan and Korea)
Venerupis japonica (Manila clam)	Japan
Venus verrucosa	Meditteranean
Paphia philippinarum	Japan
Family Myidae	
Mya arenaria	N.America, Norway, France, Japan
Family Mytilidae	
Mytilus crassitesta	Korea

Mytilus edulis	France, Spain, Germany, Italy, Netherlands, Denmark, England, Scotland, Canada
Mytilus galloprovincialis (Meditteranean mussel)	Italy, Tunisia, Greece
Mytilus smaragdinus (Green bay mussel)	Thailand, Philippines
Family Pectinidae	
Pecten laqueatus	Japan
FamilyPteriidae	
Pinctada fucata	Japan
Pinctada margaritifera (Black lip pearl oyster)	Indo-Pacific, Japan, Philippines
Pinctada martensii (Japanese pearl oyster)	Japan, Sudan, Red sea, Australia
Pinctada maxima (Siver lip, Gold lip)	Australasia
Pteria penguin (Wing shell)	Japan
Family Anomiidae	
Placuna placenta (Window pane shell)	Philippines

Molluscan aquaculture in the world

Mussels

The major species of mussels cultivated in the world are the blue mussel, *Mytilus edulis*, and the Meditteranean mussel, *Mytilus galloprovincialis*. The blue mussel is cultivetd by long line culture in China, by raft culture in Spain and by 'Bouchot' culture in France. The raft method is generally practiced in protected areas, with steep coastal profiles and considerable tidal oscillations. Very high levels of production are obtained by raft culture in the submerged river valleys or fjords of Galicia in Spain. China leads the production in mussels, followed by Spain, Italy and France. The other species of importance include the green mussel, *Perna viridis*, in Thailand and Malaysia and the New Zealand mussel, *Perna canaliculatus*. The black mussel, *Mytilus crassitesta* is also of some importance in China.

Oysters

The major species contributing to the oyster production by aquaculture is the Pacific cupped oyster, *Crassostrea gigas*, contributing to more than 95 % of the total production of oysters. The other species of importance are the European flat oyster, *Ostrea edulis* and the American cupped oyster, *Crassostrea virginica*. The major oyster producing countries of the world are China, Japan, republic of Korea and France. In China the long line method of culture is followed, whereas in Japan both on bottom and off bottom culture is done, the off bottom culture is practiced in rafts and long lines. An interesting variation of the conventional bottom culture method for *Ostrea edulis* in France is the "Claire method" of fattening and greening of oysters as final preparation for market. Claries are small, shallow, artificial ponds, 0.1 to 0.2 ha in size constructed on marshland adjacent to the sea. In this

process, by the deposition of glycogen, the oyster meat increases in size and weight, the colour of meat becomes creamy white and the flavour becomes sweet.

The other oyster species of commercial importance, cultured are The Portuguese oyster, *Crassostrea angulata*, in Portugal, Spain and Atlantic coast of France, Sydney rock oyster, *Crassostrea commercialis*, in Australia and NewZealand, *Crasspostrea eradelie*, the slipper oyster in Philippines, and the mangrove oyster, *Crassostrea rhizophorae*, in Cuba and Venezuela.

Clams and Cockles

The major cultivated species comes under two families, Arcidae and Veneridae. The major species cultivated are The Japanese carpet shell, *Venerupis japonica*, the Quahog, *Mercenaria mercenaria* and the blood cockle, *Anadara granosa*. Of this *V. japonica* contributes to more than 65 % of the total clam production by aquaculture, mainly by China and United States Clams are mainly produced by on bottom culture in intertidal areas. The hard clam *Mercenaria mercenaria* is spawned in commercial hatcheries on the east coast of USA and juveniles from the nursery are transplanted to nursery grow out systems until they are approximately 25 mm before being planted out into the natural shellfish beds for further grow out and eventual harvest.

Investigations into culturing the giant clam, *Tridacna* species have occurred in the South Pacific since the late seventies and currently there are four hatcheries that supply juveniles that are transplanted throughout the Indo-Pacific area.

Scallops

The major species culture is the Yesso scallop, *Patinopecten yessoensis*, of which more than 95% is contributed by China and Japan. The larvae collected on spat collectors are reared in hanging cages or holding ponds until the scallops exceed 3 cm shell length (after 7 months), and are then released into favourable grounds. In off bottom culture lantern nets are used. Other species used for culture are *Chlamys* farreri in china. Commercial culture of a larger species *Chlamys nobilis* is also done in southern China. The bay scallop *Argopecten* irradians has been spawned in hatcheries and the seed grown to market size in pens in the USA. It is also being cultured in China.

Pearl oysters

The major species used for commercial pearl culture include, *Pinctada fucata* in Japan, which produces pearls in the 4-8mm range, *Pinctada maxima*, the silver lip or the gold lip pearl oyster, in Australia (12-18mm size pearls) and the black lip pearl oyster *Pinctada margaritifera* in French Polynesia (10-14mm pearls). Japan dominated pearl production till the advent of the Chinese with their fresh water pearls. The fresh water pearl producing oysters in Chins are *Cristaria* and *Hyriopsis*. The genus *Pteria* is also tried for pearl production and has also been attempted in the abalone

Gastropods

Abalone is the major species cultivated. The wrinkled abalone *Haliotis discus hannai* is cultured in China in cages and raceways, the blacklip abalone, in

Australia and the Pelemoen abalone in South Africa. The Taiwan province of China is by far the leading producer of cultured abalones. Other gaspods cultured include the snails, *Helix pomatia* and *Helix aspera* in France, the queen conch, *Strombus gigas* cultivated on an experimental scale in the Carribean. The top shells *Trochus cornutus* is commercially cultured on the Seowipo coast of Korea and the Japan coast and *Trochus niloticus* on an experimental basis in the Caroline Islands.

Molluscan aquaculture in India

The potential and prospects of coastal aquaculture of molluscs in India was realized as early as the seventies and concerted efforts made to develop suitable technologies for scientific farming, which could be easily adopted by the coastal fishermen. Several research programmes were taken up by National Research Laboratories, Universities and Department of Fisheries of maritime states during the past 25 years for development of coastal aquaculture in the country. Coastal aquaculture and Mariculture occupy an area 120000 ha providing employment to more than 200000 people. However as the present region under production forms only 10% of the identified potential area in the coastal belt, there is great scope for further development in aquaculture.

The bivalve resources of India comprising the pearl oysters and the protein rich mussel and edible oyster, have become an important source of income to coastal villagers. The revival of the pearl industry, which had flourished in the earlier times, has become possible only through the development of a full-fledged pearl culture technology by the Central Marine Fisheries research Institute, Cochin.

Pearl Culture Programme

Pinctada fucata, distributed in the Gulf of Mannar, Palk Bay and Gulf of Kutch and the black lip pearl oyster, *Pinctada margaritifera* in the Andaman and Nicobar islands, constitute the two major pearl producing ousters in India. The pearl culture programme was started by CMFRI in 1972, in response to the dwindling natural pearl fisheries. A research programme on pearl culture was organized by CMFRI in collaboration with the Government of Tamil Nadu as an *ad-hoc* scheme on pearl culture under the ICAR, from 1973 to 1978, leading to the establishment of a pearl farm in Krusadai Island by the Government of Tamil Nadu. With indigenous developments in pearl culture technology, the CMFRI over the years has adopted an open policy of training and the institute has implemented training programmes in pearl culture technology and hatchery production of spat since 1976. Along the Tamil Nadu coast, Tamil Nadu fisheries development corporation (TNFDC) and Southern petrochemicals industries corporation Ltd (SPIC) took up a joint commercial project on pearl production in 1983 with technical know how of CMFRI. Pearl culture was developed as a rural upliftment programme in the early nineties. Industrialization of pearl culture is progressing with several private companies (ITAP Ltd, Tuticorin, Orkay company, Mandapam, Master Pearls Ltd, Chirala, Pearl Beach hatcheries, Visakhapatnam) taking up commercial level pearl culture.

Mussel Farming

Marine mussels form one of the most dominant cultivable species all over the world and give the highest conversion of primary producers (phytoplankton) to human food. The culture of mussels in column waters can increase seafood production several fold. The two species considered for culture in India are *Perna viridis,* the green mussel and *Perna indica*, and the brown mussel. The culture season for mussels is during December to May, when the estuaries are in the marine phase. The culture methods include Rack method (estuaries and shallow seas), Long line method (unprotected sea conditions) and Raft culture (calm open sea conditions). To popularize mussel culture demonstration units were set up by CMFRI at Andhakaranazhi (Long Line), Njarakkal (raft) and central Kerala (integrated culture of mussels and oysters). An estuarine farm was also set up at Padanna (Kasargod). An extensive community development programme started by CMFRI led to loans from Government development agencies like DWRCA (Development of women and children in rural areas), TRYSEM (Training of Rural Youth in Self Employment) and Farmers Co-operative banks to newly formed village mussel farming groups resulting in the setting up of several mussel farms in the region. The establishment of mussel farms in Kerala state led to a dramatic increase in farmed mussel production (more than 500 tonnes in 1998).

Edible Oyster Farming

Of the six species of edible oysters found along the Indian coast, The Indian backwater oyster, *Crassostrea madrasensis*, is the dominant species used for aquaculture. Since the early seventies, CMFRI has taken up R&D programmes on all aspects of oyster culture resulting in a complete package of the technology. The culture methods followed in the country are Rack and Ren method, Rack and Tray method for commercial production and Stake, Raft and Long Line culture are tried at an experimental scale. The first commercial farming area was developed in Kerala in Ashtamudi Lake (Dalavapuram) during 1995-1996. In Kerala the BFFDA now gives financial assistance to farmers to set up oyster farms as recognition to the fact that planners have recognized oyster culture as a viable project ideal for rural development and income generation.

Recent trends in Molluscan Mariculture

The production of triploid and tetraploid oysters for increasing production has been attempted. Genetic manipulation such as ploidy induction, gene transfer and selective breeding are the recent developments in bivalve farming to increase productivity through bio techniques.

Treatment of shrimp and fish farm effluents and reclaiming can be done by biological means like culture of molluscs (eg: oysters in Hawaii and mussels in Thailand), utilizing the filter feeding property of molluscs to filter out aquatic wastes.

Edible oyster and mussels are being experimented in integrated farming. Integrated farming of edible oyster and mussel from the same rack structure was introduced in the Ashtamudi lake ecosystem. Similarly, preliminary experiments

are being conducted for testing the feasibility of growing finfishes; shrimp and crabs in cages suspended in the same farm and have given encouraging results.

Pearl culture has also undergone significant upgradation in farming and nucleation techniques. The production of large sized pearls ie > 6mm is being achieved in both east and west coasts. A technique for onshore pearl production is being developed at Visakhapatnam laboratory and the results are encouraging. The *in vitro* pearl production and the colour manipulation or make-up pearl production in Tuticorin laboratory are some of the significant achievements of the Central Marine Fisheries Research Institute recently.

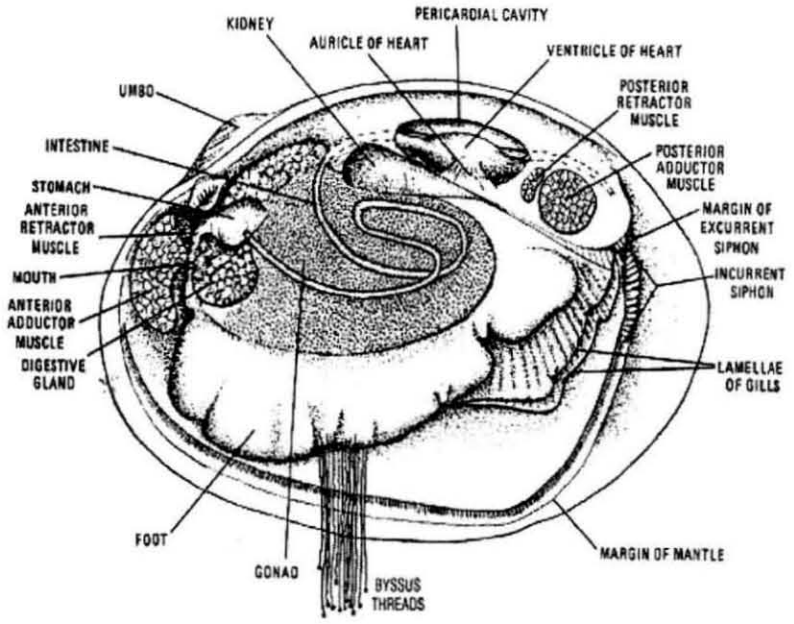
The institute has also recently developed technologies for *Haliotis* (Abalone) seed production and also squid and cuttlefish seed production.

3 Bivalve Taxonomy and Biology

P.K. Ashokan, R C of CMFRI, Veraval

The Bivalvia (also known as Lamellibranchia and Pelecypoda) represents the second largest class of Mollusca. Compared to the gastropods, the bivalvia are a more restricted group and have a specialisation devoted to a narrower range of pattern. Bivalves are more sedentary though in most of them the foot is still well developed. Very few however crawl over the substrate in the primitive molluscan way. Many species burrow into soft sand and mud, or even bore into rock and wood, a large number are permanently anchored to the ground, and among these the foot is usually reduced and sometimes quite lost.

Bivalves are bilaterally symmetrical, laterally compressed molluscs with extensive lateral mantle lobes, which secrete a single shell of two valves and a ligament, which joins them dorsally at a hinge. The head is rudimentary and lacks a radula and most of the sensory structures are located in the mantle border. They are mostly ciliary feeders, with sieving and sorting mechanisms on labial palps and a large leaf like ctenidium. The foot is compressed and adopted for burrowing, except in sedentary forms where it is rudimentary. Majority are marine. Freshwater representatives are less and there are no terrestrial forms. Fertilisation is usually external, followed by trochophore and veliger stages and a metamorphosis to adult form.



Typical bivalve anatomy

The class Bivalvia can be divided into three Sub-classes:

Sub-Class 1 Protobranchia:

These are primitive bivalves in which the ctenidia are posteriorly placed and consists of almost flat, horizontally placed, non-reflected filaments which divide the mantle cavity on each side into lower inhalant chamber and an upper exhalent chamber that contains hypobranchial glands. Foot not compressed ventrally but flattened to a crawling sole, always with two adductor muscles. Feeding primarly by means of enlarged labial palps, often provided with palp proboscides. Includes the family Nuculidae, Nuculanidae, Solemyidae and Malletiidae.

Sub-Class 2 Lamellibranchia:

Bivalves in which emphasis on feeding relates to ctenidia rather than to the palps, which are reduced; ctenidial filaments elongate and reflected to form two-sided lamellae usually being united by interlamellar junctions. Adjacent filaments linked by ciliary junctions (filibranchiate) or by vascular interfilamentar junctions (eulamellibranchiate). Adductor muscle two and equal (isomyarian) two and unequal (hetromyarian) or reduced to one (posterior) (monomyarian).

Lamellibranchia has six orders namely Taxodonta, Anisomyaria, Heterodonta, Schizodonta, Adapedonta and Anomalodesmata. Among these orders, Anisomyaria and Heterodonta are the most varied and of considerable economic importance.

Order Anisomyaria: Gills usually filibranch and with vascular interlamellar junctions; adductor musculature heteromyarian or monomyarian resulting in drastic changes in symmetry; hinge variable; mantle lobes free except for separation of exhalent aperture; usually no siphons; foot reduced or absent; many species sedentary. Includes families Mytilidae, Pteridae, Pinnidae, Pectinidae, Limidae, Anomiidae, Ostreidae.

Order Heterodonta: Gills eulamelliranchia, adductor muscle similar; hinge dentition heterodont (with cardinal and lateral teeth); mantle edges usually united at one or more points posteriorly, leading to development of siphons. Includes families Astardae, Carditidae, Sphaeriidae, Corbiculidae, Cyprinidae, Dressenidae, Lucinidae, Chamidae, Cardiidae, Tridacnidae, Veneridae, Mactridae, Amphidesmatidae, Donacidae, Tellinidae, and Solecurtidae.

Sub-Class 3 Septibranchia:

Gills in the form of a muscular septum which pumps water through the mantle cavity; mantle edges mostly free; adductor muscles equal; hinge weakly denticulate or edentate; macrophagous feeders Incledes the families Verticordiidae, Poromyidae, Cuspidariidae.

Edible Oysters

Edible oysters' belonging to the family Ostreidae.are found in hard substratum in the bays and creeks near coastal waters. They are attached permanently to the to the substratum.

Taxonomy

In India, six species of oysters are reported. They are the Indian backwater oyster *Craassostrea madrasensis* (Preston), Chinese oyster *C.rivularis* (Gould), west coast oyster *C.gryphoides* (Schlotheim), Indian rock oyster *Saccostrea cucullata* (Born), Bombay oyster *Saxostrea cucullata* (Awati and Rai) and the giant oyster *Hyostissa hyotis* (Linnaeus) are found.

Craassostrea madrasensis (Preston): Shell straight, shape irregular, covered by numerous foliaceous laminae, left valve deep, right one slightly concave, hinge narrow and elongated, adductor scar sub central, dark purple in colour, inner surface of valve white, glossy and smooth, purplish black colouration on the inner margin of the valve.

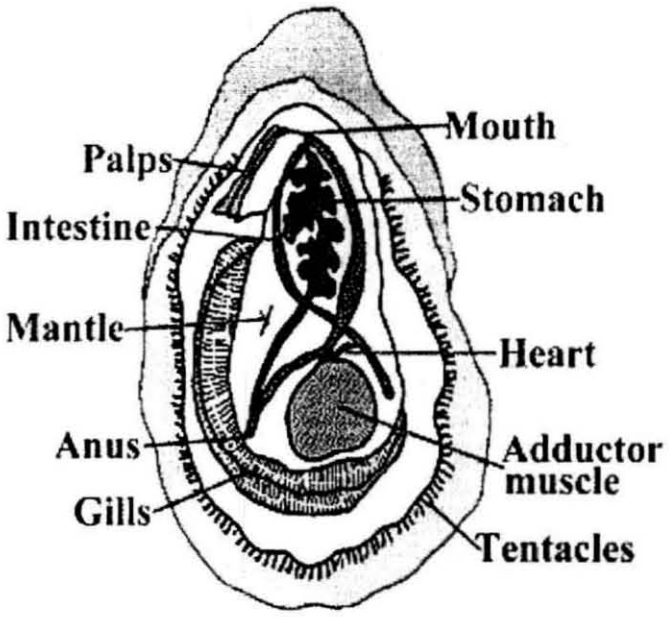
C.gryphoides (Schlotheim): Shell oblong narrow in the anterior margin and broader in the posterior margin, laminated, lower valve very thick, especially in the anterior region below the ligamental area. Muscle scar more or less heart shaped and pearly white. Upper valve thin flat and opercular, no denticles on the margin.

C.rivularis (Gould): Shell valves large, roughly round, flat, thick and with a shallow shell cavity. Left valve is thick and slightly concave and the right one is about eh same size or slightly larger. Adductor muscle scar is oblong and white or smoky white in colour.

Saccostrea cucullata (Born): Shell more or less trigonal, sometimes oblong, extremely hard and plaited. The margins of both the valves have well developed angular folds sculptured with laminae. Small tubercles present along the inner margin of the right valve and there are corresponding pits in the left valve. Adductor scar is kidney shaped.

Biology

Being sedentary and attached to the substratum by the cupped lower valve, the upper valve acts as a lid. The food consists of organic detritus and phytoplanktons. The growth of C.madrasensis has been studied in different locations showing variations. In Kakinada Bay it grew from 27mm to 72mm in 8 ½ months. In Adyar estuary it attains 50.6 mm length in 13 months. In Vellar estuary it attains 48.8, 85 and 111.7 mm atages 1-3 respectively. N theTuticorin Bay the oyster grows to 87 mm at the end of first year. I the Cochin backwaters, spat of 10mm modal length grow to 55mm modal length in about 6 months. In the Kakinada Bay, C.madrasensis spawns during January-June. In Adyar estuary, it spawns during October-December and again in March-April. At Tuticorin biannual spawning takes place during July-September and February-April.



General anatomy of Edible oyster - Diagrammatic representation

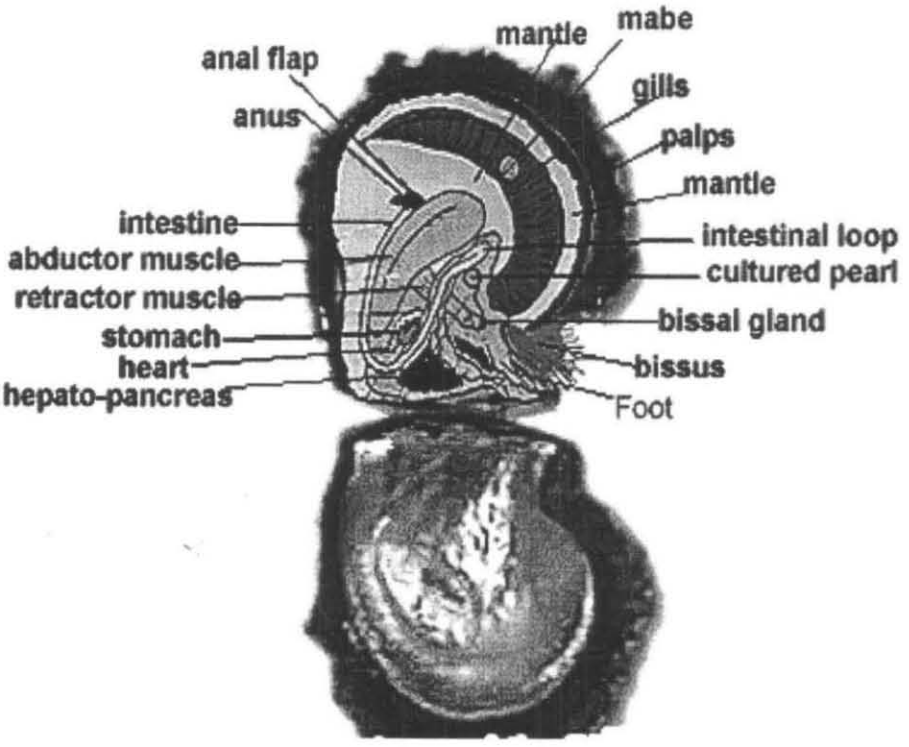
Mussels

The genus Perna comes under the Family Mytilidae. In India, there are two species, the green mussel *Perna viridis* and the brown mussel *Perna indica*. Apart from the colou of the periostracum, the mantle margin is brown colour in *P.indica* wheras in *P.viridis* it is yellowish green in colour. In *P.viridis* the anterior end of the shell is pointed with the beak turned down and in *P.indica* it is pointed and strainght. In *P.viridis*, the hinge teeth show two small on the left valve and one on the right valve and in *P.indica* there is one large tooth on the left valve and a corresponding depression on the right valve. The mussels have a foot, which secretes the byssal threads. It can discard it and produce fresh threads enabling it to change the location of attachment.

They are filter feeders of phytoplanktons. In Kakinada bay, *P.viridis* in the natural bed grows 63 mm in 6 months, 91.5 mm in one year, 117 mm in 2 years, 129 mm in 3 years and 135 mm in 4 years. When cultured on ropes in the raft, the growth was from 21.7mm to 66.6mm in 5 months. At Calicut the green mussel of 23.6 mm average length suspended from the raft in the open sea in rope culture attaned 888.2 mm in 5 months whereas in the natural bed during the same period grew to 66.9mm. P.viridis attains sexual maturity at 15.5 to 28 mm. The spawning period in the Kakinada bay s from December to July with peak activity between January and May. At Calicut spawning takes place during July to November with peak activity in August – October.

The green mussel *Perna viridis*

Pearl oysters



Pearl oyster: Internal anatomy

Taxonomy

The true pearl oyster belongs to the genus Pinctada (Roding) under the family Pteriidae, order Dysodonta. Members belonging to the Pteriidae family are characterized by a straight hinge with 1–2 small tooth-like thickening, a cavity below the anterior angle for the byssus, and usually a scaly surface of the outer shell valves. The family includes the pearl oysters belonging to the genus Pinctada and the winged oyster shells of the Pteria genus. In Pteria spp. the shell width is much longer than the height and the hinge angle is prominent and pronounced.

In Pinctada spp. the hinge is rather long and straight, the long axis of the shell is at a right angle to the hinge, the left valve is slightly deeper than the right and there is a byssal notch on each valve at the base of the anterior lobe. The colouration of periostracum varies and is often brownish with radial markings.

Six species of pearl oysters, *Pinctada fucata* (Gould), *P. margaritifera* (Linnaeus), *P. chemnitzii* (Philippi), *P. sugillata* (Reeve), *P. anomioides* (Reeve) and *P. atropurpurea* (Dunker) occur along the Indian coasts. Their morphological characteristics are as follows:

Pinctada fucata (Gould)

The hinge is fairly long and its ratio to the broadest width of the shell is about 0.85 and that to the dorsoventral measurement is about 0.76. The left valve is deeper than the right. Hinge teeth are present in both valves, one each at the anterior and posterior ends of the ligament. The anterior ear is larger than in the other species, and the byssal notch, at the junction of the body of the shell and the ear, is slit-like. The posterior ear is fairly well developed. The outer surface of the shell valves is reddish or yellowish-brown with radiating rays of lighter colour. The nacreous layer is thick and has a bright golden-yellow metallic lustre.

Pinctada margaritifera (Linnaeus)

The hinge is shorter than the width of the shell and is devoid of teeth. The anterior border of the shell extends in front of the anterior lobe. The byssal notch is broad. The anterior ear is well developed while the posterior ear and sinus are absent. The posterior end of the shell meets the hinge almost at a right angle. Shell valves are moderately convex. Externally, the shell is dark grayish-brown with radially disposed white spots. The nacreous layer is iridescent with a silvery lustre except distally where it is black. This pearl oyster is also known as the Black-lip pearl oyster due to the dark marginal colouration of the shell. The width of the nacreous region at the hinge is about 2/3 that of the broadest part of the valves.

Pinctada chemnitzii (Philippi)

The shell is very similar to that of *P.fucata* except that the posterior ear is better developed and the convexity of the valves is much less. The anterior ear is well developed and the byssal notch is slit like. The hinge is almost as long as the antero-posterior measurement of the valves. Both the anterior and posterior hinge

teeth are present, the former is small and rounded and the latter prominent and ridge-like starting a little in advance of the posterior region of the hinge ligament. The posterior ear and the posterior sinus are well developed. The shell valves are yellowish externally with about four or more light brownish radial markings from the umbo to the margin of the shell. The growth lines of the shell are broad. The nacreous layer is thin and bright, while the non-nacreous layer is yellowish-brown.

Pinctada sugillata (Reeve)

The hinge is considerably shorter than the antero-posterior axis of the shell with a ratio of 1:1.3. The anteroposterior measurement is almost equal to the dorso-ventral measurement. The anterior ear in both valves is small and the byssal notch is a moderately wide slit. The anterior ears are slightly bent towards the right. The posterior ear and sinus are poorly developed. The convexity of the valves is not prominent, especially that of the right valve. The hinge teeth are small and the posterior one is slightly elongated. The shell valves are reddish-brown with six yellowish radial markings.

Pinctada anomioides (Reeve)

The hinge is shorter than the width of the broadest region of the antero-posterior axis of the shell with a ratio of 1:1.2–1.5. The hinge and dorso-ventral axis have a ratio of 1:1.4. Hinge teeth are absent or poorly developed. The anterior ear is moderately developed and the byssal notch at its base is deep. The posterior ear and sinus are absent. The shell valves are translucent and externally yellowish or grayish. Some shells have faint radial markings. The nacreous layer is slightly iridescent.

Pinctada atropurpurea (Dunker)

The shell is roundish and its hinge narrow. The valves are thin, translucent and moderately convex. A poorly developed anterior hinge tooth is present in some oysters. The shell valves are copper coloured.

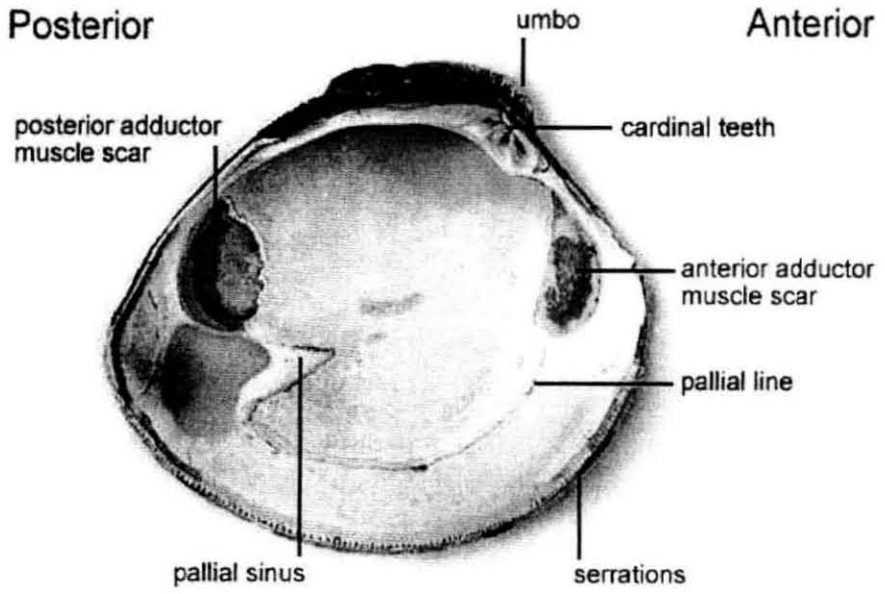
Biology

Pearl oyster is a filter feeder. The food sorting mechanism of pearl oysters is considered not very efficient as several organisms that cannot be digested find its way into the stomach and intestine. Sexes are separate but cannot be distinguished externally. The reproductive system consists of a pair of gonads, which spreads over the hepatopancreas and intestine in mature condition. It is pale yellow in males and deeper shade in females. *P.fucata* attains sexual maturity at about 15.5 mm size at eh age of 3-4 months. In the Gulf of Mannar, it spawns twice a year during June-August and November- January coinciding with the monsoons. At the Kusadai Island, *P.fucata* grows to 45 mm, 55mm, 60mm, and 65mm and70mm during five year period. Hatchery produced pearl oysters has been reported to attain model size of 47.5, 64.5 and 75 mm at the end of first, second and third years.

Distribution

In the Indian waters, six species of pearl oysters occur but only *P. fucata* has contributed to the pearl fisheries in the Gulf of Mannar and Gulf of Kutch. In the Gulf of Mannar, the pearl oysters occur in large numbers on the submerged rocky or hard substrata known as paars. The paars lie at depths of 12 to 25 m off the Tuticorin coast along a stretch of 70 km. In the Palk Bay, *P. fucata* occurs sporadically on loose sandy substratum attached to submerged objects in littoral waters. In the Gulf of Kutch, the pearl oysters are found as stray individuals on the intertidal reefs known as khaddas. In the southwest coast of India at Vizhinjam, Kerala coast, large numbers of spat of *P. fucata* have been collected from mussel culture ropes. The blacklip pearl oyster, *P. margaritifera* is confined mostly to the Andaman Islands where it is common in some places. From Lakshadweep, settlement of spat of *P. anomioides* has been observed on the ridges of rocks and corals.

Clams



Clam :Inner surface of left valve

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Cephalopod Taxonomy and Biology

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Introduction

Cephalopods are marine molluscs and there are about 600 species in the world oceans, which are diverse in form, size and nature. Of these less than a hundred species are of commercial importance. Cuttlefishes, squids and Octopods are the three major groups of cephalopods, which belong to the highly evolved class of the phylum Mollusca, namely Cephalopoda, animals with feet around head. There are about 80 species of cephalopods of commercial and scientific interest distributed in the India Seas.

Taxonomic Position of exploited and potentially important cephalopods of India

Class	CEPHALOPODA	
Sub class	NAUTILOIDEA	
Family	Nautilidae	
Genus	*Nautilus*	*Nautilus pompilius*
Subclass	COLEDIDEA	
Order	Sepiodidea	
Family	Sepiidae	
Genus	*Sepia*	*Sepia pharaonis*
		Sepia aculeata
		Sepia trygonina
		Sepia brevimana
		Sepia elliptica
		Sepia prashadi
Genus	*Sepiella*	*Sepiella inermis*
Family	*Sepiolidae*	
Genus	*Euprymna*	*Euprymna stenodactyla*
Order	TEUTHOIDEA	
Suborder	Myopsida	
Family	Loliginidae	
Genus	*Loligo*	*Loligo duvaucelii*
		Loligo uyii
Genus	*Doryteuthis*	*Doryteuthis singhalensis*
		Doryteuthis sibogae
Genus	*Sepioteuthis*	*Sepioteuthis lessoniana*
Genus	*Loliolus*	*Loliolus investigatoris*
Suborder	Oegopsida	
Family	Onychoteuthidae	
Genus	*Onychoteuthis*	*Onychoteuthis banksii*
Family	Ommastrephidae	
Subfamily	Ommastrephinae	
Genus	*Ommastrephes*	*Ommastrephes bartrami*
Genus	*Symplectoteuthis*	*Symplectoteuthis oulaniensis*
Family	*Thysanoteuthis*	*Thysanoteuthis rhombus*

Order	OCTOPODA	
Suborder	Incirrata	
Family	Octopodidae	
Genus	*Octopus*	*Octopus dollfusi*
		Octopus aegina
		Octopus membranaceous
Genus	*Cistopus*	*Cistopus indicus*
Genus	*Hapalochalaena*	*Hapalochalaena maculosa*
Genus	*Berrya*	*Berrya keralensis*
Family	Argonautidae	
Genus	*Argonauta*	*Argonauta argo*
		Argonauta hians

1. SUBCLASS NAUTILOIDEA

Shell external, coiled and chambered, more than 10 (63 to 94) circumoral appendages without suckers, two pairs of gills, funnel bilobed.

2. SUBCLASS COLEOIDEA

Shell internal, embedded in tissue, calcareous, chitinous or cartilaginous, 8 or 10 circumoral appendages with suckers, only one pair of gills, funnel tube-like.

1. Order Sepiodea

Internal shell (sepion) calcareous and either straight and laminated or coiled and chambered or vestigial and chitinous or absent; eyes covered with skin and a supplementary eyelid present; eight sessile arms; two tentacular arms contractile and retractile into pockets; suckers without stalks; fin lobes free posteriorly.

2. Order Teuthoidea

Internal shell (gladius or pen) chitinous feather or rod-shaped, eight sessile arms; two tentacular arms contractile but not retractile, pockets absent, tentacles lost secondarily in some, suckers stalked and with or without hooks; fin lobes fused posteriorly. Eyes either covered or open and without supplementary eyelid.

3. Order Octopoda

Internal shell vestigial and cartilaginous except in females of *Argonauta* which has an external, calcified shell. Eight arms, suckers without stalks and without chitinous rings; tentacles absent; fins absent except in a few deep water species; light organs absent.

Key to the Identification of Genera and Species of Commercially Exploited Cephalopods of the Indian Seas

ORDER SEPIOIDEA

The salient features to be examined for the identification of genera and species of cuttlefishes are as follows:

1. Cuttlebone: General shape, nature of the dorsal surface, structure of the inner cone, number and nature of grooves and ridges on the ventral side, the nature of growth lines found in the striated area and the spine
2. Tentacular clubs: Number of transverse rows of club suckers and their relative size (diameter), the nature of protective membrane on the sides of the clubs.

3. Hectocotylization: Structure of the hectetocotylized arm with regard to the modified portion, the number and arrangement of normal and modified suckers and the extent of modification of the arm.
4. Shape and disposition of fins along the mantle.
5. In some species the characteristic external colouration and colour pattern of the mantle, head and arms noticeable in fresh material.

1. Body either elongate and broad or very slender an dorsoventrally flattened; fins marginal and narrow, extending all along mantle on either side; internal shell (sepion) present; head free from dorsal mantle; light organs absent (Family: Sepiidae).....2

Body saccular, wide, round bottomed; fins circular; internal shell lacking; dorsal mantle and head united by a nuchal commissure; saddle-shaped light organ presnt on ink sac. (Family: Sepiolidae)...*Euprymna stenodactyla*

2. Body without a glandular pore at posterior extremity; cuttlebone mostly with a spine (rostrum) at posterior end. (Genus: *Sepia*) 3

Body with a distinct glandular pore at posterior extremely on ventral side; with brownish fluid oozing out; cuttlebone devoid of spine (Genus: *Sepiella*).....*Sepiella inermis*

3. Body small and narrow, broadest part of body excluding fins distinctly less than half mantle length; fins narrow and marginal; cuttlebone very much slender and lanceolite in shape 8

Body wide and muscular, ovoid or elliptical in shape; broadest part of body excluding fins equal or distinctly more than half of mantle length; fins marginal, moderate to wide; cuttlebone chalky, elongate, wide and nearly ovoid in shape 4

4. Tentacular clubs with suckers of unequal size, a few in the manus very much enlarged; mantle, head and arms with transverse stripes 5

Tentacular clubs with smaller and subequal suckers but none greatly enlarged; no transverse stripes on mantle, head and arms 6

5. Body robust, fins braod commencing from edge of anterior mantle margin; tentacular clubs moderately long and well expanded; 5 or 6 suckers in middle row of manus greatly enlarged; cuttlebone broad, thick and with a midventral groove flattening anteriorly in striated area; striae '^' shaped; inner cone forms a conspicuous yellowish flat ledge; a sharp thick spine present; when live, body brownish, tigerstripe pattern prominent. ... . *Sepia pharaonis*

Body not robust; fins narrow commencing a few mm behind edge of anterior mantle margin; tentacular clubs short, expanded; not more than 3 suckers in middle row of manus greatly enlarged; cuttlebone narrow, midventral groove narrow and distinct, striae anteriorly broadly truncate

with lateral corners slightly produced forward; dorsal surface pinkish in colour; a sharp thin spine present. When live, dusty brownish, transverse stripes less distinct .....*Sepia prashadi*

6. Tentacular clubs very long, with 10-14 rows of minute subequal SUCKERS. Cuttlebone broad and thick with a median longitudinal ridge with a faint groove running medially on striated area; inner cone forms a ledge-like callosity ..... *Sepia aculeata*

Tentacular clubs either short or moderately long, with 6-10 rows of small suckers. Cuttlebone thin and elliptical of acuminate in shape with or without ridges on striated area, innercone without callosity 7

7. Tentacular clubs short with 6-8 small subequal suckers. Cuttlebone flat and distinctly acuminate anteriorly, dorsal surface rugose, a shallow median groove in the striated area, the striae '^' shaped with a median shallow groove broadening anteriorly; inner cone and its limbs pinkish in colour; spine small, sharp and slightly curved.....*Sepia brevimana*

Tentacular clubs moderately long, with 10 rows of small suckers of uniform size. Cuttlebone thin, elliptical in shape, dorsal surface smooth; two conspicuous lateral ridges more prominent anteriad resulting in three longitudinal furrows in striated area; spine thick, sharp, long and well curved .....*Sepia elliptica*

8. No fleshy projections on head; fins extend upto end of mantle; tentacles with short clubs, suckers in eight rows, about five in third row enlarged. Cuttlebone lanceolite with acuminate anterior tip with edges of outer cone winged giving an arrow head appearance; spine small. .....Sepia *trygonina*

ORDER TEUTHOIDEA

The various characters used in identifying the different species of neritic and oceanic squids (Order Teuthoidea) are given below. The definitions and details of important characters and terms are given in the glossary of technical terms.

1. General shape of the mantle
2. The shape and proportion of fins, the contour of the anterior and posterior margins of the fin lobes; position of fins on the mantle viz. terminal or marginal; united or separated at the posterior end.
3. The relative size of head and arms; size, shape, number and arrangement of suckers on the arms and tentacular clubs; the nature and dentition of the chitinous rings of the suckers.
4. Presence of hooks and / or suckers on the arms and tentacular clubs.
5. Details of hectocotylization, the number and arrangement of normal and modified suckers and the extent of other modifications affecting the arm.
6. Presence or absence of light organs (photophores), their shape, number and position.
7. Nature of the funnel locking apparatus.

8. Presence or absence of accessory nidamental glands.
9. Shape of gladius.
10. Shape of eggs and egg clusters.

1. Eyes completely covered with a corneal membrane (MYOPSIDA: Neritic Squids) 2

   Eyes not covered with a corneal membrane and open to the surrounding medium (OEGOPSIDA: Oceanic squids). 7

2. Body elongate, cylindrical in outline; fins marginal, wide and muscular, very long almost running along entire length of mantle; elliptical in shape .....*Sepioteuthis lessoniana*

   Body elongate, narrow, either slender or stout, sides parallel or tapering; fins narrow, terminal running less than 65 per cent of mantle length and either rhombus (*Loligo*) or heart-shaped *Loliolus*) 3

3. Body elongate or short and stocky, posterior end of mantle blunt; fins broad, rhombic or heart-shaped, with head and arm crown more than 50 per cent of mantle length; vane of gladius broad with thin curved margins 4

   Body narrow and slender, posterior end of mantle pointed; head with arm crown distinctly less than 50 per cent of mantle length 6

4. Small squids. Mantle length of adults less than 60 mm; fins heart shaped; vane of gladius conspicuously broad at midlength .....*Loliolus investigatoris*

   Moderately large squids; fins typically rhomboid; vane of gladius narrow throughout. 5

5. Body elongate, mid-rib of gladius clearly visible through mantle skin; fins 50-57 per cent of mantle length; tentacular clubs large median manal sucker ring with 14-17 teeth; in males distal half of left ventral arm hectocotylized, papillae not fused.....*Loligo duvaucelii*

   Body short and stout; mid rib of gladius clearly visible through dorsal mantle skin as a median dark line; fins 55-65 per cent of mantle length; Tentacular clubs have median manal suckers with smooth rings; in males left ventral arm hectocotylized almost the entire arm; papillae on ventral margin fused with membrane ..... *Loligo uyii*

6. Mantle very long and slender with a ridge along midline in males; fins wide and long and more than 60 per cent of mantle length; more than half of left ventral arm hectocotylized distally in males; gladius narrow with almost straight margins and tapering gradually to a narrow point ..... *Doryteuthis singhalensis*

Mantle long, narrow and slender, no ridge but chromatophore concentration ventrally along midline; fins narrow and less than 60 per cent of mantle length; less than half of left ventral ventral arm hectocotylized distally in males; gladius narrow, sharply acuminate posteriorly ..... *Doryteuthis sibogae*

7. Oceanic squids with muscular body; head with nuchal folds on dorsal side at posterior end; rachis of gladius visible as a longitudinal ridge middorsally along the entire length of mantle; tentacular clubs with two rows of hooks, marginal suckers lacking. .....*Onchoteuthis banskii*

   Oceanic squids with muscular body; head without nuchal folds on dorsal side at posterior end; rachis of gladius not visible through dorsal mantle; tentacular clubs without hooks 8
8. Funnel locking cartilage ' ' shaped consisting of a narrow longitudinal groove and a short transverse groove branching from it medially. Fins broad and rhombus-shaped occupying nearly entire length of mantle ..... *Thysanoteuthis rhombus*

   Funnel locking-cartilage ' ' shaped consisting of a vertical groove and a transverse groove at right angles to it posteriorly. Fins terminal and less than 60 per cent of mantle length 9

9. Funnel and mantle cartilages of the locking apparatus fused together. An oval photophoric patch present middorsally near anterior margin of mantle; muscle of mantle ventrally without embedded light organs; two intestinal photophores present .....*Symplectoteuthis oualaniensis*

ORDER OCTOPODA

1. Cephalopods with eight arms; without an external shell; internal shell either vestigial or lacking; no great disparity between males and females in size; benthic in habit (Family Octopodidae) 2

   Cephalopods with eight arms; external shell present (in females); sexual dimorphism very marked, males very small; pelagic in habit (Family Argonautidae) 6

2. Right third arm in males hectocotylized with well developed ligula, calamus and spermatophoric groove; no water pores and embedded pouches between arm bases.....3

   Hectocotylized arm only slightly modified, ligula small about 3 per cent of arm. Small water pores leading to embedded pouches between bases of arms on oral surface ..... *Cistopus indicus*

3. Body either globular or slightly elongate and or firm consistency; arms long and tapering with moderately developed web between them; funnel not fused with head.....4

4. Body elongated oval; moderately large in size; dorsal surface of body and arm with reticulate pattern; no concentric rings of chromatophores on the body; ligula bout 5 to 10 per cent of arm; penis and diverticulum long 5

   Body globular smaller in size; skin smooth without reticulate pattern; while fresh dusty brown in colour with prominent bluish rings on mantle, head, web and arms .....*Haplochlaena maculosa*

5. Eyes prominent; a single large cirrus posterior to each eye. Ligula small, 5 to 8 per cent of arm; with shallow groove; penis and diverticulum together form U-shaped loop; spermatophores long and unarmed .....*Octopus aegina*

   Eyes inconspicuous; no eye cirrus. Ligula 8 to 10 percent of arm; with well formed groove and calamus. Long penis and short diverticulum together form reversed 6-shape; spermatophores long and armed with spines .....*Octopus dollfusi*

In addition to the above mentioned species, some more species of octopods such as *Octopus cyaneus*, *O. globosus, O. membranaceous, O. macropus, O. vulgaris, O. tetricus, Scaeurgus unicirris* are also known to occur in the Indian Seas and other parts of the Indian Ocean.

Biology of Exploited Species

All investigations on cephalopod biology centre around the commercially exploited species such as the Palk Bay squid, *Sepioteuthis lessoniana* (Rao, 1954), *L. duvauceli* (Kore and Joshi, 1975; Oommen, 1977; Silas et al., 1985; Rao, 1988; Mohamed, 1993), *Sepia pharaonis*, *S. aculeata*, *Sepiella inermis* (Oommen, 1977; Unnithan, 1982; Silas et al., 1985) and *Octopus dollfusi* (Sarvesan, 1969). The aspects of biology of cephalopods detailed here pertain mainly to *L. duvauceli*, *S. pharaonis* and *S. aculeata*.

Food and Feeding: Adult cephalopods are voracious and active carnivores feeding mainly on fishes and crustaceans. Fish always occurs in the diet of *L. duvauceli* of all sizes. The preference to crustacean meal declines with increase in size and there is evidence of cannibalism above 80 mm DML (Kore and Joshi, 1975; Oommen, 1977). Cephalopods are preyed upon by a variety of marine fishes (including tunas and billfishes) and cetaceans (Silas et al., 1985). Many workers have noticed the predominance of empty stomachs in samples and slackness in feeding during spawning period (Oommen, 1977). This may be due to the partial ingestion; fragmentation and rapid digestion of prey (Pierce et al., 1994).

Age and Growth: The relationship between length and weight of Indian cephalopods has been reported to be allometric with the 'b' value of the regression near to 2 than 3 (Meiyappan et al., 1993; Nair et al., 1993; Rao et al, 1993). This relationship is also significantly different for males and males and females (Mohamed, 1996).

Growth in cephalopods has been perceived to be linear, exponential, asymptotic and/or oscillating and Pauly (1985) advocated the use of VBGF model with seasonal oscillation as a means of standardising growth estimates of different cephalopods allowing comparative studies to be made. Studies on the growth of Indian cephalopods have been made by using the asymptotic (VBGF) model (Kasim, 1985; Philip and Ali, 1989; Meiyappan and Srinath, 1989; Meiyappan et al., 1993; Nair et al., 1993; Rao et al, 1993; Mohamed, 1996) and the seasonally oscillating version of VBGF (Mohamed and Rao, 1997). Clear sexwise difference in growth rate has been reported from Indian waters. In the case of *L. duvauceli* and *S. pharaonis* females grow faster than males, while in the case of *S. aculeata* males grow faster than females. A comparison of the results of various studies carried out in India is given in Table 2.

Size at First Maturity: Pioneering work on the reproductive biology of the Palk bay squid *Sepioteuthis lessoniana* has been made by Rao (1954). Later, Silas et al. (1985) reported on the maturity of three species of squids and six species of cuttlefishes. They reported that in *L. duvauceli* males attained sexual maturity earlier than females and in all species spawning is prolonged. The size at first maturity of male and female squids and cuttlefishes along west and eastcoast of India is shown in Table 3.

Maturity Stage and Spawning: Silas (1985) described and standardised the maturity stages for biological studies of squids and cuttlefishes. He described a simple 4 point (Immature, Maturing, Mature and Spent) maturity scale, which has since been used by all workers on Indian cephalopods. Rao (1988) gave detailed descriptions of *L. duvauceli* maturity stages on the above line.

Similar to other tropical marine resources, cephalopods along the Indian coast are reported to spawn almost throughout the year. Information on this aspect is scanty, but the peak spawning period of some of the studied species is given in Table 4.

The squid *L. duvauceli* spawns throughout the year along both the coast, but along the westcoast, peak spawning has been observed during post monsoon i.e., Sep-Nov (Kore and Joshi, 1975; Silas et al., 1985; Mohamed, 1993). This species forms large schools (consisting of fully mature animals, 80% males) during this season, and becomes vulnerable to the purse seine fleet operating along Karnataka coast (Mohamed, 1993) and also to cast netters along coastal knee-deep water of Alleppey (Meiyappan and Srinath, 1989). Based on this observation, Mohamed (1993) opined that the squids congregate for spawning (copulation) in near shore areas after which the females migrate to the shallow subtidal regions with hard substratum for laying the fertilized eggs. Asokan and Kakati (1991) have collected such eggs from the subtidal areas of Karwar for rearing. From the sex ratio (M 80:

F 20) of such squid schools it would be easy to conclude that female die after spawning (semelparity is common among cephalopods world-wide). However, based on the relatively low GSI levels and the occurrence of mature females over a wide range of size classes, Mohamed (1993) concluded that this species is a multiple spawner and not a semelparous species. More evidence needs to be gathered to reach a final conclusion. Similar studies on other commercial cephalopods are lacking.

Fecundity: Estimates on the fecundity of Indian cephalopods are few. Unnithan (1982) reported that in the spineless cuttlefish *S. inermis* the total number of ripe eggs of individuals between 69-71 mm DML was from 470 to 850 (average 14.9 eggs/g body weight). In the squid *L. duvauceli* Rao (1988) reported that on an average an individual produced 5300 eggs and that there was good correlation between length, ovary weight and fecundity. Mohamed and Nagaraja (1997) estimated the fecundity of the same species varied between 2000 to 14000 eggs (average 65 eggs/ g body weight). In general, fecundity is low in cephalopods because of the absence of a larval stage and the hatchlings are virtually miniature adults.

Stock Assessment and Management

Ever since the CMFRI initiated a major research project on the biology and stock assessment of cephalopod resources of India, a number of research papers have been published on the subject. Mostly F based models have applied to study cephalopod stocks. In the first study on Indian cephalopod stocks, Silas et al (1985), used length cohort analysis to estimate stock sizes. Later studies (Meiyappan et al., 1993; Nair et al., 1993 and Rao et al., 1993) also used cohort analysis to estimate mortality and stock and the yield and biomass estimates were obtained with length based Thomson and Bell analysis. Mohamed (1996) used the yield per recruit model to estimate MSY for Mangalore populations of *L. duvauceli*. Later Mohamed and Rao (1997) assessed the squid yield along Karnataka coast using the TB model to derive MSY and MSE. They also studied the relationship between spawning stock and recruitment of squids to assess the productivity of the population in terms of recruitment. They found that Ricker's stock recruitment curve could adequately explain the variation in recruitment with respect to spawning stock biomass (SSB).

Most of these studies indicated that cephalopods were either under exploited (e.g. *S. pharaonis* and *S. aculeata* along eastcoast) or optimally exploited (Table 2). While Mohamed (1996) and Mohamed and Rao (1997) found squid stock along Karnataka coast to be marginally over exploited.

Cephalopods are not a targeted fishery along the Indian coast (excepting seasonally along the SW coast) and therefore, it is difficult to set management targets and many of the models applied would have little relevance. Yet, Rosenberg et al (1990) suggests that the most effective means of managing cephalopod fisheries is by regulating fishing effort, which will reduce the risk of recruitment overfishing. The present ban on trawl fishing during the monsoon as variously practised by different maritime states along the westcoast is in effect a means of regulation of fishing effort and should be continued.

Utilization and Marketing

There is very little internal market demand for cephalopods and consequently almost all the catch is exported. While the quantity peaked in 1995, when cephalopods formed about the 45% of the total quantity exported, the annual average is about 25%. However, the value of cephalopods in total marine exports has remained at 15% from 1992 onwards without much variation. In 1996 the value of cephalopods exported amounted to more than Rs 8500 million. Categorywise, squid products are the maximum in all years followed by cuttlefish products. The products include dried, frozen whole, filleted, tentacles, rings, roe, wings, IQF and bones and ink. Octopus products exported are meagre, but from 1994 onwards there is rising trend in its exports. The main markets for export of Indian cephalopods are Europe, Japan and China.

5 Ecology of Pearl Oyster Beds and Pearl Fisheries of India.

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World distribution

The pearl oysters belong to the genus *Pinctada (Roding)* come under the family Pteriidae. They occur in almost all the seas of the tropical and sub tropical belt. They inhabit the sea bottom from low tide level to depths down to 80 m. Although 28 species of pearl oysters are reported, only 3 species have been found to produce pearls of gem quality and have commercial value. They are *Pinctada maxima* (Jameson), *P. margaritifera* (Linnaeus) and *P. fucata* (Gould). The pearl oysters occur in the Persian Gulf (Bahrain, Kuwait, Dubai, Muscat and Bushira), Red sea (Farasan Islands, South of Sabia and Jidda, West of Mecca and Sudan), Philippines, Japan, China, Korea, Myanmar, Indonesia, Papua New Guinea, French Polynesia, Cook Islands, Australia, Gulf of California, Mexico, Panama and Venezuela.

Distribution in Indian Waters

In the Indian waters, six species of pearl oysters namely *Pinctada fucata* (Gould), *P. margaritifera* (Linnaeus), *P. chemnitzii* (Philippi), *P. sugillata* (Reeve) and *P. atropurpurea* (Dunker) have been recorded. Among these, *P. fucata* is the most dominant species. It occurs in large numbers in pearl banks known as 'paars' in the Gulf of Mannar and in the intertidal reefs known as 'Khaddas' in the Gulf of Kutch. *P. fucata* is the only species which has contributed to the pearl fisheries in these two gulf regions. In the southwest coast in India at Vizhinjam, large numbers of spat of *P. fucata* have been collected from mussel culture ropes. *P. margaritifera* is confined mostly to the Andaman Islands where it is common in some places. From Lakshadweep, spat of *P. anomoides* has been recorded on the ridges of rocks and corals.

Topography

The pearl oysters are always found attached by byssus to some hard substratum such as rocks, dead coral outcrops or sand grit covered with marine organisms. In the Gulf of Mannar, the areas of occurrence of pearl oysters are known as pearl banks or "paars'. The Gulf has about 65 such pearl banks located between Kanyakumari and Rameswaram. These banks lie at a distance of about 12 to 20 km away from the coast at depths of 15 to 25 m. These paars are divided into three divisions viz., Northern or Kilakarai Division extending from Adam's bridge to Vaipar, the Central at Tuticorin Division extending from Vaipar to Manapad and the Southern or Kanyakumari Division extending from Manapad to Kanyakumari. Of these the central division is the most productive one in view of the fact that out of the 40 fisheries that had taken place between 1663 and 1961, 39 fisheries had

been in the paars located in this division. A notable feature of these fisheries is their irregular character, fishing sometimes being conducted after long intervals. This is due to the periodical decline of fishable quantities of pearl oyster population in the pearl banks for a number of years. The probable factors responsible for the decline of oyster population are failure of spatfall, pests like weaving mussels and boring worms, predation by gastropods, octopi, sharks, rays and sea breams, overgrowth of algae, changes in the oceanographic conditions, occasional silting and non-availability of sufficient number of breeding population. In the Gulf of Kutch, the pearl oyster reefs are scattered along the southern part of the Gulf of Kutch. There are about 42 known pearl oyster reefs covering an area of 24,000 ha located between Sachana on the east and Ajad on the west. These beds are located in the intertidal region and are exposed at receding tides.

Primary Production

In the Gulf of Mannar area where the pearl oyster beds are situated the productivity has been observe the reach 7.3 g c/m^2/day which appears to be fairly high when compared to the values obtained in other areas of east coast near shore waters.

Wind, Water Movement and Current

Wind velocity shows a trimodal oscillation with maximum in June, August-September and December and maximum in March-April. The velocity is greater in southwest monsoon period of water movement. Devanesan and Chidambaram (1956) state that the water drift and current over the pearl banks of Ceylon and India may carry the larvae of pearl oyster from one coast to the other, thus holding the view of interdependence of the pearl banks of Ceylon and India for getting replenishment of oyster population. There is another possibility also. Depending on the direction and rapidity of water movement the pearl oyster larvae, at the plantigrade stage, might reach such areas in the sea with unsuitable sea bottom where they perish after settlement. All these factors thus play a vital role.

Turbidity

Flood water discharged from east coast rivers during the northeast monsoon rains in October-November, carry with it considerable silt which creates great turbidity over the pearl beds, particularly over the shoreward lying banks. This introduces a new dimension to the problem of growth and survival of oyster population met within 12 - 15 m depth range.

Temperature

Unlike Japan, the variation in temperature is not much pronounced in the pearl banks of Gulf of Mannar. The general temperature of the seawater in the paar varies from 23.8°C (January) to 33.5° C (May); the monthly average ranging from 25.9°C to 31.5°C. There appears to be some correlation between temperature and the breeding behaviour of the pearl oyster. The breeding season is more restricted in higher latitudes and occurs during warmer months.

Salinity

The salinity of the Gulf of Mannar normally varies from 30 to 35 ppt. If salinity falls below 15 ppt, and if such condition is prolonged, it may lead to mortality. This may happen during unusual heavy rain and heavy discharge of freshwater by rivers in the farm.

Dissolved Oxygen

Values ranging from 6.84 ml/l in October to 3.4 ml/l in September appear to be common in pearl oyster beds. A trimodal curve has been noticed with distinct peaks in June, October and January with a decline in April, September and November. It looks as though the oxygen saturation is greater in northeast monsoon months and less in southwest monsoon months.

Associated Fauna and Flora

The very fact that the fauna and flora of the pearl banks comprise the whole assemblage of more than 2,700 species of animals and 200 species of plants, small and large, makes the study of interrelationship among them very complicated although it is well recognized that the nature and density of such animate surroundings have a profound effect on the well being of the stock of oysters in the beds.

Characteristic of the area is the dense growth of sponges, especially in the northern Vaipar area. *Aulo-sponges tubulatus* (Bowerbank). *Phakellia donnani*, *Siponochalina communis* (Carter), Iotrochota spp., *Clathria procera* (Ridley), C. *indica* Dendy, *Mycale grandis* Gray, *Zygomycale parishii* (Bowerbank), *Phyllo-spongia* spp., *Spongionella* spp. and *Suberites* spp. are abundant. Dense forest-like growth of the gorgonid *Juncella juncea* Pallas and *J. gemmacea* (Valenciennes) is noticed in the northern area.

The growth of the coral *Heteropsammia* sp. is characteristic of the paars. *Montipora* sp. and *Echinopora* sp. are the other corals in addition to *Porites* sp.

The molluscan fauna is mostly represented by myriad number of *Modiolus* spp. spreading like mattress on the bottom. Large *Pinna* spp. are found in good numbers rooted in this layer of sand covering the rock in many places. *Cypraea tigrinus* are seen in rocky pits. *Oliva* spp., *Conus* spp., *Nassa* sp. and *Bulla ampulla* are the other common shells..

Among the echinoderms *Lamprometra palmata palmata* (J. Muller) and *Comanthus (Comanthussis timorens)* (J. Muller) are the most common under rocky crevices and over the gorgonids and sponges. *Holothuria edulis* Lesson, *Protoreaster linki* (Blainville) and tests of *Clyspeaster humilis* (Leske) are the other common species.

The fish fauna is fairly rich and consists of *Scolopsis bimaculatus* Ruppell, *S. vosmeri* (Block), *Abalistes stellaris* (Bloch), *Upeneoides* spp., *Chaetodon* spp., *Pomacanthodes annularis* (Bloch) and *Lutjanus lineoiatus* (Ruppell). Large fishes

like *Gaterin* spp., *Ennaeacentrus miniatus* (Forsk), *Epinephelus* spp., *Lethrinus* spp. and *Siganus* spp. are abundantly seen.

The flora is poor in the southern area but in the Vaipar area *Gracilaria* spp., *Hypnea* spp. and *Sargassum* spp. are common.

The oysters are often found in clusters piled together in such profusion so as to interfer with one another's growth and stunting many.

Pearl Fisheries

The pearl fisheries of Madras State are known from time immemorial. All the ancient literature in Sanskrit and Tamil refer to these fisheries. The existence of the pearl fisheries on the Tamil Coast is evidenced by foreign writers. South India had much commercial intercourse and political relations with the countries of the East and West. Indian pearls were used extensively in Rome, Egypt, Babylon, Persia and Greece before the 4th century A.D. Between the 4th to 10th centuries A.D., the Arabs, Chinese, Egyptians and Greeks were trading in Indian pearls during that period. From the 10th to 12th century the Chola Empire monopolished the entire pearl fisheries of this area. From the 13th to 16th centuries, the pearl fisheries prospered under the Pandyas and the moors. The Portuguese had control over the pearl fisheries from 1524 to 1658, when the Dutch dispossessed them. The latter managed the pearl fisheries until the British took over in 1790 and controlled them until India attained independence in 1947.

The pearl banks came under the control of the British in 1796, from which year records of pearl fisheries were fairly well kept.

The pearl fisheries were placed under the control of the Madras State Fisheries Department in 1908 for proper conservation and development.

The Gulf of Mannar and the Gulf of Kutch are the well known haunts of this resource and pearl fisheries had been organized in the past from Tuticorin and Jamnagar respectively. The paars of Gulf of Mannar have yielded to very valuable fisheries in the past, the most successful of which has been the fishery series of 1955-1961.

Since 1961 the pearl banks have again become barren and the present indication are that there are no prospects of a pearl fishery in the immediate future. Even so it should be possible to collect oysters for experimental purposes.

While the pearl fishery of the Gulf of Mannar has been in existence from time immemorial, in the adjacent Palk bay only one pearl fishery was held in 1914 off Tondi. This did not prove commercially successful and attempts to find pearl oysters in fishable quantities in the subsequent years only yielded negative results.

In the Palk Bay area, the seabed does not appear to be conducive for the settlement of the oysters exept for a small stretch of ten kilometers distance from Dhanushkodi to Rameswaram where rocky patches occur at depths ranging from 7

meters to 13 meters. Apart from a freak fishery in 1914 held at Tondi lasting for 20 days there is no record of any other fishery having been conducted in the Palk Bay. Hence the Gulf of Mannar grounds are considered to be more important and productive.

The pearl fishery of the Gulf of Mannar have been of an irregular character and in the span of three centuries from 1663 to 1961 there have been only 38 official fisheries Table 1.

The Pearl fishery was conducted by the Department of Fisheries, Madras State, with Tuticorin as the base of operations. When the Departmental survey revealed the availability of fishable quantities of pearl oysters over 3 years age and when the evaluation of pearl content shows satisfactory results, a pearl fishery is declared to the public. The fishing season lasts for a month or two depending on the favourable weather conditions and the strength of the oyster population.

Table 1: Available Records Show that from 1663, there were 38 Pearl Fisheries

S. No.	Year	Gross Revenue
1	1663	Fl 18,000
2	1669	No Particulars
3	1691	No Particulars
4	1700	Very meager
5	1708	£ 9,000
6	1747	£ 5,000
7	1748	£ 9,560
8	1749	£ 42,477
9	1784	Rs. 39,109
10	1787	Rs. 63,000
11	1792	Rs. 42,525
12	1805	Rs. 39,109
13	1807	Rs. 2,91,539
14	1810	Rs. 2,38,897
15	1815	Nil
16	1818	Rs. 1,69,708
17	1822	Rs. 1,5,693
18	1828	Rs. 70,127
19	1830	Rs. 1,01,639
20	1860-61	Rs. 2,50,276
21	1862	Rs. 1,29,003
22	1889	Rs. 1,89,984
23	1890	Rs. 25,061
24	1900	Rs. 9,461
25	1908	Rs. 10,218
26	1914	Rs. 16,542
27	1926 Feb-Mar	Rs. 2,25,498
28	1926 Nov-Dec	Rs. 31,387
29	1927 Feb-Apr	Rs. 2,54,497
30	1927-28 Nov-Jan	Rs. 2,54,497
31	1928 Mar-Apr	Rs. 1,95,039
32	1955 Mar-May	Rs. 1,46,138
33	1956 Feb-Mar	Rs. 44,795
34	1957 Mar-May	Rs. 1,46,138
35	1958 Mar-May	Rs. 4,74,096
36	1959 Feb-May	Rs. 8,65,130
37	1960 Mar-May	Rs. 2,53,339
38	1961 Mar-Apr	Rs. 3,18,234

6

Natural Pearl Production and Pearl-sac Theory

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India is endowed with rich resources of pearl oysters both in Gulf of Mannar adjoining Tamil Nadu coast, Andaman and Nicobar Islands, and Gulf of Kutch along north Western Gujarat coast and Vizhinjam in the Southwest coast of India is also blessed with the settlement of pearl oyster spat in spat collecters. The marine pearl oyster and fresh water mussel from rivers, ponds and fields was used to produce pearls. The pearl oyster *Pinctada fucata* (Gould) belongs to the Phylum Mollusca, class pelecypoda, order pseudolamellibranchiae, family Pteriidae and genus *Pinctada*. In the Andaman and Nicobar group of Islands, the black-lip pearls oyster *Pinctada margaritifera* is available in stray numbers. There are four more species of pearl oysters *Pinctada sugillata*, *P.chemnitzii, P.atropurpurea* and *P.anomioides* from Indian waters. In Gujarat coast pearl oysters are found along the intertidal zones "Khaddas" of Jam Nagar Districts.While in Gulf of Mannar the oysters are always found under water on submerged reefs or rocky areas at a depth of 10-20 m at distance of 11-16 km from the coast. The oyster beds are locally known as "paars" and the total number of such paars is more than 60 in Gulf of Mannar.

Pearl Fishery

The pearl oyster resources have been fished for pearls and mother- of-pearl oyster shells since 13th centaury. In a period of 298 years commencing from 1663-1961, a total of 40 pearl fisheries had taken place along the Tamil Nadu coast from which pearls worth several million rupees had been obtained. In the Gulf of Kutch, there have been 25 pearl fisheries during 1913-1967. Pearl fishery of Gulf of Mannar came to an end in by 1966 due to lack of adult oysters in the natural beds. During 1967 pearl fishery activities at Gujarat coasts came to stand still due to the paucity of adult/old pearl bearing oysters for a sustainable fishery. To asses the pearl oyster resources of the Gulf of Mannar, an under water survey programme was under taken in 1958 by the Central Marine Fisheries Research Institute in collaboration with the Department of fisheries, Government of Tamil Nadu using Modern method of SCUBA diving. And the cause of depletion of oysters was mainly due to over fishing, biological, environmental, predation, siltation and now pollution and dynamite fishing.

Since there is no scope for obtaining natural pearls just like other countries India also started pearl culture actually for producing pearls from pearl oysters collected and grown in farms off Veppalodai as a ad- hock scheme during 1971 in collaboration with State Fisheries, Tuticorin Tamil Nadu.And now India has achieved tremendous progress in pearl culture and associated research programmes in pearl oysters and pearl production.

Pearl Formation
Natural Pearl

Most accepted theory to be known as the pearl-sac theory explains that a pearl is formed when the pearl –secreting cells of the mantle migrate into the body of the oyster under the stimulus of a foreign body (undischarged eggs of the oyster; sand grains got into the shells and formed pearls; and that parasites or other eggs or other organic matter formed the core of the pearls); and form a pearl- sac that secretes nacre which gets deposited on the foreign body and in course of time a pearl is produced according to the shape of the foreign body.

Cultured Pearl

The term "cultured pearl " was used for the first time in 1920 for the pearls produced in Japanese pearl oyster "akoya gai" and marketed in Europe.The name Mikimoto is the first man when cultured pearls are mentioned, the Australian Saville-Kent is now believed to deserve the credit for the original development of the technique. His technique involved taking a piece of mantle tissue from one oyster and implanting it in another. The term 'artificial pearl' does not denote a cultured pearl, but would refer to cheap imitations made of plastics, glass etc; by using the extract " guanine" from fish scales for artificial shine. The tissue culture pearls may be in trade in large quantities from some of the countries the secrets are not exposed wherein large quantities of pearls could be produced from the isolated cells of the mantle in the controlled laboratory conditions. For the production of a cultured pearl a shell bead nucleus is implanted into the gonad of the oyster along with a mantle graft tissue by a skillful surgery. The core material called shell bead nucleus is produced from the fresh water mussel shells from America which is imported to Japan, China Thailand and Australia where they produce the spherical beads of 2 –22 mm size according the size of oysters to be used for pearl production. Necessary surgical tools are designed and developed by CMFRI.The "Mabe" image pearls are produced by implanting the images of required object in between the mantle and shell cavity without affecting the mantle. This technique has been developed by CMFRI, during 2002.The tissue culture pearl production is under perfection.

Implantation Technique

The healthy adult pearl oysters are anesthetized using mentholated seawater in closed containers or pegging in between shells with wooden pieces. A passage is made from the base of the foot towards the gonad of the host oyster without damaging any of the vital organs of the oyster, for which it is mounted on an oyster stand. After that skillful surgery, the mantle piece (graft tissue) and the shell bead (nucleus) are implanted into the gonad (through the passage already made) to lie in contact and proper orientation. The oysters are maintained in the laboratory for two to three days for convalescence with sufficient fresh seawater and aeration for healing of the wound. The care is given to form the pearl-sac over the nucleus by implanted grafted mantle piece to get a good quality pearl from each oyster.

The operated oysters are put in iron cages with lid netted with synthetic threads /plastic baskets/netlon bags and suspended from the raft, rack, long line or kept on the under water platforms land bases culture tanks with sufficient water air and feed etc; according to the area in an air conditioned room without contamination.

In Indian pearl oyster the nucleus of 2-8 mm can be used and the duration for sufficient coating of nacre on the implanted nucleus varied from 4-22 months. The oysters could be checked after 3 months to assess the retention of the nucleus by narcotizing the oysters or by X-ray screening. The X-ray screening is expensive in the case of small *P. fucata* pearls while it in the case of *P.margaritifera* and *P.maxima* pearls for which are costly it is possible.

Harvesting, Grading, Processing and Marketing

The pearls are harvested by cutting and separating the two valves and squeezing out the pearl from the gonad of the oyster. The harvested pearls are washed in distilled water, polished with concentrated salt solution and again washed in distilled water wiped with soft cloth and dried and stored. The percentage of pearl production varies with efficiency of the operation, environmental and health conditions of the animal. The pearls are graded according to this format "A "with spherical and good lustre, "B" some times a pimple like spot with good shining and all the characters of "A" and "C" with good one to three teats with spot and shining.

In the international market pearls of larger size are highly valued. India is importing pearls worth Rs.29 crores every year. The major countries involved are Bahrain, Hong Kong, Japan, the UAE and the U.K. In the present condition in India some private companies have produced pearls and sold internally and exported very little. Based on the packages developed, CMFRI has been offering regular training to officials from State Government, Universities, Research Institutes, Krishi Vigyan Kendras, industry and progressive farmers on pearl oyster hatchery, pearl culture and **SCUBA** diving for studying the under water ecology of pearl oyster beds and resources. India is offering pearl culture training to candidates from other nations. The Swaminathan Foundations have come foreword to finance the fishermen of the coastal villages of Ramnad District in Tamil Nadu to do pearl culture and CMFRI, is giving training in pearl culrutre and also supplying implanted oysters to the farmers and they grow them in the rack constructed in the sea. The final harvest was encouraging and this will certainly give an impact to subsidies the fishermen/women groups in other parts of the country .The Central Marine Fisheries Research Institute is now handling an NATP Project worth 1.3 Crores under World Bank in "Breeding culture of pearl oyster and pearl production and The Department of Ocean Development is funding a Project cost 1. at Andaman on "Production black pearls in *Pinctada margaritifera* " if succeeds the black pearl production will increase export income of the country. India has to go forward to commercialize the pearl culture programme and production of large quantity of bigger and quality pearls both marine and freshwater to compete in the world pearl trade market.

Recently CMFRI has conducted First Indian pearl congress and exposition during 5-8th February, 2003 inviting all the pearl workers in the country to discuss and sort out the problems encountered in the pearl research and production of pearls in India.

The researchable issues are production of pearl oysters to hold larger nucleus 6-8 mm dia and the preliminary works already started. Since the pearl production is a long term process the diversification of the process to hatchery/production of young ones from nature. Mother oyster culture, implantation and convalescence, post operative culture, harvest of pearls and processing, marketing/jewellary products, by products etc. and export. All these aspects come into limelight if marine Pearl Park is identified and demarcated to avoid communal and socioeconomic conflict in the sea based aquaculture programmes.

Formation of a Pearl

In the simplest way, it may be stated that a pearl is formed when a foreign body, such as a grain of sand or parasite, lodges into the soft tissue of the pearl oyster. Since the oyster cannot always get rid of this irritant, it protects itself by secreting nacreous substance that gets deposited over the foreign body in thin micro layers, thus forming a pearl. Since only the outer epithelial cells of the mantle are capable of secreting nacreous substance the chances of pearl formation are limited only to those cases where the foreign body is in contact with the mantle epithelium. The mantle epithelium at the point of contact of foreign body under goes changes. The outer epithelium regenerates a new layer of cells, which spreads over the foreign body and covers it completely. This layer is called the pearl-sac. The pearl-sac epithelium secretes nacre around the foreign body, which becomes the nucleus or core material of the pearl. The pearl oysters learn to live with the encysted pearl. The pearl grows in size as the oyster grows.

As to the nature of the foreign body that acts as the irritant it may be of organic or inorganic origin. Sand grain is the common inorganic material that finds an entry into the pearl oyster. The larval forms of parasitic cestodes and trematodes, and minute plank tonic organisms form the organic core material around which pearls are formed.

Blister Pearl

Ma y takes place when foreign body lodges itself between the shell and mantle. Polydora boring, sponge boring, foreign body attaches to the shell and mantle secretes nacre on that and a blister pearl is formed..

Free Pearl

Under certain conditions, the foreign body gets embedded in the connective tissue of the mantle. The invading body as it breaks through the outer epithelium of the mantle carries a few epithelial cells, which would regenerate and grow into complete pearl-sac around the foreign body. Besides shell and mantle pearls are

found in other soft tissues of the pearl oyster such as adductor muscle, gills and pallial muscles.

Pearl Without Nucleus

Given this accepted theory of pearl formation with a pearl-sac and a nucleus, the term "pearl without nucleus" would seem to be a contradiction. It is supposed the size of the nucleus may even be as a few microns. It is possible that a few decayed cells blood epithelial cells or epithelial cells might provide the basis for pearl formation but subsequently disintegrate totally. Such pearls, when cut and examined, would not reveal any nucleus and would appear to be formed entirely of mother of – pearl layers without recognizable nucleus.

7

Pearl Nucleation Techniques

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Introduction

Natural pearl is produced between mantle and shell of a live pearl oyster in its natural environment. The formation of natural pearl is influenced by foreign bodies accidentally entering the body of oyster. Under stimulus of the foreign body, the outer epithelium of the mantle invaginates and forms a pearl sac. In this case production of free and spherical pearls is rare and also percentage pearl production is less. The cultured pearl production technology ensures the production free and spherical pearls and guarantees large-scale production of pearls. The technology was developed in the year 1973 in India. The techniques involved in cultured pearl production are explained below.

Surgical Tools for Nucleation

Oyster knife	:To open live pearl oyster shell valves
Incision blade cum grafting needle	:Used to make incision and to lift graft tissue
Nucleus cup	:To lift nucleus
Spatula	:To brush aside organs before implantation and to clean excised mantle piece
Hook	:To hold foot during surgery
Graft cutting knife	:To cut mantle strip
Forceps	:To hold mantle strip during graft tissue preparation
Speculum	:To keep open shell valves
Oyster clamp	:To hold narcotized oyster during surgery

Selection of Oysters

Selection of oysters is an important process, which ensures production of quality pearls. The factors such as the weight of oyster, reproductive phase and health are considered during selection. Oysters with weight of 25 g are found to be ideal for implantation. However oysters with 20 g weight may be considered for implantation of smaller size of nucleus. Fully mature oysters are not suitable as the gametes flow out during operation. Hence oysters in the post-spawning/ recovery phase and also in the early phase of gamatogenesis may be selected. Oysters free of

blisters caused by polychaetes and sponges and trematode infection may be selected for surgery.

Graft Tissue Preparation

Healthy donor oysters are selected for mantle tissue preparation. The shell valves are separated by inserting a knife between valves and severed the adductor muscle. The mantle strip of both valves is used. The mantle is cut and removed with minimum disturbance. Careless removal may cause shrinkage of the strip and may not be useful for graft preparation. The mantle strip is placed on a moist wooden block without changing the side and stretched sufficiently by holding both ends. A gentle cleaning is done with soft sponge to remove adhered particles and mucus. The pallial organs at the free end of the mantle strip and muscular connective tissue at the lower end are removed. Final cleaning is done with soft wet sponge and the strip is reversed so as to expose the outer phase of mantle and is cut into small pieces of 2-3 mm. The size of the piece has to be in proportion to the size of nucleus. The processed pieces of mantle are kept moist with a very dilute solution of water-soluble eosin using a brush. The eosin solution stains the grafts red, keeps the cells unaltered and provides aseptic condition.

Nucleus

Spherical shell beads are necessary as core material for production of round cultured pearls. Shell beads are prepared out of thick shells of fresh water mussel and other molluscs. Requirement of shell bead nuclei in India is currently fulfilled by import from Japan and Hong kong. However indigenous technology for preparation of shell bead nuclei has been developed in the Central Institute of Fisheries Technology (CIFT), Cochin utilizing shells of molluscan forms. The beads are cleaned in distilled water and dried before use.

Conditioning of Oysters

Natural physical methods are safer and inexpensive. Selected oysters are arranged vertically by dorsal side down and half immersed in seawater. Depletion of oxygen in the limited water makes oysters to wide open their valves for want of oxygen. Such oysters are plugged with wooden peg0 and fitted in an oyster clamp for surgery.

Synchronized narcotisation is also practiced using menthol crystals. In this case oysters are immersed fully in seawater in a tub and menthol powder is sprinkled over the seawater. The oysters narcotized under the effect of menthol in about 60-80-minutes. These oysters are then taken for surgery on by one. Keeping oysters in menthol water for prolonged period causes physiological upset leading to death. Duration of about 30-45 minutes immersion in menthol water is the safer limit.

Surgery

Gonad is the primary and best site for nucleus implantation. Single implantation is performed at this site. In double implantation, the larger nucleus is

placed at this site and the smaller nucleus at the dorsal region of gonad close to hepatopancreas. Multiple implantations are carried out at many sites in the visceral region.

Conditioned oysters with speculum are mounted on the oyster clamp keeping its anterior side to right side of the technician. To start with the gills are pushed aside with spatula so as to expose the site of incision. The foot is held by needle hook and pulled slightly to elevate the base of the foot. A sharp incision is made at the base of the foot and through which a passage is created below the outer skin by incision needle up to the site of implantation at the gonad region.

A piece of graft tissue is inserted through the passage and placed at the gonad. Now the outer epithelium of the graft tissue is facing the passage. In the similar way nucleus also inserted and placed in touch with the graft tissue.

Convalescence

The nucleated oysters are placed either in a gentle flow through water system or in a tub containing well aerated filtered seawater for convalescence. In the latter system the water is changed frequently to overcome the effect of narcotisation. On placing in seawater oysters would shut their valves within a few minutes and slowly resumes its normal shell activity. The incision would be healed off in two to three days. If the incision and the passage are large the nucleus may slip out through the passage.

Post-operative culture

The operated oysters are retained in the lab for 3-4 days under observation before they are returned to farm. The oysters that have ejected nucleus are taken back to mother oyster culture to be used again. Dead oysters, if any, are removed and the rest of oysters are placed in a cage at low density. The oysters should not be disturbed too frequently. They are suspended from the raft in the farm at greater depths. The greater depths ensure the production of quality pearls and less fouling. The post-operative culture varies as per the size of nucleus used. The range is about 3-24 months for 2-7 diameter nuclei under tropical conditions where the growth of pearl is faster than temperate conditions. Periodic maintenance of operated oysters is highly essential to promote good growth of oysters and pearls. Test harvest may be made at periodic intervals to assess pearl maturity. Premature harvest of pearls would result in poor lustre and iridescence. The pearl having a minimum of 0.5 mm nacre thickness is valued as pearl at international market.

Production of Cultured Pearls

The rate of production of cultured pearls depends on many variables. Formation of cultured pearls is a biological process as it is governed by the pearl oyster after nucleus implantation. Human control is restricted to the success of surgery and to provide suitable environmental conditions.

Mortality of oysters can take place due to effect of surgery and infection and due to many other factors such as disease, shell boring and biofouling. Annual mortality should be kept within 10 % of the stock through proper farm management.

Nucleus rejection is a common feature. This should be avoided by skilful operation and adopting advanced surgical procedures.

All nucleated oysters may not produce pearls, as some oyster may have only nucleus with out pearl coating. This is due to failure in orientation of nucleus and graft tissue. This may happen by wrong placement of graft tissue to nucleus. Such failures should be kept within 5 % level through greater care in surgery.

Gross production is the number of cultured pearls produced by the surviving operated oysters in the farm. In single implantation, production rate achieved is about 65 %. In multiple implantations production achieved is about 180 % with reference to number of nuclei implanted. These rates can be improved further.

Quality Improvement

Attention should be given to improve the quality of cultured pearls. The size, colour and lustre determine the quality and value. Individual pearls of exceptional quality would fetch high price. The quality of pearl can be improved through appropriate care at surgery and farming. Colour and lustre of pearls are partly decided by genetic character of individual oyster and partly by environmental factors. Genetic differences are evident between different species of pearl producing molluscs. The genetic character of nacreous later of each species is reflected in pearl colour and lustre. It is evident that the colour and luster differ among the species of pearl oysters. In general, pearls produced by pearl producing molluscs have the same colour and lustre as the nacreous layer of their shells.

Environmental factors play a major role in determining the colour and lustre of nacre. Depth is one of the important factors as quality of pearl is produced in deeper waters. Low fouling and low temperature in deeper waters promote production of quality pearls.

Temperature also influences the growth and quality of pearls. Higher temperature accelerates the metabolic rate of oyster, which causes rapid deposition of nacre. Rapid deposition of nacre causes poor quality pearl. Slow and steady deposition of nacre as thin layers result in the production of quality pearls. Hence it is evident that pearl harvest may be executed during the period of low temperature and pH.

Minerals and trace elements in seawater are considerably important as these influence the colour of pearls. Hence the chemical factors of farming sites should be thoroughly understood. The quality and quantity of phytoplankton ingested by the oyster determine the colour and lustre of pearls

8 Hatchery Production of Pearl Oyster Seeds

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Introduction

In India the pearl oysters occur in the natural beds of Gulf of Mannar and Gulf of Kutch. Depletion of natural stock caused great concern to the development of pearl culture industry in the country. The grave situation warranted an urgent need to develop hatchery system for the production of seeds under controlled condition. The Central Marine Fisheries Research Institute established shellfish hatchery laboratory at the Tuticorin Research Centre, Tuticorin. A breakthrough was achieved in the breeding and production of seeds of pearl oyster, *Pinctada fucata* in 1981. The success laid foundation for the production of seeds of other bivalves like the edible oysters, mussels, clams, windowpane oysters and the gastropods like the abalones, chanks. The techniques involved in the hatchery system were standardized and mass production of seeds was achieved.

The hatchery system has two phases i) Brood stock maintenance and spawning and ii) larval rearing and spat settlement.

Brood stock maintenance and conditioning

Maintenance of brood stock is a vital part in the hatchery that ensures ready supply of ripe oysters throughout the year. Sexually ripe oysters are maintained under controlled condition at low temperature around 22-25° C in an air-conditioned room. The oysters are adequately fed at 4 litres of mixed algae per oyster per day. The mixed algae contained mostly *chaetoceros* sp. Aeration is provided throughout the conditioning period. Under such conditions ripeness of gonad is retained for a prolonged period. The brood stock of oysters is taken to hatchery for spawning as and when required.

Spawning

i) **Natural spawning**: There are two spawning seasons (June-August & November-January) in a year for pearl oysters in the Gulf of Manner. During spawning season most of the pearl oysters would be sexually ripe and may spawn naturally when there is slight change in water temperature or water pressure. When the oysters are not spawning naturally, they are induced to spawn through thermal stimulus or chemical means.

ii) **Induced spawning**

a) **Thermal stimulation**: This is one of the best devices that it is universal factor in natural environment for inducing spawning not only in pearl oysters but also in other animals. When the oysters

conditioned at 22-25 °C are suddenly dipped in higher water temperature having even a difference of 1 or 2 ° C, the slight change in water temperature stimulates the ripe oysters to spawn. Gradual increase of water temperature also causes spawning in oysters. The maximum temperature at which the oysters can tolerate is 34° C.

b) **Chemical stimulation**: When the oysters are not responded to spawning in thermal stimulation they are resorted to chemical inducement.
   i) Seawater with pH 9.0 is prepared using TRIS buffer (Hydroxymethyl aminomethane). Sexually ripe pearl oysters are immersed in the alkaline seawater for 1-2 hours. If these oysters are transferred to normal seawater after treatment spawning is effected in about 75-80 % of oysters.
   ii) Seawater with pH 9.5 is prepared using Sodium hydroxide. When the oysters are treated in the alkaline medium 70-75 % of oysters spawned after transferring to normal seawater.
   iii) 6% hydrogen peroxide (H_2O_2) in 3.064m^M concentration in an alkaline medium (pH 9.0) using TRIS buffer had induced 62.5 % spawning in pearl oysters after 4 hours treatment.
   iv) 6% hydrogen peroxide (H_2O_2) in 3.064m^M concentration in an alkaline medium (pH 9.0) using TRIS buffer had induced 62.5 % spawning in pearl oysters after 4 hours treatment.
   v) 6% hydrogen peroxide (H_2O_2) in 6.128 m^M concentration in an alkaline medium (pH 9.0) using sodium hydroxide had resulted in 9.5 % spawning in pearl oysters after 4 hours treatment.
   vi) Injection of 0.2 ml of 0.1 N solution of ammonium hydroxide (NH_4OH) into adductor muscle or foot of ripe pearl oyster had induced spawning in about 50 % of oysters.

Embryonic Development of Larvae

Sexes are separate in pearl oysters. Invariably the males initiate spawning in normal conditions. The sperm loaded water is consumed by female oysters and stimulated the females to release eggs 45 minutes after male spawning. Fertilization takes place immediately in the water medium. The fertilized eggs having polar body settle down and cell division starts 45 minutes after fertilization. Two-celled stage is obtained with a micromere and a macromere and now the polar body is placed at the furrow of the cleavage. During the second cleavage the micromere and macromere released one micromere each. The stage having three micromeres and one macromere is called trefoil stage. The macromere does not take part in any divisions. Further divisions are taking place only in micromere resulting in to 4-, 8-, 16-, 32-, 64-celled stages and so on. The resultant stage is called morula. The stage is reached 4 hours after fertilization. It contained a ball of small micromeres and each micromere is developed itself a tiny cilium. The morula having such cilia starts its first movement in water column. The morula exhibits phototrophism that facilitated collecting of viable embryo leaving bottom debris in the spawning container.

By reorientation of cells a blastula stage is reached after 5 hours having a blastocoel and a blastopore. The cells convolute in through the blastopore and form dermal layers namely ectoderm, mesoderm and endoderm along with archenterons. The stage is called gastrula that took 7 hours to reach the stage. After gastrulation the embryo is transformed into trochopore larva after 10 hours. The larva develops a long single flagellum at its apical end surrounded by a pre-oral band of cilia. The post-oral band of cilia is situated at its rear end. The larva swims with the help of flagellum and cilia.

The outer ectodermal cells of the trochophore larva secretes the first embryonic shell material called prodissoconch I. The site where the prodissoconch I starts is formed as straight hinge line. When the formation of shell material is continued, the larva assumes D shape having a straight hinge line. The stage is called veliger or straight hinge stage or D shape larva reaching between 18-20 hours. The single flagellum disappears and a powerful locomotor organ called velum forms. Now the larva measures 67.5µm along the antero-posterior axis and 52.5 µm along dorsoventral axis.

Larval development

Veliger: The veliger larvae are collected, estimated and stocked at a density of 2 larva/ml in FRP tanks. The larva is fed with a micro alga *Isochrysis galbana* at the rate of 5000-cells/larva/ day. During larval phase no aeration is provided.

Umbo stage: The veliger larva reaches umbo stage in 10- 12 days. It measures 135 x 130 µm. The shell growth beyond veliger is by the addition of prodissoconch II. The shell valves are equal and mantle folds develop. Feeding is given at 10,000 cells/larva/day.

Eyed stage: Eyespot is developed on 15th day when the larva measures 190 x 180 µm. The eyespot is situated at the base of the foot primordium. Larva develops ctenidial ridges. Feeding is increased to 15,000 cells /larva/day.

Pediveliger larva: Foot is developed on 18th day at the size of 200 x 190 µm. At transitional stage of swimming to crawling phase, the larva has both velum and foot. When the foot becomes functional and attaches to a substratum, the velum gets reduced. Gill filaments developed. The rate of feeding at this stage is 20,000 cells/larva/day.

Plantigrade stage: The stage is reached on 20th day when it is 220 x 200 µm. Labial palps and additional gill filaments develop. Extra shell growth is noticed all along the globular shell margin except the umbo region. By the addition of such shell growth the plantigrade transforms in to adult stage. The stage needs 25,000 cells of Isochrysis per day.

Spat: The spat has again developed a hinge line, anterior and posterior ears or auricles and a byssal threads. The left valve is slightly concave than the right one. The spat attaches itself to substratum by byssal threads. The typical spat measures 300 µm on 24th day. Feeding is given at 30,000 cells/spat/day.

Larval rearing systems

Water quality management and other conditions

Larvae obtained from same brood show differential growth rates and time in settlement. Mortality of larvae would be high up to umbo stage and less afterwards. Factors like high larval concentration, colour of culture tanks, aeration and overfeeding may affect larval growth and survival. Rearing of larvae at a concentration of two per ml gave better growth and high spat set. Larvae prefer darkness and dark surfaces. Aeration is harmful to larvae. Selection of fast growing larvae by culling would yield better survival rate in spat after transplantation to farm.

Water Change

In static water system, change of water is done once in two days. Water is siphoned out through appropriate sieves. 40-µm sieve is used up to umbo stage, 80-µm sieve up to eyed stage and 140 µm afterwards until settlement. Larvae are washed gently by keeping in the sieve. Before releasing, the tank is cleaned neatly and filled with fresh seawater. The tanks are covered with dark cloth to avoid light and dust. No antibiotic is used during larval rearing.

Feeding Schedule

Feeding is commenced to the larvae on second day with unicellular micro alga *Isochrysis galbana.* Ten cells per ml are found to be optimum for veliger larvae up to umbo stage. Feeding doubled from umbo stage and tripled from pediveliger up to settlement. Other micro algae such as *Pavlova lutheri, Chromulina freibergensis* and *Dicrateria sp.* are also acceptable to the larvae. Feeding is given once in a day.

Spat Set

Normally spat set occurs between 18 and 20th day. In exceptional cases the spat set is advanced to 14th day. High percentage of spat set is obtained in 2 larvae per concentration. Dark coloured surfaces enhance spat set and aeration during larval phase affects spat set.

Survival

5 % production is obtained on the initial stock of larvae in 500 litres of seawater in FRP tank. Negligible mortality of spat is experienced in normal conditions.

Spat Rearing

Spats are kept in the hatchery for two months after settlement. Isochrysis feeding is continued for one month after setting and gradually changed over to mixed algae. The spat reaches the size of 3mm in a period of 60 days and are transferred to farm at this size. Aeration is needed for the spat after settlement. As

the spats are sedentary in habit, recirculation of seawater is provided. No spat collectors are used for spat collection. The spats are allowed to set in the tanks. The spat mortality is minimum in the hatchery phase as long as the environmental and hydrological conditions are favourable.

Nursery Rearing

Spats with an average size of 3 mm are reared in box-type net cages of 40x40x10 cm with iron frame and encased in a retrievable synthetic velon screen bag of 0.5 mm mesh. The cage is again covered with old fish net with 10 mm mesh. This serves as a protection to the velon screen bag and to the spat from predators like crabs and fishes. The bags can be washed and reused. The initial stocking density of spat of 3mm size is 10,000 nos. per cage. After a month of rearing the spat are transferred to another cage with 1.0 mm mesh size. The density is reduced to 5000 nos. When the spat reaches 10-15 mm size, they are transferred to a cage with 1.5 mm mesh size. Now the density is kept at 2000 nos per cage.

Juvenile Rearing

The juveniles are reared in 40x40x10 cm cages netted with 1.5 mm nylon thread having 10 mm mesh size. The density is kept at 750-1000 per cage. Further thinning is done when they reach 40-45 mm in a period of 12-15 months.

Spat Survival in the Farm

When the spat is transferred to farm at 3 mm size, 50 % mortality experienced. The mortality is reduced to 20 % and 10 % in the second and third months respectively. By then the spat grows to 10-15 mm. Mortality is negligible beyond the stage. The juveniles reach 45-50 mm in 12-15 months of farm rearing. The overall survival of 30 % of the initial stock can be expected. Survival rate can be enhanced through proper management of farm.

9 Pearl Farm Management (Fouling, Boring), Predation and Control Measures

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A Glimpse to Pearl Culture in India

According to Hornell, the marine pisciculture work accomplished in Madras during the financial year 1St. April 1911 to 31st March 1912. Hermann and Hornell as reported by Hermann (1903) have studied the early development of the pearl oyster. Observations made on pearl oysters in the pearl oyster farm near Krusadai Island, (Devanesan and Chidambaram, 1956) and at Tuticorin, Chacko (1954, 1956, 1957) shown that the oysters grew to a height of about 36 mm in 6 months, 35-45 mm, 50-55 mm, 55-60 mm, 60-65 mm and 65-70 mm respectively at the end of year one to fifth year. The pearl oysters have been estimated to have longevity of 5-5.5 years in the natural beds and to live upto 7 years when reared in the farm in cages .(Narayanan and Mickael, 1968) worked on the relation between age and linear measurements of the pearl oyster *P. vulgaris* (Schumacher) of the Gulf of Kutch. (Alagaraja, 1962) while working on the linear relationships of length (DVM) and weight of pearl oysters found that the growth was significantly different for each year group. (Anantharaman, 1967; Chellam, 1978, 1987) studied the growth of pearl oysters in the Gulf of Mannar and Veppalodai respectively. (Appukuttan, 1987) reported the aspects of pearl culture experiments conducted at Vizhinjam Harbour and faster growth of young oysters was highlighted. (Achary, 1980, 1986 and 1998) explained the introduction of biological associates and it has stated that it is possible to attract desirable cultivable animals to settle down and grow. He fabricated the multipurpose cage for culturing pearl oysters and mussels to grow without the help of a raft at Vizhinjam. (Chellam, 1988) studied the growth and biometric relationship of the pearl oyster *Pinctada fucata* (Gould) produced in the hatchery. (Velayudhan, 1987) suggested the selective breeding between laboratory bred oysters with oysters from the wild stock may help in obtaining improved quality pearl oysters. (Velayudhan *et al* 1996) has produced 4 generations of pearl oyster and found that the morphometric characters of the four filial generations showed values of high significance as there were differences in the morphometric relationship within the generations and between the filial generations.

Hornell, (1922) listed a total number of 72 pearl banks known as paars in the Gulf of Mannar. From 1663 onwards to 1961 there have been only 38 pearl fisheries in the Gulf of Mannar. The gap of nonproductive period extended for 27 years between 1928 and 1955, and a similar 27 years so far since 1961 with no sign of pearl fishery in the coming years. A revival of the paars has been observed in 1998 (after 27 years) in which by SCUBA diving 1 lakh oyster/2 divers/day/boat was collected. The oysters were almost at the age and size of implantation; (Personal communication, Jesuraj and Muthukrishnan, Divers, Tuticorin RC of CMFRI, Tuticorin).

There are about 42 important pearl oyster reefs, known as Khaddas, in the intertidal area of Gulf of Kutch. The Gulf of Kutch fishery used to be half almost every year or alternate years from 1913 to 1939. Subsequently, it was held every 3-4 years. There have been 25 pearl fisheries during 1913 to 1967 and the last one being in 1967.

Since there is no scope for obtaining natural pearls just like other countries India also started pearl culture actually for producing pearls from pearl oysters collected and grown in farms off Veppalodai as a ad- hock scheme during 1971 in collaboration with State Fisheries, Tuticorin Tamil Nadu.And now India has achieved tremendous progress in pearl culture and associated research programmes in pearl oysters and pearl production. The further achievements will be highlighted in the connected chapters of lecture.

Pearl Oyster Farming

Farm Site

Pearl oyster farms are ideally located in sheltered bays, which offer protection to the rafts. They can also be set up in the coastal waters where sea conditions do not get too rough. The operations are year round in a pearl culture farm. Depth should be around 3-10m and silting should be minimal. The ambient tropical sea temperature and salinity are suitable for pearl oyster. If salinity falls below 15 ppt, it might lead to mortality under prolonged low saline conditions as during unusual heavy precipitation of rain and heavy discharge of rivers in the vicinity of farms. Areas rich in phytoplankton, which is consumed by 'the oyster as food, are good but it should not lead to noxious blooms. A mild current of about 2 knots helps bringing fresh food as well as removal of metabolic products, faecal matter and other farm droppings.

Raft Culture

Raft culture is the typical method of pearl oyster farming in 'sheltered bays. Long-line culture is better suited for open coastal conditions. The raft structure is illustrated in Fig. 7 a. The overall dimensions of a raft are variable and are decided based on convenience of handling. A raft of about 6 m x 6 m may be a standard size. The raft is constructed of logs (any second class wood as venteak or casuarina pole) of about 10 cm diameter tapering to 6 cm of chosen lengths. The logs are coated with coal tar. These are arranged as illustrated in Fig. 7 a and lashed with nylon ropes. Floats are attached to the raft to give buoyancy, their number being usually 4 for a standard raft which may b;: increased if there is sagging. Sealed empty diesel drums (200 1 capacity) with fibreglass coating, mild steel barrels given coatings of anticorrosive paints, or more modern Styrofoam or FRP/synthetic floats are used for buoyancy (Pl. I A). The choice depends on cost and long-term economics. The raft is individually moored at the farm site with anchors (grapnel or admiralty type) on opposite sides connected to raft with tested quality chain. The long-line system uses floats, spherical or cylindrical, which are connected by synthetic rope or chain (Fig. 7 b).

Pearl oysters are carried in varied types of nets/cages, which are suspended from the raft or long-line by synthetic rope at appropriate depths. The typical ones are the frame net (Fig. 7 c) and the nylon-mesh cage (Fig. 7 d). The frame-net is useful toavoid crowding of oysters and to follow the performance of individual oyster post-operative (Pl. I B). The cage is good for general mother oyster culture. All iron frames used are given anticorrosive treatment with paint. All structures in the farm should be periodically checked and maintenance repairs done which would help in extending the life of rafts and materials to a period of 3-5 years or even more. Recently lantern type cages with or without compartments made of old fishnets or new nets of required sizes which are collapsible and easily transported and cheaper. Another type is pedestal cages of 1m X 1m, 8mm steel rod fabricated to cube or .75 m X .74 m frame with 4 compartments and oysters are released in these compartments.The Cage with legs in all corners with rests to avoid damages to the cage as well as oysters and from fouling & poaching is very comparatively less than the smaller cages those can be lifted easily .

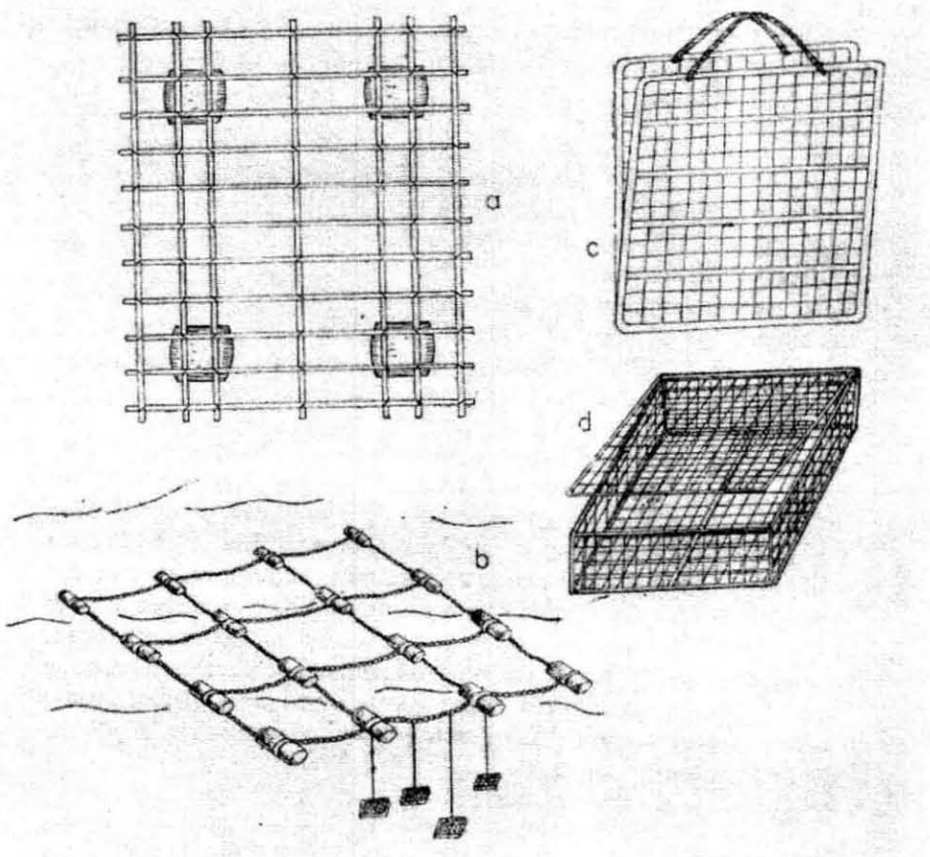


Fig. 7. Structures of pearl oyster farm. a-log raft; b-long-line; c-frame net and d-cage.

Mother Oyster Culture

Mother-oyster culture refers to the farming of pearl oysters from the time they are brought to the farm till they are used in surgery. The source for pearl oysters are (a) the natural beds, (b) spat collected III the sea and (c) pearl-oyster hatchery.

The pearl oyster populations in the natural beds, the 'paars' of Gulf of Mannar and' 'khaddas' of Gulf of Kutch, are subject to wide fluctuations. The unproductive spells are far more numerous than the productive periods. If and when the stocks are good, they should be collected and used for pearl culture, In the Gulf of Mannar, the collection is done by diving up to a depth of 20 m. SCUBA-diving (Pl. I C) enables search of a wider area and good collections as compared to skin- diving. In the Gulf of Kutch, they are collected from the intertidal flat by band picking. .

Spat collection using cedar leaves, hyze film or old fishing nets supplies almost the entire requirement of mother oysters for the Japanese pearl culture industry. In India, it has not so far been successful due to the open conditions of the natural beds. In inshore regions, particularly in the recently constructed harbour basins as Tuticorin and Vizhinjam, some spalfall of pearl oyster takes place, but it is of multispecies composition, *P. fucata* component being very small.

The source of hatchery is more reliable and can supply the required stock for pearl culture. Recently, the techniques for hatchery production of pearl oyster have been developed in India and it remains to be commercialized.

The pearl oysters are grown in the farm till they reach a suitable size. a minimum of 20 g in weight. for use in the surgery for graft and nucleus implantation. The oysters draw nutrition from the phytoplankton in the sea and no artificial feeding is necessary and possible in the farm. Mortality of stock should be kept to the lowest minimum level through appropriate farm management.

Pearl Culture Establishment

Pearl culture in Japan is carried out by small-scale units, on cooprative or family basis, save for a few large-scale operations by companies. In the peak period of production (1966), there were 4710 pearl culture units of which 49.8% were operating 1-14 rafts, 20.8% 15-29 rafts, 12.0% 30-49 rafts and the remaining 17.4% more than 50 rafts. The total number of units came down to about 2500 by 1973. This would show that small-scale operations are the mainstay in pearl culture. The Japanese pearl culturist has the advantage that he can buy the mother-oysters for his farm from those who are solely engaged in seed collection and mother- oyster culture. In India such small-scale operations at the family level can become possible only if commercial hatcheries produce pearl oysters and sell them to pearl culturists.

In Ramanathapuram District of Tamil Nadu Dr.M.S.Swaminathan Foundation initiated to take up marine pearl farming involving coastal women folk by 2002 onwards providing financial support and CMFRI has given technical support by supplying implanted pearl oysters from CMFRI, Mandapam Research

Centre on payment to the operated from ICAR, Revolving funded project. In 1991 CMFRI had given training in pearl production by selecting progressive fishermen from Coastal Valinokkam Village of Ram Nad District of Tamil Nadu and the pearls produced were distributed to them.

The activities, major inventory and manpower of a pearl culture establishment is summarised briefly to give an overview for an easy understanding of the nature of this industry. Major work is in the sea involving pearl oyster collection and farming. Details of hatchery are not included for reasons already stated. Manpower [' needs and inventory items would vary according to the scale of operation. These are not strictly applicable to family-based operations, which are not feasible in India for the present.

Raw Material: Pearl Oyster (*Pinctada fucata*)

Oysters from Natural Bed

Activity- Seasonal survey of beds and collection by diving.
Inventory- Boats; self-contained underwater breathing apparatus (SCUBA) and diving accessories such as fins, masks, snorkel, depth-gauge, knife and belt; compressed air charging units, (main and portable compressors) ; collection kit and oyster bins.
Manpower- Boat crew, navigator, divers, diving assistants.

Oysters from Spat Collection

Activity- Collection of pearl oyster spat by suspending spat collectors from rafts at suitable sites in the sea / bay.
Inventory- Rafts, lighted buoys, anchors, chain, and spat collectors; linked with item 1.1 seasonally.
*Manpower-*Linked with item 1.1 seasonally and farm labour.

Pearl Oyster Hatchery

Pearl Oyster Farm Management

Activity- Mother-oyster culture, post-operative culture, farm maintenance and stock. Maintenance.
Inventory- Log-rafts, Long-lines, lighted buoys, floats,
Anchors, chain, rope, cages, frame nets, dinghy, out-board motor,
Floating sheds and miscellaneous tools; linked with
*Manpower-*Farm superintendent, technical assistants,
farm labour; linked with.

Shore Establishment

Surgical Unit
*Activity-*Pearl oyster surgery and convalescence.
*Inventory-*Surgical tools and accessories, furniture, shell-

bead nuclei, chemicals, glassware, plasticware, ultraviolet lamps and raceway.
Manpower-Chief technician and technicians.

Farm house
Activity-Shore support for maintenance of farm and farm stock.
Inventory- Oyster cleaning tools, farm structure maintenance requirements (repairs and maintenance of raft, long-line, floats, anchors, chain, cages, frame nets) and oyster tanks.
Manpower-Linked with items in seasonal basis

Pearl Collection Centre

Activity- Collection of cultured pearls and incidental natural pearls.
Inventory-Plasticware, chemicals, oyster knife, vats.
Manpower-Technical assistants.

Pearl Processing Centre

Activity-Cleaning, sorting and grading of pearls; treatment of pearls for removal .of minor blemishes; bleaching, dyeing and colour improvement.
Inventory-Sorting trays, miscellaneous tools, chemicals and glassware.
Manpower -Pearl processing expert and technical assistants.

General Services

Seawater Supply

Activity-Supply of qualify seawater to surgery, raceway and oyster tanks. .
Inventory-Pump House, filterbed, sump, overhead tank supply channels with-. regulators; air blowers with air supply tubings and regulators.
Manpower-Electrical supervisor and assistant.

Power and Freshwater Supply

Laboratory

Activity- Monitoring of oyster healtl1. and condition; sea water analysis; advice to farm superintendent and chief technicians ; feed-back; to research. system.
Inventory-. General biological laboratory equipment and analytical equipment for seawater analysis.
Manpower- Biologist, chemist, laboratory technicians.

By-products Unit

Activity- Conversion of by-products of pearl culture to value-added items.

Inventory- If the unit is self-contained, all items required for utilisation of shell and meat; otherwise, collection, preservation and storage of materials until. sale to outside agencies.
Manpower- Specific manpower for handling by-products processing work, if self-contained; otherwise linked with other items.

Management and Administration

Activity-Planning, execution and administration of project.
Manpower-General Manager, administration, accounts and stores staff.

Biofoullng, Boring and Predation

Major problems in maintenance of pearl oyster stocks in the farm are the biofouling organisms, which settle and grow on the shells, the boring organisms which riddle through the shells and render them weak and friable and the predatory organisms which feed upon the pearl oysters. Singly, or in combination, these factors can cause heavy mortality to the farm stock through physiological stress and disease. Routine control measures should be adopted periodically and against specific problems.

Biofouling

The dominant fouling organisms are the barnacles (*Balanus amphitrite*) (Pl. II A), bryozoans (*Membranipora* sp., *Thalamoporella* sp. and *Lagenipora*), simple and compound ascidians (Pl. II B), the spat of bivalves (*Avicula vexillum* and *Crassostrea madrasensis*) and hydro ids. The weaving mussel *Modiolus* sp. Forms extensive carpet-like colonies over the natural pearl oyster beds but has not been a serious threat in the farm. Encrusting tubicolous polychaetes (Pl. II C) may be dominant in seasons. Seaweeds (Pl. II D) settle and grow on the oysters and cages. Others noticed are amphipods, isopods, sponges, polyclad worms, nematodes, opisthobranchiate molluscs, *Pinna* sp., egg capsules of gastropods and crinoids. While barnacle settlement is noticed almost throughout the year, settlement of others are seasonal. In inshore waters, under hanging culture at shallow depths, fouling load is always moderate to heavy.

Boring Organisms

The boring organisms include the two dominant groups of serious pests, the polychaetes and sponges. The boring polychaetes *Polydora ciliate* and other sp., *Cirratulus cirratus* are the major borers and causes mortality and members of families Syllidae, Nereidae and Terebellidae burrow through the shells and donot cause cause extensive blisters on the nacreous layers as done by earlier culprits (Pl. III A).

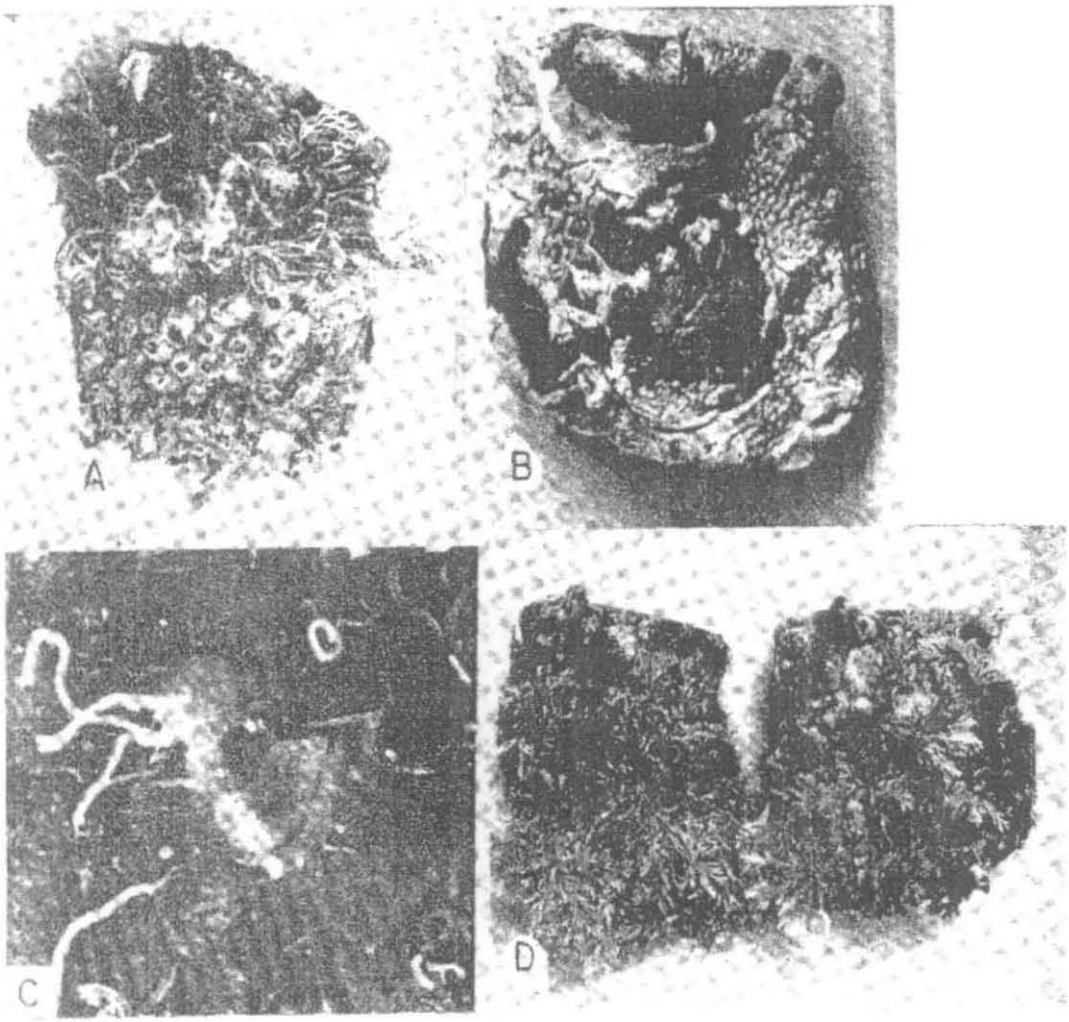


PLATE II. A—Fouling of pearl oyster by barnacle; B—Compound ascidians; C—Encrusting tubicolous polychaetes; D—Seaweed fouling on oyster.

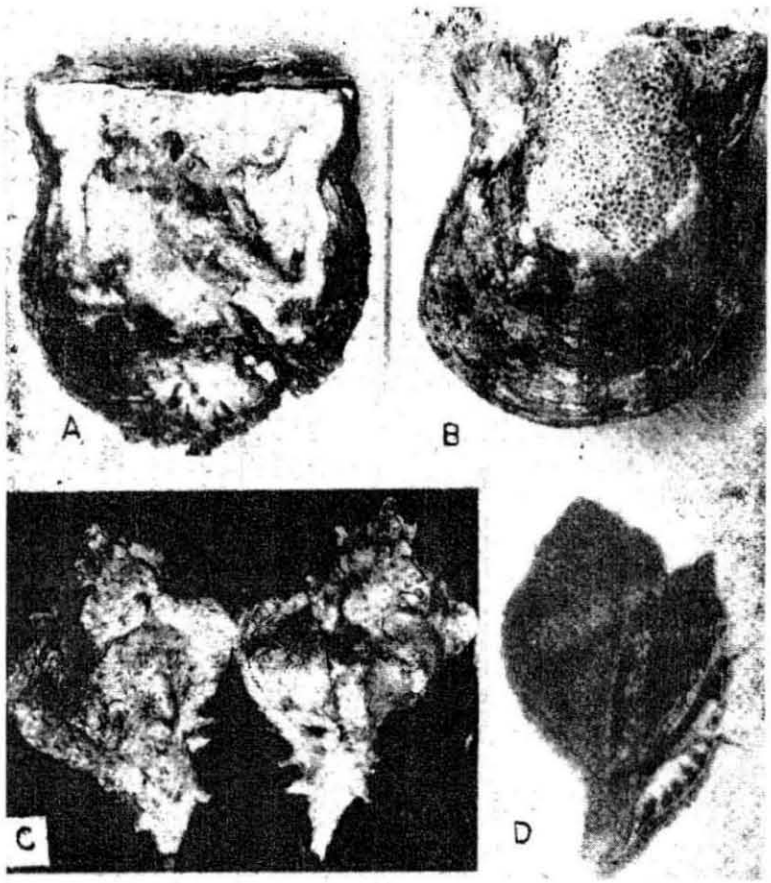


PLATE III. A—Pearl oyster shell with extensive polychaete blisters; B—Shell showing boring by sponge; C—Gastropod predator *Murex virgineus*; D—Predator *Cymatium cingulatum* attacking oyster.

The boring sponge *Cliona vastifica* and *C. celata* form honey-comb-like ramifications in the shell with numerous openings on the nacreous surface (Pl. III B). The above two groups cause great physiological strain to the oyster, while attempting to repair the shell damage, and cause mortality to the farm stock. The boring molluscs *Lithophaga* sp. and *Martesia* sp. and isopod *Sphaeroma* sp. are also occasionally found on the shells.

Predators

The major predators of pearl oysters in the farm are the gastropods *Murex virgineus* and *Cymatium cingulatum* (Pl. Ill C, D). The rate of predation by these gastropods is about an oyster per day per animal. They make their appearance seasonally. Crabs *Charybdis lucifera* and *Atergatis integerrisimus* also prey upon the pearl oysters. In the natural beds, rays fishes such as rock-perch and trigger fish and octopus may be notorious predators but these are not generally found in the shallow farm areas. Perch fishes, rays, eels, octopus, lobsters even Xancus also found to attack oysters kept in captivity

Control

The fouling organisms can be controlled only through periodic cleaning and scraping of foulers or through judicious choice of depths for growing the oysters. The deeper waters are relatively less loaded with the foulers. The intense spawning season of the major fouling organisms should be avoided while introducing new stocks in the farm.

The boring polychaetes are killed by immersing the oysters in freshwater for 6 hours. Treatment in a saturated solution of common salt for 40 minutes can also eliminate the polychaetes. Brushing the affected oysters externally with 1 % formalin kills the boring sponge and *Martesia* sp. These techniques of control should be carefully applied in each situation without causing mortality of the pearl oyster.

10 Pearl Harvesting and Grading

ACC Victor, R C of CMFRI, Tuticorin.

Pearl Harvest

Harvesting of cultured pearl is usually carried out manually. The oysters are brought to the laboratory from the farm. A sharp knife is inserted in between the two valves of the oyster upto the adductor muscle and the latter is cut vertically. The pearl is then squeezed out of the gonad region. In case oysters need to be reused for a second time, the oyster's valves are gently opened without damaging the adductor muscle and the pearl is carefully removed with the aid of instruments. The oysters are then returned to the farm for recovery and after a certain length of time they can be operated for a second time to produce additional pearls.

Pearl harvest or beaching of pearls is done during the cooler periods of the year during which time the pearl coating in thin and fine. During the post operative culture some oysters die due to natural causes and surgery. Also some reject the nucleus. In a study conducted by C.M.F.R.I. at Valinokkam by scaling of the operation, out of a total of 9414 oysters implanted (single implantation with 3 to 5 mm nuclei) mortality during one year post operative culture was 2108 (22.39%). On harvest, the remaining 7306 oysters (74.69%) did not contain pearls due to rejection of nuclei or non deposition of nacre. In the earlier studies at Veppalodai, gross production of 62.8% in single implantation and 68.3% in multiple implantation with reference to the number of oysters used have been achieved. This variation in the production may be due to differences in the location of the farms and the resultant environmental conditions, reproductive phase and the health of the oysters at the time of nucleus implantation, skill of the technicians and post-operative culture. The variation in the production of the pearls in these two studies can be considered as indicative of the range of production under variable conditions.

Grading of Pearls

The Pearls are sorted by size, shape, colour, lustre and surface quality. Some of the pearls may be perfectly spherical in shape and of outstanding colour and lustre, many are inferior in quality and some are totally value less.

Quality

The quality of cultured pearls is much relevant to the economics of pearl culture. The quality of pearls is dependent upon the thickness of the nacre, iridescence, lustre, colours, size, shape and flaws.

Thickness of Nacre

If the deposition of nacre on the nucleus is thick, then the pearl is more valuable and will give the required luster and iridescence. The nacre is composed of thousands of very thin layers. A good quality pearl is decided according to the homogeneity, evenness and the thinness of these layers.

Iridescence

The iridescence of a pearl is due to its optical characteristic. Light is refracted from the multitude of prisms of aragonite crystals. When the individual layer of organic matrix is thin, the light penetrates well into the translucent crystals. It is refracted in each layer of nacre and the rays that re-emerge together make interference effects, which decomposed the light spectrum into rainbows. The faces of aragonite crystals farm regular grooved – ripple marks on the pearl's surface and enhance the iridescence with defraction fringes.

Lustre

The brilliance of a pearl depends on its lustre. It is considered as the most important factor in evaluating pearls. Good lustre and refraction indicate that the nacre is composed of pure aragonite crystals. Even mis-shaped pearls are sometimes considered as valuable or gems if the luster is good.

Colour

The Indian pearls show diversity in colours. Colouration of pearls is mainly due to the physiological condition o the oyster. The environmental factors also play a predominant role in determining the colour of nacre. Minerals and trace elements in the seawater also influence the colour of pearl. Pearls are yellow, golden half white, ivory white, cream, grey, black, silver, light blue, green and light pink colours are seen. The marine culture pearls produced in Mandapam have different colours of the rainbow. The pink and green colour pearls are considered as most valuable pearls. Colours like pink, blue and green are a rarity.

Size

Cultured pearls from the Indian pearl oyster *P. fucata* are produced in the diameter range of 3 to 8 mm. The size is very important in deciding the price of a pearl. The bigger pearls fetches higher price.

3.5 - 4.5 mm : extra small
5.0 - 6.0 mm : small
6.5 - 8.0 mm :P medium
8.5 - 9.0 mm : large
9.5 - 10.0 mm: very large

Most natural pearls are small in size not more than 2 to 3 mm.

Shape

The shape of natural pearls generally follows that of the foreign substance, which has formed its nucleus. Hence the shape of the natural pearl is mostly irregular and no two natural pearls will be alike in shape. On the other hand since

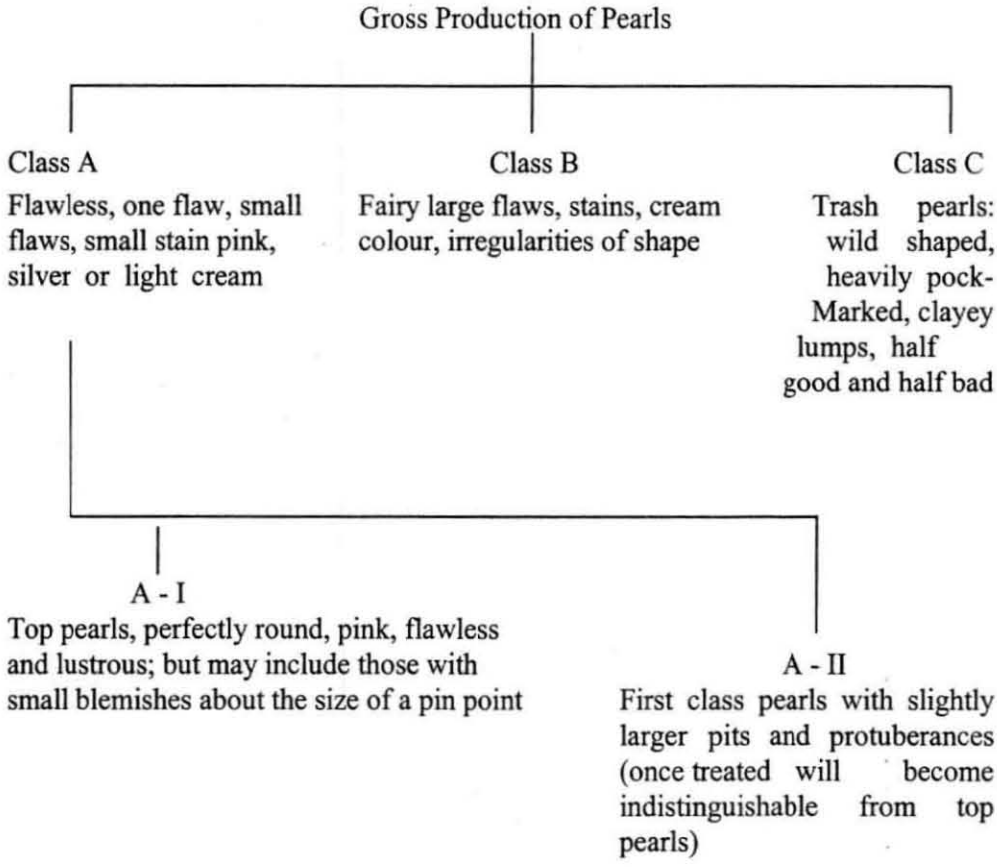
the shell bead nucleus is spherical and the size is large as predetermined, the cultured pearls is also spherical with the exception of malformation and is large in size. Hence cultured pearls of identical shape and size are very common.

Flaws

Production of flawless pearls totally depends on the oyster itself. Due to its rareness the price is always higher. The price will be less if there are many flaws. If the orientation of graft tissue and the nucleus are not properly set, the pearl produced will have teats and black dots.

Hence the above factors determine the quality of cultured pearls and for grading these pearls one can understand not only the colour or the shape or size is important, but the luster adds more value for these pearls.

Shirai (1970) classified the pearls as follows:



In India, in the experimental production, the composition was

Class A	37.6 %
Class B	37.6 %
Class C	24.8 %

However the percentage composition would vary from batch to batch depending upon several factors, which include i) selection of oysters ii)

conditioning process iii) graft tissue preparations iv) implantation v) maintenance of tools vi) skill of the technician and vii) post-operative culture.

Processing of Pearls

It is rather a common practice of the trade to improve the quality of cultured pearls through processing.

The process consists of bleaching and dyeing for colour adjustment. Bleaching the pearls is done by hydrogen peroxide in a fixed strength as bleaching agent. Only the drilled pearls are created. The organic impurity in the pearls are removed with hydrogen peroxide.

Surface polishing of pearls is done with salt. The pearls are mixed with powdered salt in equal volumes and placed in a tub with small amount of water. Then they are taken out and washed with distilled water. The residual mucus on the surface of the pearls will be removed by rubbing with salt to obtain good lustre.

Subsequent to bleaching the colour adjustment is done if required according to needs. Alkali based, oil based, acid and straight dyes are used for this purpose.

By Products and their Utilization

Class C category of cultured pearls cannot be used in jewellery. In such case the nacreous layer is ground off the nuclei and the powder is then dissolved in phosphoric acid with the final products being separated by additional chemical processes. Pearl calcium tablets are marketed in Japan. It is also reported that some Japanese companies have gained the technology to extract high quality calcium from the shell, which is marketed as pearl shell medicine.

Large shells are used in shell craft for their mother of pearl layer. Small broken shells can be used as ingredients in poultry feed.

Pricing of Cultured Pearls

Cultured pearls are priced according to size, shape, weight and quality. Unlike the bullion market where quality is precisely defined and prices fluctuate according to international market, the price of pearls eludes any standardization because of infinite differences in quality and preference variations of customers.

The size and shape of the cultured pearls depends on the size and shape of the introduced nucleus. Coupled with size of the bead the number of layer of nacre and their shape determine the value of the pearls. Because natural pearls have a much smaller irritant the nacre thickness tends to be much greater. Hence the value is considerably higher.

In Indian market the prices are by carat rat. (1 gm is equal to 5 carats). The pricing is different for pearls of different origin viz., fresh water non-nucleated pearls, fresh water nucleated (round) pearls, marine *P. fucata* pearls, south sea

white pearls, south sea black pearls and the blisters of *P. maxima*. While the fresh water non-nucleated pearls fetch the lowest, the south sea pearls fetch the highest price.

The pearls produced in C.M.F.R.I. are being marketed in three grades. At present the top quality pearls are sold at the rate of Rs. 1,500/- per gram and next quality of at the rate of Rs. 1,000/- per gram and the third quality at the rate of Rs. 500/- per gram. Pearls being a biological product, it is rather difficult to find homogenity or uniformity in size and quality among them.

11

Pearl Culture as a Societal Programme

ACC Victor, R C of CMFRI, Tuticorin.

Introduction

Gulf of Mannar is rich in biodiversity and bioresources. An estimate says that about 3,600 species of flora and fauna exist in the Gulf of Mannar, which includes extensive coral reefs, sea grass meadows, seaweed beds pearl oyster and chank beds and mangrove wetlands. Apart from this, Gulf of Mannar acts as a home for the endangered marine mammal sea cow and marine turtles. The breeding and feeding grounds created by these ecosystems and complex food web formed by various marine flora and fauna resulted in high fishery production.

Annually about 1 lakh tones of fish including fin fishes, prawns, crabs, lobsters etc. are harvested from the Gulf of Mannar. About 1.5 lakh fishers living in about 90 fishing hamlets dependent on this fishery resources and seaweed resources for their livelihood. However, due to over fishing and increased fishing population and damage to the coral reefs, sea grass beds and other ecosystems by trawlers fish catch is declining, leading to poverty among the fisher folk.

Creating alternative livelihoods and additional sources of income for the poor fishes is one of the options for the sustainable management of the fishery resources as well as conservation of the biodiversity of the Gulf of Mannar.

Alternative income source for the fisherfolk could be achieved by adopting some of the mariculture technologies perfected as a co activity of their livelihood ie., Fishing. There are few technologies readily available with the R & D institutions in India. One among them is Marine Pearl Culture, even though it is an intricate technology consisting various components among the molluscan mariculture practices.

Marine Pearl Culture – a Theoretical Look

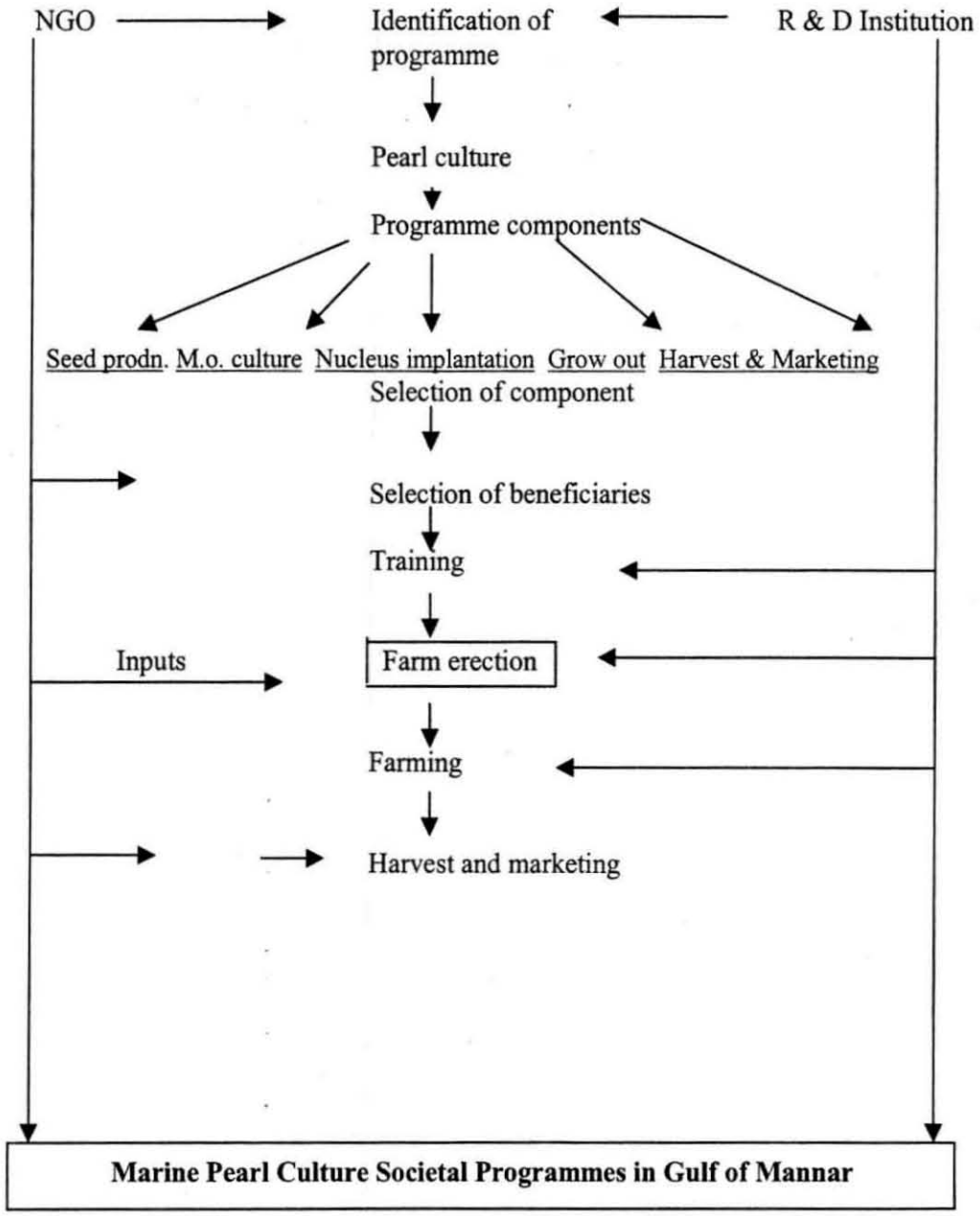
The success story of marine pearl production in India was achieved way back in early 1970's. The two breakthroughs achieved in pearl oyster seed production and culture pearl production is the backbones of the technology. In brief, pearl culture technique involves three different components and require technical competency.

They are, 1) Hatchery seed production of pearl oysters, farming and mother oyster production, (involving about 1.5 years + establishment of hatchery + technical manpower) 2) Surgical implantation of nucleus (a trained team of technician) and 3) culture and production of pearls (farm structures + technical man power + labour). All these components need both trained manpower with higher

degree of technical competency. Hence, taking up marine pearl culture as a whole needs higher inputs, manpower and money.

Marine pearl culture can be taken up as societal programme by fisherfolk with funding and technical assistance from NGO's and R&D Institutions.

Schematic diagram of societal programme - Marine pearl culture



Fishermen and CMFRI

Marine pearl culture as a societal programme has been thought of and implemented by CMFRI way back in 1993 at Valinokkam on a small scale involving Fisherman and CMFRI. A floating raft of 6 x 6 m size was floated with 100 cages suspended in it. A total of 9,414 oysters were nucleated and cultured. The detailed economics is given in the latter section.

Fishermen, NGO and CMFRI

As an one step further of the above activity, during 2002-'03 a societal programme consisting the role of all the three partners was conceived and successfully demonstrated at Mundalmunai Village, Pamban, Ramnad District.

Lead Discussion

As a beginning to the implementation of the programme, the NGO namely, MSSRF, Chennai held detailed discussions with the Scientists of the CMFRI on the various aspects of marine pearl culture, its technicalities, input components, possibility of handling by the fisher folk. The whole discussion focused on which component the fisher folk have to take up as a societal programme as pearl culture is a multifaceted one. After a couple of meetings and discussions, a schedule of activities was designed and responsibilities were determined for each of the participating units.

Mind Set Conversion of Fisher Folk

In general, fishermen are mostly conservative and are reluctant in involving in such activities. Further their financial statuses also don't encourage them to venture out in new avenues. Hence, they have to be first enlightened and encouraged by way of group discussions and all their doubts cleared.

Responsibilities of each of the participating groups

MSSRF

The primary and lead agency responsible for overall planning and execution, identification of beneficiaries, logistic supplies etc. The detailed responsibilities of NGO are detailed below:

1. Mobilize the community and organize them into a pearl culture society
2. Developing an organizational structure and management procedures for the proper functioning of the society
3. Getting necessary permission from the Tamil Nadu Forest Department to construct pearl farm in the Gulf of Mannar
4. Providing financial support to the society for training, purchase of implanted pearl oysters and farming

CMFRI

The brain of the societal programme and has responsibility in planning, training and guidance throughout the programme. The responsibilities are listed:

1. Helping MSSRF in mobilizing the villagers by providing technical and economics details of pearl culture to the villagers
2. Providing technical inputs and participating in identifying suitable site for pearl farming
3. Providing training on pearl farming to the villagers
4. Providing technical inputs for preparing a micro plan for pearl farming
5. Providing technical inputs for constructing pearl culture farm
6. Supply of 100000 nucleated pearl oysters to the society on payment
7. Periodical supervision of the farm and technical advise till harvest of pearls

Fisher Folks

The backbone of the programme is completely involved in executing the activities in consulting with the other two units. The following are the detailed responsibilities:

1. Constructing and managing pearl farm
2. Growing nucleated pearl oysters in the farm
3. Protecting pearl oysters against predators, growth of epiphytes and epifauna
4. Protecting pearl oysters from poaching
5. Protecting pearl farm from natural calamities lime cyclone
6. Harvesting and marketing of pearls with the help of MSSRF

Implementation and Progress of the Programme

As a first step, a village level society namely, "Mundalmunai Pearl culture society" was formed and registered. Twenty members of the society were taken to CMFRI, Mandapam laboratory and were given one week on hand training mainly focused on Farming of implanted oysters, precautions to be taken and farm management. A suitable site for pearl culture, located nearby the village was identified and necessary permission was obtained from the Tamil Nadu Forest Department. Construction of culture structures – racks was done in 15x10 m area. Periodically, oysters were operated at CMFRI laboratory and transplanted to the farm at Mundalmunai village for further grow out and pearl production.

Data on economics of pearl culture – Valinokkam experience

Method: Cages suspended from a 6 x 6m raft

	Input cost (for two years)		**Rupees**
1.	Cost of teakwood poles, floats, anchor chains		13,000
2.	Cages (100 nos.) for rearing 10355 oysters		10,000
3.	Cost of 10355 pearl oysters at Rs. 1.40/seed		14,500
4.	Cost of 9494 shell bead nuclei at Re 1/bead		9,500
5.	Cost of menthol, glasswares, plastic wares, Surgical instruments etc.		5,000
6.	Labour charges for pearl oyster surgery		3,000
	Total		55,000
	Production and Revenue		
	Total pearls produced	Nos	1849
1.	Sale proceeds of 1296 pearls (wt. 138.28g)	Rs.	73,133
2.	Cost of 250 pearls distributed to fishermen in lieu of their labour		12,500
	Total earnings		**85,633**

Anticipated Outcome of the Societal Programme

The experience gained and successful completion of the societal programme would enable many such units to venture for similar programmes and all such units could unit together to form a total cooperative structure including supplies of input and purchase and marketing of pearls. Apart from monetary benefits, trained manpower development; local employment generation is also foreseen.

Constraints

Any societal programme is not an easy job to begin with and successfully complete, the Mundalmunai attempt is one such thing. To add to this, are the legal status prevailing in the State government also proves to be a stumbling block. In the absence of clear cut legal issues, policies and guidelines from the state government, it would be worse. Hence, a high level meeting of Scientist, planners and administrators of state government should sit together and work out the modalities and guidelines for such programme as oysters and seafront are the monopoly of state.

Conclusion

From the above experiences, it is evident that 'Pearl culture' can be adopted as a societal programme for the alternate income generation for the fisher folk inspite of the multifaceted and high technical competence requirement. With successful completion of few similar programmes will infuse blood to form "Cooperative societies for marine pearl farming" in Gulf of Mannar area in the future. This would enable building of the economy of the poor fisher folk. The interest and involvement shown by the fisherfolk in making the programme successful are encouraging and exemplary.

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Pearl production in Abalone

ACC Victor, R C of CMFRI, Tuticorin.

Abalones are one among the few mollusks known for producing gem quality pearls and highly priced meat. The nacre of abalone shell is often multihued in tunes of silver, orange, pink, green, blue and lavender. The abalone pearls are superior to pearls produced from freshwater mussels and comparable to best marine pearls. History of abalone pearls dates back to 5000 BC. The first recorded reference occurs in one of the Japanese oldest historical writing, the Kojiki. (cs 800 AD). Production of pearls from cultured abalones is of recent origin. The French scientist Louis Boutan carried out pioneering work on abalone pearl production in 1897. He successfully produced semi-spherical pearls from abalones. Later, several workers refined his technology and in mid 1950s, Dr. Kan Uno was very successful in growing hemispherical pearls in several abalone species. But attempts to produce free pearl did not give any encouraging results. Now abalone pearl farms producing blister pearls are existing in various countries.

Half Pearl Production

CMFRI achieved initial success in the half pearl production from abalones during 1998-'99 at its regional center, Mandapam. Earlier attempts to produce pearls in abalone by fixing a nucleus on the inner side of the shell of the animal was not successful due to dislodgment of the nucleus by powerful foot movement of the animal. Due to sustained efforts a comprehensive method was developed and pearl production became a reality.

Abalones of good health, without any physical injury and unaffected by borers are segregated from the natural collection and maintained in the laboratory with seaweed *Ulva* sp. as feed. The abalones are taken out from the tank and air dried for 10 minutes prior to nucleus fixing process. This enables the easy retrieval of the foot muscle for drilling at the appropriate site.

Drilling is done on the inner side of the shell, by pushing the mantle to the maximum possible extent, using an electrically operated hand drill with a fine drill bit (3 mm). Extreme care should be taken to avoid any sort of physical injury to the animals. Drilling is done in one swift action and the drilled abalones are returned to a recovery tank containing well aerated seawater immediately after drilling. This enables the abalones to recover from the drilling shock as well as getting rid of the drill dust.

After half an hour, the drilled abalones are taken out from the recovery tank, their mantle is pushed aside with the aid of a sterile scalpel's blunt end and the inner shell is wiped with cotton. The commercial grade adhesive Anabond is used as a fixative. A drop of the glue is placed in the hole and spread on the edges of the

drill hole, immediately followed by placing a shell bead (used for marine pearl culture) of required size (4 mm) with fine tweezers and gentle pressure is applied on the nucleus till the adhesive is completely dried. The animal is returned to FRP tank with running seawater and aeration.

Active abalones having nucleus are collected on subsequent day and stocked in conventional box type cages knitted with appropriate size mesh and suspended into sea from the rack. Feeding is done at bi-weekly intervals by placing seaweed *Ulva* sp. inside the cage. Monthly observation is done to check the nacre coating, mortality etc. At the end of first month, slight nacre coating could be observed over the nucleus. The stocked abalones are harvested on 4th month when the nucleus has thick and uniform nacre coating. About 40% of the abalones had good nacre coating in the experiments.

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Marine Pearl Production through Tissue Culture

S. Dharmaraj, R C of CMFRI, Tuticorin

Introduction

Tissue culture, in general, is being carried out in various fields of medical and agricultural research. The study has been commenced since long back to understand the cell type, cell behaviour, cell structure, cell multiplication, cell reaction to drugs etc. It has become a vital tool in micro pathological and immunological studies aiming at finding solutions to certain diseases. Plant tissue culture has reached an advanced stage of achieving an entire plant from a single cell. All these studies are concerned with plants / animals which are related to freshwater species. Marine invertebrate tissue culture is not only a new origin but also a new field of research concerned with marine animals. Primary aim of the study is to formulate suitable culture media specific to each species and to establish cell lines. Later the study is extended to commercial aspects of producing *in-vitro* pearl from pearl oysters in Japan. Extensive works have been carried out on cell proliferation and its behaviour in a medium developed specifically for the species and to formulate suitable medium based on the results obtained. The countries like Japan, China, United States and Canada initiated marine invertebrate tissue culture. Among these countries, Japan is the pioneer country carrying out research in pearl oyster for the purpose producing *in-vitro* pearl through tissue culture. Visualizing the importance of the work in view of deterioration of natural environment India too entered in to the field of marine invertebrate tissue culture research, as it is one of the pearl producing countries in the world. Expertise in the field of research has already been developed and a fully functional marine invertebrate tissue culture laboratory has been established at Tuticorin for the first time in India. Cultures are organized since 1996.

Setup of Tissue Culture Laboratory

Generally the tissue culture laboratory should be compact with different modules so as to contain contamination by effectively maintaining high-grade hygienic conditions. It is fully air-conditioned. The entrance room is the one where the records are maintained and discussions are held prior to organization of cultures. Animal sterilization room is arranged on the left side of entrance room having U.V. sterilization unit and provisions for running seawater supply. The entrance room leads to preparation room where preparation of culture media, saline solutions, extracts, tissue culture materials etc., are carried out. The preparation room proceeds to dressing room and to operation room or clean room. A dark chamber or otherwise called 'Pass Box' is situated in between the preparation room, dressing room and clean room. It has three doors with a U.V.light on its top to keep the

materials always sterile. The doors are arranged in such a way that one door is facing preparation room through which sterile materials are placed inside, the other on the dressing room from where the dress materials are taken out and the third one on the operation room from where the materials are taken during organization of cultures.

Preparation of Culture Media

The marine mollusc medium (MMM) is constituted based on the composition of haemolymph of each species. Refinement of medium is done periodically based on the results obtained in the cell culture. There are a few media developed for marine molluscs such as Medium M199, P35, L-15 and Ham's F 12. The media are commercially available along with formula. The media can be prepared based on the formula.

Preparation of Balanced Salt Solution

The balanced salt solution (BSS) is prepared in the following manner.

Na. K. solution	125 ml
Mg. Solution	50 ml
Triple distilled water	200 ml
These are mixed, autoclaved and taken to clean room	
Ca. solution	50 ml
Triple distilled water	50 ml

These two solutions are mixed, autoclaved and taken to clean room. Mixing of the above two solutions and the following is done on the clean bench.

Glucose	5 ml
$NaHCO_3$	5 ml
NaH_2PO_4	5 ml
Kanamycin	0.5 ml
Penicillin	5 ml
Fungizon	5 ml
Total	500 ml

Preparation of animals and tissues

The test animals are depurated for a minimum period of 3 days in U.V.treated running seawater. The depurated animals are wiped with externally with 70 % alcohol and taken to clean room. The mantle tissues of test animals are excised and washed several times BSS to get rid of mucus and other adhering particles. If needed the tissues are treated in antibiotic solution containing 1000 µg/ml streptomycin and 2000 IU/ml penicillin. The tissues are cut in to tiny pieces of 1 square mm in size.

Culture Techniques

Flask and Petri dish Cultures:

Before introducing the fragments of tissue in to the culture flask the mouth of the flask is shown to isopropanol flame for sterilization. Tissues are placed inside the flask with the help of a needle. The tissues are allowed to stick on to the flask and 3 ml of medium is added. A similar inoculation is made in petri dishes also. The culture plates are placed in CO_2 incubator and maintained at 25-28°C.

Cell Well Culture:

The cell well is otherwise called as micro plates. There are different types of cell wells. The size of 24 wells is 16 mm in diameter and 17 mm in height and the size of 96 wells is 6.4 mm diameter and 11 mm height. The cell well is provided with a cover. The cell well is used to culture single cell for the purpose of cloning. 3 to 4 drops of medium are added to each well. The cell wells are kept in CO_2 incubator at 25-28°C.

Medium Change:

Medium change is normally done on alternative days. Periodicity of medium change is determined by observing the condition of the cultures. Culture flasks are taken to clean bench after wiping with 70 % alcohol. When the flask is opened, it is shown to flame. Much care is taken during medium change. A separate pipette is used for each flask. Half of the medium is changed during first and second time and subsequently the whole medium is changed. At times cell suspension is centrifuged and fresh inoculations are made. In some established cell lines the cells are active and hence the entire medium is changed.

Organisation of Cultures

1. Primary culture: The processed tissue is treated with trypsin for the purpose releasing the cells from the tissue. To effect this the cut pieces of tissues are placed in trypsinisation flask containing 30 ml on marine mollusc calcium magnesium free phosphate buffer solution (MM CMF PBS) with 0.05 % trypsin. A Teflon stirrer is used in the flask for proper dissociation of tissues and dispersion of cells. The stirring is done for 10-15 minutes at 1200 rpm. The cell suspension is first filtered through 150-µm sieve and then through 60-µm sieve. The filtrate is centrifuged at 4°C for 5 minutes at 800 rpm and the supernanent solution is removed gently without disturbing the precipitate. A drop of medium is added to the precipitate and mixed well. The mixture containing free cells is distributed to different flasks or petri dishes by means of Pasteur's pipette. 3 ml of medium is added to each flask and the flasks are placed in CO_2 incubator at 25-28°C.

2. Explant culture: For explant culture of tissues, fragments of tissues are processed in balanced salt solution (BSS) and inoculated in the flasks or petri dishes. 3 ml of medium is added to each flask. The cells from the

explant proliferate in large numbers and migrate away by adhering to the bottom of the flask. The round epithelial-like cells and fibroblast-like cells are seen in the cultures. The cells do multiply in *in-vitro* cultures and increase in numbers forming cell sheet. When a cell sheet is fully formed, it is due for subcultures or for cryopreservation of cells. At ideal conditions the cells develop pseudopodia and form a network to cover the entire surface of the flask as organic matrix. The migrated cells are stationed at places and formed pearl sac. The organic matrix induces the cells to secrete crystals.

3. Organ culture: The processed fragments of tissues are placed on a raft in petri dishes. The raft may be at any form as per the requirement of the experiment. In organ culture the explant tissue is not immersed in the medium but it is kept in such a way that the medium is filled up to the lower phase of the tissue leaving the upper phase with air contact. In such case the cells are kept intact without dislodging their positions. The interaction and integration of the cells perform their original functions of forming organic matrix and pearl sac. The cells secrete nacreous crystals and deposit on the matrix. As the mantle cells are responsible for the formation of shell, the cells secrete prismatic layer in hexagonal form. Each hexagonal segment is bordered by interlamellar organic matrix.

4. Cell proliferation and cell types: In the explant cultures the cells do proliferate in large numbers from all sides and migrate away from the explant. There are two types of cells i) granular cells and ii) agranular cells. These cells develop pseudopodia and form organic matrix. On completion of the matrix crystal deposition starts to form nacre layer. If the nacre layers were continuously formed, an in-vitro pearl would be formed.

Preservation of Cells

Cells to be preserved by freezing would be released from the culture flask by adding 0.25 % of trypsin. The cell suspension with 3 to 6 ml of medium is centrifuged for five minutes at 1200 rpm at 4°C. Supernanent water is removed and 2 ml of medium and 2 ml of Minimum Essential Medium (MEM) with Dimethyl Sulfoxide (DMSO) 7.5 % mixture were added drop by drop. The 4 ml suspension is divided into four parts and kept in four freezing vials. After the vials are sealed and labeled, there are frozen at the rate of -1°C at every minute. Freezing is done at three stages, first at 0°C for 30 minutes, then at -20°C for 60 minutes and thirdly at -70°C for 6 months and finally at- 196°C for one or two years in liquid nitrogen.

In order to protect cells from damages during storage, DMSO 7.5 % and glycerine 10 % are used along with medium. Freezing of cells is done mainly for three reasons.

1. During cell line the cells may change their enzyme activity, chromosome number etc. Therefore it is essential to freeze these cells at a particular stage of cell line and then rejuvenated.
2. There may be contamination in cell line. To prevent this cells are frozen at periodic intervals.

3. In an established cell line the cells can be cultured to a maximum of 50 times. In Some other cell line, cells are likely to die at any time. Such cell lines can be sub cultured only for 30 times. Freezing of these cells may extend the period of cell line.

Application of Tissue Culture Techniques

There is an increasing use of tissue culture in various fields of biological research. Tissue culture techniques are being adopted in Marine Invertebrates since in recent years. By conducting tissue culture, valuable information could be collected on aspects of like cell structure, cell division, cytogenetics, cell physiology and cell viability. Tissue culture techniques are useful in studying the structural as well as functional aspects of cells, tissues or organs by culturing them *in-vitro*. The techniques are employed in investigating the effect of chemicals and radioactive elements on normal tissues and cancer cells and in microbiology, pathology and in the production of vaccines. Results obtained may help in finding out methods of curing several diseases. Careful studies in tissue culture will help in transplantation of tissues and cells among members of a species or from one species to another species. In recent years tissue culture technique is being used in the production of *in-vitro* pearls from pearl producing molluscs.

14

Value Added Pearls – Mabe, Baroque and Keshi

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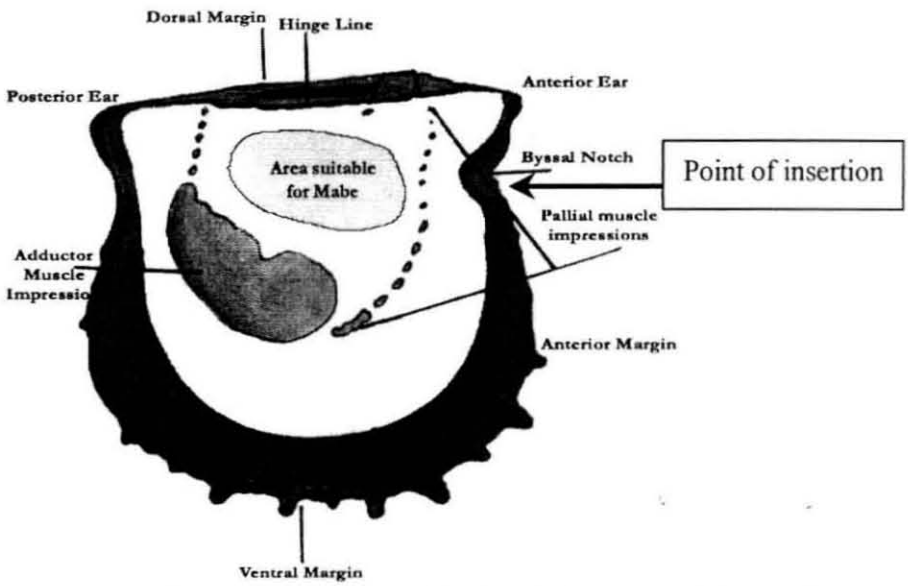
There has never been a period in history when pearls were not in vogue. They go well with any style, in any place on any person. There are many types of pearls available today than ever before. They offer a wide variety of colours, shapes and sizes and a wide range in price. The variety available results from the use of different types of oysters, the physical environment in which they live, and the varying cultivation techniques used by producers. Some of the types of marine value added pearls are presented here.

Mabe Pearls

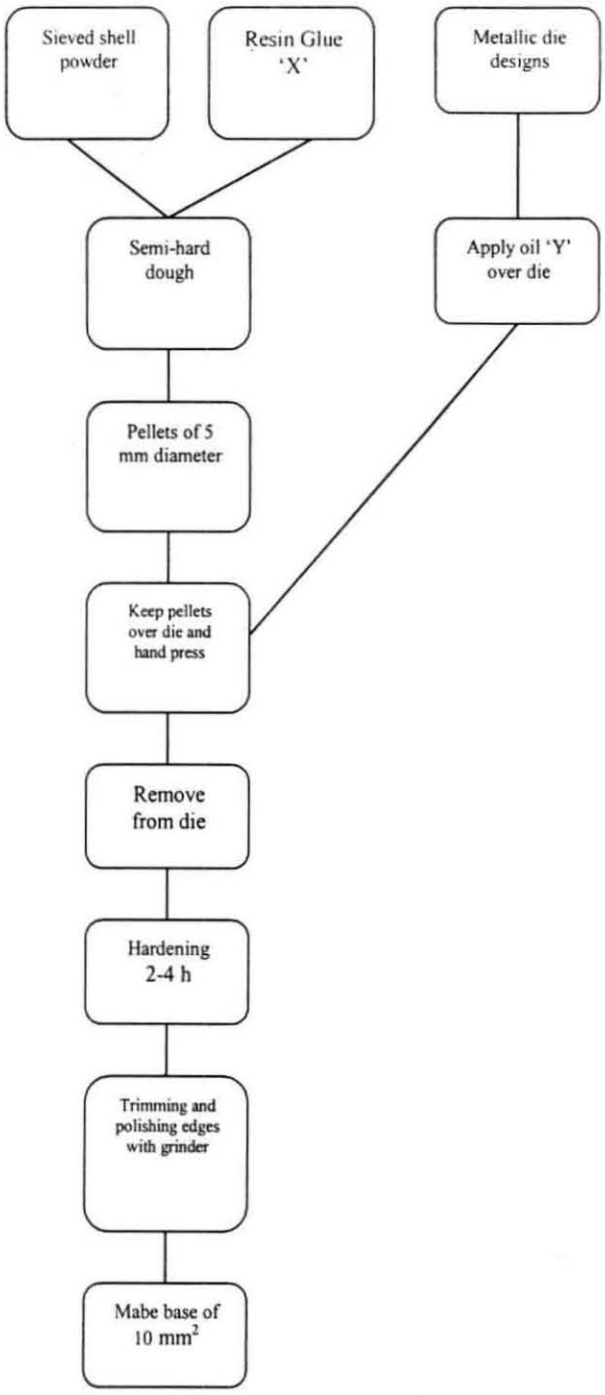
A mabe pearl is a dome shaped or image pearl produced by placing a hemisphere or miniature image against the side of the oyster shell interior. In India, the technology for producing mabe pearls is already developed in the freshwater mussel. Other than half pearls and blister pearls, images have not been tried in the marine species *Pinctada fucata*. Trials were made in *P. fucata* using base images (10 mm^2) made of shell powder and resin, plastic images and camel bone images. Rearing of oysters was done at CMFRI's Port Kollam raft farm.

Oysters suitable (> 45 mm DVM) for insertion of images were selected and placed in a shallow pan with their hinge down. Oysters with open valves were pegged with wooden splits, and using an oyster speculum, the shell gape was gently



widened. The oyster was held with the cupped left valve in the palm of the hand.

The base image was picked with a fine angled forceps and inserted face up through the anterior end near the byssal notch, where the gap is the widest. The pallial muscles offer slight resistance, and the image was slid through under the mantle so as to lie in the deep sinus close to the dorsal hinge. The image is therefore bound by the hinge, pallial muscles and the adductor muscles and therefore cannot be easily dislodged. The oyster is immediately placed in fresh seawater with hinge down and ventral margin facing up.



Individual oysters were then placed in specially made velon screen (large mesh) pouches made into strips, again taking care to see that the ventral margin is at the top. Up to 6-7 oysters can be placed individually in pouches in one velon cage. The cage is then suspended from the raft with suitable weight to keep it upright.

The base images in plastic and bone material were rejected within a month. Only base image made with shell powder gave satisfactory results. Observations (Mohamed et al., 2003) indicate that within 15-20 days, the nacre coating is initiated. This is substantiated by the observation of nacre secretion on strips under tissue culture from day 7 onwards (S.Dharmaraj, Pers. Comm.). Fusing of the image to the shell was complete by day 20. By the end of 60 days it was possible to get complete and adequate nacre coating on the image so as to produce a mabe. Rejection and mortality was high (100%) when the image size exceeded 10 mm^2. The survival of oysters and percentage recovery of Mabe after 82 days of rearing is shown in Table 1. Longer period of incubation resulted in the masking of finer details of the image. Flow chart of the process is shown in fig.

Table 1. Percentage survival and percentage recovery of Mabe pearls in *P. fucata* at Port Kollam after 82 days of rearing

Treatment	Stocked No.	Survival %	% Mabe Recovered
Strip 1	7	43	29
Strip 2	6	100	67
Strip 3	7	86	57
MEAN		76	51

Anil et al., (2004) also described a method by which mabe images were stuck to the inner shell edge using cyanacrylate glue to produce mabes.

Baroque Pearls

The rarest pearls are round pearls and round pearls in fine quality are very costly. A baroque pearl technically is any pearl that is not round and has an interesting irregular shape. Baroque pearls should not be confused with pearls that are simply 'out-of-round'. They should have a distinctive enough shape to be interesting and attractive. Baroque pearls can be produced by both saltwater and freshwater molluscs, and can be natural or cultured. They have a distinctive appeal, because of their very beautiful tints of colour and iridescent flashes, which are the results of pools of nacre (where the Baroque shape creates an area in which the nacre can collect and is deeper than along other parts of the pearl). Baroque pearls with their distinctive irregular shapes are more common than round pearls, which make them more affordable, but they can make beautiful jewelry. Baroques are often obtained in CMFRI's pearl farms.

Seed Pearls and Keshi Pearls

Seed pearls are very tiny, round, natural pearls usually under 2mm in size. They are rare today, but often seen in antique jewelry. They are sometimes cut in half to create a larger supply for particular jewelry creation, or to remove blemishes or a misshapen side: these are much less expensive than full seed pearls. Seed pearls can be produced by freshwater and saltwater molluscs.

Keshi pearls also called "chance" pearls are interesting baroque pearls accidentally produced in seawater oysters used for cultured pearl production. Sometimes an oyster rejects its bead implant, but particles of the accompanying mantle tissue used alongside the bead remain; these particles of the mantle tissue stimulate the production of nacre, resulting in the wonderful interesting pearl known as keshi. They are unusual because, like natural pearls, they are essentially all nacre and all natural.

Japanese keshi are usually very small. The word "keshi" actually comes from the Japanese word meaning a tiny particle and was used to refer to "poppy" pearls, a fitting image for the strands of minuscule pearls they describe, very tiny

pearls that might be confused with natural seed pearls. At one time it was not unusual to see necklaces comprised of 20, 50, or as many as 100 strands of these tiny pearls strung together, the strands being so delicate they look like silken thread.

The keshi pearl now attracting the attention of collectors however is the south sea variety, which is much larger, 8-10 millimeters and up. Virtually always baroque in shape, they offer a variety of unusual shapes, often oblong, and lend themselves to very distinctive jewelry creations. They occur in virtually all shades of colour from silvery-white to cream, gray to black, yellow to gold, even mauve and lilac tones. One of the most striking characteristics of the south sea keshi is its very intense luster and iridescence, far greater than what is normally seen in even the finest round cultured pearls

They are very popular in Europe and the Middle East. For Moslems they are particularly desirable because like natural pearls they are an all – natural creation and by comparison to the cost of natural pearls very affordable.

But keshi pearls are disappearing. Japanese and south sea pearl producers are trying to reduce the number of keshi pearls being produced because the production of keshi creates a costly problem. As nature would have it, the oyster can only produce a certain amount of nacre: if keshi are consuming nacre, that leaves less for the culture pearl being produced simultaneously within the same oyster. This means that the more keshi pearls, the fewer the fine, round cultured pearls. As the cultured pearl growers succeed in reducing the number of these "chance" pearls, fewer keshi will be available. Predictions are that they will become scarcer in the years ahead, which is sparking serious attention from connoisseurs.

Ringed or Circle Pearls

When a concentric ring encircles the surface of a pearl we say it is "ringed" or "circled": This is a type of surface characteristic that can occur on any variety of pearl, when a pearl exhibits numerous concentric rings from top to bottom. However it creates a very interesting and distinctive looking pearl. Usually off-round baroque in shape and much less expensive than round pearls or symmetrical baroques, these "ringed" or "circle" pearls have a special allure and are being used increasingly in jewelry especially those from the south pacific occurring in shades of white, gray to black and aubergine. Artistic designers find circle pearls an exciting choice for distinctive and dramatic creations.

Akoya Pearls

This is the pearl that comes to mind the moment anyone mentions "pearl"-lustrous round white pearls. The finest Akoyas originally produced in Japan are more perfectly round (7-10 mm diameter) than most other pearls and have the highest luster, which makes them especially desirable. Unfortunately for those who prefer very large pearls they rarely exceed 10 millimeters in diameter and when they do they command exceptionally high prices. In addition to Japan, China is now a major producer of Akoya.

In India, attempts have been made to produce fucata pearls similar to Japanese Akoya by implanting larger oysters grown in Kollam Bay along the southwest coat of India (Kripa et al., 2003). The largest cultured pearl obtained in this experiment had a diameter of 7.88 mm weighing 0.68 g and the average nacre thickness was 1.37 ± 0.27 mm. A nacre thickness of 0.5 mm is acceptable as a pearl and the minimum is 0.35 mm for good lustre and color. Assuming a uniform coating of 0.129 mm/month, it can be inferred that a pearl with 0.5 mm nacre will be formed in 4 to 5 months. Earlier work done along the east coast has shown that under tropical conditions, acceptable pearls are produced within 4-5 months with nuclei of 2–3 mm diameter and in 15-18 months with nuclei of 6-7 mm diameter. However, this study shows that along the southwest coast of India, the nacre production is faster and the period of rearing nucleated oysters can be considerably reduced to produce Akoya type pearls in India.

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On - Shore Pearl Culture Techniques

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Intrduction

Marine pearl is the most important bioproduct of gem value. It is revered from ancient times mostly for sentimental/aesthetic importance, although it has no resale value like gold. When compared to the quality, value etc. the freshwater pearls now available plenty in India have no comparison with marine pearls. Since marine pearls are not actively traded in India, and most of the people cannot afford them, cheap freshwater pearls have invaded the Indian markets from China. The technology of marine pearl culture is very old and is in vogue for the past 100 years. At present Japan, China, Australia, Polynesian islands and Indonesia are the leading commercial producers of pearls. The species that produce the valued pearls are *Pinctada fucata, P. margaritifera* and *P. maxima* in order of abundance. However, with regard to the value of the pearls produced, the order is *P. maxima, P. margaritifera* and *P. fucata.* In all the countries pearl culture is conducted in the inter island areas or sheltered bays and is exclusively a sea based activity.

In the sea based pearl culture practiced at present, the pearl oyster collected from the sea or produced in the hatchery are grown in the sea by suspending them from rafts or rens depending on the location, depth etc. The depth also varies from place to place and from season to season. As depth increases the fouling and boring problems become minimum. However, the availability of food also decreases resulting in poor growth and mortality. In a farm, the cleaning activity is a daily affair and the operation and maintenance of a suitable boat/vessel with labour force is a routine work consuming major part of recurring expenditure. Thus the operation cost is high compared to the capital expenditure.

At present pearl culture work is mostly confined to areas where there are natural beds. In India pearl oyster beds exist around Tutucorin area and research work is also largely confined to this region. The sea based technology of pearl culturewas developed at Tuticorin about three decades back. However, till date there is no active commercial production of marine pearl in India. Both the coasts of peninsular India experience rough sea at frequent intervals making it difficult to float any rafts over a long period for commercial operations of any nature.

The On - Shore Pearl Culture Technology

In the light of the above constraints, on shore pearl culture studies were initiated from Visakhapatnam in 1996. Experiments were conducted on several aspects and many related parameters were refined and standardised. A small demonstration cum research facility of onshore pearl culture has been established in

the premises of CMFRI, Visakhapatnam. The salient features of the technology developed and adopted are as follows.

The spat of *P. fucata* of 5 mm dorso ventral measurement (DVM) grows to about 60 mm in about 12 months, suitable for implantation with 5-7 mm and above nuclear beads. The oysters are grown in suitable cement tanks specially designed for this purpose. The tanks are covered with dark covers and provided with ventilators on all sides to keep water temperature at optimum level throughout the day. They are then spread out at varying densities from the spat to oysters of implantation stage depending on their size and are frequently thinned in tune with their growth.

The seawater for this purpose is drawn from the sea by a standard intake system of suitable capacity. The water system has a filter at the source permitting only semi filtered water from the sea by avoiding sand particle but retaining micro algal cells. This seawater is directly pumped into the pearl oyster tanks. About 10% of the water is exchanged daily and 100% water exchange is effected at every 10 days interval.

Three species of microalgae viz. *Chaetoceros calcitrans, Isochrysis galbana* and *Nanochloropsis salina* were identified as best combination for good growth. They are grown separately and mixed in the ratio 7:2:1 at the time of feeding. The ratio varies from spat to adult. The mixed micro algal feed is supplied to the pearl oyster tanks through a low energy drip flow system, the flow of which can be adjusted to the desired level of algal cell concentration. The algal cell concentration varies from 10,000 cells/ml to 75,000 cells/ml for the size range of 5 mm to 60 mm DVM and also suitably adjusted to ambient seasonal changes in temperature.

The salinity ranges from a low of 16 ppt for about few days during the northeast monsoon period to the normal salinity of 35 ppt. It has been observed that this wide range did not affect the growth, survival or algal production. Chemicals or antibiotics are not used at any stage, even for cleaning the tanks, which makes this technology eco friendly. Fouling, boring and predation was totally avoided in this system. With continuous food supply more than 80% survival from spat to the stage of adult was achieved. Even post-implantation mortalities were negligible, resulting in better pearl yield.

Maturation of the captive oysters and broodstock management technology has been developed. Brood stocks of pearl oysters were maintained in fully mature and ready to spawn condition, making it possible to conduct hatchery operation on a predetermined schedule. The brood stock development and spawning were tested successfully and spawning can be easily induced at any time of the year. In sea based activity one may have to wait for the oysters to attain natural maturity, which occurs only in certain seasons.

A record growth of above 100 mm was achieved for *Pinctada fucata*. Good quality pearls ranging from 6 to 9 mm were produced in about 12-15 months. Many pearl oyster of above 6 years are still active.

There are some misconceptions regarding onshore pearl culture, which need to be analysed in their proper perspective. One opinion often expressed is that in onshore pearl culture the quantity of algal requirement is huge and its production is costly. In reality it is one of the easiest process involving cheap inputs. Due to lack of practical Knowledge, some professionals feel that it is a costly input. In fact, once the algal culture facility is established, it becomes inexpensive and forms only one of the routine inputs. This is demonstrated by large number of prawn hatcheries around Visakhapatnam, which carry out algal production on a large scale and efficiently using minimum inputs.

It is time for us to realise and accept the natural limitations of our open sea conditions, which restrict sustainable farming at sea. There is therefore a need to switch over to "seawater based activity" rather than sea based activity to overcome this problem, instead of making improper comparisons with inter island, calm and protected areas as in Japan, Indonesia etc. A careful look at the recent success of commercial mussel culture along the west coast of India clearly indicates that the success is primarily due to shifting of the activity from the sea into the safe backwaters.

Apart from P.fucata, other species adopted for onshore (land based) system are *Pinctada maxima, Pinctada margaritifera* and *Pinctada chemnitz*.

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Site Selection for Bivalve Culture

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Introduction

In India, most of the bivalves produced are fished from the natural stocks. At present mussels, edible oysters and pearl oyster are the bivalves cultivated mainly from Kerala and Tamil Nadu. Cultured mussels form about 7% of the total catch. Production of bivalves by mariculture in India is very little compared to the rest of the world. In India, the cultivated bivalves are the green mussel *Perna viridis*, the edible oyster *Crassostrea madrasensis* and the pearl oyster *Pinctada fucata*. Except for the pearl oysters, the major source for collection of the seeds of the bivalves is through wild collection or through laying of cultch material as in edible oysters. There is considerable possibility for development of bivalve cultivation industry in India.

The success of mollusc mariculture depends largely on the proper selection of culture sites. In selecting a site for mollusc culture, considerations should be carefully given to a number of factors, which can be grouped under primary and secondary factors. Physical, ecological and biological factors (primary factors) are of prime importance in the selection of suitable culture sites, while factors such as risk and economics and legal usually follow in terms of importance. It is important to understand that if the primary factors are not fully satisfied, the particular site under consideration should be discarded whether or not all secondary factors are satisfied.

The selection of a culture site is initially determined on what bivalve species is intended to be cultured and consequently on the tolerance range of the above species to a number of environmental parameters (e.g. salinity tolerance range). In addition, the site will have to be suitable to the culture method or system intended to be practiced.

Primary Factors

Area Location

Locating the culture site would vary according to species and culture system. For strictly bottom dwellers such as the blood cockle *Anadara granosa*, the ark shell *Arca broughtonii* and the carpet shell *Venerupis japonica* the culture grounds should be located in protected areas where strong winds (eg. monsoon) do not prevail seriously over the area. Deposition of mud and fast siltation rates are often related to water turbulence partly induced by wind action. Although cockles and clams can actively move within their substrate, heavy mud deposition can cause serious mortality either by physically trapping the organism below the soil/water

interface or by raising turbidity to a level where filtering activity is completely hindered. For species that can be cultured by using different systems such as pole, rack or hanging, the location of a suitable area will depend on the culture system intended to be established. Oyster long-line culture as practiced in the Republic of Korea allows the exploitation of areas, which would be otherwise unsuitable for other bivalve culture systems. Long-lines can withstand relatively strong wind and wave action due to the flexibility of the system itself. The only limitation of the above offshore system is the depth of the water column, which will determine the length of the rens.

The length of the rens or of any other hanging structures used like lantern nets, pearl nets, etc. usually determine whether a long-line or raft structure would be economical to establish. The length of these culture units is usually limited to the upper water layers which abound in phytoplankton cells, however a minimum length is necessary in order to economically justify the initial investment and running cost of the above facilities

Substrate

Substrate composition and stability is a major environmental parameter to be considered during the selection of a culture site suitable for benthic species such as cockles and clams or where bottom culture is intended to be carried out. Substrate composition will determine the suitability of an area for a particular species. Cockles are usually found on muddy or silty-clay bottoms, with the highest population densities found on soft intertidal mud flats bordering mangrove swamp forests (Observations on cockle spatfall showed that they settle mainly on fine, soft, blackish mud. In Penang Island, Malaysia the well known Bagan Jermal bed adjoins areas of sand, sandy-mud and stiff black mud with sand, shell and plant debris. In this area spatfall has taken place every year with the greatest concentration always on fine soft mud.

Oyster bottom culture is limited to areas where the sea floor is firm enough to support some kind of cultch and where siltation is not excessive. This traditional culture method, although not as productive as other culture systems such as the raft method, is sometimes the only system that can be adopted either due to a number of unfavorable environmental conditions or limited funds. This method is in fact the most inexpensive as it relies exclusively on the availability of stones, empty oyster shells or similar materials on which the oysters settle and grow. This method is widely adopted in many areas around the world. In Thailand rocks are usually piled in groups of 5–10 and spread in rows approximately 50 cm apart in each direction. This technique is used in areas with hard, sandy or sandy-mud bottoms firm enough to support the rocks, however bamboo mats or platforms are commonly used in soft bottom areas to prevent the rock from sinking. The use of bamboo mat adds to the initial investment cost and needs to be replaced quite frequently. If bottom culture is the only possibility, substrate nature in terms of firmness needs to be carefully examined in order to carry out a correct cost/benefit analysis.

Water Depth

Water depth is not usually a limiting factor in mollusc culture, however it will determine what culture method can be used. Probably the most important aspect with regard to water depth is to avoid long exposure periods during the extreme low water spring tides when benthic molluscs such as cockles and clams are cultured. Long exposure periods increase the culture period due mainly to the fact that during these periods the molluscs burrow into the substrate and stop feeding. However, one advantage may be during the harvesting phase particularly where it is carried out manually. With cockle culture, where planting and harvesting is carried out from a vessel, the culture area should have a water depth of about 1 to 2 m mean tide level.

The Manila clam, *Tapes semidecussatus* is a highly valued species in Europe, particularly in Spain, France and Italy. They are cultured in mud flats in enclosed bays, lagoons, man-made ponds and areas bordering estuaries. Preparation of the ground is necessary to enable the clams to dig themselves in. Usually, a protective fence is built around the culture site. Removal of predators, especially crabs, is necessary and carried out periodically. Harvesting is generally done manually although harvesting and cleaning machines have been developed. All these activities are usually carried out during the exposure period. Therefore, sites, which are selected for this kind of culture generally, become exposed for short periods during the tidal cycle.

With regard to mussel and oyster culture the water depth depends on the culture method and it can be in the range of 1–15 m mean tide level. In areas where the mean tide level is usually less than 1.5 m, bottom culture on rocks or other materials can be practiced. For raft and hanging method, the water depth can be a limiting factor as usually a minimum water column height is essential during the low water spring tides. In the above two culture methods, the hanging rens should never touch the bottom mainly to prevent predators from reaching the bivalves, avoid exposing the molluscs to high water turbidity near the seabed, and avoid losing the bivalves at the end of the rens as a result of their friction with the ground. Culture ropes should be above the sea floor at least 1 m during extreme low water spring tides.

Exposure

Marine molluscs are unable to function when removed from their water medium and long exposure periods usually lead to death. Exposure is one of the major environmental conditions that influences the growth and mortality of marine molluscs. Both growth and mortality rate vary according to shore elevation. Growth performance of a mollusc located at higher levels is usually lower compared to one located at lower levels, due to prolonged exposure periods and subsequently reduced feeding time.

Exposure to sun is one of the physical parameters, which need to be taken into account when selecting a potential culture ground in shallow coastal areas. In

raft or long-line culture, exposure is not a problem as the cultured organisms are always below the water surface. Exposure however has a number of advantages, particularly with regard to the mortality rate. There is in fact evidence that mortality increases markedly with depth due to a greater degree of predation at the lower levels, presumably as a result of longer access time of predators in the culture grounds. It has been suggested that optimum sites for culturing benthic bivalves are areas, which become exposed for periods lasting 2–3 hours. A further example where limited exposure is an advantage can be clearly seen in the mussel culture industry in the venetian lagoon, Italy. Mussel (*Mytilus edulis*) is extensively cultured by using the rack hanging method. During late spring and summer month the suspended ropes bearing the mussels (known as "pergolari") become heavily encrusted with fouling organisms, such as seasquirts and seaweeds. The presence of these organisms is undesirable because they compete for food and space and critically increase the weight of each hanging unit.

There is, therefore, a need to remove these fouling organisms. This laborious process, however, is not required in this particular site, as the adequate exposure time of the mussel ropes causes all encrusted organisms to dry up. In other areas such as Taranto, in the south of Italy, mussel aquaculturists have to routinely suspend the mussel ropes and remove the fouling organisms manually. This process is time consuming and labour intensive. Labour effort and growth period are therefore related to exposure.

Water Movement

Bivalve culture sites should not be in the vicinity of strong currents particularly where bottom culture is practiced as strong currents usually generate high turbidity and high siltation rates. However, moderate currents are needed to provide adequate food supply. Currents of 0.02–0.1 m/sec have been reported to be suitable for cockle cultures, while stronger currents are usually required for the hanging method due to the intensive culture nature of this method. In the hanging method, slow water movement usually results in slow growth of the bivalves due to the poor replenishment of food. Slow currents also promote the settling of organic and inorganic particulate materials on the cultured organisms. Potential sites should have a current speed within the range of 0.1–0.3 m/sec.

Turbidity

High turbidity levels due to the presence of finely suspended matter such as clay, sand, and other organic and inorganic particulate materials at the culture site is usually undesirable as it causes ill effects on the bivalves being cultured and often resulting in high mortalities. The presence of suspended materials above a certain level hinders the filtering activity of the bivalve, which often remain closed to avoid tissue damage and becoming clogged. In addition, low primary productivity is often the case in sites of high turbidity due to the reduced penetration of sunlight in the water column. As a result poor growth results due to reduced feeding time and limited food available. It has been reported that water containing a high suspended load of more than 400 mg/l have a lethal effect on the grow-out of mussels. The maximum suspended load tolerable level varies according to species. A practical

method for determining the turbidity level is with the use of the Secchi-disc. Sites having a disc reading less than 15 cm are usually considered unsuitable for bivalve culture.

Salinity

Although most species of molluscs tolerate a certain range of salinity levels, some species tend to be more euryhaline than others. When the salinity value falls below or above the range of a certain species for prolonged periods, high mortalities generally occur. Decrease in salinity levels is usually the major and frequent problem, mainly caused by the influx of large volumes of fresh water from rivers or land runoff during the rainy season. With regard to the blood cockle, *Anadara granosa* a number of field surveys and laboratory trials have shown that adult specimens function relatively efficiently at salinities above 25 ppt, although young specimens seem to be able to continue normal feeding activity at a lower salinity than older specimens are. Very young individuals apparently remain active at salinities as low as 18–19 ppt. Although feeding efficiency and activity generally decrease substantially at salinities less than 20 ppt, *A. granosa* is capable of acclimating to salinities as low as 12 ppt, at least in the short term. These results are consistent with the known distribution of *A. granosa* in areas where the salinity is usually in the range of 26–31 ppt, but which are subject to large, short-term fluctuations.

Bottom Slope

The degree of bottom slope is one factor, which needs to be considered particularly when the bivalve species is cultured directly on the substrate. Suitable culture beds should have a moderate seaward slope between 5 –15 degree. Slopes exceeding 15 degrees often cause cockles to be shifted from their original site due to wind and wave action. On the other hand, if the slope gradient is too little the culture area is often exposed for too long between tides.

Food Organisms

All bivalves are filter feeders, mainly feeding on a wide range of phytoplankton species. The presence of suitable micro algae species is usually not a problem, however, problems do arise when the availability of food is limited. . It has been estimated that when bivalves are grown under similar conditions at different sites, up to 85% of any difference in growth observed between sites can be attributed to water temperature and primary productivity. Studies have shown that the growth of small scallop spat is positively related to the concentration of chlorophyll in the water. This indicates the importance of primary productivity for growth of cultivated bivalves, yet it is the most difficult factor to assess for a given site. It is usually measured as the total organic weight of algae produced in a year for each square metre of sea surface area (to include the water column beneath). The carrying capacity of a body of water, (ie the biomass of animals that the algae food it contains can support) can be exceeded by overstocking, leading to reduced growth Bivalve intensively cultured in rafts may be affected by the length of the

culture period when food is scarce. In the above example, poor growth is usually the result of poor water movement (ie. low current) rather than food availability.

Another problem related to food organisms are the sudden blooms of certain phytoplankton organisms, usually in coastal waters. This phenomena is known as red tide as the organisms become so dense that the seawater takes on a brown, red or yellow coloration. Unfortunately, it is often difficult to predict if any area is prone to be affected by these toxic blooms, however, during the site selection process, one should ask about the past history of the area. Bivalves affected with red tides are not usually killed, but tend to accumulate toxic substances in their flesh. Depuration studies have shown that those bivalves can be depurated, however the longer depuration time required would make it very uneconomical. Another problem which arises from food organisms are shellfish which are harvested or cultured in estuaries or coastal areas which are used as repositories for untreated domestic sewage. Shellfish from such areas are known to accumulate bacteria and viruses which are pathogenic to man. Major diseases are typhoid and paratyphoid fever, salmonellosis, *Vibrio parahaemolyticus* infection, cholera, viral Hepatitis type A and viral gastroenteritis. Contaminated bivalves can be made edible by: 1) re-laying or transferring the shellfish to pollution free waters or 2) depuration. These processes are time, labour and cost intensive. Therefore, during site selection it is important to bear in mind that being filter-feeders, they can accumulate pathogenic organisms, toxins as well as heavy metals at levels which can be lethal to humans.

Source of Seed

Bivalve culture needs a regular supply of spat or seed is one factor, which may affect site selection decisions. However, if it has to be transported from elsewhere, it should be transported to the farm site within a reasonable time and cost. This factor has to be considered, as it will affect the cost and returns analysis. Transportation itself is not only costly, but usually negatively affects the bivalve seed due to abnormal and stressful conditions. The mussel (*P.viridis*) seed can remain without water for about 24 hrs and seeds are transported to areas where there is short supply. At Padanna, the mussel farmers get seeds of mussel from Calicut, Malpe and Karwar. The region of abundant seed availability may not be the ideal areas for grow out.

Pests

Bivalves may be eaten by various predators particularly crabs, fishes and gastropods. Bivalves grown on bottom are more vulnerable to various predators. The predatory gastropod, *Cymatium cingulatum*, is found in the edible oyster farm at Tuticorin during July to December preying on oysterlings causing upto 15% mortality. At Vizhinjam, in the raft culture of *Perna indica*, predation by the fish Rhabdosargus and lobsters were reported. At Parappanangadi, the green crab *Scylla serrata* destroyed the seeded mussel ropes in the rack culture. In the pearl oyster culture racks, crabs, polychaetes and fouling organisms like tunicates pose problem for constant maintenance. During site selection it is difficult to determine whether an area would eventually become affected by this problem, however it is good

practice to survey the area for potential predators. In pearl culture farms as well as in natural pearl oyster beds, *Cymatium cingulatum* and *Murex virgeneus* have been found to be serious predators in natural oyster beds. In culture sites crabs are the worst predators of the spats. *Charybdis lucifera*, *Atergatis integerrisimus*, *Leptodius exaratus*, *Neptunus* spp. and *Thalamita* spp. are some of the crabs commonly found inside the pearl oyster cages in the Indian oyster farms.

Secondary Factors

Pollution

Waters with heavy industrial contamination such as trace metals and organic compounds are unsuitable for bivalve cultivation. The development of intensive agriculture, heavy industries along the coastal areas and increasing number of urban settlements have increased the pollution load into the biologically productive coastal waters. Domestic wastes carry detergents, solids and various toxic substances. Agriculture pollution involves animal waste, solids, insecticides, herbicides etc. Bivalves are known to accumulate trace metals and pollutants. This renders it unpalatable due to the unpleasant flavour they impart like the copper and oil tainting. In the 1980's the biocide tributyl tin (TBT) was highly toxic to bivalves. Banning of TBT in July 1987 helped in reviving the oyster industry. In areas with untreated effluents discharges as is done in many developing countries, the location of these sites could affect the production as well as the product quality. In Jakarta Bay and Manila Bay, due to pollution and numerous heath incidences related to consumption of molluscs reared in these areas, the molluscan culture enterprise have suffered severe losses as the market demand was reduced. The EU standards to be met for export of mussel products are given in Table 1 and the criteria for classifying shellfish harvesting areas are given in Table 2.

Poaching

The problem of pilfering and damage is common in aquaculture. Constant supervision of the culture is the only effective answer. Living near the culture site is obviously the most advantageous situation for keeping constant watch over the stock and facilities. When located away from these grounds, a small guardhouse in the culture site is constructed. However, this adds to the production cost.

Resource Competition

Conflicting activities of the common users of the sea may pose problems for stocking suitable culture sites .The proximity of the culture sites to navigation channels, recreational activities and industrial activities may expose the farm to a series of problems generated by the normal activities of the common users. The wave action created by vessels, which may have a disturbing or destructive effect on both the cultured organisms and rearing facilities.

Economic Considerations

While considering the different options of culturing (eg. bottom, raft, rack, long-line, etc.) the species, a cost benefit analysis is to be done when the site is selected. Culturists interested in commercially growing oysters, as the selected bivalve species, will be confronted with the initial capital investment required to set up the operation. The various culture systems, which may be set up to culture the oysters, require different levels of investments depending on the complexity of the system itself.

Potential culturists with adequate financial resources may well consider investing in a more capital intensive system such as the raft culture or the long-line method. If the financial needs do not pose any major problem, the investor will direct his efforts in selecting sites suitable for establishing long-line facilities, therefore excluding all other sites unsuitable for this culture method.

Conclusion

The prospective cultivator may be looking for a site on which to cultivate particular types of bivalve mollusc. Or he may already have a site in mind, and needs to decide which species would perform best and be most profitable for that site. Careful consideration of the criteria discussed above will help him to arrive at the most suitable choice. It is wise to approach site selection with caution, since once committed; any errors in judgement may prove expensive. Environmental data and other information on sites may be obtained from various organisations. When looking at environmental data, it is well to remember that there will be a certain amount of variation within and between years for the same site. Very few sites, if any, are likely have the perfect blend of qualities for the cultivation of the chosen bivalve species. Choice of site will also be restricted by availability. Growers should avoid sites where several environmental factors provide less than optimum conditions, as each may impose a small stress on the bivalves, which together result in poor growth and possible mortality. Where circumstances permit, the cultivator should evaluate the suitability of a number of sites in a pilot study with trial plantings of the chosen bivalve species. Growth differences between sites usually reflect differences in conditions, which may be fairly specific to the sites. These conditions may vary widely between and within years, requiring long-term studies of at least one year and preferably longer, to get an accurate picture of the suitability of the site for cultivation. Finally, it should be remembered that a successful and profitable bivalve cultivation operation requires good husbandry and management of the stock as well as the selection of a suitable site.

Suggested reading:

1. Bivalve culture in Asia and the Pacific. 1982. Proceedings of a workshop held in Singapore. (Eds.) F.Brain Davy and Michael Graham. Intenational Development Research Centre, Ottawa, Canada.

2. James, P.S.B.R. and K.A.Narasimham.1997.Molluscs. Handbook on aquaculture farming. MPEDA. 91 pp.

Table 1. European Union (EU) standards to be met for export of mussel products

	Parameters in farm site	Mandatory level
1.	Colour	> 1mg Pt/l
2.	Temperature	± 2 °C from normal sea temperature
3.	pH	7 – 9
4.	Salinity	2 – 48 ppt
5.	Dissolved oxygen (Saturation)	>80 %
6.	Suspended solids (mg/l)	30 %
7.	Petoleum hydrocarbons	Should not be deposited in the flesh.
8.	Organo-halogenated substances	Should not exceed harmful levels in shellfish and larvae
Bacteriological parameters: Maximum permissible limit (Nos./100ml)		
1.	Faecal coliforms	< 300 in the shellfish & intervalvular liquid
Heavy Metals in tissue: Maximum permissible residual level (ppm)		
1.	Mercury	1.0
2.	Cadmium	3.0
3.	Arsenic	75
4.	Lead	1.5
5.	Tin	250
6.	Nickel	80
7.	Chromium	12
Pesticides in tissue: Maximum permissible residual level (ppm)		
1.	BHC	0.3
2.	Aldrin	0.3
3.	Dieldrin	0.3
4.	Endrin	0.3
5.	DDT	5.0
Antibiotics and other Pharmacologically active substances in tissue: Maximum permissible residual level (ppm)		
1.	Tetracycline	0.1
2.	Oxytetracycline	0.1
3.	Trimethoprim	0.05
4.	Oxolinic acid	0.3

Table 2. Criteria for classifying shellfish harvesting areas

Classification category	Faecal coliform bacteria (*E.coli*) per 100 g shellfish flesh	Comment
A	All samples less than 300 (230)	Suitable for consumption. Can be marketed.
B	Less than 6,000 (4,600) in 90% of samples.	Depuration needed (or relaying in catergory A area or cooking by an approved method).
C	All samples less than 60,000 (46,000)	Relaying (minimum of two months in approved relaying area or cooking by an approved method).
Prohibited	Above 60,000 (46,000)	Cannot be taken for placing n the market.

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Clam Culture : Global Scenario

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Bivalves such as oysters, mussels, clams and cockles are widely distributed, both in the tropical and temperate waters. In recent years, they have emerged as delicacy and luxury food item in Japan, USA and Western Europe.

The total global mollusk production through exploitation of the wild stocks was 6684 thousand t in 2002 forming nearly 5 % to the total world fish production (132989 thousand tonnes). The total global aquaculture production of fish, crustaceans and mollusks for 2002 was estimated to be 39.8 million tones with a farm gate value of US $ 53.8 billion. The total mollusk production through aquaculture was estimated at 11784 thousand t by quantity and US $ 10,512 million by value, in 2002. Due to depletion of the intensely exploited wild stocks, there is increased demand for farm grown products (FAO Fisheries Statistics 2002).

The world landings of clams/cockles/ark shells have been increasing steadily for the past few years totaling 826 thousand t in 2002 forming nearly 12.4 % of the total mollusk production. The clam/cockles/ark shells production through aquaculture in 2002 was 3431 thousand t forming 8.6 % of the total aquaculture production. The major mollusk producers include China, Japan, Korean Republic, France, Spain, and the USA, Italy, Malaysia, Netherlands and other Asian countries. The major clam producing countries include China, Japan, Malaysia, Korean Republic and Thailand (FAO Fisheries Statistics 2002).

Among bivalves, clams are by far, the most abundant and widely distributed resources. They are commercially important and fished in fairly large quantities in several countries. In India, clams form subsistence fisheries all along the coast. Clam meat is nutritious and is a cheap source of protein rich seafood. Clam culture is practiced in several countries such as Taiwan, Thailand, Malaysia, Indonesia, Singapore, and U. K. Australia. However, it is not as advanced an art, as is the case with oysters or mussels. In clam culture, the seed is generally collected from natural grounds and replanted in areas with a suitable substratum but where seed is not abundant. They are then allowed to grow to market size.

1. Selection of Site

Clams are cultured on the bottom and therefore site selection depends on the substrate. The occurrence of natural clam populations is indicative of the suitability of the site with particular reference to the tide level, substratum and water salinity. Clam farms are located in estuaries, bays and other sheltered close to the shore. About 1-2 hrs exposure at low tide is desirable as it is easy to remove the predators.

Too long an exposure results in poor growth due to reduced feeding and in summer there may be mortality due to desiccation. Farms located further in sub tidal area have the disadvantage when predators are to be eradicated.

The type of substratum preferred varies with the species. For example, *M. casta* thrives well on sandy bottom, while *Anadara granosa* prefers mud flats. Also the salinity range tolerated differs between species. While *V. cyprinoides* prefers low saline waters few species tolerate prolonged low saline conditions which are generally prevalent in areas subjected to heavy rains and freshwater drain from the land. Clam farms are located in areas where there is little wave action. Areas prone to frequent changes of contour and vulnerable to pollution are avoided.

2. Hatchery Production of Spat

Mature clams of 35-45 mm shell length are used as brood stock. The brood stock is conditioned in unfiltered seawater of 25 ppt, and at 22-24°C and then transferred to spawning troughs. Spawning is induced by thermal cycling: the spawning through is part filled with cooler water to a depth of 10 cm and a small amount of cultured microalgae (*Isochrysis galbana*) to stimulate the clams to extend their siphons and start pumping activity. After 15-30 minutes, the water is drained and replaced with water at 28-30 C, again with small addition of algae. This water is drained after a similar period of time and replaced with cooler water and the procedure is repeated. The number of cycles, which are necessary to induce spawning, depends on the readiness of the clams to spawn.

Eggs are separated through a 60 μm nylon mesh sieve and transferred to filtered seawater at 25° C. The larvae are grown in flat-bottomed vessels, or in conically based fiberglass thanks fitted with bottom drains, at 15,000-20,000/1 but optimum density for good growth is 10,000/1.

Good aeration at the rate of 2001/h depending on the size of the container and filtered and sterilized seawater of 25 ppt and 24-26°C provides optimum conditions for larval growth. Unicellular alga such as *Chaetoceros calcitrans, Isochrysis galbana, Tetraselmis suecica* and *Thalasiosira pseudonana* are suitable food species. Diets of mixed alga are beneficial. A suitable diet for D-shelled larvae is a mixture of *Chaetoceros calcitrans* and *Isochrysis galbana,* the most suitable cell densities are 125 cells /ml respectively.
Airlift down welling recirculation systems of 100 l capacity are generally used for growing 0.5 million spat to size retained on 440 μm mesh size.

3. Nursery Rearing

Nursery upwelling systems are generally used in several European countries for spat rearing. Water flow is induced through cylinders (30 cm x diameter) placed in a 30 l-header tank, by creating a difference in the head of the water. The header tank is placed over the 500-10000 L fiberglass reservoir tank, valves are fitted to the over flow of the upwelling cylinders, since spat growth is strongly influenced by water flow rate. Flow rates of 20-50 ml/minute per gram are used.

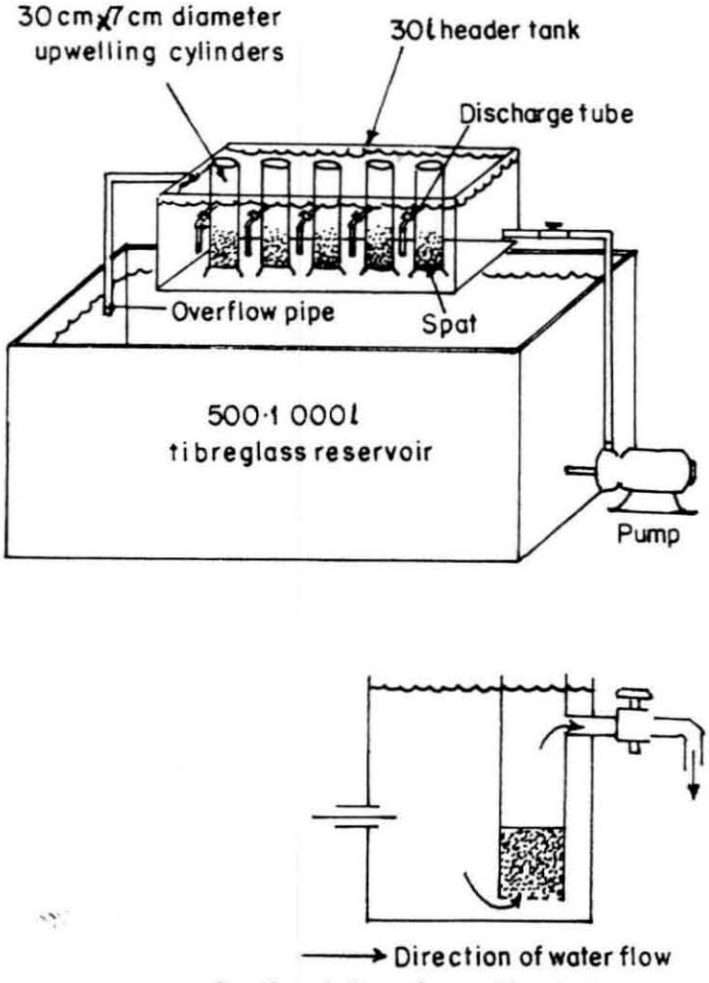

Fig 1. A nursery upwelling system used at the Fisheries Laboratory at Conwy

Seawater coarsely filtered through a 45 / μm mesh is used so that the spat are benefited from presence of naturally occurring algae, in addition to those offered as food.

Nursery upwelling systems need relatively large volume, since only small biomasses of spat can be grown successfully per unit volume of water.

4. Growout Culture and Production

The ground is leveled and cleaned of predators such as boring gastropods, starfishes, crabs and skates. Bamboo poles are planted on the boundary of the farm as markers. The movements of the clams are limited and in many areas fencing is not necessary. Synthetic fibre net pens are erected to protect clams against strong water currents in the USA; bamboo stakes with nylon netting are used in Taiwan.

At high tide, seed measuring 10-25 mm in length are taken in a boat and planted in the farm, taking care to get even dispersal as far as possible. Uneven distribution is set right at the next low tide. In Malaysia, *A. granosa* is stocked at 1000-2000/m^2 and thinned more than once to achieve final density of 300-600/m^2. The stocking density varies with the species and a stocking density of 400/m for 10 mm seed and 300/m^2 for 20 mm seed is usually optimal.

After seeding the farm, 10 mm mesh synthetic netting is laid and held in position by stakes driven into the substratum at the periphery of the farm, to offer protection to the young clams. Except for watch and ward and eradication of predators, no other maintenance job is necessary during the grow-out phase. The clams are harvested after 5 or 6 months either by hand picking or by hand operated dredge.

In Malaysia, wild seed of *A. granosa* are sown in prepared coastal mud flats, generally bound by natural landmarks and where these are lacking, are marked by other means. The sowing density is between 2-6.5-kg/seed/m^2. An average production of 40 t/ha is obtained.

In Thailand also the same method is followed for *A. granosa* and 50 cm long bamboo stakes are used to fence the inter tidal mud flats to prevent escape of clam from the culture beds. Clam seed are also sown in the central elevated areas of shrimp ponds and fenced with bamboo stakes. The seed used here are larger than those used for the intertidal flats. These methods yield 31-109 t/ha annually.

In China, *Sinonovacula constricta* (razor clam), *Arca (Anadara) granosa* and *Tapes philippinarum* (small necked clam) are cultivated. Seed clams (1 cm long) of *S. constrictare* collected and sown in rearing beds during January at 9-18 x 10^6 clam seeds / ha. The average yield is 15-22 t/ha. After 6-7 months. *A granosa* seed are also raised from natural spat and reared in enclosed water pools. They are thinned several times, transplanted to rearing grounds in the lower tidal zone. It takes 2-3 years to reach marketable size of 2cm and yield is 22.5–60 t /ha. The small necked clam is also cultivated on pre-prepared culture beds by stocking 1.4 cm seed clams at 1.8 x 10^6 seed /ha. The yield is about 18.7 t /ha. But sometimes as high as 45 t/ ha.

Clams are rarely grown in ponds, but in recent years due to adverse impact of viral diseases in shrimp culture, there is growing interest in many southeast Asian countries to utilize the shrimp ponds for clam culture. In Taiwan, *Meretrix lusoria* is grown in ponds formerly used for milkfish and shrimps and also in the outlet and inlet canals of these ponds.

5. Giant Clam culture

In Giant clam culture, four phases are involved.

a) Hatchery phase: Rearing of larvae from eggs in indoor or out door tanks. Six out of the eight known species of giant clams have been successfully spawned in the Philippines by injecting serotonin into the gonad of mature clams and also by introduction of macerated gonad materials into the mantle cavity through the exhalent siphon. The development stages are similar to those in other clams and settlement takes place in about 7-10 days after spawning. *Isochrysis galbana* is fed to the larvae. The spat attach with the byssus but they may break attachment and creep along the substrate. At this stage the symbiosis with Zooxanthellae is established. The larval rearing is done in both indoor and outdoor tanks.

b) Nursery phase: Rearing juvenile clams in onshore tanks for metamorphosis (0.2mm) to about nine months of age and 20+ mm shell length (seed clams). The tanks are provided with flow of raw seawater. The clams acquire the zooxanthellae from the seawater in about 3 weeks after fertilization and they become increasingly autotrophic.

c) Ocean nursery phase: Rearing juvenile clams in protective containers in the field from about 20 mm shell length to 200 + mm shell length.

d) Grow out phase: Rearing clams of 200+ mm shell length without protection in the field to market size.

Tridacna gigas, the largest among the giant clams grows to 18.6 mm (total wt 0.55 g) in 0.83 years, 121.1 mm (193.8 g) in 2 years, 206.4 mm (923.3 g) in 2.66 years and 221.3 mm (1.15 kg) in 3 years. The wet meat forms 12% of the total weight in the 18.6 mm clams and it increases to 26% in 221.3 mm clams. A production of 29 t of wet meat/ha has been estimated in *T gigas* culture for three-year-old clams. The field culture techniques, survival and production for various giant clam species are still in experimental stage.

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Clam Culture in India

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Clams are bivalves, which burrow into the substratum with the help of a usually well-developed foot. A few clam species are also known to attach to hard substrates with byssus threads. Among bivalves Clams are by far, the most abundant and widely distributed resource.

1. Resources and Distribution

A number of clam species belonging to the families Arcidae, Veneridae, Corbuculidae, Tridacnidae, Solenidae, Mesodesmatidae, Tellinidae and Donacidae are exploited along the Indian coast. The cultivable species mostly belong to the first four of the above-mentioned families.

Arcidae: The arcid clams are called blood clams, as their blood is red due to presence of haemoglobin. A single species *Anadara granosa* is important. It occurs all along the Indian coast in soft muddy substratum and forms a fishery of some magnitude in the Kakinada Bay.

Veneridae: The venerid clams are the most sought after in the clam fisheries of India and three genera namely *Meretix, Paphia,* and *Marcia* are important.

Along the Maharastra coast, *Meretrix meretrix (Linnaues), Marcia opima (Gmelin)* and *Paphia laterisulca* are the dominant species. In Goa, *M casta* forms a fishery. Along Karnataka coast, there are 14 estuaries with varying abundance of clams. *M meretix* is found in all the estuaries. *Meretrix meretrix* in the Kalinadi and Coondapur estuaries, *Paphia malabarica* in the Mulky, Gurpur, Udyavara and Coondapur estuaries. *Marcia opima* is found in Coondapur, Uppunda and Sita estuaries. Along the Kerala coast, *Paphia malabarica* forms a fishery in Koduvally, Azhikkal, Karyamgod and Chittari estuaries and Ashtamudi Lake. Other Venerid clams form fisheries in several estuaries in Kerala State. Along the east coast, *M casta* occurs at several places and forms a fishery at Vellar estuary, Pulicat lake and Bhimunipatnam backwaters. *M opima, P malabarica* and *M meretrix* contribute to the clam fisheries in the Kakinada Bay. Along the Orissa coast, *Meretrix* sp occur in the Chilka Lake and Sonapur backwaters.

Corbuculidae: The black clam *Villorita cyprinoides* is the major resource in the Vembanad Lake and is also exploited in several backwaters, lakes and estuaries of Kerala. It also contributes to the fisheries in Goa and in the Nethravathi, Gurpur, Udyavara, Swarna and Coondapur estuaries in Karnataka.

Tridacnidae: Represented in by *Tridacna maxima, T crocea, T squamosa* and *Hippopus hippopus*, they occur in the Andaman, Nicobar and Lakshadweep islands.

2. Exploitation

In India, clams form subsistence fisheries all along the coast. Clam meat is nutritious and is a cheap source of protein rich seafood. Clams are fished by men, women and children, all along the Indian coast. They are collected from the intertidal region to about 4 m depth. They are hand picked and also a hand operated dredge is used. Plank built non-powered boats are used for transport. Clams under one year contribute in considerable numbers to the fishery. At many centers, November to April is the peak fishing season as the new recruits become available to the fishery.

In India, the production of clams and the species break up through harvest of the wild populations is not regularly monitored. However, estimated annual landings of commercially important bivalves by Central Marine Fisheries Research Institute for 2002-2003 are summarized.

Six species of clams viz., *Villorita cyprinoides, Paphia malabarica, Sunetta scripta, Meretrix casta, M meretrix, Marcia opima* and the cockle *Anadara granosa* contribute to the commercial fisheries. Of the estimated total annual production (2002-2003) of 38800 t of clams, the black clam *Villorita cyprinoides* is the major clam species landed (20666 t) forming 56% of the total clam production. Major production center was Vembanad Lake. It is also fished from Korapuzha and Chaliyar estuaries in north Kerala but is seasonal from April to June only.

Paphia malabarica, the second most abundant clam resource accounted for 6903 t and the major fishing area was Ashtamudi Lake (6277 t) in Kerala. It is also fished from Dharmadom estuary in north Kerala. In Karnataka 622 t were landed from Gangoli backwaters (340 t) and Kali estuary (282 t). In Andhra Pradesh, 4 t were landed from the Kakinada bay. *Sunetta scripta* landed 4486 t from Cochin and utilized mainly for shell.

The total estimated landing of *Meretrix casta* was 3571 t. In Kerala, *M casta* fishery was more prevalent in northern Kerala in Kottakal (940 t) and Chaliyar (491 t) and Dharmadom (41 t). In central Kerala, significant depletion of the stock in Chettuva has been reported. In Karnataka (1477 t) of *M casta* were landed mostly from Mulki estuary.

Meretrix meretrix accounted for 200 t from the Agnashini estuary in north Karnartaka and two regions Bhimili (70 t) and Kakinada Bay (16 t) in Andhra Pradesh. The fishery for *Marcia opima* was reported (5 t) only at Gangoli in south Karnataka. *Anadara granosa* accounted for 873 t from the Kakinada Bay.

3. Distinctive Characters of Cultivable Species

Anadara granosa: - Shell thick, inflated and dark brown. This species differs from other clams in having taxodont dentition. (Small teeth in a single straight series) and about 20 elevated ribs bearing rectangular nodules.

In venerid clams, the hinge usually bears three cardinal teeth and a single anterior lateral tooth on the left valve and a corresponding depression on the right valve. Two adductor muscle impressions, slightly unequal in size are present.

Paphia malabarica: - Shell slightly inflated, surface concentrically grooved. Pallial sinus is U shaped and very deep. Lunule relatively short. Shell length only one and one third times longer than height. Hinge area short with narrowly diverging teeth, shell yellowish brown in color, indistinctly rayed with grayish brown bands or mottled with brownish angular markings.

Meretrix casta: - Shell thick smooth devoid of any sculpture and triangularly ovate. Outer surface pale yellowish brown tined with dark grey posterior and very faintly rayed with grayish radial lines.

M. meretrix: - It differs from *M. casta* in having less elongated lateral tooth and more ovate shell. Also it grows to a larger size.

Marcia opima: - shell thick, inflated, smooth triangularly ovate. Pallial line deeply sinuate, Apex of the pallial sinus is bluntly angular. Lunule distinct, flattened and rather broad. Area behind umbones is well defined, flattened and greatly elongated reaching almost upto the hind margin of the shell. Outer surface of shell polished, pale yellowish brown or straw coloured, variously mottled and rayed with purplish grey markings.

Villorita cyprinoides: - Shell thick, ovately triangular with strong concentric ridges; ridges more strongly developed in the anterior half. Umbones prominent, well elevated, hinge margin short and thick, always with three oblique cardinal teeth of which the anterior in the right valve and posterior in the left valve are obsolete. Pallial sinus small, lunule narrow and ligament large. Periostracum dark olive brown to blackish brown.

The Tridacnid clams have large massive shells with broad radial ribs, sometimes bearing large fluted scales. Edges of valves usually scalloped.

Tridacna crocea **(Crocus or Boring clam)**: - Smallest of the giant clams, grows upto 15 cm, Large thick, triangularly ovate shell with large byssal gape, 6-10 broad flattened ribs with concentric ridges, Shell grayish white, flushed with yellow or pinkish orange.

T. maxima: - (Rugose giant clam): Shell strongly in equilateral. Resembles *T crocea* but its 6-12 broad radial ribs have much more strongly developed concentric scales. Large byssal gape with distinct plicate at edges. Ventral margin of the valve often deeply scalloped. Shell grayish white, sometimes tinged with yellow or pinkish orange. Grows to about 35 cm.

T. squamosa **(Fluted or scaly clam)**: - Large, thick strongly inflated shell with small or medium sized byssal gape. 4-12 strongly convex ribs with rib lets in inter spaces. Broad, sometimes long fluted scales on ribs, which may

project beyond ventral margin considerably. Grayish white, sometimes fringed with yellow. Grows to 40 cm.

4. Biology Of Cultivable Species

Like oysters and mussels, the clams are filter feeders.

A. granosa: Comprehensive information is available on the biology from the Kakinada bay. It attains 41.1, 55.3 and 66.3 mm on completion of 1,2 and 3 years respectively. It attains a maximum length of 72 mm. Males attain first maturity at 20 mm and females at 24 mm length. It spawns throughout the year and there can be 2-4 reproductive cycles in a year. The condition index based on the percentage of wet flesh weight in total weight varies from 15.1 to 23.1 and expressed as percentage of dry flesh weight in wet flesh weight ranges from 17.2 to 24.2. About 10.85% of the clams are infested by the pea crab *Pinnotheres alcocki.*

M. meretrix: In the velar estuary it grows to 47 mm in the first year and 651.5 mm in the second year. At Tuticorin, growth is slow and it reaches 29.0, 48.3 and 62.3 mm in 1^{st}, 2^{nd} and 3^{rd} years respectively. It grows to a maximum length of 91 mm and the life span is estimated at 7-8 years. The condition index expressed as percentage of wet meat wt in total wt varies from 7.6 to 16.1 with an average of 12.2. First maturity is attained at 21-26 mm length. Along the Bombay coast spawning is from March to June, in the Vellar estuary from February to September and at Tuticorin in January-April and June-October. It withstands a low salinity of 10.5 ppt under laboratory conditions.

Meretrix casta: This species grows fast in the Mulky estuary, attaining 36.5mm in 6 months and 42.6 mm at the end of first year. In the Adyar estuary the growth is comparable while it is slow with the monthly average growth rate at 2 to 2.6 mm in the velar estuary, 2.7 mm in Goa and 2.9 mm in the kali estuary. It grows up to 55 mm in length. The wet meat forms 7.6 to 16.0 % of the total weight and is usually high before spawning. Length at first maturity is between 11 to 17 mm. In the Adayar, Mandapam, Goa and Kali estuary areas it spawns throughout the year with 1-3 peaks. In the Mulky estuary spawning is prolonged, extending from September to March. In the same estuary the clams are often found infested by crabs.

Paphia malabarica: In the Mulky estuary it grows to 36.3 mm in 6 months, 43.1 mm in 9 months and 49.1 mm in one year. The largest clam in the commercial catches measured 51 mm. In the Kakinada bay clams measuring 65 mm in length are often found. The wet meat forms 11.8 to 15.4 % of the total weight. The length at first maturity is 20 mm. It spawns during October-February in the Mulky estuary and September –January in the Ashtamudi Lake.

Marcia opima: In the Adayar estuary it attains 26-33.8 mm in length in over one year and 38.8-43.5 mm length in 2 years. In the Kalbadevi estuary it grows to 22, 31 and 43 mm during 1-3 years respectively. In the Velar

estuary it grows from 5.6 mm to 33.3 mm in 8 months. Wet meat forms 7.9 to 12.5% of the total weight. It attains maturity at 11.20 mm length. In the Adayar estuary spawning begins in December when the bar mouth is open and lasts for a month. In Kalbadevi, a major spawning during October-November and a minor spawning during March-April takes place. Under laboratory conditions it tolerates a low salinity of 14 ppt when transferred suddenly and 7.5 ppt on acclimatization.

Villorita cyprinoides: This species tolerates near freshwater conditions and occurs in the upper reaches of the estuaries and backwaters. In the Cochin backwaters it grows to 30 mm in one year and 41 mm in the second year. In the Nethravathi estuary it attains comparables length at the end of first year and in Goa it grows from 20.4 mm to 33.2 mm in one year. Wet meat forms 10.9 to 16.5% of the total weight. Length at first maturity is 11-15 mm. In the Cochin backwaters it spawns twice a year, from late May to August/September and January to March. In the Nethravathi estuary spawning is from December to March.

Tridacnid clams: In India no work has been done on these clams. The unique feature of the giant clams is their symbiotic relationship with the dinoflagellate algae, *zooxanthellae* in their mantle tissues. They retain the filter feeding habit and food is supplemented by the nutrients, gained from the photosynthesis of *zooxanthellae.* They mature as males at two or more years of age and latter develop female gonads also. The initial growth of the giant clams is slow and they reach 2-4 cm in shell length after a year. There after growth is rapid in larger species. Estimations of the life spans of giant clams have been speculative and some of them do live for a few decades. Giant clams are the only known auto tropic (in this case get their food by symbiotic association) farm animals known to man.

5. Clam Culture in India

The Central Marine Fisheries Research Institute has developed the technology for culture of the blood clam *Anadara granosa* in the Kakinada bay. Although complete package of technology including seed production under controlled conditions has been developed in the country for the blood clam *A. granosa* and the venerid clam *P. malabarica*, it is yet to be commercialized.

i) Site Selection

Clams are cultured on the bottom and therefore site selection depends on the substrate. The occurrence of natural clam populations is indicative of the suitability of the site with particular reference to the tide level, substratum and water salinity. Clam farms are located in estuaries, bays and other sheltered close to the shore. About 1-2 hrs exposure at low tide is desirable as it is easy to remove the predators.

Too long an exposure results in poor growth due to reduced feeding and in summer there may be mortality due to desiccation. Farms located further in sub tidal area have the disadvantage when predators are to be eradicated.

The type of substratum preferred varies with the species. For example, *M. casta* thrives well on sandy bottom, while *Anadara granosa* prefers mud flats. Also the salinity range tolerated differs between species. While *V. cyprinoides* prefers low saline waters few species tolerate prolonged low saline conditions which are generally prevalent in areas subjected to heavy rains and freshwater drain from the land. Clam farms are located in areas where there is little wave action. Areas prone to frequent changes of contour and vulnerable to pollution are avoided.

ii) Hatchery Production of Seed

Hatchery production of seed technology has been developed for *A. granosa*, *M. meretrix M. casta* and *P. malabarica*. In clams, spawning occurs both at elevated water temperature of about 34° C and also at the lower temperature of about 24°C on transfer to the conditioning room, after the thermal shock. Spat settlement takes place between 7th and 26th day after spawning in different clam species studied. The clam spat attain 2-3 mm in length in the hatchery in two months after fertilization and are transferred to the nursery. A survival rate 15-20% in spat production in the hatchery is considered as satisfactory. In the hatchery the micro algae *Isochrysis galbana* is given as food to the larvae and mixed micro algae, reared in outdoor tanks as food to the spat.

iii) Nursery Rearing

The 2-3 mm hatchery produced clam seed are transferred to 40 x 10 cm box type cages. These cages are covered with fine velon screen mesh and for additional protection against damage by crabs and fishes; a 10 cm mesh nylon fish net is stitched over the cage. The cage is suspended from racks in shallow calm waters. They are periodically cleaned of silt, predators and foulers, which enter the cages as larvae. In 6-8 weeks, the clams grow to about 10 mm in length and are ready for planting on the grow-out grounds.

Recently rearing of the hatchery produced spat of *P. malabarica* (2-3mm length) in 25x 25 mm nylon bags of 1-3 mm mesh at density of 1000 spat/bag and suspended from a rack in the Tuticorin bay gave highly encouraging results. This method is cost effective when compared to rearing in cages.

iv) Growout and Production

In the blood clams, *A. granosa* culture at Kakinada, seed clams of 21.8 -25.1 mm average length (5.53-7.08 g average wt) were stocked at 240-175/m. They attained 39.2 to 42.7 mm average length and 25.53 to 32.9 g average weight at harvest. The retrieval is 83.4% to88.6% when pen enclosures are used and 41.5% without pen. Production rates of 39.0-41.6t/ha/5.5 months are obtained when pen culture is practiced and 21 t/ha/6 months when pen is not used. Thus, both retrieval and production rates are reduced by about 50% in the blood culture if pen is not used. At a stocking density of 300/m the production is estimated at 70 t /ha with pen enclosure.

Growth of *M. casta* observed by Durve (1970) in the Marine Fish Farm of C.M.F.R.I., at Mandapam was 11.4 mm from 27.5 mm to 38.9 mm in 19 months at an average increase of 0.6 mm per month. The corresponding weight increase was 20.39 g from 7.51 g to 27.90 at an average of 1.10 g per month. He noted that growth was continuous, but there was slacking from May to September, when hyper saline conditions prevailed. During that time, the water in the farm was more or less stagnant resulting in poor quantity of phytoplankton food available for the clam. The period of slow growth also coincide with period of sexual activity. However, Even after prolonged resting, there was spurt in the growth when favorable conditions returned. The slow growth observed was attributed to the nature of the species, which is a true backwater clam and purely marine conditions found in the fish farm was not conducive to growth. In another experiment, he observed a growth of 14.4 mm in *M.casta* in 5 months from November to April at an average growth of 2.9 mm per month.

In the experiments conducted by Rao and Rao (1983) in pens at Mulki estuary, *M. casta* was stocked in three pens and *M. Meretrix* in one pen. Stocking density ranged from 60 to 250 seed per sq. m. for *M. casta* while it was 177 per sq. m. for *M, meretrix*. Seed size ranged from 6 mm to 28 mm in the case of *M casta* there was 6.5 mm growth in the mean size from January to June, at an average of 1.3 mm per month. The average weight of the clams increased from 5.29 g to 12.58g showing an increase of 7.29 g and the meat weight increased from 0.53 g to 2.52 g in the same period. Survival rate of the clams recorded was 80.5% of the stocking density.

In the case of *M. meretrix*, there was rapid growth with increase from the mean size of 23.6 mm to 34.0 mm, between January and March and later retarded to 35.6 mm and 37.5 mm during April and May respectively, thus showing overall increase of 13.9 mm in four months at an average of 3.5 mm growth per month. The average weight of the clam showed an increase of 12.30 g from 5.30 g in January to 17.60 g in May and meat weight showed a gain of 1.36 g from o.91 g to 2.31 g in the above period. Of the clams stocked, 75.5% survived till the end of observations in May.

Sreenivasan (1983 b) observed growth of *M. casta* in Vellar estuary to be from 7.5 mm to 41.4 mm in 13 months with a net increase of 34 mm at an average of 2.6 mm per month. Corresponding increase in weight was 31.07 g at an average of 2.39 g per month. Growth was slowed down in October-December, when low saline conditions prevailed in the estuary. Growth was fast during January-March, when there was rise in the salinity and temperature. Growth was moderate from April-September, during which months there was intense spawning activity by the clam in Vellar estuary. Growth of the transplanted clam was observed to be much faster than those in the natural bed.

In the later experiments, seed of *M. casta* transplanted in two pens, one below LTL and another above MTL at the rate of 1 kg/sq. m. There was differential growth among the seed grown over a period. Observed growth was 21 mm in 12 months in the pen below LTL, but was only 9 mm in 10 months in the pen above MTL. In a fully submerged multi-tier rack, the clam seed showed a

growth of 27 mm in 13 months. This clearly indicated that period of exposure and submergence play a major role in growth of the clams. Prolonged period of submergence helped the clam with long period of feeding since the clam is a continuously filer feeder and also reduced period of desiccation by exposure to air.

Growth of *M opima*, determined by transplantation experiment was 27.6 mm in 8 months from 5.6 mm to 33.2 mm during March- November. Growth rate of *M. opima* was observed to be comparatively faster than that of *M. casta* in Vellar estuary.

In a ranching experiment in Ashtamuddi Lake in Kerala, *P. malabarica* seed of 11.5 mm average length and 0.27 g average weight were stocked at 3566 nos/m. They attained an average length of 31.58 mm and 8.54 g average weight at harvest after 3.5 to 5.5 months. The retrieval was 7.5%. At Munambam, *P. malabarica* seed of 2.4 mm average length and 0.2 g average weight was stocked at 1500 nos./ m. After 4.5 months, they attained 34.6 mm average length and 9.05 g average weight. The retrieval was 17.64%. The production works out to be 1.5 to 2 kg/m.

6. Depuration, Processing, By - products and Utilization

Depuration: Clams like other filter feeding bivalve, accumulate pathogenic organisms in their body. By depuration the bacterial load is brought down to permissible levels; also faeces, sand particles and silt are removed from the alimentary canal. Clams are depurated in the same way as other bivalves. They are placed for 24 hrs in cleaning tanks under a flow of filtered seawater. About 10-20% of the seawater is continuously replaced. At the end of 12 hrs the water in the tank is drained and the clams are cleaned by a strong jet of water to remove the accumulated faeces. The tanks are again filled with water to remove the accumulated faeces. They are further held on filtered seawater for 12 hrs and for about one hr in 3 ppm chlorinated seawater, washed once again in filtered seawater before processing. In several countries, they are eaten raw and also steamed and eaten.

Processing: The various techniques followed in processing the clam meat are similar to those used for other bivalve mollusks. The clam meat is frozen as blocks or individual quick frozen, canned and smoked. Other products are clam juice, clam stripes, clam streaks, stuffed clams, clam pickle and chowder.

The adductor muscle is the valued part of the giant clam. In a 20 cm clam, it weighs about 500 g. Except the liver all parts of the soft body of the giant clams are eaten. The mantle of the giant clam is used to the Japanese salads, spaghetti, marinara, clam crackers and minced clam.

Byproducts and utilization: In clam culture, shell is the byproduct. It is used in the manufacture of cement, calcium carbide sand-lime bricks and lime. The shell lime is used for manuring coffee plantations, as a mortar in building constructions, in the treatment of effluents, as pesticide by mixing with copper sulphate and in glass, rayon, polyfibre, paper and sugar industries. The shells of several clams have ornamental value and are used in making curious. Truckloads of blood clamshells

are transported from Kakinada to southern Tamil Nadu districts for use in the shell craft industry.

Giant clamshells currently find a ready marked as decorative objects, trays, salad bowls and washbasins. *T. Squamosa* shells are most valued in this trade. Philippines is the center for shell craft industry.

Export Market for Clams: The export demand for clam meat has been increasing over the past few years, particularly from Japan, Western Europe and the USA. The clam meat export from India has increased from meager 371 t in 1989 to 900 t in 1993. In terns of value, almost fivefold increase has been recorded at Rs. 63.02 lakhs in 1989 to Rs, 292,25 lakhs in 1993.

7. Prospects and Constraints

The prospects for developing clam culture in India on commercial lines are very bright and the advantages are given below.

1. Clam feed low in the food web on detritus and phytoplankton and give high production per unit area. They are efficient converters of primary production into nutritious seafood, suitable for human consumption.

2. Clam culture is essentially a relaying practice of collecting the seed from high-density areas and stocking them in suitable grow out areas. The farm management involves periodic site inspection and eradication of predators: the technology is simple and easy for adoption by the farmers.

3. On bottom clam culture does not involve high labor or cost input.

4. Clam culture can easily be blended with capture fisheries and can be taken up as an income and employment generation programme in rural areas.

5. In the export market there is demand for some specious of clams only. From India there is insatiable demand for the frozen meat of *P. malabarica*. There are large tracts of derelict water bodies such as the Kakinada bay and they can be utilized for the culture of this species.

6. In clam culture fertilizers and feeds are not used and it is eco friendly. Clams are good biological filters and the introduction of clams in areas of high eutrophication such as shrimp ponds helps to reduce the pollution due to high load of suspended matter.

7. After the outbreak of shrimp disease in Taiwan, the farmers have switched over to culture of the clam *Mercenaria lusoria* in shrimp ponds for export to Japan. Similar practice can be followed in Andhra Pradesh and Tamil Nadu. Also fattening of the clams in shrimp ponds as followed in Thailand deserves merit.

Constraints

1. The major constraint for the large-scale propagation of clam culture in the country is the absence of laws to allot water bodies to prospective farmers.
2. Mapping of sites suitable for clam culture, based on species site interaction are needed for developing culture.
3. Consumption of clams is still localized; close to the production centers and only a small segment of the population take them as food. They still remain as non-conventional food. Vigorous extension drive is needed to make them popular.

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Giant Clam Culture

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Giant clams or tridacnids (Class Bivalvia: Family Tridacnidae) occur naturally in only the tropical or sub tropical marine waters of Indian and Pacific Oceans. However, they are confined to the western portion of the Pacific Ocean and do not occur on the western coast of the Americas or Hawaii. They are well adapted to tropical clear waters such as those that favor the growth of corals.

The giant clams are a traditional food source for the people of Indo-Pacific. The flesh of giant clams is eaten by many communities and the shells are used either as ornaments or for utilitarian purposes. In recent times, giant clams have become significant specimens for the aquarium trade. As a result over exploitation has led to the extermination of many of the local species. Thus, in the 1970s and 1980s research into the reproduction and larval culture of giant clams became important. The Micronesian Mariculture Demonstration Centre (MMDC) in Palau played a key role in developing and promoting mass culture of giant clams. The University of Papua New Guinea involved in giant clam larviculture. The Australian Centre for International Agricultural Research (ACIAR) funded giant clam project began with Australia, Fiji, Philippines and Papua New Guinea in the mid 1980s and in the late 1980s in included Tonga, Cook Islands, Kiribati and Tuvalu.. The International Centre for Living Aquatic Resources Management (ICLARM) also in the late 1980s set up a coastal aquaculture centre near Honiara, Solomon Islands for giant clams. Other hatcheries and Ocean nurseries have been started notable in Micronesia and Tonga and Cook islands.

Species

There are eight extant species of giant clam (Family Tridacnidae) within two genera:

Tridacna gigas (Linnaeus 1758): The true Giant clam is the largest extant bivalve and attains weights of over 200 Kg of which 55-65 Kg is living tissue; reaches 1370 mm in length; white fan shaped with deep radiating ribs.

Tridacna derasa (Roding 1819): The smooth shell giant clam or the Southern clam is the second largest tridacnid, reaches about 500 mm; low primary and radial sculpture, variable shape, massive umbonal area, smooth white shell.

Tridacna squamosa (Lamark1819): the fluted or scaly clam, reaches about 400 mm; elongate shell with conspicuous fluted scales on its radial ridges, valves white and occasionally tinged with orange; mantle yellowish green.

Tridacna maxima (Roding 1798): The rugose or the small giant clam, partially burrowing species, reaches 200 mm; mantle color highly variable, from blue to brown.

Tridacna crocea (Lamark1819): The boring or crocus clam is the smallest of the tridacnids, reaches 150 mm; valves grayish, white often fringed with orange or yellow both inside and outside, triangularly ovate; mantle predominantly blue but shows great variability.
Tridacna tevoroa: The deep water devil clam, a rare species lives in the deep water (20-30 +m) habitat in the eastern Fiji islandsand northern Tonga islands; only recently described.
Hippopus hippopus (Linnaeus 1758): The horse's hoof, bear paw or strawberry clam, reaches approximately 400 mm in length; valves thick, heavy, triangular in shape, often covered with reddish spots and obscured by encrustations; mantle deep yellow-green, irregularly mottled at the periphery and in the centre.
Hippopus porcellanus (Rosewater 1982): The China clam recently described species; shell thinner and smoother than *Hippopus hippopus*, no pigmentation, more semi circular; mantle similar to *Hippopus hippopus.*
The larger giant clams are listed by the International Union for Conservation of Nature (IUCN) as threatened species.

Special Features of the Giant Clams

All the species are limited in their distribution to the shallow, sunlit waters of Indo-Pacific coral reefs. Giant clams have solved the problem of poor plankton availability in Oceanic waters by "farming" enormous numbers of dinoflagellate *Symbiodinium microadriaticum* in the inter haemal sinuses of their enlarged siphonal tissues. The algal symbionts, zooxanthellae are probably responsible for the larger sizes of these giant clams. The ideal features for the Mari culture of these species are therefore, the self feeding capability and photo trophy, rapid growth rates and high market value.

All tridacnids form byssal attachments to the reef early in life. As in other bivalves, the giant clam byssus serves to prevent physical displacement from the substrate. Its important function, however is to maintain the clam in an upright position, ensuring a favorable orientation to sunlight. The larger giant clams (*T. gigas, T derasa, T squamosa* and *H. hippopus*) eventually lose their byssus, presumably because the weight of the valves provide sufficient ballast to prevent displacement and to maintain an upright position. The smaller species *T. maxima* and *T crocea* remain strongly byssate throughout life actively burrowing via mechanical and chemical means into coral substrates.

Culture

Early attempts to culture giant clams were by several groups simultaneously; Stephenson (1934) in Australia, Rosewater (1965) and Hardy and Hardy (1969) in Palau. However, these were unsuccessful. Wada (1954) conducted extensive spawning trials on Palauan tridacnids. La barbera (1975) could carry out the first successful culture of *T maxima* in Fiji. Jameson (1976) reared *T crocea,, T maxima* and *H. hippopus* in the Palau and guan islands. Murakoshi (1978) reared T crocea in Okinawa. Since then larval rearing and grow out culture of giant clams are been carried out in many of the Indo Pacific islands. Methods for culturing gianrt clams continue to evolve and a wide range of production techniques is now available.

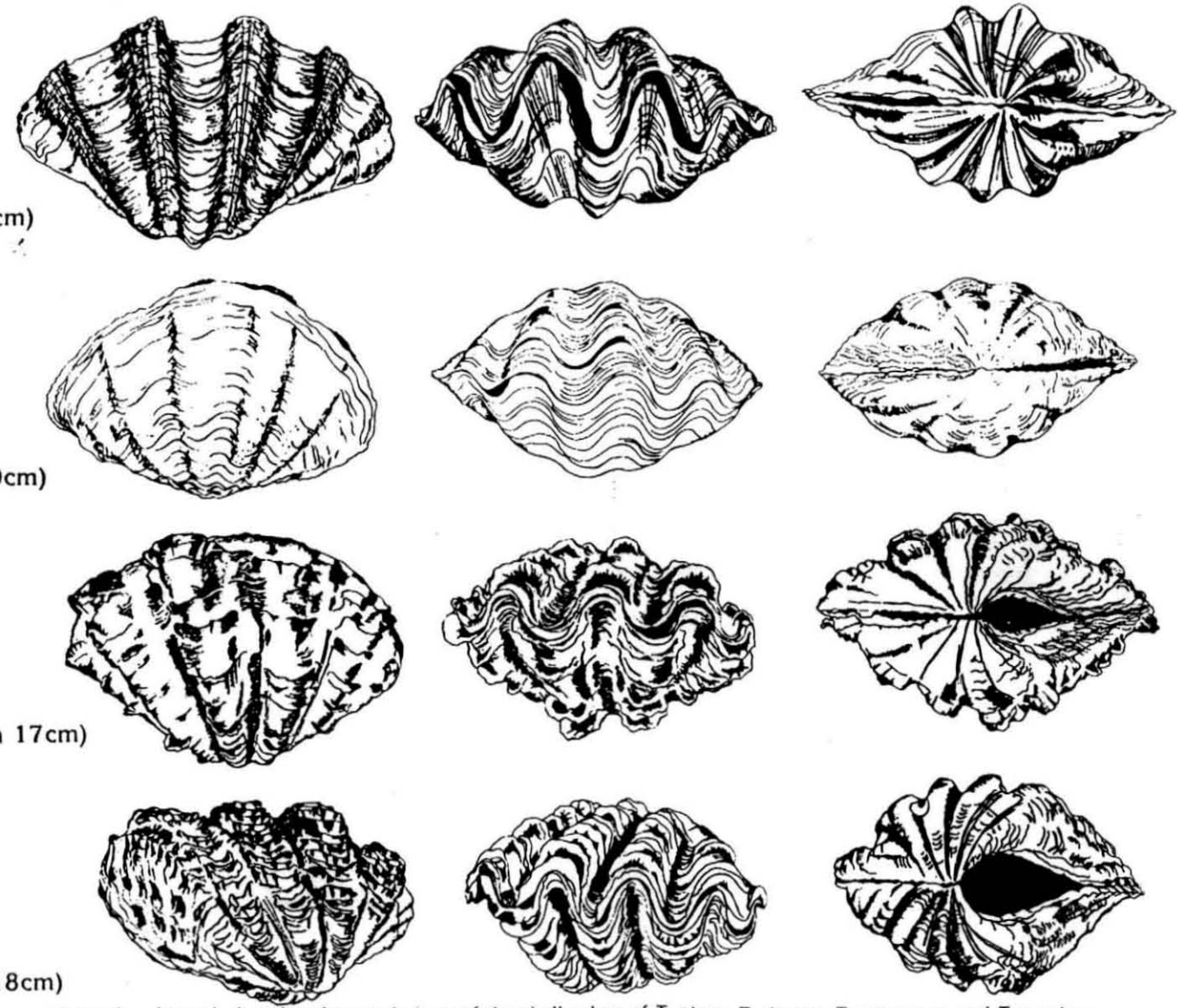


Figure 1 Lateral, dorsal and ventral views of the shell valves of *T. gigas*, *T. derasa*, *T. squamosa* and *T. maxima*.

Present Hatchery Techniques

In giant clams, four phases are involved.

i) Hatchery phase: Rearing the larvae from eggs in indoor/outdoor tanks. Six of the eight known species have been successfully spawned in Philippines by injecting serotonin in to the gonad of mature clams and also by introduction of macerated gonad materials into the mantle cavity through exhalent siphons.
The larval development stages are similar to those in other clams and settlement takes place in about 7-10 days after spawning. *Isochrysis galbana* is fed to the larvae. The spat attach with the byssus but they may break attachment and creep along the substrate. At this stage the symbiosis with Zooxanthellae is established. The larval rearing is done in both indoor and outdoor tanks.
Culture is also done in raceways containing fiberglass shellfish rearing trays wherein seed clams of 10 mm size are counted and redistributed.

ii) Nursery phase: Rearing juvenile clams in onshore tanks for metamorphosis (0.2mm) to about nine months of age and 20+ mm shell length (seed clams).
The tanks are provided with flow of raw seawater. The clams acquire the zooxanthellae from the seawater in about 3 weeks after fertilization and they become increasingly autotrophic.

iii) Ocean nursery phase: Rearing juvenile clams in protective containers (ocean nursery trays) in the field from about 20 mm shell length to 200 + mm shell length. (8-9 months post fertilization stage to 2 years.
The trays are free standing modular units with 60 mm clearance above the substrate and a 25 mm mesh polyethylene lid. Each tray is filled with 5-10 kg of basalt chips, which provide ballast as well as serve as substrate for byssal attachment.
Stocking densities of 100/tray for 30 mm and 24/tray for 120 mm size seed clams are adopted. The mesh lids exclude predators, the muricid gastropod *Cymatium muricinum* that causes extensive damage by crawling into the byssal orifice and feeding on the soft tissues.

iv) Grow out phase: Rearing clams of 200+ mm shell length without protection in the field to market size of 250 mm which takes as long as 2-3 years. No care is required and survival is over 90%.

Reef seeding: Tridacnid juveniles survive minimum of 24 hrs out of water and large numbers can be air shipped inexpensively over large distances and for reef seeding purposes.

There are many variations to the techniques for the production of clams. However, three different methods of culture of giant clams may be recognized based on the degrees of reliance on land based and ocean based operation (see schematic diagram). Based on the hatchery/ nursery phases, using any of the three methods, three methods of Giant clam larval culture are recognized.

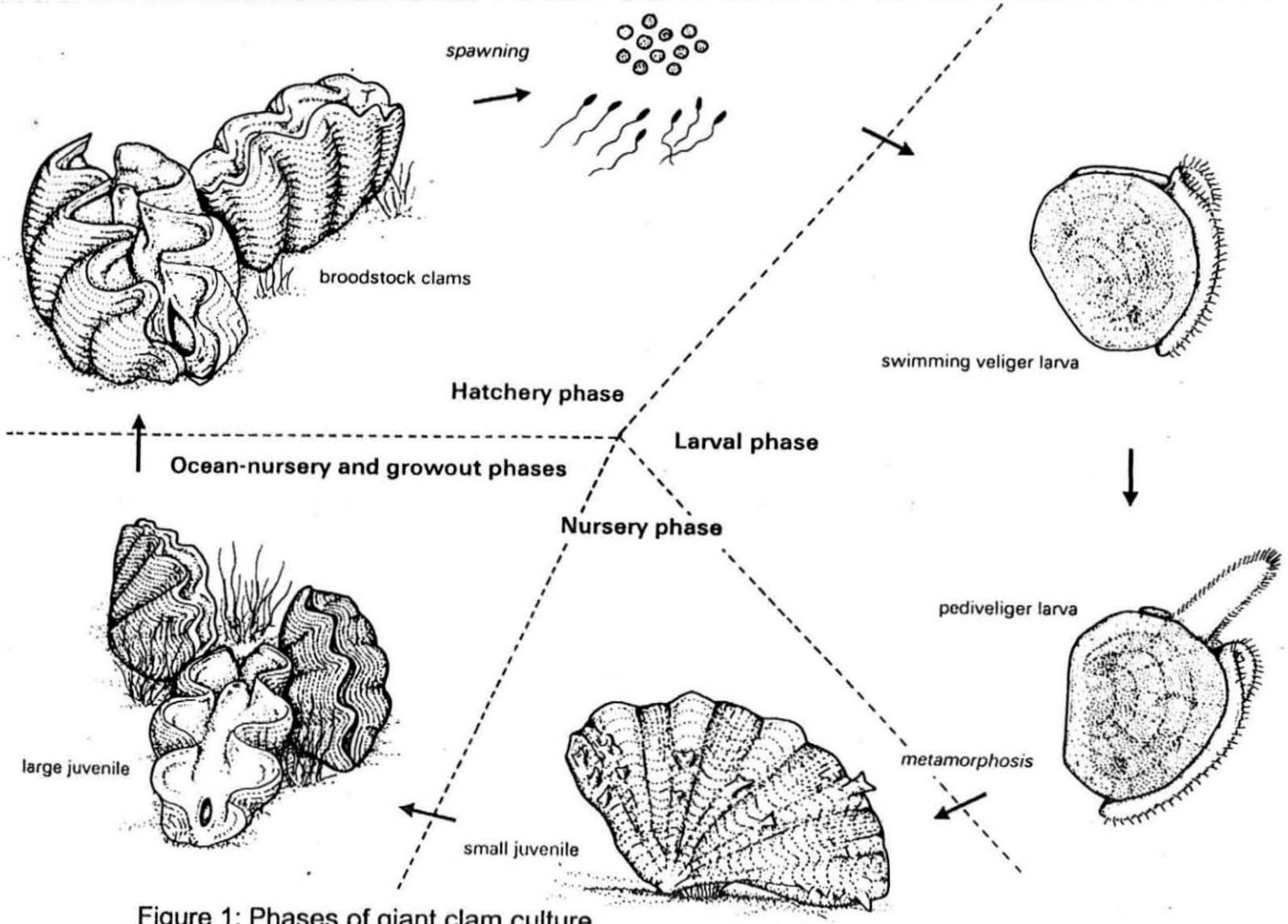
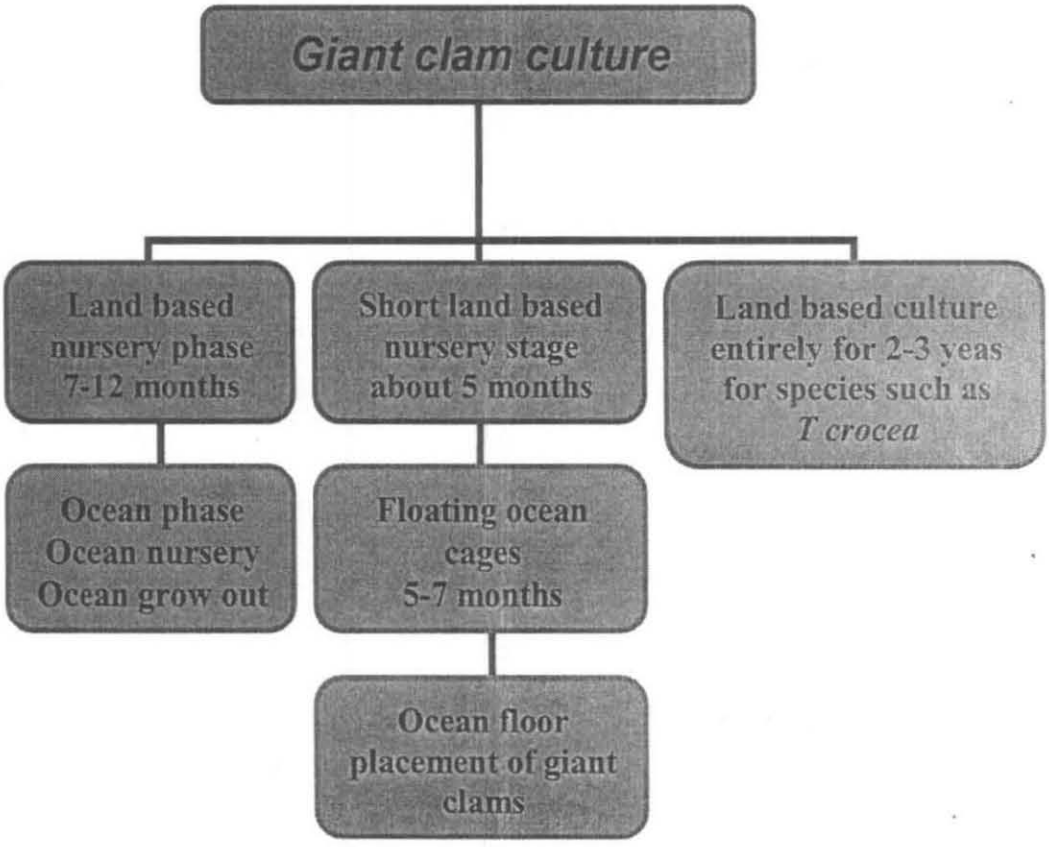


Figure 1: Phases of giant clam culture

a) Extensive: Fertilized eggs added to sea water which has been allowed to develop a local phytoplankton bloom (3000-10000 liter tanks)
b) Semi-extensive: swimming larvae stocked and fed cultured unicellular algae (3000-10000 liter tanks)
c) Intensive: selected swimming D – stage veligers stocked into 500-2000 liter tanks and fed unicellular algae, later released to settlement/nursery tanks.



Three different methods of giant clam culture involving different degrees of reliance based on land based and ocean based operations.

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Mussel Farming Methods and Seed Collection

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Introduction

Green mussel, *Perna viridis* and brown mussel, *Perna indica* are the two candidate species available along the Indian coasts and Andaman and Nicobar Islands.

Mussels are sedentary animals growing attached to hard substrates by means of byssus. They can be transplanted from their natural habitats to any artificial hard objects. This unique character of mussels is taken advantage of culturing these animals. Mussel culture is being practiced in France since the beginning of thirteenth century. From here it has spread to other countries like Spain, Holland, Belgium, Italy, Philippines, U.S.A, Australia and New Zealand, where different techniques are adopted depending on the hydrographic, social and economic conditions.

In terms of production and consumption, the European Union plays a dominant role with a production of 500,000 tonnes / year. Spain is the largest producer among EU countries followed by Netherlands, Italy, France and New Zealand. In Asia, China is the larger producer of mussel. In India the total production during 2002 from captures and culture fishery of mussels amounts to 15,066 tonnes. Taking Kerala as a model the mussel culture has been taken up by other coastal states of India.

In India mussel culture has been introduced only very recently. In 1971 The Central Marine Fisheries Research Institute, initiated culture of brown mussels at Vizhinjam Bay and later successful experiments were conducted at Kozhikode and Chennai in 1975, to study the possibilities of culturing mussels in the open sea. Backwater culture was started in Padanna (Kasargod district) in 1996. From a production of 20 t in 1996, the culture production increased to 1350 t in 2002 by mussel faming in the estuaries of Kerala. Karnataka and Maharashtra have also initiated mussel farming from 2002 onwards.

Distribution

Approximately 17 species of edible mussels are harvested or cultured worldwide. The blue mussels, *Mytilus edulis* and *M. galloprovincialis*, are the most common species in Spain. China, have *M.edulis*, black mussel *M.crasitesta* and green mussel *M.smaragdinus* are found in China, which tops in mussel production in the world. Srilanka, Singapore, Thailand, Philippines, Indonesia, Malaysia, Burma and Fiji have the green mussel *Perna viridis*. In New Zealand large, green-lipped mussels, known as green shell mussel is cultured. In California

M.californianus is cultured. The brown mussel *P. Perna* is available in Srilanka, which is the same as that found in the India region. In India green mussel *Perna viridis* and the brown mussel *Perna indica* are cultured.

Green mussel is widely distributed along the intertidal coasts of India and found extensively around Kollam, Alappuzha, Kochi, Kozhikode, Kannur and Kasargod in Kerala and in small beds in Chilka lake,Orissa, Vishakhapatnam, Kakinada Chennai, Pondichery, Cudalloor, Mangalore, Karwar, Goa, Ratnagiri and in Gulf of Kutch and also in Andaman and Nicobar Islands. *Perna indica* has a restricted distribution and is found along the southwest coast from Varkala to Kanyakumari and on southeast coast from Kanyakumari to Tiruchendur.

Mussel Farming

Sites for mussel farming has been identified in Kerala, Karnataka, Maharashtra Goa, Tamil Nadu, Andhra Pradesh, Pondichery, Gujarat, Orissa and Andaman and Nicobar islands. The seed availability and environmental conditions plays a critical role in mussel farming.

Seed Collection

Success of mussel farming depends on the availability of good quality seed. In areas where natural seeds are available is considered as the primary source of seed. Though the technology for hatchery production of seed has been studied, it is not economically viable. Seed collection requires accurate forecasting of spat fall. Various types of materials are used as spat collectors namely tiles, ropes, asbestos, shading material, frilled polypropylene rope etc. Selection of spat collector depends on the efficiency, local availability and durability of the material and initial cost of investment. Commonly polypropylene ropes are used for seeding of mussel. In Kerala, spat settlement is observed from July to August. During October this settled spat attains a length of 20-25mm size and weighs 1.5-2.0gm.

Seeding Method

Seeds collected from the submerged (sub tidal) areas will be healthier. After removing other organisms and weeds, the seeds may be washed thoroughly in

seawater. About 1000gm of seed is required for seeding on one-meter length rope of 12-14mm or 15-20mm. Cotton mosquito netting is used for enclosing the seed to the rope. The cloth will disintegrate within 2-3 days. By this time the seeds will secrete byssus thread and will get attached to the rope. Duration of mussel farming is 4-6months. Mussels attain 80-88mm size by 5months with an average weight of 36 - 40g and an average production of 10 - 12 kg/m of rope is obtained.

Site Selection

Open sea and estuarine areas free from strong wave action may be selected for farming. Clear sea water with high plankton production (17-40µg chlorophyll /l) is ideal for mussel culture. Moderate water current (0.17-0.25m/s at flood tide and 0.25-0.35m/s at ebb tide) will bring the required planktonic food and will carry away the excessive build-up of pseudofaeces and silt in the culture area. The water should have a salinity of 27-35 ppt and temperature of 26 – 32°C. Site selected should be free from domestic and industrial sewage. In shallow waters, sea and estuaries rack and stake (Bouchot) method can be adopted. For deeper regions, raft or long line method is ideal.

Open Sea Farming

Open sea farming is practiced in areas with depth of 5-20m. The area of culture should be free of strong wave action, less turbulent and with high productivity. Long line, and raft culture techniques are ideal for sea farming. Disadvantages of this type of farming are poaching, unpredicted climatic changes and predation.

Estuarine Farming

Compared to open sea, the estuarine ecosystems are less turbulent and shallow (<4m). Stake and rack culture (horizontal and vertical) are ideal for estuarine conditions. Fluctuation in salinity during monsoon season and pollution through domestic and industrial waste are the main constraints in estuarine mussel farming. On - bottom culture by relaying of mussel seed in pen enclosures is also practiced.

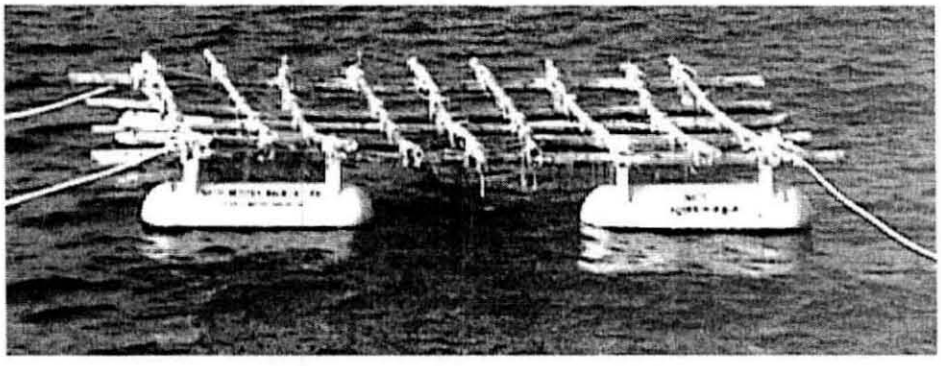
Culture Methods

Culture methods are divided into two categories viz. bottom and off bottom culture. In bottom culture the mussel seeds are relayed and left to grow until harvest. Off-bottom culture comprises of providing a structure to which the mussel attaches by byssal threads. In this method ropes or other materials are used as substratum for the mussel to settle and grow. Types of off-bottom culture are:

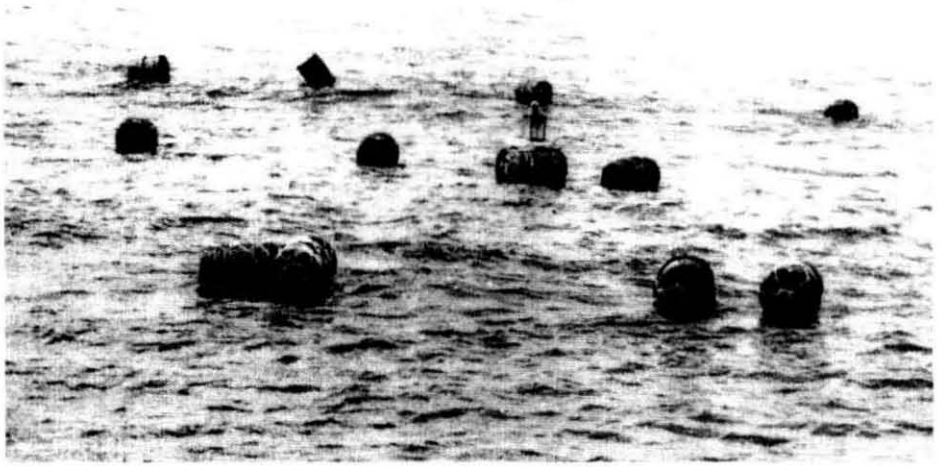
Rack method: Suitable for estuaries and shallow seas. Bamboo or Casuarina poles are driven into the bottom spaced 1-2m apart to form a lattice network of frame. Seeded ropes are suspended from these frames. A modified version of off-bottom culture is the horizontal rope culture method where the seeded ropes are suspended horizontally. This method is practiced in shallow waters where the depth is <1m.

Due to the effective utilization of the productive column water this type of culture gives better yield.

Raft method: Ideal for open sea conditions, which are not rough. Square or rectangular rafts are made with sturdy bamboo or casuarina poles. Buoyancy for the raft is provided by tying together 5 barrels of 200 liter capacity (metal oil barrel painted with anticorrosive paint or synthetic barrel). Ideal size of the raft is 5 x 5 m. The rafts are to be positioned at suitable site in the sea using anchors (grapnel, granite, concrete).



Long-line method: Considered ideal for unprotected open sea conditions. Synthetic rope of 16-20mm diameter is used for the long-line (main line). The main line is supported with 220 litre barrels tied to it, spaced at 5m. The seeded ropes are suspended from the main line 1.5-2m apart. The long –lines and barrels are anchored in position at either ends using concrete blocks and nylon ropes or metal chain.



Stake / "Bouchot" culture: Culturing mussel on stakes is carried out extensively in the intertidal mud flats along the Atlantic coast. Initially rows of poles are placed in the intertidal region to allow mussel spat to settle and grow. When the spat grows slightly bigger they are transferred to " bouchot " placed in shallow waters in the same region. The mussels attain marketable size on the poles. Periodical thinning of mussel is necessary to prevent overcrowding

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Innovations in Increase in Mussel Farming

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The Central Marine Fisheries Research Institute (CMFRI) by setting up demonstration farms and conducting training programmes to fishers was able to popularize bivalve farming in Kerala State. Simultaneously, through interactions with the State government officials and members of local governing bodies, project proposals on oyster and mussel farming were recognized as financially viable schemes for rural development and self-employment. Since 1995, edible bivalves like the Indian backwater oyster, *Crassostrea madrasensis* and the green mussel, *Perna viridis* are farmed on a commercial scale in the estuaries of Kerala. Along the Malabar Coast, especially in the estuaries of Kozhikode, Malappuram and Kasaragod districts mussel farming (by rope culture from racks) is now a popular seasonal vocation through which about 2500 tonnes of mussels are produced annually.

Extraordinary growth performance, natural abundance, adaptability to new environments and fairly simple culture techniques make mussels of the genus *Perna* an ideal shellfish crop in many Asian countries (Vakily, 1989). Although the profitability of mussel farming operations in Indian waters can be high (Qasim *et al.*, 1977; Mohamed *et al.*, 1998; Asokan *et al.*, 2001), the returns obtained by farmers largely depend on the input costs. The rope method are also affected by losses (varying from 30 to 80%) due to fall-out or slipping of seeded mussels from ropes. Further, it has been reported (Velayudhan *et al.*, 2000, Asokan *et al.*, 2001) that the polyethylene rope used for seeding accounts for almost 40% of the investment cost. Presently the seeding method involves placing the mussel rope on biodegradable cotton netting, uniformly distributing the mussel seed and then manually stitching the netting around the rope and mussel seed. This process is labour intensive and seeding costs are estimated to range from 15-20% of the operating cost. The mussel tubing socks, widely used in Europe, have not been tried in India. The possibility of using alternate seeding material and methods to reduce costs due to seeding on polyethylene ropes has been studied. The quality of the alternate seeding material and method were assessed in terms of growth, production and percentage fall-out of mussels from the rope.

1. Use of Pre-stitched Mussel Tubing

Results of the study carried out by Mohamed et al., (2003) are presented here. New methods were attempted by transplanting *Perna viridis* (L.) seed to 12 mm diameter polyethylene ropes on which biodegradable cotton net was wrapped and stitched together (control), 12 mm frilled polyethylene rope (Fuzzy™) with white fully degradable tubing socks (FuW) and grey semi-degradable synthetic

tubing socks (FuG) and 5 cm broad flexible plastic strips (FPS) kept inside pre-stitched biodegradable cotton net. The treatments, which were replicated, were suspended from a fixed rack in a shallow tropical estuary (Ashtamudi Lake). The specific growth rate (SGR) in length and weight, fallout percentage and production in different treatments were compared. There was no significant difference in the SGR in length and weight, while the fallout percentage was significantly ($P<0.05$) lower and production significantly ($P<0.05$) higher in FPS and control treatments. Since the FPS and control treatments did not show any difference in terms of growth and production, the economic performance of these two methods were compared. The economic analysis indicated that the use of FPS together with pre-stitched biodegradable cotton net reduced the investment costs by 34% and increased the rate of return by 48% over that of the control.

Table 1. Comparative economics of mussel farming by existing and improved methods. Area – 0.0025 ha; 100 seeded ropes of 1 m length, cultivation period - five months (All amounts in Indian Rupees).

	Criteria	**Existing method**	**Improved method**
A	Investment		
	Bamboo poles – 15 nos	1500	1500
	Polyethylene rope for seeding	1020	-
	Others	500	500
	TOTAL	3020	2000
B	Annual Fixed Costs		
	Interest (@ 15% per annum)	453	300
	Depreciation		
	1. Bamboo poles (50% per annum)	750	750
	2. Polyethylene rope (50% per annum)	510	-
	3. Others (50% per annum)	250	250
	TOTAL	1963	1300
C	Operating Costs		
	Annual lease	500	500
	Labour for rack construction	750	750
	Biodegradable cotton netting	750	750
	Mussel seed – 150 kg	900	900
	Flexible plastic strip	-	200
	Canoe hire charges	750	750
	Labour for seeding	1200	700
	Harvesting and marketing	1500	1500
	TOTAL	6350	6050
D	Total Cost (B + C)	8313	7350
E	Production (kg)	1260	1150
F	Income @ Rs.8/kg	10080	9200
G	Net Operating Income (F-C)	3730	3150
H	Net Profit (F-D)	1767	1850
I	Break-even price –Rs./kg (D/E)	6.6	6.4
J	Rate of Return (%)	73	108

The advantage in FPS treatment was the ease of filling up the pre-stitched cotton biodegradable tubes with mussel seed as compared to the manual drudgery of stitching. This directly resulted in halving of the labour cost involved in seeding. Results of the economic analysis indicate that by using the improved method, marked gains (by 48%) could be made in the rate of return. The polyethylene ropes used presently is 10 times more expensive than FPS and this study has shown that there is no significant difference in the production and fallout percentage due to its use. Although the FPS is a 'use and throw' type of material, its life could be extended by another year through careful use. The use of FPS as seeding substrate and pre-stitched cotton biodegradable net tubes can therefore be recommended for use to estuarine mussel farmers. Furthermore, use of pre-stitched tubes opens up the method for mechanisation of the seeding process.

2. Development of Semi-automatic Mussel Seeder

Seeding is one of the most critical activities in mussel farming. The process which is physically demanding (as farmers have to kneel and bend down to do it) is crucial to the success of farming as the uniform attachment of mussel seed around the rope is dependant on how well it is done. Now, to reduce the physical strain and to increase efficiency during this process, a semi automated mussel seeder has been designed and developed.

The seeder was field tested at the CMFRI's demonstration mussel farm in the Ashtamudi Lake in Kollam district, Kerala. The efficiency of the seeder was evaluated by comparing the time taken for seeding 1 m length using the conventional method and the seeder. The uniformity of attachment of mussel seeds around the central core material was judged by visual examination after 1 week when the mussel seeds were attached. The seeder (Fig.1) made from quality hardwood consists of the following parts.

PVC pipe: PVC pipes of 1m lengths are for providing rigidity to the pre stitched cotton tubing during seeding. The diameter of the pipe is decided based on the size of the mussel seed. For smaller seed of length 20-25 mm PVC pipe of 6 cm diameter and for 25-30 mm seed 7.5 cm diameter pipes have to be used. Aluminum couplings of appropriate diameter are used to hold the PVC pipes to the seed holder.

Mussel Seed Holder: A wooden rectangular basin (75 x 50 x 6 cm) with two circular openings of 9 cm diameter, which are spaced 16 cm apart, is used for placing the mussel seed. The circular openings are for holding the top part of the PVC pipe. To hold these pipes tightly, detachable aluminum couplings are used. Two hooks are provided on the wider side of the seed holder diametrically opposite the circular opening.

Base plate: The base plate (75 x 50 cm) is a wooden board for supporting the lower end of the PVC pipe. It has also two elongated slits of length 25 cm and width 1.5 cm through which the lower end of the core material can be passed and locked. A semicircular girdle with height of 2 cm outside the elongated slit prevents tilting of the PVC pipe.

Vertical support: The seeder is held together with the help of vertical supports. The two legs on each side are joined horizontally on top and bottom. The seed holder rests on this. The base plate is bolted to the bottom portion of the legs. The height between the seed holder and the base plate is 1m so that the PVC pipe can be inserted between these and held tightly.

Top Stand: The top wooden stand of height 105 cm from the seed holder consists of two vertical poles connected by a horizontal pole which can be fixed to the sides of the mussel seed holder. The horizontal pole is provided with two metal rings, which are aligned to the center of the circular opening on the seed holder. The rings on the top stand, opening in the mussel seed holder and the end of the elongated slit on the base plate are aligned so that the core material can be held vertically in the center of the PVC pipe.

All the above-mentioned 5 parts can be easily assembled within 5 minutes at the farm site with the help of nuts and bolts and are detachable making the seeder a portable unit. The cost of a single unit of mussel seeder made of Mahogany wood is Rs.2500.

Although 12 mm diameter nylon ropes are conventionally used as core material, the use of 5 cm width flexible plastic strips (FPS), which are used to make camp cots, and chairs has been recommended as a cheaper and durable substitute for seeding[2]. FPS is commercially available as 100 m rolls.

Items necessary for seeding are the core material such as FPS and the pre-stitched cotton tubes. The pre-stitched tubes are prepared from biodegradable cloth (e.g. cotton mosquito net) that are cut into required length (1.25 m) and width (slightly larger than outer width of the PVC tube) and machine stitched together longitudinally. These are kept ready before the seeding process is initiated.

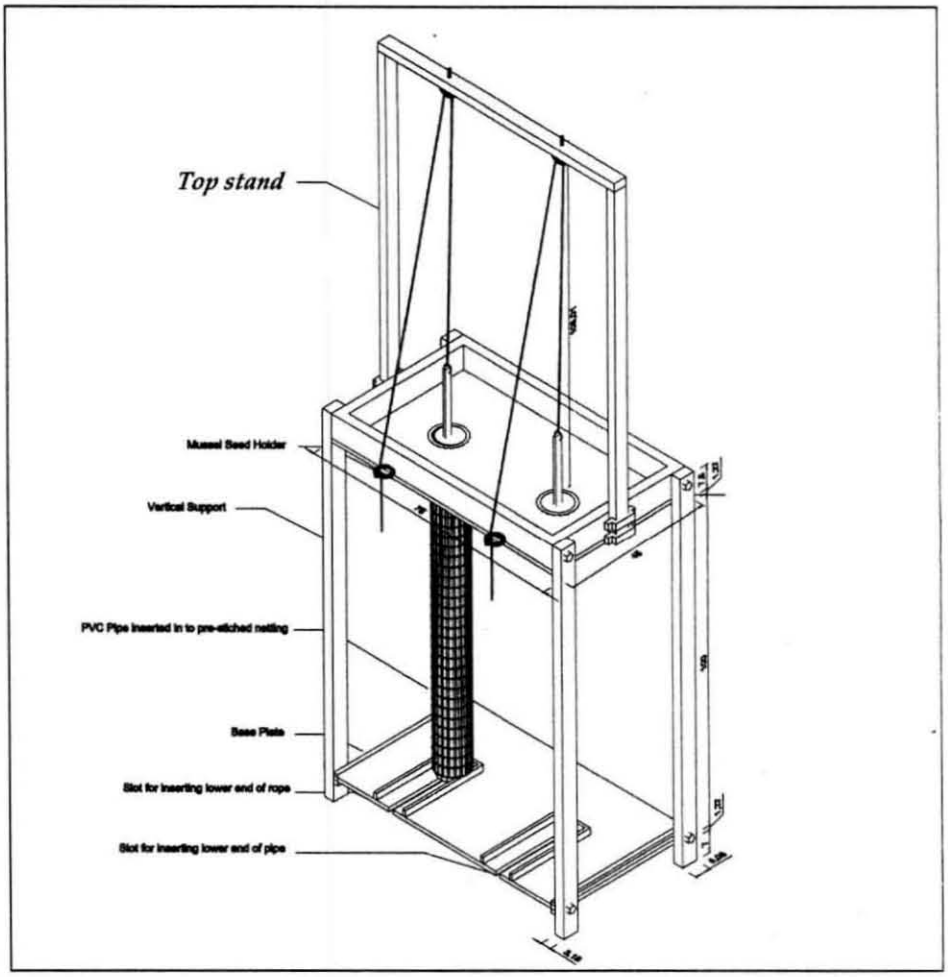
The pre-stitched tube is first pulled over the PVC pipe and the FPS is passed through the PVC pipe. The lower end of the pipe is closed by tying the pre stitched tube and the FPS. Then the PVC pipe covered by the pre-stitched tube is inserted into the mussel seeder between the mussel seed holder and the base plate. The lower knotted end is slid under the elongated slit and the knot holds the PVC pipe and the pre-stitched tube in position. The upper end of the PVC pipe is aligned to the circular opening on the mussel seeder and is held in position by the coupling.

The upper end of the FPS is tied to a 3 mm nylon rope, which is passed through the ring on the top stand and tightly tied to the hook provided on the side of the mussel seed holder. Cleaned, separated and sorted mussel seeds are placed in the seed holder from where the seed can be slipped into the pipe. When it is filled to the brim, the PVC pipe alone is lifted up slowly until it is above the seed holder. Then the knot on the hook is loosened enabling PVC pipe to be slipped out. Finally, the seeded mussel tubing can be easily slid out of the seeder and knotted at the top. These tubes can be stocked immediately in the farm. If depth is more and if

horizontal method of stocking is followed, then these can be joined by tying the ends to one another to get the required length.

The relative advantages of the developed mussel seeder in terms of time taken to do the seeding, uniformity of attachment of mussel seeds and relative physical exertion are given in Table 2.

After successful field trials, the seeder was demonstrated to mussel farmers and panchayat officials at Korapuzha in Kozhikode district and Vallikunnu in



Malappuram district in North Kerala. The response of the farmers was graded as good considering the advantage of reduction in time taken for seeding and the resulting decrease in expenditure on labour. Farmers were of the opinion that the seeder can be used as a common facility by all mussel farmers in a village unit. The village panchayat officials have included the seeder in the subsidy component given to mussel farmers.

Table 2. Advantages of the mussel seeder

Innovation	Use	Advantages
Mussel seeder	Semi-automation of the process of filling the seed – seeding	**Reduction in labour and time**. The time taken for manual stitching of 1m rope by the conventional method is 8 minutes whereas in the seeder the same can be accomplished in 2 minutes. **Uniformity in attachment of mussel seeds around the FPS**. Visual examination revealed that mussel seeds were more evenly attached around the FPS than in the conventional method. **Reduction in physical exertion** – In the new method the seeding can be done easily without kneeling or bending for long durations thereby reducing or completely eliminating the physical stress. This is especially advantageous for women who mostly do the seeding work.

3. Cost Reduction in Rack Structure

The constant replacement of bamboo or casurina poles used for fabrication of grow out structure due to fouling and boring is the main recurring expenditure in bivalve farming using the rack method in estuaries. PVC poles of 2-inch diameter filled with concrete were used instead of bamboo poles in 1997 (Kripa et al., 2001). These have withstood 3 seasons without any fouling / boring or natural degradation. Though the capital investment in the first year is high, continuous replacement / maintenance work of farm owing to collapse of farm structure on account of natural calamities like strong wind or rain can be avoided.

4. Integrated Culture of Finfish in Bivalve Farms

To utilize the space in between the rack farm and as a means of improving the profit, integrated farming of finfish together with bivalves was attempted (Kripa et al., 2001). Two nylon net cages (1.3 x 1.3 x 1.5 m, 1.5 cm mesh) were tied to the vertical poles in the rack farm and stocked with young ones of the pearl spot *Etroplus suratensis*, which is a favoured food fish of the region.

The mean seed size was 6.6 cm (6.8 g) and the stocking density was 22/m^2. The fishes were fed with dried clam meat and pellet feed through a feeding tray at 5% of body weight. The growth observed was good and there was 100% survival. The average growth was estimated as 10.3 mm/ month, which is considerably more than that observed for this fish in pond culture (CIBA, 1995). The average production obtained was 1.6 kg/ m^2 from an initial 0.15 kg/ m^2 within less than 8 months.

Since pearl spot fetches a high price (Rs. 70/kg) in the local markets, it is clear that cage farming of quality food fishes in estuarine bivalve rack farms would form a significant source of additional income to farmers.

Suggested Reading

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Kripa V, Mohamed K.S, Velayudhan, T.S, Laxmilatha P, Radhakrishnan P, Joseph M, Alloycious P.S, Sharma J & Appukuttan K.K., Recent developments in edible bivalve farming in India. *Aquaculture Asia*, 6 (2001), 40-43.

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Mussel Farming as a Village Linked Programme

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Introduction

Mussel culture was started in the early seventies but this could not be popularised, as these were operation limited to the open sea with raft culture. Such activities could be done only with the help of fishermen and a large scale operation had logistic problems too. With the success of mussel culture in the backwaters, the practice has been continued for the past seven years. The simple methods employed for mussel farming was transferred to progressive farmers who took up mussel culture in the backwaters. Soon they found the venture profitable as unlike the raft culture the cost of production was low and the risk of losing the raft to inclement weather was not there. Demands came from new entrepreneurs for training and mussel farming spread from Kasaragod to Ponnani. From 250 tonnes of mussels harvested in 2000, the total harvest estimated for the year 2004 is 2500 tonnes.The growth has been ten times.

Mussel culture in the backwaters of Kerala was first started in Padanna and Cheruvattur Panchayats in Hosdurg Taluk of Kasaragod district. Later it was taken to Elathur in Calicut district and Vallikunnu and Ponnani in Malappuram district. This has happened mainly due to the popularisation efforts by the CMFR Institute. This year the Padanna farmers go for the eight harvests.

Initially this low cost technology of farming was transferred to five groups with 15 to 21 members at Cheruvattur and Valiyaparamba. Financial assistance was provided by the North Malabar Gramin Bank and Cheruvattur Farmers Co-operative Bank. They provided a loan of Rs.2,60,200/= for the implementation of the project with a subsidy component of 50% subsidy. These groups harvested 67.4 tonnes of mussels during May-June 1997. A portion of the harvested and shucked meat (2000-Kg) was sold to the Integrated Fisheries Project, Cochin at a rate Rs.45 per Kg. The remaining harvest was sold in the domestic market. The groups could realise Rs.3, 34,555/= from the harvest with a net profit of Rs.1,04,455/= within a period of 6 months.

Major Areas of Mussel Culture

Kasaragod District

The Cheruvattur panchayat has an area of 18.37 sq km with a population of 24,504 of which 18.631 are literate. About 150 families are engaged in fishing activities and about 300 families are engaged in ancilliary activities. Padanna panchayat has an area of 13.08 sq km with a population of 17,961 out of which

12,746 are literate. About 200 families are engaged in fishing as main activity and about 400 families as part time occupation.

The culture is done in the Padanna backwater systems in the Hosdurg Taluk. In Cheruvattur Panchayat, five groups were formed at Koyambram, Kavunchira, Kayuthakadu and Paranthamadu and in Padanna panchayat three groups were formed at Badkekad, Ori and Thekkekadu. At Padanna it was individuals who have done mussel culture. At Koyambram and Paranthamadu, there are 13 members in each group and at Kavunchira and Kayuthakadu 15 members each in the groups.

Malappuram District

TTC (Trainers training centre) training was given to 45 trainees at Vallikunnu panchayat during September1999 by CMFRI. Subsequently, during January 2001, training was imparted to 60 trainees of Malappuram district under the self-help group (SHG) training programme of the State fisheries Department. This training was conducted at Balathurithi. These trainees did mussel culture in Vallikunnu and Puthuponnani. TTC training was given to 20 trainees at Puthuponnani. They have taken up mussel farming under the auspecies of the Organisation named 'Youth power'. At Vallikunnu panchayat in Malappuram district, farming is done in the Kadalundi estuarine system. The total production from this area during this year is estimated to be 150 tonnes. Most of the units are done by groups.

Here the panchayat took the initiative in taking up mussel culture activity. A subsidy of Rs. 1200/= was given to each person of the group by the Panchayat. The total production by the trainees was 5 tonnes. This was the first time that a commercial scale operation was done at Vallikunnu. The markets were the nearby areas of Calicut and Malappuram district at Kotta, Althani, Kottakadavu, Kootimadu and Manavala.

Kozhikode District

Mussel culture is being done in the Korapuzha estuarine system. Training was imparted to 20 persons under the self-help group (SHG) training programme of the State fisheries Department. Initially at Elathur three units were doing mussel culture. Two units by individuals and the third by a group of 10 members. Now the ADAK (Agency for development of aquaculture Kerala) started giving subsidies for the past two years. Each year about 40 groups are identified.

Production

The total green mussel production by capture fisheries from Malabar area was 6317 tonnes during 2001. The total production from culture was 400 tonnes. This forms only 6.3 % of the total mussel production from Malabar, which had increased from 4.62 % during 1999. Now the production from the wild is increased to about 9000 tonnes but the total production from mariculture has increased to 2500 tonnes forming 27% of the total production.

The yields obtained during 1999 by the groups and the numbers of ropes suspended are given below:

Sl.No.	Place	Total yeild (tonnes)	No. of ropes	Yield/rope(Kg.)
1.	Koyambram	22.75	700	32.5
2.	Kayuthakadu	36.22	900	40.24
3.	Kavunchira	25.2	900	28
4.	Paranthamadu	12.75	300	42.5
5.	Badkekad	18.75	625	30
6.	Ori	13.5	482	28
7.	Thekkekadu	22	760	29
Total		151.75	4667	32.89 (Av.)

Thirteen persons started mussel culture on individual enterprise. The total production from these farms was 97.5 tonnes and the total production from Kasaragod districtwas 248.97 tonnes. During the year 2004, the numbers have dramatically increased and the total production from Malabar is estimated at 2500 tonnes. The number of groups and individuals engaged in mussel culture has gone up dramatically. An interesting to note that the number of single holding has increased and most of them are male members.

Financial assitance

Mussel culture was initiated in Kasaragod district through the DWCRA (Development of Women and Children in Rural Areas) scheme. Loan amount sanctioned was Rs.8800/= per member with a subsidy component of 50%. The amount was to be paid back in five years and the rate of interest was 12.5% per annum. A revolving fund of Rs. 5000/= without interest is also provided. Now these schemes come under the SGSY (Swarnajayanthi Gramaswa Rojgar Yojana) which takes care of economic empowerment of weaker sections of the society. The women's self help groups were the major players in the mussel culture activities of this area.

At Vallikunnu, a subsidy of Rs. 1200/= was given to each farmer of the group by the Panchayat. The total production by the trainees was 5 tonnes. This was the first time that a commercial scale mussel culture operation was done at Vallikunnu. During the previos years only a demonstration culture was done. The harvest was sold in the local markets as wellas among the growers themselves. The markets were the nearby areas of Calicut and Malappuram district. This year, the estimated production is 300 tonnes. Some of the trainees have trained other members also and they have done mussel culture on their own.

At Elathur, although the mussel culture is developing very fast, no financial assistance was given to the farmers. A few of them are engaged in sand mining and coir making for additional income. Two culture units started as individual enterprise is also being operated successfully at Elathur. Now the ADAK is giving subsidies in the form of materials like bamboo poles, nylon ropes, netting cloth. Rs.1000 is given to each group for the purchase of mussel seed.

Inputs

In Kasargod, the net operating profit ranged from Rs. 7,646/= in Kayambram to Rs. 16,413/= at Badkekad. The cost analysis of mussel culture at Padanna showed that the major cost was that of Nylon rope (34%), Bamboo (20%) and seed (20%). The other expenditres involved cloth (7%), construction cost (5%), harvesting (4%), seeding (4%) and coir rope (3%).

Constraints

1. Availability of seed: The seeds required for culture is presently collected from traditional fishing areas and these are often causing conflicts between farmers and mussel fishermen. Hence it is essential that additional spat collectors has to be established along the coast to ensure supply of seeds to the farmers.

2. Marketing: The harvesting seasons of cultured mussels is mostly during April – May months and farmers are forced to sell their crop before the onset of monsoon to avoid mass mortality of mussels due to freshwater influx into the backwater system. At present only a few processing plants purchases cultured mussels from the farmers and as a result the local market are flooded with cultured mussels during these months resulting in fall in the prices and thereby affecting the profitability of the operation.

3. Depuration system: The main constraint in the export of cultured mussels is the lack of proper depuration techniques. Depuration plants are needed at regular intervals along the coast to depurate the cultured mussels for export processing.

4. Storage facility: If sufficient cold storage facility is provided, cultured mussels can be depurated, shucked and stored not only for export market but also for local market throughout the year. This will increase the profitability of the culture operation.

5. Post harvest technology: Value added products of longer shelf life need to be developed from mussel meat to increase the revenue realization from cultured mussels. Mussel fry, mussel pickle etc. are some of the best examples for value added products. More studies are needed to develop ethnic cuisines with longer shelf life.

6. Siltation of backwaters: Some areas in the backwater system have very high siltation levels especially during rainy season. This often results in mortality of mussels in the farms. Hence, scientific feasibility studies are required to demarcate potential culture sites.

Prospects

1. Backwater mussel culture is a recent phenomenon along the Malabar coast and opens immense potential for resource and employment generation among coastal communities especially women living below poverty line.

2. Mussel culture is a low investment activity with very good returns. If promoted properly, mussel farming can be used as a tool for women empowerment in the coastal areas and can stimulate a healthy socio-economic development in the area.

3. Better post harvest technologies can develop attractive value added products. Since very good export markets are available for mussels, they can be taken up as a challenging opportunity by technicians and scientists.

In the western countries, mussel is considered as poor man's oyster. But in India, mussel can be considered as tool for the upliftment of the poor people living in the coastal areas especially along the Malabar Coast.

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Socio Economics of Mussel Farming: Case Studies

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Introduction

Rational utilization of common property resources for sustainable development without endangering the environment is possible through community participation. Mussel farming offers good scope for development in our open waters for enhancing food and livelihood security of the stakeholders in our coastal agro climatic zones. Mussel farming has already been proved as one of the profitable enterprises in the coastal belts as a subsidiary income-deriving source of rural fishermen community. The experimental trials conducted by CMFRI have proved the techno-economic feasibility of mussel farming (Asokan et al, 2001 and Vipinkumar.V.P et al, 2001). Here an attempt has been made on exploration of two case studies in Kerala and Karnataka on socio economics of Self Help Groups of fisherfolk engaged in Mussel Farming.

A Self Help Group (SHG) consists of members linked by a common bond like caste, sub-caste, community, place of origin, activity etc. The Group Dynamics of these SHG's refer to the interaction of forces between the members. It is the internal nature of the groups as to how they are formed, what their structures and processes are, how they function and affect the individual members and the organization. (Lewin *et al.*1960). In an intensive study of Group Dynamics, Pfeiffer and Jones (1972) identified the Group Dynamics factors as to how the group is organised, the manner in which the group is led, the amount of training in membership and leadership skills, the tasks given to the groups, its prior history of success or failure etc. In a detailed study of Group Dynamics, Hersey and Blanchard (1995) gave emphasis on helping and hindering roles individuals play in groups such as establishing, aggressive, persuading, manipulative, committing, dependent, attending and avoidance.

Case Study 1

Kasargod, the extreme north district of Kerala is particularly notable for mussel farming as it has been successfully accomplished by the women's Self Help Groups (SHGs) for the past few years. These groups were given financial assistance in the scheme namely, SGSY (Swarnajayanthi Gramaswa Rosgar Yojana) by the state government which takes care of economic empowerment of weaker sections (Vipinkumar, 2001). Subsidies, bank loans etc are the part and parcel of it and it essentially focuses attention on poverty alleviation through organised Self Help Groups. This programme looks into training, credit, marketing, technical knowledge and basic facilities necessary for the upliftment of the poor to bring them above the poverty line within three years in such a way that they should have a monthly earnings of at least Rs 2000 /-. It would be pertinent to have a look into

the consequences of adoption and cost dynamics of mussel farming by the women's Self Help Groups in Kasargod district.

This district possesses an area of 1992 km^2 with a population of 10, 71508 as per 1991 census. The district with a population density of 538 km^2 has an average growth rate of 22.78 and 82.51 % literacy rate. Majority of the villagers earns their livelihood by agriculture, fishing, coir retting, coconut husk, toddy tapping etc. There is tremendous potential for aquaculture diversification in Kasargod coastal belts. Water bodies in these coastal belts have amble scope for the judicious utilisation of finfish culture, prawn and crab farming in Kasargod. (Asokan et al 2001)

Methodology

This study was undertaken in two major panchayaths namely Cheruvathur and Padanna in Kasargod district. The study area, Cheruvathur panchayath has an area of 18.37 km^2 with a population of 24, 504 out of which 18, 631 people are literate. Agriculture is the main occupation of the majority and about 150 families are engaged in fishing as the main occupation and about 300 families as subsidiary occupation.

Similarly, Padanna panchayath has an area of 13.08 km^2 with a population of 17, 961 out of which 12, 746 people are literate. About 200 families are engaged in fishing as main occupation and about 400 families as part time occupation. The brackish water estuary systems of these panchayaths are extremely suitable for mussel culture.

Six Self Help Groups of women (three each from both panchayaths) were selected as the sample and the data were gathered as explorative case studies through personal interviews of the respondents. For the study, the Group Dynamics of members of Self Help Groups was measured by developing an index called Group Dynamics Effectiveness Index (GDEI). Group Dynamics Effectiveness was operationally defined for the study as the sum-total of the forces among the member of SHG based on the sub-dimensions, such as participation, influence & styles of influence, decision making procedures, task functions, maintenance functions, group atmosphere, membership, feelings, norms, empathy, interpersonal trust and achievements of SHG. (Vipinkumar, 1998)

For the computation of the Group Dynamics Effectiveness Index (GDEI) the scores obtained for each of the above mentioned sub-dimensions were first made uniform and then multiplied by the corresponding weightage assigned to each as by expert judges. These scores were then added up to get the GDEI score of each respondent.

It was also ensured that all the sub-dimensions identified as components of GDE were of high significance on the basis of the coefficient of agreement in judges rating as well as the statistical evidence from the results of the pilot study. The measurement device developed for the dependent variable *i.e.*, GDE was ascertained for its content validity.

Measurement of Sub-dimensions

A. Participation: For the present study, participation was operationally defined as the degree to which the farmer is involved in group meetings, discussions and group activities of SHG.

B. Influence & Style of Influence: Influence was operationally defined as the degree to which a farmer can influence other member of SHG in a desirable way. Style of influence was operationalised as the manner in which the member attempts to influence other members of SHG. The four different styles included were autocratic style, peacemaker style, laissez-faire style and democratic style.

C. Decision Making Procedures: This is operationally defined as the degree to which farmer makes a decision with involvement of other group member of SHG, makes decisions without topic drifting, supports other members' decisions in consensus, feels the majority's decisions valid in the SHG, attempts to get all members participate in decisions of SHG and feels the gains of recognition for his contribution in decision making process.

D. Task Functions: This is operationalised as the degree to which the farmer makes suggestions to tackle a problem in the SHG, summarises what has been covered in the group, tries to give or ask for facts, ideas, opinions, feelings, feed back etc. and keeps the group on target.

E. Maintenance Functions: This is operationalised as the extent to which farmer helps others into group activities of SHG, helps/interrupts him in group discussions, feels the other members are co-operative and listening, perceives other members help in clarifying the ideas of all members, feels good or bad when ideas are accepted or rejected and the extent to which other members attempt to maintain task functions of SHG.

F. Group Atmosphere: This is operationalised as the extent to which the group member prefers friendly congenial atmosphere in the SHG, attempts to suppress conflict or unpleasant feelings in the group, feels other members are involved and interested and feels satisfied from the work climate.

G. Membership: This is operationally defined as the degree to which a group member feels accepted or included in the SHG, feels sub-grouping in the SHG and feels himself or other members to be outside the group.

H. Feelings: This is operationally defined as the degree to which the farmer feels anger/irritation, frustration, warmth, affection, excitement/boredom and competitiveness while performing the group activities of SHG.

I. Norms: This is operationalised as the extent to which the farmer feels the standards or ground rules and regulations are in operation that controls the behaviour of group members for the smooth functioning of the SHG.

J. Empathy: This is operationally defined as the degree to which the respondent is able to make out other person's feelings and thereby to understand it as he feels.

K. Interpersonal trust: This is operationally defined as the degree to which the respondent trusts the other members of the group as well as the faith other members has in him as perceived by the respondent.

L. Achievements of SHG: This is operationalised as the level of performance of SHG as perceived by the farmer as well as the performance of the farmer himself as the group member.

All these sub-dimensions were measured by a set of inventories containing appropriate questions arranged in a three-point continuum of always, sometimes and never with scoring pattern 2,1 and 0 for positive and vice versa for negative questions.

The cost estimates of all the selected Self help Groups were also computed and by taking in to consideration of major expenditure required for mussel farming is for the materials such as bamboo, nylon rope, coir, cloth, seed, etc. and labour costs essentially cover construction, seeding, harvesting etc. the Net Operating Profit and B: C ratio also were calculated for different SHG's to draw valid inferences.

Results and Discussion

The study, focused attention on Group Dynamics Effectiveness as a trait of Self Help Groups resulted by the joint influence of individual members of the group generated out of skills and orientations from the past life experiences. It definitely varies from person to person, place to place, time to time, situation to situation and in turn from group to group. This might be the probable reason for the differential degree of GDEI observed among respondents.

Profile of Cost Estimates of Mussel Farming

The major expenditure required for mussel farming is for the materials such as bamboo, nylon rope, coir, cloth, seed, etc. and labour costs essentially cover construction, seeding, harvesting etc. The women's' groups constituted in the scheme DWCRA started mussel farming as early as 1996-97 and are assisted by loan amount worth Rs 8800 / -per member with a subsidy amount worth Rs 4400 / - which looks quiet fascinating. The duration of the loan is 5 years and the rate of interest is 12.5 % per annum. In addition to this, a revolving fund of Rs 5000 /- was also provided without interest. When the SHGs are economically empowered with the provision of loan facilities, the returns from mussel farming help them to repay the loan slowly.

The loan was granted through Farmers' Service Cooperative Banks and North Malabar Gramin Banks in Cheruvathur and Padanna panchayaths of Kasargod district. Majority of the SHGs' showed considerable progress in repayment of the loans, which can be concluded as an indication of the profitability of Mussel farming. The expenditure details of the selected SHGs in the initial year of mussel cultivation are shown in the Table 1.

The Net Operating Profit in all the six SHG's was computed and found as substantially good which proves the profitability of Mussel farming in the initial trial itself and since during the subsequent years, material costs such as those of bamboo, rope, cloth and labour cost in construction etc. are negligible, this ensures reasonable profit as a major consequence of adoption of Mussel farming enterprise bringing about economic empowerment of rural women through organised Self Help Groups.

Table 1. Cost estimate of the SHG's in mussel farming in Kasargod district.

	SHG1	SHG 2	SHG 3	SHG 4	SHG 5	SHG 6
No.of ropes	500	800	600	750	900	725
Items						
Bamboo	6400	9600	7980	9000	11437	7800
Nylon rope	9954	17500	12000	15000	18000	14500
Coir rope	1100	1500	1200	1587	2000	1450
Cloth	3000	3250	1700	3338	3600	2250
Seed	6500	10000	8700	9000	10800	9770
Labour						
Construction	1600	2400	2170	2250	2700	2200
Seeding	1500	2565	1500	1875	2500	1800
Harvesting	1300	2000	1500	2000	2750	1875
Miscellaneous	1000	1600	1200	1500	1800	1450
Total Cost	32,354	50,415	37,950	45,550	55,587	43,095
Returns	40,000	64,000	48,000	60,000	72,000	58,000
Net Operating Profit	7,646	13,585	10,050	14,450	16,413	14,905
B : C Ratio	1.236	1.269	1.265	1.317	1.295	1.346
GDE Index	52.78	54.33	53.91	57.32	55.68	59.14

Experiences and observations already indicated that for a group to be developed as an SHG it requires a period of at least 36 months and it is a hectic process. It has to pass through various phases such as Formation phase, Stabilisation phase and Self Helping phase. These Self Help Groups promote a cooperative and participative culture among the members, which ensures the empowerment culture of the Self Helping phase.

The loan sanctioning, utilisation, accounts maintenance and timely repayment of loans etc. are all perfectly accomplished with proper maintenance of the documented records by the group members. This ascertains the fulfillment of norms and standards of the SHG leading to economic empowerment of the members.

Case Study 2

Self Help Groups (SHGs') of fisherfolk were mobilised in *Karwar* and *Bhatkal* locations of Karnataka coastal belts. Three SHG's of 15 members each comprising a total of 45 were mobilised in Majali (Open Sea) of Dhandebag and three SHG's of 15 members each comprising a total of 45 were mobilised in Sunkeri of Kali estuary in Karwar coastal belts in Uttar Kannada district of Karnataka state. Training and demonstration on mussel farming was undertaken in these SHGs.'. Initially, two training and demonstration programmes in these two sites in Karwar were undertaken, one for *raft culture* in open sea in *Majali* of *Dandebag* and one for *rack culture* in *Sunkeri* of *Kali* estuary. The training was imparted to 45 members of three Self Help Groups, each possessing 15 members in 2 sites separately comprising a total of 90 participants. At *Majali* in open sea, a 5 x

5 metre raft and at *Sunkeri* of Kali esturay, a 5 x 5 metre rack were constructed for mussel farming.

Similarly In *Mundalli* river of *Bhatkal* estuary in Karnataka, 4 Self Help Groups of 15 members each exclusively of women fisherfolk mobilised under the NGO, ' Snehakunja ' comprising a total of 60 participants were trained on mussel farming. They initiated a trial in 5 x 6 metre rack mussel culture by long line method.

The sample design for observation including the number of SHGs' trained, beneficiaries and method of culture is given in Table 2.

Table 2: Mussel culture interventions in Karnataka state

Site	No.Of SHG's Trained	No. of beneficiaries	Method of culture	Size of the rack / raft
Sunkeri of Kali estuary	3	45	Rack culture	5 x 5 m
Majali of Dhandebag	3	45	Raft culture	5 x 5 m
Bhatkal of Mundalli estuary	4	60	Raft culture	5 x 6 m

Data were gathered from these 10 Self Help Groups through personal interviews of the respondents. For the study, the Group Dynamics of members of Self Help Groups was again measured by developing an index called Group Dynamics Effectiveness Index (GDEI). The growth parameters were monitored every week in all the sites and the yield particulars of mussel during harvesting in each SHG was also noted.

Results and Discussion

The major expenditure required for mussel farming is for the materials such as bamboo, nylon rope, coir, cloth, seed, etc. and labour costs essentially for construction, seeding, harvesting etc. The SHGs' of *Majali* and *Sunkeri* were mobilized by the project team of CMFRI and the SHG's of *Bhatkal* were mobilized by a NGO namely *Snehakunja.* The first two trials and demonstrations were under the funding of CMFRI and for the last one, only the technical helps during the training and demonstration were offered by CMFRI. The Yield particulars in all the ten SHG's was noted and found as substantially good which proves the profitability of mussel farming in the subsequent trials because the material costs such as those of bamboo, rope, cloth and labour cost in construction etc. are negligible, this ensures reasonable profit as a major consequence of adoption of Mussel farming enterprise bringing about economic empowerment of rural women through organised Self Help Groups.

The yield in Kg per metre length of the rope recorded in all SHGs' as Average Yield showed a positive relationship with GDEI score. The correlation (r = 0.958139) was found significant owing to the 't' value 9.465624 at 1% level of significance. (Table 3.)

Experiences and observations already indicated that for a group to be developed as an SHG it requires a period of at least 36 months and it is a hectic

process. It has to pass through various phases such as Formation phase, Stabilisation phase and Self Helping phase. These Self Help Groups promote a cooperative and participative culture among the members, which ensures the empowerment culture of the Self Helping phase.

The utilization of fund sources, accounts maintenance etc. are all perfectly accomplished with proper maintenance of the documented records by the group members. This ascertains the fulfillment of norms and standards of the SHG leading to economic empowerment of the members.

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Table 3. Relationship of Yield and GDEI of SHGs'

SHG	Yield in Kg / m	GDEI score	Correlation Coefficient (r)	't' value
SHG 1	9.2	53.71	0.958139	9.4656248**
SHG 2	9.1	52.31		
SHG 3	8.9	51.91		
SHG 4	12.6	57.32		
SHG 5	12.7	56.68		
SHG 6	12.5	57.14		
SHG 7	13.6	60.01		
SHG 8	13.1	59.98		
SHG 9	13.8	61.29		
SHG 10	13.2	60.02		

Constraints Faced by the Fisherfolk in Mussel Farming

Mussel farming faces a number of impediments like water salinity, seed availability, selection of location / site, climatic vagaries, identification of proper beneficiaries and proper monitoring opportunities. The major problems and constraints faced by the fisherfolk in mussel cultivation are as follows

- Unpredictable seed availability.
- Mortality of seeds during transportation.
- Reduced growth during certain years.
- Meat shucking problems.
- Marketing of mussels.

- Social constraints like caste splits, conflicts, politics etc.to a limited extent.

The open sea mussel culture in this particular case met with the impediment of unfortunate sabotage of the seeded mussel by some miscreants. It was rectified by reseeding, but the yield was not that much conspicuous compared to the trials undertaken in estuaries. All the SHG members are of unanimous opinion that the government agencies should come forward with improved marketing facilities, as marketing of the mussel was perceived as one of the biggest constraints. Provision of loans with reduced interest rates and freezer facility for storage of harvested mussels can bring about a breakthrough in this sector in the near future.

Conclusions and Remarks

An attempt has been made to assess the socio economic impact of mussel farming by mobilizing Self Help Groups in Kerala and Karnataka coastal belts. Mussel farming is slowly achieving considerable significance because of its profitability. But it is inevitable to take care of the selection of suitable sites fulfilling the essential parameters for undertaking mussel culture trials. It would be pertinent to have study on the effect of coir retting zones on growth and attachment of mussel seeds to the strings, which often found to be not suitable by experiences and observations. Laboratory experiments should be widened to study the effect of coir retting zones on growth of mussel.

Similarly, export potential of mussel can be promoted through value addition experiments on depuration plants in filtered seawater. Organised fishermen's cooperatives can play a vital in various stages of seeding, harvesting, sorting, grading, packing, and marketing with an intention of export potential.

The study emphatically disclosed the deep rooted influence of Group Dynamics network among the farmer folk as influenced by their participation, influence & styles of influence, decision making procedures, task function, maintenance function, group atmosphere, membership, feelings, norms, empathy, interpersonal trust and achievements of SHG.

Irrespective of the location specific problem oriented resource based alternative programmes for income generation, this study emphasises on the economic empowerment of rural women through mussel farming as a means of poverty eradication through Self Help Groups because, poverty can only be alleviated by mobilising the poor to solve their actual problems in the form of organised SHGs'. In the impact assessment, the correlation analysis revealed, a proportional relationship between the Group Dynamics Effectiveness and Average Yield obtained for each SHG, which ensures reasonable profit as a major consequence of adoption of Mussel farming enterprise bringing about economic empowerment of fisherfolk through organised Self Help Groups.

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Seed Production of Whelk

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Babylonia sp., commonly known as 'Whelk,' 'Spiral Babylon' and 'Puramuttai chank' (Dove egg shell) in local parlance and 'Baigai' in the trade sector, is a marine edible gastropod. It is widely distributed in the Indo Pacific region. In India, it is well represented in the Indian Peninsula at places such as Gulf of Mannar, Poompuhar, Nagapattinam, Madras and the waters around Andaman and Nicobar islands (Ayyakannu, 1994). *Babylonia* is a much sought after marine gastropod and it fetch a good foreign exchange. This edible gastropod is an important food species in the Indo-pacific region (Ayyakannu, 1994). The total quantity of whelk trade during 1993-94 was 300 tonnes and it increased to 500-600 tonnes during 1995-96.

Global trade in the whelk meat and by-products, although presently meager, has considerable potential. Whelk or "Baigai" is exported frozen shell-on to Australia, France, Hong Kong, Japan, Singapore and Thailand. Export of shell-on frozen Baigai from India was 703 t in 2001 and fetched revenue of Rs 386 lakhs. Japan is the leading importer of frozen Baigai from India (689 t in 2001) followed by Hong Kong (11.3t in 2001). Live whelk is exported to Hong Kong and Thailand (Statistics of Marine Products Exporters 2001).

Economic Importance of *Babylonia* spp

Baigai (*Babylonia)* have received considerable attention due to their economic importance and the increased demand for meat of these snails in the western countries. The boiled meat of the snail was Rs 40/kg (Ayyakannu, 1994), though it is not available in the market nowadays. Presently it is exported mainly to Japan. *Babylonia zeylanica* is sold at Rs. 30/kg shell on where as *Babylonia spirata* fetches Rs. 10/kg in the local. The shell has ornamental value and the operculum has medicinal importance and therefore no part of the whelk is wasted.

The shells of *Babylonia* are used for interior decorations after cleaning, processing and polishing. A well polished whelk shell fetches Rs 3 / shell. Beautiful items such as curtains, pen stands, mementos, key chains and other novelties are made out of small shells. There is a good market for them not only in India but also in western countries. Handicraft items made from *Babylonia* shells are widely sold in almost all cities and tourists centres in India.

The operculum popularly known as 'fish nail' is an important by- product for export and is valued at Rs.400 / kg. 100 kg gastropod shells yield 1 kg of operculum. The total export of operculum for 1992-93 is 2 t worth Rs. 4.14 lakhs (Statistics of Marine Products Export, 2001).

Two species of Babylonia occur in the Indian waters; *Babylonia spirata* and *Babylonia zeylanica*. These together form nearly 56% of the shrimp by catch. From the by-catch, *Babylonia spirata* and *Babylonia zeylanica* are segregated and separately auctioned due to their market preferences and considered as an emerging resource. In view of its high economic value and the increasing fishing pressure on the present stock, efforts were initiated for the seed production and farming of the whelks. The breeding, spawning and larval development of *Babylonia spirata* has been successfully carried out in the Central Marine Fisheries research Institute and is detailed below.

Breeding, Spawnimg and Larval Rearing of *Babylonia spirata*

i) Collection and Transportation of the Brood Stock

The brood stock of *Babylonia spirata* and *B. zeylanica* were collected from landing centers of Neendakara and Sakthikulangara and transported to molluscan hatchery at CMFRI, Cochin for breeding and spawning studies. The best method to minimize the transportation stress and ensure complete survival the whelks were kept in cotton moistened with seawater or gunny sacs presoaked in seawater.

ii) Management of Brood Stock in the Hatchery for Spawning

Live and healthy specimens were transferred to 1 t FRP tanks with pure filtered seawater and provided with aeration. 70% of the fresh seawater was exchanged daily. The snails were allowed to acclimatize in the hatchery for two days after transportation. During this period no food was given to them. After acclimatization, they were fed with clam, squid, prawn, annelids, etc. From the observations, it was found that within five minutes they were able to locate the food and extended the proboscis to take the food. After two days of acclimatization, they were transferred to the tanks with sandy substratum for providing them a natural environment. The FRP tanks were provided with sand as the substrate bottom. The tanks were fitted with two biological filters to maintain water quality. The environmental parameters like salinity, temperature and pH were regularly monitored and kept with in the range of 32-35ppt, 26-29°C and 8-8.3 respectively. *Babylonia spirata* had a distinct preference for clay (51.9%) as their substrate. The order of preference for other substrate was coarse silt (24.7%), coarse sand (17.3%), and gravel (6.1%).

iii) Spawning

The acclimatized brooders took average 15 days to spawn in the hatchery though some took nearly two months to show the spawning activities. The average size of the spawners was 36mm. Spawning occurred during night and continued up to the early morning hours. An erect position of spawners by pressing its foot in the substratum indicated spawning and any slight disturbance halted the spawning activity. The average number of capsules per spawner was 35-40 with 350-800 eggs per capsule.

Egg capsules: The eggs were laid in transparent vasiform capsules. Due to the transparent nature of the egg capsules, the eggs were visible and could be counted

externally. The apical portion of the egg capsule was concave in appearance and the membrane in this region was thinner than the walls. The stalk of the egg capsule was firmly attached to the substratum to hold it in an erect position till the larvae hatch out. The average total length of the egg capsule was 27.8 ± 2.5mm and the capsular length excluding the stalk showed variation. The average width of the capsule at the apical region was 8.4 ± 1.5 mm. The average diameter of the fertilized egg was 275µm, irrespective of the size of the capsule and number of eggs in the egg capsule. There was positive linear correlation (r= 0.8764) between the average length of the egg capsule and average number of eggs in the capsule. The average size of the fertilized eggs in the egg capsule was 260-280 µm.

Hatching, Larval Rearing and Larval Development

The capsules attached to the substratum with the help of the hold fast were transferred from the spawning tanks to the hatching tanks of 50 lit capacities with fresh filtered seawater and provided with gentle aeration. The salinity was maintained at 32 ppt, pH 8±0.2 and temperature 28±2°C.

i) **Fertilized Eggs to Planktonic Larvae:**

First polar body was released within 60 minutes after the release of fertilized egg capsule. The release of second polar body commenced at 90th minute. The first cleavage occurred 30 minutes after the release of the second polar body, which was followed by the second cleavage after one hour. The divisions were clearly visible up to 16-cell stage. Subsequently, it becomes an opaque mass due to large quantity of yolk in the egg. After 24 hours of spawning, the divisions completed and the embryo transformed into the morula stage with marginal cells at the anterior region. Further development resulted in the rotation of the morula and this stage lasted for about 48 hours.

On the 3rd day, the cilia were visible at the top and transformed to trochophore larva. On 4th day the larval size increased to 380 µm. Subsequently the larval size increased to 420 µm on 5th day and developed velum boarded by two rows of fast beating cilia along its margin. On 6th day, the velar lobes become enlarged and a thin, transparent larval shell was clearly visible. From this day onwards veliger larvae were fully developed and concentrated at the tip of the egg capsule. Though the exact mechanism of the releasing of the larvae is not known, the apical part splits and releases the larvae from the egg capsule. The average hatching percentage of larvae from each capsule was 90 and all of them were released by 7th and 8th day after spawning. The larvae are plank tonic, swim towards the surface of the water and exhibit photo tactism. The larvae are transparent; possess bi-lobed velum fringed with cilia. The larval shell is fully developed. Eyespot is also developed.

ii) **Larval Rearing**

On the 7th day, the larvae were transferred from the hatching tanks to the rearing tanks (Perspex/glass tanks) by filtering through a sieve of 400µm and stocked seawater in the rearing tank at a density of 150 larvae/liter. The rearing

conditions were salinity 32±1ppt, pH 8±2 and temp 28±2°C. Prior to stocking, the water for rearing was treated with hypochlorite and potassium permanganate solution to eliminate the unwanted microorganisms. Different algal feeding regimes were tried. Poor growth and heavy larval mortality occurred when fed with *Tetraselmis* sp. and *Nannochloropsis* sp. Pure cultures of *Isochrysis* and *Chaetoceros* were provided to the larvae up to the 17th day and larvae settled as juvenile. The larvae were fed at the rate of 7000 cells/ml/hr. Almost 95% of the larvae hatched out from the egg capsule and the survival rate was 60% from the hatching to settlement.

***iii)* Metamorphosis and Settlement**

The larvae feed and swim actively with the fast movement of cilia along the rim of the velum. Eyespot becomes clearer. This stage lasts up to the 13th day. The larval shell is fully developed and the foot protrudes out Operculum is seen as a scar; pair of tentacles develop at the base of the tentacles. Velum is 4-lobed, as a folding along the horizontal position. Active feeding of phytoplankton continues up to the 17th day. The plank tonic lifestyle begins to change and the larvae begin creeping and crawling along the bottom, actively searching for food and become carnivorous in nature, feeding on shrimp, clam squilla meat etc. Settlement begins when they attain the average size of 895 µm. At this stage the velum is shed, radula and digestive tract is developed and the juveniles secrete mucus along their path.

iv) Rearing of Juveniles

Metamorphosis of the larvae completed 17-19 days after the release of the capsule. The settled juveniles were transferred to 5 liter beakers provided with filtered seawater and gentle aeration. After settlement, the feeding habit changes and they become carnivorous and the juveniles begin to creep and crawl along the bottom. Algae settled on glass slides, shrimp feed, agar based feed (composition agar 0.25gms, shrimp 1.5 gms, soyabean 0.25 gms and boiled egg albumin 0.25gms,in 100ml sea water) egg yolk; egg albumin, tubifex and rotifer were tried as food for the juveniles. However, only shrimp feed found as better for the growth and survival of the young ones. The survival rate after settlement was 70%. During the settlement stage, they attained 800-1000µ shell lengths. The juveniles had well developed radula and digestive tract suitable for carnivorous life and fully developed shell and operculum for protection.

The growth of Juveniles was recorded from day1 to 18 months of growth. The average total length on the 1st day was 1.5 mm. On the 15th day, the average total growth was 2.218 mm. After 1 month, an average total length of 2.3 mm was attained. After 45 days of growth, the average total length was 2.82 mm. At 2 months 3.84 mm of average total length was recorded. And after 75 days, 4.06 mm was attained.

After 6 months, the average total length was 14.41mm, average width was 9.87mm and average weight gain was 0.92g. After 10 months the average total length was 23.33 mm average width 15.06 mm and average weight gain was 3.2 g. After 14 months, the average total length was 28.7mm average 29.15 mm average

width 20.07 mm and average weight gain was 8.8 g. After 18 months, the average total length was 30.98 mm average width 21.29 mm and average weight gain was 9.99 g.

Preference of Microalgal Feed by Larvae of *B. spirata*

Although the larvae showed the general acceptance of Isochrysis galbana, Chaetoceros calcitrans and Tetraselmis gracilis as feed, Chaetoceros calcitrans proved to the most preferred micro algal feed by the larvae followed by Isochrysis galbana. Tetraselmis gracilis found poor acceptance due to the fact that algae was not available to the larvae, since it remained at the surface of the water column. Nannochloropsis salina did not find acceptance at all, since there was no settlement and complete mortality occurred on the 2nd day it self.

Larval Stocking Density

Larval survival, settlement and growth at different stocking densities was studied. Babylonia spirata larvae were maintained in 4 lit containers in pure, filtered sea water 0f 32 ppt, at 8 different stocking densities 75/lit, 100/lit, 125 /lit, 150/lit, 200/lit, 225/lit, 300/lit, 325/lit. They were provided with Chaetoceros calcitrans at the rate of 10,000 cells/ml/hr and gentle aeration. The growth and percentage of settlement were recorded till 17 days when complete settlement was observed.

The optimum stocking density was found to be 150 nos/lit, giving very high settlement rate and good growth compared to other stocking densities. Lower stocking densities viz, 75/lit, 100/lit and 125/lit showed of better growth compared to that of 150 /lit, however the settlement was very poor in these stocking densities 49.5%, 44%and 52% respectively. Higher stocking densities 250 nos/lit, 225 nos/lit 300 nos /lit and 325 nos/lit resulted poor growth and very low settlement.

Elimination of Vorticella Infestation on the Larvae

Vorticella infestation occurred in the veliger and juvenile stages of growth and lead to extensive mortality. Vorticella was found on the velar lobes and shell of the larvae cover the opercular opening of the shell, causing mortality. Experiments were conducted to eliminate the infestations on the larvae. It was found that 20ppm formalin was effective in eliminating nearly 85% of Vorticella.

Effect of Salinity on Hatching of Larvae

The egg capsules of Babylonia spirata were maintained in different 20, 30, 35 and 40‰ salinities in 2 lit pure filtered seawater in 3 lit capacity plastic containers and provided with gentle aeration. Complete water exchange was done on alternate days. Three egg capsules were introduced into each container of average size 26 ± 2mm total length; 16 ± 2mm capsule length and 10 ± 2 mm capsule width.

From the experiment it was observed that no hatching occurred at lower salinities of 20 and 25 ppt. 50-70% hatching occurred on the 7th day in the other

salinity ranges, 30, 35 and 40 ppt and 25-30% hatching occurred on the 8th day. Thus the ideal salinity range for the hatching of the eggs is 30-40 ppt.

The larvae were reared in different salinities ranging from 5 to 50‰ to study the effect of salinity on settlement % and growth of juvenile on the settlement stage. The larvae were stocked at the density of 150 larvae/ lit in different salinities in duplicates and growth were recorded till the day of settlement on 17th day. In the 5, 10, 15 20 and 50‰ salinities, the larvae did not survive and there was total mortality. The settlement percentage was very low at 40‰ salinity (14%) and no settlement was observed in 45‰-till17th day. Good growth and maximum percentage of settlement obtained at 30‰ salinity (56%). So the ideal salinity required for the larval growth was confirmed as 30‰ with a pH ranging between 8.1-8.3 and the temperature 26-28^0 C.

Effect of Water Change

Maximum settlement of 57.3% and growth was recorded when complete water exchange was carried out on alternate days of the experiments. Daily change of 25% of water also provided good settlement (50.5%). Fifty percentage of water exchange on alternate days resulted in poor settlement (35.5%) while no exchange of water through out the experimental period did not facilitate settlement and growth. Thus for achieving maximum settlement of B.spirata larvae, complete water exchange on alternate days is ideal.

Feed Preference of *Babylonia spirata* Juvenile in the Hatchery

The survival of juveniles highest among those fed with shrimp feed (92.5%), followed by those fed with squilla (85%). The survival when fed with squid was 65% and those fed on clam was only 50%. Egg custard was found to be unsuitable as feed for juvenile as the total mortality observed after 10 days. Growth was highest among those fed with shrimp feed. However squid was more acceptable than squilla in terms of growth although there was better survival when fed with squilla.

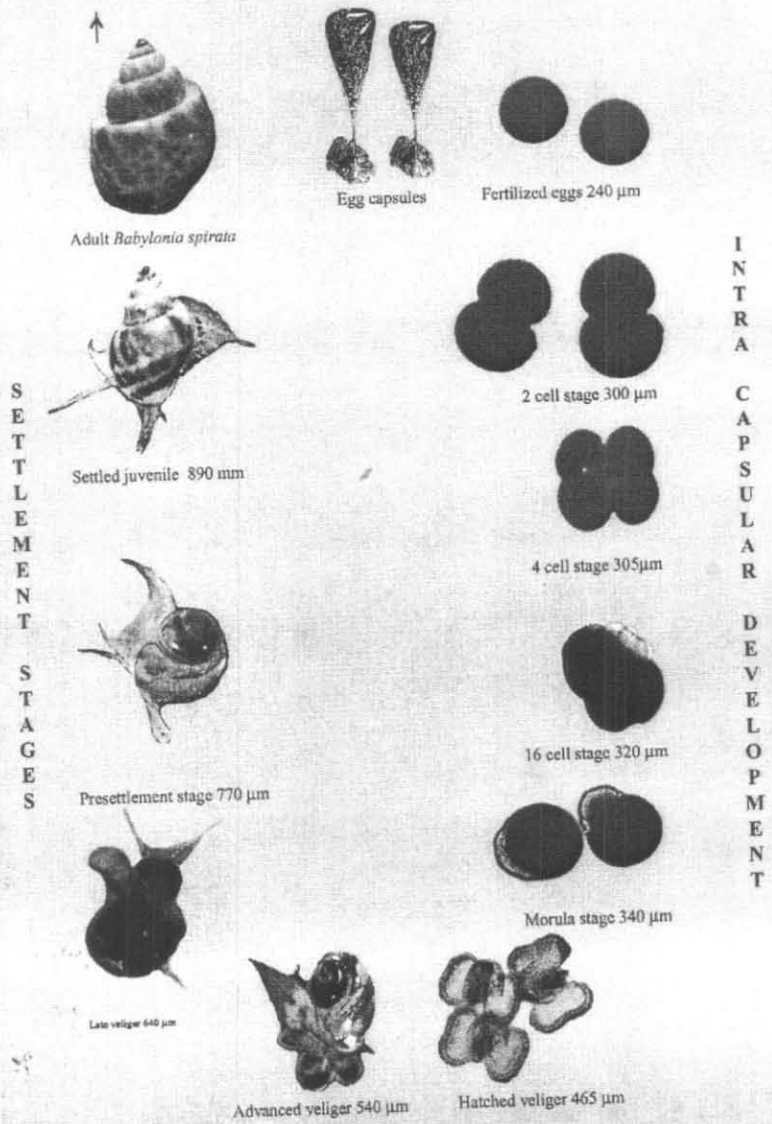
The life cycle of Babylonia spirata is appended.

The up gradation of the present larval rearing and hatchery technique will help to develop whelk farming on commercial basis in India, which will ultimately help in increasing production besides reducing the fishing pressure in the natural whelk stocks.

Juvenile *B. spirata*

Spawning of *Babylonia spirata* in the hatchery

Figure 34: Life cycle of *Babylonia spirata*



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Phytoplankton Culture

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Introduction

The floating microscopic plant components of the seawater are the phytoplankton or the micro algae, which forms the basic food of almost all the larval organisms either crustaceans, molluscs or fishes. They are the primary producers of the sea, constituting various classes of Algae. The important components of micro algae are the Diatoms, Dinoflagellates, Silicoflagellates (Phytoflagellates), Coccolithophores, blue-green algae and the 'hidden flora', the nannoplankters. Among these, the diatoms and phytoflagellates are significant organisms since they forms the primary link in the food chain of the sea. It is known that the hatchery operations depend mainly on the availability of the basic food, the phytoplankton.

Mass Culture of phytoplankton has been in prevalence in many research Institutions, universities and hatcheries the world war, since the past 50 years. As is well known, the success of any hatchery system, prawns, oysters, fishes or sea cucumbers, entirely depend or the availability of the suitable live feed, the phytoplankton. In the natural environment, the larvae feed on any minute plant components, which are readily available to them. But in a hatchery, the organisms which are acceptable to the larvae for their growth and further development have to be identified and isolated. In the early critical stages of the rearing larvae of finfishes and shellfishes, the phytoflagellates (species of *Isochrysis*, *Dicrateria*, *Chromulina* and *Tetraselmis*) and other nannoplankters (species of *Chlorella, Nannochloropsis* etc) forms the basic food. But in the post-larval stages of crustaceans and post Juvenile stages of molluscs, the diatoms (species of *Chaetoceros*, *Skeletolema* and *Thalassiosiara*) forms the primary food. Hence the culture of phytoplankton is an essential pre-requisite for the rearing operations of economically important cultivable organisms in a hatchery system.

Methodology – Isolation

Isolation of the required species of phytoplankton can be done by the following methods.

1. Pipette Method: Larger organisms can be pipetted out using a micro- pipette under microscope and transferred to culture tubes, having suitable culture media.

2. Centrifuge or Washing Method: By repeated centrifuging of the samples in different revolutions and by inoculating the deposits, we may get different

organisms. Transferring the deposits in various culture media, different organisms can be isolated.

3. By exploiting the Phototactic Movements: By this method, most of the phytoflagellates can be isolated. Make a dark chamber with a small hole on one side and keep the sample in a beaker nearer to the hole. Place a candle near to the hole outside. Since the phytoflagellates have a tendency to move towards the light, it is visible after some time that these organisms crowded near to the candlelight. By pipetting we can separate these organisms, and by tube culture method, can be raised to a pure culture.

4. By Agar Plating Method: For preparing the agar medium, 1.5gm of agar is added to one litre of suitable culture medium or even natural seawater. This agar solution is sterilized in an autoclave for 15 minutes under 120 lbs pressure and 100°C temperature. Now this medium is poured in sterilized 15cm petridishes and keep for 24 hours. For the isolation, the required species can be picked up by pipette or needle or loop under microscope and streaked on the surface of agar plate. After inoculation, these petridishs are placed in an incubation chamber for 7-8 days providing light (1000 lux) and constant temperature (25°C). Within this time, the required species, if it has grown into a colony removed by platinum loop under microscope and transferred to culture tubes. Further, from the culture tube to small conical flasks and larger containers, the algae can be grown and keep as stock culture and later for mass culture.

5. Serial dilution culture method: This method is used mainly for the isolation of nannoplankters and phytoflagellates (Sournia, 1971). In this method mainly 5 dilution steps (the inocula corresponding to 1, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} or 4 steps 0.001, 0.01,0.1 and 1 ml) are involved for the isolation of the required species. For the serial dilution technique, nearly 25 culture tubes (15ml 'Borosil') are required. After filtering the seawater through 10 – 40 μ seive, the filterate has to be inoculated to 5 series of culture tubes in various concentrations. This has to be kept under sufficient light (1000 lux) and uniform temperature (25^0C) conditions. After 8 to 10 days some discoloration can be seen in the culture tubes, due to the growth of micro algae. Further purification of this culture can be done by sub culturing it in 500ml and 1 litre conical flasks. Once the culture is fully purified, it can be transferred to 3 or 4 litre Hafkin culture flasks and maintained as stock culture.

Culture Media

For the successful culturing of the micro algae, either diatoms or flagellates, various chemical culture media have been used depending on the type of organisms cultured and their growth phases. Though Schreiber's and Miquel's media (Miquel, 1892) were found to be very effective for culturing the diatoms and nannoplankters, several other media also came into existence with the addition of trace metals, vitamins and other organic and inorganic salts. Usually for culturing the flagellates, Conway or Walne's medium (Walne, 1974) is used in the laboratory for the maintenance of the stock culture as well as mass culture. The important culture media used for the phyto plankton culture are:

1. Schreiber's medium

Potassium nitrate	..... 0.1gm
Sodium ortho phosphate	.....0.02gm
Soil extract	.....50 ml
Filtered and sterilized sea water	.....1 litre

Soil extract is prepared by boiling 1kg of garden soil in 1 litre of fresh water for one hour. After 24 hours, decant the clear water and keep in a bottle. This is the soil extract. 50ml can be added to each litre of sterilized seawater.

2. Miquel's medium

A.	Potassium Nitrate	.....20.2 gm
	Distilled Water	.....100 ml
B.	Sodium Ortho phosphate	.....4 gm.
	Calcium Chloride	.....2gm
	Ferric Chloride	..... 2 gm
	Hydrochloric acid	..... 2ml
	Distilled Water	.....100ml

Add 0.55ml of A and 0.50ml of B to one litre of filtered and sterilized seawater.

3. Conway or Walne's medium

A.	Potassium nitrate	.....100gms.
	Sodium Orthophosphate	.....20gm
	EDTA (Na)	.....45 gm
	Boric Acid	.....33.4gm
	Ferric chloride	.....1.3gm
	Manganese chloride	.....0.36gm
	Distilled Water	.....1 litre
B.	Zinc chloride	.....4.2gm
	Cobalt chloride	.....4.0gm
	Copper Sulphate	.....4.0gm
	Ammonium Molybdate	.....1.8gm
	Distilled water	.....1 litre
C.	Thiamine(B_1)	.....200 mg in 100 ml DW
	Cyanocobalamine (B_{12})	.....25 mg in 100ml

Prepare A, B and C (each) in different reagent bottles. Add 1ml of A 0.5ml of B and 0.1ml of C to 1 litre of filtered and sterilized seawater.
For the preparation of mixture of various phytoplankton in the open tanks, using direct sun light, the following medium can be used:

4. Mixture Culture Medium

Potassium Nitrate	.....1.2 gm
Sodium Orthophosphate	.....0.66 gm
EDTA (Na)	.....0.66 gm
Sodium Nitrate	..... 0.66 gm

Besides the above mentioned laboratory prepared chemicals which serve as nutrients, commercial feelings can be used for the mass culture of diatoms and nanoplankters, in open tanks for economy purposes. The media used for the open culture are:

5. Fertilizing Medium

Urea 46	..... 10 mg/l
16.20.0 (NPK)	.....10 mg/l
20.0.0	.....100 mg/l

Growth Phases of the Algal Culture

The usual way of the laboratory culture of phytoplankton is one in which a limited volume of medium containing the necessary inorganic and organic nutrients is inoculated with a relatively small number of cells and these exposed to suitable conditions of light, temperature and aeration. Increase in cell numbers in such a culture follows a characteristic pattern in which the following phases of growth may usually be recognized:

1. Lag or induction Phase: The cells taken from the stock culture room are inoculated to a new flask have to acclimatise the surroundings or in the new medium. Hence there will be no cell division for a few hours and this stage is known as lag phase.

2. Exponential Phase: Once the cells are acclimatized to the surroundings it starts multiplication and grows rapidly. This growing phase is known as exponential phase.

3. Declining Phase: Once the cells reached the maximum concentration, the growth and multiplication will be arrested and slowly show the symptom of declining. This arrested growth of the cells in the culture is known as declining phase.

4. Stationary Phase: After the arrested growth, the culture will be stationary without any further cell division for a few days. In the stationary phase, if the cells get a new environment, they may start further growth and reproduction.

5. Death Phase: After a long period in the stationary phase, the cells may lose its viability and started to die and thus the culture will become useless, either for reculturing or for feeding.

Harvest of the Culture

The fully grown culture should be harvested during the exponential phase of the phytoplankton after determining the cell concentration. If the culture has entered the declining or stationary phase, the metabolite will be very high and the cells may not be in healthy condition. The rearing larval organisms may not show the required growth if fed with this feed.

Preservation of the Culture

The maintenance of the culture and constant supply of the same whenever required is a problem in the hatchery especially during adverse weather conditions. In this case preservation of the algae either by freezing or by sun drying could be done in the sense that during scarcity of the feed, the rearing operations may be successfully controlled. For the method of freezing, the culture has to be flocculated either by adding lime or by adjustment of pH using Sodium Hydroxide. After knowing the quantity of the culture to be flocculated, measure the volume of Sodium Hydroxide solution needed to flocculate to get one degree raise in pH. Suppose the pH of the culture is 8.4, rise to 9.4 by adding sufficient quantity of Sodium Hydroxide solution. After vigorous stirring, leave the culture for one hour to settle the algal mass at the bottom of the tank. Decant slowly the clear water and collect the mass in a plastic bucket. Then bring the pH of the mass to the original level of pH by slowly adding dilute HCl. Now the algae are ready for freezing or sun drying. Drying of the algae can be done by pouring the mass in white enamel trays and keep it in glass bottles. Before freezing the algal mass, some protective reagents like Dimethyl Sulphoxide or Glycerol (few drops) can be added. Then pour the concentrate into polythene bags after measuring. Label the polythene bags and keep the same in deep-freezer. The frozen algae may not have the same protein content as in the live condition. Whenever adverse conditions arise the frozen algae can be used for rearing the larval organisms.

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26 Health Management in Bivalve Larval Rearing Systems

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Suitable hatchery techniques for producing pearl oyster *Pinctada fucata* spat were perfected in India dring 1981 at C.M.F.R.I (Alagarswami *et al.*, 1987). In general, the larvae grow through the straight – hinge, umbo, eyespot and pediveliger stages in the pelagic phase, before metamorphosing into plantigrade and setting on a suitable substratum as spat. Large scale differences in survival and larval growth within and between different rearing conditions were noticed and reflected in several research reports. This could be attributed to health conditions of the bivalve young ones.

The available space, feed and microbial pathogens are critical factors, which determine the survival of bivalve larvae, as their defense mechanisms are age - related. For example, the adult oysters can tolerate exposure to much higher populations of bacteria. *Crassostrea virginia* exposed to high densities of *Aeromoas* and *Vibrio* may be unaffected while the lower concentrations in larvae produce disease and mortality. Though bacteria and protozoa infect the weak and dying larvae, systematic investigations on diseases are scanty. The environmental and other factors responsible for the lower survival rate of larvae have to be monitored so as to devise suitable disease management strategies. Observations also indicated that twenty to thirty percentage of spat production compared to the initial stock at veliger stage could be only obtained in mass production in one-ton capacity tank.

Ex. 1: Vibriosis or bacillary necrosis of bivalve's larvae in culture environments.
Seasonal changes in the abundance of vibrios and other related genera.
The exotoxin produced by Vibrio, kills the developing oyster larvae.
Bacterial swarming around mantle margins of larvae is a reliable indicator of epizootics. Mortality may reach up to 100%.
Out break of vibriosis in oyster larvae, with associated mortalities were correlated with peaks in abundance of Vibrio in inflow water and in culture water.

Ex. 2: *Pseudomonas* has also been implicated in disease outbreaks.
Larvae of *Ostrea edulis*, *Crassossstrea virginica* and *C. gigas* were susceptible. Juveniles were less susceptible.
Effects on larvae vary with the particular strain of isolate of bacteria.
Ex: abnormal embryonic development, leading to incomplete shell formation or velum protrusion, decreased growth or death of larvae in veliger stage.

Three types of pathogenesis could such as:
1. Progressive mantle disruption,
2. Severe velar deformation and damage, and
4. Progressive visceral lesions and atrophy.

Physiological stress during spawning also predisposes them to bacterial infections.

Management by Manipulating the Available Space/stocking Density

The stocking density in the rearing system played a key role in larval development (Krishnan and Alagarswami, 1993). Oyster larvae are generally reared in static water in dense numbers and fed with the required density of unicellular algae (Alagarswami *et al.*, 1987). These conditions are also favorable for the proliferation of heterotrophic bacteria such as *Acinetobacter*, *Aeromonas*, *Pseudomonas* and *Vibrio* (Colwell and Sparks, 1967). All these bacteria are reported as opportunistic pathogens, which induce epizootics in hatcheries. According to Skjermo (1999), the combination of high larval densities, debris from dead larvae and high load of organic matter and bacteria due to addition of live food stimulates selection and growth of such opportunistic bacteria in larval tanks.

In an investigation into the cause of high mortality of the pearl oyster, *P. maxima* in the northwest of Western Australia revealed that the majority of diseased oysters were infected with marine *Vibrio* bacteria. Among them the common isolate *Vibrio harveyi*, induced the disease similar to that seen in the field (Pass *et al.*, 1987). Apart from causing mortalities, bacteria have long been reported to be associated with decreased growth of bivalve larvae. The findings of Lipton *et al.*, (2003) indicated that hatchery production of *Pinctada fucata* was seriously affected by massive larval mortalities caused by *Vibrio* sp.

After a series of research investigations, it is noted that the higher survival of 41.02 % of pearl oyster *Pinctada fucata* larvae could be achieved in low stocking density of 200/L compared to 10.26 and 0.82 % in the increased density of 1000 and 5000 larvae/L respectively at an ambient mean temperature of 28.6 °C. Although the larvae were fed with *Isochrysis galbana* at the recommended cell densities and the rearing pH, salinity, dissolved oxygen contents were similar in the three rearing densities the microbial load was high with 5.8 $x10^3$ cfu/ml in the high stocking density. In the low-density culture system, the microbial load fluctuated between 6.0 x 10^1 and 4.0 x 10^2 cfu/ml. The total number of spat produced in 200 and 1000 larvae/L stocking density was more or less similar. Considering the management methods and cost, the lower stocking density is advantageous as it reduces microbial load and possible water exchange thereby augmenting higher survival as well as spat settlement. It is also probable that the lower growth and lower survival in high stocking densities could be attributed to frequent collisions among larvae and increased metabolites. Thus the lower stocking of larvae reduces the mechanical stress.

Antibiotic Exposure to Minimise Microbial Load in Live Feed

High microbial load noted in the rearing water, tissue samples and in the micro algal feed, resulted in poor spat production of less than 3.0% in *Pinctada fucata* hatchery. In general, the pathogenic microbes invade the hatcheries, by three principal routes viz., the seawater, brood stock and algal food. Prophylactic antibiotic usage has been suggested to reduce bacterial load in live feed. However, the exposure time and the minimum dose of antibiotic agent required to reduce the proliferation of bacteria in the mass culture of micro algae has to be evaluated.

Example:

Experiments were conducted in the Marine Biotechnology Laboratory of Central Marine Fisheries Research Institute, Vizhinjam (South India). *Isochrysis galbana* was inoculated in one litre flask and maintained under constant illumination for growth. Log phase culture (100 ml) was aseptically dispensed in four 250 ml conical flasks. Chloramphenicol (Hi Media) was added at 10, 100 and 1000 mg/L to each 250 ml conical flask respectively and one flask was kept as control along with replicates.

The bacterial load in the *I. galbana* culture was determined by the plate count method. The algal samples were collected aseptically at four different time intervals viz., immediately after the application of antibiotic, after fixed hours. Each sample was serially diluted, plated in nutrient agar and incubated at 37°C for 24 h. The viability of *I. galbana* was examined using hemocytometer at the respective time intervals.

The use of chloramphenicol in *I. galbana* culture resulted in decreased bacterial load with the increase in time (Fig. 1). After three hours of exposure, 88.7, 90.6 and 94.3 % reduction was noted at 10, 100 and 1000 mg/L respectively. The algal cells in the three experimental groups were active. After six hours of exposure, the reduction in bacterial load was 75, 42 and 93 % at 10, 100 and 1000 mg/L respectively. The algal cells at 10 and 100 mg/L were actively moving while at 1000 mg/L 25 % of algae were inactive or dead, indicating the adverse effect of antibiotic. In the control group, bacterial population increased with the advancing culture period. The load almost doubled at the end of 12 h as could be seen from Fig. 1.

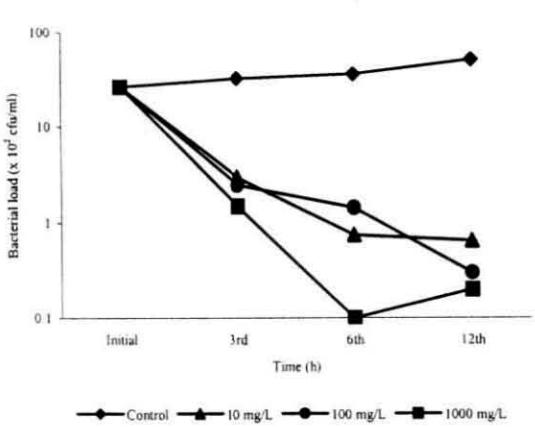

Fig. 1 Effect of antibiotic on the reduction of bacterial load in *Isochrysis galbana* at different time interval

Management by Incorporating Probiotic Microbes

Experiments indicated that hatchery reared *Crassostrea gigas* larvae are susceptible to attack by three strains of sucrose fermenting *Vibrio* initially isolated from diseased larvae. Administration of 10^5 cells/ml of any of these strains led to the collapse of culture within 48 hours. Larval oysters (*Crassostria virginica*) could be experimentally infected with isolates of pure cultures of marine Vibrio species. All the inoculated groups demonstrated decreased growth and/or high mortality.

These research findings indicated that the artificial production of bivalve seed has been seriously affected by the occurrence of massive larval mortalities of which one probable cause has been infection by Vibrio species. One of the usual methods of controlling proliferation of pathogens in hatcheries has been by using antibiotics. Though these antibiotics have been used as feed additives, the associated toxicity, allergy, residues in food and resistance obtained after long-term administration of low doses makes their use worthy of second thought. Also the indiscriminate use of broad – spectrum antibiotics may alter the normal gut flora by suppressing its growth and cause an over growth of pathogenic bacteria. Hence, the use of probiotics such as food additives is preferred over the use of antibiotics.

Jory (1998) defined probiotics from aquaculture point of view as culture (single or mixed) of selected strains of bacteria that are used in culture and production systems (tanks, ponds and others) to modify or manipulate the microbial communities in water and sediment, reduce or eliminate selected pathogenic species of microorganisms, and generally improve growth and survival of the targeted species.

The mechanism of action of probiotic, which include depletion of nutrients, production of acids and antimicrobial substances as well as competition for adhesion receptors in the intestine and immunostimulation create an environment incompatible to the growth of pathogens. Apart from this, very little work has been carried out on the effects of potential probiotic strains on bivalves. The research work by (Riquelme *et al.,* 1997) revealed that among a total of 506 bacterial isolates, obtained from laboratory and hatchery sources, one strain (*Vibrio* species), when used as a pre-treatment, protected the scallop larvae against subsequent experimental infections with the *Vibrio anguillarum* – related (VAR) larval pathogens. Preliminary investigations in CMFRI indicated that the probiotic bacteria *Lactobacillus* offered good health and survival rate to the pearl oyster larvae even in adverse/unfavorable conditions

Therefore further research is needed towards standardization of this beneficial practice of addition of probiotics. The first question unanswered in many cases, is the fate of the probiotics in the rearing medium and in gastrointestinal tract. In this context, immunological and molecular probes will be useful tools to trace the probiotics cells. It is essential to investigate the best way of introduction and the optimal dose of the probiotics. Technological solutions are required, especially to keep the probiotics alive in dry pellets. The potential strains of probiotics species of microorganisms have to be identified and evaluated through extensive trials.

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High Density Phytoplankton Cultures and use of Probiotics in Bivalve Larval Rearing

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Introduction

Unicellular marine algae are widely used as food in the hatchery production of commercially valuable fish and shellfish. Bivalves and their larvae feed by filtering them from seawater. Rotifers and brine shrimps also ingest microalgae, which are then used as food for larval fish and prawns. In some systems algae are added to the water containing fish or prawns to improve quality.

Microalgae can be cultured using closely controlled methods on laboratory bench top, with a few litres of algae, to less predictable methods in outdoor tanks, containing thousands of litres. Coutteau (1996) described three basic types of phytoplankton culture systems. a) **Batch culture** is a system where the total culture is harvested and used as food. b) **Semi-continuous culture** is a system where part of the culture is harvested and used as food and the amount taken is replaced with fresh culture medium. After allowing 2-3 days for the remaining cells to grow and divide, the process is repeated. c) **Continuous culture** in which the number of algal cells in the culture is monitored and as the cells divide and grow an automatic system keeps the culture density at a pre-set level by diluting the culture with fresh medium.

Although batch culture is relatively easy to carryout, its efficiency is very poor and the cultures are prone to crashes. Considering the advantages of continuous and semi-continuous culture systems over the traditional batch culture systems, a number of workers (Persoone and Sorgeloos, 1975; Boussiba et al., 1988; James and Al-Khars, 1990; Fabregas et al., 1996) have reported on several designs for continuous production of algae in high densities. Published works on microalgal culture in India are sparse. Gopinathan (1982) has described the batch culture method for culturing marine phytoplankton for use in shellfish hatcheries. In batch culture technique adopted presently in various Indian hatcheries and laboratories, production of microalgae is highly inconsistent, with frequent collapse of cultures due to ciliate infestation and consistent high production are never achieved.

Using 2.5 times the normal nutrient concentration and limited supply of CO_2 the culture density could be increased from an average 1.5 million cells/ml concentration in traditional batch culture systems to an average of 13.7 million cells/ml in continuous systems (Lambade and Mohamed, 2002). Besides the increased biomass production, the duration of culture was enhanced to 30 days without crashing. The semi-continuous system was up-scaled (to 60 l capacity) with an internal illumination system to yield 528 litres of average 3.2 million cells/ml within 36 days. The velocity of cell growth during the logarithmic phase in

doublings/day ranged from 0.21 in control (batch culture) to 2.51 in the up-scaled semi-continuous system. Furthermore, the costs of production, even with additional inputs, are comparable or even less than the traditional batch cultures. The economic efficiency was highest in continuous systems as compared to semi-continuous systems (Rs. 0.03 and 0.017 /billion cells as against Rs. 0.082 and 0.037 /billion cells).

The results of this study reveal that algal cultures in the laboratory can be more efficiently carried out using continuous and semi-continuous systems. The advantage of sustained higher biomass production and use of limited laboratory space are evident. Moreover, the costs of production, even with additional inputs, are comparable or even less than the traditional batch cultures.

Table 1. Details of production, input costs and cost of production per billion cells of *Chaetoceros* in different treatments. Assuming charges are same for facilities like container, treated seawater, aeration, illumination, etc. (From Lambade & Mohamed, 2002)

Experiment/ Particulars	**Batch culture (Control)**	**Outdoor batch culture (Control)**	**Cont. with CO_2**	**Cont.**	**Semi-cont with CO_2**	**Semi-cont.**	**Semi-cont culture Outdoor (60 litres)**
Duration (Days)	30 (5 batches)	36 (6 batches)	27	21	30	26	36
Total volume harvested (litres)	20	360	11	8	26.2	17.5	530
Average cell density (million cells/ml)	1.5	1.0	13.7	8.5	4.2	4.0	3.2
Total cells harvested (Billion cells)	30	360	150	68	110	70	1696
Cost (Rs.)of production							
1) Chemicals	1.1824	21.78	1.6258	1.1824	3.8724	2.5865	78.334
2) Carbon dioxide	-	-	2.916	-	5.832	-	12.000
Total	1.1824	21.78	4.5418	1.1824	9.7056	2.5865	90.334
Cost (Rs.) of production per Billion cells	**0.039**	**0.059**	**0.030**	**0.017**	**0.082**	**0.037**	**0.053**

Use of Probiotics

The origin of the term probiotic is attributed to Parker (1974) who defined them as organisms and substances, which contribute to intestinal microbial balance. However, the concept of microbial manipulation was first appreciated by Metchnikoff during the early 1900s when he viewed the consumption of yoghurt by Bulgarian peasants as conferring a long span of life. Although evidence for a link between longevity and ingestion of fermented milk products has not been proven yet, some workers have claimed that its therapeutic value is related to viable bacteria, in particular *Lactobacillus* sp. Although a strict definition of probiotics is difficult to come by, Tannock (1997) proposed it as "*living microbial cells administered as dietary supplements with the aim of improving health*". Gatesoupe (1999) reviewed the state of probiotic usage in aquaculture and stated that the first application of probiotics in aquaculture is relatively recent, but the interest in such environmentally friendly treatments is increasing rapidly.

There now exist a growing number of scientific papers, which deal specifically with use of probiotics in aquatic animals. Yet, more questions have been raised as to whether probiotics have any relevance in the aquatic environment (Gatesoupe, 1999). Aquatic animals are quite different from land animals for which the probiotic concept was developed. Live-bearing endotherms undergo embryonic development within an amnion, whereas the larval forms of most fish and shellfish are released into the external medium at an early ontogenetic stage. Thus the latter are exposed to all types of microflora available in the medium, while the former develop a particular type (obligate or facultative anaerobes) of gastrointestinal microbiota. Most identified probiotics belong to the dominant or sub-dominant genera of *Bifidobacterium*, *Lactobacillus* and *Streptococcus*. On the other hand environmental microbes like *Vibrio* and *Pseudomonas* are the most common genera in crustaceans (Moriarty, 1990), marine fish (Sakata, 1990) and bivalves (Prieur et al., 1990).

Although the use of probiotic bacterial strains in microalgal cultures does not come within the strict definition of probiotic usage, recent work by Avendano and Riquelme (1999) and Gomez-Gil et. al., (2002) has shown the significance of such probiotic addition in marine larviculture. One of the obvious advantages of such treatments is that microalgal cultures can be used as vectors for the delivery of bacterial antagonists to bacterial pathogens in marine larviculture.

Avendano and Riquelme (1999) established the feasibility of incorporating bacteria with the ability to produce inhibitory substances (BPI) into axenic cultures of *Isochrysis galbana* with the object of using this microalga as a vector for transmitting BPI into cultures of larval bivalves as antagonists of pathogenic bacteria in these cultures. As a first step, the ability of seven strains of BPI to grow in extracellular products of *I. galbana* was evaluated, with positive results with four of these (334,C33, 11, and 77). Subsequently, the effect of the addition of these strains on the growth of I. galbana was evaluated. Comparison of growth rates of I. *galbana* with and without the addition of BPI showed no significant differences ($P>0.05$). A stable and persistent inhibitory capacity of strain C33 on the pathogen *Vibrio anguillarum* was also observed. Finally studies were made on the ingestion of BPI by larvae of *Argopecten purpuratus* (Lamarck 1819). Results demonstrated significant ingestion of strain 11 ($p>0.05$), when it was inoculated directly into the water, and bacterium C33, when delivered in conjunction with the microalga. Upon evaluating incorporation and maintenance of BPI strains 11 and C33 after 5 days of larval culture, we observed the major presence of strain C33 ($3x10^2$ CFU/larva) compared with strain 11 (90 CFU/larva). The results obtained suggested that it was feasible to use microalgal cultures as vectors for the introduction of bacterial antagonists to bacterial pathogens in molluscan larval culture.

Gomez-Gil et. al., (2002) studies made to evaluate the performance of the microalga *Chaetoceros muelleri* then cultured with a potential probiotic bacterium *Vibrio alginolyticus* strain C7b as compared when both are cultured alone in medium f/2. Strain C7b grew significantly better and lasted longer when grown with the microalga than when grown alone. The microalgal density was not affected by the presence of the bacteria compared when grown alone. *C. muelleri*

and the bacterial strain C7b can be cultured together for up to 9 days to achieve a high density ($5.15x10^6$ and $6.63x10^4$ cell/ml, respectively) and then fed to the protozoeal and mysis stages of penaeid shrimp.

A recent study by Rajiv (2003) showed that generally, more number of bacterial colonies was observed in *Isochrysis galbana* than in *Chaetoceros* cultures. In both these cultures, the mean total aerobic count was less (10^3-10^5 CFU/ ml) during the exponential and stationary phases than in the declining phase (10^6-10^7 CFU/ ml) when the cultures were either senescent or dying. This study therefore shows that if microalgal cultures are used when they are in the log and stationary phase rather than in the declining phase, the amount of bacterial added to the larval culture medium can be reduced by 3-4 orders of magnitude.

In both the algal species tested the Simpson Diversity index was at the maximum at peak log phase, indicating that one or two species were dominating the bacterial community in the algal culture medium when the growth rate was high. The Margalef's species richness index showed an inverse proportionality with Simpson diversity in *Chaetoceros* culture. This relationship was not evident in *I. galbana* culture. The hierarchical cluster analysis clearly established the dissimilarity in bacterial taxa occurring during the different phases of growth of *Chaetoceros* and *I. galbana*. There was marked clustering of bacterial taxa during the initial lag phase, early log phase and peak log phase and death phase.

The addition of the probiotic yeast *S. boulardii* as a single addition to *Chaetoceros* culture resulted in significantly ($P < 0.01$) improved (162% increase in maximum algal density) algal growth rates with prolonged stationary period when compared to the control. The daily addition of the same yeast yielded very poor algal density.

The addition of the probiotic yeast *S. boulardii* as a single addition helped in keeping low the total aerobic bacterial count in the medium to between 10^4 and 10^5 CFU/ml as compared to control, which had counts of $10^7$and 10^8 CFU/ml. *S. boulardii* treatment as a single addition also helped to keep the vibrios in TCBS at lower level than control (10^2 vs 10^4 CFU/ml on Day 21 and nil on Day 28). The mean total aerobic flora showed a steeply increasing trend in control and DA treatments, while the trend in SA treatment was that of slow increase.

In hierarchical cluster analysis there was a marked increase in the similarity percentage of clusters indicating a much better discrimination of bacterial taxa in treatment SA as compared to control. It is likely that such heightened discrimination helped in prolonging the culture in SA treatment.

28 Rearing of Baby Chanks and Mark – Recovery Studies

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The sacred chank, *Xancus pyrum* is a gregarious, large, marine gastropod and its dwelling places form distinct chank beds (Nayar and Mahadevan, 1974, Lipton *et al.* 1996a). In addition to the ornamental purposes, the recent demand for chank shells, flesh and operculum led to the increased exploitation. Chank flesh is rich in protein and minerals (Chari, 1966) and the values compared favorably with those of fishes.

In the live condition, the shell of the chank is covered by a surface skin, called periostracum, which protects the shell from several environmental factors including corrosive effects. The periostracum in live animals is brown in colour, soft and velvety, which peels off after the animal dies. Upon removal of the periostracum, the shell shows its characteristic milky white appearance.

Although, there are restrictions by the respective state Fisheries Departments, specific exploitation of chanks by long-lines in Kerala (Appukuttan *et al.* 1980) and by modified trawl nets along Rameswaram coasts in Tamil Nadu (Lipton *et al.* 1996 b) have been reported. Such intense bottom trawl activities also led to depletion of population of chanks in the traditional chank bed areas.

Chank Bed Areas

In Gulf of Mannar and Palk Bay, the depth ranging from 5 to 6.5 m with substratum such as dead coral reefs, sand mixed with mud and algae supported the chank settlement and the resulting chank bed area. In addition to the traditional practice of chank diving, chanks were also exploited using a modified trawl net (=chanku madi). The details are presented in a paper (Lipton *et al.* 1996 b). With the operation of such modified trawl nets (which comprises large number of sinkers), the bottom biota is disturbed. Discussions with the traditional chank divers revealed that in Rameswaram area six traditional chank beds (`paars') adjacent to the coral reefs are totally destroyed by the operation of the `chnku madi'. During the chank diving season, which extends from January to March, they find almost barren seabed, which was earlier flourishing with chanks, holothurians, corals and other mollusks. In addition, the size of chanks obtained from these chank bed areas are also decreased and thus fetch lesser rates. This information is very important in the conservation aspects of chanks.

Morphometric Characteristics of the Sacred Chank

The morphometric measurements revealed two well distinguished sub-species of the chank viz., *Xancus pyrum* var. *acuta and Xancus pyrum* var. *obtusa.* In the *Xancus pyrum* var. *acuta*, the profile of whorls in the spires is convex. In the

case of *Xancus pyrum* var. *obtusa*, the profile of whorls in the spires in very short and the shell appears as a `top'. In addition to these two well marked sub-species, which are also recorded earlier in literature, there are two more sub-species could be distinguished viz., *Xancus pyrum* var. *comorinensis* and *Xancus pyrum* var. *irupiravi*. However, it could be inferred from the data on the collection of chanks that these two latter sub-species formed less than 5.0% of the total chanks either landed or collected by diving.

Breeding of Chanks

The laboratory-reared (maintained) adult chanks exhibited breeding behaviour during the different months. Upon close observation on their breeding behaviour, the males and females can be marked individually and subsequently transferred and reared in the `brood stock' tanks. The `brood tanks' are made of FRP with a water holding capacity of 500 lit. Washed sand was provided at the bottom of the tanks up to 20 cm as substratum. Seawater flow rate was adjusted at a rate of 500 ml/ min. They were fed *ad-libitum* with live clams (*Donax cuneatus* and *D. faba*). The sand substratum was changed every month. During the breeding time, the mating behaviour was recorded carefully. After their mating, the females start releasing the characteristic `ram-horn' shaped egg capsules. The release of egg capsules by the female chank takes a few hours to almost 3 days in some cases. Initially, they secrete a holdfast and paste it to the bottom surface of the tank. Then the female (mother) secretes and makes individual chamber and carefully lays the eggs in to the chamber, which is sealed and this process is repeated till the eggs last. Subsequent to the complete release of the egg capsule the egg capsule stands erect.

In general, the mean length of egg capsules of *Xancus pyrum* was about 224 mm, depending on the size of the mother chank. The width of the egg chamber ranged from 9.64±0.81 (minimum) to 33.0±4.79 mm (maximum). Examination of the total number of chambers in each capsule indicates that they vary between 20 and 33 per capsule. From each egg capsule, 99 to 275 (average 222) babies hatch out.

Release of Baby Chanks from the Egg Capsules

Depending on the hydrological conditions of the water and after 30 to 35 days of release of egg capsules, babies hatch out from the egg capsules. Regarding the hatching mechanism, the juveniles of *Xancus pyrum* rasp the wall of egg chamber with their radula and then come out from their respective chamber. The juveniles of *Xancus pyrum* are benthic and very active in creeping movement. At the time of their release, the baby chanks actively move on the surface of the egg capsule and subsequently on the substrata of the rearing tanks.

Rearing of Baby Chanks

The babies of the *Xancus pyrum* are carnivores. They feed on very small/young ones of polychaete worms up to 2 months. After two months, according to the size of baby chanks they prey on live earthworm and Neries. After

eight months, the baby chanks feed live clams. The growth obtained in experimental studies is given below:

Growth of Baby Chanks, *Xancus pyrum*

The baby chanks, which hatch out from the egg capsule, are of about 09.09 mm in length. After 120 days, they attain an average length of 42.88 mm and after eight months they reach 53.66 mm in length. They attain about 62.0mm after one year of their release from the egg capsule.

Tagging and Recapture

In order to detect the natural growth rate and the migratory behaviour the chanks were tagged using Letro labels with araldite and sea ranched in the Gulf of Mannar and Palk Bay. The results indicated that:

- With the relaxations of chank fishing restrictions, the sacred chank is over-exploited (using modified trawls).
- It is a non-migratory species, which lives in restricted chank beds.
- The sacred chank, *Xancus pyrum* is a slow growing species with an MSD-wise growth is about 8.0 mm/year.
- Its fecundity is also not very high and it breeds once in a year.

Suggested Reading

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29

Oyster Farming Methods

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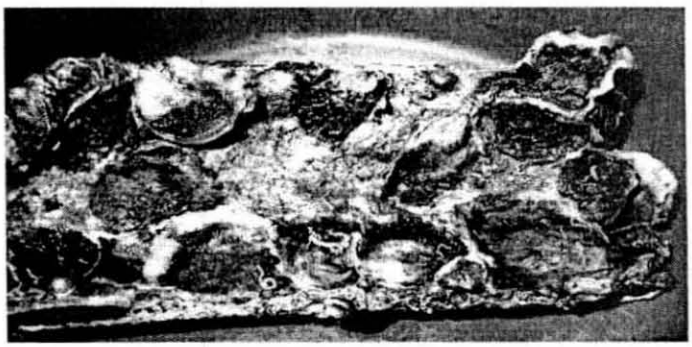
Edible oyster is one of the most widely cultivated bivalves. As early as the first century BC, the Romans practiced simple method of oyster culture by collection oyster seeds and growing them for food. The important oyster producing countries are Japan, Korea, France and China and together they contribute 78.7% of the total oyster production by culture. In India, Hornell (1910) initiated experiments on spat collections of oyster *Crassostrea madrasensis* at Pulicate Lake. Realising the resources potential, nutritive and commercial values of edible oysters the Central Marine Fisheries Research Institute made attempts to evolve suitable farming techniques for edible oysters from 1970.

The technique of oyster farming involves two important phases namely 1. Oyster seed collection/production, and 2. Rearing seed oysters to marketable size.

1. Seed Collection from Wild

The seed required for culture is met either from natural spat collection or through hatchery system. For collection of spat from nature suitable spat collectors or cultch materials are provided at appropriate time. The spat collectors should be able to retain the oysters till they reach marketable size or upto the size at which they could be scrapped for further rearing. The choice of spat collectors depends on the culture method adopted, local availability, and economic and practical consideration. In culture experiments at Tuticorin, cultch materials viz. semi-cylindrical roofing tiles, oyster, mussel and coconut shells, asbestos sheet, netlon and automobile tyre pieces were used. The tiles are given lime coating for roughness. The oyster shells are made into strings on a GI wire or synthetic rope. The collectors are laid on the racks. Of these collectors, lime coated shell (with an average of 34 spat/tile) (Fig.1) and oyster shell (with an average 7 spat/shell) were found suitable for large scale spat collection from wild.



Spat settled on lime coated tiles (average 35 per tile)

2. Spat Fall Prediction

The prediction of spat fall is essential for collecting seed oysters in the appropriate time with minimum foulers interference. This time is called as cultching time. The prediction of spat fall is based on the study of maturation and spawning of ripen gonads in the oyster population or by the appearance of oyster larvae in the plankton samples of the area. The collectors are exposed just a week before spawning period. Large scale spat collection experiments showed the abundance of seed oysters in intertidal areas, creeks and bays. The method, season of spat collection and the type of spat collectors to be used vary from place to place, depending on the local conditions.

3. Seed Production through Hatchery System

On the establishments of a shellfish hatchery in 1980, the Central Marine Fisheries Research Institute succeeded in mass production of both clutched and cultch free spat.

4. Site Selection

- The following requirements are essential in the selection of farm site.
- Sheltered areas offering protection from strong wave with a depth ranging from 2-5 m.
- Salinity range of 22 to 35 ppt.
- Temperature range is 21-31°C
- Area with pollution free water.

5. Methods of Culture

The farming methods are broadly divided into i) on-bottom and ii) off-bottom culture; the seed oysters are sown on the ground. This method is substrata specific and the area sown is free from silting and predators. When oysters are grown by off-bottom methods, the advantages lie in better growth and good condition of the meat. The methods involved in off-bottom culture are 1) rack and tray 2) rack and string 3) stake and 4) raft

On-bottom Culture

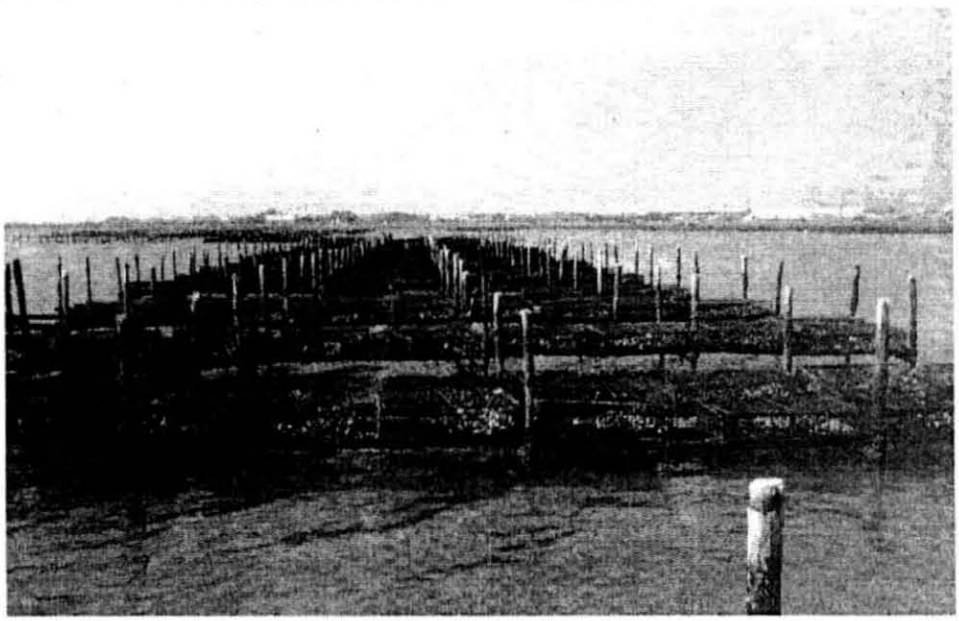
This method is practiced in France. The seed oysters are sown on the ground. This method is substrata-specific and the area should be free from silting and predators. In France oyster seed are stocked at the rate of 20-50 oysters/m^2 in the intertidal culture areas called 'parks' of 0.5 – 1 ha. The production rate is 0.9 kg/m^2. In America, oyster seed are sown in the sub-tidal areas with a depth of 5-18 feet.

Off-bottom Method

The methods involved in off-bottom culture are (1) rack and tray (2) rack and string (3) stake and (4) raft.

Rack and Tray Method

The spat attached on lime coated tiles on attaining 25 mm were scrapped or cultchless seeds produced in the hatchery are stocked in box cages. The cages are of 40 x 40 x 10 cm size made of 6 mm mild steel rod and webbed with 2.5 mm synthetic twine. For nursery rearing of hatchery produced cultch free seed (of 5-10 mm) the cages are covered with velon screens. The cages are suspended from single rack system. After 2-3 months rearing, oysters of 50 mm and above are transferred to reactangular trays. Each tray is of 90 x 60 x 15 cm size accommodating 150 – 200 oysters. Twenty such trays are placed on a rack. Rack, a wooden platform for placing the rearing trays is constructed using eucalyptus poles (Fig.2). Each rack occupies an area of 25 sq.m and holds 3000 – 4000 oysters. The estimated production rate is 110 t/ha (Fig.2).

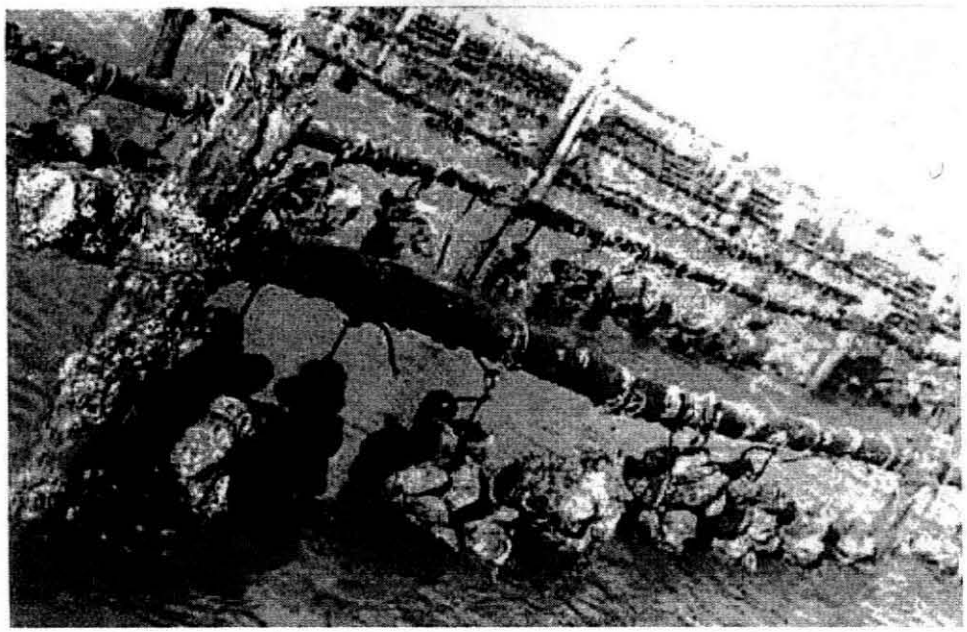


View of Oyster farm by Rack & Tray method Production 110 t/ha (Meat 9 ton)

Rack and String Method

The oyster spat collected on shells are made into strings having six shells using 5-6 mm synthetic rope. These strings 2-3 are kept inside a velon screen bag and suspended from racks for nursery rearing. Racks for this purpose are a series of vertical poles driven into the bottom in rows and horizontal poles are connected on

top of the poles. The oyster shell strings are suspended from the horizontal poles of the rack with a space of 10 cm between two strings. The production rate is 80 t/ha. Total racks is one ha is 125. No. of strings/rack = 90 No. of oysters/string = 40 Fig.3



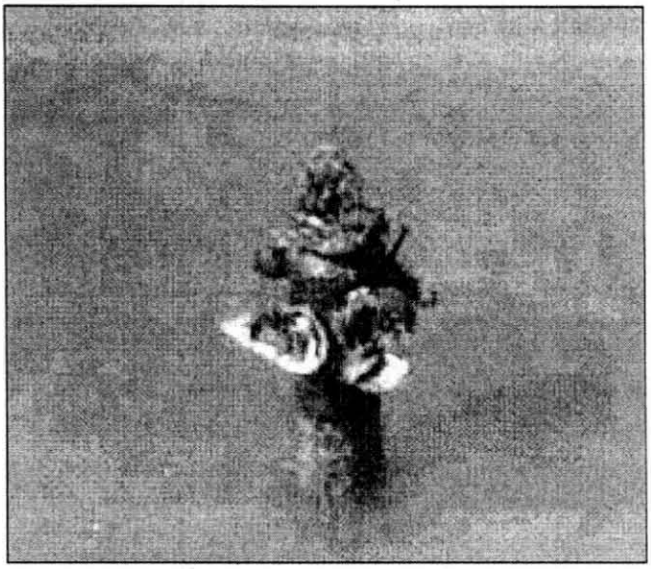
View of rack and string farm production rate 80 t/ha

Stake Method

Stake method is adopted if the culture site is soft and muddy. Each stake, casuarina or eucalyptus poles of 1½ m length with a nail on top and two nails on the sides is driven into the ground. The nail holds a shell with spat. To protect the spat against crab predation, initially the top of the stake is covered with a piece of velon screen. Once oysters attain 25 – 30 mm the velon screen is removed and oysters are grown on stakes upto harvestable size of 70 – 80 mm in length.

No of spat / shell	= 16
No of oysters / stake	= 14
No of stakes / ha	= 17500 – 18000
Production rate	= 20 t / ha

Fig. 4



3 to 4 Shells nailed on a stake and erected in muddy bay

Raft Method

In this method, oysters are suspended from floating rafts. Rafts are constructed using bamboo or wooden poles and are floated with empty oil drums or wooden barrels. Once raft is positioned by anchors, shell strings with attached spat are hung from raft. Where wave action is more, series of small floats are joined by synthetic ropes. The line is anchored at both ends. From the ropes, the strings are suspended. These methods are also called as floatation methods. The production rate is 12-15 kg/ m^2.

6. Growth of oysters

Stage	Growth rate (mm/month)
Initial growth Upto 4 months	10.2 – 10.6
4 – 8 months	6.7
8-12 months	5.5
Overall growth rate	6.6 mm/month

7. Foulers, Predators and Diseases

Fouling organisms such as barnacles, ascidians, sponges and algae settle on rearing trays and oyster and compete for food and space. They are periodically cleaned. Woodborers like *Martesia* sp. and *Teredo* spp. Damage the wooden farm structures. Crabs, fishes, starfishes and gastropods are the oyster predators. Predatory gastropods Cymatium spp. Causes 13% mortality of oyster in the farm. Apart from these, diseases caused by Haplosporidians such as Perkinsus *marinus, Minchinia spp.* Cause considerable large scale mortalities of oysters in temperature waters. Some of the termatodes notably Bucephalids cause castration of gonads.

8. Harvesting

Oysters reach harvestable size (above 80 mm) within 10-12 months. They are harvested when the condition of meat reaches high value. The condition factor of *C. madrasensis* at Tuticorin reaches maximum of 170 during pre-spawning periods (February-March and July-August) Harvesting is done manually.

9. Depuration and Shucking

Harvested oysters are kept for 10 – 12 hours in the tanks under a flow of filtered seawater. As a result the bacterial load of the shellfish is reduced. The depurated oysters are taken for shucking. Shucking is the removal of meat from the oyster. Depurated oysters are kept in a gunny bag and held for 3 minutes in boiling seawater. This treatment makes the meat removed easy with a shucking knife. Shucked meat is washed and dipped for 10 minutes in salt solution containing citric acid. The meat is weighed and packed in polythene bags as 2 kg units. These are quick frozen at -30°C, using horizontal contact plate freezer. The frozen meat is transported to canning factory and marketing. Live oysters could also be transported them in wet gunny bags.

10. Utilisation of oyster shell

Since oyster shells on ignition contains 52% Calcium oxide are used in manufacturing calcium carbide & lime. The shells crushed to suitable size are used as grit along with the poultry feed.

30 Remote Setting and Upwelling Systems

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Introduction

The first report of successful artificial oyster spawn was in 1879 when Dr. Brooks produced "free-swimming" oyster larvae by stripping eggs and sperm from ripe adult oysters. Following this several other researchers attempted to rear larvae in their laboratories; however, it was not until 1920 that W.F. Wells successfully reared and set oyster larvae. The development of modern shellfish hatchery methods has evolved over the last hundred years. To solve the problems related to transportation of huge quatities of shell clutches, the modern method of remote setting was developed. Remote setting is the method of transporting eyed larvae of bivalves in cool and moist condition to distant areas where ambient conditions are provided to setting. The rapid growth of remote setting in the last few years has now almost replaced the unreliable and costly collection of natural spat. Currently, over 40 remote setting operations are in production within British Columbia, more than any other region of the world.

Bivalve Larval Settlement

Oyster larval settlement involves two phases:(1) attachment to substrate and (2) metamorphosis. Settlement is a general term to describe the transition from larva to juvenile. During transition larvae cease feeding, search for a suitable substrate and finally attach to their chosen location. At the time of attachment metamorphosis is initiated.

After the larva has attached to the substratum, the velum and foot are no longer needed. Their disappearance marks the transition from free-swimming to a sedentary mode of life. During the metamorphosis the larval organs disappear and there is an anatomical reorganization of the permanent organs. At this time the relative size of the organs and their orientation are changed. The recent practice of remote setting of larvae a considerable distance from hatcheries or natural setting areas has caused dramatic changes in the oyster industry. Since many growers shuck their own oysters, large amounts of discarded shell are on hand for cultch. In the past this material had to be hauled to and from the natural setting locations or hatcheries. Now, instead of hauling cultch long distances, cultch can be artificially set with spat close to the grow-out site.

Setting Methods

Ready-to-set larvae are now available to remote setting operations from several hatcheries. The basic method for producing setting larvae is as follows though the basic procedure of oyster setting is frequently fine-tuned by hatcheries and setting operations to suit. Prior to shipment larvae are sieved out of the tank, wrapped in nylon cloth and moist paper towels to prevent dehydration and transported in an insulated container at 50 C. Two and a half million eyed-larvae are the approximate volume of a golf ball. The study by Carlson (1981) showed that holding eyed larvae in this way at 50 C for five to eight days before setting appears to have no effect on the viability of the larvae, However, this length of storage is not recommended since this stress may contribute to post-set mortality problems.

Larvae should be put into the setting tank of seawater within 24 hours of leaving the hatchery. Setting normally starts as soon as the larvae are put into the setting tank with cultch, which can be either oyster shell or plastic collectors. For best success, cultch should be aged for one year in the ocean and be thoroughly cleaned. Some growers add algae to their setting water, although the feeding of setting larvae hasn't yet been proven to be beneficial. When metamorphosed larvae are fed there is greater initial survival. After the setting process is complete, the cultch is placed on the beach in the inter-tidal zone (covered to protect from the effects of the sun), or hung subtidally.

Seawater used for setting should be filtered with a 50 or 100 micron filter bag or sand filter while the larvae are swimming. After the set has occurred, raw or filtered seawater is pumped into the tank to feed the larvae. If algae are being cultured, it is usually added at a concentration that the larvae can consume in less than a day. It has not yet been proven whether feeding with algae improves the setting density but some growers consider it to be a good idea and is not harmful if done properly. Aeration can be provided by any compressor, air pump or blower that does not produce an oil mist. Oyster larvae do not require aeration for extra oxygen, aeration is provided to create water movement to evenly distribute larvae throughout the tank and to prevent uneven heating of water. The air pump must be positioned above the setting tank so that it will not fill with water when shut off. Tank water should be only aerated gently, not at a boil and only while adding larvae or algae or intermittently during setting. Excessive aeration can cause the larvae to separate out of the water into the foam.

Cultch (whatever is used for a setting substratum) must be clean and leached for one year or longer by pre-exposing it to seawater or by placing it high in the intertidal.zone. During the conditioning process the cultch becomes covered with a thin film of bacteria or micro fouling that seems to attract the larvae at time of settlement.

Set Inducers

Once the environmental conditions are satisfactory for larvae to set, competent larvae appear to respond to a chemical cue to settle and attach themselves. In nature this cue is reported to be a pigmented bacterium called LST,

which adheres strongly to surfaces like oyster shell. Simple chemical compounds trigger attachment and metamorphosis of many larval marine invertebrates. DOPA (L-3, 4-dihydroxyphenylalanine), an amino acid, has been used to induce the setting of oysters. The use of chemical cues appears to be most applicable to cultchless setting systems where there is no natural attractant.

Cultch Material

Different materials are used as cultch material. The most common cultch used by the oyster industry is given below.

- Shells: Vexar bagged shells are used as the cultch material, shells must be clean and aged for at least two year s before being used.
- French plastic pipe: These are 2 meter pipes with 25mm diameter (hollow tubes) with longitudinal groves 2mm deep on the outer surface. With the older pipes (ones that have been used before) it is possible to get sets of 2,000 to 3,000 larvae per pipe. New pipes leach for about two or three months and the maximum setting have been found to be 400 to 500.
- Shell chips: The shell chips are spread over the tank bottom and the larvae added to the tank. This is a simple and easy method of producing single seed.
- 'Chinaman hats" (to produce single seed oysters): These plastic spat collectors are dipped in a slurry of cement at least six months prior to setting. The mixture of the slurry is 1/3 Portland cement to 2/3 sand. Water is added till it has a consistency that will leave a 1/8 inch coating on a stick.

Metamorphosis is a critical stage for oyster larvae because this is the point at which mobility is lost and the internal organs are modified to adapt to a sedentary existence. This process of change in morphology reduces the ability to filter feed for 24 to 48 hours and as a direct response to the reduced feeding activity growth rates are also reduced.

Recommended Algal Feeding Rates

A recent development in the feeding of bivalve larvae is the use of centrifuged algae subsequently made into a paste. Algae paste is made by passing dense algae cultures through a continuous flow centrifuge called a separator or clarifier. This spins the algae cells out of the culture water and deposits them on the rotating bowls of the clarifier. These packed algae cells have the consistency of toothpaste and can be refrigerated for several weeks without any loss of nutritional value. *Thalassiosira pseudonana* clone 3H), a diatom, is an excellent food for large larvae and spat. One liter of 3H will spin down to 0.2 grams of paste. One gram of paste contains roughly 1X10, or 10,000,000,000 cells.

Larvae Density

The number of larvae added to the tank depends on the density of set required. Since most growers seem to prefer 10 to 20 spat per shell, we recommend a larval density of 150 larvae per shell. A tank containing 300 cultch bags would

take 5 million larvae. Underestimating the amount of larvae needed for a set is a common mistake made by beginning setters. The optimum density on unbroken seed was determined to be between 20 and 25 spat per shell. These survival studies also found that initial density must be at least 6 spat per shell to be economically viable.

Post-Settlement Handling and Survival

Postset survival is the largest problem facing the remote setter. The main facters affecting the spat are heat and drying during transfer from the set tank to the nursery area. Freshly set spat can be killed in less than an hour at room temperature by drying. Some cultch types hold moisture better than others. Freshly set spat are very delicate and severe mortalities can be caused by exposure to heat and sun when the cultch is removed from the setting tank. The transfer should be done in cool temperatures, (early mornings or rainy weather) and as quickly as possible.

In the absence of silt, seed mortalities could be substantially reduced. It appears that during times of poor seawater quality, survival is better if the spat are kept in the intertidal zone rather than hanging subtidally.

Remote setting method has helped to revitalize the oyster industry. Larvae users are apparently learning from their past mistakes and now the mortality problems that occur are usually post set problems. The most common setting problems are still: (1) toxicity of the tank surface, (2) cultch condition (too dirty or not conditioned) and (3) water temperature (too hot or too cold). Several years ago the future of oyster farming seemed dependent on a "reliable source of seed". Now that there is an adequate supply of larvae and the methods and procedures for its use are established and results consistent, the new problem appears to be water quality degeneration

Floating Upweller Shellfish Nursery System

Land-based upweller nurseries for shellfish culture have been around since the 1960's.Upwelling is an efficient way to pass water vertically through a three dimensional mass of shellfish seed resting on a mesh in order to culture them from a hatchery size to a field nursery orgrowout size during the first season of growth. Land-based systems use valuable waterfront real estate, operate at relatively high heads, which require expensive to operate centrifugal pumps and are limited in the number of seed the shallow "silos" of the majority of such systems can hold. A floating upweller system (FLUPSY) takes the silos and places them floating just above the surface of a water body or floating tank, thereby reducing the "head" that makes pumping water so expensive in land-based systems. (FLUPSY)'s move water through the use of tidal flow, airlifts, paddlewheels or pumps.

31

Bivalves and Harmful Algal Blooms

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Introduction

An algal bloom is the rapid growth of one or more species which lead to an increase in biomass of the species. Its defined as 'those which are noticeable, particularly to the general public, directly or indirectly through their effects such as visible discoloration of the water, foam production, fish or invertebrate mortality or toxicity to humans' (ICES, 1984). It is estimated that globally approximately 300 people die due to consumption of shellfish contaminated with toxin produced by phytoplankton.

Of the 4000 marine planktonic microalgae described to date, approximately 80 toxic species and 200 noxious species have been implicated in the formation HAB's. Sournia (1995) has remarked that the major toxin producing algae are dinoflagellates followed by diatoms. Though toxic blooms have been known to occur for centuries, there has been a phenomenal increase in the HAB records in coastal waters in the recent years. With the advancement of scientific research and enhancement of human activities in the coastal zone more information about the HAB has been documented. The increase in biomass is specific for each species and may vary considerably in space and time depending on the environmental conditions.

Most harmful species become hazardous only when their concentration exceeds a threshold level, which varies with species. The diatom *Chaetoceros concavicornis* and *C.convolutus* which have long siliceous spine become a cause for fish mortality due to lesions produced on the gill tissues even at low concentration of 5 cells ml^{-1} while some like the *Phaeocystics* become noxious only when they reach very high concentration. This micro algae which is a normal component in the temperate areas, at high concentrations affects the fisheries, confer bad taste to fish, deviate the herring migration patterns and in some cases produce slime and foam. Similarly very dense concentration of non toxic diatoms like *Cosinidiscus* spp, *Thalassiosira mala* have caused discoloration or fish gill clogging.

Types of Algal Bloom

There are 3 different types of algal blooms.

1. Blooms which are basically harmless water discolorations (under exceptional condition they may cause mortality of aquatic organism)
   Gonyaulax polygramma, Noctiluca scintillans, Scrippsiella trochoidea, Trichodesmium erythreum.

2. Blooms which produce potent toxins

   Alexandrium spp., and *Pyrodinium bahamense* – PSP; *Dinophysis acuminata* – DSP *Nizschia pungens* – ASP; *Gambierdiscus toxicus* - Ciguatera fish poisoning
   Gymnodinium breve - NSP

3. Blooms which are non toxic to humans but toxic to fish and invertebrates by damaging or clogging gills and they are problematic especially in intensive aquaculture systems/mariculture system.

   Chaetoceros convolutus, Gymnodinium mikimotoi, Chrysochromulina polylepsis, prymnesium parvum, heterosigma carterae.

Under favorable conditions following algal groups produce bloom

- Diatoms (Bacillariophyceae)
- Dinoflagellates (Dinophyceae)
- Members of Green algae (Chlorophyceae)
- Blue green algae (Cyanophyceae).

Dinoflagellate bloom

Dinoflagellates occur in both salt and freshwater and can be both planktonic and benthic. These are the protist group with the largest number of harmful species. Dinoflagellates show a great range of forms, they can be grouped into 5 basic types as bloom causing agents.

1. **Gymnodiniods And Noctilucoids:** *Gymnodium breve, G. stein, G. catenatum, G.mikimotoi, G. pulchellum, G. veneficum. Gyrodinium Noctiluca scintillans* (*N. miliaris*)
2. **Peridinoids** : *Peridinium polonicum*
3. **Gonyaulacoids**: *Alexandrium/Gonyaulax catenella, A. angustitabulatum, A. cohorticula, A. hiranoi, A. minutum. Pyrodinium bahamense, Gambierdiscus toxicus, Ostreopsis lenticulatris, Ceratium fusus*
4. **Dinophysoids**: *Dinophysis acuta, D. acuminata.D. sacculus*
5. **Prorocentroids**: *Prorocentrum concavum, P. emarginatum, P. lima etc*

Diatom bloom.

Diatoms are one of the largest algal groups known.The diatoms are found in all types of aquatic habitats and in marine plankton at all latitudes and through out all season. Harmful events observed when blooms of *Cosinodiscus concinnus and*

C. centralis, Thallassiosira mara, Rhizosolania chunii and Cheatoceros spp. occurred.

Most algal bloom represent useful contributions to plankton production but some periodically produce harmful results.

Physical damage: dense concentration of tide may suffocate fish by clogging or irritating their gills. In 1962, mortality of more than 100 tons of fish in False Bay was attributed to gill clogging by the Dinoflagellate Gonyaulax *polygramma*. Oxygen depletion can kill indirectly by depleting the oxygen dissolved in the water.

Direct poisoning: toxins of dinoflagellates are more potent, which disrupt normal nerve functions. This has caused numerous marine fish and shellfish mortalities. In 1980 entire mussel population of Elands Bay was destroyed by *Gonyaux catenella* and in 1989 in Japan, 30 tons of abalone were washed up due to *G. nagasakiense.*

Indirect poisoning: Mussel, clams and oysters which are filter feeders-accumulate toxins in the digestive system-cause illness or death to consumers such as birds, marine mammals and man. Four different types of indirect poisoning have been identified as harmful to man.

Paralytic Shellfish Poisoning (PSP)

PSP was discovered in 1700. Most serious of the shellfish poisoning. Several hundreds human deaths have been recorded worldwide during past 300 years. Wide spread in U. S., west coast, Maine to NewYork. Mussels, clams, oysters, scallops, herring, sardines, marine mammals, birds are directly affected. Humans are affected by eating contaminated shellfish which contains the toxin-Saxitoxin which disrupts normal nerve function.The toxic molecule inhibits the passage of sodium ions causing numbness, paralysis, respiratory failure, death.*Alexandrium spp.*, and *Pyrodinium bahamense* are some PSP producing alage

Diarrhetic Shellfish Poisoning (DSP)

The causative organism: *Dinophysis acuminata*. Which produces the toxin Okadaic acid. It is reported from South African waters, no report in US. The chemicalaffects proteins that control the sodium secretion by intestinal cells, causing nausea, vomiting, abdominal pain and diarrhea in humans.

Neurotoxin Shellfish Poisoning (NSP)

It is common in Gulf of Maxico coast, Florida, North caorolina, South Carolina and South African coast. Mantaees, bottlenose dolphins, oysters, fish, clams and birds are affected. Humans may be affected by breathing sea foam or eating contaminated shellfish. Toxin molecule induces greater flux of sodium ions causing diarrhea vomiting, tingling in lips, dizziness.

Amnesic shellfish Poisoning (ASP)

ASP was recorded first time off the coast of Canada in 1987. 3 deaths and over 100 confirmed cases of acute intoxications followed the consumption of cultured mussels. The main causing organism is *Nizschia pungens*. The toxin produced is domoic acid. Razor clams, dungeness crabs, scallops, mussels, anchovies, sea lions, brown pelicans and cormorants are affected. Humans may be affected by eating contaminated shellfish. Toxin molecule attacks human central nervous system causing vomiting, abdominal cramps, diarrhea, short-term memory loss etc.

Ciguatera fish poisoning (CFP)

Gambierdiscus toxicus is the main phytoplankton and it affects reef fish and their predater like Barracuda, snapper, amberjack, grouper, and kingfish. Humans may experience vomiting, cramps, diarrhea, headache, weakness, numbness.

Blooms and Bivalve Utilisation

It is essential to avoid areas, which have previous records of blooms, or if such a bloom occurs, the harvest should be postponed. The retention level for diffrernt toxins varies between species and for the same toxin clearance rates are different (Table 1). The sample from the bloom site should be analyzed for toxin and only if it is within the safe levels it should be harvested and marketed. The levels set by different countries for PSP and DSP is given in Table.2. The total ASP content should not exceed 20µg of domoic acid per gram using the HPLC method.

Table 1. The toxic retention time for different bivalves

Species	Toxin sources	Retention time
Anadara maculosa	*Pyrodinium bahamense*	6weeks
Arctica islandica	*Protogonyaulax tamarensis*	2 months *in vivo*
Choromytilus meridionalis	*Gonyaulax catenella*	3 months
Clinocardium nuttalli	*Gonyaulax acatenella*	9 weeks
Crassostrea cucullata	Not specified, probably *Pyrodinium bahamense*	2 months
Crassostrea echinata	*Pyrodinium bahamense*	3 weeks in closed system; longer period *in vivo*
Crassostrea gigas	*Gonyaulax acantenella*	1-9 weeks
Crassostrea iridescens	*Gymnodinium catenatum*	> 1 month
Crassostrea virginica	*Gymnodinium breve*	2-6 weeks
Modiolus auriculatus	*Pyrodinium bahamense*	6 weeks
Modiolus modiolus	*Gonyaulax tamarensis*	Up to 60 days
Mya arenaria	*Gonyaulax acatenella*	5 weeks
Mytilus californianus	*Gonyaulax catenella*	Up to 45 days
Mytilus edulis	*Protogonyaulax tamarensis* *Gonyaulax acatenella* *Gonyaulax excavata*	10days-7 weeks up to 50 days 11 weeks 2-3 weeks
Patinopecten yessoensis	*Protogonyaulax tamarensis*	6 weeks to 5 months

Protothaca staminea	*Protogonyaulax acatenella*	5 weeks
Saxidimus solidissima	*Gonyaulax catenella*	< 1 month
Spisula solidissima	*Protogonyaulax tamarensis*	Up to 1 year
Spondylus sp.	*Pyrodinium bahamense*	Still highly toxic after months
Tresus capax	*Gonyaulax acatenella*	11 weeks
Venerupis japonica	*Gonyaulax acatenella*	5 weeks

Table 2. Regulations of paralytic shellfish poisons in various countries

Country	Product	Toxins	Tolerable level	Responsible Authority
Australia	Shellfish	Saxitoxin	80 $\mu g100g^{-1}$	State Authority under supervision of the Australian Quarantine and Inspection Service
Austria	Shellfish	Saxitoxin	40$\mu g100g^{-1}$	Ministry of Public Health and provincial authorities
Canada	Molluscs	PSP	<80$\mu g100g^{-1}$	Dept of Fisheries and Oceans; Dept of Health and welfare
		DSP	0.2μgg^{-1}	
European Union	Bivalve molluscs	PSP	80$\mu g100g^{-1}$	Various
Hong Kong	Shellfish	PSP	400MU$100g^{-1}$	Dept of Health: Agriculture and Fisheries Dept
Japan	Bivalve	PSP	400 MU$100g^{-1}$	Ministry of Health &welfare, Bureau of Environmental Health
		DSP	5 MU$100g^{-1}$	
Korea	Bivalve	Gonyautoxin	400MU$100g^{-1}$	Ministry of Health & social services
		DSP	5 MU$100g^{-1}$	
Singapore	Bivalve	Saxitoxin	80 $\mu g100g^{-1}$	Ministry of National Development
Norway	All types of mussel	PSP	40-80 $\mu g100g^{-1}$	Food Council Authority
		DSP	5 –7 MU$100g^{-1}$	

Economic Impact

1. Economic loss of due to closure and mortalities.
2. Consumer fear of purchasing seafood
3. Fear of investing in aquaculture business
4. Discoloration of water - aesthetically unpleasant
5. Loss of marine recreational opportunities including tourism, fishing, swimming and sunbathing.

Monitoring and Managemnet

1. A surveillance programme should be established-to monitor bloom and physicochemical parameters to predict bloom.
2. A research design should be setup systematically collect and analyze information on variables (contributors) associated with increased kills of fish.
3. Development of antibody and DNA "probes" that are being used to detect HAB and toxins in natural waters.
4. Development of methods to utilize satellite imagery of costal waters to follow HABs and water masses with which they are associated.
5. Educational strategy to the general population should receive special attention.

32 Post Harvest Processing and Depuration of Farmed Bivalves

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The edible bivalves like the mussel and the oysters are harvested when the condition index is high i.e., when the gonad is ripe and the meat occupies the entire shell cavity. In temperate countries, mussel and oyster harvest is mechanised while in India the 'mussel ropes' and oyster strings are collected manually and brought to the shore. Mussels are normally marketed shell-on. Harvested oysters, which are kept under moist and cool conditions, survive for several days. However, it is desirable that they reach the consumer within three days of harvest. Studies indicate that oysters packed in wet gunny bags can be safely transported for 25-30 hours without mortality and in good condition. Oysters are eaten in fresh condition in the half shell in many countries. Removal of meat from the shell is termed shucking. Live mussels and oysters are shucked easily using stainless steel knives or by gently heating to open the shell. Remnants of mussel byssus thread if any are removed before marketing the meat. The post-harvest procedures for mussel and oysters are shown in Fig.

By-products and Value Addition

A variety of products have been developed in India from mussels and oysters (Table). These products have been developed by the R & D activities of the Central Institute of Fisheries Technology (CIFT), Cochin.

- **Icing:** Fresh oysters and mussels can be preserved in ice in organoleptically acceptable condition up to 9 days. Fresh frozen oyster and mussel meat remains acceptable for 40 weeks.

- **Canning:** Cleaned meat after blanching in 5% brine for 5 minutes can be canned. The blanched meat with medium is canned by heat processing in steam at 115°C for 20 minutes.

- **Smoking:** Smoking improves flavour of the meat. The blanched bivalve meat after drying to a moisture level of 40-45% is smoked at 80-90 °C for 30 minutes. It is then dried further to bring the moisture level to 10%. The shelf life of smoked oyster and mussel in room temperature is six months.

- **Drying:** Blanched meat can be sun-dried or dried in an electrical dryer to bring down the moisture content to 10-15%. Shelf life of the dried meat is six months in room condition.

Table. Value added mussel and oyster products from India

Mussel products	*Pearl Oyster Products*	*Oyster products*
▪ Iced and frozen mussel ▪ Canned mussels ▪ Smoked mussels ▪ Dried mussels ▪ Marinated mussels ▪ Mussel pickle ▪ Mussel chutney powder	▪ Pearl powder ▪ Pearl liquid ▪ Seed pearls ▪ Mother-of-pearl shell	▪ Frozen oysters ▪ Canned oysters ▪ Smoked oysters ▪ Oyster stew

For further economic utilization, value added products of mussels like seafood cocktails are also prepared and marketed by many seafood export firms from India. The export of these items from India has by and large, been showing an increasing trend.

The two shell valves constitute about 85% of the total weight of oyster and contain about 52-55% calcium oxide. They are used in the manufacture of calcium carbide, lime, fertilisers and cement. Larger oyster shells are useful spat collectors in oyster culture. The shells are broken to pieces and also used as poultry grit. The mussel shell finds use as a liming agent in coconut plantations. Another important economic use of bivalve shell is in the making of curios, a small-scale industry which is rapidly developing along the east coast of India, and in the Andaman and Nicobar Islands.

Marketing

India is presently a net importer of cultured pearls and the pearl trade is centred in the city of Hyderabad. Export of Indian cultured marine pearls is of recent origin (Fig. 3) and the main country to which it exports is Hong Kong (@ US $ 8.5 per gram). Besides, there is a growing internal market for cultured marine pearls.

The edible bivalve market channel is a relatively straightforward and fresh and frozen farmed mussel and oysters have a healthy and growing domestic demand in maritime regions of the country. There is now increasing appreciation of the fine texture and taste of mussel and oyster meat and these comparative new products look set to captivate the urban connoisseurs. New strategies need to be developed to fully exploit the domestic markets. On the export front, in the case of mussels, Indian products have found a place in the markets of UAE, Germany and Republic of South Africa, and the list is growing. Although live oysters are an expensive gourmet food in Europe and America, they have not found such a niche in India. Markets are limited to few isolated pockets like the Parsi community in Mumbai, who have a specific preference to smoked oysters marketed in cans.

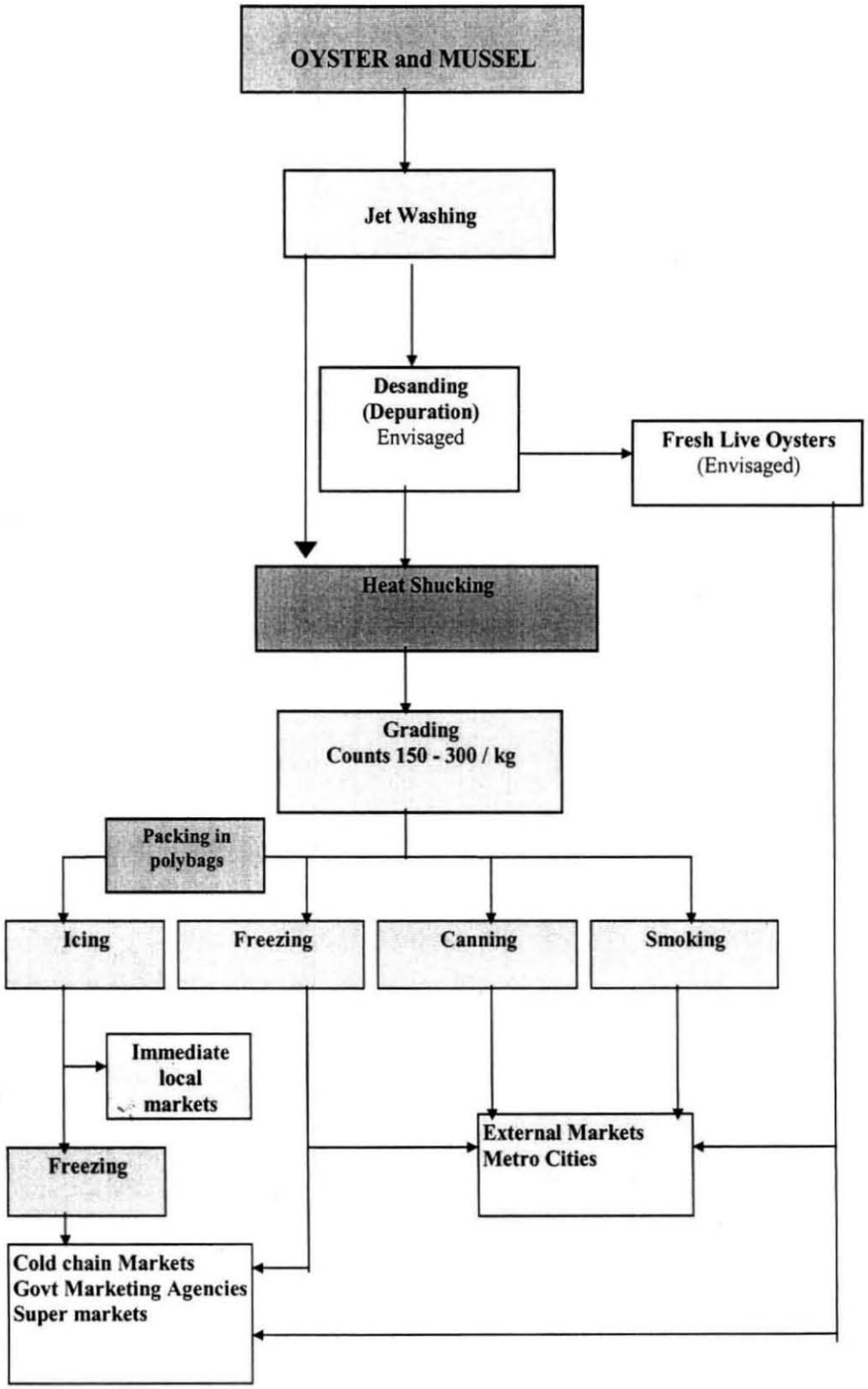


Fig. Bivalve Post-Harvest procedures/ processing

The product is exportable if it meets basic quality and sanitation standards. Through export to western markets the farmers can get better price for their produce, thereby, increasing their profit margins. However, European Union markets are very stringent about the quality of bivalve products that they import from Asian markets. To ascertain and maintain the quality of bivalve products depuration is essential.

What is Depuration?

Bivalves are filter feeders in their feeding habit. During this process they accumulate all suspended biological materials including harmful microorganisms. Before the product reaches the market, these materials have to be removed from their gut. The process of such purification is called depuration.

Simple depuration can be achieved by starving the bivalves in clean and filtered seawater/ brackishwater for a certain period of time. More effective depuration can be achieved by using disinfected water in the depuration process.

Even a simple and small depuration unit will be beyond the capabilities of the small-scale farmers, and hence, it is proposed to have depuration plants where bivalve farmers are concentrated, thus enabling the farmers to use it as a common facility for a price to be determined later.

Depuration Process

1. Requirements

(a) The basic principle for controlled purification or depuration of bivalve involves providing clean and purified seawater in tanks, whereby the bivalve filter and pump such water for a period of 24 hours or more if required.

(b) Ideally a depuration plant should be located near the least polluted source of water in the vicinity of bivalve farms. Also the physical characteristics (salinity, temperature, dissolved oxygen etc.) of the seawater used in the depuration plant should not be radically different from that of the bivalve farming areas. Care should be taken such that the level of dissolved oxygen should not be allowed to drop below 2 mg/l.

(c) A concrete seawater storage tank of the dimension 20 x 8 x 8 m (capacity 160 tonnes) should be constructed at a level above that of the depuration tank to facilitate gravity flow into the depuration tank (see figure). The water to be used will be first pumped into a rapid sand filter (preferably 2, arranged serially) to remove all suspended material.

(d) The choices for disinfection of seawater are chlorination, ozonation and UV light irradiation. The latter two are expensive, and hence chlorination (@ 3 ppm) is the method chosen for this project. After chlorinating, the water will be dechlorinated using vigorous aeration and / or neutralization with Sodium thiosulphate.

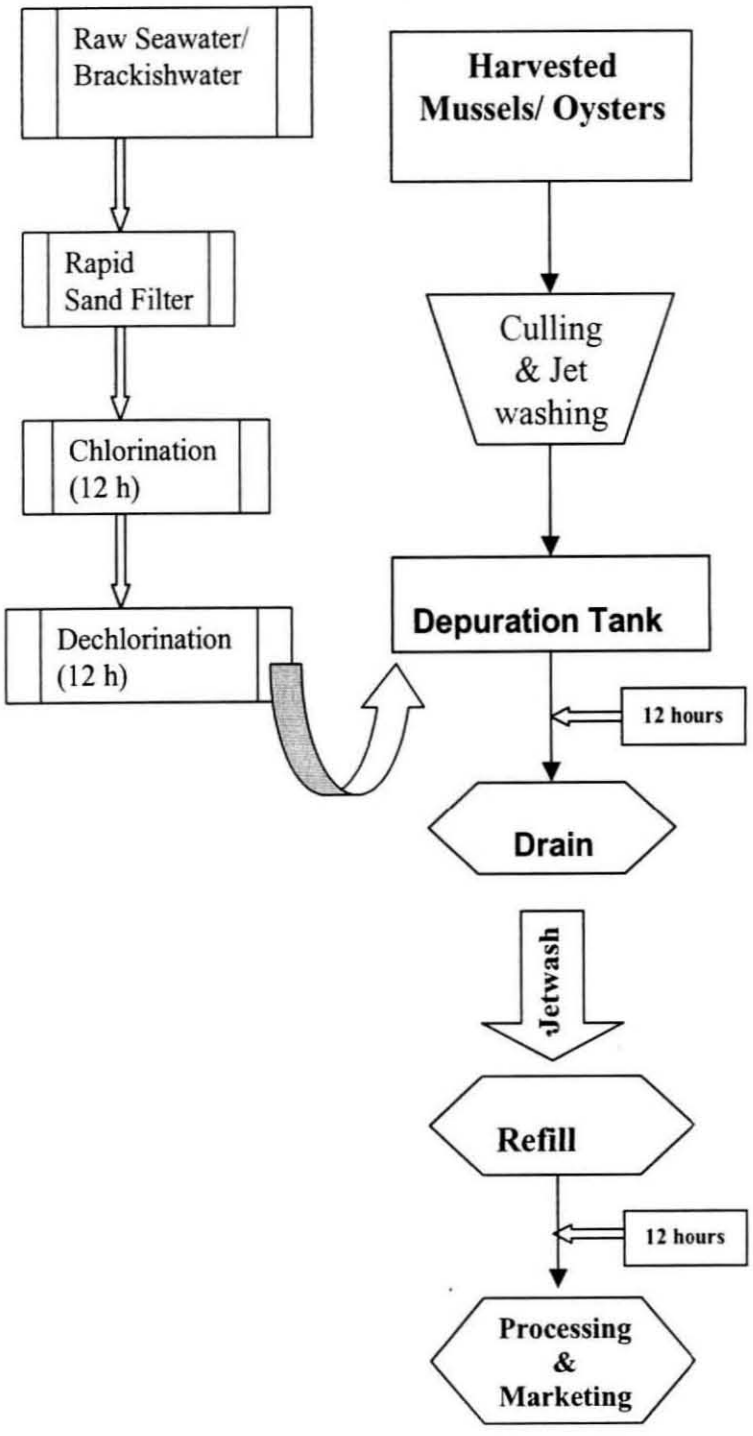
(e) Most depuration plants use flow through, once through or fill and draw principles. It is proposed here to use the batch process (fill and draw), wherein seawater is drawn from the supply treated with predetermined amount of disinfectant to reduce bacterial levels, stored for a time, then pumped to the tank containing bivalves. The process will be repeated once to ensure complete depuration (see flow chart).

(f) Each depuration unit will consist of two concrete tanks of the size 15 x 4 x 1 m with a gradient of 3% to hold bivalves. Bivalves will be placed in perforated plastic trays of standard size. The trays in a single tier will be raised from the tank bottom with the help of PVC pipe runners. The tank will have a drain plugs at the lower end to facilitate cleaning and flushing.

2. Run Duration and Capacity

(a) The duration of the run will be 24 h, in two cycles with one complete flushing for both mussels and oysters (see flow chart). The unit has the capacity to hold 1.0 tonnes of mussels and 0.62 tonnes of oysters per run. The water requirement per run will be 144 tonnes.

Depuration Protocol



33 Environmental Impact Assessment of Suspended Bivalve Culture

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Bivalves are sedentary organisms that require substrate for spat settlement and subsequent growth during which time they filter feed on phytoplankton, detritus, protozoans and bacteria. It is well known that large scale aquaculture can pose complex ecological socio-economic and management problems. As commented by Hastings and Helnte in the introduction to the dedicated issue on "Effects of Aquaculture in the Estuarine Environment (Estuaries Vol. 18 (1)-1995)- *the potential for increased farming of coastal marine waters is considerable but the potential for significant environmental degradation associated with such activities is also large*". Considerable work has been done on the variations in the hydrological, sediment and benthic faunal composition due to mussel and oyster farming by off bottom methods. The main impacts of suspended bivalve farming are given below.

Effect on primary productivity: Commercial large scale bivalves farming will consume substantial quantities of phytoplankton particularly when there is a high density of culture units over a large area, resulting in reduction in primary productivity of the area. In Japan, the culture of 50,000 to 60,000 oysters reduced the amount of seston (predominantly phytoplankton) by 76.95%. Suspended culture of green mussels in New Zealand has been found to remove upto 60% of the available food as the water flows through the farm. A mussel raft in Spain has been found to remove 35-40% of plankton and detritus, whereby 30% of the carbon,42% of the nitrogen and 60% of the chlorophyll *a* of the particulate organic matter is retained. However it has also been suggested that primary productivity may be stimulated by an increase in nutrient cycling although field evidence of increased primary production in the farm vicinity is still lacking. Bivalve culture competes with other planktonic herbivores which has been shown for the Spanish Ria de Arousa where suspended mussel culture replaced copepods as the main pelagic grazing organism.

Effects on current velocity and water movements: Bivalve farm structures modify current velocity and direction of water movements. In turn, these movements may alter patterns of erosion and sedimentation of particulate matter. Reduced water flow may result in decrease in natural erosion by wave action, which in turn is followed by siltation and accumulation of suspended matter in cultured areas.

Effects on sedimentation: Bivalves produce pseudofaeces (mucous-bound particles expelled without passing through the gut) in addition to the normal faeces (biodeposition) which constitutes organic –rich particulate waste. For example in Hiroshima Bay a raft holding 420 000 oysters generate 16 m tons of faeces and pseudo faeces in about a 9 month period and several such farms have been found to have a major impact on the sediment deposition in the bay. Studies have shown that

for a farm covering an area of 1500 m^2 the sedimentation of dry matter would amount to about 10 t, and sediment under the raft would accumulate to 10 cm per farming season.

Table 1. Faecal waste production and from bivalve farming

Species	System	Faecal production
Mytilus galloprovincialis		14.3 –149.3 mgDW/individual/24h
Mytilus edulis	Natural shore population	1.76gDW/gDW/mussel/yr 0.13gC/gDW/mussel/yr 0.0017 g N/ gDW mussel/yr 0.00026 g P/ gDW mussel/yr
Mytilus edulis	Rafts	9.5 kg carbon /sqm/yr 1.1 kgnitrogen/sqm/yr

Sediment accumulation below bivalve farms				
Species	System	Depth	Current velocity	Sediment accumulation
M.edulis	longline	8-13 m	App 3cm/sec	10-15 cm
M.edulis		11-13 m	Very weak	7-30 cm
M.edulis	Rafts	>15m	Upto 200cm/sec	No sig biodeposition, shells present

Effect on benthic productivity: The deposition of particulate organic wastes can result in physico-chemical changes of the substrate, particularly in the immediate vicinity of the culture site. The enrichment of sediment with organic material stimulates microbial activity resulting in deoxygenation of the substrate and bottom waters due to reduced interstitial oxygen concentration and increased oxygen consumption, increased sulphate reduction, increased denitrification and increased release of inorganic nutrients such as nitrate, nitrite, ammonium, silicate and phosphate from mussel beds. The regeneration of potentially limiting nutrients may increase primary productivity.

Effect on benthic community structures: Benthic communities beneath suspended farms may be affected. Macro fauna may be lacking entirely in the area directly under the culture site. Species richness is reduced and opportunistic enrichment tolerant species become predominant. A relatively large number of detailed studies of fine sediment deposition have been carried out. A range of responses of the sea-floor biota have been identified, from little or no community modification after low levels of nutrient enrichment, through to major alterations and the dominance of small polychaetes and absence of larger animals such as molluscs and urchins after high levels.

Introduction of predaters: Introductions of bivalves have negative ecological effects, particularly when parasites and diseases are also introduced. A typical

example is the introduction of the Japanese oyster drill and flatworm to North America from Japan

Impact on birds: The structures could have several impacts on birds. The rope system could impede diving and the pursuit of prey and possibly cause injury to birds. However, there is no evidence that this is a problem, the ropes being coarse and very visible, and birds have been observed feeding within farms on occasions.

Creation of new habitat: The mussel lines can be considered to be new temporary habitats created in the water column for a range of animals in addition to mussels. The epifaunal community on mussels has been recorded to consist of over 100 different species. There is also deposition of live shells, mussel shell litter, and the remains of other associated biota below a farm. 'Shell drop', the deposition of shells, live mussels and associated biota, largely affects the area directly below the farm, typically to 20 m from its boundaries. The value of shell drop in creating a reef-like substrate seems very variable; under some farms the litter is barren, whereas under others there can be a rich biota, including sponges, ascidians, anemones, tube worms together with starfish, sea cucumbers and crabs

Effects on water column: The column is frequently stratified due to the separation of water layers with different densities associated with changes in salinity or temperature. The impacts of a farm can be considered in terms of nitrogen alone, which can at times be at such low levels as to limit plankton growth. The harvesting of mussels will periodically remove nitrogen from the aquatic system. It has been calculated that, based on an average turnover of mussels of two years, denitrification was 68% higher at the farm study site compared to a nearby reference site. Further research is in progress on the role of nitrogen in limiting phytoplankton growth and thus in turn mussel growth. A positive response was seen from adding nitrogen to the water in summer. 'Fertilising' the sea in this way could become a management practice and flow-on effects on zooplankton and fish

Increase in pelagic resources: Farms exclude trawlers from areas, and this has resulted in enhanced numbers of scallops and horse mussels at sites. There is debate about the extent to which the mussel lines and their attached.

Though the impacts are not as large as shrimp and cage culture, the intensity of negative impact cannot be neglected. Bivalve farming practices are simple and are known to provide employment opportunities and promote development of ancillary industries in coastal areas. Hence it is essential that proper management practices be stipulated for sustainable development of bivalve farming industry.

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Selective Breeding of Bivalves

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Introduction

Recently a variety of approaches have been introduced in the field of genetics of molluscs, including, Mendelian genetics, cytogenesis, quantitative genetics, cytogenesis, quantitative genetics, biochemical genetics and hybridization. Wada (1975a, b, 1985) has estimated the response to selection for several attributes of Pinctada fucata for variance of full siblings. He analysed the genetic variability and genefrequencis at three loci in two strains selected for four to five generations. The change of frequencies of colour of nacre in the selected lines of pearl oyster to yellow prismatic layer for five generations has been studied (Wada, 1985), Wada (1976, 1985) and Wada and Komaru (1985) have studied the chromosome morphology of different species of bivalves.

Research Achievements in India

Variations in Indian pearl oyster, edible oyster etc has been noticed as in the case of European, American, and Japanese oyster. Alagarswami et.al, (1983) and (1986) artificially produced Pinctada fucata and P.margaritifera pearl oysters from wild brood. Velayudhan et.al (1996) produced 4 generations of pearl oysters and studied the heritability and noticed inbreeding depression in the hatchery produced pearl oysters at the same time noticed the increase in the percentage of yellow nacre bearing oysters which produced the Golden yellow pearls. He again noticed the nacre thickness in the second generation stock from the hatchery produced stock. The triploid edible oysters were produced in India. Recently it was proved that the pearl oysters from Gujarat had more thickness than from all other regions, Tuticorin, Vizhinjam and Mandapam. The oysters from Vizhinjam had more shell cavity, grow faster and produced larger pearls of 8 mm in diameter. The faster growth and more shell thickness will be suitable for producing larger good quality pearls. Production of triploids, transgenic oysters also will enhance the pearl production in India. The bio-technological approach in Molluscan research will increase the production and quality of molluscs produced in India.

Haley (1977) as reported by Newkirk (1980) has been following the frequency of changes in the 5 full sib families of *Crassostrea virginica*. Matsui (1958), in Pinctada martensii the right shell is slightly convex, where as the left shell is more strongly so. The degree of convexity of shell is very important from the practical point, because oysters with more strongly convex shells harbour larger pearls. Singh and Green (1984) have reported that the relative mortality of the heterozygote (faster growers) of *Macoma bathica* during the larval period is expected to vary from year to year depending on the environmental conditions particularly then relative abundance of the phytoplankton blooms and faster

particularly then relative abundance of the phytoplankton blooms and faster growing heterozygotes with higher food requirements have relatively higher mortality. Triploids in *Macoma bathica*, transgenic forms in Manila clams are also some of the good achievements in Molluscan genetics.

Selection of animals is important in the selective breeding programmes

The more phenotypic variation in a trait the more intense the selection from the natural stock/ populations. The selected oysters are then mated according to a prearranged programme. Unless there are sufficient numbers of spawners, at least 50 numbers, significant inbreeding may occur and a number of stocks can be taken and performance evaluation could be done during the first generation.

Second, one can take a number of stocks and do performance evaluation during the first generation

The third is to cross males and females from different populations to form mixed population. This can be done if parents from a number of stocks spawned together. If we keep each generation (50 males and 50 females) of each stock or line, the inbreeding rate will be 0.5 % per generation and total accumulation of inbreeding after 5, 10 and 20 generations is 1, 3 and 5 % respectively (Newkirk, 1983). More control can be kept exercised and consequently less inbreeding will occur if separate lines are maintained at least in the first generation.

- Inbreeding rate after the first generation with the magnitude of over estimation decreasing as the effective population size increases
- Inbreeding coefficient of matings:

• Half-sib	fx =1/2 (1/2)² or ½(0.25)	Inbreeding coefficient of individual x %12.50
• Full -sib	=1/2(0.5)	25.00
• Sire X daughter	=1/2(0.5)	25.00
• Sire X daughter with Sire inbred	= 1/2[0.50(1.25)]=1/2(0.625)	31.25

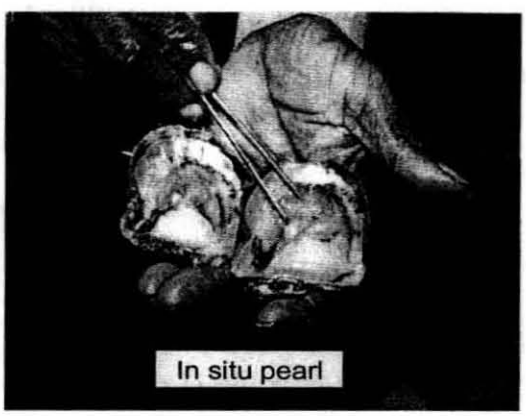
1/8 Nm +1/8 Nf = Inbreeding coefficient				
	Number of male parfents			
Number of female parents	1	10	20	50
1	0.2500	0.1375	0.1312	0.1262
3	0.1667	0.0541	0.0478	0.0425
5	0.1500	0.0375	0.0312	0.0262
10	0.1375	0.0250	0.0187	0.0137
20	0.1312	0.0187	0.0125	0.0075
25	0.1300	0.0175	0.0113	0.0063
50	0.1275	0.0150	0.0088	0.0038
75	0.1267	0.0141	0.0079	0.0029
100	0.1262	0.0137	0.0075	0.0025
150	0.1258	0.0133	0.0071	0.0021
200	0.1256	0.0131	0.0069	0.0019
250	0.1255	0.0130	0.0068	0.0018

BROODSTOCK MANAGEMENT

- Useful to keep the identity of the progeny groups and for providing evaluation of their performance

Needs much care to maintain adults without inbreeding , interbreeding and loss of genetic variation

Tagged oysters



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Ploidy Manipulation in Bivalves

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Introduction

The number of chromosomes is generally specified for each species. Irregularities during meiosis, mitosis or fertilization may produce cells with variations in the number of chromosomes generally specified for the species. Such variations in chromosome number generally specified for the species is called ploidy. Ploidy may occur through duplication or loss of complete set of chromosome **(euploidy)** or a part of the chromosome complement (**aneuploidy**)..

Euploidy: The term euploidy designates genomes containing whole sets of chromosomes. The euploids are those organisms, which contain whole set or sets of chromosomes (genomes) in any number, in their body cells. The euploidy is of following types:

Monoploidy: The monoploid organisms have one-full set of cghromosome in their body cells and is represented by the notation (**n**). When monoploidy occurs in gametes (sperms and eggs) it is termed as ***haploidy***. Most micro-organisms (*e.g.*, bacteria, fungi and algae); gametophytic generation of plants (*e.g.*, bryophytes and other plants); sporophytic generation of some higher angiospermic plants (*e.g.*, *Sorghum, Triticum, Hordeum, Datura,* etc.) and certain hymenopteran male insects (*e.g.*, wasps, bees, etc.) have one genome in their body cells, hence are monoploids. They are usually smaller and less vigorous than their diploid prototypes. Characteristically, monoploid plants are sterile. The reason of sterility is that the chromosomes have no regular pairing partners (homologous chromosomes) during meiosis and meiotic products are deficient in one or more chromosomes. For instance a monoploid maize will have 10 chromosomes and in a gamete it can range from 0 - 10. Consequently, considerable sterility will be found in monoploid maize.

Diploidy: The diploidy is characterized by two genomes (2n) in each somatic cell of the diploid organisms. Most animals and plants are diploids. The diploidy is related with fertility, balanced growth, great vigorsity, adaptability and survivability of the diploid organisms.

Polyploidy: The organisms with more than two genomes are called polyploids. Among plants and animals, the polyploidy occurs in a multiple series of 3,4,5,6,7,8, etc., of the basic chromosomes or genome number and thus causing triploidy, tetraploidy, pentaploidy, hexaploidy, heptaploidy, octaploidy, respectively. Ploidy levels higher than tetraploid are not commonly encountered in natural populations, but our most important crops and ornamental flowers are polyploid, e.g., wheat (hexaploid, 6n), strawberries (octaploid, 8n), may commercial fruit and ornamental plants, liver cells of man, etc. Polyploidy is rare in animals, however has been

reported in *Ascaris, Daphina, Cypris, Drosophila, Bombyx mori, Hbrobracon,* bees, wasps, *Artemia, Echinus, Rana esculenta, Rana pipiens, Ambystoms, Jeffersoniarium, Cricetus cricetus* (hamaster) *etc.*

Polyploidy occurs naturally in somespecies and it can be induced artificially also. Fish like Mahseers, Common carp, Gold fish and some mullets are considered as natural tetraploids. First extensive studies on artificially produced triploid fish were done on three-spine stickleback, *Gasterosteus aculeatus* by Swarup (1959a,b). He reared triploid fishes to adulthood and compared growth rate and fertility with diploids. Mass production of tetraploids was achieved for the first time in Rainbow trout by using pressure shock. To produce polyploid seed on a commercial scale standard protocol for the ploidy induction and efficient screening method for their identification is necessary. Amenability of some fish species (*M. anguillicaudatus*) to chromosome multiplication is amazing! Experimentally produced penta, hexa and heptaploids are reported in in Japanese loach *Misgurnus anguillicaudatus*; and rainbow trout (*O.mykiss*).

Kinds of polyploidy: Following two main kinds of polyploidy, auto and allopolyploidies have been distinguished on the basis of the source of chromosomes.

(1) **Autopolyploidy** - The prefix "auto" indicates that the ploidy involves only homologous chromosome set and

(2) **Allopolyploidy** - The prefix "allo" indicates that non-homologous sets of chromosomes are involved. The union of unreduced or diploid or polyploid gametes from different diploid or polyploid species could produce in one step, an amphipolyploid or allopolyploid.

For example:

Species A x Species B

Diploid chromosome (AA) ↓

AB
(Diploid sterile)

↓ (Induction polyploidy) with cytochalacin B/ colchicine

AABB
(Allotetraploid)

Allopolyploids behave like new species.

Aneuploidy

An aneuploid is an individual whose chromosome numbers differs from the wild type (diploid number) by part of a chromosome set. Generally the aneuploid chromosome set differs from that of wild type only by one (or) a small number of

chromosomes. Aneuploids can have a chromosome number either greater or smaller than that of the wild type. Non-disjunction during mitosis (or) meiosis is the cause of most of the aneuploidies.

Type of aneuploidy

1. Monosomy – The diploid organisms which lack only one chromosome from a single homologous pair is called monosomic.
   (General formula = 2n-1)
   A monosomic produce two types of gametes i.e. (n) and (n-1)
   Turner syndrome in human is a monosomic condition involving" X "chromosome i.e. 46-1, the absent one being an X chromosome.

2. Nullisomic – The diploid organisms that have lost a pair of homologous chromosome are called nullisomic.
   (General formula = 2n-2)
   Nullisomy is mostly lethal. If they survive, the individual will generally exhibit reduced vigour, fertility, survivability etc.

3. Trisomy - Diploid organism having an extra chromosome, i.e. 2n+1. It can produce two types of gametes i.e. (n) and (n+1).
   Klinefelter syndrome is a condition in males which has trisomy involving an X chromosome, i.e. 44Autosomes + XXY. A male has an extra X chromosome.
   Down syndrome: is due to trisomy of chromosome 21.

4. Tetrasomy – One homologous pair is duplicated i.e. 2n+2. Gametes only one type (n+1).

5. Double trisomy – In a diploid organism two different chromosomes have an extra i.e. 2n+1+1

Chromosome Manipulation for Induction of Polyploidy

Chromosome sets can be manipulated by meticulous application of temperature (cold and heat) shock, pressure shock or chemical shock in dividing cells. Shock treatment disrupts the normal cycles of mitosis and meiosis. In freshly fertilized embryos it suppresses the extrusion of second polar body or arrests the first cleavage division and can lead to induction of triploidy and tetraploidy.

Shock Treatment

(a) **Thermal Shock**: Cold shocks are usually applied near 0^0C for cold water species and somewhat at higher temperature (5-12 ^{0}C) for warm water species. Heat shock is applied at a lower temperature (around 26-28^{0}C) in the case of cold water species than in warm water species (37-42^{0}C).

(b) **Pressure Shock**: This method is simple to administer. The pressure range varies between 7000 to 9000 pascals (Psi). The hydrostatic pressure is applied by a specialized instrument designed by mechanical engineering method. Pressure shock

is supposed to have fewer side effects than the thermal shock. But given in a sub-optimal intensity, pressure shock produces aneuploid offspring and

(c) **Chemical Shock**: Colchicine and cytochalasin-B have the potential to disrupt cell division and induce ploidy induction. But the results are inconsistent and unsatisfactory. Anesthetics such as nitrous oxide and Freon 22 have also been tried to induce triploidy. Of late 6-Dimethyl aminopurine (6-DMAP) has been proved to be an ideal chemical for induction of ploidy.

Significance of Polyploidy

Polyploidy has great genetic, taxonomical, evolutionary and economical significance. The genetic significance is that it helps in understanding dosage effect. The taxonomical and evolutionary significance is that it forms new species. Autopolyploidy is less significant in this respect than allopolyploidy, because it adds no new alleles to the genome. Allopolyploidy on the other hand offers great opportunities for production of new adaptive gene combinations and since it accumulates diverse genomes, it provides better adaptability to the species for a wider variety of habitats, which consequently increases the chance of being successful in natural selection. In plants polyploidy at least causes gigantism and accumulation of greater quantities of vitamins etc in the cells and therefore polyploidy provides various economically important food, fruits and ornamental plants.

Triploids are generally infertile and it has many advantages. Triplody therefore results in better growth rate in animals as no energy is wasted for reproduction. In plants triploidy is used to produce seedless fruits.

Procedure optimized for Inducing Triploidy in Edible Oysters

Triploid oysters have been successfully produced at CMFRI. Protocol for induction of triploidy by pplication of heat & cold shock and chemical (cytochalasin-B & DMAP) shock have been optimized. This leads to the retention of the I or II polar body resulting in the production of Meiosis I (M I) triploids or Meiosis II (M II) triploids respectively. Procedure optimized for inducing triploidy in edible oysters at CMFRI is presented below.

Collection and Conditioning of Oysters

Oysters in the size range 60-90 mm of which about thirty percent belong to zero year class or just one year old are recommended. Mature oysters in the size range 60-90 mm are collected and samples of 10 oysters opened for checking the gonad maturity stage. If gonad is ripe the oysters are induced for spawning. If the gonadal condition is in maturing stage, the oysters are kept in the conditioning room, where the oysters are intensively fed with mixed algal culture at 22 ± 1°C (Nayar *et al.*, 1987). After 10-15 days on assessing the gonadal condition the oysters are used for induced spawning experiments.

Brood stock conditioning plays an essential role in successful production of triploids. Ripe eggs uniformly conditioned for fertilization will go through meiosis synchronously and arrive together at the time of treatment. Unripe eggs will produce asynchronous finish. Synchrony is important in the eggs collected from different females. Brood stocks of fully mature males are selected so that the sperms are not only active but will fertilize the egg instantaneously to promote synchronous development. All the females should be at the same stage of maturity so that development in all the eggs from a single female and all the eggs pooled from all the females are synchronous.

Induction of Spawning

Gametes are collected by natural spawning or by stripping the mature oysters. The mature oysters are thoroughly washed and transported to a 100 litre perspex spawning tank containing about 50 litre of sea water at a temperature of 2-4°C above the ambient water temperature with proper aeration. If spawning did not occur within an hour, fresh sperms stripped from a sexually ripe male are introduced in the tank containing the brood stock to induce sympathetic spawning. Once an oyster starts spawning it is transferred to a glass tray containing filtered seawater. One oyster is placed in each tray. Each individual oyster is allowed to complete its spawning in the tray. The gametes in the trays are filtered through a 100μ sieve into 10 litre glass beakers. At this stage mild aeration is given to ensure sufficient supply of oxygen. Care is taken to prevent inadvertent contamination (fertilisation). The eggs are fertilized by adding the sperm. Diluted sperms suspension is used as the dilute suspension will facilitate the dispersion of the sperm throughout the egg and promote synchronous development.

Triploidy Induction

The method generally suggested for the induction of triploidy is by blocking the extrusion of the second polar body through the interference with the second meiotic division of the freshly fertilized eggs. Freshly fertilized eggs were exposed to all the above agents to arrest II PB extrusion and induce triploids. Trials were carried out with different concentrations of DMAP and CB and different temperatures with varying durations of exposure to optimize the protocol for inducing triploidy with each of them. Triploids were identified by the larval chromosome count in the metaphase plates.

The ideal time suggested for treatment of the freshly fertilized egg for arresting second polar body and inducing triploidy is when 50% of first polar bodies have been extruded. In the present study the extrusion of 50% first polar body was achieved 16minutes post fertilization at 28-29°C (normal room temperature). Hence treatments were initiated 17 minutes post fertilization to arrest II PB extrusion using both physical and chemical agents. General scheme of the protocol optimized in the present study for induction of triploidy in edible oyster is shown in (Fig.1). The optimum dosage and duration of treatment standardized for each of the inducing agents are presented in table 1.

Table 1. Dosage and duration of treatment for optimum triploidy in *C.madrasensis* (Thomas *et al.* 2004)

Inducing agent	Concentration / Temperature	Duration
Heat	37°C	5 minutes
Cold	5°C	10 minutes
Cytochalascin- B	0.05mg/l	3 minutes
6-DMAP	100µM	8 minutes

Among the various physical and chemical treatments tried for producing II meiotic triploids, 6-DMAP yielded the highest percentage of 66.6% at larval stage and 61.82% at "D" stage. The results indicate that 6-DMAP is the ideal for inducing triploidy in *C.madrasensis*.

Detection of Polyploid Individuals

The production frequency of polyploidy individuals varies if the shock treatment is not given in proper dosage at appropriate time. It may so happen that a mixture of diploid, triploid, tetraploid and mosaic offspring may be produced. The diploid and polyploid fishes are difficult to differentiate on the basis of external morphology but can be distinguished by following methods:

i) Chromosome analysis is the simplest and appropriate method for determining the ploidy level. But it is very much labour intensive and time-consuming.

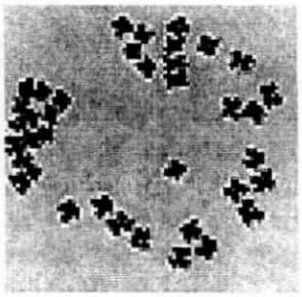
ii) By the measurement and comparison of the nuclear volume and cell volume of the erythrocytes, the ploidy level can be distinguished. This can be done with the help of light microscopy. The electronic instruments like Coulter counter can be used to measure the cell size rapidly.

iii) Flow cytometer is an instrument that helps to detremine the DNA content and cell size. Flow cytometry involves staining the cells with a DNA specific fluorescent dye, propdium iodide, followed by quantification of fluorescence upon laser excitation.

iv) Counting of the number of nucleoli after silver staining method that can be applied in those species having a constant number of nuclear organizer regions (NORs) per cell. However, it is not a suitable methods if the number of NOR varies per cell.

v) Isozyme analysis can be applied insome cases. For example, electrophoretic examination of Phospho Gluco Isomerase (PGI). Esterase and other allozymes in Brown trout (*Salmo trutta fario)* and in some Cyprinids could distinguish the diploid and triploid individuals

Plate. 1 Metaphase plate of triploid edible oyster (3n=30)



The duration and dose of thermal or pressure shock and the optimum conditions for each species have to be found out by trial and error process. Table 2 summarizes the conditions applied for manipulating the chromosome sets in some species of fishes.

Table 2. Conditions applied for manipulating chromosome sets in some species of fishes

Species	Method (shock/duration/time after fertilization)	Reference
A. Triploidy: Retention of second polar body		
Common carp	40 or 41^{0}C/2 or 1.5 min/6 min	Recoubratsky *et al.*, 1992
Grass carp	5-7^{0}C/25-30 min/2.0 - 4.5 min	Cassani & Caton, 1985
Laboe rohita	42^{0}C/1-2 min/ 7 min	Reddy *et al.*, 1990
O.mossambicus	42^{0}C/3 min/2.5-4.5 min	Varadaraj & Pandian, 1990
Ictalrus punctatus	7500 psi/2-5min/2.5 min 0^{0}C/1 hr/5min	Wolters *et al.*, 1981
Heteropneustes fossillis	4^{0}C/30 min/ 2min	Tiwary *et al.*, 1997
B Tetraploidy: Prevention of first cleavage		
Rainbow trout	490 Kg-cm^2 4 min/ 5.8 hr	Chourrout, 1984
O.aureus	11.0^{0}C/ 60 min /80-104 min	Don & Avtalion, 1988
l. Punctatus	40^{0}C/80-90 min/ 3 min	Bidwell *et al.*, 1985
Plecoglossus altivelis	650 kg-cm^{-2}/ 6 min/80 min	Taniguchi *et al.*, 1990

Figure. 1. Scheme for triploid induction in Edible oyster

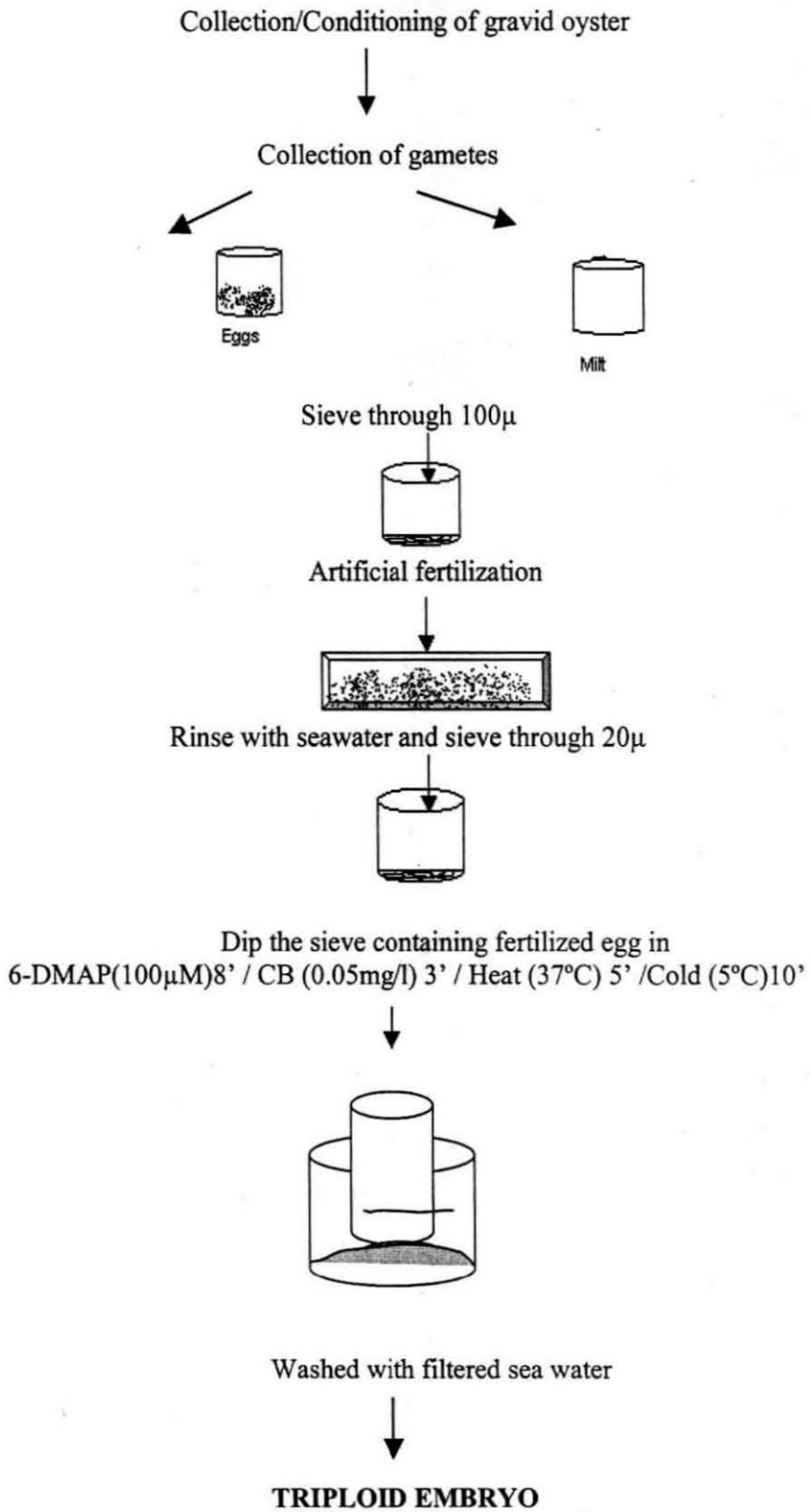
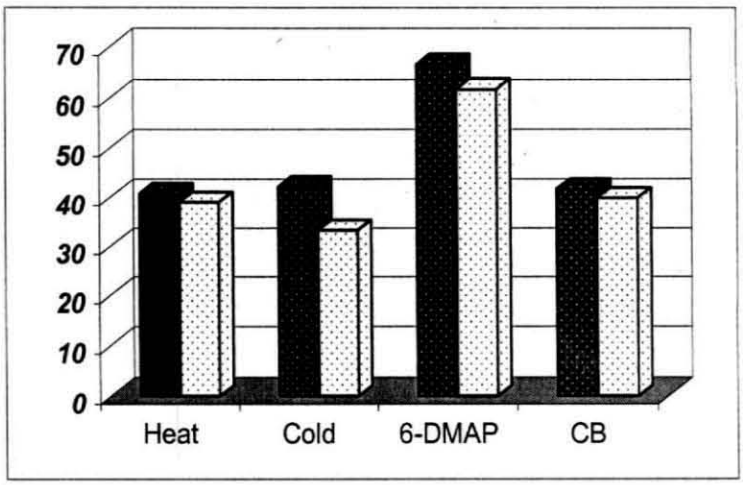


Figure 2. Relative efficiency of triploidy inducing agents in *C.madrasensis*



Applications of polyploidy in aquaculture

Polyploidization may enable an animal to adapt to varying environmental conditions. Common carp and the catostomid fishes are naturally polyploid species. It is believed that the polyploid genome enables these species to live in diverse ecological conditions. Triploid hybrids are more viable than diploid hybrids. Triploids are also more heterozygous and heterozygosity helps in maintaining their developmental stability.

Triploidy led to sterility in some fishes like Grass carp and Channel catfish. Triploid Grass carp cannot breed in natural waters and thus does not pose any environmental problem if transplants into larger water bodies for weed control. The culture of triploid Channel catfish is more profitable.

Sterile triploid progeny can be produced by crossing tetraploids with diploid ones. Triploid Rainbow trout produced by mating of tetraploid female with diploid male exhibited higher growth rate and survival than artificial triploids. Diploid spermatozoa of tetraploid males are useful for producing androgenetic offspring.

In India research on induction of triploidy is mainly undertaken at the Central Institute of Freshwater Aquaculture (CIFA), Bhubaneshwar, where triploids of most of the major carps and their hybrids have been produced. In the Central Marine Fisheries Research Institute (CMFRI), Cochin, the technology has been perfected for the production of triploid edible oysters (*Crassostrea madrasensis*) by arresting the release of both first and second polar bodies by chemical (6-DMAP) treatment They observed 30% increase in growth and 2 fold increase in glycogen and lipid content in triploid oysters.

36 Concept of Integrated farming - Bivalves as Bio-filters

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Background

Environmental concerns have surfaced in the recent years and it has been proved that for long term growth of aquaculture industry both ecologically sound practices and sustainable resource management are a pre requisite. Among the aquaculture practices, semi intensive and intensive farming of shrimp and finfish have done considerable damage to the environment. The excessive use of supplementary feeds and the metabolic wastes from high-density farms have made the effluent pond water quality detrimental. Very high levels of suspended solids, organic carbon and frequent algal blooms are all indicative of the ecological imbalance – signs of negative impacts. Suspended solids are essential for growth of shrimps and absence of suspended solids may also adversely affect shrimp.

Intensive fish culture systems also have produced detrimental impacts. The feed required to produce 1 tonne of fish contains 110 to 130 kg N, of this 20-25% is retained in the fish, and remainder is either not ingested or converted to waste products. Similarly high concentration of Chlorophyll *a* has been reported within 500 m of the salmon farms. To utilise excess algae and suspended solids, farming bivalves and seaweeds has been suggested. Bivalves low in the food chain are filter feeders and seaweeds are autotrophic utilising the dissloved nutrients.

Bivalves as Biological Filters

Bivalves subsist mainly on particles filtered from the surrounding water, which they pump through the lamellae of their gills. Filtration rate can be termed as the volume of water from which all particles are removed in a given period of time. Filtering rate is equivalent to pumping rate if all the suspended particles are removed from the water passing through the filtering mechanism. The filtration rate of a bivalve depend on a) size of the species b) environmental conditions like temperature, salinity, pH etc c) water movement and d) particle size and their concentration/ density. Some of the particles are utilized while others are rejected as pseudofaeces. Studies have also shown that bivalves remove more cells from flowing water than from stagnant water.

Phytoplankton has been identified as the main component of bivalve feed. Apart from phytoplankton suspended solids are also observed to play a positive role in bivalve growth. Several studies have indicated that considerable weight gain is observed in oysters when a small quantity of suspended solids is added to the oyster diet. Wyban *et al* (1988) has found that diatoms, which are excellent food,

dominated the algal blooms in shrimp ponds. Considering these it is suggested that the solution to shrimp pond water effluent control may well in the utilization of the effluent instead of the current practice of discharging it into the open waters

Bivalves like mussels, oysters and clams are considered a delicacy in the temperate countries and the possibilties of utilising the nutrient rich water from the shrimp pond for farming oysters was researched since the 70s (see Table 1).

1. Hhigher growth rate and survival of bivalves grown with fishes and prawns.
2. Low fouling and good shape for the farmed oysters indicating their suitability for half shell trade
3. Sustainable production of shrimp.
4. Possible to reduce the input of fresh water by 50% (through reduction in phytoplankton concentration) in fishfarms when *oysters* were stocked with the fish.
5. Bivalve farmed in effluent drainage canals can reduce the concentration of organic matter by approximately 50%. Can significantly reduce the concentration of Ammonia –nitrogen, nitrite -nitrogen, phosphate and total suspended and bacterial numbers solids per tonne of shrimp pond water.

Model Systems

Reduction of particulate organic matter by sedimentation and microseiving has been found to be relatively expensive requiring regular maintenance. The biological treatment of sewage by algae and bivalves has proved to be efficient and expensive but the questionable quality of the cultured organisms as food has lead to the discontinuation of this method in many areas. Such objections do not arise for biofilter organisms cultured in fish/ shrimp pond effluents as long as the fish/ shrimp consume commercial feed and the water source is clean.

The concept of developing an environmentally clean aquaculture practice based on an integrated fish –mollusk- seaweed system has been tried at the National Center for Maiculture in Israel. In the model the water from the fishponds drains through an earthern sedimentation pond, a bivalve filtration unit and a seaweed filtration /production unit and is finally discharged back into the sea. An additional loop recirculates water from the sedimentation pond through a bivalve production unit. The performance of each of the component in terms of total nitrogen budget is: fish yield, 26% of N introduced in the feed; bivalve yield , 14.5%; seaweed yield,22.4%; settled feces,32.8% suspended and dissolved discharge back into the sea, 4.25%. Folke and Kautsky (1992) have also proposed a model for integrated coastal aquaculture linking species from different tropic level such as salmon, mussels and seaweeds. Building on this model Newkrik (1992) has suggested that environmental impact can be further reduced by including a benthic species such detritus consuming bivalves, bait worms etc.

Feasibility of Integrated Shrimp Aquaculture with Bivalve Farming in India

India has a diverse range of cultivable bivalve species among the Indian backwater oyster like *Crassostrea madrasensis* and the green mussel *Perna virides*

is commercially farmed. Though these are euryhaline, tolerance levels, the lower limit of salinity tolerance varies widely. Integration of shrimp culture with green mussel was done in a shrimp farm along the Gujarat coast with technical guidance from Central Marine Fisheries Research Institute, Cochin. The farm had a waterspread area of 9.36 ha with 9 ponds of 0.5 ha and 5 ponds of 0.25 ha independently fed through a feeder canal and dained into a drain canal. The drain water was collected in a waste settling pond of 0.5ha before disposal. The pond was provided with paddle wheel aerators. About 306 kg of mussel was obtained from 48m^2 area (32.6% meat percentage). With a stocking density of 60,000 per ha it was possible to obtain 330 kg Penaeus *monodon* in 150 days (Subramanyan and Gopalakrishnan 2000). Similar results were obtained in Goan shrimp farms also.

Growth and survival of bivalves are location specific and information on their tolerance ranges and filtration rates is essential before stocking them with shrimp. Rajesh *et al* 2000 has found that the filtration rates of these commercially important species vary with the salinity of the environment, the concentration of the algal species and the size of the species. Oysters were found to be having higher filtration rates than mussels and clams. Experiments conducted at the demonstration farm of CMFRI at Ashtamudi Lake, Kerala has shown that the brown mussel, *Perna indica* is not suitable for farming in the estuaries. Though the salinity range falls within the tolerance limit, their growth and survival was very low. Location testing studies have shown that the oyster *Crassostrea madrasensis* can survive in low salinities even below 10 ppt. while for the mussels it is on the higher side; between 15 and 18ppt. Based on these it is possible to work out a model for different coastal regions of India.

Feasibility of integration of shrimp and finfish in a bivalve farm was experimentally tried in Ashtamudi Lake, Kerala with the main objective of increasing the profit obtained from the bivalve farm. In the estuarine systems the usual grow out system is the wooden rack. In this farm where mussels and oysters are the main crops, shrimp seed, *Peneus monodon* and *Etroplus suratensis* were stocked in separate closed cages in the space between the vertical poles. High growth rates and survival were observed for both the species. Though only very few studies have been conducted in this line, the preliminary results have indicated the scope for developing an integrated approach in the aquaculture practices of India.

Though bivalves can be considered as natural clearing agents of blooms, they filter other substances like calcium from the system. Since calcium carbonate is the major component of clam and oyster shells it would undoudtely be depleted from the water faster than other salt. Galtsoff (1964) found that *Crassostrea virginica* (size unspecified) held in flowing seawater deposited a median of 1.4 mg of shell material /cm^2 of shell surface per day during peak growing season. Excessive pseudofeces production coupled with low water movement can damage the benthic habitat structure and ecological web. Before taking up integrated farming it is essential that the information be gathered.

1. The rate of consumption of food, oxygen and dissolved chemicals by the animals
2. The rate of production of wastes by the animals

3. The tolerance of the animals to various water qualities conditions and particularly those resulting from accumulation of their own wastes.

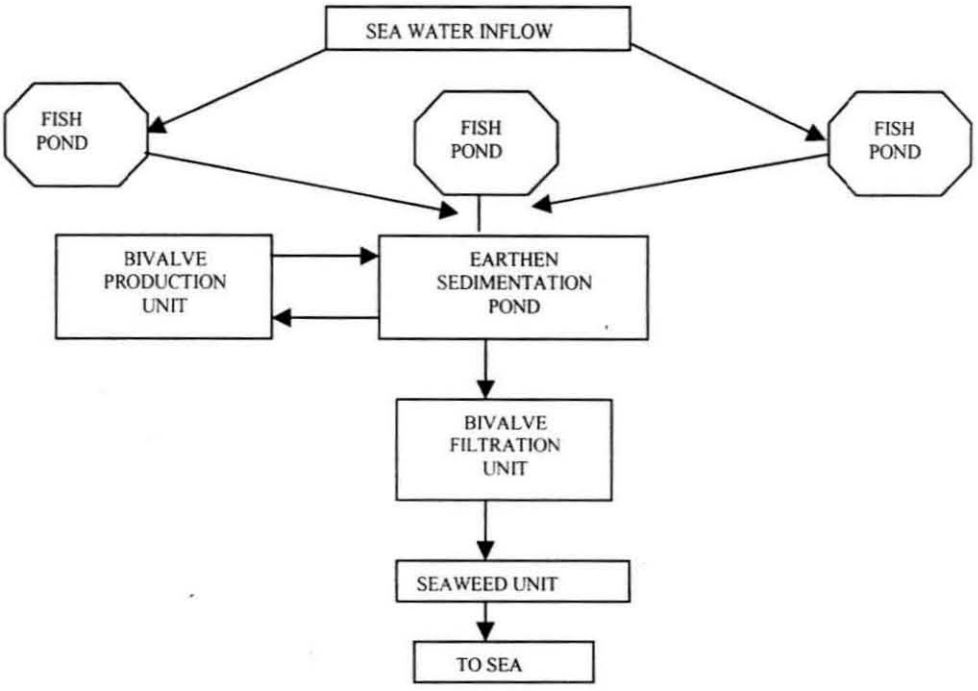
Among the bivalves, oysters and mussels will be better than clams. Studies have shown that placing bivalves on bottom will effect their survival (Hopkins *et al*, 1993). Most clams are infaunal and their growth and survival depends on the nature of the substratum in habit. Moreover shrimps are mostly benthic and their feeding habits also do not recommend the use of clams in shrimp ponds. Suspended culture of oysters and mussels will be beneficial to both bivalves and shrimps.

Table.1. Details regarding the integrated farming experiments

Candidate groups	Observations
Crassostrea gigas with fishes and prawns	Higher growth and survival of oysters but very little gonad development, oyster shape was good for half shell trade Oysters free from fouling.
Oyster with shrimp, *Penaeus vannamei*	Growth and survival of both species high, Oysters free from Dermo, *Perkinsus marinus*
Crassostrea virginica in commercial shrimp pond water	Capable of producing excellent half-shell oysters in Hawai. High growth rate (0.1 g to 54.2g in 198 days) survival 96%, meat to shell ratio 16.3 and condition facter 14.9%
Ulva lactuca grown in fish pond effluent in Israel	10 m^3 of Ulva can remove 90% of the ammonia produced by approximately 75 kg of fish
Seaweed in shrimp farm effluent water	In 24 hrs ammonia –nitrogen was absorbed by seaweed at 100% efficiency and BOD_5^{20} reduced by 39%
Abalone,(*Haliotis rufescens*), mussels , (*M. californianus*), and spot prawns, (*Pandalus platyceros*)	Growth of abalones and prawns were significantly higher in Polyculture systems
Crassostrea gigas with chinook salmon, *Oncorhynchus tshawytcha*	Growth and condition indices of oysters near the fish farms three times higher, growth increments were dependent on POM and Chlorophyll a
Sparus aurata (gilthead seabream) with *Crassostrea gigas*	Reduction in phytoplankton level possible to reduce the input of fresh water by 50%
Artemia and green mussel	In 24 hrs could reduce ammonia nitrogen, chlorophlll a, total suspended soloids and BOD in effluents with an efficiency of 67,87,13 and 77% respectively
Peneaus monodon and mussel	Good production of mussel and shrimp, mussel culture component played played a significant role in sustainable shrimp production
Prawns and clams	Net profit increased by 169.53% compared with monoculture

Penaeus vannamei, Clams, *Mercenaria mercinaria*, and oyster *Crassostrea madrasensis*	Growth and survival of shrimp not affected by the bivalves Low survival of clams and oysters placed on bottom,, high,95% survival of oysters placed in trays
Crassostrea virginica grown in effluent water from shrimp pond in Hawaii	High growth rate of oysters – hydrographic parameters not studied.
Crassostrea gigas grown in fish farm effluent	Better growth rate, condition indices in oysters grown in the effluent water Reasons attributed are Higher algal diversity, additional nutritious food consisting of benthic diatoms and stable algal concentrations
Green mussel in Shrimp effluent drainage canals	1kg of mussel for an effluent load of 4 tons per day reducing the concentration of of organic matter by approximately 50%
Penaeus japonicus and *Ostrea rivularis*	Shrimp yield increased by 30%, survival rate of oysters raised by 17%, meat percentage increased by20.3%, High economic benefit
Perna virides grown in effluent water from shrimp ponds.	One kilogram of mussel significantly decreased the concentration of Ammonia – nitrogen, nitrite – nitrogen, dissolved oxygen phosphate and total suspended solids per ton of effluent.
Saccostrea cucullata and *Penaeus japonicus*	Reduced the effluent total suspended solids to 49%, Bacterial numbers to 58%, Total nitrogen to 80%, Total phosphorous to 67%, Chlorophyll a to 8%
Crassostrea madrasenis in shrimp farm effluent	Higher level of reduction of suspended solids and chlrophyll concentration possible with larger oysters

Fig.1. Schematic diagram of the integrated system proposed by Shpigel et al (1993)



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37 Concept of Organic bivalve farming

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Organic farming is based on holistic production management systems, which promote and enhance agro-ecosystem health, including biodiversity, biological cycles and biological activity. Complete lack of application of fertilizers and feed including antibiotics, vaccines and growth hormones during the farming period either to promote growth or to increase survival is one of the advantages of molluscan shellfish farming. Molluscan shellfish are biofilters, which have the beneficial secondary effect of taking up nutrients and purifying the water column, thereby "enhancing ecosystem health." In addition to this the farm structures, provide cover and forage for other species - enhancing biodiversity, biological cycles and biological activities through the creation of critical habitat.

Organic certification addresses the processes involved in production rather than the qualities of the product itself. The molluscan shellfish farming processes adhere to the principles of organic farming by default. However, bivalves being grazers or filter feeders can accumulate pollutants, pathogens and algal biotoxins, which pose a threat to the health of the consumer. Hence the public health safety standards on which the shellfish industry is based will be applied to the concept of organic farming in molluscan mariculture. Monitoring of the growing areas, its classification, certification of farm sites in terms of water quality are essential to evaluate the quality / safety of farmed molluscs. Periodic residue testing is also permitted.

Fundamental Organic Certification

Water quality is an essential part of Organic farming. The main pollutants, which hamper the quality of bivalves, are microbial pollutants, biotoxins and trace metals. Simultaneous observations of the level of these pollutants in the environment and the farmed animal are essential for organic farm certification. Targeted research on this aspect for a period of three years is essential for certification.

Protocols of bivalve farming, which are conventionally followed, have been critically evaluated and modifications at various levels such as method of farming, source of seed, method of seed collection from natural bed, type of cultch material used, pest control, waste management stocking density, method of harvest and processing have been suggested.

Impact on the Environment should be minimal and it has to be proved that the farming has not hampered the normal ecological and biological processes taking place in the environment. Its impact on the naturally occurring species should be documented. This is vital for certification.

The general guidelines currently available are intended for bivalve farming in temperate countries. Comparison of bivalve farming under the Indian conditions and the temperate waters show significant variation *viz.* short grow out period of 5 to 7 months in India compared to 36 to 48 months in temperate regions, occurrence of non toxic blooms in nearshore areas rather than toxic blooms as in temperate waters, dependence on natural seed by Indian farmers rather than hatchery produced spat or genetically modified or triploid / tetraploid spat etc.

Status of Organic Farming

Molluscan shellfish industry has been subjected to considerable threat and public criticisms in the beginning of last century due to human fatalities resulting from consumption of shellfish. To safeguard the interests of this industry, the Government and the industry jointly initiated several programs in several parts of the world. One of the main programs started in 1920 in the United States is the National Shellfish Sanitation Program (NSSP) a cooperative program among the Food and Drug Administration (FDA), State and the foreign Shellfish Regulatory Authorities. The public health regulations governing the shellfish farming and harvesting are among the strictest imposed upon any food producer in the U.S., and pose an excellent foundation that aligns well with the organic standards. Under the NSSP, each growing area must be tested for pathogens for several months before they are classified. Growing areas are classified based on the water quality Quayle and Newkirk (1989) and harvesters to use tags on each container the company name, harvest location and date and it is illegal to sell shellfish to the public without proper commercial certification.

With the development of global interest in organic farming, the question of developing standards for molluscan farming arose before the Certifying agencies. Unlike shrimp farming, the open nature of the bivalve farming, without closed boundaries posed several problems.

In the US, a report by Goldberg and Triplett (1997) titled "Murky waters: Environmental Effects of Aquaculture in the United States" documented the adverse impacts some types of aquaculture are having on the environment. A strong recommendation which came out of this is the need to develop organic or ecocertification programs that empower consumers to chose aquaculture products grown in an environmentally friendly sound manner and that give aquaculturists incentives to produce products which can bring higher prices.

One of the guidance documents which has critically considered the various points regarding the certification of bivalve farming is the: "White paper : Developing Organics standards for Mollusk Shellfish" by Dr.Robin Downey, Executive Director, Pacific Coast Shellfish Growers Association, USA (Reference: wed site http://www.pcsga.org/). This document considers the principles of Organic Agriculture as it applies to Shellfish farming and lists some points for consideration while formulating standards that can be further developed based on the shellfish industry and organics experts to assure equitable and implementable standards. One of the major points suggested in the document is reproduced below in the box.

Areas for consideration in establishing criteria and management plans for organic molluscan shellfish standards: To obtain organic molluscan shellfish certification:

1 a) Require shellfish growing areas to be in the "Approved" classification status for three consecutive years and require periodic lot testing at intervals of 6 months.

OR

b) For Conditionally Approved growing areas, and only during the "open" status, require lot testing once a month during "dry" weather periods or once a week during periods of intermittent rainfall. Following periods of closure due to rainfall, require testing on the first lot harvested once the areas achieves "open" status again.

2. Require shellfish growers to adopt Environmental Codes of Practice that include farm plans that explicitly describe their sustainable and conservation management farming and processing systems.

Source: "White paper: Developing Organics standards for Mollusk Shellfish" by Dr.Robin Downey, Executive Director, Pacific Coast Shellfish Growers Association, USA (Ref wed site http://www.pcsga.org/).

One of the certifying agencies for Organic Aquaculture is the " ***Naturland"***, a member of the international umbrella organization- International Federation of Organic Agriculture Movement (IFOAM), which issues binding standards in the fields of both production and processing. They have elucidated standards for culture of marine mussels (Ref: website www.naturland.de - Section I: general guidelines and Section III C). Importance has been given to site selection, type and origin of stock and culture systems. Site is required be Class IA, wherein the faecal *Eschererichia coli in* the farmed mussel should be ≤ 3 counts /g tissue), the origin of the seed be traceable to the area of collection and on bottom faring is prohibited. Another organization the BIO-GRO has set standards for New Zealand (Ref website: www.bio-gro.co.nz) and as per the document revised in April 2001 (version 1:30 under Module 4.7.) importance has been given to site and presence of biotoxin.

The organics movement in New Zealand incorporated the marine environment and there are several BIO-GRO oyster farmers substantiating the fact that demand for organic products is reaching aquaculturists (Ref :Organics and Aquaculture , Paper by Dr.Sean Handly). In support of the organic aquaculture production, the NZ has in place some of the most stringent export testing regimes facilitating the farmers to sell their products in the most challenging overseas market like the U.S. and Japan. The NZ Mussel Industry Council has also produced its own Environmental Code of Practice- the first marine farmers to do so in the world.

In London a charity has launched world's first seafood products under the " Marine Stewardship Council " standards (MSC) – an initiative with links to the

Dutch organic certifier "Skal" and the World Wide Fund for Nature WWF in the Netherlands (MSC-2000, Agro- Eco Consultancy 1999). One of the first seafoods to be launched under the MSC standards is the mussel and cockle fishery in the Waddensea and this is under pre certification process (MSC 2000, agro-Eco Consultancy 1999)

Table.1. Guidelines set for Oyster Farming (in British Columbia) and Mussel (for details refer COABC site)

Bivalve Farming Feature	**Required**	**Prohibited**
Grow out method	Off bottom like rack, long line, stake	On bottom
Material for mussel seeding	Net /rope should be appropriate for reuse. They should be decomposed or recycled after use	
Location of farm site	Maximum possible turn over from open sea	Mussel culture in immediate proximity to shore or close to nutrient inflows is not permitted
Water quality	• Should meet the criteria of sites classified as "Approved ' in terms of general water quality, trace metal content, biotoxin levels and microbial load • Should be monitored monthly and recorded for reference	Polluted water bodies, areas with history of toxic algal blooms and high loads of enteric bacteria
Seed	From natural bed or hatchery	Triploid or Genetically Modified
Setting of larvae in the hatchery		Use of epinephrine
Collection of wild spat	Collecting activity must be documented and traceable to respective collecting area, quantity of seed collected, name of seed collector,	Collecting activity should not cause lasting damage to the natural ecosystem
Type of cultch material	Shellfish shell, food grade plastic, cement and French tubes made of allowable material	Tires, plastics that are not food grade quality, plastics that have previously contained toxic or harmful material, new PVC French tubes
Pest control	Should have only minimal impact on the fish and wildlife habitat	Fungicides, traps etc
Waste management	Shells and other wastes must be disposed of and should not attract vermin &insects	
Shellfish stocking density	Must reflect health of the organism	Should not exceed the sustainable yield of the ecosystem in which the farm is situated
Harvest	Producers must only harvest shellfish within the boundary of their production site	Dredging
Visual quality	Tidy and uniformly laid out site Floatation devices must be of uniform shape and colour	

Important Web Sites

Agro-Eco Conultancy.1999. MSC pre-certification of mussel and cockle fisheries-the Netherlands. http://antenna.nl/agrroeco/welkom.htm

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38

Cephalopod Culture

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Introduction

Cephalopods are the largest and most active invertebrates. About 1, 17, 278 tonnes of cephalopods are exploited during 2003 in India (Annam *et al.*, 2004). During 2002-2003 India has exported 41,381 tonnes of frozen cuttlefish and 37838 tonnes of frozen squid valued at US$ 166.2 million to countries such as Japan, USA and the European Union (Anon, 2003). Cephalopods are unique because they are 85% protein by dry weight (16-21% by wet weight) (Lakshmanan and Balachandran, 2000) and are considered a delicacy in seafood restaurants.

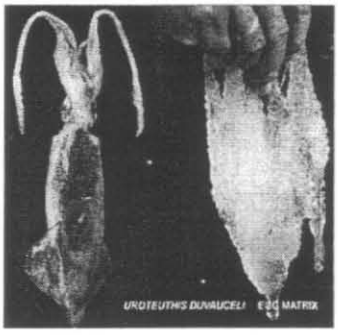
Recent years have witnessed a significant amount of research interest in cephalopod culture, in order to develop technology for commercial farming as well as to produce multiple laboratory generations for research in neurobiology (Minton *et al.* 2001). They are highly promising biomedical models because of their giant axons and are of interest to neurobiologists. Squids 4 months old have giant axons larger than 450μm in diameter. Studies have shown that the ultrastructure and physiology of these systems rival the sophistication of their vertebrate counterparts, the vestibular end organs and the vestibulo-oculomotor system. In detail, many parallels exist, e.g., the dynamic response characteristics (gain and phase lag values) of the cephalopod angular acceleration receptor systems are similar to those of the vertebrate semicircular canals, the putative transmitters in the afferent and efferent fiber systems are similar, and the cephalopod brain pathways involved in oculomotor control have vertebrate-like organizations. Thus, these systems are interesting invertebrate models that can substantially contribute to our understanding of the basic principles of morphology, physiology and pathology of these systems in higher vertebrates, including man.

Choe and Oshima (1963) and Choe (1966) reared three species of the genus *Sepia*, the squid *Sepioteuthis lessoniana* and the sepiolid *Euprymna berryi* from egg to adult size. Nabhitabhata and co-workers of Rayong Brackish water Fisheries Station have conducted pioneering research work on the culture of several species of commercially important cephalopods in Thailand (Nabhitabhata, 1978a, b, Nabhitabhata *et al.*, 1984 and Nabhitabhata and Nilaphat, 1999). *Sepia pharaonis* was successfully bred under laboratory conditions in Thailand as well as the USA using sophisticated, temperature controlled recirculation systems (Nabhitabhata, 1994, Minton *et al.*, 2001).

In India our first major success in Cephalopod Mariculture was realized in 1999 with the cuttlefish *Sepiella inermis* (Sivalingam, 1999) at Tuticorin Research Centre of CMFRI. Since that time we have worked on squids *Uroteutis duvauceli*, *Sepioteuthis lessoniana*, *Doryteuthis singhalensis*, cuttlefish *Sepia pharaonis*,

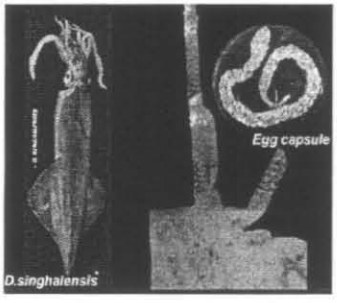
Sepiella inermis and Octopus *Octopus dollffusi*. However, for the past three years we have focused our efforts on developing the potential of the cuttlefish *Sepia pharaonis* and squid, *Sepioteuthis lessoniana*.

Egg Masses of Different Species of Cephalopod

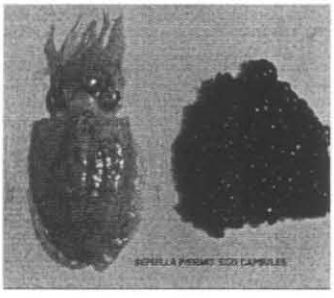


Egg mass of *Uroteutis duvauceli*

Egg mass of *S.lessoniana*

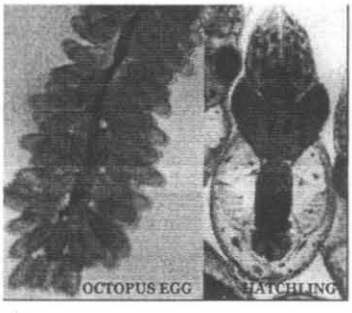


Egg mass of *D. singhalensis*



Egg mass of *S. inermis*

Egg mass of *S. pharaonis*



Egg mass of *O. dollffusi*

Rearing of Cephalopods

Cephalopods require high standards of water quality while feeding at high rates and producing copious quantities of ammonia and ink.

Water Quality

The water quality criteria for cephalopod culture in both nursing and grow-out phases regardless of species, are as follows: -

Dissolved oxygen:	>5 mg/l
Salinity	: 30-35 ppt
Temperature	: 27-32^{0}c
P^H	: 7.0-8.5
Ammonia	: <0.005 mg/lit
Nitrate	: <25 mg/ml.

Food and Feeding

The limitation is that the cephalopods are carnivorous and selective feeders; they require live feed with a specific size, shape and movement. Feed without these characteristics will be ignored and the cephalopods will starve to death. The degree of selectivity is higher in the early stages compared to the adults. After a stage they can be trained to accept low value fish.

Brine shrimp nauplii, which is used as live feed for most of the cultivated marime fishes and shellfishes, is unfortunately not suitable for cephalopods. But adult brine shrimp can be used as a feed supplement. Mysid shrimp collected from natural waters is used world over to rear cephalopod hatchlings. Experiments conducted in Thailand and India have shown that live prawn postlarvae can be used as feed for Cephalopod but will substantially increase the production cost. In USA the first successful defined diet formulated specifically for cephalopods.

At Karwar Research Centre of CMFRI, spineless cuttlefish *Sepiella inermis* was successfully reared from the egg mass collected from wild. They mated under captivity and spawed on 86th day at a size of 60 mm mantle length producing 214 viable eggs. Only live food organisms, consisting of mysids, shrimp post larvae, and juvenile fishes formed the diet of these animals in different stages. The initial average size of hatchling was 4mm ML (0.02g) that increased to 69 mm (54.67g) on 110th day. Average survival was 43, 37 and 28% at the end of first, second and third months (Anil, 2003).

At Vizhinjam Research Centre of CMFRI, Pharaoh cuttlefish (*Sepia pharaonis*) was successfully reared from egg to an average size of 168 mm mantle length (ML) and weight of 521 g in 226 days in the laboratory, using simple biological filtration systems. The period of egg incubation was 15 days at a temperature range of 27-31 C. Food items given were live mysids, *Artemia salina*, juveniles of fishes and prawns. Subsequently, the juveniles were slowly acquainted with food items such as dead caridian prawns and small fishes. Hatchlings were reared at a stocking density of one animal/litre during the first month, and subsequently stocking density was reduced as the growth proceeded. The study shows that the pharaoh cuttlefish can be reared under captivity with a survival rate

of 40% with the use of live feed limited to the initial phase of 50 days. (Anil *et al.*2004).

At Vizhinjam the PallkBay squid *Sepioteuthis lessoniana* was also successfully bred under captivity. The squids reared from egg masses collected from wild, in rearing systems containing biological filtration units, successfully mated and spawned on 105th day of rearing.

With the use of cage type of rearing systems in open waters and with better feeding schedules, commercial culture systems with good survival rate and growth can be developed. The future of cephalopod culture depends on the development of mass culture techniques of mysids for feeding hatchlings with *Artemia* as supplement and artificial feed for the adults. The recent success achieved in feeding the young ones with *Artemia* as supplement and acquainting the cuttlefish to food items other than live feed such as anchovies and sardines which can be obtained in large quantities are steps in this direction.

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CMFRI

Winter School on

Recent Advances in Mussel and Edible Oyster Farming & Marine Pearl Production

Compiled and Edited by

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Technical Notes

CONTENTS

1 Production trends in Indian marine Fisheries and need for Mariculture

N.G. K. Pillai, Pelagic Fisheries Division
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India is one among the top ten fish producing countries in the world contributing over 3% (6 million tonnes (mt)) of the world marine fish production. The fisheries sector in India contributes nearly Rs.22, 000 crores to the total national income and form about 1.4% of total national gross domestic product (GDP) and 4.5% of agricultural GDP. The sector provides employment and income to over 10 million fishers and fish farmers. The marine fisheries sector in the country contributes about 50% of the total fish production.

Among the countries bordering the Indian Ocean, India, endowed with a coastline of 8129 km, 2.02 million km^2 of EEZ and 0.5 million km^2 of continental shelf has a catchable annual marine fishery potential of 3.93 million tonnes occupies a unique position. Besides, there are vast brackish water spread areas all along the coastline, which offer ideal sites for seafarming and coastal mariculture. Among the Asian countries, India ranks second in culture and third in capture fisheries production, and is one of the leading nations in seafood export earning annually over Rs.6500 crores (forming about 29% of agri. exports). Marine fisheries sector occupies a very important place in the socio-economic development of the country. The sector has been recognized as a powerful instrument to generate income and employment as it stimulates growth of a number of subsidiary industries and is source of cheap and nutritious food besides being a foreign exchange earner. At the same time it is an instrument of livelihood for a large section of economically backward coastal population of the country.

The fisheries research during the last five decades together with the technological advancements in the harvest and post-harvest areas have accelerated the process of transformation of a traditional, subsistence oriented marine fisheries into a market driven multicrore industrial sector. With the result the marine fish production has made great leaps through successive stages, first with a change from natural to synthetic fibers in gear fabrication and a concurrent introduction of mechanised trawlers in fifties, second with the introduction of mass harvesting gear, the purse seine along the southwest coast in 70s and immediately followed by the introduction of motorisation (outboard engine) of country crafts and the subsequent proliferation of innovative gears like ring seine in late eighties, and introduction of multiday fishing in 90s and the yield reached around 2.7 million t during the year 1997. This production remains almost static since 1997, probably waiting for another technological breakthrough in the harvesting sector.

The availability and distribution pattern of marine fishery resources in India are typical of tropical waters. The fishery resource is constituted by a large variety of species coexisting in the same ground. There are nearly 1570 species of finfishes and about 1000 species of shellfishes known from our seas. The multispecies

fishery comprises of over 200 commercially important finfish and shellfish species (Table 1). The important varieties belonging to the pelagic groups such as the sardines, anchovies, mackerel, carangids, Bombay duck, ribbonfishes, seerfishes, tunas; demersal finfish groups such as the sharks, rays, croakers (sciaenids) perches, silverbellies, lizardfishes, catfish; crustaceans such as the penaeid and non-penaeid shrimps, crabs and lobsters; and cephalopods *viz.*, squids and cuttlefishes are common. The abundance of these stocks varies from region to region and from season to season with large pelagics like tunas being more abundant around Island Territories and small pelagics like sardines and mackerel supporting a fishery of considerable magnitude along the southwest and southeast coasts (Fig.1). The Bombay duck and non-penaeid shrimps form a good fishery along the northwest coast, while perches are dominant in the southwest and southeast coasts, especially in the Gulf of Mannar, Palk Bay and Wadge Bank. Among this, species/groups contributing to more than one lakh tonnes a year are oil sardine, mackerel, Bombay duck, ribbonfishes, carangids, perches, croakers, shrimps and cephalopods.

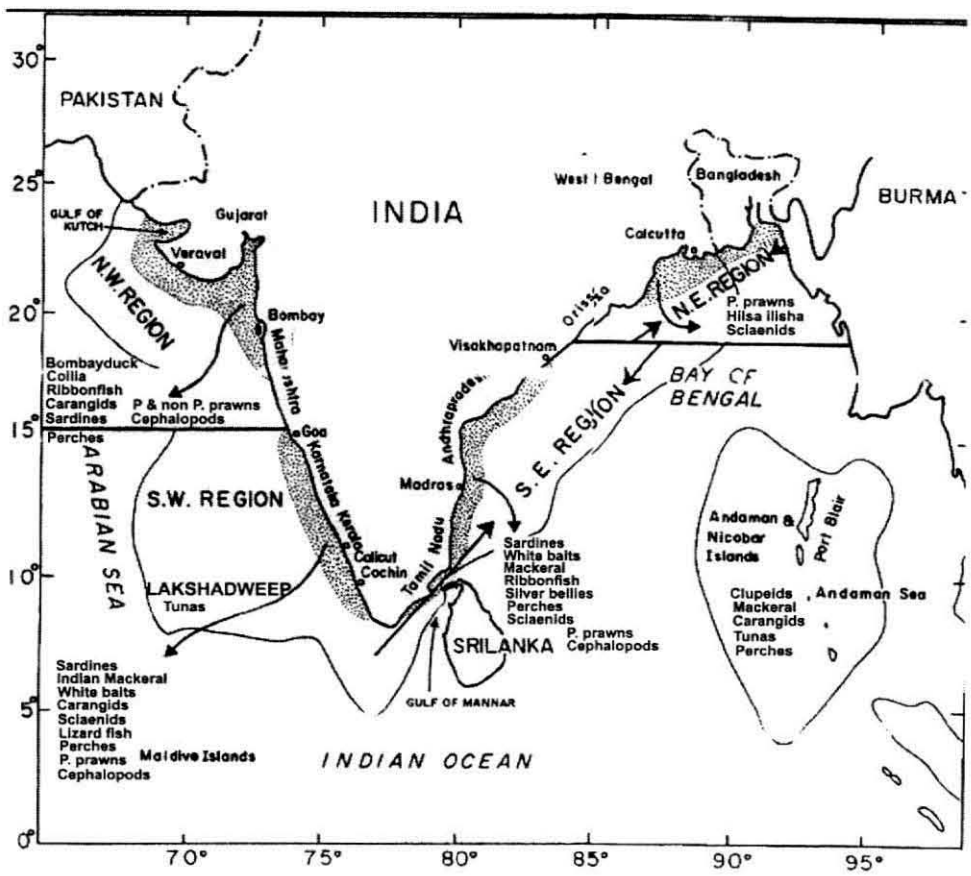

Fig.1. EEZ of India and geographical distribution of major fishery resources

Table 1. Catch trends and potential yield estimates of different groups

Group	Average catch (t)		Group Contribution (%)	Potential yield* (t)
	1985-89	1999-2003		
Elasmobranchs	54027	62799	2.46	71408
Oil sardine	141831	319419	12.53	294869
Other sardines	76541	101130	3.97	101490
Anchovies	68630	115598	4.53	141817
Other clupeoids	132626	43987	1.73	78932
Bombay duck	93185	105601	4.14	116227
Ribbonfishes	78384	172102	6.75	193670
Carangids	111040	120608	4.73	238148
Indian mackerel	123832	128430	5.04	295040
Seerfishes	35171	48905	1.92	61719
Coastal tunas	34185	50337	1.97	65472
Barracudas	-	17125	0.67	20849
Catfishes	50630	53711	2.11	51255
Eels	6317	9637	0.38	9081
Croakers	102934	141933	5.57	273027
Perches	90083	189093	7.42	226793
Flatfishes	29612	45482	1.78	47304
Silverbellies	60766	53849	2.11	67247
Pomfrets	37356	38378	1.51	46088
Penaeid shrimps	143073	196464	7.70	194192
Non-penaeid shrimps	48057	142929	5.61	138711
Stomatopods	-	43663	1.71	120351
Lobster	-	1938	0.08	3874
Cephalopods	39799	107415	4.21	101259
Others	40034	239327	9.39	975594
Total	1598113	2549860		3934417

Source: Modified CMFRI, 1997a *Anon, 2000

The annual catchable potential yield in the Indian EEZ is validated by a Committee as 3.93 mt consisting of 2.02 mt of demersal, 1.67 mt of pelagic and 0.24 mt of oceanic resources (Anon, 2000). This Working Group for the first time estimated the potential yield of as many as 68 species/groups of fishes occurring in the EEZ. The present annual average production of about 2.55 mt forms 64.8% of the revalidated fishery potential.

The coastal fisheries exploit a large number of species using different crafts and gears mostly in the depth range of 0 to 50 m. In recent years, however, the depth of operation has been extended upto about 120 m in some regions. Being a multigear fishery (gillnets, drift nets, hooks & line, pole & line, troll line, bag nets, ringseines, purseseines, trawls, etc.), fishing practices vary between different regions, depending on the nature of the fishing grounds and the distribution of the fisheries resources. The marine fish production in the country progressively

increased from 0.58 mt in 1950 to 2.73 mt in 1997 showing an average annual growth rate of 6.4% over a period of 4 decades (Fig.2). The annual growth rate during the different decades commencing from 1950, declined from 6.5% during 1950-60 to 2.3% during 1960-70; increased to 4.3% during 1970-80 and to 4.8% during 1980-90 but declined to 4.0% during 1990-96. This fall in the growth rate is reflected in the annual catch attaining the optimum level in the inshore fishing grounds extending upto a depth of 50 m. As could be seen from Figure 2 the marine fish production has reached a plateau since 1991, which is because the fishing effort is mainly concentrated in the 0-100 m depth zone. Over these years the trawling effort has increased considerably leading to excess pressure in the coastal waters.

The annual average landing during 1999-2003 was 2.55 mt against an annual catchable potential yield of 3.93 mt principally constituted by oil sardine (12.5%), penaeid prawns (7.7%), perches (7.4%), ribbonfishes (6.7%), non-penaeid prawns (5.6%), croakers (5.6 %), mackerel (5.1%), carangids (4.7%), anchovies (4.5%), cephalopods (4.2%), and Bombay duck (4.1%) (Table 1).

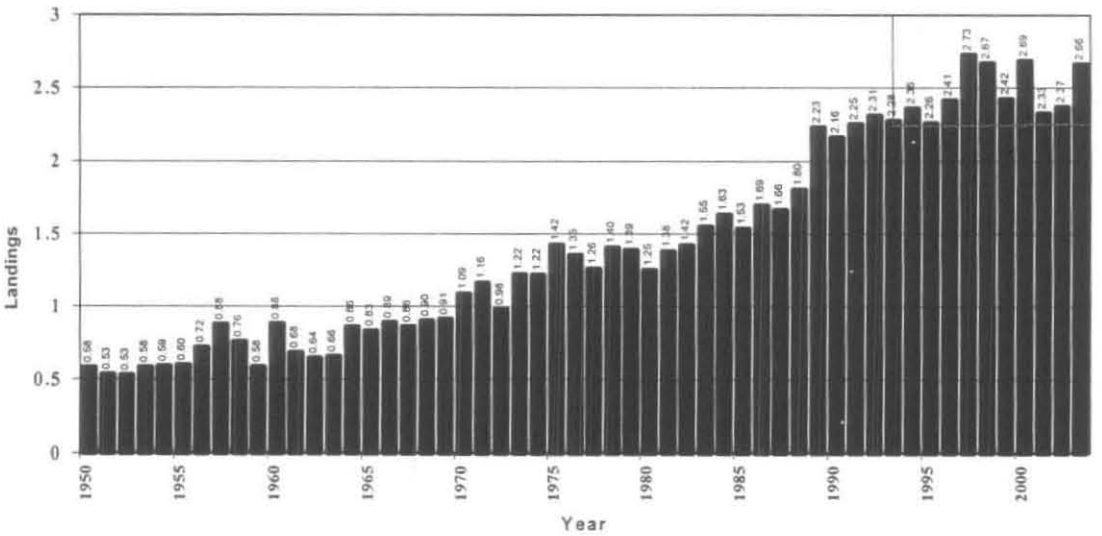

Fig. 2. All India marine fish landing (in mt.) during 1950-2003.

The mechanised sector accounted for 67.9%, motorised sector 25.0% and artisanal sector 7.1% of the total production. The sector-wise landings in different regions during 2003 are given in Figure 3. Comparative output of the marine fishing sector of different coastal states in 1985 and 2000 are given in Table 2. Catch trend during 2003 (Fig.4) indicate that the northwest coast contributed 34% to the total marine fish production followed by southwest coast (33%), southeast coast (23%) and the remaining (10%) by northeast coast (CMFRI, 2003).

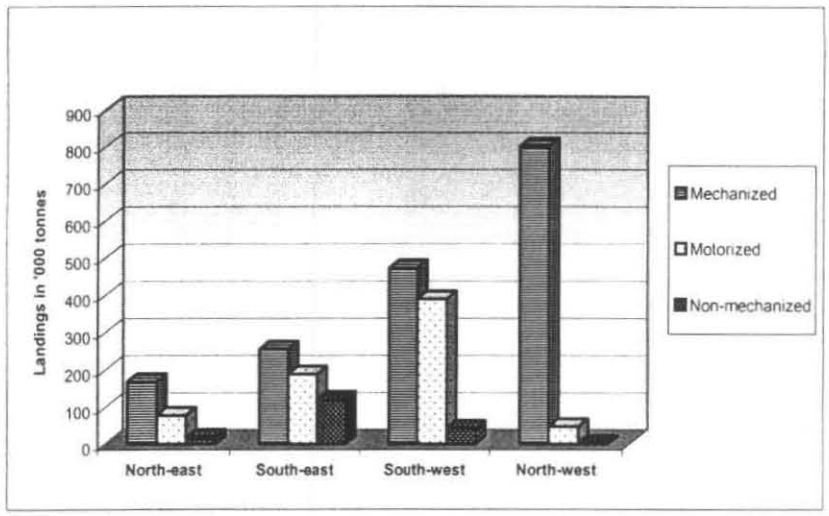

Fig.3.Sector-wise landings in different regions during 2003

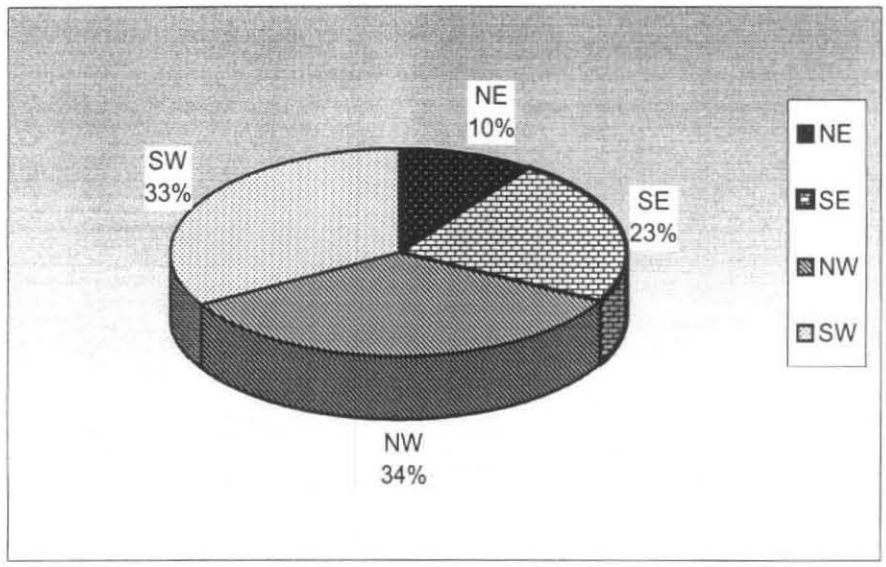

Fig.4. Regionwise fish landings in India during 2003

The increase or decrease in the annual marine fish production of the country by and large depends on the success or failure of oil sardine, mackerel, Bombay duck and shrimp fisheries (Pillai, 2003). The wide fluctuations in the annual yield of oil sardine and mackerel are well known and are generally due to factors such as spawning success, recruitment strength and environmental factors. However, in the case of the shrimp fishery, particularly the penaeid prawns, which are much, sought after by the export trade, the landings have been fluctuating from year to year with no definite trend. In most of the years, the margin of fluctuation has been varying from 10 to 15%. Further, the data on production, CPUE and other parameters of the coastal shrimp fishery at centres such as Sassoon Dock, Karwar, Mangalore, Calicut, Cochin, Neendakara, Mandapam and Chennai have indicated that further increase of effort may not yield increased production as the exploitation has reached the optimum level.

Table 2. Comparative output (in tonnes) of the primary marine fishing industry of different coastal States/Union Territories of India in 1985 and 2000

States/Union Territories	1985		2000	
	Output	Rank	Output	Rank
Andhra Pradesh	1,26,848	6	1,66,482	6
Gujarat	2,88,500	3	6,70,951	1
Goa	39,927	8	61,460	9
Karnataka	2,00,828	5	1,65,653	7
Kerala	2.95,339	2	5,75,500	2
Maharashtra	3,88,088	1	3,97,901	3
Orissa	49,205	7	1,25,935	8
Tamilnadu	2,57,000	4	3,93,000	4
West Bengal	39,350	9	1,71,500	5
Andamans	6,304	11	28,147	11
Lakshadweep	4,676	12	13,600	13
Pondicherry	19,913	10	38,620	10
Daman and Diu			15,946	12

Source: Korakandy, 1994 and Sudarsan, 2000

Although the achievements were tremendous, slowly but gradually, this common property was stressed and led to over harvest of at least a few easily vulnerable and target species and degradation of fish habitats perhaps to the extent of denudation by the unbridled human greed.

Status of exploitation of dominant species-stocks along the Indian coast in the 0-50m depth zone is given in Table 3. It is evident from the table that exploitation of many of the species at different regions have reached optimum level and in the case of certain prime species, the exploitation rate has even crossed the maximum sustainable level. The substantial increase in the effort over the last 4 decades resulted in the decrease in per capita area per active fisherman and per boat in the inshore fishing grounds, and also in the catch per unit effort. It also gave rise to conflicts among different categories of fishermen, particularly between the artisanal and mechanised sectors. Ultimately the sustainability of many resources in the coastal areas has been jeopardized by the incessant fishing pressure coupled with the impacts of pollution, and other anthropogenic causes. Such a critical situation warrants effective management of the exploited stocks in the coastal waters for sustaining the current production and to augment it further by focusing attention on the deep sea and oceanic sector.

Table 3. Status of exploitation of different species-stocks along the Indian coast in the 0-50 m depth zone

Species	State of Exploitation		
	Full	Over	Under
Sardinella longiceps	All along	-	-
S. gibbosa	SW coast	-	West coast
Hilsa ilisha	NE coast	-	-
Encrassicolina devisi	-	-	All along
Stolephorus waitei	-	-	-
Rastrelliger kanagurta	All along	-	-
Scomberomorus commerson	-	SE&SW coast	-
Euthynnus affinis	All along	-	-
Thunnus tonggol	All along	-	-
A. rochei	-	-	All along
Katsuwonus pelamis	-	-	All along
Megalaspis cordyla	-	-	SW coast
Decapterus russelli	-	-	All along
Selaroides leptolepis	SE coast	-	-
Atropus atropus	NW coast	-	-
Alepes kalla	SW coast	-	-
Atule mate	-	-	SW coast
Caranx carangus	SE coast	-	-
Parastromateus argenteus	-	West coast	-
Formio niger	-	SW coast	-
Trichiurus lepturus	-	East coast	West coast
Harpadon nehereus	NW coat	-	-
Nemipterus japonicus	All along	-	-
Nemipterus mesoprion	All along	-	-
Leiognathus bindus	East coast	-	-
L. dussumieri	Tamil Nadu	-	-
L. jonesi	Tamil Nadu	-	-
Secutor insidiator	East coast	-	-
Tachysurus tenuispinis	-	West coast	-
T. thalassinus	-	W&NE coast	-
Otolithus cuvieri	NW coast	-	-
Johnius macrorhynus	NW coast	-	-
J. vogleri	NW coast	-	-
J. sina	SW coast	-	-
J. carutta	SE coast	-	-
Penaeus monodon	East coast	-	-
P. indicus	-	East coast	-
P. semisulcatus	-	SE coast	-
Metapenaeus monoceros	All along	-	-
M. dobsoni	All along	-	-
Acetes indicus	NW coast	-	-
Panilurus polyphagus	-	NW coast	-

Loligo duvauceli	All along	-	-
Sepia aculeata	East coast	-	West coast
S. pharaonis	East coast	-	West coast

Source: Murty & Rao, 1996

There is increasing awareness in recent years among researchers, policy planners and management experts that any additional increase in fish production has to be obtained from offshore, deep sea and oceanic waters beyond the harvesting range of coastal fishing fleet. The estimated potential yield from deeper areas in the EEZ beyond 50 m depth is 1.69 mt. This includes several conventional and non-conventional resources. Oceanic resources consist of tunas (*Thunnus albacares, T.obesus, Katsuwonus pelamis*), billfishes, myctophids (*Benthosema* spp., *Myctophum* spp. and *Diaphus* spp.) and oceanic squids (*Symplectoteuthis oualaniensis*, *Onychoteuthis banskii*, *Thysanoteuthis rhombus*). But there is no directed fishery for these species, except marginal exploitation by chartered vessels, which operated under the deep sea fishing schemes in the nineties but were later suspended. Longline surveys conducted by Fishery Survey of India (FSI) have revealed abundant resources of skipjack (*K.pelamis*) and yellowfin *(T.albacares)* tunas and pelagic sharks in our waters (Somavansi, 2001). For exploitation and management of tuna resources of the coastal areas and the high seas, separate strategies should be evolved (Pillai *et al.*, 2002).

Among the multigears, gillnets, drift nets and bag nets of varied mesh sizes are widely employed by traditional fishermen along both the coasts while ring seines, purse seines and mechanized gillnets are confined to the southwest coast. Bottom trawlers upto 13 m OAL are operated along the entire coast, while the second-generation large trawlers 13-17m are operated from selected harbours along both the east and west coasts.

The growth of the fleets shows that the artisanal fleet (including the motorized) increased by about 110% from the 1960s to the 1990s and the mechanized fleet by about 570% during the same period (CMFRI, 1997) and has resulted in an over capacity of fleet operating in the inshore waters.

Currently 2251 traditional landing centres, 33 minor and six major fishing harbours serve as base for 208 thousand traditional nonmotorised crafts, 55,000 small scale beach landing motorised crafts, 51,500 mechanised crafts (mainly bottom trawlers, drift gillnetters and purse seiners) and 180 deep sea fishing vessels of 25 m OAL (Anon, 2001). The development of harbours and landing jetties, motorization of artisanal crafts and rapid expansion of mechanized fishing have contributed towards a significant increase in fish production, employment generation and revenue earnings. It has also resulted in declining per capita area for the boats (Table 4) and given rise to serious conflicts between artisanal and mechanised sectors in the inshore waters where CPUE for most of the fisheries and especially the shrimp are showing a declining trend. The pattern of marine fish landings in India during the past fifty years clearly reveals that the contribution by the artisanal sector to the total production was significant only up to 1960s while presently, the contribution by the mechanized and motorized sector accounts for 93% of the marine fish catch (CMFRI, 2003). Under these circumstances adoption

of sustainable fishing practices, diversified multi-gear and resource specific fishing and complementary mariculture practices are being advocated.

Table 4. Change in per capita area in ha/boat (non-mechanised + mechanised) in the shore areas (0-50 m) and offshore shelf areas (50-200 m) during successive periods

State	1961-62		1973-77		1980		1990	
	Inshore	Offshore	Inshore	Offshore	Inshore	Offshore	Inshore	Offshore
Gujarat	1453	2214	1095	1669	862	1314	499	760
Maharashtra	257	852	251	833	205	680	108	359
Goa	3030	7070	229	534	87	204	94	220
Karnataka	114	244	109	233	89	190	51	109
Kerala	59	123	57	118	44	92	40	84
Tamilnadu	78	55	74	53	52	36	53	38
Pondicherry	-	-	82	55	77	51	25	17
Andhra Pradesh	84	69	64	53	46	38	31	25
Orissa	528	599	317	359	147	166	96	109
West Bengal	1503	626	599	249	234	97	192	80
Lakshadweep	-	-	-	-	-	-	-	347
Andamans	-	-	-	-	-	-	-	3043

Source: *CMFRI Vision 2020*

Ornamental fish and fisheries

Besides the marine fishery resources for human consumption, there are certain resources of commercial value. Marine aquarium fish trade is gaining increasing popularity the world over with an estimated value of 4.5 billion US$ (Srivastava, 1994). The Gulf of Mannar, Palk Bay, Gulf of Kutch, southwest coast and the Lakshadweep and Andaman group of islands are known to be rich in ornamental fishes (Murty *et al.*, 1989, Murty 2002). The wrasses, damselfish, surgeons, butterflyfish, moorish idol, squirrelfish, triggerfish, rabbitfish, parrotfish, angels, goatfish and pufferfish are the major aquarium fishes represented by nearly 180 species. Most of these fishes are abundant and offer scope for live fish export and development of home aquariculture in the country. The results of the survey and assessment of marine ornamental fishes of Lakshadweep (nine islands) implemented by the Central Marine Fisheries Research Institute indicate an annual potential yield of 25 million fish consisting mainly of wrasses (38.0%), damsel fishes (32.7%), goat fish (8.4%), parrot fish (7.4%), squirrel fish (4.9%), surgeon fish (4.8%), butterfly fish (2.1%), trigger fish (0.8%) and others. Their exploitation, utilization and trade should be exercised with caution without trampling the habitat or other co-habitants of ecological value.

Key issues in marine fisheries sector

- Multi-gear, multi-species, Open Access Fisheries
- Increased and excessive fishing pressure in the coastal areas up to about 50 m depth zone
- Optimal exploitation of resources in the inshore waters
- Indiscriminate exploitation of juveniles of many commercially important species by reducing the mesh size
- Damage to the benthos and benthic ecosystem by continuous sweeping of the same ground by shrimp trawlers
- Decrease in area available in the sea per active fisherman and boat for conducting fishing operations
- Conflicts among different categories of fishermen particularly between the artisanal and mechanised groups of fishermen
- Conflicts between those engaged in coastal artisanal fishing and coastal aquaculture
- Ecosystem degradation
- Lack of proper fishery management system (Participatory Fisheries Management)
- Lack of National Marine Fisheries Policy
- Need for popularisation of Code of Conduct for Responsible Fisheries
- Absence of informed management regime

The annual growth rate of marine fisheries production increased from 4.3% during 1970s to 4.8% during 1980s and declined to 4.0% during 1990s(CMFRI, 1997a) and lowering down in growth rate is reflected in the annual catch attaining the optimum levels in the inshore fishing grounds up to a depth of 50m. The substantial increase in fishing effort since the 1970s has resulted in the decrease in per capita area per active fishermen and per boat in the inshore fishing grounds and also in the CPUE, which, in turn has given rise to intra/inter sector conflicts among difference categories of fishermen, especially artisanal and mechanized sectors (Sathiadas, 1996). Such a critical situation warrants effective management of the exploited stocks in the coastal waters for sustaining the current production and to augment it further by focusing attention on the offshore sector and on seafarming and coastal mariculture. There is also urgent need to formulate national and state level regulations/policies in marine capture and culture fisheries in conformity with the objectives of the FAO Code of Conduct for Responsible Fisheries and other relevant global conventions and regulations, within the ambit of the prevailing socio-political and economic objectives.

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2

Molluscan Mariculture – Global status

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Aquaculture has been in existence for centuries as a traditional practice in Europe and Asian countries mainly for edible oysters, freshwater fin fishes and brackish water shrimps. Farming of molluscs is one of the earliest form of mariculture especially, farming of oysters in the western hemisphere from Roman times. Since eighties scientific shrimp farming commenced in the country with traditional, extensive and semi-intensive farming techniques. Experimental farming of Mussels, pearl oyster and pearl production and clams were initiated in the early seventies in the country by national research laboratories. Since marine fish production is stagnating at an optimum level with further increase in production not envisaged, there is increase in awareness in the country to produce more seafood through scientific aquaculture.

Global Scenario

Global aquaculture production has shown a continuous and steady increase over the years, increasing from 3.9% of the total fish production by weight in 1970 to 29.8%in 2002. Of the total world fisheries production of 132.9 million metric tonnes in 2002, aquaculture contributed to 39.7 million tonnes. Of the total world aquaculture production in 2002, molluscs, mainly consisting of oysters, mussels, scallops, clams and cockles contributed 11.27 million mt, *ie* 28.3%. Among mulluscs, oysters dominated contributing to 36.67%, followed by clams and cockles (29.15%) and mussels (12.2%). Asian countries contributed 87.98% of the total aquaculture production .The percentage of molluscs in the total world aquaculture production was 28.3%.

Scientific pearl culture was initiated by Japan and till recently the international market for cultured pearls were controlled by them. However in recent years several other nations have started utilizing their pearl oyster resources and Japan lost its monopoly in the production and trade of cultured pearls. In 1998, the world pearl exports, valued at 396 million US dollars were controlled by French Polynesia (28%), Australia (20%), Japan (14%) and Indonesia (14%).

The world production of edible oysters in 2002 was 4317380 t and the important contributions were by China (83.9 %), Japan (5.1 %) and Republic of Korea (3.9 %).

The world production of mussels by aquaculture was 1444734 t dominated by China (67.1%), Italy (9.3 %) and France (7.3%).

The total clam production by aquaculture was 3430820 tonnes forming 30.3% of the total molluscan production by aquaculture and the leading countries were China, (92.5%), Malaysia (2.2%) and Italy (1.2%).

The total production of scallops by aquaculture was 1226568 tonnes, of China contributed 76.2%, and followed by Japan contributing 22.1%.

Cultured gastropods contributed 2816 tonnes and the major contributors were Taiwan province of China (82.56%) and South Africa (7.81%).

Cephalopods, mostly experimental culture, contributed 14 tonnes, mostly by Spain.

2002- World Aquaculture Production (FAO)

Group	Production (Mt)	Pecentage
Fish	1201060	5.72
Crustaceans	246525	1.17
Molluscs	11770659	56.09
Sea weeds	151387	0.72
Others	7616891	36.29
Total	**20986522**	

2002- World Aquaculture Production- Molluscs (FAO)

Group	Production (Mt)	Pecentage
Abalones, Winkles & Conchs	2816	0.02
Oysters	4317380	36.68
Mussels	1444734	12.27
Scallops, Pectens	1226568	10.42
Clams, Cockles, Ark shells	3430820	29.15
Squids, Cuttlefishes, Octopuses	14	0.0001
Miscellaneous marine mollusks	1348327	11.45
Total	**11770659**	

Methods of Bivalve culture

Bivalves, including mussels, oysters and clams contribute to the bulk of production of molluscs. The different farming techniques used for bivalve mariculture are

a) **On bottom culture**: It involves the transfer of young seed mussels from areas of great abundance were growth is poor owing to over crowding, to areas of good growth and fattening. This practice is followed in Holland, Denmark and Germany

b) **Pole culture**: Rows of poles interwoven with branches are used. Conducted in the intertidal mudflats along the Atlantic coast, mostly in France

c) **Raft culture**: Both floating and anchored rafts are used from which rens or cages holding oysters are hung. Eg: culture of mussels in Spain, Southern France, Yugoslavia and Italy.

d) **Long line culture:** Shallow waters of 10-15 m depth. It is able to withstand severe monsoon along the southwest coast of India.

Major Molluscan Species Cultured

Family Arcidae	
Anadara broughtonii	Japan (Experimental culture)
Anadara granosa	Tropical Pacific, Thailand, Malaysia, Korea
Anadara granosa bisinensis	Korea
Anadara inflata	Banten (West Java)
Anadara subcrenata	Japan
Arca granosa	Pacific islands, Japan
Family Ostreidae	
Crassostrea angulata (Potuguese oyster)	France, Tunisia
Crassostrea commercialis (Sydney rock oyster)	Australia, Hawaii
Crassostrea cucullata (Indian rock oyster)	Japan, Oyster, New Zealand
Crassostrea gigas (Pacific oyster)	Japan, Korea, N.America, Tasmania, New Zealand
Crassostrea glomerata (Rock oyster)	New Zealand
Crassostrea rhizophorae	Cuba, Jamaica, Puerto Rico
Crassostrea rivularis	Japan
Crassostrea virginica (Atlantic oyster)	USA
Ostrea edulis (European flat oyster)	Spain, France, Tunisia, Greece, Scotland, Ireland
Ostrea iredalie	Philippines
Ostrea lurida (Olympia oyster)	Puget Sound (USA)
Pycnodonta numisma	Thailand
Family Mactridae	
Mactra sulcataria	Japan
Family Mercenaridae	
Mercenaria mercenaria	Gulf of St Lawrence to Gulf of Mexico, USA
Family Veneridae	
Meretrix lamarckii	Japan
Meretrix lusoria	Japan, Korea
Meretrix meretrix	Japan, Korea
Protothaca staminea	N. America
Saxodomus giganteus (Butter clam)	USA
Tapes decussatus (Meditteranean clam)	Meditteranean
Tapes semidecussata (Japanese little neck clam)	Pacific coast of Asia (Japan and Korea)
Venerupis japonica (Manila clam)	Japan
Venus verrucosa	Meditteranean
Paphia philippinarum	Japan
Family Myidae	
Mya arenaria	N.America, Norway, France, Japan
Family Mytilidae	
Mytilus crassitesta	Korea

Mytilus edulis	France, Spain, Germany, Italy, Netherlands, Denmark, England, Scotland, Canada
Mytilus galloprovincialis (Meditteranean mussel)	Italy, Tunisia, Greece
Mytilus smaragdinus (Green bay mussel)	Thailand, Philippines
Family Pectinidae	
Pecten laqueatus	Japan
FamilyPteriidae	
Pinctada fucata	Japan
Pinctada margaritifera (Black lip pearl oyster)	Indo-Pacific, Japan, Philippines
Pinctada martensii (Japanese pearl oyster)	Japan, Sudan, Red sea, Australia
Pinctada maxima (Siver lip, Gold lip)	Australasia
Pteria penguin (Wing shell)	Japan
Family Anomiidae	
Placuna placenta (Window pane shell)	Philippines

Molluscan aquaculture in the world

Mussels

The major species of mussels cultivated in the world are the blue mussel, *Mytilus edulis*, and the Meditteranean mussel, *Mytilus galloprovincialis*. The blue mussel is cultivetd by long line culture in China, by raft culture in Spain and by 'Bouchot' culture in France. The raft method is generally practiced in protected areas, with steep coastal profiles and considerable tidal oscillations. Very high levels of production are obtained by raft culture in the submerged river valleys or fjords of Galicia in Spain. China leads the production in mussels, followed by Spain, Italy and France. The other species of importance include the green mussel, *Perna viridis*, in Thailand and Malaysia and the New Zealand mussel, *Perna canaliculatus*. The black mussel, *Mytilus crassitesta* is also of some importance in China.

Oysters

The major species contributing to the oyster production by aquaculture is the Pacific cupped oyster, *Crassostrea gigas*, contributing to more than 95 % of the total production of oysters. The other species of importance are the European flat oyster, *Ostrea edulis* and the American cupped oyster, *Crassostrea virginica*. The major oyster producing countries of the world are China, Japan, republic of Korea and France. In China the long line method of culture is followed, whereas in Japan both on bottom and off bottom culture is done, the off bottom culture is practiced in rafts and long lines. An interesting variation of the conventional bottom culture method for *Ostrea edulis* in France is the "Claire method" of fattening and greening of oysters as final preparation for market. Claries are small, shallow, artificial ponds, 0.1 to 0.2 ha in size constructed on marshland adjacent to the sea. In this

process, by the deposition of glycogen, the oyster meat increases in size and weight, the colour of meat becomes creamy white and the flavour becomes sweet.

The other oyster species of commercial importance, cultured are The Portuguese oyster, *Crassostrea angulata*, in Portugal, Spain and Atlantic coast of France, Sydney rock oyster, *Crassostrea commercialis*, in Australia and NewZealand, *Crasspostrea eradelie*, the slipper oyster in Philippines, and the mangrove oyster, *Crassostrea rhizophorae*, in Cuba and Venezuela.

Clams and Cockles

The major cultivated species comes under two families, Arcidae and Veneridae. The major species cultivated are The Japanese carpet shell, *Venerupis japonica*, the Quahog, *Mercenaria mercenaria* and the blood cockle, *Anadara granosa*. Of this *V. japonica* contributes to more than 65 % of the total clam production by aquaculture, mainly by China and United States Clams are mainly produced by on bottom culture in intertidal areas. The hard clam *Mercenaria mercenaria* is spawned in commercial hatcheries on the east coast of USA and juveniles from the nursery are transplanted to nursery grow out systems until they are approximately 25 mm before being planted out into the natural shellfish beds for further grow out and eventual harvest.

Investigations into culturing the giant clam, *Tridacna* species have occurred in the South Pacific since the late seventies and currently there are four hatcheries that supply juveniles that are transplanted throughout the Indo-Pacific area.

Scallops

The major species culture is the Yesso scallop, *Patinopecten yessoensis*, of which more than 95% is contributed by China and Japan. The larvae collected on spat collectors are reared in hanging cages or holding ponds until the scallops exceed 3 cm shell length (after 7 months), and are then released into favourable grounds. In off bottom culture lantern nets are used. Other species used for culture are *Chlamys* farreri in china. Commercial culture of a larger species *Chlamys nobilis* is also done in southern China. The bay scallop *Argopecten* irradians has been spawned in hatcheries and the seed grown to market size in pens in the USA. It is also being cultured in China.

Pearl oysters

The major species used for commercial pearl culture include, *Pinctada fucata* in Japan, which produces pearls in the 4-8mm range, *Pinctada maxima*, the silver lip or the gold lip pearl oyster, in Australia (12-18mm size pearls) and the black lip pearl oyster *Pinctada margaritifera* in French Polynesia (10-14mm pearls). Japan dominated pearl production till the advent of the Chinese with their fresh water pearls. The fresh water pearl producing oysters in Chins are *Cristaria* and *Hyriopsis*. The genus *Pteria* is also tried for pearl production and has also been attempted in the abalone

Gastropods

Abalone is the major species cultivated. The wrinkled abalone *Haliotis discus hannai* is cultured in China in cages and raceways, the blacklip abalone, in

Australia and the Pelemoen abalone in South Africa. The Taiwan province of China is by far the leading producer of cultured abalones. Other gaspods cultured include the snails, *Helix pomatia* and *Helix aspera* in France, the queen conch, *Strombus gigas* cultivated on an experimental scale in the Carribean. The top shells *Trochus cornutus* is commercially cultured on the Seowipo coast of Korea and the Japan coast and *Trochus niloticus* on an experimental basis in the Caroline Islands.

Molluscan aquaculture in India

The potential and prospects of coastal aquaculture of molluscs in India was realized as early as the seventies and concerted efforts made to develop suitable technologies for scientific farming, which could be easily adopted by the coastal fishermen. Several research programmes were taken up by National Research Laboratories, Universities and Department of Fisheries of maritime states during the past 25 years for development of coastal aquaculture in the country. Coastal aquaculture and Mariculture occupy an area 120000 ha providing employment to more than 200000 people. However as the present region under production forms only 10% of the identified potential area in the coastal belt, there is great scope for further development in aquaculture.

The bivalve resources of India comprising the pearl oysters and the protein rich mussel and edible oyster, have become an important source of income to coastal villagers. The revival of the pearl industry, which had flourished in the earlier times, has become possible only through the development of a full-fledged pearl culture technology by the Central Marine Fisheries research Institute, Cochin.

Pearl Culture Programme

Pinctada fucata, distributed in the Gulf of Mannar, Palk Bay and Gulf of Kutch and the black lip pearl oyster, *Pinctada margaritifera* in the Andaman and Nicobar islands, constitute the two major pearl producing ousters in India. The pearl culture programme was started by CMFRI in 1972, in response to the dwindling natural pearl fisheries. A research programme on pearl culture was organized by CMFRI in collaboration with the Government of Tamil Nadu as an *ad-hoc* scheme on pearl culture under the ICAR, from 1973 to 1978, leading to the establishment of a pearl farm in Krusadai Island by the Government of Tamil Nadu. With indigenous developments in pearl culture technology, the CMFRI over the years has adopted an open policy of training and the institute has implemented training programmes in pearl culture technology and hatchery production of spat since 1976. Along the Tamil Nadu coast, Tamil Nadu fisheries development corporation (TNFDC) and Southern petrochemicals industries corporation Ltd (SPIC) took up a joint commercial project on pearl production in 1983 with technical know how of CMFRI. Pearl culture was developed as a rural upliftment programme in the early nineties. Industrialization of pearl culture is progressing with several private companies (ITAP Ltd, Tuticorin, Orkay company, Mandapam, Master Pearls Ltd, Chirala, Pearl Beach hatcheries, Visakhapatnam) taking up commercial level pearl culture.

Mussel Farming

Marine mussels form one of the most dominant cultivable species all over the world and give the highest conversion of primary producers (phytoplankton) to human food. The culture of mussels in column waters can increase seafood production several fold. The two species considered for culture in India are *Perna viridis,* the green mussel and *Perna indica*, and the brown mussel. The culture season for mussels is during December to May, when the estuaries are in the marine phase. The culture methods include Rack method (estuaries and shallow seas), Long line method (unprotected sea conditions) and Raft culture (calm open sea conditions). To popularize mussel culture demonstration units were set up by CMFRI at Andhakaranazhi (Long Line), Njarakkal (raft) and central Kerala (integrated culture of mussels and oysters). An estuarine farm was also set up at Padanna (Kasargod). An extensive community development programme started by CMFRI led to loans from Government development agencies like DWRCA (Development of women and children in rural areas), TRYSEM (Training of Rural Youth in Self Employment) and Farmers Co-operative banks to newly formed village mussel farming groups resulting in the setting up of several mussel farms in the region. The establishment of mussel farms in Kerala state led to a dramatic increase in farmed mussel production (more than 500 tonnes in 1998).

Edible Oyster Farming

Of the six species of edible oysters found along the Indian coast, The Indian backwater oyster, *Crassostrea madrasensis*, is the dominant species used for aquaculture. Since the early seventies, CMFRI has taken up R&D programmes on all aspects of oyster culture resulting in a complete package of the technology. The culture methods followed in the country are Rack and Ren method, Rack and Tray method for commercial production and Stake, Raft and Long Line culture are tried at an experimental scale. The first commercial farming area was developed in Kerala in Ashtamudi Lake (Dalavapuram) during 1995-1996. In Kerala the BFFDA now gives financial assistance to farmers to set up oyster farms as recognition to the fact that planners have recognized oyster culture as a viable project ideal for rural development and income generation.

Recent trends in Molluscan Mariculture

The production of triploid and tetraploid oysters for increasing production has been attempted. Genetic manipulation such as ploidy induction, gene transfer and selective breeding are the recent developments in bivalve farming to increase productivity through bio techniques.

Treatment of shrimp and fish farm effluents and reclaiming can be done by biological means like culture of molluscs (eg: oysters in Hawaii and mussels in Thailand), utilizing the filter feeding property of molluscs to filter out aquatic wastes.

Edible oyster and mussels are being experimented in integrated farming. Integrated farming of edible oyster and mussel from the same rack structure was introduced in the Ashtamudi lake ecosystem. Similarly, preliminary experiments

are being conducted for testing the feasibility of growing finfishes; shrimp and crabs in cages suspended in the same farm and have given encouraging results.

Pearl culture has also undergone significant upgradation in farming and nucleation techniques. The production of large sized pearls ie > 6mm is being achieved in both east and west coasts. A technique for onshore pearl production is being developed at Visakhapatnam laboratory and the results are encouraging. The *in vitro* pearl production and the colour manipulation or make-up pearl production in Tuticorin laboratory are some of the significant achievements of the Central Marine Fisheries Research Institute recently.

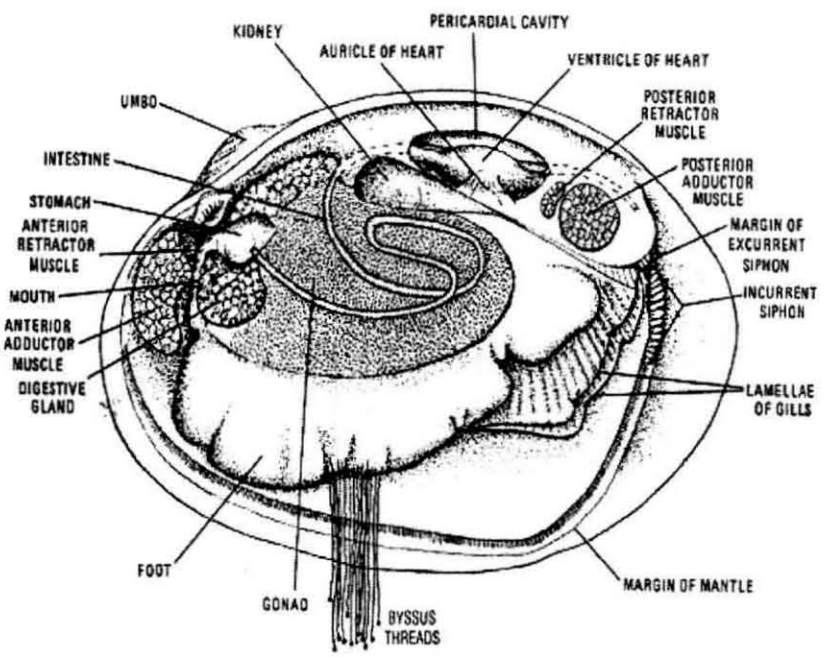
The institute has also recently developed technologies for *Haliotis* (Abalone) seed production and also squid and cuttlefish seed production.

3 Bivalve Taxonomy and Biology

P.K. Ashokan, R C of CMFRI, Veraval

The Bivalvia (also known as Lamellibranchia and Pelecypoda) represents the second largest class of Mollusca. Compared to the gastropods, the bivalvia are a more restricted group and have a specialisation devoted to a narrower range of pattern. Bivalves are more sedentary though in most of them the foot is still well developed. Very few however crawl over the substrate in the primitive molluscan way. Many species burrow into soft sand and mud, or even bore into rock and wood, a large number are permanently anchored to the ground, and among these the foot is usually reduced and sometimes quite lost.

Bivalves are bilaterally symmetrical, laterally compressed molluscs with extensive lateral mantle lobes, which secrete a single shell of two valves and a ligament, which joins them dorsally at a hinge. The head is rudimentary and lacks a radula and most of the sensory structures are located in the mantle border. They are mostly ciliary feeders, with sieving and sorting mechanisms on labial palps and a large leaf like ctenidium. The foot is compressed and adopted for burrowing, except in sedentary forms where it is rudimentary. Majority are marine. Freshwater representatives are less and there are no terrestrial forms. Fertilisation is usually external, followed by trochophore and veliger stages and a metamorphosis to adult form.



Typical bivalve anatomy

The class Bivalvia can be divided into three Sub-classes:

Sub-Class 1 Protobranchia:

These are primitive bivalves in which the ctenidia are posteriorly placed and consists of almost flat, horizontally placed, non-reflected filaments which divide the mantle cavity on each side into lower inhalant chamber and an upper exhalent chamber that contains hypobranchial glands. Foot not compressed ventrally but flattened to a crawling sole, always with two adductor muscles. Feeding primarly by means of enlarged labial palps, often provided with palp proboscides. Includes the family Nuculidae, Nuculanidae, Solemyidae and Malletiidae.

Sub-Class 2 Lamellibranchia:

Bivalves in which emphasis on feeding relates to ctenidia rather than to the palps, which are reduced; ctenidial filaments elongate and reflected to form two-sided lamellae usually being united by interlamellar junctions. Adjacent filaments linked by ciliary junctions (filibranchiate) or by vascular interfilamentar junctions (eulamellibranchiate). Adductor muscle two and equal (isomyarian) two and unequal (hetromyarian) or reduced to one (posterior) (monomyarian).

Lamellibranchia has six orders namely Taxodonta, Anisomyaria, Heterodonta, Schizodonta, Adapedonta and Anomalodesmata. Among these orders, Anisomyaria and Heterodonta are the most varied and of considerable economic importance.

Order Anisomyaria: Gills usually filibranch and with vascular interlamellar junctions; adductor musculature heteromyarian or monomyarian resulting in drastic changes in symmetry; hinge variable; mantle lobes free except for separation of exhalent aperture; usually no siphons; foot reduced or absent; many species sedentary. Includes families Mytilidae, Pteridae, Pinnidae, Pectinidae, Limidae, Anomiidae, Ostreidae.

Order Heterodonta: Gills eulamelliranchia, adductor muscle similar; hinge dentition heterodont (with cardinal and lateral teeth); mantle edges usually united at one or more points posteriorly, leading to development of siphons. Includes families Astardae, Carditidae, Sphaeriidae, Corbiculidae, Cyprinidae, Dressenidae, Lucinidae, Chamidae, Cardiidae, Tridacnidae, Veneridae, Mactridae, Amphidesmatidae, Donacidae, Tellinidae, and Solecurtidae.

Sub-Class 3 Septibranchia:

Gills in the form of a muscular septum which pumps water through the mantle cavity; mantle edges mostly free; adductor muscles equal; hinge weakly denticulate or edentate; macrophagous feeders Incledes the families Verticordiidae, Poromyidae, Cuspidariidae.

Edible Oysters

Edible oysters' belonging to the family Ostreidae.are found in hard substratum in the bays and creeks near coastal waters. They are attached permanently to the to the substratum.

Taxonomy

In India, six species of oysters are reported. They are the Indian backwater oyster *Craassostrea madrasensis* (Preston), Chinese oyster *C.rivularis* (Gould), west coast oyster *C.gryphoides* (Schlotheim), Indian rock oyster *Saccostrea cucullata* (Born), Bombay oyster *Saxostrea cucullata* (Awati and Rai) and the giant oyster *Hyostissa hyotis* (Linnaeus) are found.

Craassostrea madrasensis (Preston): Shell straight, shape irregular, covered by numerous foliaceous laminae, left valve deep, right one slightly concave, hinge narrow and elongated, adductor scar sub central, dark purple in colour, inner surface of valve white, glossy and smooth, purplish black colouration on the inner margin of the valve.

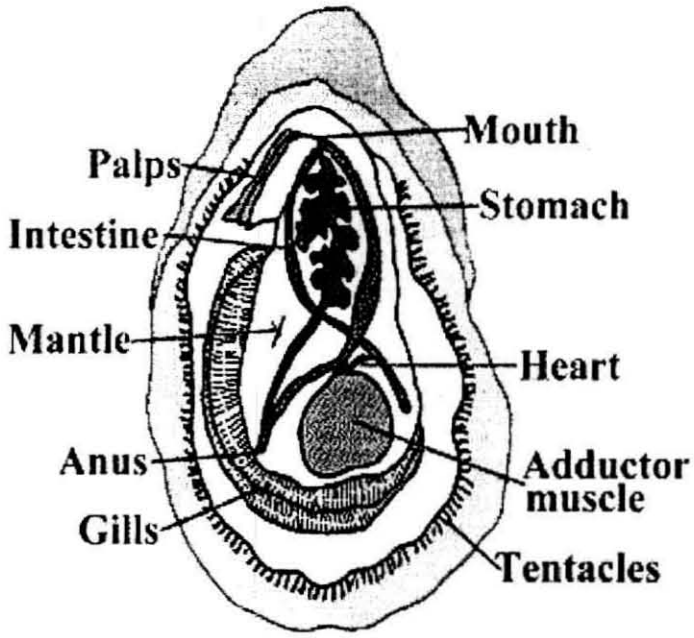
C.gryphoides (Schlotheim): Shell oblong narrow in the anterior margin and broader in the posterior margin, laminated, lower valve very thick, especially in the anterior region below the ligamental area. Muscle scar more or less heart shaped and pearly white. Upper valve thin flat and opercular, no denticles on the margin.

C.rivularis (Gould): Shell valves large, roughly round, flat, thick and with a shallow shell cavity. Left valve is thick and slightly concave and the right one is about eh same size or slightly larger. Adductor muscle scar is oblong and white or smoky white in colour.

Saccostrea cucullata (Born): Shell more or less trigonal, sometimes oblong, extremely hard and plaited. The margins of both the valves have well developed angular folds sculptured with laminae. Small tubercles present along the inner margin of the right valve and there are corresponding pits in the left valve. Adductor scar is kidney shaped.

Biology

Being sedentary and attached to the substratum by the cupped lower valve, the upper valve acts as a lid. The food consists of organic detritus and phytoplanktons. The growth of C.madrasensis has been studied in different locations showing variations. In Kakinada Bay it grew from 27mm to 72mm in 8 ½ months. In Adyar estuary it attains 50.6 mm length in 13 months. In Vellar estuary it attains 48.8, 85 and 111.7 mm atages 1-3 respectively. N theTuticorin Bay the oyster grows to 87 mm at the end of first year. I the Cochin backwaters, spat of 10mm modal length grow to 55mm modal length in about 6 months. In the Kakinada Bay, C.madrasensis spawns during January-June. In Adyar estuary, it spawns during October-December and again in March-April. At Tuticorin biannual spawning takes place during July-September and February-April.

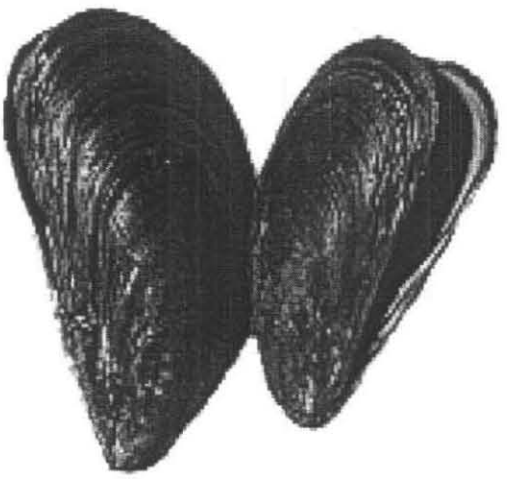


General anatomy of Edible oyster - Diagrammatic representation

Mussels

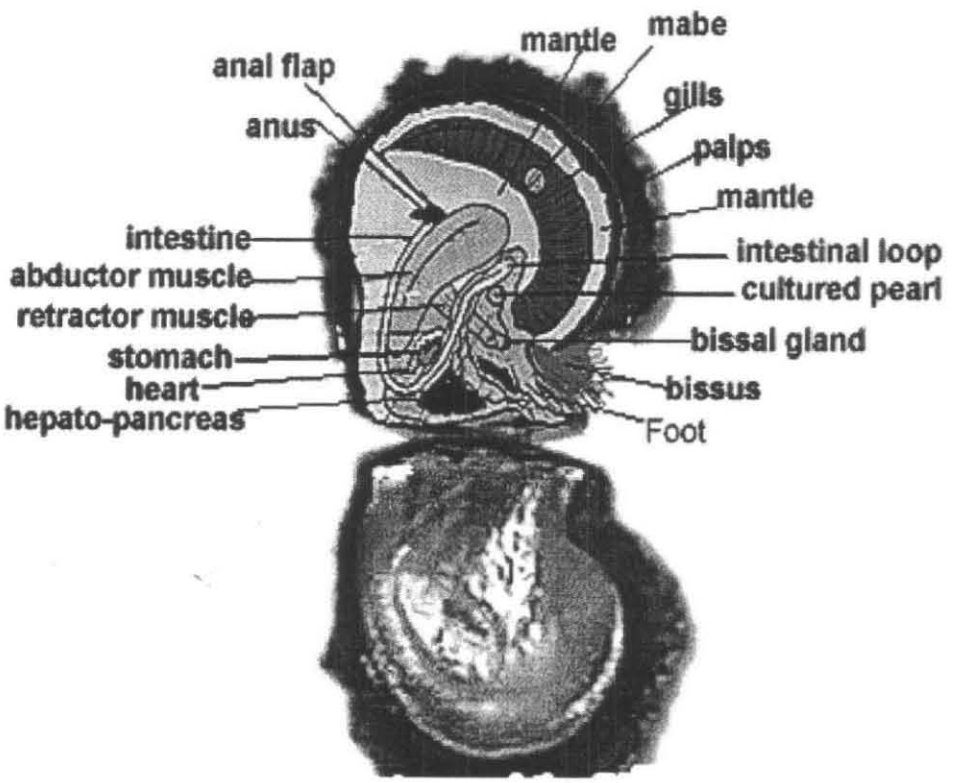
The genus Perna comes under the Family Mytilidae. In India, there are two species, the green mussel *Perna viridis* and the brown mussel *Perna indica*. Apart from the colou of the periostracum, the mantle margin is brown colour in *P.indica* wheras in *P.viridis* it is yellowish green in colour. In *P.viridis* the anterior end of the shell is pointed with the beak turned down and in *P.indica* it is pointed and strainght. In *P.viridis*, the hinge teeth show two small on the left valve and one on the right valve and in *P.indica* there is one large tooth on the left valve and a corresponding depression on the right valve. The mussels have a foot, which secretes the byssal threads. It can discard it and produce fresh threads enabling it to change the location of attachment.

They are filter feeders of phytoplanktons. In Kakinada bay, *P.viridis* in the natural bed grows 63 mm in 6 months, 91.5 mm in one year, 117 mm in 2 years, 129 mm in 3 years and 135 mm in 4 years. When cultured on ropes in the raft, the growth was from 21.7mm to 66.6mm in 5 months. At Calicut the green mussel of 23.6 mm average length suspended from the raft in the open sea in rope culture attaned 888.2 mm in 5 months whereas in the natural bed during the same period grew to 66.9mm. P.viridis attains sexual maturity at 15.5 to 28 mm. The spawning period in the Kakinada bay s from December to July with peak activity between January and May. At Calicut spawning takes place during July to November with peak activity in August – October.



The green mussel *Perna viridis*

Pearl oysters



Pearl oyster: Internal anatomy

Taxonomy

The true pearl oyster belongs to the genus Pinctada (Roding) under the family Pteriidae, order Dysodonta. Members belonging to the Pteriidae family are characterized by a straight hinge with 1–2 small tooth-like thickening, a cavity below the anterior angle for the byssus, and usually a scaly surface of the outer shell valves. The family includes the pearl oysters belonging to the genus Pinctada and the winged oyster shells of the Pteria genus. In Pteria spp. the shell width is much longer than the height and the hinge angle is prominent and pronounced.

In Pinctada spp. the hinge is rather long and straight, the long axis of the shell is at a right angle to the hinge, the left valve is slightly deeper than the right and there is a byssal notch on each valve at the base of the anterior lobe. The colouration of periostracum varies and is often brownish with radial markings.

Six species of pearl oysters, *Pinctada fucata* (Gould), *P. margaritifera* (Linnaeus), *P. chemnitzii* (Philippi), *P. sugillata* (Reeve), *P. anomioides* (Reeve) and *P. atropurpurea* (Dunker) occur along the Indian coasts. Their morphological characteristics are as follows:

Pinctada fucata (Gould)

The hinge is fairly long and its ratio to the broadest width of the shell is about 0.85 and that to the dorsoventral measurement is about 0.76. The left valve is deeper than the right. Hinge teeth are present in both valves, one each at the anterior and posterior ends of the ligament. The anterior ear is larger than in the other species, and the byssal notch, at the junction of the body of the shell and the ear, is slit-like. The posterior ear is fairly well developed. The outer surface of the shell valves is reddish or yellowish-brown with radiating rays of lighter colour. The nacreous layer is thick and has a bright golden-yellow metallic lustre.

Pinctada margaritifera (Linnaeus)

The hinge is shorter than the width of the shell and is devoid of teeth. The anterior border of the shell extends in front of the anterior lobe. The byssal notch is broad. The anterior ear is well developed while the posterior ear and sinus are absent. The posterior end of the shell meets the hinge almost at a right angle. Shell valves are moderately convex. Externally, the shell is dark grayish-brown with radially disposed white spots. The nacreous layer is iridescent with a silvery lustre except distally where it is black. This pearl oyster is also known as the Black-lip pearl oyster due to the dark marginal colouration of the shell. The width of the nacreous region at the hinge is about 2/3 that of the broadest part of the valves.

Pinctada chemnitzii (Philippi)

The shell is very similar to that of *P.fucata* except that the posterior ear is better developed and the convexity of the valves is much less. The anterior ear is well developed and the byssal notch is slit like. The hinge is almost as long as the antero-posterior measurement of the valves. Both the anterior and posterior hinge

teeth are present, the former is small and rounded and the latter prominent and ridge-like starting a little in advance of the posterior region of the hinge ligament. The posterior ear and the posterior sinus are well developed. The shell valves are yellowish externally with about four or more light brownish radial markings from the umbo to the margin of the shell. The growth lines of the shell are broad. The nacreous layer is thin and bright, while the non-nacreous layer is yellowish-brown.

Pinctada sugillata (Reeve)

The hinge is considerably shorter than the antero-posterior axis of the shell with a ratio of 1:1.3. The anteroposterior measurement is almost equal to the dorso-ventral measurement. The anterior ear in both valves is small and the byssal notch is a moderately wide slit. The anterior ears are slightly bent towards the right. The posterior ear and sinus are poorly developed. The convexity of the valves is not prominent, especially that of the right valve. The hinge teeth are small and the posterior one is slightly elongated. The shell valves are reddish-brown with six yellowish radial markings.

Pinctada anomioides (Reeve)

The hinge is shorter than the width of the broadest region of the antero-posterior axis of the shell with a ratio of 1:1.2–1.5. The hinge and dorso-ventral axis have a ratio of 1:1.4. Hinge teeth are absent or poorly developed. The anterior ear is moderately developed and the byssal notch at its base is deep. The posterior ear and sinus are absent. The shell valves are translucent and externally yellowish or grayish. Some shells have faint radial markings. The nacreous layer is slightly iridescent.

Pinctada atropurpurea (Dunker)

The shell is roundish and its hinge narrow. The valves are thin, translucent and moderately convex. A poorly developed anterior hinge tooth is present in some oysters. The shell valves are copper coloured.

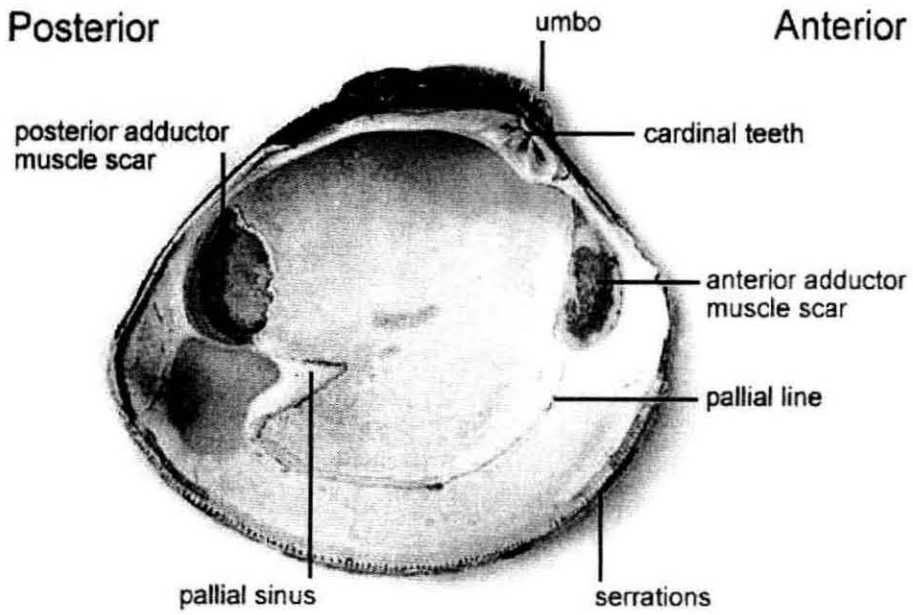
Biology

Pearl oyster is a filter feeder. The food sorting mechanism of pearl oysters is considered not very efficient as several organisms that cannot be digested find its way into the stomach and intestine. Sexes are separate but cannot be distinguished externally. The reproductive system consists of a pair of gonads, which spreads over the hepatopancreas and intestine in mature condition. It is pale yellow in males and deeper shade in females. *P.fucata* attains sexual maturity at about 15.5 mm size at eh age of 3-4 months. In the Gulf of Mannar, it spawns twice a year during June-August and November- January coinciding with the monsoons. At the Kusadai Island, *P.fucata* grows to 45 mm, 55mm, 60mm, and 65mm and70mm during five year period. Hatchery produced pearl oysters has been reported to attain model size of 47.5, 64.5 and 75 mm at the end of first, second and third years.

Distribution

In the Indian waters, six species of pearl oysters occur but only *P. fucata* has contributed to the pearl fisheries in the Gulf of Mannar and Gulf of Kutch. In the Gulf of Mannar, the pearl oysters occur in large numbers on the submerged rocky or hard substrata known as paars. The paars lie at depths of 12 to 25 m off the Tuticorin coast along a stretch of 70 km. In the Palk Bay, *P. fucata* occurs sporadically on loose sandy substratum attached to submerged objects in littoral waters. In the Gulf of Kutch, the pearl oysters are found as stray individuals on the intertidal reefs known as khaddas. In the southwest coast of India at Vizhinjam, Kerala coast, large numbers of spat of *P. fucata* have been collected from mussel culture ropes. The blacklip pearl oyster, *P. margaritifera* is confined mostly to the Andaman Islands where it is common in some places. From Lakshadweep, settlement of spat of *P. anomioides* has been observed on the ridges of rocks and corals.

Clams



Clam :Inner surface of left valve

4

Cephalopod Taxonomy and Biology

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Introduction

Cephalopods are marine molluscs and there are about 600 species in the world oceans, which are diverse in form, size and nature. Of these less than a hundred species are of commercial importance. Cuttlefishes, squids and Octopods are the three major groups of cephalopods, which belong to the highly evolved class of the phylum Mollusca, namely Cephalopoda, animals with feet around head. There are about 80 species of cephalopods of commercial and scientific interest distributed in the India Seas.

Taxonomic Position of exploited and potentially important cephalopods of India

Class	CEPHALOPODA	
Sub class	NAUTILOIDEA	
Family	Nautilidae	
Genus	*Nautilus*	*Nautilus pompilius*
Subclass	COLEDIDEA	
Order	Sepiodidea	
Family	Sepiidae	
Genus	*Sepia*	*Sepia pharaonis*
		Sepia aculeata
		Sepia trygonina
		Sepia brevimana
		Sepia elliptica
		Sepia prashadi
Genus	*Sepiella*	*Sepiella inermis*
Family	*Sepiolidae*	
Genus	*Euprymna*	*Euprymna stenodactyla*
Order	TEUTHOIDEA	
Suborder	Myopsida	
Family	Loliginidae	
Genus	*Loligo*	*Loligo duvaucelii*
		Loligo uyii
Genus	*Doryteuthis*	*Doryteuthis singhalensis*
		Doryteuthis sibogae
Genus	*Sepioteuthis*	*Sepioteuthis lessoniana*
Genus	*Loliolus*	*Loliolus investigatoris*
Suborder	Oegopsida	
Family	Onychoteuthidae	
Genus	*Onychoteuthis*	*Onychoteuthis banksii*
Family	Ommastrephidae	
Subfamily	Ommastrephinae	
Genus	*Ommastrephes*	*Ommastrephes bartrami*
Genus	*Symplectoteuthis*	*Symplectoteuthis oulaniensis*
Family	*Thysanoteuthis*	*Thysanoteuthis rhombus*

Order	OCTOPODA	
Suborder	Incirrata	
Family	Octopodidae	
Genus	*Octopus*	*Octopus dollfusi*
		Octopus aegina
		Octopus membranaceous
Genus	*Cistopus*	*Cistopus indicus*
Genus	*Hapalochalaena*	*Hapalochalaena maculosa*
Genus	*Berrya*	*Berrya keralensis*
Family	Argonautidae	
Genus	*Argonauta*	*Argonauta argo*
		Argonauta hians

1. SUBCLASS NAUTILOIDEA

Shell external, coiled and chambered, more than 10 (63 to 94) circumoral appendages without suckers, two pairs of gills, funnel bilobed.

2. SUBCLASS COLEOIDEA

Shell internal, embedded in tissue, calcareous, chitinous or cartilaginous, 8 or 10 circumoral appendages with suckers, only one pair of gills, funnel tube-like.

1. Order Sepiodea

Internal shell (sepion) calcareous and either straight and laminated or coiled and chambered or vestigial and chitinous or absent; eyes covered with skin and a supplementary eyelid present; eight sessile arms; two tentacular arms contractile and retractile into pockets; suckers without stalks; fin lobes free posteriorly.

2. Order Teuthoidea

Internal shell (gladius or pen) chitinous feather or rod-shaped, eight sessile arms; two tentacular arms contractile but not retractile, pockets absent, tentacles lost secondarily in some, suckers stalked and with or without hooks; fin lobes fused posteriorly. Eyes either covered or open and without supplementary eyelid.

3. Order Octopoda

Internal shell vestigial and cartilaginous except in females of *Argonauta* which has an external, calcified shell. Eight arms, suckers without stalks and without chitinous rings; tentacles absent; fins absent except in a few deep water species; light organs absent.

Key to the Identification of Genera and Species of Commercially Exploited Cephalopods of the Indian Seas

ORDER SEPIOIDEA

The salient features to be examined for the identification of genera and species of cuttlefishes are as follows:

1. Cuttlebone: General shape, nature of the dorsal surface, structure of the inner cone, number and nature of grooves and ridges on the ventral side, the nature of growth lines found in the striated area and the spine
2. Tentacular clubs: Number of transverse rows of club suckers and their relative size (diameter), the nature of protective membrane on the sides of the clubs.

3. Hectocotylization: Structure of the hectetocotylized arm with regard to the modified portion, the number and arrangement of normal and modified suckers and the extent of modification of the arm.
4. Shape and disposition of fins along the mantle.
5. In some species the characteristic external colouration and colour pattern of the mantle, head and arms noticeable in fresh material.

1. Body either elongate and broad or very slender an dorsoventrally flattened; fins marginal and narrow, extending all along mantle on either side; internal shell (sepion) present; head free from dorsal mantle; light organs absent (Family: Sepiidae).....2

Body saccular, wide, round bottomed; fins circular; internal shell lacking; dorsal mantle and head united by a nuchal commissure; saddle-shaped light organ presnt on ink sac. (Family: Sepiolidae)...*Euprymna stenodactyla*

2. Body without a glandular pore at posterior extremity; cuttlebone mostly with a spine (rostrum) at posterior end. (Genus: *Sepia*) 3

Body with a distinct glandular pore at posterior extremely on ventral side; with brownish fluid oozing out; cuttlebone devoid of spine (Genus: *Sepiella*).....*Sepiella inermis*

3. Body small and narrow, broadest part of body excluding fins distinctly less than half mantle length; fins narrow and marginal; cuttlebone very much slender and lanceolite in shape 8

Body wide and muscular, ovoid or elliptical in shape; broadest part of body excluding fins equal or distinctly more than half of mantle length; fins marginal, moderate to wide; cuttlebone chalky, elongate, wide and nearly ovoid in shape 4

4. Tentacular clubs with suckers of unequal size, a few in the manus very much enlarged; mantle, head and arms with transverse stripes 5

Tentacular clubs with smaller and subequal suckers but none greatly enlarged; no transverse stripes on mantle, head and arms 6

5. Body robust, fins braod commencing from edge of anterior mantle margin; tentacular clubs moderately long and well expanded; 5 or 6 suckers in middle row of manus greatly enlarged; cuttlebone broad, thick and with a midventral groove flattening anteriorly in striated area; striae '^' shaped; inner cone forms a conspicuous yellowish flat ledge; a sharp thick spine present; when live, body brownish, tigerstripe pattern prominent. ... . *Sepia pharaonis*

Body not robust; fins narrow commencing a few mm behind edge of anterior mantle margin; tentacular clubs short, expanded; not more than 3 suckers in middle row of manus greatly enlarged; cuttlebone narrow, midventral groove narrow and distinct, striae anteriorly broadly truncate

with lateral corners slightly produced forward; dorsal surface pinkish in colour; a sharp thin spine present. When live, dusty brownish, transverse stripes less distinct .....*Sepia prashadi*

6. Tentacular clubs very long, with 10-14 rows of minute subequal SUCKERS. Cuttlebone broad and thick with a median longitudinal ridge with a faint groove running medially on striated area; inner cone forms a ledge-like callosity ..... *Sepia aculeata*

Tentacular clubs either short or moderately long, with 6-10 rows of small suckers. Cuttlebone thin and elliptical of acuminate in shape with or without ridges on striated area, innercone without callosity 7

7. Tentacular clubs short with 6-8 small subequal suckers. Cuttlebone flat and distinctly acuminate anteriorly, dorsal surface rugose, a shallow median groove in the striated area, the striae 'Λ' shaped with a median shallow groove broadening anteriorly; inner cone and its limbs pinkish in colour; spine small, sharp and slightly curved.....*Sepia brevimana*

Tentacular clubs moderately long, with 10 rows of small suckers of uniform size. Cuttlebone thin, elliptical in shape, dorsal surface smooth; two conspicuous lateral ridges more prominent anteriad resulting in three longitudinal furrows in striated area; spine thick, sharp, long and well curved .....*Sepia elliptica*

8. No fleshy projections on head; fins extend upto end of mantle; tentacles with short clubs, suckers in eight rows, about five in third row enlarged. Cuttlebone lanceolite with acuminate anterior tip with edges of outer cone winged giving an arrow head appearance; spine small. .....Sepia *trygonina*

ORDER TEUTHOIDEA

The various characters used in identifying the different species of neritic and oceanic squids (Order Teuthoidea) are given below. The definitions and details of important characters and terms are given in the glossary of technical terms.

1. General shape of the mantle
2. The shape and proportion of fins, the contour of the anterior and posterior margins of the fin lobes; position of fins on the mantle viz. terminal or marginal; united or separated at the posterior end.
3. The relative size of head and arms; size, shape, number and arrangement of suckers on the arms and tentacular clubs; the nature and dentition of the chitinous rings of the suckers.
4. Presence of hooks and / or suckers on the arms and tentacular clubs.
5. Details of hectocotylization, the number and arrangement of normal and modified suckers and the extent of other modifications affecting the arm.
6. Presence or absence of light organs (photophores), their shape, number and position.
7. Nature of the funnel locking apparatus.

8. Presence or absence of accessory nidamental glands.
9. Shape of gladius.
10. Shape of eggs and egg clusters.

1. Eyes completely covered with a corneal membrane (MYOPSIDA: Neritic Squids) 2

   Eyes not covered with a corneal membrane and open to the surrounding medium (OEGOPSIDA: Oceanic squids). 7

2. Body elongate, cylindrical in outline; fins marginal, wide and muscular, very long almost running along entire length of mantle; elliptical in shape .....*Sepioteuthis lessoniana*

   Body elongate, narrow, either slender or stout, sides parallel or tapering; fins narrow, terminal running less than 65 per cent of mantle length and either rhombus (*Loligo*) or heart-shaped *Loliolus*) 3

3. Body elongate or short and stocky, posterior end of mantle blunt; fins broad, rhombic or heart-shaped, with head and arm crown more than 50 per cent of mantle length; vane of gladius broad with thin curved margins 4

   Body narrow and slender, posterior end of mantle pointed; head with arm crown distinctly less than 50 per cent of mantle length 6

4. Small squids. Mantle length of adults less than 60 mm; fins heart shaped; vane of gladius conspicuously broad at midlength .....*Loliolus investigatoris*

   Moderately large squids; fins typically rhomboid; vane of gladius narrow throughout. 5

5. Body elongate, mid-rib of gladius clearly visible through mantle skin; fins 50-57 per cent of mantle length; tentacular clubs large median manal sucker ring with 14-17 teeth; in males distal half of left ventral arm hectocotylized, papillae not fused.....*Loligo duvaucelii*

   Body short and stout; mid rib of gladius clearly visible through dorsal mantle skin as a median dark line; fins 55-65 per cent of mantle length; Tentacular clubs have median manal suckers with smooth rings; in males left ventral arm hectocotylized almost the entire arm; papillae on ventral margin fused with membrane ..... *Loligo uyii*

6. Mantle very long and slender with a ridge along midline in males; fins wide and long and more than 60 per cent of mantle length; more than half of left ventral arm hectocotylized distally in males; gladius narrow with almost straight margins and tapering gradually to a narrow point ..... *Doryteuthis singhalensis*

Mantle long, narrow and slender, no ridge but chromatophore concentration ventrally along midline; fins narrow and less than 60 per cent of mantle length; less than half of left ventral ventral arm hectocotylized distally in males; gladius narrow, sharply acuminate posteriorly ..... *Doryteuthis sibogae*

7. Oceanic squids with muscular body; head with nuchal folds on dorsal side at posterior end; rachis of gladius visible as a longitudinal ridge middorsally along the entire length of mantle; tentacular clubs with two rows of hooks, marginal suckers lacking. .....*Onchoteuthis banskii*

   Oceanic squids with muscular body; head without nuchal folds on dorsal side at posterior end; rachis of gladius not visible through dorsal mantle; tentacular clubs without hooks 8

8. Funnel locking cartilage ' ' shaped consisting of a narrow longitudinal groove and a short transverse groove branching from it medially. Fins broad and rhombus-shaped occupying nearly entire length of mantle ..... *Thysanoteuthis rhombus*

   Funnel locking-cartilage ' ' shaped consisting of a vertical groove and a transverse groove at right angles to it posteriorly. Fins terminal and less than 60 per cent of mantle length 9

9. Funnel and mantle cartilages of the locking apparatus fused together. An oval photophoric patch present middorsally near anterior margin of mantle; muscle of mantle ventrally without embedded light organs; two intestinal photophores present .....*Symplectoteuthis oualaniensis*

ORDER OCTOPODA

1. Cephalopods with eight arms; without an external shell; internal shell either vestigial or lacking; no great disparity between males and females in size; benthic in habit (Family Octopodidae) 2

   Cephalopods with eight arms; external shell present (in females); sexual dimorphism very marked, males very small; pelagic in habit (Family Argonautidae) 6

2. Right third arm in males hectocotylized with well developed ligula, calamus and spermatophoric groove; no water pores and embedded pouches between arm bases.....3

   Hectocotylized arm only slightly modified, ligula small about 3 per cent of arm. Small water pores leading to embedded pouches between bases of arms on oral surface ..... *Cistopus indicus*

3. Body either globular or slightly elongate and or firm consistency; arms long and tapering with moderately developed web between them; funnel not fused with head.....4

4. Body elongated oval; moderately large in size; dorsal surface of body and arm with reticulate pattern; no concentric rings of chromatophores on the body; ligula bout 5 to 10 per cent of arm; penis and diverticulum long 5

   Body globular smaller in size; skin smooth without reticulate pattern; while fresh dusty brown in colour with prominent bluish rings on mantle, head, web and arms .....*Haplochlaena maculosa*

5. Eyes prominent; a single large cirrus posterior to each eye. Ligula small, 5 to 8 per cent of arm; with shallow groove; penis and diverticulum together form U-shaped loop; spermatophores long and unarmed .....*Octopus aegina*

   Eyes inconspicuous; no eye cirrus. Ligula 8 to 10 percent of arm; with well formed groove and calamus. Long penis and short diverticulum together form reversed 6-shape; spermatophores long and armed with spines .....*Octopus dollfusi*

In addition to the above mentioned species, some more species of octopods such as *Octopus cyaneus*, *O. globosus*, *O. membranaceous*, *O. macropus*, *O. vulgaris*, *O. tetricus*, *Scaeurgus unicirris* are also known to occur in the Indian Seas and other parts of the Indian Ocean.

Biology of Exploited Species

All investigations on cephalopod biology centre around the commercially exploited species such as the Palk Bay squid, *Sepioteuthis lessoniana* (Rao, 1954), *L. duvauceli* (Kore and Joshi, 1975; Oommen, 1977; Silas et al., 1985; Rao, 1988; Mohamed, 1993), *Sepia pharaonis*, *S. aculeata*, *Sepiella inermis* (Oommen, 1977; Unnithan, 1982; Silas et al., 1985) and *Octopus dollfusi* (Sarvesan, 1969). The aspects of biology of cephalopods detailed here pertain mainly to *L. duvauceli*, *S. pharaonis* and *S. aculeata*.

Food and Feeding: Adult cephalopods are voracious and active carnivores feeding mainly on fishes and crustaceans. Fish always occurs in the diet of *L. duvauceli* of all sizes. The preference to crustacean meal declines with increase in size and there is evidence of cannibalism above 80 mm DML (Kore and Joshi, 1975; Oommen, 1977). Cephalopods are preyed upon by a variety of marine fishes (including tunas and billfishes) and cetaceans (Silas et al., 1985). Many workers have noticed the predominance of empty stomachs in samples and slackness in feeding during spawning period (Oommen, 1977). This may be due to the partial ingestion; fragmentation and rapid digestion of prey (Pierce et al., 1994).

Age and Growth: The relationship between length and weight of Indian cephalopods has been reported to be allometric with the 'b' value of the regression near to 2 than 3 (Meiyappan et al., 1993; Nair et al., 1993; Rao et al, 1993). This relationship is also significantly different for males and males and females (Mohamed, 1996).

Growth in cephalopods has been perceived to be linear, exponential, asymptotic and/or oscillating and Pauly (1985) advocated the use of VBGF model with seasonal oscillation as a means of standardising growth estimates of different cephalopods allowing comparative studies to be made. Studies on the growth of Indian cephalopods have been made by using the asymptotic (VBGF) model (Kasim, 1985; Philip and Ali, 1989; Meiyappan and Srinath, 1989; Meiyappan et al., 1993; Nair et al., 1993; Rao et al, 1993; Mohamed, 1996) and the seasonally oscillating version of VBGF (Mohamed and Rao, 1997). Clear sexwise difference in growth rate has been reported from Indian waters. In the case of *L. duvauceli* and *S. pharaonis* females grow faster than males, while in the case of *S. aculeata* males grow faster than females. A comparison of the results of various studies carried out in India is given in Table 2.

Size at First Maturity: Pioneering work on the reproductive biology of the Palk bay squid *Sepioteuthis lessoniana* has been made by Rao (1954). Later, Silas et al. (1985) reported on the maturity of three species of squids and six species of cuttlefishes. They reported that in *L. duvauceli* males attained sexual maturity earlier than females and in all species spawning is prolonged. The size at first maturity of male and female squids and cuttlefishes along west and eastcoast of India is shown in Table 3.

Maturity Stage and Spawning: Silas (1985) described and standardised the maturity stages for biological studies of squids and cuttlefishes. He described a simple 4 point (Immature, Maturing, Mature and Spent) maturity scale, which has since been used by all workers on Indian cephalopods. Rao (1988) gave detailed descriptions of *L. duvauceli* maturity stages on the above line.

Similar to other tropical marine resources, cephalopods along the Indian coast are reported to spawn almost throughout the year. Information on this aspect is scanty, but the peak spawning period of some of the studied species is given in Table 4.

The squid *L. duvauceli* spawns throughout the year along both the coast, but along the westcoast, peak spawning has been observed during post monsoon i.e., Sep-Nov (Kore and Joshi, 1975; Silas et al., 1985; Mohamed, 1993). This species forms large schools (consisting of fully mature animals, 80% males) during this season, and becomes vulnerable to the purse seine fleet operating along Karnataka coast (Mohamed, 1993) and also to cast netters along coastal knee-deep water of Alleppey (Meiyappan and Srinath, 1989). Based on this observation, Mohamed (1993) opined that the squids congregate for spawning (copulation) in near shore areas after which the females migrate to the shallow subtidal regions with hard substratum for laying the fertilized eggs. Asokan and Kakati (1991) have collected such eggs from the subtidal areas of Karwar for rearing. From the sex ratio (M 80:

F 20) of such squid schools it would be easy to conclude that female die after spawning (semelparity is common among cephalopods world-wide). However, based on the relatively low GSI levels and the occurrence of mature females over a wide range of size classes, Mohamed (1993) concluded that this species is a multiple spawner and not a semelparous species. More evidence needs to be gathered to reach a final conclusion. Similar studies on other commercial cephalopods are lacking.

Fecundity: Estimates on the fecundity of Indian cephalopods are few. Unnithan (1982) reported that in the spineless cuttlefish *S. inermis* the total number of ripe eggs of individuals between 69-71 mm DML was from 470 to 850 (average 14.9 eggs/g body weight). In the squid *L. duvauceli* Rao (1988) reported that on an average an individual produced 5300 eggs and that there was good correlation between length, ovary weight and fecundity. Mohamed and Nagaraja (1997) estimated the fecundity of the same species varied between 2000 to 14000 eggs (average 65 eggs/ g body weight). In general, fecundity is low in cephalopods because of the absence of a larval stage and the hatchlings are virtually miniature adults.

Stock Assessment and Management

Ever since the CMFRI initiated a major research project on the biology and stock assessment of cephalopod resources of India, a number of research papers have been published on the subject. Mostly F based models have applied to study cephalopod stocks. In the first study on Indian cephalopod stocks, Silas et al (1985), used length cohort analysis to estimate stock sizes. Later studies (Meiyappan et al., 1993; Nair et al., 1993 and Rao et al., 1993) also used cohort analysis to estimate mortality and stock and the yield and biomass estimates were obtained with length based Thomson and Bell analysis. Mohamed (1996) used the yield per recruit model to estimate MSY for Mangalore populations of *L. duvauceli*. Later Mohamed and Rao (1997) assessed the squid yield along Karnataka coast using the TB model to derive MSY and MSE. They also studied the relationship between spawning stock and recruitment of squids to assess the productivity of the population in terms of recruitment. They found that Ricker's stock recruitment curve could adequately explain the variation in recruitment with respect to spawning stock biomass (SSB).

Most of these studies indicated that cephalopods were either under exploited (e.g. *S. pharaonis* and *S. aculeata* along eastcoast) or optimally exploited (Table 2). While Mohamed (1996) and Mohamed and Rao (1997) found squid stock along Karnataka coast to be marginally over exploited.

Cephalopods are not a targeted fishery along the Indian coast (excepting seasonally along the SW coast) and therefore, it is difficult to set management targets and many of the models applied would have little relevance. Yet, Rosenberg et al (1990) suggests that the most effective means of managing cephalopod fisheries is by regulating fishing effort, which will reduce the risk of recruitment overfishing. The present ban on trawl fishing during the monsoon as variously practised by different maritime states along the westcoast is in effect a means of regulation of fishing effort and should be continued.

Utilization and Marketing

There is very little internal market demand for cephalopods and consequently almost all the catch is exported. While the quantity peaked in 1995, when cephalopods formed about the 45% of the total quantity exported, the annual average is about 25%. However, the value of cephalopods in total marine exports has remained at 15% from 1992 onwards without much variation. In 1996 the value of cephalopods exported amounted to more than Rs 8500 million. Categorywise, squid products are the maximum in all years followed by cuttlefish products. The products include dried, frozen whole, filleted, tentacles, rings, roe, wings, IQF and bones and ink. Octopus products exported are meagre, but from 1994 onwards there is rising trend in its exports. The main markets for export of Indian cephalopods are Europe, Japan and China.

5 Ecology of Pearl Oyster Beds and Pearl Fisheries of India.

ACC Victor, R C of CMFRI, Tuticorin.

World distribution

The pearl oysters belong to the genus *Pinctada (Roding)* come under the family Pteriidae. They occur in almost all the seas of the tropical and sub tropical belt. They inhabit the sea bottom from low tide level to depths down to 80 m. Although 28 species of pearl oysters are reported, only 3 species have been found to produce pearls of gem quality and have commercial value. They are *Pinctada maxima* (Jameson), *P. margaritifera* (Linnaeus) and *P. fucata* (Gould). The pearl oysters occur in the Persian Gulf (Bahrain, Kuwait, Dubai, Muscat and Bushira), Red sea (Farasan Islands, South of Sabia and Jidda, West of Mecca and Sudan), Philippines, Japan, China, Korea, Myanmar, Indonesia, Papua New Guinea, French Polynesia, Cook Islands, Australia, Gulf of California, Mexico, Panama and Venezuela.

Distribution in Indian Waters

In the Indian waters, six species of pearl oysters namely *Pinctada fucata* (Gould), *P. margaritifera* (Linnaeus), *P. chemnitzii* (Philippi), *P. sugillata* (Reeve) and *P. atropurpurea* (Dunker) have been recorded. Among these, *P. fucata* is the most dominant species. It occurs in large numbers in pearl banks known as 'paars' in the Gulf of Mannar and in the intertidal reefs known as 'Khaddas' in the Gulf of Kutch. *P. fucata* is the only species which has contributed to the pearl fisheries in these two gulf regions. In the southwest coast in India at Vizhinjam, large numbers of spat of *P. fucata* have been collected from mussel culture ropes. *P. margaritifera* is confined mostly to the Andaman Islands where it is common in some places. From Lakshadweep, spat of *P. anomoides* has been recorded on the ridges of rocks and corals.

Topography

The pearl oysters are always found attached by byssus to some hard substratum such as rocks, dead coral outcrops or sand grit covered with marine organisms. In the Gulf of Mannar, the areas of occurrence of pearl oysters are known as pearl banks or "paars'. The Gulf has about 65 such pearl banks located between Kanyakumari and Rameswaram. These banks lie at a distance of about 12 to 20 km away from the coast at depths of 15 to 25 m. These paars are divided into three divisions viz., Northern or Kilakarai Division extending from Adam's bridge to Vaipar, the Central at Tuticorin Division extending from Vaipar to Manapad and the Southern or Kanyakumari Division extending from Manapad to Kanyakumari. Of these the central division is the most productive one in view of the fact that out of the 40 fisheries that had taken place between 1663 and 1961, 39 fisheries had

been in the paars located in this division. A notable feature of these fisheries is their irregular character, fishing sometimes being conducted after long intervals. This is due to the periodical decline of fishable quantities of pearl oyster population in the pearl banks for a number of years. The probable factors responsible for the decline of oyster population are failure of spatfall, pests like weaving mussels and boring worms, predation by gastropods, octopi, sharks, rays and sea breams, overgrowth of algae, changes in the oceanographic conditions, occasional silting and non-availability of sufficient number of breeding population. In the Gulf of Kutch, the pearl oyster reefs are scattered along the southern part of the Gulf of Kutch. There are about 42 known pearl oyster reefs covering an area of 24,000 ha located between Sachana on the east and Ajad on the west. These beds are located in the intertidal region and are exposed at receding tides.

Primary Production

In the Gulf of Mannar area where the pearl oyster beds are situated the productivity has been observe the reach 7.3 g c/m^2/day which appears to be fairly high when compared to the values obtained in other areas of east coast near shore waters.

Wind, Water Movement and Current

Wind velocity shows a trimodal oscillation with maximum in June, August-September and December and maximum in March-April. The velocity is greater in southwest monsoon period of water movement. Devanesan and Chidambaram (1956) state that the water drift and current over the pearl banks of Ceylon and India may carry the larvae of pearl oyster from one coast to the other, thus holding the view of interdependence of the pearl banks of Ceylon and India for getting replenishment of oyster population. There is another possibility also. Depending on the direction and rapidity of water movement the pearl oyster larvae, at the plantigrade stage, might reach such areas in the sea with unsuitable sea bottom where they perish after settlement. All these factors thus play a vital role.

Turbidity

Flood water discharged from east coast rivers during the northeast monsoon rains in October-November, carry with it considerable silt which creates great turbidity over the pearl beds, particularly over the shoreward lying banks. This introduces a new dimension to the problem of growth and survival of oyster population met within 12 - 15 m depth range.

Temperature

Unlike Japan, the variation in temperature is not much pronounced in the pearl banks of Gulf of Mannar. The general temperature of the seawater in the paar varies from 23.8°C (January) to 33.5° C (May); the monthly average ranging from 25.9°C to 31.5°C. There appears to be some correlation between temperature and the breeding behaviour of the pearl oyster. The breeding season is more restricted in higher latitudes and occurs during warmer months.

Salinity

The salinity of the Gulf of Mannar normally varies from 30 to 35 ppt. If salinity falls below 15 ppt, and if such condition is prolonged, it may lead to mortality. This may happen during unusual heavy rain and heavy discharge of freshwater by rivers in the farm.

Dissolved Oxygen

Values ranging from 6.84 ml/l in October to 3.4 ml/l in September appear to be common in pearl oyster beds. A trimodal curve has been noticed with distinct peaks in June, October and January with a decline in April, September and November. It looks as though the oxygen saturation is greater in northeast monsoon months and less in southwest monsoon months.

Associated Fauna and Flora

The very fact that the fauna and flora of the pearl banks comprise the whole assemblage of more than 2,700 species of animals and 200 species of plants, small and large, makes the study of interrelationship among them very complicated although it is well recognized that the nature and density of such animate surroundings have a profound effect on the well being of the stock of oysters in the beds.

Characteristic of the area is the dense growth of sponges, especially in the northern Vaipar area. *Aulo-sponges tubulatus* (Bowerbank). *Phakellia donnani*, *Siponochalina communis* (Carter), Iotrochota spp., *Clathria procera* (Ridley), C. *indica* Dendy, *Mycale grandis* Gray, *Zygomycale parishii* (Bowerbank), *Phyllo-spongia* spp., *Spongionella* spp. and *Suberites* spp. are abundant. Dense forest-like growth of the gorgonid *Juncella juncea* Pallas and *J. gemmacea* (Valenciennes) is noticed in the northern area.

The growth of the coral *Heteropsammia* sp. is characteristic of the paars. *Montipora* sp. and *Echinopora* sp. are the other corals in addition to *Porites* sp.

The molluscan fauna is mostly represented by myriad number of *Modiolus* spp. spreading like mattress on the bottom. Large *Pinna* spp. are found in good numbers rooted in this layer of sand covering the rock in many places. *Cypraea tigrinus* are seen in rocky pits. *Oliva* spp., *Conus* spp., *Nassa* sp. and *Bulla ampulla* are the other common shells..

Among the echinoderms *Lamprometra palmata palmata* (J. Muller) and *Comanthus (Comanthussis timorens)* (J. Muller) are the most common under rocky crevices and over the gorgonids and sponges. *Holothuria edulis* Lesson, *Protoreaster linki* (Blainville) and tests of *Clyspeaster humilis* (Leske) are the other common species.

The fish fauna is fairly rich and consists of *Scolopsis bimaculatus* Ruppell, *S. vosmeri* (Block), *Abalistes stellaris* (Bloch), *Upeneoides* spp., *Chaetodon* spp., *Pomacanthodes annularis* (Bloch) and *Lutjanus lineoiatus* (Ruppell). Large fishes

like *Gaterin* spp., *Ennaeacentrus miniatus* (Forsk), *Epinephelus* spp., *Lethrinus* spp. and *Siganus* spp. are abundantly seen.

The flora is poor in the southern area but in the Vaipar area *Gracilaria* spp., *Hypnea* spp. and *Sargassum* spp. are common.

The oysters are often found in clusters piled together in such profusion so as to interfer with one another's growth and stunting many.

Pearl Fisheries

The pearl fisheries of Madras State are known from time immemorial. All the ancient literature in Sanskrit and Tamil refer to these fisheries. The existence of the pearl fisheries on the Tamil Coast is evidenced by foreign writers. South India had much commercial intercourse and political relations with the countries of the East and West. Indian pearls were used extensively in Rome, Egypt, Babylon, Persia and Greece before the 4^{th} century A.D. Between the 4^{th} to 10^{th} centuries A.D., the Arabs, Chinese, Egyptians and Greeks were trading in Indian pearls during that period. From the 10^{th} to 12^{th} century the Chola Empire monopolished the entire pearl fisheries of this area. From the 13^{th} to 16^{th} centuries, the pearl fisheries prospered under the Pandyas and the moors. The Portuguese had control over the pearl fisheries from 1524 to 1658, when the Dutch dispossessed them. The latter managed the pearl fisheries until the British took over in 1790 and controlled them until India attained independence in 1947.

The pearl banks came under the control of the British in 1796, from which year records of pearl fisheries were fairly well kept.

The pearl fisheries were placed under the control of the Madras State Fisheries Department in 1908 for proper conservation and development.

The Gulf of Mannar and the Gulf of Kutch are the well known haunts of this resource and pearl fisheries had been organized in the past from Tuticorin and Jamnagar respectively. The paars of Gulf of Mannar have yielded to very valuable fisheries in the past, the most successful of which has been the fishery series of 1955-1961.

Since 1961 the pearl banks have again become barren and the present indication are that there are no prospects of a pearl fishery in the immediate future. Even so it should be possible to collect oysters for experimental purposes.

While the pearl fishery of the Gulf of Mannar has been in existence from time immemorial, in the adjacent Palk bay only one pearl fishery was held in 1914 off Tondi. This did not prove commercially successful and attempts to find pearl oysters in fishable quantities in the subsequent years only yielded negative results.

In the Palk Bay area, the seabed does not appear to be conducive for the settlement of the oysters exept for a small stretch of ten kilometers distance from Dhanushkodi to Rameswaram where rocky patches occur at depths ranging from 7

meters to 13 meters. Apart from a freak fishery in 1914 held at Tondi lasting for 20 days there is no record of any other fishery having been conducted in the Palk Bay. Hence the Gulf of Mannar grounds are considered to be more important and productive.

The pearl fishery of the Gulf of Mannar have been of an irregular character and in the span of three centuries from 1663 to 1961 there have been only 38 official fisheries Table 1.

The Pearl fishery was conducted by the Department of Fisheries, Madras State, with Tuticorin as the base of operations. When the Departmental survey revealed the availability of fishable quantities of pearl oysters over 3 years age and when the evaluation of pearl content shows satisfactory results, a pearl fishery is declared to the public. The fishing season lasts for a month or two depending on the favourable weather conditions and the strength of the oyster population.

Table 1: Available Records Show that from 1663, there were 38 Pearl Fisheries

S. No.	Year	Gross Revenue
1	1663	Fl 18,000
2	1669	No Particulars
3	1691	No Particulars
4	1700	Very meager
5	1708	£ 9,000
6	1747	£ 5,000
7	1748	£ 9,560
8	1749	£ 42,477
9	1784	Rs. 39,109
10	1787	Rs. 63,000
11	1792	Rs. 42,525
12	1805	Rs. 39,109
13	1807	Rs. 2,91,539
14	1810	Rs. 2,38,897
15	1815	Nil
16	1818	Rs. 1,69,708
17	1822	Rs. 1,5,693
18	1828	Rs. 70,127
19	1830	Rs. 1,01,639
20	1860-61	Rs. 2,50,276
21	1862	Rs. 1,29,003
22	1889	Rs. 1,89,984
23	1890	Rs. 25,061
24	1900	Rs. 9,461
25	1908	Rs. 10,218
26	1914	Rs. 16,542
27	1926 Feb-Mar	Rs. 2,25,498
28	1926 Nov-Dec	Rs. 31,387
29	1927 Feb-Apr	Rs. 2,54,497
30	1927-28 Nov-Jan	Rs. 2,54,497
31	1928 Mar-Apr	Rs. 1,95,039
32	1955 Mar-May	Rs. 1,46,138
33	1956 Feb-Mar	Rs. 44,795
34	1957 Mar-May	Rs. 1,46,138
35	1958 Mar-May	Rs. 4,74,096
36	1959 Feb-May	Rs. 8,65,130
37	1960 Mar-May	Rs. 2,53,339
38	1961 Mar-Apr	Rs. 3,18,234

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Natural Pearl Production and Pearl-sac Theory

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India is endowed with rich resources of pearl oysters both in Gulf of Mannar adjoining Tamil Nadu coast, Andaman and Nicobar Islands, and Gulf of Kutch along north Western Gujarat coast and Vizhinjam in the Southwest coast of India is also blessed with the settlement of pearl oyster spat in spat collecters. The marine pearl oyster and fresh water mussel from rivers, ponds and fields was used to produce pearls. The pearl oyster *Pinctada fucata* (Gould) belongs to the Phylum Mollusca, class pelecypoda, order pseudolamellibranchiae, family Pteriidae and genus *Pinctada*. In the Andaman and Nicobar group of Islands, the black-lip pearls oyster *Pinctada margaritifera* is available in stray numbers. There are four more species of pearl oysters *Pinctada sugillata*, *P.chemnitzii, P.atropurpurea* and *P.anomioides* from Indian waters. In Gujarat coast pearl oysters are found along the intertidal zones "Khaddas" of Jam Nagar Districts.While in Gulf of Mannar the oysters are always found under water on submerged reefs or rocky areas at a depth of 10-20 m at distance of 11-16 km from the coast. The oyster beds are locally known as "paars" and the total number of such paars is more than 60 in Gulf of Mannar.

Pearl Fishery

The pearl oyster resources have been fished for pearls and mother- of-pearl oyster shells since 13th centaury. In a period of 298 years commencing from 1663-1961, a total of 40 pearl fisheries had taken place along the Tamil Nadu coast from which pearls worth several million rupees had been obtained. In the Gulf of Kutch, there have been 25 pearl fisheries during 1913-1967. Pearl fishery of Gulf of Mannar came to an end in by 1966 due to lack of adult oysters in the natural beds. During 1967 pearl fishery activities at Gujarat coasts came to stand still due to the paucity of adult/old pearl bearing oysters for a sustainable fishery. To asses the pearl oyster resources of the Gulf of Mannar, an under water survey programme was under taken in 1958 by the Central Marine Fisheries Research Institute in collaboration with the Department of fisheries, Government of Tamil Nadu using Modern method of SCUBA diving. And the cause of depletion of oysters was mainly due to over fishing, biological, environmental, predation, siltation and now pollution and dynamite fishing.

Since there is no scope for obtaining natural pearls just like other countries India also started pearl culture actually for producing pearls from pearl oysters collected and grown in farms off Veppalodai as a ad- hock scheme during 1971 in collaboration with State Fisheries, Tuticorin Tamil Nadu.And now India has achieved tremendous progress in pearl culture and associated research programmes in pearl oysters and pearl production.

Pearl Formation
Natural Pearl

Most accepted theory to be known as the pearl-sac theory explains that a pearl is formed when the pearl –secreting cells of the mantle migrate into the body of the oyster under the stimulus of a foreign body (undischarged eggs of the oyster; sand grains got into the shells and formed pearls; and that parasites or other eggs or other organic matter formed the core of the pearls); and form a pearl- sac that secretes nacre which gets deposited on the foreign body and in course of time a pearl is produced according to the shape of the foreign body.

Cultured Pearl

The term "cultured pearl " was used for the first time in 1920 for the pearls produced in Japanese pearl oyster "akoya gai" and marketed in Europe.The name Mikimoto is the first man when cultured pearls are mentioned, the Australian Saville-Kent is now believed to deserve the credit for the original development of the technique. His technique involved taking a piece of mantle tissue from one oyster and implanting it in another. The term 'artificial pearl' does not denote a cultured pearl, but would refer to cheap imitations made of plastics, glass etc; by using the extract " guanine" from fish scales for artificial shine. The tissue culture pearls may be in trade in large quantities from some of the countries the secrets are not exposed wherein large quantities of pearls could be produced from the isolated cells of the mantle in the controlled laboratory conditions. For the production of a cultured pearl a shell bead nucleus is implanted into the gonad of the oyster along with a mantle graft tissue by a skillful surgery. The core material called shell bead nucleus is produced from the fresh water mussel shells from America which is imported to Japan, China Thailand and Australia where they produce the spherical beads of 2 –22 mm size according the size of oysters to be used for pearl production. Necessary surgical tools are designed and developed by CMFRI.The "Mabe" image pearls are produced by implanting the images of required object in between the mantle and shell cavity without affecting the mantle. This technique has been developed by CMFRI, during 2002.The tissue culture pearl production is under perfection.

Implantation Technique

The healthy adult pearl oysters are anesthetized using mentholated seawater in closed containers or pegging in between shells with wooden pieces. A passage is made from the base of the foot towards the gonad of the host oyster without damaging any of the vital organs of the oyster, for which it is mounted on an oyster stand. After that skillful surgery, the mantle piece (graft tissue) and the shell bead (nucleus) are implanted into the gonad (through the passage already made) to lie in contact and proper orientation. The oysters are maintained in the laboratory for two to three days for convalescence with sufficient fresh seawater and aeration for healing of the wound. The care is given to form the pearl-sac over the nucleus by implanted grafted mantle piece to get a good quality pearl from each oyster.

The operated oysters are put in iron cages with lid netted with synthetic threads /plastic baskets/netlon bags and suspended from the raft, rack, long line or kept on the under water platforms land bases culture tanks with sufficient water air and feed etc; according to the area in an air conditioned room without contamination.

In Indian pearl oyster the nucleus of 2-8 mm can be used and the duration for sufficient coating of nacre on the implanted nucleus varied from 4-22 months. The oysters could be checked after 3 months to assess the retention of the nucleus by narcotizing the oysters or by X-ray screening. The X-ray screening is expensive in the case of small *P. fucata* pearls while it in the case of *P.margaritifera* and *P.maxima* pearls for which are costly it is possible.

Harvesting, Grading, Processing and Marketing

The pearls are harvested by cutting and separating the two valves and squeezing out the pearl from the gonad of the oyster. The harvested pearls are washed in distilled water, polished with concentrated salt solution and again washed in distilled water wiped with soft cloth and dried and stored. The percentage of pearl production varies with efficiency of the operation, environmental and health conditions of the animal. The pearls are graded according to this format "A "with spherical and good lustre, "B" some times a pimple like spot with good shining and all the characters of "A" and "C" with good one to three teats with spot and shining.

In the international market pearls of larger size are highly valued. India is importing pearls worth Rs.29 crores every year. The major countries involved are Bahrain, Hong Kong, Japan, the UAE and the U.K. In the present condition in India some private companies have produced pearls and sold internally and exported very little. Based on the packages developed, CMFRI has been offering regular training to officials from State Government, Universities, Research Institutes, Krishi Vigyan Kendras, industry and progressive farmers on pearl oyster hatchery, pearl culture and **SCUBA** diving for studying the under water ecology of pearl oyster beds and resources. India is offering pearl culture training to candidates from other nations. The Swaminathan Foundations have come foreword to finance the fishermen of the coastal villages of Ramnad District in Tamil Nadu to do pearl culture and CMFRI, is giving training in pearl culrutre and also supplying implanted oysters to the farmers and they grow them in the rack constructed in the sea. The final harvest was encouraging and this will certainly give an impact to subsidies the fishermen/women groups in other parts of the country .The Central Marine Fisheries Research Institute is now handling an NATP Project worth 1.3 Crores under World Bank in "Breeding culture of pearl oyster and pearl production and The Department of Ocean Development is funding a Project cost 1. at Andaman on "Production black pearls in *Pinctada margaritifera* " if succeeds the black pearl production will increase export income of the country. India has to go forward to commercialize the pearl culture programme and production of large quantity of bigger and quality pearls both marine and freshwater to compete in the world pearl trade market.

Recently CMFRI has conducted First Indian pearl congress and exposition during 5-8th February, 2003 inviting all the pearl workers in the country to discuss and sort out the problems encountered in the pearl research and production of pearls in India.

The researchable issues are production of pearl oysters to hold larger nucleus 6-8 mm dia and the preliminary works already started. Since the pearl production is a long term process the diversification of the process to hatchery/production of young ones from nature. Mother oyster culture, implantation and convalescence, post operative culture, harvest of pearls and processing, marketing/jewellary products, by products etc. and export. All these aspects come into limelight if marine Pearl Park is identified and demarcated to avoid communal and socioeconomic conflict in the sea based aquaculture programmes.

Formation of a Pearl

In the simplest way, it may be stated that a pearl is formed when a foreign body, such as a grain of sand or parasite, lodges into the soft tissue of the pearl oyster. Since the oyster cannot always get rid of this irritant, it protects itself by secreting nacreous substance that gets deposited over the foreign body in thin micro layers, thus forming a pearl. Since only the outer epithelial cells of the mantle are capable of secreting nacreous substance the chances of pearl formation are limited only to those cases where the foreign body is in contact with the mantle epithelium. The mantle epithelium at the point of contact of foreign body under goes changes. The outer epithelium regenerates a new layer of cells, which spreads over the foreign body and covers it completely. This layer is called the pearl-sac. The pearl-sac epithelium secretes nacre around the foreign body, which becomes the nucleus or core material of the pearl. The pearl oysters learn to live with the encysted pearl. The pearl grows in size as the oyster grows.

As to the nature of the foreign body that acts as the irritant it may be of organic or inorganic origin. Sand grain is the common inorganic material that finds an entry into the pearl oyster. The larval forms of parasitic cestodes and trematodes, and minute plank tonic organisms form the organic core material around which pearls are formed.

Blister Pearl

Ma y takes place when foreign body lodges itself between the shell and mantle. Polydora boring, sponge boring, foreign body attaches to the shell and mantle secretes nacre on that and a blister pearl is formed..

Free Pearl

Under certain conditions, the foreign body gets embedded in the connective tissue of the mantle. The invading body as it breaks through the outer epithelium of the mantle carries a few epithelial cells, which would regenerate and grow into complete pearl-sac around the foreign body. Besides shell and mantle pearls are

found in other soft tissues of the pearl oyster such as adductor muscle, gills and pallial muscles.

Pearl Without Nucleus

Given this accepted theory of pearl formation with a pearl-sac and a nucleus, the term "pearl without nucleus" would seem to be a contradiction. It is supposed the size of the nucleus may even be as a few microns. It is possible that a few decayed cells blood epithelial cells or epithelial cells might provide the basis for pearl formation but subsequently disintegrate totally. Such pearls, when cut and examined, would not reveal any nucleus and would appear to be formed entirely of mother of – pearl layers without recognizable nucleus.

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Pearl Nucleation Techniques

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Introduction

Natural pearl is produced between mantle and shell of a live pearl oyster in its natural environment. The formation of natural pearl is influenced by foreign bodies accidentally entering the body of oyster. Under stimulus of the foreign body, the outer epithelium of the mantle invaginates and forms a pearl sac. In this case production of free and spherical pearls is rare and also percentage pearl production is less. The cultured pearl production technology ensures the production free and spherical pearls and guarantees large-scale production of pearls. The technology was developed in the year 1973 in India. The techniques involved in cultured pearl production are explained below.

Surgical Tools for Nucleation

Oyster knife	:To open live pearl oyster shell valves
Incision blade cum grafting needle	:Used to make incision and to lift graft tissue
Nucleus cup	:To lift nucleus
Spatula	:To brush aside organs before implantation and to clean excised mantle piece
Hook	:To hold foot during surgery
Graft cutting knife	:To cut mantle strip
Forceps	:To hold mantle strip during graft tissue preparation
Speculum	:To keep open shell valves
Oyster clamp	:To hold narcotized oyster during surgery

Selection of Oysters

Selection of oysters is an important process, which ensures production of quality pearls. The factors such as the weight of oyster, reproductive phase and health are considered during selection. Oysters with weight of 25 g are found to be ideal for implantation. However oysters with 20 g weight may be considered for implantation of smaller size of nucleus. Fully mature oysters are not suitable as the gametes flow out during operation. Hence oysters in the post-spawning/ recovery phase and also in the early phase of gamatogenesis may be selected. Oysters free of

blisters caused by polychaetes and sponges and trematode infection may be selected for surgery.

Graft Tissue Preparation

Healthy donor oysters are selected for mantle tissue preparation. The shell valves are separated by inserting a knife between valves and severed the adductor muscle. The mantle strip of both valves is used. The mantle is cut and removed with minimum disturbance. Careless removal may cause shrinkage of the strip and may not be useful for graft preparation. The mantle strip is placed on a moist wooden block without changing the side and stretched sufficiently by holding both ends. A gentle cleaning is done with soft sponge to remove adhered particles and mucus. The pallial organs at the free end of the mantle strip and muscular connective tissue at the lower end are removed. Final cleaning is done with soft wet sponge and the strip is reversed so as to expose the outer phase of mantle and is cut into small pieces of 2-3 mm. The size of the piece has to be in proportion to the size of nucleus. The processed pieces of mantle are kept moist with a very dilute solution of water-soluble eosin using a brush. The eosin solution stains the grafts red, keeps the cells unaltered and provides aseptic condition.

Nucleus

Spherical shell beads are necessary as core material for production of round cultured pearls. Shell beads are prepared out of thick shells of fresh water mussel and other molluscs. Requirement of shell bead nuclei in India is currently fulfilled by import from Japan and Hong kong. However indigenous technology for preparation of shell bead nuclei has been developed in the Central Institute of Fisheries Technology (CIFT), Cochin utilizing shells of molluscan forms. The beads are cleaned in distilled water and dried before use.

Conditioning of Oysters

Natural physical methods are safer and inexpensive. Selected oysters are arranged vertically by dorsal side down and half immersed in seawater. Depletion of oxygen in the limited water makes oysters to wide open their valves for want of oxygen. Such oysters are plugged with wooden peg0 and fitted in an oyster clamp for surgery.

Synchronized narcotisation is also practiced using menthol crystals. In this case oysters are immersed fully in seawater in a tub and menthol powder is sprinkled over the seawater. The oysters narcotized under the effect of menthol in about 60-80-minutes. These oysters are then taken for surgery on by one. Keeping oysters in menthol water for prolonged period causes physiological upset leading to death. Duration of about 30-45 minutes immersion in menthol water is the safer limit.

Surgery

Gonad is the primary and best site for nucleus implantation. Single implantation is performed at this site. In double implantation, the larger nucleus is

placed at this site and the smaller nucleus at the dorsal region of gonad close to hepatopancreas. Multiple implantations are carried out at many sites in the visceral region.

Conditioned oysters with speculum are mounted on the oyster clamp keeping its anterior side to right side of the technician. To start with the gills are pushed aside with spatula so as to expose the site of incision. The foot is held by needle hook and pulled slightly to elevate the base of the foot. A sharp incision is made at the base of the foot and through which a passage is created below the outer skin by incision needle up to the site of implantation at the gonad region.

A piece of graft tissue is inserted through the passage and placed at the gonad. Now the outer epithelium of the graft tissue is facing the passage. In the similar way nucleus also inserted and placed in touch with the graft tissue.

Convalescence

The nucleated oysters are placed either in a gentle flow through water system or in a tub containing well aerated filtered seawater for convalescence. In the latter system the water is changed frequently to overcome the effect of narcotisation. On placing in seawater oysters would shut their valves within a few minutes and slowly resumes its normal shell activity. The incision would be healed off in two to three days. If the incision and the passage are large the nucleus may slip out through the passage.

Post-operative culture

The operated oysters are retained in the lab for 3-4 days under observation before they are returned to farm. The oysters that have ejected nucleus are taken back to mother oyster culture to be used again. Dead oysters, if any, are removed and the rest of oysters are placed in a cage at low density. The oysters should not be disturbed too frequently. They are suspended from the raft in the farm at greater depths. The greater depths ensure the production of quality pearls and less fouling. The post-operative culture varies as per the size of nucleus used. The range is about 3-24 months for 2-7 diameter nuclei under tropical conditions where the growth of pearl is faster than temperate conditions. Periodic maintenance of operated oysters is highly essential to promote good growth of oysters and pearls. Test harvest may be made at periodic intervals to assess pearl maturity. Premature harvest of pearls would result in poor lustre and iridescence. The pearl having a minimum of 0.5 mm nacre thickness is valued as pearl at international market.

Production of Cultured Pearls

The rate of production of cultured pearls depends on many variables. Formation of cultured pearls is a biological process as it is governed by the pearl oyster after nucleus implantation. Human control is restricted to the success of surgery and to provide suitable environmental conditions.

Mortality of oysters can take place due to effect of surgery and infection and due to many other factors such as disease, shell boring and biofouling. Annual mortality should be kept within 10 % of the stock through proper farm management.

Nucleus rejection is a common feature. This should be avoided by skilful operation and adopting advanced surgical procedures.

All nucleated oysters may not produce pearls, as some oyster may have only nucleus with out pearl coating. This is due to failure in orientation of nucleus and graft tissue. This may happen by wrong placement of graft tissue to nucleus. Such failures should be kept within 5 % level through greater care in surgery.

Gross production is the number of cultured pearls produced by the surviving operated oysters in the farm. In single implantation, production rate achieved is about 65 %. In multiple implantations production achieved is about 180 % with reference to number of nuclei implanted. These rates can be improved further.

Quality Improvement

Attention should be given to improve the quality of cultured pearls. The size, colour and lustre determine the quality and value. Individual pearls of exceptional quality would fetch high price. The quality of pearl can be improved through appropriate care at surgery and farming. Colour and lustre of pearls are partly decided by genetic character of individual oyster and partly by environmental factors. Genetic differences are evident between different species of pearl producing molluscs. The genetic character of nacreous later of each species is reflected in pearl colour and lustre. It is evident that the colour and luster differ among the species of pearl oysters. In general, pearls produced by pearl producing molluscs have the same colour and lustre as the nacreous layer of their shells.

Environmental factors play a major role in determining the colour and lustre of nacre. Depth is one of the important factors as quality of pearl is produced in deeper waters. Low fouling and low temperature in deeper waters promote production of quality pearls.

Temperature also influences the growth and quality of pearls. Higher temperature accelerates the metabolic rate of oyster, which causes rapid deposition of nacre. Rapid deposition of nacre causes poor quality pearl. Slow and steady deposition of nacre as thin layers result in the production of quality pearls. Hence it is evident that pearl harvest may be executed during the period of low temperature and pH.

Minerals and trace elements in seawater are considerably important as these influence the colour of pearls. Hence the chemical factors of farming sites should be thoroughly understood. The quality and quantity of phytoplankton ingested by the oyster determine the colour and lustre of pearls

8 Hatchery Production of Pearl Oyster Seeds

S. Dharmaraj, R C of CMFRI, Tuticorin.

Introduction

In India the pearl oysters occur in the natural beds of Gulf of Mannar and Gulf of Kutch. Depletion of natural stock caused great concern to the development of pearl culture industry in the country. The grave situation warranted an urgent need to develop hatchery system for the production of seeds under controlled condition. The Central Marine Fisheries Research Institute established shellfish hatchery laboratory at the Tuticorin Research Centre, Tuticorin. A breakthrough was achieved in the breeding and production of seeds of pearl oyster, *Pinctada fucata* in 1981. The success laid foundation for the production of seeds of other bivalves like the edible oysters, mussels, clams, windowpane oysters and the gastropods like the abalones, chanks. The techniques involved in the hatchery system were standardized and mass production of seeds was achieved.

The hatchery system has two phases i) Brood stock maintenance and spawning and ii) larval rearing and spat settlement.

Brood stock maintenance and conditioning

Maintenance of brood stock is a vital part in the hatchery that ensures ready supply of ripe oysters throughout the year. Sexually ripe oysters are maintained under controlled condition at low temperature around 22-25° C in an air-conditioned room. The oysters are adequately fed at 4 litres of mixed algae per oyster per day. The mixed algae contained mostly *chaetoceros* sp. Aeration is provided throughout the conditioning period. Under such conditions ripeness of gonad is retained for a prolonged period. The brood stock of oysters is taken to hatchery for spawning as and when required.

Spawning

i) **Natural spawning**: There are two spawning seasons (June-August & November-January) in a year for pearl oysters in the Gulf of Manner. During spawning season most of the pearl oysters would be sexually ripe and may spawn naturally when there is slight change in water temperature or water pressure. When the oysters are not spawning naturally, they are induced to spawn through thermal stimulus or chemical means.

ii) **Induced spawning**

a) **Thermal stimulation**: This is one of the best devices that it is universal factor in natural environment for inducing spawning not only in pearl oysters but also in other animals. When the oysters

conditioned at 22-25 °C are suddenly dipped in higher water temperature having even a difference of 1 or 2 ° C, the slight change in water temperature stimulates the ripe oysters to spawn. Gradual increase of water temperature also causes spawning in oysters. The maximum temperature at which the oysters can tolerate is 34° C.

b) **Chemical stimulation**: When the oysters are not responded to spawning in thermal stimulation they are resorted to chemical inducement.
   i) Seawater with pH 9.0 is prepared using TRIS buffer (Hydroxymethyl aminomethane). Sexually ripe pearl oysters are immersed in the alkaline seawater for 1-2 hours. If these oysters are transferred to normal seawater after treatment spawning is effected in about 75-80 % of oysters.
   ii) Seawater with pH 9.5 is prepared using Sodium hydroxide. When the oysters are treated in the alkaline medium 70-75 % of oysters spawned after transferring to normal seawater.
   iii) 6% hydrogen peroxide (H_2O_2) in 3.064m^M concentration in an alkaline medium (pH 9.0) using TRIS buffer had induced 62.5 % spawning in pearl oysters after 4 hours treatment.
   iv) 6% hydrogen peroxide (H_2O_2) in 3.064m^M concentration in an alkaline medium (pH 9.0) using TRIS buffer had induced 62.5 % spawning in pearl oysters after 4 hours treatment.
   v) 6% hydrogen peroxide (H_2O_2) in 6.128 m^M concentration in an alkaline medium (pH 9.0) using sodium hydroxide had resulted in 9.5 % spawning in pearl oysters after 4 hours treatment.
   vi) Injection of 0.2 ml of 0.1 N solution of ammonium hydroxide (NH_4OH) into adductor muscle or foot of ripe pearl oyster had induced spawning in about 50 % of oysters.

Embryonic Development of Larvae

Sexes are separate in pearl oysters. Invariably the males initiate spawning in normal conditions. The sperm loaded water is consumed by female oysters and stimulated the females to release eggs 45 minutes after male spawning. Fertilization takes place immediately in the water medium. The fertilized eggs having polar body settle down and cell division starts 45 minutes after fertilization. Two-celled stage is obtained with a micromere and a macromere and now the polar body is placed at the furrow of the cleavage. During the second cleavage the micromere and macromere released one micromere each. The stage having three micromeres and one macromere is called trefoil stage. The macromere does not take part in any divisions. Further divisions are taking place only in micromere resulting in to 4-, 8-, 16-, 32-, 64-celled stages and so on. The resultant stage is called morula. The stage is reached 4 hours after fertilization. It contained a ball of small micromeres and each micromere is developed itself a tiny cilium. The morula having such cilia starts its first movement in water column. The morula exhibits phototrophism that facilitated collecting of viable embryo leaving bottom debris in the spawning container.

By reorientation of cells a blastula stage is reached after 5 hours having a blastocoel and a blastopore. The cells convolute in through the blastopore and form dermal layers namely ectoderm, mesoderm and endoderm along with archenterons. The stage is called gastrula that took 7 hours to reach the stage. After gastrulation the embryo is transformed into trochopore larva after 10 hours. The larva develops a long single flagellum at its apical end surrounded by a pre-oral band of cilia. The post-oral band of cilia is situated at its rear end. The larva swims with the help of flagellum and cilia.

The outer ectodermal cells of the trochophore larva secretes the first embryonic shell material called prodissoconch I. The site where the prodissoconch I starts is formed as straight hinge line. When the formation of shell material is continued, the larva assumes D shape having a straight hinge line. The stage is called veliger or straight hinge stage or D shape larva reaching between 18-20 hours. The single flagellum disappears and a powerful locomotor organ called velum forms. Now the larva measures 67.5µm along the antero-posterior axis and 52.5 µm along dorsoventral axis.

Larval development

Veliger: The veliger larvae are collected, estimated and stocked at a density of 2 larva/ml in FRP tanks. The larva is fed with a micro alga *Isochrysis galbana* at the rate of 5000-cells/larva/ day. During larval phase no aeration is provided.

Umbo stage: The veliger larva reaches umbo stage in 10- 12 days. It measures 135 x 130 µm. The shell growth beyond veliger is by the addition of prodissoconch II. The shell valves are equal and mantle folds develop. Feeding is given at 10,000 cells/larva/day.

Eyed stage: Eyespot is developed on 15th day when the larva measures 190 x 180 µm. The eyespot is situated at the base of the foot primordium. Larva develops ctenidial ridges. Feeding is increased to 15,000 cells /larva/day.

Pediveliger larva: Foot is developed on 18th day at the size of 200 x 190 µm. At transitional stage of swimming to crawling phase, the larva has both velum and foot. When the foot becomes functional and attaches to a substratum, the velum gets reduced. Gill filaments developed. The rate of feeding at this stage is 20,000 cells/larva/day.

Plantigrade stage: The stage is reached on 20th day when it is 220 x 200 µm. Labial palps and additional gill filaments develop. Extra shell growth is noticed all along the globular shell margin except the umbo region. By the addition of such shell growth the plantigrade transforms in to adult stage. The stage needs 25,000 cells of Isochrysis per day.

Spat: The spat has again developed a hinge line, anterior and posterior ears or auricles and a byssal threads. The left valve is slightly concave than the right one. The spat attaches itself to substratum by byssal threads. The typical spat measures 300 µm on 24th day. Feeding is given at 30,000 cells/spat/day.

Larval rearing systems

Water quality management and other conditions

Larvae obtained from same brood show differential growth rates and time in settlement. Mortality of larvae would be high up to umbo stage and less afterwards. Factors like high larval concentration, colour of culture tanks, aeration and overfeeding may affect larval growth and survival. Rearing of larvae at a concentration of two per ml gave better growth and high spat set. Larvae prefer darkness and dark surfaces. Aeration is harmful to larvae. Selection of fast growing larvae by culling would yield better survival rate in spat after transplantation to farm.

Water Change

In static water system, change of water is done once in two days. Water is siphoned out through appropriate sieves. 40-µm sieve is used up to umbo stage, 80-µm sieve up to eyed stage and 140 µm afterwards until settlement. Larvae are washed gently by keeping in the sieve. Before releasing, the tank is cleaned neatly and filled with fresh seawater. The tanks are covered with dark cloth to avoid light and dust. No antibiotic is used during larval rearing.

Feeding Schedule

Feeding is commenced to the larvae on second day with unicellular micro alga *Isochrysis galbana.* Ten cells per ml are found to be optimum for veliger larvae up to umbo stage. Feeding doubled from umbo stage and tripled from pediveliger up to settlement. Other micro algae such as *Pavlova lutheri, Chromulina freibergensis* and *Dicrateria sp.* are also acceptable to the larvae. Feeding is given once in a day.

Spat Set

Normally spat set occurs between 18 and 20th day. In exceptional cases the spat set is advanced to 14th day. High percentage of spat set is obtained in 2 larvae per concentration. Dark coloured surfaces enhance spat set and aeration during larval phase affects spat set.

Survival

5 % production is obtained on the initial stock of larvae in 500 litres of seawater in FRP tank. Negligible mortality of spat is experienced in normal conditions.

Spat Rearing

Spats are kept in the hatchery for two months after settlement. Isochrysis feeding is continued for one month after setting and gradually changed over to mixed algae. The spat reaches the size of 3mm in a period of 60 days and are transferred to farm at this size. Aeration is needed for the spat after settlement. As

the spats are sedentary in habit, recirculation of seawater is provided. No spat collectors are used for spat collection. The spats are allowed to set in the tanks. The spat mortality is minimum in the hatchery phase as long as the environmental and hydrological conditions are favourable.

Nursery Rearing

Spats with an average size of 3 mm are reared in box-type net cages of 40x40x10 cm with iron frame and encased in a retrievable synthetic velon screen bag of 0.5 mm mesh. The cage is again covered with old fish net with 10 mm mesh. This serves as a protection to the velon screen bag and to the spat from predators like crabs and fishes. The bags can be washed and reused. The initial stocking density of spat of 3mm size is 10,000 nos. per cage. After a month of rearing the spat are transferred to another cage with 1.0 mm mesh size. The density is reduced to 5000 nos. When the spat reaches 10-15 mm size, they are transferred to a cage with 1.5 mm mesh size. Now the density is kept at 2000 nos per cage.

Juvenile Rearing

The juveniles are reared in 40x40x10 cm cages netted with 1.5 mm nylon thread having 10 mm mesh size. The density is kept at 750-1000 per cage. Further thinning is done when they reach 40-45 mm in a period of 12-15 months.

Spat Survival in the Farm

When the spat is transferred to farm at 3 mm size, 50 % mortality experienced. The mortality is reduced to 20 % and 10 % in the second and third months respectively. By then the spat grows to 10-15 mm. Mortality is negligible beyond the stage. The juveniles reach 45-50 mm in 12-15 months of farm rearing. The overall survival of 30 % of the initial stock can be expected. Survival rate can be enhanced through proper management of farm.

Pearl Farm Management (Fouling, Boring), Predation and Control Measures

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A Glimpse to Pearl Culture in India

According to Hornell, the marine pisciculture work accomplished in Madras during the financial year 1St. April 1911 to 31st March 1912. Hermann and Hornell as reported by Hermann (1903) have studied the early development of the pearl oyster. Observations made on pearl oysters in the pearl oyster farm near Krusadai Island, (Devanesan and Chidambaram, 1956) and at Tuticorin, Chacko (1954, 1956, 1957) shown that the oysters grew to a height of about 36 mm in 6 months, 35-45 mm, 50-55 mm, 55-60 mm, 60-65 mm and 65-70 mm respectively at the end of year one to fifth year. The pearl oysters have been estimated to have longevity of 5-5.5 years in the natural beds and to live upto 7 years when reared in the farm in cages .(Narayanan and Mickael, 1968) worked on the relation between age and linear measurements of the pearl oyster *P. vulgaris* (Schumacher) of the Gulf of Kutch. (Alagaraja, 1962) while working on the linear relationships of length (DVM) and weight of pearl oysters found that the growth was significantly different for each year group. (Anantharaman, 1967; Chellam, 1978, 1987) studied the growth of pearl oysters in the Gulf of Mannar and Veppalodai respectively. (Appukuttan, 1987) reported the aspects of pearl culture experiments conducted at Vizhinjam Harbour and faster growth of young oysters was highlighted. (Achary, 1980, 1986 and 1998) explained the introduction of biological associates and it has stated that it is possible to attract desirable cultivable animals to settle down and grow. He fabricated the multipurpose cage for culturing pearl oysters and mussels to grow without the help of a raft at Vizhinjam. (Chellam, 1988) studied the growth and biometric relationship of the pearl oyster *Pinctada fucata* (Gould) produced in the hatchery. (Velayudhan, 1987) suggested the selective breeding between laboratory bred oysters with oysters from the wild stock may help in obtaining improved quality pearl oysters. (Velayudhan *et al* 1996) has produced 4 generations of pearl oyster and found that the morphometric characters of the four filial generations showed values of high significance as there were differences in the morphometric relationship within the generations and between the filial generations.

Hornell, (1922) listed a total number of 72 pearl banks known as paars in the Gulf of Mannar. From 1663 onwards to 1961 there have been only 38 pearl fisheries in the Gulf of Mannar. The gap of nonproductive period extended for 27 years between 1928 and 1955, and a similar 27 years so far since 1961 with no sign of pearl fishery in the coming years. A revival of the paars has been observed in 1998 (after 27 years) in which by SCUBA diving 1 lakh oyster/2 divers/day/boat was collected. The oysters were almost at the age and size of implantation; (Personal communication, Jesuraj and Muthukrishnan, Divers, Tuticorin RC of CMFRI, Tuticorin).

There are about 42 important pearl oyster reefs, known as Khaddas, in the intertidal area of Gulf of Kutch. The Gulf of Kutch fishery used to be half almost every year or alternate years from 1913 to 1939. Subsequently, it was held every 3-4 years. There have been 25 pearl fisheries during 1913 to 1967 and the last one being in 1967.

Since there is no scope for obtaining natural pearls just like other countries India also started pearl culture actually for producing pearls from pearl oysters collected and grown in farms off Veppalodai as a ad- hock scheme during 1971 in collaboration with State Fisheries, Tuticorin Tamil Nadu.And now India has achieved tremendous progress in pearl culture and associated research programmes in pearl oysters and pearl production. The further achievements will be highlighted in the connected chapters of lecture.

Pearl Oyster Farming

Farm Site

Pearl oyster farms are ideally located in sheltered bays, which offer protection to the rafts. They can also be set up in the coastal waters where sea conditions do not get too rough. The operations are year round in a pearl culture farm. Depth should be around 3-10m and silting should be minimal. The ambient tropical sea temperature and salinity are suitable for pearl oyster. If salinity falls below 15 ppt, it might lead to mortality under prolonged low saline conditions as during unusual heavy precipitation of rain and heavy discharge of rivers in the vicinity of farms. Areas rich in phytoplankton, which is consumed by 'the oyster as food, are good but it should not lead to noxious blooms. A mild current of about 2 knots helps bringing fresh food as well as removal of metabolic products, faecal matter and other farm droppings.

Raft Culture

Raft culture is the typical method of pearl oyster farming in 'sheltered bays. Long-line culture is better suited for open coastal conditions. The raft structure is illustrated in Fig. 7 a. The overall dimensions of a raft are variable and are decided based on convenience of handling. A raft of about 6 m x 6 m may be a standard size. The raft is constructed of logs (any second class wood as venteak or casuarina pole) of about 10 cm diameter tapering to 6 cm of chosen lengths. The logs are coated with coal tar. These are arranged as illustrated in Fig. 7 a and lashed with nylon ropes. Floats are attached to the raft to give buoyancy, their number being usually 4 for a standard raft which may b;: increased if there is sagging. Sealed empty diesel drums (200 1 capacity) with fibreglass coating, mild steel barrels given coatings of anticorrosive paints, or more modern Styrofoam or FRP/synthetic floats are used for buoyancy (Pl. I A). The choice depends on cost and long-term economics. The raft is individually moored at the farm site with anchors (grapnel or admiralty type) on opposite sides connected to raft with tested quality chain. The long-line system uses floats, spherical or cylindrical, which are connected by synthetic rope or chain (Fig. 7 b).

Pearl oysters are carried in varied types of nets/cages, which are suspended from the raft or long-line by synthetic rope at appropriate depths. The typical ones are the frame net (Fig. 7 c) and the nylon-mesh cage (Fig. 7 d). The frame-net is useful toavoid crowding of oysters and to follow the performance of individual oyster post-operative (Pl. I B). The cage is good for general mother oyster culture. All iron frames used are given anticorrosive treatment with paint. All structures in the farm should be periodically checked and maintenance repairs done which would help in extending the life of rafts and materials to a period of 3-5 years or even more. Recently lantern type cages with or without compartments made of old fishnets or new nets of required sizes which are collapsible and easily transported and cheaper. Another type is pedestal cages of 1m X 1m, 8mm steel rod fabricated to cube or .75 m X .74 m frame with 4 compartments and oysters are released in these compartments.The Cage with legs in all corners with rests to avoid damages to the cage as well as oysters and from fouling & poaching is very comparatively less than the smaller cages those can be lifted easily .

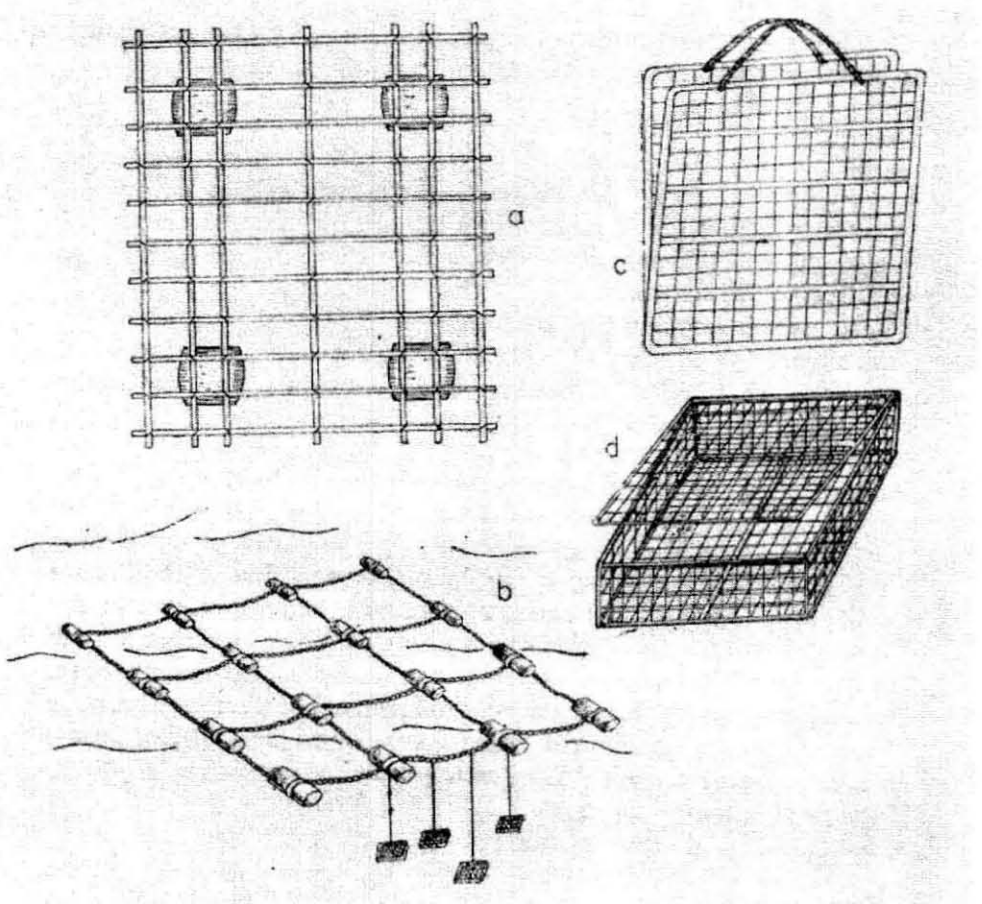


Fig. 7. Structures of pearl oyster farm. a-log raft; b-long-line; c-frame net and d-cage.

Mother Oyster Culture

Mother-oyster culture refers to the farming of pearl oysters from the time they are brought to the farm till they are used in surgery. The source for pearl oysters are (a) the natural beds, (b) spat collected III the sea and (c) pearl-oyster hatchery.

The pearl oyster populations in the natural beds, the 'paars' of Gulf of Mannar and' 'khaddas' of Gulf of Kutch, are subject to wide fluctuations. The unproductive spells are far more numerous than the productive periods. If and when the stocks are good, they should be collected and used for pearl culture, In the Gulf of Mannar, the collection is done by diving up to a depth of 20 m. SCUBA-diving (Pl. I C) enables search of a wider area and good collections as compared to skin- diving. In the Gulf of Kutch, they are collected from the intertidal flat by band picking. .

Spat collection using cedar leaves, hyze film or old fishing nets supplies almost the entire requirement of mother oysters for the Japanese pearl culture industry. In India, it has not so far been successful due to the open conditions of the natural beds. In inshore regions, particularly in the recently constructed harbour basins as Tuticorin and Vizhinjam, some spalfall of pearl oyster takes place, but it is of multispecies composition, *P. fucata* component being very small.

The source of hatchery is more reliable and can supply the required stock for pearl culture. Recently, the techniques for hatchery production of pearl oyster have been developed in India and it remains to be commercialized.

The pearl oysters are grown in the farm till they reach a suitable size. a minimum of 20 g in weight. for use in the surgery for graft and nucleus implantation. The oysters draw nutrition from the phytoplankton in the sea and no artificial feeding is necessary and possible in the farm. Mortality of stock should be kept to the lowest minimum level through appropriate farm management.

Pearl Culture Establishment

Pearl culture in Japan is carried out by small-scale units, on cooprative or family basis, save for a few large-scale operations by companies. In the peak period of production (1966), there were 4710 pearl culture units of which 49.8% were operating 1-14 rafts, 20.8% 15-29 rafts, 12.0% 30-49 rafts and the remaining 17.4% more than 50 rafts. The total number of units came down to about 2500 by 1973. This would show that small-scale operations are the mainstay in pearl culture. The Japanese pearl culturist has the advantage that he can buy the mother-oysters for his farm from those who are solely engaged in seed collection and mother- oyster culture. In India such small-scale operations at the family level can become possible only if commercial hatcheries produce pearl oysters and sell them to pearl culturists.

In Ramanathapuram District of Tamil Nadu Dr.M.S.Swaminathan Foundation initiated to take up marine pearl farming involving coastal women folk by 2002 onwards providing financial support and CMFRI has given technical support by supplying implanted pearl oysters from CMFRI, Mandapam Research

Centre on payment to the operated from ICAR, Revolving funded project. In 1991 CMFRI had given training in pearl production by selecting progressive fishermen from Coastal Valinokkam Village of Ram Nad District of Tamil Nadu and the pearls produced were distributed to them.

The activities, major inventory and manpower of a pearl culture establishment is summarised briefly to give an overview for an easy understanding of the nature of this industry. Major work is in the sea involving pearl oyster collection and farming. Details of hatchery are not included for reasons already stated. Manpower [' needs and inventory items would vary according to the scale of operation. These are not strictly applicable to family-based operations, which are not feasible in India for the present.

Raw Material: Pearl Oyster (*Pinctada fucata*)

Oysters from Natural Bed

Activity- Seasonal survey of beds and collection by diving.
Inventory- Boats; self-contained underwater breathing apparatus (SCUBA) and diving accessories such as fins, masks, snorkel, depth-gauge, knife and belt; compressed air charging units, (main and portable compressors) ; collection kit and oyster bins.
Manpower- Boat crew, navigator, divers, diving assistants.

Oysters from Spat Collection

Activity- Collection of pearl oyster spat by suspending spat collectors from rafts at suitable sites in the sea / bay.
Inventory- Rafts, lighted buoys, anchors, chain, and spat collectors; linked with item 1.1 seasonally.
Manpower-Linked with item 1.1 seasonally and farm labour.

Pearl Oyster Hatchery

Pearl Oyster Farm Management

Activity- Mother-oyster culture, post-operative culture, farm maintenance and stock. Maintenance.
Inventory- Log-rafts, Long-lines, lighted buoys, floats,
Anchors, chain, rope, cages, frame nets, dinghy, out-board motor,
Floating sheds and miscellaneous tools; linked with
Manpower-Farm superintendent, technical assistants,
farm labour; linked with.

Shore Establishment

Surgical Unit
Activity-Pearl oyster surgery and convalescence.
Inventory-Surgical tools and accessories, furniture, shell-

bead nuclei, chemicals, glassware, plasticware, ultraviolet lamps and raceway.
Manpower-Chief technician and technicians.

Farm house
Activity-Shore support for maintenance of farm and farm stock.
Inventory- Oyster cleaning tools, farm structure maintenance requirements (repairs and maintenance of raft, long-line, floats, anchors, chain, cages, frame nets) and oyster tanks.
Manpower-Linked with items in seasonal basis

Pearl Collection Centre

Activity- Collection of cultured pearls and incidental natural pearls.
Inventory-Plasticware, chemicals, oyster knife, vats.
Manpower-Technical assistants.

Pearl Processing Centre

Activity-Cleaning, sorting and grading of pearls; treatment of pearls for removal .of minor blemishes; bleaching, dyeing and colour improvement.
Inventory-Sorting trays, miscellaneous tools, chemicals and glassware.
Manpower -Pearl processing expert and technical assistants.

General Services

Seawater Supply

Activity-Supply of qualify seawater to surgery, raceway and oyster tanks. .
Inventory-Pump House, filterbed, sump, overhead tank supply channels with-. regulators; air blowers with air supply tubings and regulators.
Manpower-Electrical supervisor and assistant.

Power and Freshwater Supply

Laboratory

Activity- Monitoring of oyster healtl1. and condition; sea water analysis; advice to farm superintendent and chief technicians ; feed-back; to research. system.
Inventory-. General biological laboratory equipment and analytical equipment for seawater analysis.
Manpower- Biologist, chemist, laboratory technicians.

By-products Unit

Activity- Conversion of by-products of pearl culture to value-added items.

Inventory- If the unit is self-contained, all items required for utilisation of shell and meat; otherwise, collection, preservation and storage of materials until. sale to outside agencies.
Manpower- Specific manpower for handling by-products processing work, if self-contained; otherwise linked with other items.

Management and Administration

Activity-Planning, execution and administration of project.
Manpower-General Manager, administration, accounts and stores staff.

Biofoullng, Boring and Predation

Major problems in maintenance of pearl oyster stocks in the farm are the biofouling organisms, which settle and grow on the shells, the boring organisms which riddle through the shells and render them weak and friable and the predatory organisms which feed upon the pearl oysters. Singly, or in combination, these factors can cause heavy mortality to the farm stock through physiological stress and disease. Routine control measures should be adopted periodically and against specific problems.

Biofouling

The dominant fouling organisms are the barnacles (*Balanus amphitrite*) (Pl. II A), bryozoans (*Membranipora* sp., *Thalamoporella* sp. and *Lagenipora*), simple and compound ascidians (Pl. II B), the spat of bivalves (*Avicula vexillum* and *Crassostrea madrasensis*) and hydro ids. The weaving mussel *Modiolus* sp. Forms extensive carpet-like colonies over the natural pearl oyster beds but has not been a serious threat in the farm. Encrusting tubicolous polychaetes (Pl. II C) may be dominant in seasons. Seaweeds (Pl. II D) settle and grow on the oysters and cages. Others noticed are amphipods, isopods, sponges, polyclad worms, nematodes, opisthobranchiate molluscs, *Pinna* sp., egg capsules of gastropods and crinoids. While barnacle settlement is noticed almost throughout the year, settlement of others are seasonal. In inshore waters, under hanging culture at shallow depths, fouling load is always moderate to heavy.

Boring Organisms

The boring organisms include the two dominant groups of serious pests, the polychaetes and sponges. The boring polychaetes *Polydora ciliate* and other sp., *Cirratulus cirratus* are the major borers and causes mortality and members of families Syllidae, Nereidae and Terebellidae burrow through the shells and donot cause cause extensive blisters on the nacreous layers as done by earlier culprits (Pl. III A).

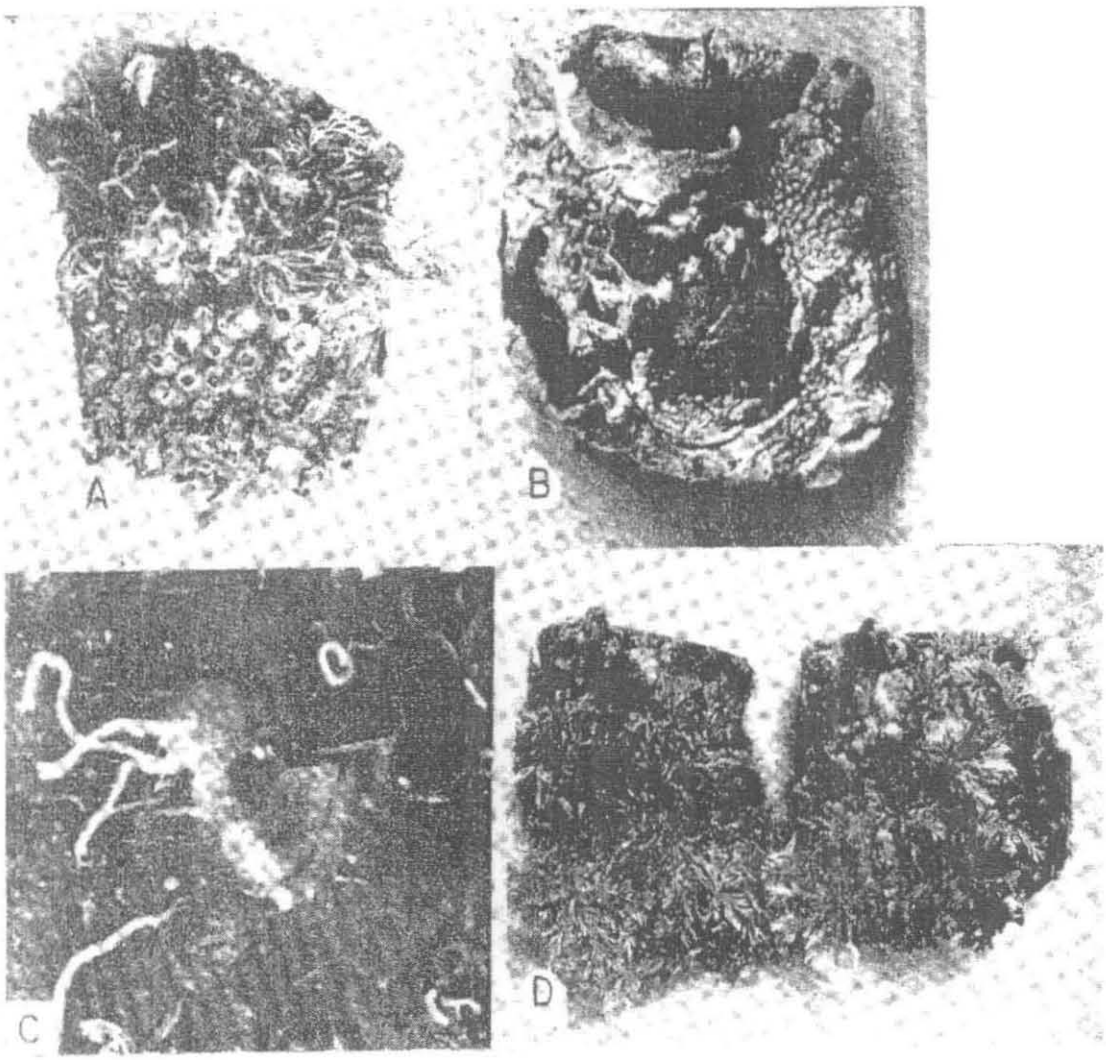


PLATE II. A—Fouling of pearl oyster by barnacle; B—Compound ascidians; C—Encrusting tubicolous polychaetes; D—Seaweed fouling on oyster.

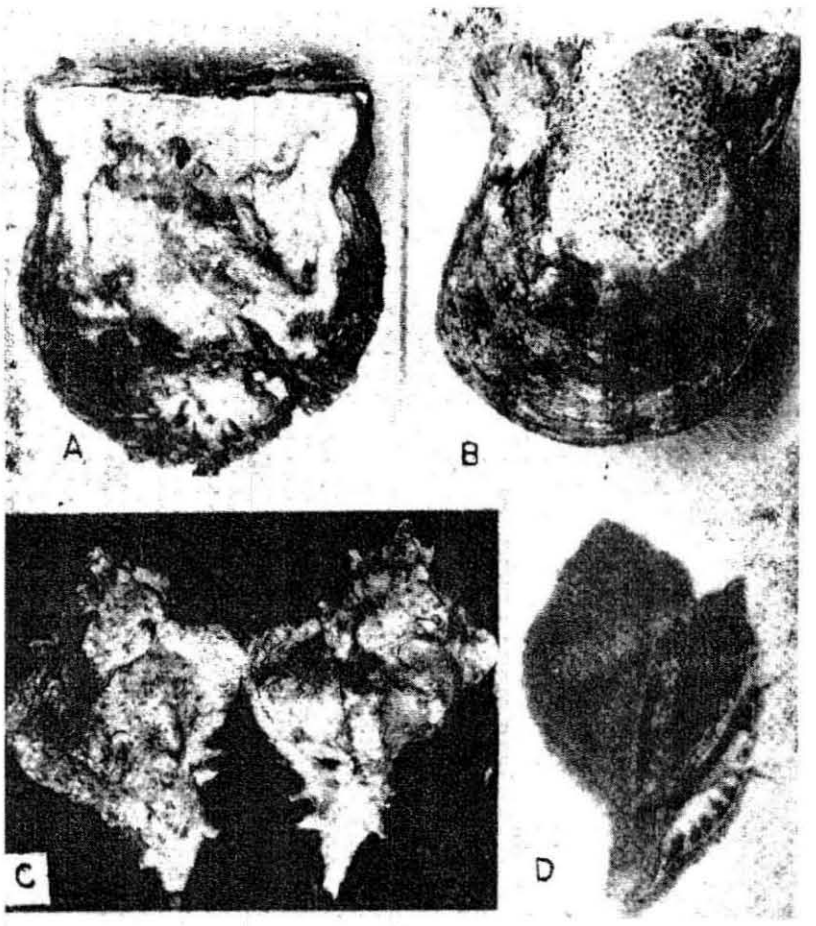


PLATE III. A—Pearl oyster shell with extensive polychaete blisters; B—Shell showing boring by sponge; C—Gastropod predator *Murex virgineus*; D—Predator *Cymatium cingulatum* attacking oyster.

The boring sponge *Cliona vastifica* and *C. celata* form honey-comb-like ramifications in the shell with numerous openings on the nacreous surface (Pl. III B). The above two groups cause great physiological strain to the oyster, while attempting to repair the shell damage, and cause mortality to the farm stock. The boring molluscs *Lithophaga* sp. and *Martesia* sp. and isopod *Sphaeroma* sp. are also occasionally found on the shells.

Predators

The major predators of pearl oysters in the farm are the gastropods *Murex virgineus* and *Cymatium cingulatum* (Pl. Ill C, D). The rate of predation by these gastropods is about an oyster per day per animal. They make their appearance seasonally. Crabs *Charybdis lucifera* and *Atergatis integerrisimus* also prey upon the pearl oysters. In the natural beds, rays fishes such as rock-perch and trigger fish and octopus may be notorious predators but these are not generally found in the shallow farm areas. Perch fishes, rays, eels, octopus, lobsters even Xancus also found to attack oysters kept in captivity

Control

The fouling organisms can be controlled only through periodic cleaning and scraping of foulers or through judicious choice of depths for growing the oysters. The deeper waters are relatively less loaded with the foulers. The intense spawning season of the major fouling organisms should be avoided while introducing new stocks in the farm.

The boring polychaetes are killed by immersing the oysters in freshwater for 6 hours. Treatment in a saturated solution of common salt for 40 minutes can also eliminate the polychaetes. Brushing the affected oysters externally with 1 % formalin kills the boring sponge and *Martesia* sp. These techniques of control should be carefully applied in each situation without causing mortality of the pearl oyster.

10 Pearl Harvesting and Grading

ACC Victor, R C of CMFRI, Tuticorin.

Pearl Harvest

Harvesting of cultured pearl is usually carried out manually. The oysters are brought to the laboratory from the farm. A sharp knife is inserted in between the two valves of the oyster upto the adductor muscle and the latter is cut vertically. The pearl is then squeezed out of the gonad region. In case oysters need to be reused for a second time, the oyster's valves are gently opened without damaging the adductor muscle and the pearl is carefully removed with the aid of instruments. The oysters are then returned to the farm for recovery and after a certain length of time they can be operated for a second time to produce additional pearls.

Pearl harvest or beaching of pearls is done during the cooler periods of the year during which time the pearl coating in thin and fine. During the post operative culture some oysters die due to natural causes and surgery. Also some reject the nucleus. In a study conducted by C.M.F.R.I. at Valinokkam by scaling of the operation, out of a total of 9414 oysters implanted (single implantation with 3 to 5 mm nuclei) mortality during one year post operative culture was 2108 (22.39%). On harvest, the remaining 7306 oysters (74.69%) did not contain pearls due to rejection of nuclei or non deposition of nacre. In the earlier studies at Veppalodai, gross production of 62.8% in single implantation and 68.3% in multiple implantation with reference to the number of oysters used have been achieved. This variation in the production may be due to differences in the location of the farms and the resultant environmental conditions, reproductive phase and the health of the oysters at the time of nucleus implantation, skill of the technicians and post-operative culture. The variation in the production of the pearls in these two studies can be considered as indicative of the range of production under variable conditions.

Grading of Pearls

The Pearls are sorted by size, shape, colour, lustre and surface quality. Some of the pearls may be perfectly spherical in shape and of outstanding colour and lustre, many are inferior in quality and some are totally value less.

Quality

The quality of cultured pearls is much relevant to the economics of pearl culture. The quality of pearls is dependent upon the thickness of the nacre, iridescence, lustre, colours, size, shape and flaws.

Thickness of Nacre

If the deposition of nacre on the nucleus is thick, then the pearl is more valuable and will give the required luster and iridescence. The nacre is composed of thousands of very thin layers. A good quality pearl is decided according to the homogeneity, evenness and the thinness of these layers.

Iridescence

The iridescence of a pearl is due to its optical characteristic. Light is refracted from the multitude of prisms of aragonite crystals. When the individual layer of organic matrix is thin, the light penetrates well into the translucent crystals. It is refracted in each layer of nacre and the rays that re-emerge together make interference effects, which decomposed the light spectrum into rainbows. The faces of aragonite crystals farm regular grooved – ripple marks on the pearl's surface and enhance the iridescence with defraction fringes.

Lustre

The brilliance of a pearl depends on its lustre. It is considered as the most important factor in evaluating pearls. Good lustre and refraction indicate that the nacre is composed of pure aragonite crystals. Even mis-shaped pearls are sometimes considered as valuable or gems if the luster is good.

Colour

The Indian pearls show diversity in colours. Colouration of pearls is mainly due to the physiological condition o the oyster. The environmental factors also play a predominant role in determining the colour of nacre. Minerals and trace elements in the seawater also influence the colour of pearl. Pearls are yellow, golden half white, ivory white, cream, grey, black, silver, light blue, green and light pink colours are seen. The marine culture pearls produced in Mandapam have different colours of the rainbow. The pink and green colour pearls are considered as most valuable pearls. Colours like pink, blue and green are a rarity.

Size

Cultured pearls from the Indian pearl oyster *P. fucata* are produced in the diameter range of 3 to 8 mm. The size is very important in deciding the price of a pearl. The bigger pearls fetches higher price.

3.5 - 4.5 mm : extra small
5.0 - 6.0 mm : small
6.5 - 8.0 mm :P medium
8.5 - 9.0 mm : large
9.5 - 10.0 mm: very large

Most natural pearls are small in size not more than 2 to 3 mm.

Shape

The shape of natural pearls generally follows that of the foreign substance, which has formed its nucleus. Hence the shape of the natural pearl is mostly irregular and no two natural pearls will be alike in shape. On the other hand since

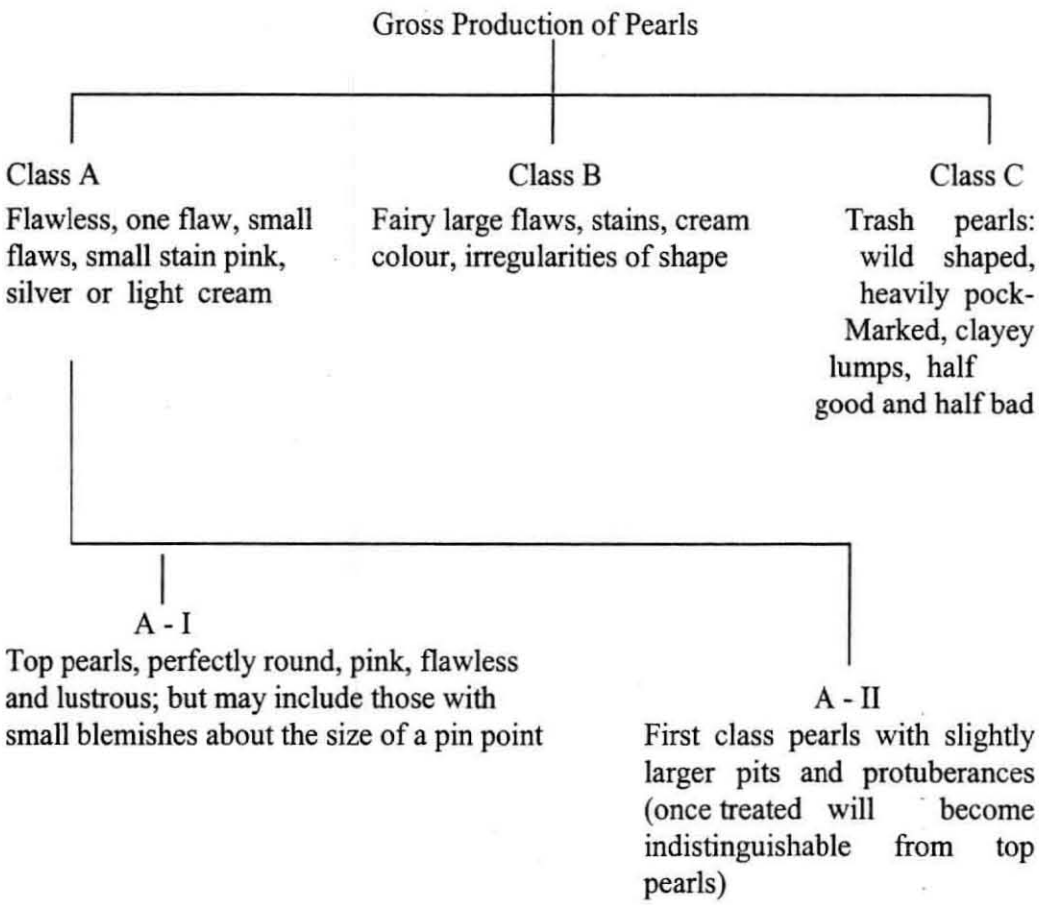
the shell bead nucleus is spherical and the size is large as predetermined, the cultured pearls is also spherical with the exception of malformation and is large in size. Hence cultured pearls of identical shape and size are very common.

Flaws

Production of flawless pearls totally depends on the oyster itself. Due to its rareness the price is always higher. The price will be less if there are many flaws. If the orientation of graft tissue and the nucleus are not properly set, the pearl produced will have teats and black dots.

Hence the above factors determine the quality of cultured pearls and for grading these pearls one can understand not only the colour or the shape or size is important, but the luster adds more value for these pearls.

Shirai (1970) classified the pearls as follows:



In India, in the experimental production, the composition was

Class A	37.6 %
Class B	37.6 %
Class C	24.8 %

However the percentage composition would vary from batch to batch depending upon several factors, which include i) selection of oysters ii)

conditioning process iii) graft tissue preparations iv) implantation v) maintenance of tools vi) skill of the technician and vii) post-operative culture.

Processing of Pearls

It is rather a common practice of the trade to improve the quality of cultured pearls through processing.

The process consists of bleaching and dyeing for colour adjustment. Bleaching the pearls is done by hydrogen peroxide in a fixed strength as bleaching agent. Only the drilled pearls are created. The organic impurity in the pearls are removed with hydrogen peroxide.

Surface polishing of pearls is done with salt. The pearls are mixed with powdered salt in equal volumes and placed in a tub with small amount of water. Then they are taken out and washed with distilled water. The residual mucus on the surface of the pearls will be removed by rubbing with salt to obtain good lustre.

Subsequent to bleaching the colour adjustment is done if required according to needs. Alkali based, oil based, acid and straight dyes are used for this purpose.

By Products and their Utilization

Class C category of cultured pearls cannot be used in jewellery. In such case the nacreous layer is ground off the nuclei and the powder is then dissolved in phosphoric acid with the final products being separated by additional chemical processes. Pearl calcium tablets are marketed in Japan. It is also reported that some Japanese companies have gained the technology to extract high quality calcium from the shell, which is marketed as pearl shell medicine.

Large shells are used in shell craft for their mother of pearl layer. Small broken shells can be used as ingredients in poultry feed.

Pricing of Cultured Pearls

Cultured pearls are priced according to size, shape, weight and quality. Unlike the bullion market where quality is precisely defined and prices fluctuate according to international market, the price of pearls eludes any standardization because of infinite differences in quality and preference variations of customers.

The size and shape of the cultured pearls depends on the size and shape of the introduced nucleus. Coupled with size of the bead the number of layer of nacre and their shape determine the value of the pearls. Because natural pearls have a much smaller irritant the nacre thickness tends to be much greater. Hence the value is considerably higher.

In Indian market the prices are by carat rat. (1 gm is equal to 5 carats). The pricing is different for pearls of different origin viz., fresh water non-nucleated pearls, fresh water nucleated (round) pearls, marine *P. fucata* pearls, south sea

white pearls, south sea black pearls and the blisters of *P. maxima*. While the fresh water non-nucleated pearls fetch the lowest, the south sea pearls fetch the highest price.

The pearls produced in C.M.F.R.I. are being marketed in three grades. At present the top quality pearls are sold at the rate of Rs. 1,500/- per gram and next quality of at the rate of Rs. 1,000/- per gram and the third quality at the rate of Rs. 500/- per gram. Pearls being a biological product, it is rather difficult to find homogenity or uniformity in size and quality among them.

11

Pearl Culture as a Societal Programme

ACC Victor, R C of CMFRI, Tuticorin.

Introduction

Gulf of Mannar is rich in biodiversity and bioresources. An estimate says that about 3,600 species of flora and fauna exist in the Gulf of Mannar, which includes extensive coral reefs, sea grass meadows, seaweed beds pearl oyster and chank beds and mangrove wetlands. Apart from this, Gulf of Mannar acts as a home for the endangered marine mammal sea cow and marine turtles. The breeding and feeding grounds created by these ecosystems and complex food web formed by various marine flora and fauna resulted in high fishery production.

Annually about 1 lakh tones of fish including fin fishes, prawns, crabs, lobsters etc. are harvested from the Gulf of Mannar. About 1.5 lakh fishers living in about 90 fishing hamlets dependent on this fishery resources and seaweed resources for their livelihood. However, due to over fishing and increased fishing population and damage to the coral reefs, sea grass beds and other ecosystems by trawlers fish catch is declining, leading to poverty among the fisher folk.

Creating alternative livelihoods and additional sources of income for the poor fishes is one of the options for the sustainable management of the fishery resources as well as conservation of the biodiversity of the Gulf of Mannar.

Alternative income source for the fisherfolk could be achieved by adopting some of the mariculture technologies perfected as a co activity of their livelihood ie., Fishing. There are few technologies readily available with the R & D institutions in India. One among them is Marine Pearl Culture, even though it is an intricate technology consisting various components among the molluscan mariculture practices.

Marine Pearl Culture – a Theoretical Look

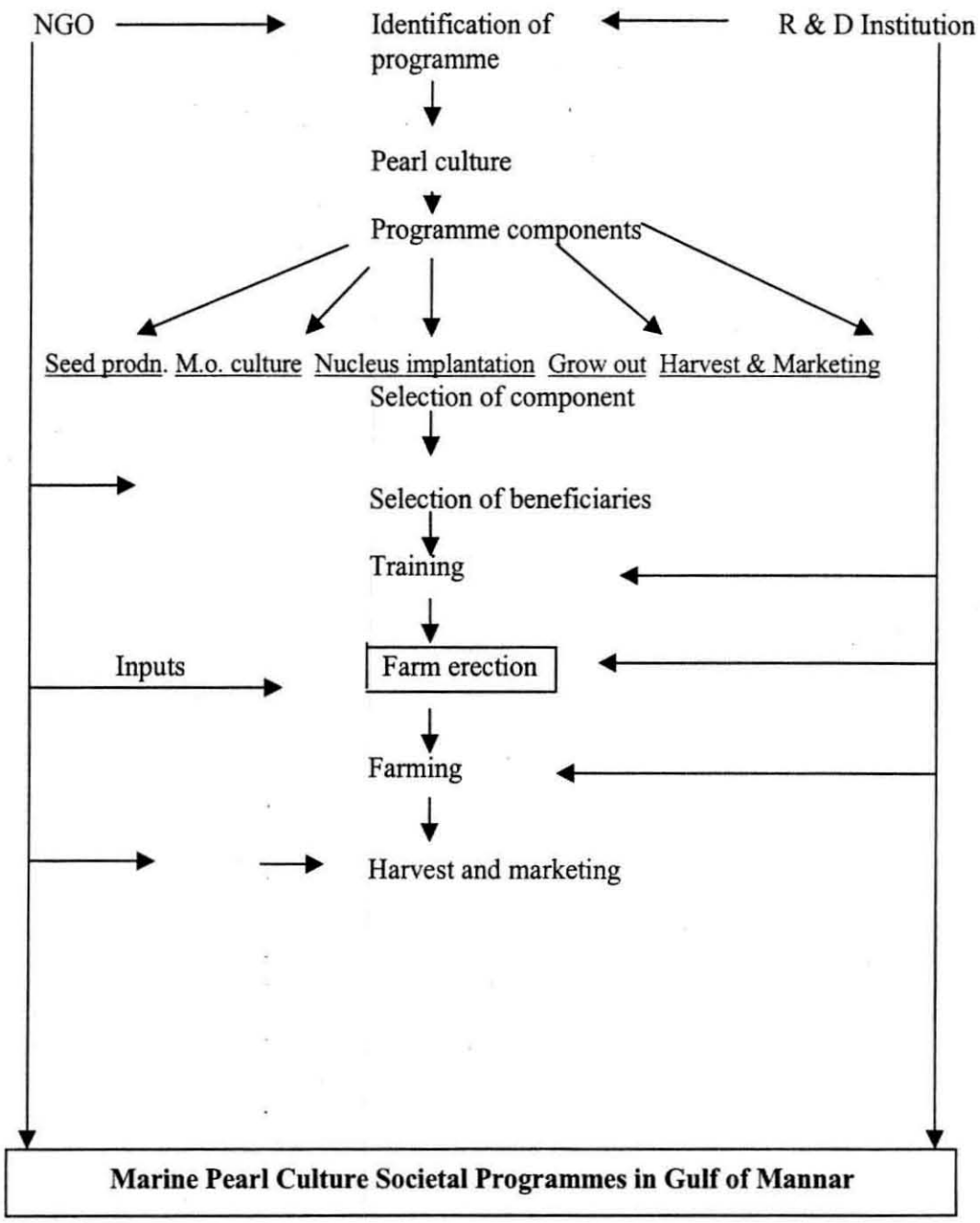
The success story of marine pearl production in India was achieved way back in early 1970's. The two breakthroughs achieved in pearl oyster seed production and culture pearl production is the backbones of the technology. In brief, pearl culture technique involves three different components and require technical competency.

They are, 1) Hatchery seed production of pearl oysters, farming and mother oyster production, (involving about 1.5 years + establishment of hatchery + technical manpower) 2) Surgical implantation of nucleus (a trained team of technician) and 3) culture and production of pearls (farm structures + technical man power + labour). All these components need both trained manpower with higher

degree of technical competency. Hence, taking up marine pearl culture as a whole needs higher inputs, manpower and money.

Marine pearl culture can be taken up as societal programme by fisherfolk with funding and technical assistance from NGO's and R&D Institutions.

Schematic diagram of societal programme - Marine pearl culture



Fishermen and CMFRI

Marine pearl culture as a societal programme has been thought of and implemented by CMFRI way back in 1993 at Valinokkam on a small scale involving Fisherman and CMFRI. A floating raft of 6 x 6 m size was floated with 100 cages suspended in it. A total of 9,414 oysters were nucleated and cultured. The detailed economics is given in the latter section.

Fishermen, NGO and CMFRI

As an one step further of the above activity, during 2002-'03 a societal programme consisting the role of all the three partners was conceived and successfully demonstrated at Mundalmunai Village, Pamban, Ramnad District.

Lead Discussion

As a beginning to the implementation of the programme, the NGO namely, MSSRF, Chennai held detailed discussions with the Scientists of the CMFRI on the various aspects of marine pearl culture, its technicalities, input components, possibility of handling by the fisher folk. The whole discussion focused on which component the fisher folk have to take up as a societal programme as pearl culture is a multifaceted one. After a couple of meetings and discussions, a schedule of activities was designed and responsibilities were determined for each of the participating units.

Mind Set Conversion of Fisher Folk

In general, fishermen are mostly conservative and are reluctant in involving in such activities. Further their financial statuses also don't encourage them to venture out in new avenues. Hence, they have to be first enlightened and encouraged by way of group discussions and all their doubts cleared.

Responsibilities of each of the participating groups

MSSRF

The primary and lead agency responsible for overall planning and execution, identification of beneficiaries, logistic supplies etc. The detailed responsibilities of NGO are detailed below:

1. Mobilize the community and organize them into a pearl culture society
2. Developing an organizational structure and management procedures for the proper functioning of the society
3. Getting necessary permission from the Tamil Nadu Forest Department to construct pearl farm in the Gulf of Mannar
4. Providing financial support to the society for training, purchase of implanted pearl oysters and farming

CMFRI

The brain of the societal programme and has responsibility in planning, training and guidance throughout the programme. The responsibilities are listed:

1. Helping MSSRF in mobilizing the villagers by providing technical and economics details of pearl culture to the villagers
2. Providing technical inputs and participating in identifying suitable site for pearl farming
3. Providing training on pearl farming to the villagers
4. Providing technical inputs for preparing a micro plan for pearl farming
5. Providing technical inputs for constructing pearl culture farm
6. Supply of 100000 nucleated pearl oysters to the society on payment
7. Periodical supervision of the farm and technical advise till harvest of pearls

Fisher Folks

The backbone of the programme is completely involved in executing the activities in consulting with the other two units. The following are the detailed responsibilities:

1. Constructing and managing pearl farm
2. Growing nucleated pearl oysters in the farm
3. Protecting pearl oysters against predators, growth of epiphytes and epifauna
4. Protecting pearl oysters from poaching
5. Protecting pearl farm from natural calamities lime cyclone
6. Harvesting and marketing of pearls with the help of MSSRF

Implementation and Progress of the Programme

As a first step, a village level society namely, "Mundalmunai Pearl culture society" was formed and registered. Twenty members of the society were taken to CMFRI, Mandapam laboratory and were given one week on hand training mainly focused on Farming of implanted oysters, precautions to be taken and farm management. A suitable site for pearl culture, located nearby the village was identified and necessary permission was obtained from the Tamil Nadu Forest Department. Construction of culture structures – racks was done in 15x10 m area. Periodically, oysters were operated at CMFRI laboratory and transplanted to the farm at Mundalmunai village for further grow out and pearl production.

Data on economics of pearl culture – Valinokkam experience

Method: Cages suspended from a 6 x 6m raft

	Input cost (for two years)		**Rupees**
1.	Cost of teakwood poles, floats, anchor chains		13,000
2.	Cages (100 nos.) for rearing 10355 oysters		10,000
3.	Cost of 10355 pearl oysters at Rs. 1.40/seed		14,500
4.	Cost of 9494 shell bead nuclei at Re 1/bead		9,500
5.	Cost of menthol, glasswares, plastic wares, Surgical instruments etc.		5,000
6.	Labour charges for pearl oyster surgery		3,000
	Total		55,000
	Production and Revenue		
	Total pearls produced	Nos	1849
1.	Sale proceeds of 1296 pearls (wt. 138.28g)	Rs.	73,133
2.	Cost of 250 pearls distributed to fishermen in lieu of their labour		12,500
	Total earnings		**85,633**

Anticipated Outcome of the Societal Programme

The experience gained and successful completion of the societal programme would enable many such units to venture for similar programmes and all such units could unit together to form a total cooperative structure including supplies of input and purchase and marketing of pearls. Apart from monetary benefits, trained manpower development; local employment generation is also foreseen.

Constraints

Any societal programme is not an easy job to begin with and successfully complete, the Mundalmunai attempt is one such thing. To add to this, are the legal status prevailing in the State government also proves to be a stumbling block. In the absence of clear cut legal issues, policies and guidelines from the state government, it would be worse. Hence, a high level meeting of Scientist, planners and administrators of state government should sit together and work out the modalities and guidelines for such programme as oysters and seafront are the monopoly of state.

Conclusion

From the above experiences, it is evident that 'Pearl culture' can be adopted as a societal programme for the alternate income generation for the fisher folk inspite of the multifaceted and high technical competence requirement. With successful completion of few similar programmes will infuse blood to form "Cooperative societies for marine pearl farming" in Gulf of Mannar area in the future. This would enable building of the economy of the poor fisher folk. The interest and involvement shown by the fisherfolk in making the programme successful are encouraging and exemplary.

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Pearl production in Abalone

ACC Victor, R C of CMFRI, Tuticorin.

Abalones are one among the few mollusks known for producing gem quality pearls and highly priced meat. The nacre of abalone shell is often multihued in tunes of silver, orange, pink, green, blue and lavender. The abalone pearls are superior to pearls produced from freshwater mussels and comparable to best marine pearls. History of abalone pearls dates back to 5000 BC. The first recorded reference occurs in one of the Japanese oldest historical writing, the Kojiki. (cs 800 AD). Production of pearls from cultured abalones is of recent origin. The French scientist Louis Boutan carried out pioneering work on abalone pearl production in 1897. He successfully produced semi-spherical pearls from abalones. Later, several workers refined his technology and in mid 1950s, Dr. Kan Uno was very successful in growing hemispherical pearls in several abalone species. But attempts to produce free pearl did not give any encouraging results. Now abalone pearl farms producing blister pearls are existing in various countries.

Half Pearl Production

CMFRI achieved initial success in the half pearl production from abalones during 1998-'99 at its regional center, Mandapam. Earlier attempts to produce pearls in abalone by fixing a nucleus on the inner side of the shell of the animal was not successful due to dislodgment of the nucleus by powerful foot movement of the animal. Due to sustained efforts a comprehensive method was developed and pearl production became a reality.

Abalones of good health, without any physical injury and unaffected by borers are segregated from the natural collection and maintained in the laboratory with seaweed *Ulva* sp. as feed. The abalones are taken out from the tank and air dried for 10 minutes prior to nucleus fixing process. This enables the easy retrieval of the foot muscle for drilling at the appropriate site.

Drilling is done on the inner side of the shell, by pushing the mantle to the maximum possible extent, using an electrically operated hand drill with a fine drill bit (3 mm). Extreme care should be taken to avoid any sort of physical injury to the animals. Drilling is done in one swift action and the drilled abalones are returned to a recovery tank containing well aerated seawater immediately after drilling. This enables the abalones to recover from the drilling shock as well as getting rid of the drill dust.

After half an hour, the drilled abalones are taken out from the recovery tank, their mantle is pushed aside with the aid of a sterile scalpel's blunt end and the inner shell is wiped with cotton. The commercial grade adhesive Anabond is used as a fixative. A drop of the glue is placed in the hole and spread on the edges of the

drill hole, immediately followed by placing a shell bead (used for marine pearl culture) of required size (4 mm) with fine tweezers and gentle pressure is applied on the nucleus till the adhesive is completely dried. The animal is returned to FRP tank with running seawater and aeration.

Active abalones having nucleus are collected on subsequent day and stocked in conventional box type cages knitted with appropriate size mesh and suspended into sea from the rack. Feeding is done at bi-weekly intervals by placing seaweed *Ulva* sp. inside the cage. Monthly observation is done to check the nacre coating, mortality etc. At the end of first month, slight nacre coating could be observed over the nucleus. The stocked abalones are harvested on 4th month when the nucleus has thick and uniform nacre coating. About 40% of the abalones had good nacre coating in the experiments.

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Marine Pearl Production through Tissue Culture

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Introduction

Tissue culture, in general, is being carried out in various fields of medical and agricultural research. The study has been commenced since long back to understand the cell type, cell behaviour, cell structure, cell multiplication, cell reaction to drugs etc. It has become a vital tool in micro pathological and immunological studies aiming at finding solutions to certain diseases. Plant tissue culture has reached an advanced stage of achieving an entire plant from a single cell. All these studies are concerned with plants / animals which are related to freshwater species. Marine invertebrate tissue culture is not only a new origin but also a new field of research concerned with marine animals. Primary aim of the study is to formulate suitable culture media specific to each species and to establish cell lines. Later the study is extended to commercial aspects of producing *in-vitro* pearl from pearl oysters in Japan. Extensive works have been carried out on cell proliferation and its behaviour in a medium developed specifically for the species and to formulate suitable medium based on the results obtained. The countries like Japan, China, United States and Canada initiated marine invertebrate tissue culture. Among these countries, Japan is the pioneer country carrying out research in pearl oyster for the purpose producing *in-vitro* pearl through tissue culture. Visualizing the importance of the work in view of deterioration of natural environment India too entered in to the field of marine invertebrate tissue culture research, as it is one of the pearl producing countries in the world. Expertise in the field of research has already been developed and a fully functional marine invertebrate tissue culture laboratory has been established at Tuticorin for the first time in India. Cultures are organized since 1996.

Setup of Tissue Culture Laboratory

Generally the tissue culture laboratory should be compact with different modules so as to contain contamination by effectively maintaining high-grade hygienic conditions. It is fully air-conditioned. The entrance room is the one where the records are maintained and discussions are held prior to organization of cultures. Animal sterilization room is arranged on the left side of entrance room having U.V. sterilization unit and provisions for running seawater supply. The entrance room leads to preparation room where preparation of culture media, saline solutions, extracts, tissue culture materials etc., are carried out. The preparation room proceeds to dressing room and to operation room or clean room. A dark chamber or otherwise called 'Pass Box' is situated in between the preparation room, dressing room and clean room. It has three doors with a U.V.light on its top to keep the

materials always sterile. The doors are arranged in such a way that one door is facing preparation room through which sterile materials are placed inside, the other on the dressing room from where the dress materials are taken out and the third one on the operation room from where the materials are taken during organization of cultures.

Preparation of Culture Media

The marine mollusc medium (MMM) is constituted based on the composition of haemolymph of each species. Refinement of medium is done periodically based on the results obtained in the cell culture. There are a few media developed for marine molluscs such as Medium M199, P35, L-15 and Ham's F 12. The media are commercially available along with formula. The media can be prepared based on the formula.

Preparation of Balanced Salt Solution

The balanced salt solution (BSS) is prepared in the following manner.

Na. K. solution	125 ml
Mg. Solution	50 ml
Triple distilled water	200 ml
These are mixed, autoclaved and taken to clean room	
Ca. solution	50 ml
Triple distilled water	50 ml

These two solutions are mixed, autoclaved and taken to clean room. Mixing of the above two solutions and the following is done on the clean bench.

Glucose	5 ml
$NaHCO_3$	5 ml
NaH_2PO_4	5 ml
Kanamycin	0.5 ml
Penicillin	5 ml
Fungizon	5 ml
Total	500 ml

Preparation of animals and tissues

The test animals are depurated for a minimum period of 3 days in U.V.treated running seawater. The depurated animals are wiped with externally with 70 % alcohol and taken to clean room. The mantle tissues of test animals are excised and washed several times BSS to get rid of mucus and other adhering particles. If needed the tissues are treated in antibiotic solution containing 1000 µg/ml streptomycin and 2000 IU/ml penicillin. The tissues are cut in to tiny pieces of 1 square mm in size.

Culture Techniques

Flask and Petri dish Cultures:

Before introducing the fragments of tissue in to the culture flask the mouth of the flask is shown to isopropanol flame for sterilization. Tissues are placed inside the flask with the help of a needle. The tissues are allowed to stick on to the flask and 3 ml of medium is added. A similar inoculation is made in petri dishes also. The culture plates are placed in CO_2 incubator and maintained at 25-28°C.

Cell Well Culture:

The cell well is otherwise called as micro plates. There are different types of cell wells. The size of 24 wells is 16 mm in diameter and 17 mm in height and the size of 96 wells is 6.4 mm diameter and 11 mm height. The cell well is provided with a cover. The cell well is used to culture single cell for the purpose of cloning. 3 to 4 drops of medium are added to each well. The cell wells are kept in CO_2 incubator at 25-28°C.

Medium Change:

Medium change is normally done on alternative days. Periodicity of medium change is determined by observing the condition of the cultures. Culture flasks are taken to clean bench after wiping with 70 % alcohol. When the flask is opened, it is shown to flame. Much care is taken during medium change. A separate pipette is used for each flask. Half of the medium is changed during first and second time and subsequently the whole medium is changed. At times cell suspension is centrifuged and fresh inoculations are made. In some established cell lines the cells are active and hence the entire medium is changed.

Organisation of Cultures

1. Primary culture: The processed tissue is treated with trypsin for the purpose releasing the cells from the tissue. To effect this the cut pieces of tissues are placed in trypsinisation flask containing 30 ml on marine mollusc calcium magnesium free phosphate buffer solution (MM CMF PBS) with 0.05 % trypsin. A Teflon stirrer is used in the flask for proper dissociation of tissues and dispersion of cells. The stirring is done for 10-15 minutes at 1200 rpm. The cell suspension is first filtered through 150-µm sieve and then through 60-µm sieve. The filtrate is centrifuged at 4°C for 5 minutes at 800 rpm and the supernanent solution is removed gently without disturbing the precipitate. A drop of medium is added to the precipitate and mixed well. The mixture containing free cells is distributed to different flasks or petri dishes by means of Pasteur's pipette. 3 ml of medium is added to each flask and the flasks are placed in CO_2 incubator at 25-28°C.

2. Explant culture: For explant culture of tissues, fragments of tissues are processed in balanced salt solution (BSS) and inoculated in the flasks or petri dishes. 3 ml of medium is added to each flask. The cells from the

explant proliferate in large numbers and migrate away by adhering to the bottom of the flask. The round epithelial-like cells and fibroblast-like cells are seen in the cultures. The cells do multiply in *in-vitro* cultures and increase in numbers forming cell sheet. When a cell sheet is fully formed, it is due for subcultures or for cryopreservation of cells. At ideal conditions the cells develop pseudopodia and form a network to cover the entire surface of the flask as organic matrix. The migrated cells are stationed at places and formed pearl sac. The organic matrix induces the cells to secrete crystals.

3. Organ culture: The processed fragments of tissues are placed on a raft in petri dishes. The raft may be at any form as per the requirement of the experiment. In organ culture the explant tissue is not immersed in the medium but it is kept in such a way that the medium is filled up to the lower phase of the tissue leaving the upper phase with air contact. In such case the cells are kept intact without dislodging their positions. The interaction and integration of the cells perform their original functions of forming organic matrix and pearl sac. The cells secrete nacreous crystals and deposit on the matrix. As the mantle cells are responsible for the formation of shell, the cells secrete prismatic layer in hexagonal form. Each hexagonal segment is bordered by interlamellar organic matrix.

4. Cell proliferation and cell types: In the explant cultures the cells do proliferate in large numbers from all sides and migrate away from the explant. There are two types of cells i) granular cells and ii) agranular cells. These cells develop pseudopodia and form organic matrix. On completion of the matrix crystal deposition starts to form nacre layer. If the nacre layers were continuously formed, an in-vitro pearl would be formed.

Preservation of Cells

Cells to be preserved by freezing would be released from the culture flask by adding 0.25 % of trypsin. The cell suspension with 3 to 6 ml of medium is centrifuged for five minutes at 1200 rpm at 4°C. Supernanent water is removed and 2 ml of medium and 2 ml of Minimum Essential Medium (MEM) with Dimethyl Sulfoxide (DMSO) 7.5 % mixture were added drop by drop. The 4 ml suspension is divided into four parts and kept in four freezing vials. After the vials are sealed and labeled, there are frozen at the rate of -1°C at every minute. Freezing is done at three stages, first at 0°C for 30 minutes, then at -20°C for 60 minutes and thirdly at -70°C for 6 months and finally at- 196°C for one or two years in liquid nitrogen.

In order to protect cells from damages during storage, DMSO 7.5 % and glycerine 10 % are used along with medium. Freezing of cells is done mainly for three reasons.

1. During cell line the cells may change their enzyme activity, chromosome number etc. Therefore it is essential to freeze these cells at a particular stage of cell line and then rejuvenated.
2. There may be contamination in cell line. To prevent this cells are frozen at periodic intervals.

3. In an established cell line the cells can be cultured to a maximum of 50 times. In Some other cell line, cells are likely to die at any time. Such cell lines can be sub cultured only for 30 times. Freezing of these cells may extend the period of cell line.

Application of Tissue Culture Techniques

There is an increasing use of tissue culture in various fields of biological research. Tissue culture techniques are being adopted in Marine Invertebrates since in recent years. By conducting tissue culture, valuable information could be collected on aspects of like cell structure, cell division, cytogenetics, cell physiology and cell viability. Tissue culture techniques are useful in studying the structural as well as functional aspects of cells, tissues or organs by culturing them *in-vitro*. The techniques are employed in investigating the effect of chemicals and radioactive elements on normal tissues and cancer cells and in microbiology, pathology and in the production of vaccines. Results obtained may help in finding out methods of curing several diseases. Careful studies in tissue culture will help in transplantation of tissues and cells among members of a species or from one species to another species. In recent years tissue culture technique is being used in the production of *in-vitro* pearls from pearl producing molluscs.

14 Value Added Pearls – Mabe, Baroque and Keshi

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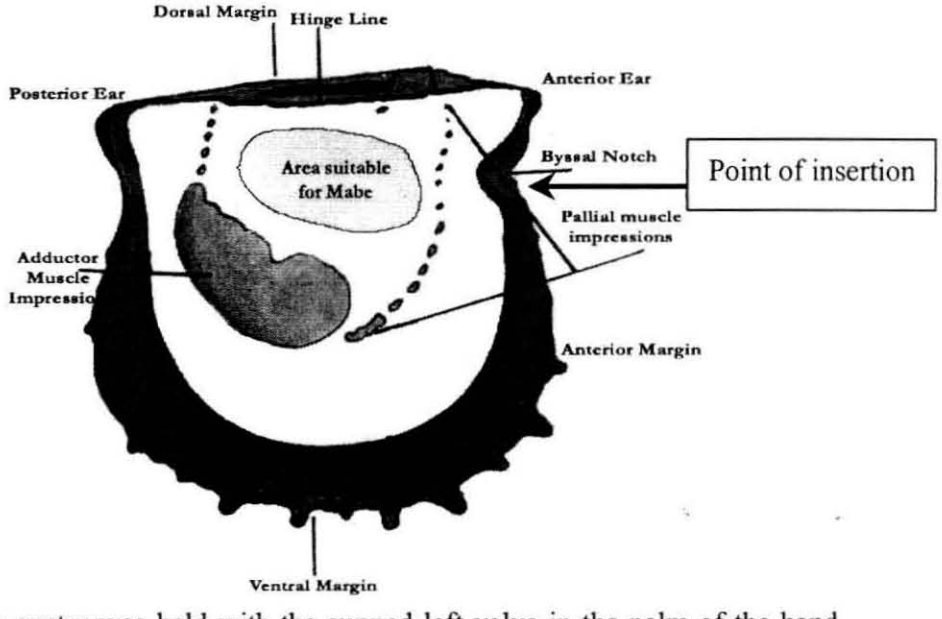
There has never been a period in history when pearls were not in vogue. They go well with any style, in any place on any person. There are many types of pearls available today than ever before. They offer a wide variety of colours, shapes and sizes and a wide range in price. The variety available results from the use of different types of oysters, the physical environment in which they live, and the varying cultivation techniques used by producers. Some of the types of marine value added pearls are presented here.

Mabe Pearls

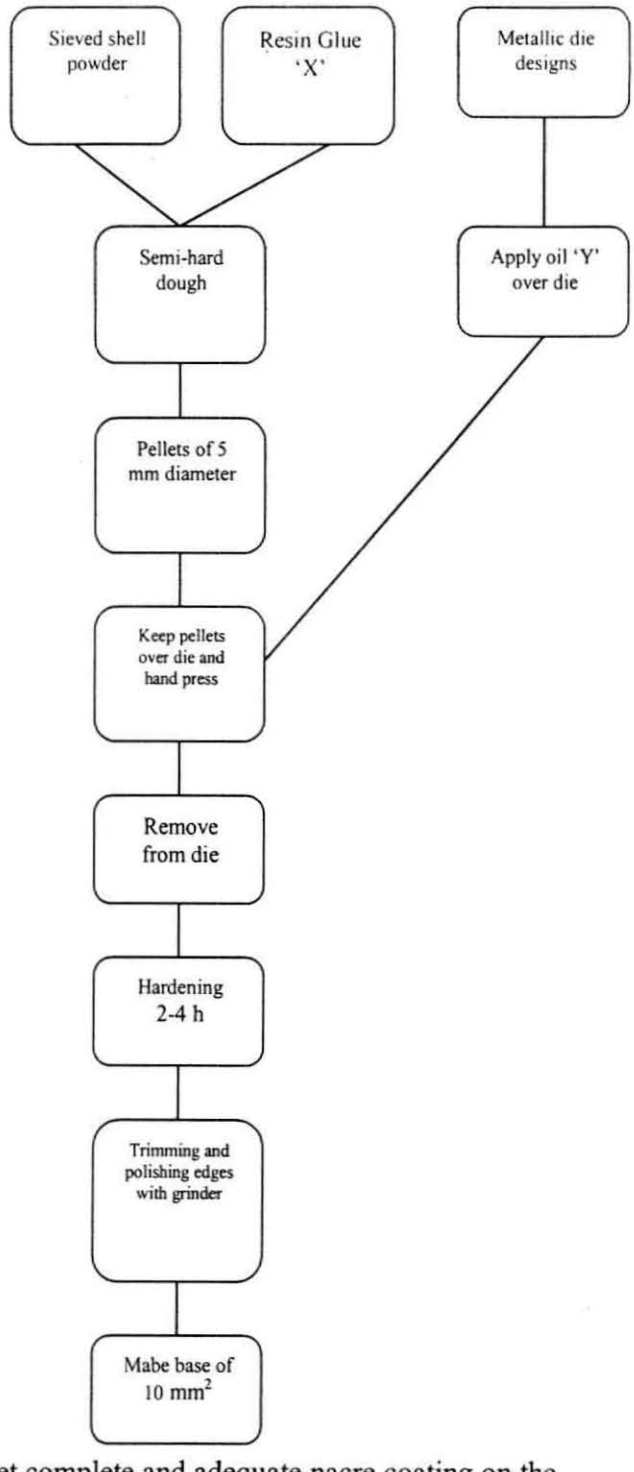
A mabe pearl is a dome shaped or image pearl produced by placing a hemisphere or miniature image against the side of the oyster shell interior. In India, the technology for producing mabe pearls is already developed in the freshwater mussel. Other than half pearls and blister pearls, images have not been tried in the marine species *Pinctada fucata*. Trials were made in *P. fucata* using base images (10 mm^2) made of shell powder and resin, plastic images and camel bone images. Rearing of oysters was done at CMFRI's Port Kollam raft farm.

Oysters suitable (> 45 mm DVM) for insertion of images were selected and placed in a shallow pan with their hinge down. Oysters with open valves were pegged with wooden splits, and using an oyster speculum, the shell gape was gently



widened. The oyster was held with the cupped left valve in the palm of the hand.

The base image was picked with a fine angled forceps and inserted face up through the anterior end near the byssal notch, where the gap is the widest. The pallial muscles offer slight resistance, and the image was slid through under the mantle so as to lie in the deep sinus close to the dorsal hinge. The image is therefore bound by the hinge, pallial muscles and the adductor muscles and therefore cannot be easily dislodged. The oyster is immediately placed in fresh seawater with hinge down and ventral margin facing up.



Individual oysters were then placed in specially made velon screen (large mesh) pouches made into strips, again taking care to see that the ventral margin is at the top. Up to 6-7 oysters can be placed individually in pouches in one velon cage. The cage is then suspended from the raft with suitable weight to keep it upright.

The base images in plastic and bone material were rejected within a month. Only base image made with shell powder gave satisfactory results. Observations (Mohamed et al., 2003) indicate that within 15-20 days, the nacre coating is initiated. This is substantiated by the observation of nacre secretion on strips under tissue culture from day 7 onwards (S.Dharmaraj, Pers. Comm.). Fusing of the image to the shell was complete by day 20. By the end of 60 days it was possible to get complete and adequate nacre coating on the image so as to produce a mabe. Rejection and mortality was high (100%) when the image size exceeded 10 mm^2. The survival of oysters and percentage recovery of Mabe after 82 days of rearing is shown in Table 1. Longer period of incubation resulted in the masking of finer details of the image. Flow chart of the process is shown in fig.

Table 1. Percentage survival and percentage recovery of Mabe pearls in *P. fucata* at Port Kollam after 82 days of rearing

Treatment	Stocked No.	Survival %	% Mabe Recovered
Strip 1	7	43	29
Strip 2	6	100	67
Strip 3	7	86	57
MEAN		76	51

Anil et al., (2004) also described a method by which mabe images were stuck to the inner shell edge using cyanacrylate glue to produce mabes.

Baroque Pearls

The rarest pearls are round pearls and round pearls in fine quality are very costly. A baroque pearl technically is any pearl that is not round and has an interesting irregular shape. Baroque pearls should not be confused with pearls that are simply 'out-of-round'. They should have a distinctive enough shape to be interesting and attractive. Baroque pearls can be produced by both saltwater and freshwater molluscs, and can be natural or cultured. They have a distinctive appeal, because of their very beautiful tints of colour and iridescent flashes, which are the results of pools of nacre (where the Baroque shape creates an area in which the nacre can collect and is deeper than along other parts of the pearl). Baroque pearls with their distinctive irregular shapes are more common than round pearls, which make them more affordable, but they can make beautiful jewelry. Baroques are often obtained in CMFRI's pearl farms.

Seed Pearls and Keshi Pearls

Seed pearls are very tiny, round, natural pearls usually under 2mm in size. They are rare today, but often seen in antique jewelry. They are sometimes cut in half to create a larger supply for particular jewelry creation, or to remove blemishes or a misshapen side: these are much less expensive than full seed pearls. Seed pearls can be produced by freshwater and saltwater molluscs.

Keshi pearls also called "chance" pearls are interesting baroque pearls accidentally produced in seawater oysters used for cultured pearl production. Sometimes an oyster rejects its bead implant, but particles of the accompanying mantle tissue used alongside the bead remain; these particles of the mantle tissue stimulate the production of nacre, resulting in the wonderful interesting pearl known as keshi. They are unusual because, like natural pearls, they are essentially all nacre and all natural.

Japanese keshi are usually very small. The word "keshi" actually comes from the Japanese word meaning a tiny particle and was used to refer to "poppy" pearls, a fitting image for the strands of minuscule pearls they describe, very tiny

pearls that might be confused with natural seed pearls. At one time it was not unusual to see necklaces comprised of 20, 50, or as many as 100 strands of these tiny pearls strung together, the strands being so delicate they look like silken thread.

The keshi pearl now attracting the attention of collectors however is the south sea variety, which is much larger, 8-10 millimeters and up. Virtually always baroque in shape, they offer a variety of unusual shapes, often oblong, and lend themselves to very distinctive jewelry creations. They occur in virtually all shades of colour from silvery-white to cream, gray to black, yellow to gold, even mauve and lilac tones. One of the most striking characteristics of the south sea keshi is its very intense luster and iridescence, far greater than what is normally seen in even the finest round cultured pearls

They are very popular in Europe and the Middle East. For Moslems they are particularly desirable because like natural pearls they are an all – natural creation and by comparison to the cost of natural pearls very affordable.

But keshi pearls are disappearing. Japanese and south sea pearl producers are trying to reduce the number of keshi pearls being produced because the production of keshi creates a costly problem. As nature would have it, the oyster can only produce a certain amount of nacre: if keshi are consuming nacre, that leaves less for the culture pearl being produced simultaneously within the same oyster. This means that the more keshi pearls, the fewer the fine, round cultured pearls. As the cultured pearl growers succeed in reducing the number of these "chance" pearls, fewer keshi will be available. Predictions are that they will become scarcer in the years ahead, which is sparking serious attention from connoisseurs.

Ringed or Circle Pearls

When a concentric ring encircles the surface of a pearl we say it is "ringed" or "circled": This is a type of surface characteristic that can occur on any variety of pearl, when a pearl exhibits numerous concentric rings from top to bottom. However it creates a very interesting and distinctive looking pearl. Usually off-round baroque in shape and much less expensive than round pearls or symmetrical baroques, these "ringed" or "circle" pearls have a special allure and are being used increasingly in jewelry especially those from the south pacific occurring in shades of white, gray to black and aubergine. Artistic designers find circle pearls an exciting choice for distinctive and dramatic creations.

Akoya Pearls

This is the pearl that comes to mind the moment anyone mentions "pearl"-lustrous round white pearls. The finest Akoyas originally produced in Japan are more perfectly round (7-10 mm diameter) than most other pearls and have the highest luster, which makes them especially desirable. Unfortunately for those who prefer very large pearls they rarely exceed 10 millimeters in diameter and when they do they command exceptionally high prices. In addition to Japan, China is now a major producer of Akoya.

In India, attempts have been made to produce fucata pearls similar to Japanese Akoya by implanting larger oysters grown in Kollam Bay along the southwest coat of India (Kripa et al., 2003). The largest cultured pearl obtained in this experiment had a diameter of 7.88 mm weighing 0.68 g and the average nacre thickness was 1.37 ± 0.27 mm. A nacre thickness of 0.5 mm is acceptable as a pearl and the minimum is 0.35 mm for good lustre and color. Assuming a uniform coating of 0.129 mm/month, it can be inferred that a pearl with 0.5 mm nacre will be formed in 4 to 5 months. Earlier work done along the east coast has shown that under tropical conditions, acceptable pearls are produced within 4-5 months with nuclei of 2–3 mm diameter and in 15-18 months with nuclei of 6-7 mm diameter. However, this study shows that along the southwest coast of India, the nacre production is faster and the period of rearing nucleated oysters can be considerably reduced to produce Akoya type pearls in India.

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On - Shore Pearl Culture Techniques

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Intrduction

Marine pearl is the most important bioproduct of gem value. It is revered from ancient times mostly for sentimental/aesthetic importance, although it has no resale value like gold. When compared to the quality, value etc. the freshwater pearls now available plenty in India have no comparison with marine pearls. Since marine pearls are not actively traded in India, and most of the people cannot afford them, cheap freshwater pearls have invaded the Indian markets from China. The technology of marine pearl culture is very old and is in vogue for the past 100 years. At present Japan, China, Australia, Polynesian islands and Indonesia are the leading commercial producers of pearls. The species that produce the valued pearls are *Pinctada fucata, P. margaritifera* and *P. maxima* in order of abundance. However, with regard to the value of the pearls produced, the order is *P. maxima, P. margaritifera* and *P. fucata.* In all the countries pearl culture is conducted in the inter island areas or sheltered bays and is exclusively a sea based activity.

In the sea based pearl culture practiced at present, the pearl oyster collected from the sea or produced in the hatchery are grown in the sea by suspending them from rafts or rens depending on the location, depth etc. The depth also varies from place to place and from season to season. As depth increases the fouling and boring problems become minimum. However, the availability of food also decreases resulting in poor growth and mortality. In a farm, the cleaning activity is a daily affair and the operation and maintenance of a suitable boat/vessel with labour force is a routine work consuming major part of recurring expenditure. Thus the operation cost is high compared to the capital expenditure.

At present pearl culture work is mostly confined to areas where there are natural beds. In India pearl oyster beds exist around Tutucorin area and research work is also largely confined to this region. The sea based technology of pearl culturewas developed at Tuticorin about three decades back. However, till date there is no active commercial production of marine pearl in India. Both the coasts of peninsular India experience rough sea at frequent intervals making it difficult to float any rafts over a long period for commercial operations of any nature.

The On - Shore Pearl Culture Technology

In the light of the above constraints, on shore pearl culture studies were initiated from Visakhapatnam in 1996. Experiments were conducted on several aspects and many related parameters were refined and standardised. A small demonstration cum research facility of onshore pearl culture has been established in

the premises of CMFRI, Visakhapatnam. The salient features of the technology developed and adopted are as follows.

The spat of *P. fucata* of 5 mm dorso ventral measurement (DVM) grows to about 60 mm in about 12 months, suitable for implantation with 5-7 mm and above nuclear beads. The oysters are grown in suitable cement tanks specially designed for this purpose. The tanks are covered with dark covers and provided with ventilators on all sides to keep water temperature at optimum level throughout the day. They are then spread out at varying densities from the spat to oysters of implantation stage depending on their size and are frequently thinned in tune with their growth.

The seawater for this purpose is drawn from the sea by a standard intake system of suitable capacity. The water system has a filter at the source permitting only semi filtered water from the sea by avoiding sand particle but retaining micro algal cells. This seawater is directly pumped into the pearl oyster tanks. About 10% of the water is exchanged daily and 100% water exchange is effected at every 10 days interval.

Three species of microalgae viz. *Chaetoceros calcitrans, Isochrysis galbana* and *Nanochloropsis salina* were identified as best combination for good growth. They are grown separately and mixed in the ratio 7:2:1 at the time of feeding. The ratio varies from spat to adult. The mixed micro algal feed is supplied to the pearl oyster tanks through a low energy drip flow system, the flow of which can be adjusted to the desired level of algal cell concentration. The algal cell concentration varies from 10,000 cells/ml to 75,000 cells/ml for the size range of 5 mm to 60 mm DVM and also suitably adjusted to ambient seasonal changes in temperature.

The salinity ranges from a low of 16 ppt for about few days during the northeast monsoon period to the normal salinity of 35 ppt. It has been observed that this wide range did not affect the growth, survival or algal production. Chemicals or antibiotics are not used at any stage, even for cleaning the tanks, which makes this technology eco friendly. Fouling, boring and predation was totally avoided in this system. With continuous food supply more than 80% survival from spat to the stage of adult was achieved. Even post-implantation mortalities were negligible, resulting in better pearl yield.

Maturation of the captive oysters and broodstock management technology has been developed. Brood stocks of pearl oysters were maintained in fully mature and ready to spawn condition, making it possible to conduct hatchery operation on a predetermined schedule. The brood stock development and spawning were tested successfully and spawning can be easily induced at any time of the year. In sea based activity one may have to wait for the oysters to attain natural maturity, which occurs only in certain seasons.

A record growth of above 100 mm was achieved for *Pinctada fucata*. Good quality pearls ranging from 6 to 9 mm were produced in about 12-15 months. Many pearl oyster of above 6 years are still active.

There are some misconceptions regarding onshore pearl culture, which need to be analysed in their proper perspective. One opinion often expressed is that in onshore pearl culture the quantity of algal requirement is huge and its production is costly. In reality it is one of the easiest process involving cheap inputs. Due to lack of practical Knowledge, some professionals feel that it is a costly input. In fact, once the algal culture facility is established, it becomes inexpensive and forms only one of the routine inputs. This is demonstrated by large number of prawn hatcheries around Visakhapatnam, which carry out algal production on a large scale and efficiently using minimum inputs.

It is time for us to realise and accept the natural limitations of our open sea conditions, which restrict sustainable farming at sea. There is therefore a need to switch over to "seawater based activity" rather than sea based activity to overcome this problem, instead of making improper comparisons with inter island, calm and protected areas as in Japan, Indonesia etc. A careful look at the recent success of commercial mussel culture along the west coast of India clearly indicates that the success is primarily due to shifting of the activity from the sea into the safe backwaters.

Apart from P.fucata, other species adopted for onshore (land based) system are *Pinctada maxima, Pinctada margaritifera* and *Pinctada chemnitz*.

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Site Selection for Bivalve Culture

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Introduction

In India, most of the bivalves produced are fished from the natural stocks. At present mussels, edible oysters and pearl oyster are the bivalves cultivated mainly from Kerala and Tamil Nadu. Cultured mussels form about 7% of the total catch. Production of bivalves by mariculture in India is very little compared to the rest of the world. In India, the cultivated bivalves are the green mussel *Perna viridis*, the edible oyster *Crassostrea madrasensis* and the pearl oyster *Pinctada fucata*. Except for the pearl oysters, the major source for collection of the seeds of the bivalves is through wild collection or through laying of cultch material as in edible oysters. There is considerable possibility for development of bivalve cultivation industry in India.

The success of mollusc mariculture depends largely on the proper selection of culture sites. In selecting a site for mollusc culture, considerations should be carefully given to a number of factors, which can be grouped under primary and secondary factors. Physical, ecological and biological factors (primary factors) are of prime importance in the selection of suitable culture sites, while factors such as risk and economics and legal usually follow in terms of importance. It is important to understand that if the primary factors are not fully satisfied, the particular site under consideration should be discarded whether or not all secondary factors are satisfied.

The selection of a culture site is initially determined on what bivalve species is intended to be cultured and consequently on the tolerance range of the above species to a number of environmental parameters (e.g. salinity tolerance range). In addition, the site will have to be suitable to the culture method or system intended to be practiced.

Primary Factors

Area Location

Locating the culture site would vary according to species and culture system. For strictly bottom dwellers such as the blood cockle *Anadara granosa*, the ark shell *Arca broughtonii* and the carpet shell *Venerupis japonica* the culture grounds should be located in protected areas where strong winds (eg. monsoon) do not prevail seriously over the area. Deposition of mud and fast siltation rates are often related to water turbulence partly induced by wind action. Although cockles and clams can actively move within their substrate, heavy mud deposition can cause serious mortality either by physically trapping the organism below the soil/water

interface or by raising turbidity to a level where filtering activity is completely hindered. For species that can be cultured by using different systems such as pole, rack or hanging, the location of a suitable area will depend on the culture system intended to be established. Oyster long-line culture as practiced in the Republic of Korea allows the exploitation of areas, which would be otherwise unsuitable for other bivalve culture systems. Long-lines can withstand relatively strong wind and wave action due to the flexibility of the system itself. The only limitation of the above offshore system is the depth of the water column, which will determine the length of the rens.

The length of the rens or of any other hanging structures used like lantern nets, pearl nets, etc. usually determine whether a long-line or raft structure would be economical to establish. The length of these culture units is usually limited to the upper water layers which abound in phytoplankton cells, however a minimum length is necessary in order to economically justify the initial investment and running cost of the above facilities

Substrate

Substrate composition and stability is a major environmental parameter to be considered during the selection of a culture site suitable for benthic species such as cockles and clams or where bottom culture is intended to be carried out. Substrate composition will determine the suitability of an area for a particular species. Cockles are usually found on muddy or silty-clay bottoms, with the highest population densities found on soft intertidal mud flats bordering mangrove swamp forests (Observations on cockle spatfall showed that they settle mainly on fine, soft, blackish mud. In Penang Island, Malaysia the well known Bagan Jermal bed adjoins areas of sand, sandy-mud and stiff black mud with sand, shell and plant debris. In this area spatfall has taken place every year with the greatest concentration always on fine soft mud.

Oyster bottom culture is limited to areas where the sea floor is firm enough to support some kind of cultch and where siltation is not excessive. This traditional culture method, although not as productive as other culture systems such as the raft method, is sometimes the only system that can be adopted either due to a number of unfavorable environmental conditions or limited funds. This method is in fact the most inexpensive as it relies exclusively on the availability of stones, empty oyster shells or similar materials on which the oysters settle and grow. This method is widely adopted in many areas around the world. In Thailand rocks are usually piled in groups of 5–10 and spread in rows approximately 50 cm apart in each direction. This technique is used in areas with hard, sandy or sandy-mud bottoms firm enough to support the rocks, however bamboo mats or platforms are commonly used in soft bottom areas to prevent the rock from sinking. The use of bamboo mat adds to the initial investment cost and needs to be replaced quite frequently. If bottom culture is the only possibility, substrate nature in terms of firmness needs to be carefully examined in order to carry out a correct cost/benefit analysis.

Water Depth

Water depth is not usually a limiting factor in mollusc culture, however it will determine what culture method can be used. Probably the most important aspect with regard to water depth is to avoid long exposure periods during the extreme low water spring tides when benthic molluscs such as cockles and clams are cultured. Long exposure periods increase the culture period due mainly to the fact that during these periods the molluscs burrow into the substrate and stop feeding. However, one advantage may be during the harvesting phase particularly where it is carried out manually. With cockle culture, where planting and harvesting is carried out from a vessel, the culture area should have a water depth of about 1 to 2 m mean tide level.

The Manila clam, *Tapes semidecussatus* is a highly valued species in Europe, particularly in Spain, France and Italy. They are cultured in mud flats in enclosed bays, lagoons, man-made ponds and areas bordering estuaries. Preparation of the ground is necessary to enable the clams to dig themselves in. Usually, a protective fence is built around the culture site. Removal of predators, especially crabs, is necessary and carried out periodically. Harvesting is generally done manually although harvesting and cleaning machines have been developed. All these activities are usually carried out during the exposure period. Therefore, sites, which are selected for this kind of culture generally, become exposed for short periods during the tidal cycle.

With regard to mussel and oyster culture the water depth depends on the culture method and it can be in the range of 1–15 m mean tide level. In areas where the mean tide level is usually less than 1.5 m, bottom culture on rocks or other materials can be practiced. For raft and hanging method, the water depth can be a limiting factor as usually a minimum water column height is essential during the low water spring tides. In the above two culture methods, the hanging rens should never touch the bottom mainly to prevent predators from reaching the bivalves, avoid exposing the molluscs to high water turbidity near the seabed, and avoid losing the bivalves at the end of the rens as a result of their friction with the ground. Culture ropes should be above the sea floor at least 1 m during extreme low water spring tides.

Exposure

Marine molluscs are unable to function when removed from their water medium and long exposure periods usually lead to death. Exposure is one of the major environmental conditions that influences the growth and mortality of marine molluscs. Both growth and mortality rate vary according to shore elevation. Growth performance of a mollusc located at higher levels is usually lower compared to one located at lower levels, due to prolonged exposure periods and subsequently reduced feeding time.

Exposure to sun is one of the physical parameters, which need to be taken into account when selecting a potential culture ground in shallow coastal areas. In

raft or long-line culture, exposure is not a problem as the cultured organisms are always below the water surface. Exposure however has a number of advantages, particularly with regard to the mortality rate. There is in fact evidence that mortality increases markedly with depth due to a greater degree of predation at the lower levels, presumably as a result of longer access time of predators in the culture grounds. It has been suggested that optimum sites for culturing benthic bivalves are areas, which become exposed for periods lasting 2–3 hours. A further example where limited exposure is an advantage can be clearly seen in the mussel culture industry in the venetian lagoon, Italy. Mussel (*Mytilus edulis*) is extensively cultured by using the rack hanging method. During late spring and summer month the suspended ropes bearing the mussels (known as "pergolari") become heavily encrusted with fouling organisms, such as seasquirts and seaweeds. The presence of these organisms is undesirable because they compete for food and space and critically increase the weight of each hanging unit.

There is, therefore, a need to remove these fouling organisms. This laborious process, however, is not required in this particular site, as the adequate exposure time of the mussel ropes causes all encrusted organisms to dry up. In other areas such as Taranto, in the south of Italy, mussel aquaculturists have to routinely suspend the mussel ropes and remove the fouling organisms manually. This process is time consuming and labour intensive. Labour effort and growth period are therefore related to exposure.

Water Movement

Bivalve culture sites should not be in the vicinity of strong currents particularly where bottom culture is practiced as strong currents usually generate high turbidity and high siltation rates. However, moderate currents are needed to provide adequate food supply. Currents of 0.02–0.1 m/sec have been reported to be suitable for cockle cultures, while stronger currents are usually required for the hanging method due to the intensive culture nature of this method. In the hanging method, slow water movement usually results in slow growth of the bivalves due to the poor replenishment of food. Slow currents also promote the settling of organic and inorganic particulate materials on the cultured organisms. Potential sites should have a current speed within the range of 0.1–0.3 m/sec.

Turbidity

High turbidity levels due to the presence of finely suspended matter such as clay, sand, and other organic and inorganic particulate materials at the culture site is usually undesirable as it causes ill effects on the bivalves being cultured and often resulting in high mortalities. The presence of suspended materials above a certain level hinders the filtering activity of the bivalve, which often remain closed to avoid tissue damage and becoming clogged. In addition, low primary productivity is often the case in sites of high turbidity due to the reduced penetration of sunlight in the water column. As a result poor growth results due to reduced feeding time and limited food available. It has been reported that water containing a high suspended load of more than 400 mg/l have a lethal effect on the grow-out of mussels. The maximum suspended load tolerable level varies according to species. A practical

method for determining the turbidity level is with the use of the Secchi-disc. Sites having a disc reading less than 15 cm are usually considered unsuitable for bivalve culture.

Salinity

Although most species of molluscs tolerate a certain range of salinity levels, some species tend to be more euryhaline than others. When the salinity value falls below or above the range of a certain species for prolonged periods, high mortalities generally occur. Decrease in salinity levels is usually the major and frequent problem, mainly caused by the influx of large volumes of fresh water from rivers or land runoff during the rainy season. With regard to the blood cockle, *Anadara granosa* a number of field surveys and laboratory trials have shown that adult specimens function relatively efficiently at salinities above 25 ppt, although young specimens seem to be able to continue normal feeding activity at a lower salinity than older specimens are. Very young individuals apparently remain active at salinities as low as 18–19 ppt. Although feeding efficiency and activity generally decrease substantially at salinities less than 20 ppt, *A. granosa* is capable of acclimating to salinities as low as 12 ppt, at least in the short term. These results are consistent with the known distribution of *A. granosa* in areas where the salinity is usually in the range of 26–31 ppt, but which are subject to large, short-term fluctuations.

Bottom Slope

The degree of bottom slope is one factor, which needs to be considered particularly when the bivalve species is cultured directly on the substrate. Suitable culture beds should have a moderate seaward slope between 5 –15 degree. Slopes exceeding 15 degrees often cause cockles to be shifted from their original site due to wind and wave action. On the other hand, if the slope gradient is too little the culture area is often exposed for too long between tides.

Food Organisms

All bivalves are filter feeders, mainly feeding on a wide range of phytoplankton species. The presence of suitable micro algae species is usually not a problem, however, problems do arise when the availability of food is limited. . It has been estimated that when bivalves are grown under similar conditions at different sites, up to 85% of any difference in growth observed between sites can be attributed to water temperature and primary productivity. Studies have shown that the growth of small scallop spat is positively related to the concentration of chlorophyll in the water. This indicates the importance of primary productivity for growth of cultivated bivalves, yet it is the most difficult factor to assess for a given site. It is usually measured as the total organic weight of algae produced in a year for each square metre of sea surface area (to include the water column beneath). The carrying capacity of a body of water, (ie the biomass of animals that the algae food it contains can support) can be exceeded by overstocking, leading to reduced growth Bivalve intensively cultured in rafts may be affected by the length of the

culture period when food is scarce. In the above example, poor growth is usually the result of poor water movement (ie. low current) rather than food availability.

Another problem related to food organisms are the sudden blooms of certain phytoplankton organisms, usually in coastal waters. This phenomena is known as red tide as the organisms become so dense that the seawater takes on a brown, red or yellow coloration. Unfortunately, it is often difficult to predict if any area is prone to be affected by these toxic blooms, however, during the site selection process, one should ask about the past history of the area. Bivalves affected with red tides are not usually killed, but tend to accumulate toxic substances in their flesh. Depuration studies have shown that those bivalves can be depurated, however the longer depuration time required would make it very uneconomical. Another problem which arises from food organisms are shellfish which are harvested or cultured in estuaries or coastal areas which are used as repositories for untreated domestic sewage. Shellfish from such areas are known to accumulate bacteria and viruses which are pathogenic to man. Major diseases are typhoid and paratyphoid fever, salmonellosis, *Vibrio parahaemolyticus* infection, cholera, viral Hepatitis type A and viral gastroenteritis. Contaminated bivalves can be made edible by: 1) re-laying or transferring the shellfish to pollution free waters or 2) depuration. These processes are time, labour and cost intensive. Therefore, during site selection it is important to bear in mind that being filter-feeders, they can accumulate pathogenic organisms, toxins as well as heavy metals at levels which can be lethal to humans.

Source of Seed

Bivalve culture needs a regular supply of spat or seed is one factor, which may affect site selection decisions. However, if it has to be transported from elsewhere, it should be transported to the farm site within a reasonable time and cost. This factor has to be considered, as it will affect the cost and returns analysis. Transportation itself is not only costly, but usually negatively affects the bivalve seed due to abnormal and stressful conditions. The mussel (*P.viridis*) seed can remain without water for about 24 hrs and seeds are transported to areas where there is short supply. At Padanna, the mussel farmers get seeds of mussel from Calicut, Malpe and Karwar. The region of abundant seed availability may not be the ideal areas for grow out.

Pests

Bivalves may be eaten by various predators particularly crabs, fishes and gastropods. Bivalves grown on bottom are more vulnerable to various predators. The predatory gastropod, *Cymatium cingulatum*, is found in the edible oyster farm at Tuticorin during July to December preying on oysterlings causing upto 15% mortality. At Vizhinjam, in the raft culture of *Perna indica*, predation by the fish Rhabdosargus and lobsters were reported. At Parappanangadi, the green crab *Scylla serrata* destroyed the seeded mussel ropes in the rack culture. In the pearl oyster culture racks, crabs, polychaetes and fouling organisms like tunicates pose problem for constant maintenance. During site selection it is difficult to determine whether an area would eventually become affected by this problem, however it is good

practice to survey the area for potential predators. In pearl culture farms as well as in natural pearl oyster beds, *Cymatium cingulatum* and *Murex virgeneus* have been found to be serious predators in natural oyster beds. In culture sites crabs are the worst predators of the spats. *Charybdis lucifera*, *Atergatis integerrisimus*, *Leptodius exaratus*, *Neptunus* spp. and *Thalamita* spp. are some of the crabs commonly found inside the pearl oyster cages in the Indian oyster farms.

Secondary Factors

Pollution

Waters with heavy industrial contamination such as trace metals and organic compounds are unsuitable for bivalve cultivation. The development of intensive agriculture, heavy industries along the coastal areas and increasing number of urban settlements have increased the pollution load into the biologically productive coastal waters. Domestic wastes carry detergents, solids and various toxic substances. Agriculture pollution involves animal waste, solids, insecticides, herbicides etc. Bivalves are known to accumulate trace metals and pollutants. This renders it unpalatable due to the unpleasant flavour they impart like the copper and oil tainting. In the 1980's the biocide tributyl tin (TBT) was highly toxic to bivalves. Banning of TBT in July 1987 helped in reviving the oyster industry. In areas with untreated effluents discharges as is done in many developing countries, the location of these sites could affect the production as well as the product quality. In Jakarta Bay and Manila Bay, due to pollution and numerous heath incidences related to consumption of molluscs reared in these areas, the molluscan culture enterprise have suffered severe losses as the market demand was reduced. The EU standards to be met for export of mussel products are given in Table 1 and the criteria for classifying shellfish harvesting areas are given in Table 2.

Poaching

The problem of pilfering and damage is common in aquaculture. Constant supervision of the culture is the only effective answer. Living near the culture site is obviously the most advantageous situation for keeping constant watch over the stock and facilities. When located away from these grounds, a small guardhouse in the culture site is constructed. However, this adds to the production cost.

Resource Competition

Conflicting activities of the common users of the sea may pose problems for stocking suitable culture sites .The proximity of the culture sites to navigation channels, recreational activities and industrial activities may expose the farm to a series of problems generated by the normal activities of the common users. The wave action created by vessels, which may have a disturbing or destructive effect on both the cultured organisms and rearing facilities.

Economic Considerations

While considering the different options of culturing (eg. bottom, raft, rack, long-line, etc.) the species, a cost benefit analysis is to be done when the site is selected. Culturists interested in commercially growing oysters, as the selected bivalve species, will be confronted with the initial capital investment required to set up the operation. The various culture systems, which may be set up to culture the oysters, require different levels of investments depending on the complexity of the system itself.

Potential culturists with adequate financial resources may well consider investing in a more capital intensive system such as the raft culture or the long-line method. If the financial needs do not pose any major problem, the investor will direct his efforts in selecting sites suitable for establishing long-line facilities, therefore excluding all other sites unsuitable for this culture method.

Conclusion

The prospective cultivator may be looking for a site on which to cultivate particular types of bivalve mollusc. Or he may already have a site in mind, and needs to decide which species would perform best and be most profitable for that site. Careful consideration of the criteria discussed above will help him to arrive at the most suitable choice. It is wise to approach site selection with caution, since once committed; any errors in judgement may prove expensive. Environmental data and other information on sites may be obtained from various organisations. When looking at environmental data, it is well to remember that there will be a certain amount of variation within and between years for the same site. Very few sites, if any, are likely have the perfect blend of qualities for the cultivation of the chosen bivalve species. Choice of site will also be restricted by availability. Growers should avoid sites where several environmental factors provide less than optimum conditions, as each may impose a small stress on the bivalves, which together result in poor growth and possible mortality. Where circumstances permit, the cultivator should evaluate the suitability of a number of sites in a pilot study with trial plantings of the chosen bivalve species. Growth differences between sites usually reflect differences in conditions, which may be fairly specific to the sites. These conditions may vary widely between and within years, requiring long-term studies of at least one year and preferably longer, to get an accurate picture of the suitability of the site for cultivation. Finally, it should be remembered that a successful and profitable bivalve cultivation operation requires good husbandry and management of the stock as well as the selection of a suitable site.

Suggested reading:

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Table 1. European Union (EU) standards to be met for export of mussel products

	Parameters in farm site	Mandatory level
1.	Colour	> 1mg Pt/l
2.	Temperature	± 2 °C from normal sea temperature
3.	pH	7 – 9
4.	Salinity	2 – 48 ppt
5.	Dissolved oxygen (Saturation)	>80 %
6.	Suspended solids (mg/l)	30 %
7.	Petoleum hydrocarbons	Should not be deposited in the flesh.
8.	Organo-halogenated substances	Should not exceed harmful levels in shellfish and larvae
Bacteriological parameters: Maximum permissible limit (Nos./100ml)		
1.	Faecal coliforms	< 300 in the shellfish & intervalvular liquid
Heavy Metals in tissue: Maximum permissible residual level (ppm)		
1.	Mercury	1.0
2.	Cadmium	3.0
3.	Arsenic	75
4.	Lead	1.5
5.	Tin	250
6.	Nickel	80
7.	Chromium	12
Pesticides in tissue: Maximum permissible residual level (ppm)		
1.	BHC	0.3
2.	Aldrin	0.3
3.	Dieldrin	0.3
4.	Endrin	0.3
5.	DDT	5.0
Antibiotics and other Pharmacologically active substances in tissue: Maximum permissible residual level (ppm)		
1.	Tetracycline	0.1
2.	Oxytetracycline	0.1
3.	Trimethoprim	0.05
4.	Oxolinic acid	0.3

Table 2. Criteria for classifying shellfish harvesting areas

Classification category	Faecal coliform bacteria (*E.coli*) per 100 g shellfish flesh	Comment
A	All samples less than 300 (230)	Suitable for consumption. Can be marketed.
B	Less than 6,000 (4,600) in 90% of samples.	Depuration needed (or relaying in catergory A area or cooking by an approved method).
C	All samples less than 60,000 (46,000)	Relaying (minimum of two months in approved relaying area or cooking by an approved method).
Prohibited	Above 60,000 (46,000)	Cannot be taken for placing n the market.

17 Clam Culture : Global Scenario

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Bivalves such as oysters, mussels, clams and cockles are widely distributed, both in the tropical and temperate waters. In recent years, they have emerged as delicacy and luxury food item in Japan, USA and Western Europe.

The total global mollusk production through exploitation of the wild stocks was 6684 thousand t in 2002 forming nearly 5 % to the total world fish production (132989 thousand tonnes). The total global aquaculture production of fish, crustaceans and mollusks for 2002 was estimated to be 39.8 million tones with a farm gate value of US $ 53.8 billion. The total mollusk production through aquaculture was estimated at 11784 thousand t by quantity and US $ 10,512 million by value, in 2002. Due to depletion of the intensely exploited wild stocks, there is increased demand for farm grown products (FAO Fisheries Statistics 2002).

The world landings of clams/cockles/ark shells have been increasing steadily for the past few years totaling 826 thousand t in 2002 forming nearly 12.4 % of the total mollusk production. The clam/cockles/ark shells production through aquaculture in 2002 was 3431 thousand t forming 8.6 % of the total aquaculture production. The major mollusk producers include China, Japan, Korean Republic, France, Spain, and the USA, Italy, Malaysia, Netherlands and other Asian countries. The major clam producing countries include China, Japan, Malaysia, Korean Republic and Thailand (FAO Fisheries Statistics 2002).

Among bivalves, clams are by far, the most abundant and widely distributed resources. They are commercially important and fished in fairly large quantities in several countries. In India, clams form subsistence fisheries all along the coast. Clam meat is nutritious and is a cheap source of protein rich seafood. Clam culture is practiced in several countries such as Taiwan, Thailand, Malaysia, Indonesia, Singapore, and U. K. Australia. However, it is not as advanced an art, as is the case with oysters or mussels. In clam culture, the seed is generally collected from natural grounds and replanted in areas with a suitable substratum but where seed is not abundant. They are then allowed to grow to market size.

1. Selection of Site

Clams are cultured on the bottom and therefore site selection depends on the substrate. The occurrence of natural clam populations is indicative of the suitability of the site with particular reference to the tide level, substratum and water salinity. Clam farms are located in estuaries, bays and other sheltered close to the shore. About 1-2 hrs exposure at low tide is desirable as it is easy to remove the predators.

Too long an exposure results in poor growth due to reduced feeding and in summer there may be mortality due to desiccation. Farms located further in sub tidal area have the disadvantage when predators are to be eradicated.

The type of substratum preferred varies with the species. For example, *M. casta* thrives well on sandy bottom, while *Anadara granosa* prefers mud flats. Also the salinity range tolerated differs between species. While *V. cyprinoides* prefers low saline waters few species tolerate prolonged low saline conditions which are generally prevalent in areas subjected to heavy rains and freshwater drain from the land. Clam farms are located in areas where there is little wave action. Areas prone to frequent changes of contour and vulnerable to pollution are avoided.

2. Hatchery Production of Spat

Mature clams of 35-45 mm shell length are used as brood stock. The brood stock is conditioned in unfiltered seawater of 25 ppt, and at 22-24°C and then transferred to spawning troughs. Spawning is induced by thermal cycling: the spawning through is part filled with cooler water to a depth of 10 cm and a small amount of cultured microalgae (*Isochrysis galbana*) to stimulate the clams to extend their siphons and start pumping activity. After 15-30 minutes, the water is drained and replaced with water at 28-30 C, again with small addition of algae. This water is drained after a similar period of time and replaced with cooler water and the procedure is repeated. The number of cycles, which are necessary to induce spawning, depends on the readiness of the clams to spawn.

Eggs are separated through a 60 µm nylon mesh sieve and transferred to filtered seawater at 25° C. The larvae are grown in flat-bottomed vessels, or in conically based fiberglass thanks fitted with bottom drains, at 15,000-20,000/1 but optimum density for good growth is 10,000/1.

Good aeration at the rate of 2001/h depending on the size of the container and filtered and sterilized seawater of 25 ppt and 24-26°C provides optimum conditions for larval growth. Unicellular alga such as *Chaetoceros calcitrans, Isochrysis galbana, Tetraselmis suecica* and *Thalasiosira pseudonana* are suitable food species. Diets of mixed alga are beneficial. A suitable diet for D-shelled larvae is a mixture of *Chaetoceros calcitrans* and *Isochrysis galbana,* the most suitable cell densities are 125 cells /ml respectively.
Airlift down welling recirculation systems of 100 l capacity are generally used for growing 0.5 million spat to size retained on 440 µm mesh size.

3. Nursery Rearing

Nursery upwelling systems are generally used in several European countries for spat rearing. Water flow is induced through cylinders (30 cm x diameter) placed in a 30 l-header tank, by creating a difference in the head of the water. The header tank is placed over the 500-10000 L fiberglass reservoir tank, valves are fitted to the over flow of the upwelling cylinders, since spat growth is strongly influenced by water flow rate. Flow rates of 20-50 ml/minute per gram are used.

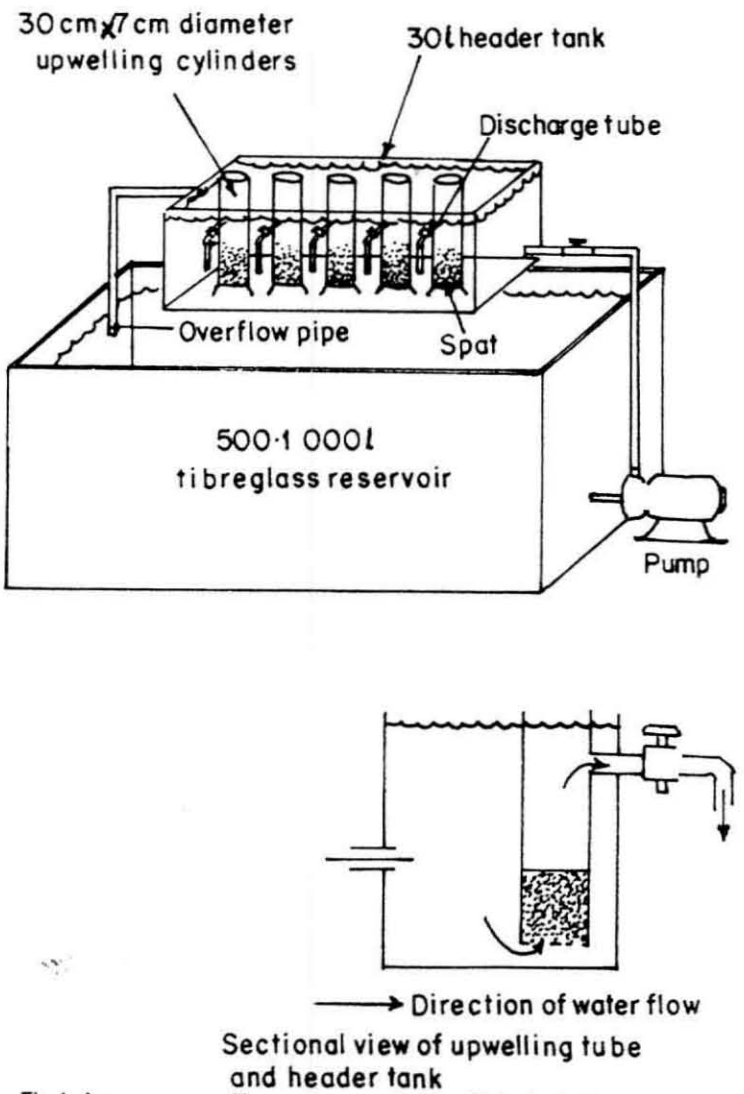

Fig 1. A nursery upwelling system used at the Fisheries Laboratory at Conwy

Seawater coarsely filtered through a 45 / μm mesh is used so that the spat are benefited from presence of naturally occurring algae, in addition to those offered as food.

Nursery upwelling systems need relatively large volume, since only small biomasses of spat can be grown successfully per unit volume of water.

4. Growout Culture and Production

The ground is leveled and cleaned of predators such as boring gastropods, starfishes, crabs and skates. Bamboo poles are planted on the boundary of the farm as markers. The movements of the clams are limited and in many areas fencing is not necessary. Synthetic fibre net pens are erected to protect clams against strong water currents in the USA; bamboo stakes with nylon netting are used in Taiwan.

At high tide, seed measuring 10-25 mm in length are taken in a boat and planted in the farm, taking care to get even dispersal as far as possible. Uneven distribution is set right at the next low tide. In Malaysia, *A. granosa* is stocked at 1000-2000/m^2 and thinned more than once to achieve final density of 300-600/m^2. The stocking density varies with the species and a stocking density of 400/m for 10 mm seed and 300/m^2 for 20 mm seed is usually optimal.

After seeding the farm, 10 mm mesh synthetic netting is laid and held in position by stakes driven into the substratum at the periphery of the farm, to offer protection to the young clams. Except for watch and ward and eradication of predators, no other maintenance job is necessary during the grow-out phase. The clams are harvested after 5 or 6 months either by hand picking or by hand operated dredge.

In Malaysia, wild seed of *A. granosa* are sown in prepared coastal mud flats, generally bound by natural landmarks and where these are lacking, are marked by other means. The sowing density is between 2-6.5-kg/seed/m^2. An average production of 40 t/ha is obtained.

In Thailand also the same method is followed for *A. granosa* and 50 cm long bamboo stakes are used to fence the inter tidal mud flats to prevent escape of clam from the culture beds. Clam seed are also sown in the central elevated areas of shrimp ponds and fenced with bamboo stakes. The seed used here are larger than those used for the intertidal flats. These methods yield 31-109 t/ha annually.

In China, *Sinonovacula constricta* (razor clam), *Arca (Anadara) granosa* and *Tapes philippinarum* (small necked clam) are cultivated. Seed clams (1 cm long) of *S. constrictare* collected and sown in rearing beds during January at 9-18 x 10^6 clam seeds / ha. The average yield is 15-22 t/ha. After 6-7 months. *A granosa* seed are also raised from natural spat and reared in enclosed water pools. They are thinned several times, transplanted to rearing grounds in the lower tidal zone. It takes 2-3 years to reach marketable size of 2cm and yield is 22.5–60 t /ha. The small necked clam is also cultivated on pre-prepared culture beds by stocking 1.4 cm seed clams at 1.8 x 10^6 seed /ha. The yield is about 18.7 t /ha. But sometimes as high as 45 t/ ha.

Clams are rarely grown in ponds, but in recent years due to adverse impact of viral diseases in shrimp culture, there is growing interest in many southeast Asian countries to utilize the shrimp ponds for clam culture. In Taiwan, *Meretrix lusoria* is grown in ponds formerly used for milkfish and shrimps and also in the outlet and inlet canals of these ponds.

5. Giant Clam culture

In Giant clam culture, four phases are involved.

a) Hatchery phase: Rearing of larvae from eggs in indoor or out door tanks. Six out of the eight known species of giant clams have been successfully spawned in the Philippines by injecting serotonin into the gonad of mature clams and also by introduction of macerated gonad materials into the mantle cavity through the exhalent siphon. The development stages are similar to those in other clams and settlement takes place in about 7-10 days after spawning. *Isochrysis galbana* is fed to the larvae. The spat attach with the byssus but they may break attachment and creep along the substrate. At this stage the symbiosis with Zooxanthellae is established. The larval rearing is done in both indoor and outdoor tanks.

b) Nursery phase: Rearing juvenile clams in onshore tanks for metamorphosis (0.2mm) to about nine months of age and 20+ mm shell length (seed clams). The tanks are provided with flow of raw seawater. The clams acquire the zooxanthellae from the seawater in about 3 weeks after fertilization and they become increasingly autotrophic.

c) Ocean nursery phase: Rearing juvenile clams in protective containers in the field from about 20 mm shell length to 200 + mm shell length.

d) Grow out phase: Rearing clams of 200+ mm shell length without protection in the field to market size.

Tridacna gigas, the largest among the giant clams grows to 18.6 mm (total wt 0.55 g) in 0.83 years, 121.1 mm (193.8 g) in 2 years, 206.4 mm (923.3 g) in 2.66 years and 221.3 mm (1.15 kg) in 3 years. The wet meat forms 12% of the total weight in the 18.6 mm clams and it increases to 26% in 221.3 mm clams. A production of 29 t of wet meat/ha has been estimated in *T gigas* culture for three-year-old clams. The field culture techniques, survival and production for various giant clam species are still in experimental stage.

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18

Clam Culture in India

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Clams are bivalves, which burrow into the substratum with the help of a usually well-developed foot. A few clam species are also known to attach to hard substrates with byssus threads. Among bivalves Clams are by far, the most abundant and widely distributed resource.

1. Resources and Distribution

A number of clam species belonging to the families Arcidae, Veneridae, Corbuculidae, Tridacnidae, Solenidae, Mesodesmatidae, Tellinidae and Donacidae are exploited along the Indian coast. The cultivable species mostly belong to the first four of the above-mentioned families.

Arcidae: The arcid clams are called blood clams, as their blood is red due to presence of haemoglobin. A single species *Anadara granosa* is important. It occurs all along the Indian coast in soft muddy substratum and forms a fishery of some magnitude in the Kakinada Bay.

Veneridae: The venerid clams are the most sought after in the clam fisheries of India and three genera namely *Meretix, Paphia,* and *Marcia* are important.

Along the Maharastra coast, *Meretrix meretrix (Linnaues), Marcia opima (Gmelin)* and *Paphia laterisulca* are the dominant species. In Goa, *M casta* forms a fishery. Along Karnataka coast, there are 14 estuaries with varying abundance of clams. *M meretix* is found in all the estuaries. *Meretrix meretrix* in the Kalinadi and Coondapur estuaries, *Paphia malabarica* in the Mulky, Gurpur, Udyavara and Coondapur estuaries. *Marcia opima* is found in Coondapur, Uppunda and Sita estuaries. Along the Kerala coast, *Paphia malabarica* forms a fishery in Koduvally, Azhikkal, Karyamgod and Chittari estuaries and Ashtamudi Lake. Other Venerid clams form fisheries in several estuaries in Kerala State. Along the east coast, *M casta* occurs at several places and forms a fishery at Vellar estuary, Pulicat lake and Bhimunipatnam backwaters. *M opima, P malabarica* and *M meretrix* contribute to the clam fisheries in the Kakinada Bay. Along the Orissa coast, *Meretrix* sp occur in the Chilka Lake and Sonapur backwaters.

Corbuculidae: The black clam *Villorita cyprinoides* is the major resource in the Vembanad Lake and is also exploited in several backwaters, lakes and estuaries of Kerala. It also contributes to the fisheries in Goa and in the Nethravathi, Gurpur, Udyavara, Swarna and Coondapur estuaries in Karnataka.

Tridacnidae: Represented in by *Tridacna maxima, T crocea, T squamosa* and *Hippopus hippopus*, they occur in the Andaman, Nicobar and Lakshadweep islands.

2. Exploitation

In India, clams form subsistence fisheries all along the coast. Clam meat is nutritious and is a cheap source of protein rich seafood. Clams are fished by men, women and children, all along the Indian coast. They are collected from the intertidal region to about 4 m depth. They are hand picked and also a hand operated dredge is used. Plank built non-powered boats are used for transport. Clams under one year contribute in considerable numbers to the fishery. At many centers, November to April is the peak fishing season as the new recruits become available to the fishery.

In India, the production of clams and the species break up through harvest of the wild populations is not regularly monitored. However, estimated annual landings of commercially important bivalves by Central Marine Fisheries Research Institute for 2002-2003 are summarized.

Six species of clams viz., *Villorita cyprinoides, Paphia malabarica, Sunetta scripta, Meretrix casta, M meretrix, Marcia opima* and the cockle *Anadara granosa* contribute to the commercial fisheries. Of the estimated total annual production (2002-2003) of 38800 t of clams, the black clam *Villorita cyprinoides* is the major clam species landed (20666 t) forming 56% of the total clam production. Major production center was Vembanad Lake. It is also fished from Korapuzha and Chaliyar estuaries in north Kerala but is seasonal from April to June only.

Paphia malabarica, the second most abundant clam resource accounted for 6903 t and the major fishing area was Ashtamudi Lake (6277 t) in Kerala. It is also fished from Dharmadom estuary in north Kerala. In Karnataka 622 t were landed from Gangoli backwaters (340 t) and Kali estuary (282 t). In Andhra Pradesh, 4 t were landed from the Kakinada bay. *Sunetta scripta* landed 4486 t from Cochin and utilized mainly for shell.

The total estimated landing of *Meretrix casta* was 3571 t. In Kerala, *M casta* fishery was more prevalent in northern Kerala in Kottakal (940 t) and Chaliyar (491 t) and Dharmadom (41 t). In central Kerala, significant depletion of the stock in Chettuva has been reported. In Karnataka (1477 t) of *M casta* were landed mostly from Mulki estuary.

Meretrix meretrix accounted for 200 t from the Agnashini estuary in north Karnartaka and two regions Bhimili (70 t) and Kakinada Bay (16 t) in Andhra Pradesh. The fishery for *Marcia opima* was reported (5 t) only at Gangoli in south Karnataka. *Anadara granosa* accounted for 873 t from the Kakinada Bay.

3. Distinctive Characters of Cultivable Species

Anadara granosa: - Shell thick, inflated and dark brown. This species differs from other clams in having taxodont dentition. (Small teeth in a single straight series) and about 20 elevated ribs bearing rectangular nodules.

In venerid clams, the hinge usually bears three cardinal teeth and a single anterior lateral tooth on the left valve and a corresponding depression on the right valve. Two adductor muscle impressions, slightly unequal in size are present.

Paphia malabarica: - Shell slightly inflated, surface concentrically grooved. Pallial sinus is U shaped and very deep. Lunule relatively short. Shell length only one and one third times longer than height. Hinge area short with narrowly diverging teeth, shell yellowish brown in color, indistinctly rayed with grayish brown bands or mottled with brownish angular markings.

Meretrix casta: - Shell thick smooth devoid of any sculpture and triangularly ovate. Outer surface pale yellowish brown tined with dark grey posterior and very faintly rayed with grayish radial lines.

M. meretrix: - It differs from *M. casta* in having less elongated lateral tooth and more ovate shell. Also it grows to a larger size.

Marcia opima: - shell thick, inflated, smooth triangularly ovate. Pallial line deeply sinuate, Apex of the pallial sinus is bluntly angular. Lunule distinct, flattened and rather broad. Area behind umbones is well defined, flattened and greatly elongated reaching almost upto the hind margin of the shell. Outer surface of shell polished, pale yellowish brown or straw coloured, variously mottled and rayed with purplish grey markings.

Villorita cyprinoides: - Shell thick, ovately triangular with strong concentric ridges; ridges more strongly developed in the anterior half. Umbones prominent, well elevated, hinge margin short and thick, always with three oblique cardinal teeth of which the anterior in the right valve and posterior in the left valve are obsolete. Pallial sinus small, lunule narrow and ligament large. Periostracum dark olive brown to blackish brown.

The Tridacnid clams have large massive shells with broad radial ribs, sometimes bearing large fluted scales. Edges of valves usually scalloped.

Tridacna crocea **(Crocus or Boring clam)**: - Smallest of the giant clams, grows upto 15 cm, Large thick, triangularly ovate shell with large byssal gape, 6-10 broad flattened ribs with concentric ridges, Shell grayish white, flushed with yellow or pinkish orange.

T. maxima: - (Rugose giant clam): Shell strongly in equilateral. Resembles *T crocea* but its 6-12 broad radial ribs have much more strongly developed concentric scales. Large byssal gape with distinct plicate at edges. Ventral margin of the valve often deeply scalloped. Shell grayish white, sometimes tinged with yellow or pinkish orange. Grows to about 35 cm.

T. squamosa **(Fluted or scaly clam)**: - Large, thick strongly inflated shell with small or medium sized byssal gape. 4-12 strongly convex ribs with rib lets in inter spaces. Broad, sometimes long fluted scales on ribs, which may

project beyond ventral margin considerably. Grayish white, sometimes fringed with yellow. Grows to 40 cm.

4. Biology Of Cultivable Species

Like oysters and mussels, the clams are filter feeders.

A. granosa: Comprehensive information is available on the biology from the Kakinada bay. It attains 41.1, 55.3 and 66.3 mm on completion of 1,2 and 3 years respectively. It attains a maximum length of 72 mm. Males attain first maturity at 20 mm and females at 24 mm length. It spawns throughout the year and there can be 2-4 reproductive cycles in a year. The condition index based on the percentage of wet flesh weight in total weight varies from 15.1 to 23.1 and expressed as percentage of dry flesh weight in wet flesh weight ranges from 17.2 to 24.2. About 10.85% of the clams are infested by the pea crab *Pinnotheres alcocki.*

M. meretrix: In the velar estuary it grows to 47 mm in the first year and 651.5 mm in the second year. At Tuticorin, growth is slow and it reaches 29.0, 48.3 and 62.3 mm in 1^{st}, 2^{nd} and 3^{rd} years respectively. It grows to a maximum length of 91 mm and the life span is estimated at 7-8 years. The condition index expressed as percentage of wet meat wt in total wt varies from 7.6 to 16.1 with an average of 12.2. First maturity is attained at 21-26 mm length. Along the Bombay coast spawning is from March to June, in the Vellar estuary from February to September and at Tuticorin in January-April and June-October. It withstands a low salinity of 10.5 ppt under laboratory conditions.

Meretrix casta: This species grows fast in the Mulky estuary, attaining 36.5mm in 6 months and 42.6 mm at the end of first year. In the Adyar estuary the growth is comparable while it is slow with the monthly average growth rate at 2 to 2.6 mm in the velar estuary, 2.7 mm in Goa and 2.9 mm in the kali estuary. It grows up to 55 mm in length. The wet meat forms 7.6 to 16.0 % of the total weight and is usually high before spawning. Length at first maturity is between 11 to 17 mm. In the Adayar, Mandapam, Goa and Kali estuary areas it spawns throughout the year with 1-3 peaks. In the Mulky estuary spawning is prolonged, extending from September to March. In the same estuary the clams are often found infested by crabs.

Paphia malabarica: In the Mulky estuary it grows to 36.3 mm in 6 months, 43.1 mm in 9 months and 49.1 mm in one year. The largest clam in the commercial catches measured 51 mm. In the Kakinada bay clams measuring 65 mm in length are often found. The wet meat forms 11.8 to 15.4 % of the total weight. The length at first maturity is 20 mm. It spawns during October-February in the Mulky estuary and September –January in the Ashtamudi Lake.

Marcia opima: In the Adayar estuary it attains 26-33.8 mm in length in over one year and 38.8-43.5 mm length in 2 years. In the Kalbadevi estuary it grows to 22, 31 and 43 mm during 1-3 years respectively. In the Velar

estuary it grows from 5.6 mm to 33.3 mm in 8 months. Wet meat forms 7.9 to 12.5% of the total weight. It attains maturity at 11.20 mm length. In the Adayar estuary spawning begins in December when the bar mouth is open and lasts for a month. In Kalbadevi, a major spawning during October-November and a minor spawning during March-April takes place. Under laboratory conditions it tolerates a low salinity of 14 ppt when transferred suddenly and 7.5 ppt on acclimatization.

Villorita cyprinoides: This species tolerates near freshwater conditions and occurs in the upper reaches of the estuaries and backwaters. In the Cochin backwaters it grows to 30 mm in one year and 41 mm in the second year. In the Nethravathi estuary it attains comparables length at the end of first year and in Goa it grows from 20.4 mm to 33.2 mm in one year. Wet meat forms 10.9 to 16.5% of the total weight. Length at first maturity is 11-15 mm. In the Cochin backwaters it spawns twice a year, from late May to August/September and January to March. In the Nethravathi estuary spawning is from December to March.

Tridacnid clams: In India no work has been done on these clams. The unique feature of the giant clams is their symbiotic relationship with the dinoflagellate algae, *zooxanthellae* in their mantle tissues. They retain the filter feeding habit and food is supplemented by the nutrients, gained from the photosynthesis of *zooxanthellae.* They mature as males at two or more years of age and latter develop female gonads also. The initial growth of the giant clams is slow and they reach 2-4 cm in shell length after a year. There after growth is rapid in larger species. Estimations of the life spans of giant clams have been speculative and some of them do live for a few decades. Giant clams are the only known auto tropic (in this case get their food by symbiotic association) farm animals known to man.

5. Clam Culture in India

The Central Marine Fisheries Research Institute has developed the technology for culture of the blood clam *Anadara granosa* in the Kakinada bay. Although complete package of technology including seed production under controlled conditions has been developed in the country for the blood clam *A. granosa* and the venerid clam *P. malabarica*, it is yet to be commercialized.

i) Site Selection

Clams are cultured on the bottom and therefore site selection depends on the substrate. The occurrence of natural clam populations is indicative of the suitability of the site with particular reference to the tide level, substratum and water salinity. Clam farms are located in estuaries, bays and other sheltered close to the shore. About 1-2 hrs exposure at low tide is desirable as it is easy to remove the predators.

Too long an exposure results in poor growth due to reduced feeding and in summer there may be mortality due to desiccation. Farms located further in sub tidal area have the disadvantage when predators are to be eradicated.

The type of substratum preferred varies with the species. For example, *M. casta* thrives well on sandy bottom, while *Anadara granosa* prefers mud flats. Also the salinity range tolerated differs between species. While *V. cyprinoides* prefers low saline waters few species tolerate prolonged low saline conditions which are generally prevalent in areas subjected to heavy rains and freshwater drain from the land. Clam farms are located in areas where there is little wave action. Areas prone to frequent changes of contour and vulnerable to pollution are avoided.

ii) Hatchery Production of Seed

Hatchery production of seed technology has been developed for *A. granosa*, *M. meretrix M. casta* and *P. malabarica*. In clams, spawning occurs both at elevated water temperature of about 34° C and also at the lower temperature of about 24°C on transfer to the conditioning room, after the thermal shock. Spat settlement takes place between 7th and 26th day after spawning in different clam species studied. The clam spat attain 2-3 mm in length in the hatchery in two months after fertilization and are transferred to the nursery. A survival rate 15-20% in spat production in the hatchery is considered as satisfactory. In the hatchery the micro algae *Isochrysis galbana* is given as food to the larvae and mixed micro algae, reared in outdoor tanks as food to the spat.

iii) Nursery Rearing

The 2-3 mm hatchery produced clam seed are transferred to 40 x 10 cm box type cages. These cages are covered with fine velon screen mesh and for additional protection against damage by crabs and fishes; a 10 cm mesh nylon fish net is stitched over the cage. The cage is suspended from racks in shallow calm waters. They are periodically cleaned of silt, predators and foulers, which enter the cages as larvae. In 6-8 weeks, the clams grow to about 10 mm in length and are ready for planting on the grow-out grounds.

Recently rearing of the hatchery produced spat of *P. malabarica* (2-3mm length) in 25x 25 mm nylon bags of 1-3 mm mesh at density of 1000 spat/bag and suspended from a rack in the Tuticorin bay gave highly encouraging results. This method is cost effective when compared to rearing in cages.

iv) Growout and Production

In the blood clams, *A. granosa* culture at Kakinada, seed clams of 21.8 -25.1 mm average length (5.53-7.08 g average wt) were stocked at 240-175/m. They attained 39.2 to 42.7 mm average length and 25.53 to 32.9 g average weight at harvest. The retrieval is 83.4% to88.6% when pen enclosures are used and 41.5% without pen. Production rates of 39.0-41.6t/ha/5.5 months are obtained when pen culture is practiced and 21 t/ha/6 months when pen is not used. Thus, both retrieval and production rates are reduced by about 50% in the blood culture if pen is not used. At a stocking density of 300/m the production is estimated at 70 t /ha with pen enclosure.

Growth of *M. casta* observed by Durve (1970) in the Marine Fish Farm of C.M.F.R.I., at Mandapam was 11.4 mm from 27.5 mm to 38.9 mm in 19 months at an average increase of 0.6 mm per month. The corresponding weight increase was 20.39 g from 7.51 g to 27.90 at an average of 1.10 g per month. He noted that growth was continuous, but there was slacking from May to September, when hyper saline conditions prevailed. During that time, the water in the farm was more or less stagnant resulting in poor quantity of phytoplankton food available for the clam. The period of slow growth also coincide with period of sexual activity. However, Even after prolonged resting, there was spurt in the growth when favorable conditions returned. The slow growth observed was attributed to the nature of the species, which is a true backwater clam and purely marine conditions found in the fish farm was not conducive to growth. In another experiment, he observed a growth of 14.4 mm in *M.casta* in 5 months from November to April at an average growth of 2.9 mm per month.

In the experiments conducted by Rao and Rao (1983) in pens at Mulki estuary, *M. casta* was stocked in three pens and *M. Meretrix* in one pen. Stocking density ranged from 60 to 250 seed per sq. m. for *M. casta* while it was 177 per sq. m. for *M, meretrix*. Seed size ranged from 6 mm to 28 mm in the case of *M casta* there was 6.5 mm growth in the mean size from January to June, at an average of 1.3 mm per month. The average weight of the clams increased from 5.29 g to 12.58g showing an increase of 7.29 g and the meat weight increased from 0.53 g to 2.52 g in the same period. Survival rate of the clams recorded was 80.5% of the stocking density.

In the case of *M. meretrix*, there was rapid growth with increase from the mean size of 23.6 mm to 34.0 mm, between January and March and later retarded to 35.6 mm and 37.5 mm during April and May respectively, thus showing overall increase of 13.9 mm in four months at an average of 3.5 mm growth per month. The average weight of the clam showed an increase of 12.30 g from 5.30 g in January to 17.60 g in May and meat weight showed a gain of 1.36 g from o.91 g to 2.31 g in the above period. Of the clams stocked, 75.5% survived till the end of observations in May.

Sreenivasan (1983 b) observed growth of *M. casta* in Vellar estuary to be from 7.5 mm to 41.4 mm in 13 months with a net increase of 34 mm at an average of 2.6 mm per month. Corresponding increase in weight was 31.07 g at an average of 2.39 g per month. Growth was slowed down in October-December, when low saline conditions prevailed in the estuary. Growth was fast during January-March, when there was rise in the salinity and temperature. Growth was moderate from April-September, during which months there was intense spawning activity by the clam in Vellar estuary. Growth of the transplanted clam was observed to be much faster than those in the natural bed.

In the later experiments, seed of *M. casta* transplanted in two pens, one below LTL and another above MTL at the rate of 1 kg/sq. m. There was differential growth among the seed grown over a period. Observed growth was 21 mm in 12 months in the pen below LTL, but was only 9 mm in 10 months in the pen above MTL. In a fully submerged multi-tier rack, the clam seed showed a

growth of 27 mm in 13 months. This clearly indicated that period of exposure and submergence play a major role in growth of the clams. Prolonged period of submergence helped the clam with long period of feeding since the clam is a continuously filer feeder and also reduced period of desiccation by exposure to air.

Growth of *M opima*, determined by transplantation experiment was 27.6 mm in 8 months from 5.6 mm to 33.2 mm during March- November. Growth rate of *M. opima* was observed to be comparatively faster than that of *M. casta* in Vellar estuary.

In a ranching experiment in Ashtamuddi Lake in Kerala, *P. malabarica* seed of 11.5 mm average length and 0.27 g average weight were stocked at 3566 nos/m. They attained an average length of 31.58 mm and 8.54 g average weight at harvest after 3.5 to 5.5 months. The retrieval was 7.5%. At Munambam, *P. malabarica* seed of 2.4 mm average length and 0.2 g average weight was stocked at 1500 nos./ m. After 4.5 months, they attained 34.6 mm average length and 9.05 g average weight. The retrieval was 17.64%. The production works out to be 1.5 to 2 kg/m.

6. Depuration, Processing, By - products and Utilization

Depuration: Clams like other filter feeding bivalve, accumulate pathogenic organisms in their body. By depuration the bacterial load is brought down to permissible levels; also faeces, sand particles and silt are removed from the alimentary canal. Clams are depurated in the same way as other bivalves. They are placed for 24 hrs in cleaning tanks under a flow of filtered seawater. About 10-20% of the seawater is continuously replaced. At the end of 12 hrs the water in the tank is drained and the clams are cleaned by a strong jet of water to remove the accumulated faeces. The tanks are again filled with water to remove the accumulated faeces. They are further held on filtered seawater for 12 hrs and for about one hr in 3 ppm chlorinated seawater, washed once again in filtered seawater before processing. In several countries, they are eaten raw and also steamed and eaten.

Processing: The various techniques followed in processing the clam meat are similar to those used for other bivalve mollusks. The clam meat is frozen as blocks or individual quick frozen, canned and smoked. Other products are clam juice, clam stripes, clam streaks, stuffed clams, clam pickle and chowder.

The adductor muscle is the valued part of the giant clam. In a 20 cm clam, it weighs about 500 g. Except the liver all parts of the soft body of the giant clams are eaten. The mantle of the giant clam is used to the Japanese salads, spaghetti, marinara, clam crackers and minced clam.

Byproducts and utilization: In clam culture, shell is the byproduct. It is used in the manufacture of cement, calcium carbide sand-lime bricks and lime. The shell lime is used for manuring coffee plantations, as a mortar in building constructions, in the treatment of effluents, as pesticide by mixing with copper sulphate and in glass, rayon, polyfibre, paper and sugar industries. The shells of several clams have ornamental value and are used in making curious. Truckloads of blood clamshells

are transported from Kakinada to southern Tamil Nadu districts for use in the shell craft industry.

Giant clamshells currently find a ready marked as decorative objects, trays, salad bowls and washbasins. *T. Squamosa* shells are most valued in this trade. Philippines is the center for shell craft industry.

Export Market for Clams: The export demand for clam meat has been increasing over the past few years, particularly from Japan, Western Europe and the USA. The clam meat export from India has increased from meager 371 t in 1989 to 900 t in 1993. In terns of value, almost fivefold increase has been recorded at Rs. 63.02 lakhs in 1989 to Rs, 292,25 lakhs in 1993.

7. Prospects and Constraints

The prospects for developing clam culture in India on commercial lines are very bright and the advantages are given below.

1. Clam feed low in the food web on detritus and phytoplankton and give high production per unit area. They are efficient converters of primary production into nutritious seafood, suitable for human consumption.

2. Clam culture is essentially a relaying practice of collecting the seed from high-density areas and stocking them in suitable grow out areas. The farm management involves periodic site inspection and eradication of predators: the technology is simple and easy for adoption by the farmers.

3. On bottom clam culture does not involve high labor or cost input.

4. Clam culture can easily be blended with capture fisheries and can be taken up as an income and employment generation programme in rural areas.

5. In the export market there is demand for some specious of clams only. From India there is insatiable demand for the frozen meat of *P. malabarica*. There are large tracts of derelict water bodies such as the Kakinada bay and they can be utilized for the culture of this species.

6. In clam culture fertilizers and feeds are not used and it is eco friendly. Clams are good biological filters and the introduction of clams in areas of high eutrophication such as shrimp ponds helps to reduce the pollution due to high load of suspended matter.

7. After the outbreak of shrimp disease in Taiwan, the farmers have switched over to culture of the clam *Mercenaria lusoria* in shrimp ponds for export to Japan. Similar practice can be followed in Andhra Pradesh and Tamil Nadu. Also fattening of the clams in shrimp ponds as followed in Thailand deserves merit.

Constraints

1. The major constraint for the large-scale propagation of clam culture in the country is the absence of laws to allot water bodies to prospective farmers.
2. Mapping of sites suitable for clam culture, based on species site interaction are needed for developing culture.
3. Consumption of clams is still localized; close to the production centers and only a small segment of the population take them as food. They still remain as non-conventional food. Vigorous extension drive is needed to make them popular.

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Giant Clam Culture

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Giant clams or tridacnids (Class Bivalvia: Family Tridacnidae) occur naturally in only the tropical or sub tropical marine waters of Indian and Pacific Oceans. However, they are confined to the western portion of the Pacific Ocean and do not occur on the western coast of the Americas or Hawaii. They are well adapted to tropical clear waters such as those that favor the growth of corals.

The giant clams are a traditional food source for the people of Indo-Pacific. The flesh of giant clams is eaten by many communities and the shells are used either as ornaments or for utilitarian purposes. In recent times, giant clams have become significant specimens for the aquarium trade. As a result over exploitation has led to the extermination of many of the local species. Thus, in the 1970s and 1980s research into the reproduction and larval culture of giant clams became important. The Micronesian Mariculture Demonstration Centre (MMDC) in Palau played a key role in developing and promoting mass culture of giant clams. The University of Papua New Guinea involved in giant clam larviculture. The Australian Centre for International Agricultural Research (ACIAR) funded giant clam project began with Australia, Fiji, Philippines and Papua New Guinea in the mid 1980s and in the late 1980s in included Tonga, Cook Islands, Kiribati and Tuvalu.. The International Centre for Living Aquatic Resources Management (ICLARM) also in the late 1980s set up a coastal aquaculture centre near Honiara, Solomon Islands for giant clams. Other hatcheries and Ocean nurseries have been started notable in Micronesia and Tonga and Cook islands.

Species

There are eight extant species of giant clam (Family Tridacnidae) within two genera:

Tridacna gigas (Linnaeus 1758): The true Giant clam is the largest extant bivalve and attains weights of over 200 Kg of which 55-65 Kg is living tissue; reaches 1370 mm in length; white fan shaped with deep radiating ribs.

Tridacna derasa (Roding 1819): The smooth shell giant clam or the Southern clam is the second largest tridacnid, reaches about 500 mm; low primary and radial sculpture, variable shape, massive umbonal area, smooth white shell.

Tridacna squamosa (Lamark1819): the fluted or scaly clam, reaches about 400 mm; elongate shell with conspicuous fluted scales on its radial ridges, valves white and occasionally tinged with orange; mantle yellowish green.

Tridacna maxima (Roding 1798): The rugose or the small giant clam, partially burrowing species, reaches 200 mm; mantle color highly variable, from blue to brown.

Tridacna crocea (Lamark1819): The boring or crocus clam is the smallest of the tridacnids, reaches 150 mm; valves grayish, white often fringed with orange or yellow both inside and outside, triangularly ovate; mantle predominantly blue but shows great variability.
Tridacna tevoroa: The deep water devil clam, a rare species lives in the deep water (20-30 +m) habitat in the eastern Fiji islandsand northern Tonga islands; only recently described.
Hippopus hippopus (Linnaeus 1758): The horse's hoof, bear paw or strawberry clam, reaches approximately 400 mm in length; valves thick, heavy, triangular in shape, often covered with reddish spots and obscured by encrustations; mantle deep yellow-green, irregularly mottled at the periphery and in the centre.
Hippopus porcellanus (Rosewater 1982): The China clam recently described species; shell thinner and smoother than *Hippopus hippopus*, no pigmentation, more semi circular; mantle similar to *Hippopus hippopus.*
The larger giant clams are listed by the International Union for Conservation of Nature (IUCN) as threatened species.

Special Features of the Giant Clams

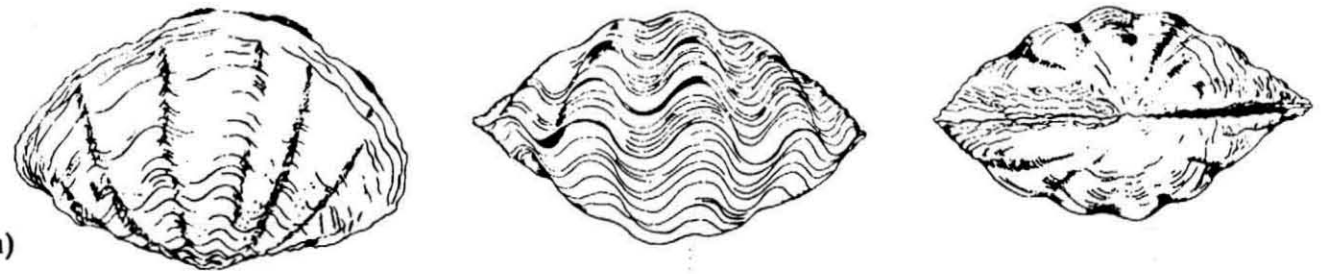
All the species are limited in their distribution to the shallow, sunlit waters of Indo-Pacific coral reefs. Giant clams have solved the problem of poor plankton availability in Oceanic waters by "farming" enormous numbers of dinoflagellate *Symbiodinium microadriaticum* in the inter haemal sinuses of their enlarged siphonal tissues. The algal symbionts, zooxanthellae are probably responsible for the larger sizes of these giant clams. The ideal features for the Mari culture of these species are therefore, the self feeding capability and photo trophy, rapid growth rates and high market value.

All tridacnids form byssal attachments to the reef early in life. As in other bivalves, the giant clam byssus serves to prevent physical displacement from the substrate. Its important function, however is to maintain the clam in an upright position, ensuring a favorable orientation to sunlight. The larger giant clams (*T. gigas, T derasa, T squamosa* and *H. hippopus*) eventually lose their byssus, presumably because the weight of the valves provide sufficient ballast to prevent displacement and to maintain an upright position. The smaller species *T. maxima* and *T crocea* remain strongly byssate throughout life actively burrowing via mechanical and chemical means into coral substrates.

Culture

Early attempts to culture giant clams were by several groups simultaneously; Stephenson (1934) in Australia, Rosewater (1965) and Hardy and Hardy (1969) in Palau. However, these were unsuccessful. Wada (1954) conducted extensive spawning trials on Palauan tridacnids. La barbera (1975) could carry out the first successful culture of *T maxima* in Fiji. Jameson (1976) reared *T crocea,, T maxima* and *H. hippopus* in the Palau and guan islands. Murakoshi (1978) reared T crocea in Okinawa. Since then larval rearing and grow out culture of giant clams are been carried out in many of the Indo Pacific islands. Methods for culturing gianrt clams continue to evolve and a wide range of production techniques is now available.

T. gigas shell (shell length 35cm)



T. derasa shell (shell length 20cm)

T. squamosa shell (shell length 17cm)

T. maxima shell (shell length 18cm)

Figure 1 Lateral, dorsal and ventral views of the shell valves of *T. gigas*, *T. derasa*, *T. squamosa* and *T. maxima*.

Present Hatchery Techniques

In giant clams, four phases are involved.

i) Hatchery phase: Rearing the larvae from eggs in indoor/outdoor tanks. Six of the eight known species have been successfully spawned in Philippines by injecting serotonin in to the gonad of mature clams and also by introduction of macerated gonad materials into the mantle cavity through exhalent siphons.
The larval development stages are similar to those in other clams and settlement takes place in about 7-10 days after spawning. *Isochrysis galbana* is fed to the larvae. The spat attach with the byssus but they may break attachment and creep along the substrate. At this stage the symbiosis with Zooxanthellae is established. The larval rearing is done in both indoor and outdoor tanks.
Culture is also done in raceways containing fiberglass shellfish rearing trays wherein seed clams of 10 mm size are counted and redistributed.

ii) Nursery phase: Rearing juvenile clams in onshore tanks for metamorphosis (0.2mm) to about nine months of age and 20+ mm shell length (seed clams).
The tanks are provided with flow of raw seawater. The clams acquire the zooxanthellae from the seawater in about 3 weeks after fertilization and they become increasingly autotrophic.

iii) Ocean nursery phase: Rearing juvenile clams in protective containers (ocean nursery trays) in the field from about 20 mm shell length to 200 + mm shell length. (8-9 months post fertilization stage to 2 years.
The trays are free standing modular units with 60 mm clearance above the substrate and a 25 mm mesh polyethylene lid. Each tray is filled with 5-10 kg of basalt chips, which provide ballast as well as serve as substrate for byssal attachment.
Stocking densities of 100/tray for 30 mm and 24/tray for 120 mm size seed clams are adopted. The mesh lids exclude predators, the muricid gastropod *Cymatium muricinum* that causes extensive damage by crawling into the byssal orifice and feeding on the soft tissues.

iv) Grow out phase: Rearing clams of 200+ mm shell length without protection in the field to market size of 250 mm which takes as long as 2-3 years. No care is required and survival is over 90%.

Reef seeding: Tridacnid juveniles survive minimum of 24 hrs out of water and large numbers can be air shipped inexpensively over large distances and for reef seeding purposes.

There are many variations to the techniques for the production of clams. However, three different methods of culture of giant clams may be recognized based on the degrees of reliance on land based and ocean based operation (see schematic diagram). Based on the hatchery/ nursery phases, using any of the three methods, three methods of Giant clam larval culture are recognized.

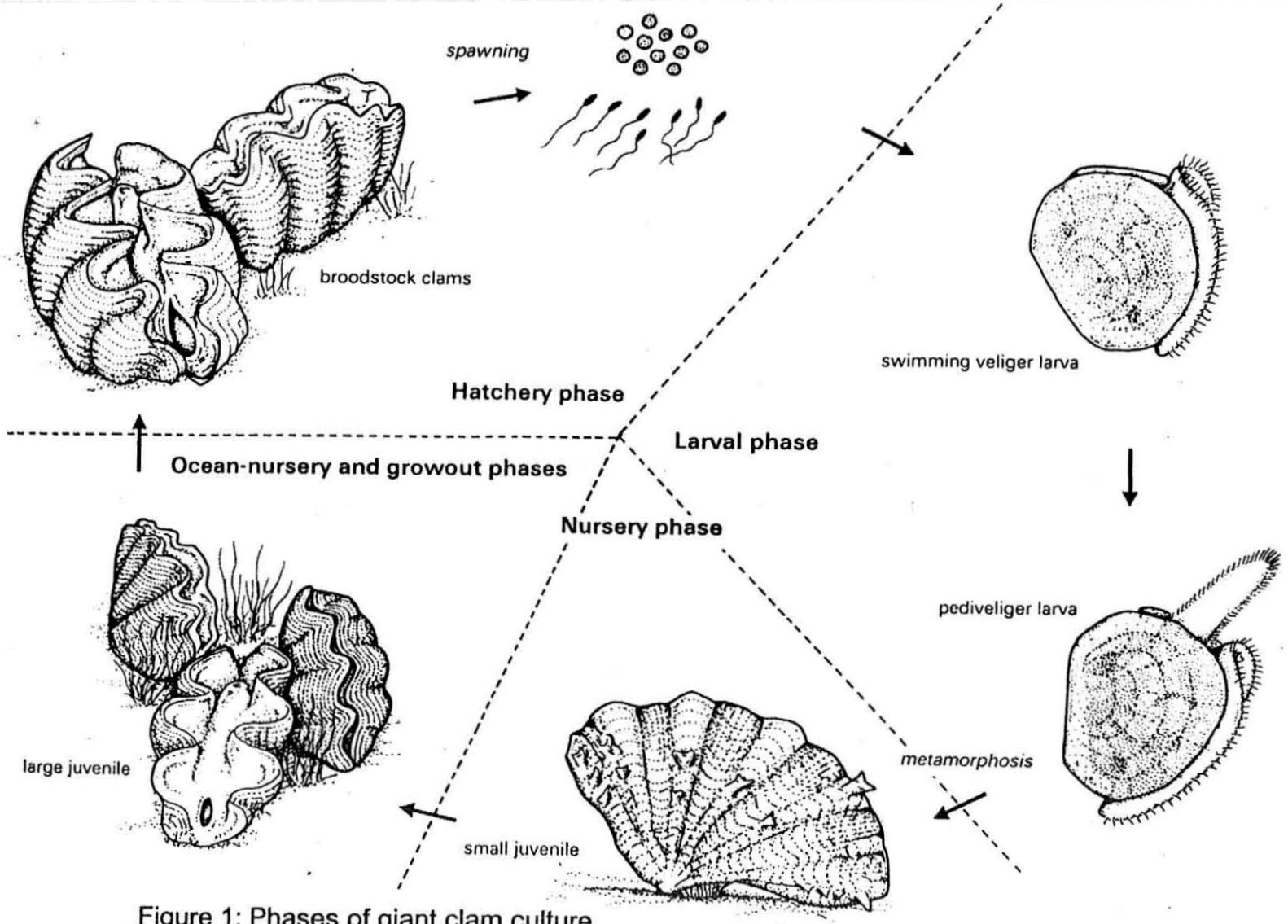
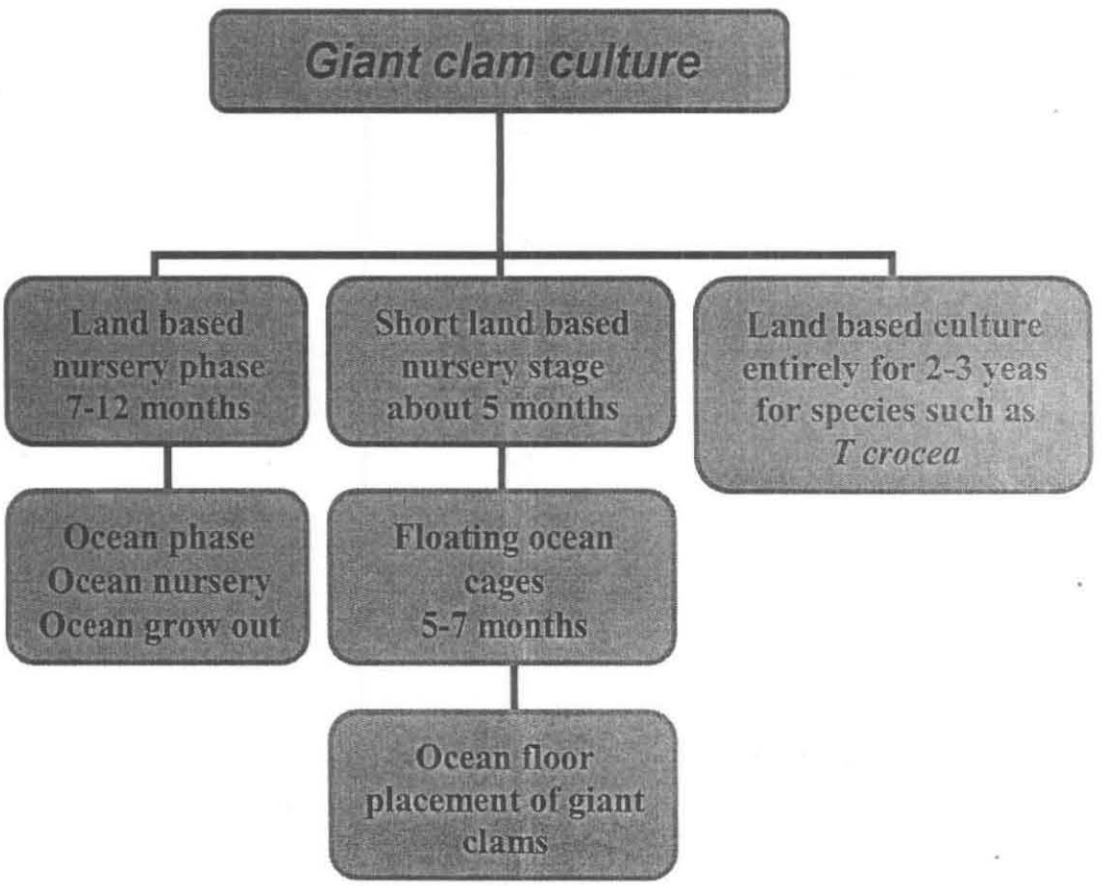


Figure 1: Phases of giant clam culture

a) Extensive: Fertilized eggs added to sea water which has been allowed to develop a local phytoplankton bloom (3000-10000 liter tanks)
b) Semi-extensive: swimming larvae stocked and fed cultured unicellular algae (3000-10000 liter tanks)
c) Intensive: selected swimming D – stage veligers stocked into 500-2000 liter tanks and fed unicellular algae, later released to settlement/nursery tanks.



Three different methods of giant clam culture involving different degrees of reliance based on land based and ocean based operations.

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20 Mussel Farming Methods and Seed Collection

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Introduction

Green mussel, *Perna viridis* and brown mussel, *Perna indica* are the two candidate species available along the Indian coasts and Andaman and Nicobar Islands.

Mussels are sedentary animals growing attached to hard substrates by means of byssus. They can be transplanted from their natural habitats to any artificial hard objects. This unique character of mussels is taken advantage of culturing these animals. Mussel culture is being practiced in France since the beginning of thirteenth century. From here it has spread to other countries like Spain, Holland, Belgium, Italy, Philippines, U.S.A, Australia and New Zealand, where different techniques are adopted depending on the hydrographic, social and economic conditions.

In terms of production and consumption, the European Union plays a dominant role with a production of 500,000 tonnes / year. Spain is the largest producer among EU countries followed by Netherlands, Italy, France and New Zealand. In Asia, China is the larger producer of mussel. In India the total production during 2002 from captures and culture fishery of mussels amounts to 15,066 tonnes. Taking Kerala as a model the mussel culture has been taken up by other coastal states of India.

In India mussel culture has been introduced only very recently. In 1971 The Central Marine Fisheries Research Institute, initiated culture of brown mussels at Vizhinjam Bay and later successful experiments were conducted at Kozhikode and Chennai in 1975, to study the possibilities of culturing mussels in the open sea. Backwater culture was started in Padanna (Kasargod district) in 1996. From a production of 20 t in 1996, the culture production increased to 1350 t in 2002 by mussel faming in the estuaries of Kerala. Karnataka and Maharashtra have also initiated mussel farming from 2002 onwards.

Distribution

Approximately 17 species of edible mussels are harvested or cultured worldwide. The blue mussels, *Mytilus edulis* and *M. galloprovincialis*, are the most common species in Spain. China, have *M.edulis*, black mussel *M.crasitesta* and green mussel *M.smaragdinus* are found in China, which tops in mussel production in the world. Srilanka, Singapore, Thailand, Philippines, Indonesia, Malaysia, Burma and Fiji have the green mussel *Perna viridis*. In New Zealand large, green-lipped mussels, known as green shell mussel is cultured. In California

M.californianus is cultured. The brown mussel *P. Perna* is available in Srilanka, which is the same as that found in the India region. In India green mussel *Perna viridis* and the brown mussel *Perna indica* are cultured.

Green mussel is widely distributed along the intertidal coasts of India and found extensively around Kollam, Alappuzha, Kochi, Kozhikode, Kannur and Kasargod in Kerala and in small beds in Chilka lake,Orissa, Vishakhapatnam, Kakinada Chennai, Pondichery, Cudalloor, Mangalore, Karwar, Goa, Ratnagiri and in Gulf of Kutch and also in Andaman and Nicobar Islands. *Perna indica* has a restricted distribution and is found along the southwest coast from Varkala to Kanyakumari and on southeast coast from Kanyakumari to Tiruchendur.

Mussel Farming

Sites for mussel farming has been identified in Kerala, Karnataka, Maharashtra Goa, Tamil Nadu, Andhra Pradesh, Pondichery, Gujarat, Orissa and Andaman and Nicobar islands. The seed availability and environmental conditions plays a critical role in mussel farming.

Seed Collection

Success of mussel farming depends on the availability of good quality seed. In areas where natural seeds are available is considered as the primary source of seed. Though the technology for hatchery production of seed has been studied, it is not economically viable. Seed collection requires accurate forecasting of spat fall. Various types of materials are used as spat collectors namely tiles, ropes, asbestos, shading material, frilled polypropylene rope etc. Selection of spat collector depends on the efficiency, local availability and durability of the material and initial cost of investment. Commonly polypropylene ropes are used for seeding of mussel. In Kerala, spat settlement is observed from July to August. During October this settled spat attains a length of 20-25mm size and weighs 1.5-2.0gm.

Seeding Method

Seeds collected from the submerged (sub tidal) areas will be healthier. After removing other organisms and weeds, the seeds may be washed thoroughly in

seawater. About 1000gm of seed is required for seeding on one-meter length rope of 12-14mm or 15-20mm. Cotton mosquito netting is used for enclosing the seed to the rope. The cloth will disintegrate within 2-3 days. By this time the seeds will secrete byssus thread and will get attached to the rope. Duration of mussel farming is 4-6months. Mussels attain 80-88mm size by 5months with an average weight of 36 - 40g and an average production of 10 - 12 kg/m of rope is obtained.

Site Selection

Open sea and estuarine areas free from strong wave action may be selected for farming. Clear sea water with high plankton production (17-40µg chlorophyll /l) is ideal for mussel culture. Moderate water current (0.17-0.25m/s at flood tide and 0.25-0.35m/s at ebb tide) will bring the required planktonic food and will carry away the excessive build-up of pseudofaeces and silt in the culture area. The water should have a salinity of 27-35 ppt and temperature of 26 – 32°C. Site selected should be free from domestic and industrial sewage. In shallow waters, sea and estuaries rack and stake (Bouchot) method can be adopted. For deeper regions, raft or long line method is ideal.

Open Sea Farming

Open sea farming is practiced in areas with depth of 5-20m. The area of culture should be free of strong wave action, less turbulent and with high productivity. Long line, and raft culture techniques are ideal for sea farming. Disadvantages of this type of farming are poaching, unpredicted climatic changes and predation.

Estuarine Farming

Compared to open sea, the estuarine ecosystems are less turbulent and shallow (<4m). Stake and rack culture (horizontal and vertical) are ideal for estuarine conditions. Fluctuation in salinity during monsoon season and pollution through domestic and industrial waste are the main constraints in estuarine mussel farming. On - bottom culture by relaying of mussel seed in pen enclosures is also practiced.

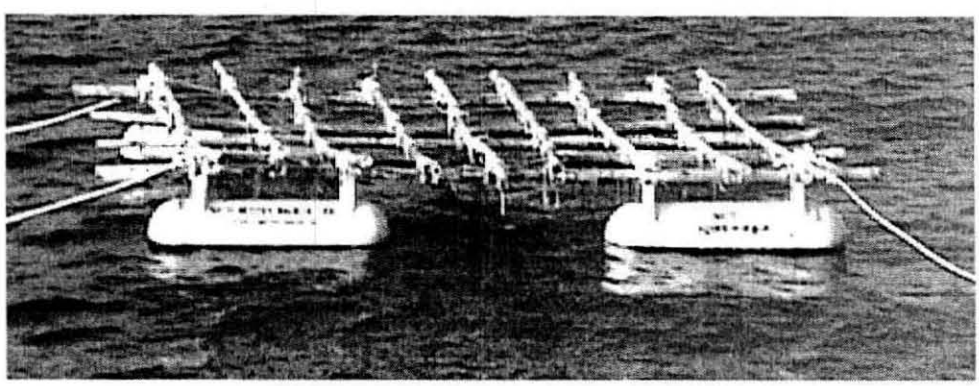
Culture Methods

Culture methods are divided into two categories viz. bottom and off bottom culture. In bottom culture the mussel seeds are relayed and left to grow until harvest. Off-bottom culture comprises of providing a structure to which the mussel attaches by byssal threads. In this method ropes or other materials are used as substratum for the mussel to settle and grow. Types of off-bottom culture are:

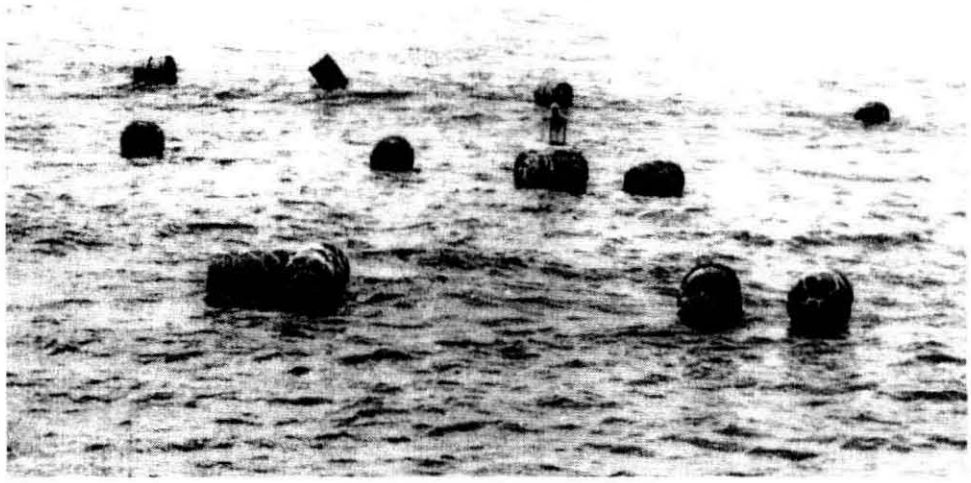
Rack method: Suitable for estuaries and shallow seas. Bamboo or Casuarina poles are driven into the bottom spaced 1-2m apart to form a lattice network of frame. Seeded ropes are suspended from these frames. A modified version of off-bottom culture is the horizontal rope culture method where the seeded ropes are suspended horizontally. This method is practiced in shallow waters where the depth is <1m.

Due to the effective utilization of the productive column water this type of culture gives better yield.

Raft method: Ideal for open sea conditions, which are not rough. Square or rectangular rafts are made with sturdy bamboo or casuarina poles. Buoyancy for the raft is provided by tying together 5 barrels of 200 liter capacity (metal oil barrel painted with anticorrosive paint or synthetic barrel). Ideal size of the raft is 5 x 5 m. The rafts are to be positioned at suitable site in the sea using anchors (grapnel, granite, concrete).



Long-line method: Considered ideal for unprotected open sea conditions. Synthetic rope of 16-20mm diameter is used for the long-line (main line). The main line is supported with 220 litre barrels tied to it, spaced at 5m. The seeded ropes are suspended from the main line 1.5-2m apart. The long –lines and barrels are anchored in position at either ends using concrete blocks and nylon ropes or metal chain.



Stake / "Bouchot" culture: Culturing mussel on stakes is carried out extensively in the intertidal mud flats along the Atlantic coast. Initially rows of poles are placed in the intertidal region to allow mussel spat to settle and grow. When the spat grows slightly bigger they are transferred to " bouchot " placed in shallow waters in the same region. The mussels attain marketable size on the poles. Periodical thinning of mussel is necessary to prevent overcrowding

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Innovations in Increase in Mussel Farming

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The Central Marine Fisheries Research Institute (CMFRI) by setting up demonstration farms and conducting training programmes to fishers was able to popularize bivalve farming in Kerala State. Simultaneously, through interactions with the State government officials and members of local governing bodies, project proposals on oyster and mussel farming were recognized as financially viable schemes for rural development and self-employment. Since 1995, edible bivalves like the Indian backwater oyster, *Crassostrea madrasensis* and the green mussel, *Perna viridis* are farmed on a commercial scale in the estuaries of Kerala. Along the Malabar Coast, especially in the estuaries of Kozhikode, Malappuram and Kasaragod districts mussel farming (by rope culture from racks) is now a popular seasonal vocation through which about 2500 tonnes of mussels are produced annually.

Extraordinary growth performance, natural abundance, adaptability to new environments and fairly simple culture techniques make mussels of the genus *Perna* an ideal shellfish crop in many Asian countries (Vakily, 1989). Although the profitability of mussel farming operations in Indian waters can be high (Qasim *et al.*, 1977; Mohamed *et al.*, 1998; Asokan *et al.*, 2001), the returns obtained by farmers largely depend on the input costs. The rope method are also affected by losses (varying from 30 to 80%) due to fall-out or slipping of seeded mussels from ropes. Further, it has been reported (Velayudhan *et al.*, 2000, Asokan *et al.*, 2001) that the polyethylene rope used for seeding accounts for almost 40% of the investment cost. Presently the seeding method involves placing the mussel rope on biodegradable cotton netting, uniformly distributing the mussel seed and then manually stitching the netting around the rope and mussel seed. This process is labour intensive and seeding costs are estimated to range from 15-20% of the operating cost. The mussel tubing socks, widely used in Europe, have not been tried in India. The possibility of using alternate seeding material and methods to reduce costs due to seeding on polyethylene ropes has been studied. The quality of the alternate seeding material and method were assessed in terms of growth, production and percentage fall-out of mussels from the rope.

1. Use of Pre-stitched Mussel Tubing

Results of the study carried out by Mohamed et al., (2003) are presented here. New methods were attempted by transplanting *Perna viridis* (L.) seed to 12 mm diameter polyethylene ropes on which biodegradable cotton net was wrapped and stitched together (control), 12 mm frilled polyethylene rope (Fuzzy™) with white fully degradable tubing socks (FuW) and grey semi-degradable synthetic

tubing socks (FuG) and 5 cm broad flexible plastic strips (FPS) kept inside pre-stitched biodegradable cotton net. The treatments, which were replicated, were suspended from a fixed rack in a shallow tropical estuary (Ashtamudi Lake). The specific growth rate (SGR) in length and weight, fallout percentage and production in different treatments were compared. There was no significant difference in the SGR in length and weight, while the fallout percentage was significantly ($P<0.05$) lower and production significantly ($P<0.05$) higher in FPS and control treatments. Since the FPS and control treatments did not show any difference in terms of growth and production, the economic performance of these two methods were compared. The economic analysis indicated that the use of FPS together with pre-stitched biodegradable cotton net reduced the investment costs by 34% and increased the rate of return by 48% over that of the control.

Table 1. Comparative economics of mussel farming by existing and improved methods. Area – 0.0025 ha; 100 seeded ropes of 1 m length, cultivation period - five months (All amounts in Indian Rupees).

	Criteria	**Existing method**	**Improved method**
A	Investment		
	Bamboo poles – 15 nos	1500	1500
	Polyethylene rope for seeding	1020	-
	Others	500	500
	TOTAL	3020	2000
B	Annual Fixed Costs		
	Interest (@ 15% per annum)	453	300
	Depreciation		
	1. Bamboo poles (50% per annum)	750	750
	2. Polyethylene rope (50% per annum)	510	-
	3. Others (50% per annum)	250	250
	TOTAL	1963	1300
C	Operating Costs		
	Annual lease	500	500
	Labour for rack construction	750	750
	Biodegradable cotton netting	750	750
	Mussel seed – 150 kg	900	900
	Flexible plastic strip	-	200
	Canoe hire charges	750	750
	Labour for seeding	1200	700
	Harvesting and marketing	1500	1500
	TOTAL	6350	6050
D	Total Cost (B + C)	8313	7350
E	Production (kg)	1260	1150
F	Income @ Rs.8/kg	10080	9200
G	Net Operating Income (F-C)	3730	3150
H	Net Profit (F-D)	1767	1850
I	Break-even price –Rs./kg (D/E)	6.6	6.4
J	Rate of Return (%)	73	108

The advantage in FPS treatment was the ease of filling up the pre-stitched cotton biodegradable tubes with mussel seed as compared to the manual drudgery of stitching. This directly resulted in halving of the labour cost involved in seeding. Results of the economic analysis indicate that by using the improved method, marked gains (by 48%) could be made in the rate of return. The polyethylene ropes used presently is 10 times more expensive than FPS and this study has shown that there is no significant difference in the production and fallout percentage due to its use. Although the FPS is a 'use and throw' type of material, its life could be extended by another year through careful use. The use of FPS as seeding substrate and pre-stitched cotton biodegradable net tubes can therefore be recommended for use to estuarine mussel farmers. Furthermore, use of pre-stitched tubes opens up the method for mechanisation of the seeding process.

2. Development of Semi-automatic Mussel Seeder

Seeding is one of the most critical activities in mussel farming. The process which is physically demanding (as farmers have to kneel and bend down to do it) is crucial to the success of farming as the uniform attachment of mussel seed around the rope is dependant on how well it is done. Now, to reduce the physical strain and to increase efficiency during this process, a semi automated mussel seeder has been designed and developed.

The seeder was field tested at the CMFRI's demonstration mussel farm in the Ashtamudi Lake in Kollam district, Kerala. The efficiency of the seeder was evaluated by comparing the time taken for seeding 1 m length using the conventional method and the seeder. The uniformity of attachment of mussel seeds around the central core material was judged by visual examination after 1 week when the mussel seeds were attached. The seeder (Fig.1) made from quality hardwood consists of the following parts.

PVC pipe: PVC pipes of 1m lengths are for providing rigidity to the pre stitched cotton tubing during seeding. The diameter of the pipe is decided based on the size of the mussel seed. For smaller seed of length 20-25 mm PVC pipe of 6 cm diameter and for 25-30 mm seed 7.5 cm diameter pipes have to be used. Aluminum couplings of appropriate diameter are used to hold the PVC pipes to the seed holder.

Mussel Seed Holder: A wooden rectangular basin (75 x 50 x 6 cm) with two circular openings of 9 cm diameter, which are spaced 16 cm apart, is used for placing the mussel seed. The circular openings are for holding the top part of the PVC pipe. To hold these pipes tightly, detachable aluminum couplings are used. Two hooks are provided on the wider side of the seed holder diametrically opposite the circular opening.

Base plate: The base plate (75 x 50 cm) is a wooden board for supporting the lower end of the PVC pipe. It has also two elongated slits of length 25 cm and width 1.5 cm through which the lower end of the core material can be passed and locked. A semicircular girdle with height of 2 cm outside the elongated slit prevents tilting of the PVC pipe.

Vertical support: The seeder is held together with the help of vertical supports. The two legs on each side are joined horizontally on top and bottom. The seed holder rests on this. The base plate is bolted to the bottom portion of the legs. The height between the seed holder and the base plate is 1m so that the PVC pipe can be inserted between these and held tightly.

Top Stand: The top wooden stand of height 105 cm from the seed holder consists of two vertical poles connected by a horizontal pole which can be fixed to the sides of the mussel seed holder. The horizontal pole is provided with two metal rings, which are aligned to the center of the circular opening on the seed holder. The rings on the top stand, opening in the mussel seed holder and the end of the elongated slit on the base plate are aligned so that the core material can be held vertically in the center of the PVC pipe.

All the above-mentioned 5 parts can be easily assembled within 5 minutes at the farm site with the help of nuts and bolts and are detachable making the seeder a portable unit. The cost of a single unit of mussel seeder made of Mahogany wood is Rs.2500.

Although 12 mm diameter nylon ropes are conventionally used as core material, the use of 5 cm width flexible plastic strips (FPS), which are used to make camp cots, and chairs has been recommended as a cheaper and durable substitute for seeding[2]. FPS is commercially available as 100 m rolls.

Items necessary for seeding are the core material such as FPS and the pre-stitched cotton tubes. The pre-stitched tubes are prepared from biodegradable cloth (e.g. cotton mosquito net) that are cut into required length (1.25 m) and width (slightly larger than outer width of the PVC tube) and machine stitched together longitudinally. These are kept ready before the seeding process is initiated.

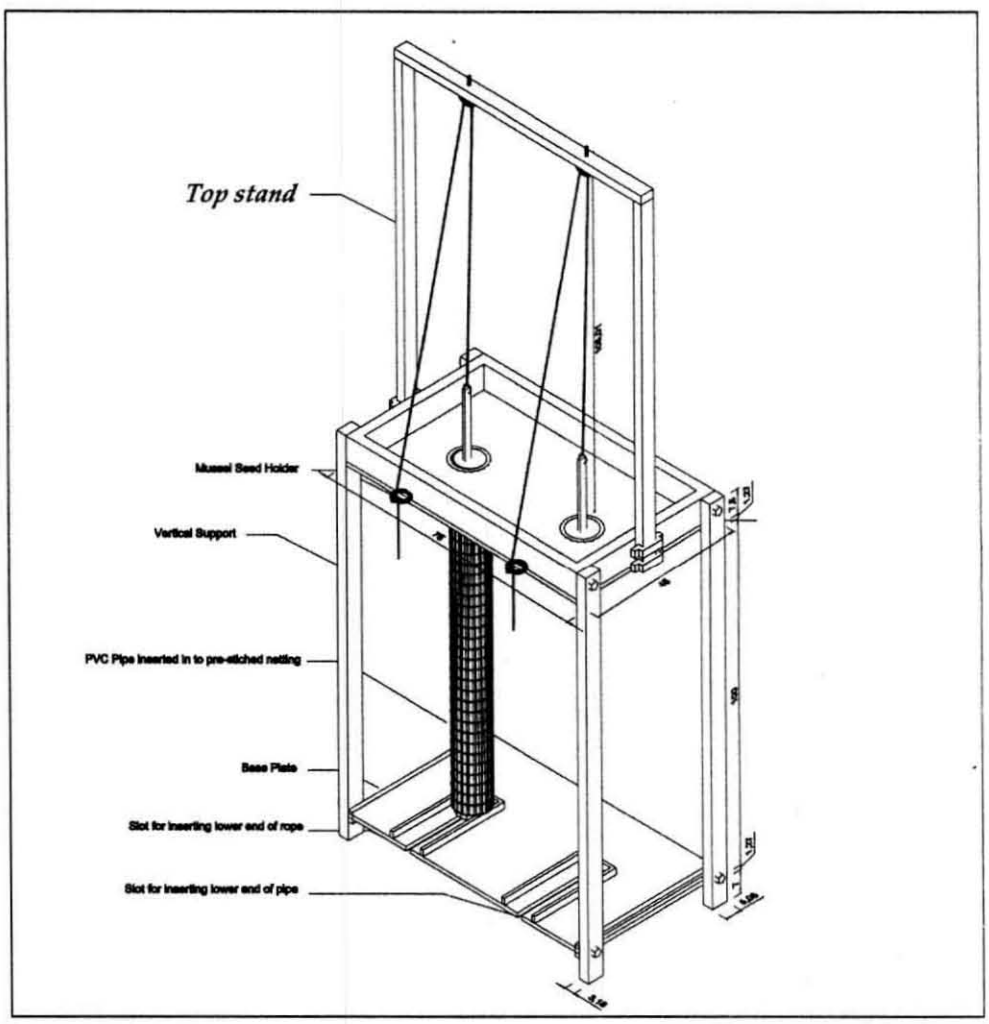
The pre-stitched tube is first pulled over the PVC pipe and the FPS is passed through the PVC pipe. The lower end of the pipe is closed by tying the pre stitched tube and the FPS. Then the PVC pipe covered by the pre-stitched tube is inserted into the mussel seeder between the mussel seed holder and the base plate. The lower knotted end is slid under the elongated slit and the knot holds the PVC pipe and the pre-stitched tube in position. The upper end of the PVC pipe is aligned to the circular opening on the mussel seeder and is held in position by the coupling.

The upper end of the FPS is tied to a 3 mm nylon rope, which is passed through the ring on the top stand and tightly tied to the hook provided on the side of the mussel seed holder. Cleaned, separated and sorted mussel seeds are placed in the seed holder from where the seed can be slipped into the pipe. When it is filled to the brim, the PVC pipe alone is lifted up slowly until it is above the seed holder. Then the knot on the hook is loosened enabling PVC pipe to be slipped out. Finally, the seeded mussel tubing can be easily slid out of the seeder and knotted at the top. These tubes can be stocked immediately in the farm. If depth is more and if

horizontal method of stocking is followed, then these can be joined by tying the ends to one another to get the required length.

The relative advantages of the developed mussel seeder in terms of time taken to do the seeding, uniformity of attachment of mussel seeds and relative physical exertion are given in Table 2.

After successful field trials, the seeder was demonstrated to mussel farmers and panchayat officials at Korapuzha in Kozhikode district and Vallikunnu in



Malappuram district in North Kerala. The response of the farmers was graded as good considering the advantage of reduction in time taken for seeding and the resulting decrease in expenditure on labour. Farmers were of the opinion that the seeder can be used as a common facility by all mussel farmers in a village unit. The village panchayat officials have included the seeder in the subsidy component given to mussel farmers.

Table 2. Advantages of the mussel seeder

Innovation	Use	Advantages
Mussel seeder	Semi-automation of the process of filling the seed – seeding	**Reduction in labour and time**. The time taken for manual stitching of 1m rope by the conventional method is 8 minutes whereas in the seeder the same can be accomplished in 2 minutes. **Uniformity in attachment of mussel seeds around the FPS**. Visual examination revealed that mussel seeds were more evenly attached around the FPS than in the conventional method. **Reduction in physical exertion** – In the new method the seeding can be done easily without kneeling or bending for long durations thereby reducing or completely eliminating the physical stress. This is especially advantageous for women who mostly do the seeding work.

3. Cost Reduction in Rack Structure

The constant replacement of bamboo or casurina poles used for fabrication of grow out structure due to fouling and boring is the main recurring expenditure in bivalve farming using the rack method in estuaries. PVC poles of 2-inch diameter filled with concrete were used instead of bamboo poles in 1997 (Kripa et al., 2001). These have withstood 3 seasons without any fouling / boring or natural degradation. Though the capital investment in the first year is high, continuous replacement / maintenance work of farm owing to collapse of farm structure on account of natural calamities like strong wind or rain can be avoided.

4. Integrated Culture of Finfish in Bivalve Farms

To utilize the space in between the rack farm and as a means of improving the profit, integrated farming of finfish together with bivalves was attempted (Kripa et al., 2001). Two nylon net cages (1.3 x 1.3 x 1.5 m, 1.5 cm mesh) were tied to the vertical poles in the rack farm and stocked with young ones of the pearl spot *Etroplus suratensis*, which is a favoured food fish of the region.

The mean seed size was 6.6 cm (6.8 g) and the stocking density was 22/m^2. The fishes were fed with dried clam meat and pellet feed through a feeding tray at 5% of body weight. The growth observed was good and there was 100% survival. The average growth was estimated as 10.3 mm/ month, which is considerably more than that observed for this fish in pond culture (CIBA, 1995). The average production obtained was 1.6 kg/ m^2 from an initial 0.15 kg/ m^2 within less than 8 months.

Since pearl spot fetches a high price (Rs. 70/kg) in the local markets, it is clear that cage farming of quality food fishes in estuarine bivalve rack farms would form a significant source of additional income to farmers.

Suggested Reading

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Mussel Farming as a Village Linked Programme

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Introduction

Mussel culture was started in the early seventies but this could not be popularised, as these were operation limited to the open sea with raft culture. Such activities could be done only with the help of fishermen and a large scale operation had logistic problems too. With the success of mussel culture in the backwaters, the practice has been continued for the past seven years. The simple methods employed for mussel farming was transferred to progressive farmers who took up mussel culture in the backwaters. Soon they found the venture profitable as unlike the raft culture the cost of production was low and the risk of losing the raft to inclement weather was not there. Demands came from new entrepreneurs for training and mussel farming spread from Kasaragod to Ponnani. From 250 tonnes of mussels harvested in 2000, the total harvest estimated for the year 2004 is 2500 tonnes.The growth has been ten times.

Mussel culture in the backwaters of Kerala was first started in Padanna and Cheruvattur Panchayats in Hosdurg Taluk of Kasaragod district. Later it was taken to Elathur in Calicut district and Vallikunnu and Ponnani in Malappuram district. This has happened mainly due to the popularisation efforts by the CMFR Institute. This year the Padanna farmers go for the eight harvests.

Initially this low cost technology of farming was transferred to five groups with 15 to 21 members at Cheruvattur and Valiyaparamba. Financial assistance was provided by the North Malabar Gramin Bank and Cheruvattur Farmers Co-operative Bank. They provided a loan of Rs.2,60,200/= for the implementation of the project with a subsidy component of 50% subsidy. These groups harvested 67.4 tonnes of mussels during May-June 1997. A portion of the harvested and shucked meat (2000-Kg) was sold to the Integrated Fisheries Project, Cochin at a rate Rs.45 per Kg. The remaining harvest was sold in the domestic market. The groups could realise Rs.3, 34,555/= from the harvest with a net profit of Rs.1,04,455/= within a period of 6 months.

Major Areas of Mussel Culture

Kasaragod District

The Cheruvattur panchayat has an area of 18.37 sq km with a population of 24,504 of which 18.631 are literate. About 150 families are engaged in fishing activities and about 300 families are engaged in ancilliary activities. Padanna panchayat has an area of 13.08 sq km with a population of 17,961 out of which

12,746 are literate. About 200 families are engaged in fishing as main activity and about 400 families as part time occupation.

The culture is done in the Padanna backwater systems in the Hosdurg Taluk. In Cheruvattur Panchayat, five groups were formed at Koyambram, Kavunchira, Kayuthakadu and Paranthamadu and in Padanna panchayat three groups were formed at Badkekad, Ori and Thekkekadu. At Padanna it was individuals who have done mussel culture. At Koyambram and Paranthamadu, there are 13 members in each group and at Kavunchira and Kayuthakadu 15 members each in the groups.

Malappuram District

TTC (Trainers training centre) training was given to 45 trainees at Vallikunnu panchayat during September1999 by CMFRI. Subsequently, during January 2001, training was imparted to 60 trainees of Malappuram district under the self-help group (SHG) training programme of the State fisheries Department. This training was conducted at Balathurithi. These trainees did mussel culture in Vallikunnu and Puthuponnani. TTC training was given to 20 trainees at Puthuponnani. They have taken up mussel farming under the auspecies of the Organisation named 'Youth power'. At Vallikunnu panchayat in Malappuram district, farming is done in the Kadalundi estuarine system. The total production from this area during this year is estimated to be 150 tonnes. Most of the units are done by groups.

Here the panchayat took the initiative in taking up mussel culture activity. A subsidy of Rs. 1200/= was given to each person of the group by the Panchayat. The total production by the trainees was 5 tonnes. This was the first time that a commercial scale operation was done at Vallikunnu. The markets were the nearby areas of Calicut and Malappuram district at Kotta, Althani, Kottakadavu, Kootimadu and Manavala.

Kozhikode District

Mussel culture is being done in the Korapuzha estuarine system. Training was imparted to 20 persons under the self-help group (SHG) training programme of the State fisheries Department. Initially at Elathur three units were doing mussel culture. Two units by individuals and the third by a group of 10 members. Now the ADAK (Agency for development of aquaculture Kerala) started giving subsidies for the past two years. Each year about 40 groups are identified.

Production

The total green mussel production by capture fisheries from Malabar area was 6317 tonnes during 2001. The total production from culture was 400 tonnes. This forms only 6.3 % of the total mussel production from Malabar, which had increased from 4.62 % during 1999. Now the production from the wild is increased to about 9000 tonnes but the total production from mariculture has increased to 2500 tonnes forming 27% of the total production.

The yields obtained during 1999 by the groups and the numbers of ropes suspended are given below:

Sl.No.	Place	Total yeild (tonnes)	No. of ropes	Yield/rope(Kg.)
1.	Koyambram	22.75	700	32.5
2.	Kayuthakadu	36.22	900	40.24
3.	Kavunchira	25.2	900	28
4.	Paranthamadu	12.75	300	42.5
5.	Badkekad	18.75	625	30
6.	Ori	13.5	482	28
7.	Thekkekadu	22	760	29
Total		151.75	4667	32.89 (Av.)

Thirteen persons started mussel culture on individual enterprise. The total production from these farms was 97.5 tonnes and the total production from Kasaragod districtwas 248.97 tonnes. During the year 2004, the numbers have dramatically increased and the total production from Malabar is estimated at 2500 tonnes. The number of groups and individuals engaged in mussel culture has gone up dramatically. An interesting to note that the number of single holding has increased and most of them are male members.

Financial assitance

Mussel culture was initiated in Kasaragod district through the DWCRA (Development of Women and Children in Rural Areas) scheme. Loan amount sanctioned was Rs.8800/= per member with a subsidy component of 50%. The amount was to be paid back in five years and the rate of interest was 12.5% per annum. A revolving fund of Rs. 5000/= without interest is also provided. Now these schemes come under the SGSY (Swarnajayanthi Gramaswa Rojgar Yojana) which takes care of economic empowerment of weaker sections of the society. The women's self help groups were the major players in the mussel culture activities of this area.

At Vallikunnu, a subsidy of Rs. 1200/= was given to each farmer of the group by the Panchayat. The total production by the trainees was 5 tonnes. This was the first time that a commercial scale mussel culture operation was done at Vallikunnu. During the previos years only a demonstration culture was done. The harvest was sold in the local markets as wellas among the growers themselves. The markets were the nearby areas of Calicut and Malappuram district. This year, the estimated production is 300 tonnes. Some of the trainees have trained other members also and they have done mussel culture on their own.

At Elathur, although the mussel culture is developing very fast, no financial assistance was given to the farmers. A few of them are engaged in sand mining and coir making for additional income. Two culture units started as individual enterprise is also being operated successfully at Elathur. Now the ADAK is giving subsidies in the form of materials like bamboo poles, nylon ropes, netting cloth. Rs.1000 is given to each group for the purchase of mussel seed.

Inputs

In Kasargod, the net operating profit ranged from Rs. 7,646/= in Kayambram to Rs. 16,413/= at Badkekad. The cost analysis of mussel culture at Padanna showed that the major cost was that of Nylon rope (34%), Bamboo (20%) and seed (20%). The other expenditres involved cloth (7%), construction cost (5%), harvesting (4%), seeding (4%) and coir rope (3%).

Constraints

1. Availability of seed: The seeds required for culture is presently collected from traditional fishing areas and these are often causing conflicts between farmers and mussel fishermen. Hence it is essential that additional spat collectors has to be established along the coast to ensure supply of seeds to the farmers.

2. Marketing: The harvesting seasons of cultured mussels is mostly during April – May months and farmers are forced to sell their crop before the onset of monsoon to avoid mass mortality of mussels due to freshwater influx into the backwater system. At present only a few processing plants purchases cultured mussels from the farmers and as a result the local market are flooded with cultured mussels during these months resulting in fall in the prices and thereby affecting the profitability of the operation.

3. Depuration system: The main constraint in the export of cultured mussels is the lack of proper depuration techniques. Depuration plants are needed at regular intervals along the coast to depurate the cultured mussels for export processing.

4. Storage facility: If sufficient cold storage facility is provided, cultured mussels can be depurated, shucked and stored not only for export market but also for local market throughout the year. This will increase the profitability of the culture operation.

5. Post harvest technology: Value added products of longer shelf life need to be developed from mussel meat to increase the revenue realization from cultured mussels. Mussel fry, mussel pickle etc. are some of the best examples for value added products. More studies are needed to develop ethnic cuisines with longer shelf life.

6. Siltation of backwaters: Some areas in the backwater system have very high siltation levels especially during rainy season. This often results in mortality of mussels in the farms. Hence, scientific feasibility studies are required to demarcate potential culture sites.

Prospects

1. Backwater mussel culture is a recent phenomenon along the Malabar coast and opens immense potential for resource and employment generation among coastal communities especially women living below poverty line.

2. Mussel culture is a low investment activity with very good returns. If promoted properly, mussel farming can be used as a tool for women empowerment in the coastal areas and can stimulate a healthy socio-economic development in the area.

3. Better post harvest technologies can develop attractive value added products. Since very good export markets are available for mussels, they can be taken up as a challenging opportunity by technicians and scientists.

In the western countries, mussel is considered as poor man's oyster. But in India, mussel can be considered as tool for the upliftment of the poor people living in the coastal areas especially along the Malabar Coast.

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Socio Economics of Mussel Farming: Case Studies

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Introduction

Rational utilization of common property resources for sustainable development without endangering the environment is possible through community participation. Mussel farming offers good scope for development in our open waters for enhancing food and livelihood security of the stakeholders in our coastal agro climatic zones. Mussel farming has already been proved as one of the profitable enterprises in the coastal belts as a subsidiary income-deriving source of rural fishermen community. The experimental trials conducted by CMFRI have proved the techno-economic feasibility of mussel farming (Asokan et al, 2001 and Vipinkumar.V.P et al, 2001). Here an attempt has been made on exploration of two case studies in Kerala and Karnataka on socio economics of Self Help Groups of fisherfolk engaged in Mussel Farming.

A Self Help Group (SHG) consists of members linked by a common bond like caste, sub-caste, community, place of origin, activity etc. The Group Dynamics of these SHG's refer to the interaction of forces between the members. It is the internal nature of the groups as to how they are formed, what their structures and processes are, how they function and affect the individual members and the organization. (Lewin *et al.*1960). In an intensive study of Group Dynamics, Pfeiffer and Jones (1972) identified the Group Dynamics factors as to how the group is organised, the manner in which the group is led, the amount of training in membership and leadership skills, the tasks given to the groups, its prior history of success or failure etc. In a detailed study of Group Dynamics, Hersey and Blanchard (1995) gave emphasis on helping and hindering roles individuals play in groups such as establishing, aggressive, persuading, manipulative, committing, dependent, attending and avoidance.

Case Study 1

Kasargod, the extreme north district of Kerala is particularly notable for mussel farming as it has been successfully accomplished by the women's Self Help Groups (SHGs) for the past few years. These groups were given financial assistance in the scheme namely, SGSY (Swarnajayanthi Gramaswa Rosgar Yojana) by the state government which takes care of economic empowerment of weaker sections (Vipinkumar, 2001). Subsidies, bank loans etc are the part and parcel of it and it essentially focuses attention on poverty alleviation through organised Self Help Groups. This programme looks into training, credit, marketing, technical knowledge and basic facilities necessary for the upliftment of the poor to bring them above the poverty line within three years in such a way that they should have a monthly earnings of at least Rs 2000 /-. It would be pertinent to have a look into

the consequences of adoption and cost dynamics of mussel farming by the women's Self Help Groups in Kasargod district.

This district possesses an area of 1992 km^2 with a population of 10, 71508 as per 1991 census. The district with a population density of 538 km^2 has an average growth rate of 22.78 and 82.51 % literacy rate. Majority of the villagers earns their livelihood by agriculture, fishing, coir retting, coconut husk, toddy tapping etc. There is tremendous potential for aquaculture diversification in Kasargod coastal belts. Water bodies in these coastal belts have amble scope for the judicious utilisation of finfish culture, prawn and crab farming in Kasargod. (Asokan et al 2001)

Methodology

This study was undertaken in two major panchayaths namely Cheruvathur and Padanna in Kasargod district. The study area, Cheruvathur panchayath has an area of 18.37 km^2 with a population of 24, 504 out of which 18, 631 people are literate. Agriculture is the main occupation of the majority and about 150 families are engaged in fishing as the main occupation and about 300 families as subsidiary occupation.

Similarly, Padanna panchayath has an area of 13.08 km^2 with a population of 17, 961 out of which 12, 746 people are literate. About 200 families are engaged in fishing as main occupation and about 400 families as part time occupation. The brackish water estuary systems of these panchayaths are extremely suitable for mussel culture.

Six Self Help Groups of women (three each from both panchayaths) were selected as the sample and the data were gathered as explorative case studies through personal interviews of the respondents. For the study, the Group Dynamics of members of Self Help Groups was measured by developing an index called Group Dynamics Effectiveness Index (GDEI). Group Dynamics Effectiveness was operationally defined for the study as the sum-total of the forces among the member of SHG based on the sub-dimensions, such as participation, influence & styles of influence, decision making procedures, task functions, maintenance functions, group atmosphere, membership, feelings, norms, empathy, interpersonal trust and achievements of SHG. (Vipinkumar, 1998)

For the computation of the Group Dynamics Effectiveness Index (GDEI) the scores obtained for each of the above mentioned sub-dimensions were first made uniform and then multiplied by the corresponding weightage assigned to each as by expert judges. These scores were then added up to get the GDEI score of each respondent.

It was also ensured that all the sub-dimensions identified as components of GDE were of high significance on the basis of the coefficient of agreement in judges rating as well as the statistical evidence from the results of the pilot study. The measurement device developed for the dependent variable *i.e.*, GDE was ascertained for its content validity.

Measurement of Sub-dimensions

A. Participation: For the present study, participation was operationally defined as the degree to which the farmer is involved in group meetings, discussions and group activities of SHG.

B. Influence & Style of Influence: Influence was operationally defined as the degree to which a farmer can influence other member of SHG in a desirable way. Style of influence was operationalised as the manner in which the member attempts to influence other members of SHG. The four different styles included were autocratic style, peacemaker style, laissez-faire style and democratic style.

C. Decision Making Procedures: This is operationally defined as the degree to which farmer makes a decision with involvement of other group member of SHG, makes decisions without topic drifting, supports other members' decisions in consensus, feels the majority's decisions valid in the SHG, attempts to get all members participate in decisions of SHG and feels the gains of recognition for his contribution in decision making process.

D. Task Functions: This is operationalised as the degree to which the farmer makes suggestions to tackle a problem in the SHG, summarises what has been covered in the group, tries to give or ask for facts, ideas, opinions, feelings, feed back etc. and keeps the group on target.

E. Maintenance Functions: This is operationalised as the extent to which farmer helps others into group activities of SHG, helps/interrupts him in group discussions, feels the other members are co-operative and listening, perceives other members help in clarifying the ideas of all members, feels good or bad when ideas are accepted or rejected and the extent to which other members attempt to maintain task functions of SHG.

F. Group Atmosphere: This is operationalised as the extent to which the group member prefers friendly congenial atmosphere in the SHG, attempts to suppress conflict or unpleasant feelings in the group, feels other members are involved and interested and feels satisfied from the work climate.

G. Membership: This is operationally defined as the degree to which a group member feels accepted or included in the SHG, feels sub-grouping in the SHG and feels himself or other members to be outside the group.

H. Feelings: This is operationally defined as the degree to which the farmer feels anger/irritation, frustration, warmth, affection, excitement/boredom and competitiveness while performing the group activities of SHG.

I. Norms: This is operationalised as the extent to which the farmer feels the standards or ground rules and regulations are in operation that controls the behaviour of group members for the smooth functioning of the SHG.

J. Empathy: This is operationally defined as the degree to which the respondent is able to make out other person's feelings and thereby to understand it as he feels.

K. Interpersonal trust: This is operationally defined as the degree to which the respondent trusts the other members of the group as well as the faith other members has in him as perceived by the respondent.

L. Achievements of SHG: This is operationalised as the level of performance of SHG as perceived by the farmer as well as the performance of the farmer himself as the group member.

All these sub-dimensions were measured by a set of inventories containing appropriate questions arranged in a three-point continuum of always, sometimes and never with scoring pattern 2,1 and 0 for positive and vice versa for negative questions.

The cost estimates of all the selected Self help Groups were also computed and by taking in to consideration of major expenditure required for mussel farming is for the materials such as bamboo, nylon rope, coir, cloth, seed, etc. and labour costs essentially cover construction, seeding, harvesting etc. the Net Operating Profit and B: C ratio also were calculated for different SHG's to draw valid inferences.

Results and Discussion

The study, focused attention on Group Dynamics Effectiveness as a trait of Self Help Groups resulted by the joint influence of individual members of the group generated out of skills and orientations from the past life experiences. It definitely varies from person to person, place to place, time to time, situation to situation and in turn from group to group. This might be the probable reason for the differential degree of GDEI observed among respondents.

Profile of Cost Estimates of Mussel Farming

The major expenditure required for mussel farming is for the materials such as bamboo, nylon rope, coir, cloth, seed, etc. and labour costs essentially cover construction, seeding, harvesting etc. The women's' groups constituted in the scheme DWCRA started mussel farming as early as 1996-97 and are assisted by loan amount worth Rs 8800 / -per member with a subsidy amount worth Rs 4400 / - which looks quiet fascinating. The duration of the loan is 5 years and the rate of interest is 12.5 % per annum. In addition to this, a revolving fund of Rs 5000 /- was also provided without interest. When the SHGs are economically empowered with the provision of loan facilities, the returns from mussel farming help them to repay the loan slowly.

The loan was granted through Farmers' Service Cooperative Banks and North Malabar Gramin Banks in Cheruvathur and Padanna panchayaths of Kasargod district. Majority of the SHGs' showed considerable progress in repayment of the loans, which can be concluded as an indication of the profitability of Mussel farming. The expenditure details of the selected SHGs in the initial year of mussel cultivation are shown in the Table 1.

The Net Operating Profit in all the six SHG's was computed and found as substantially good which proves the profitability of Mussel farming in the initial trial itself and since during the subsequent years, material costs such as those of bamboo, rope, cloth and labour cost in construction etc. are negligible, this ensures reasonable profit as a major consequence of adoption of Mussel farming enterprise bringing about economic empowerment of rural women through organised Self Help Groups.

Table 1. Cost estimate of the SHG's in mussel farming in Kasargod district.

	SHG1	SHG 2	SHG 3	SHG 4	SHG 5	SHG 6
No.of ropes	500	800	600	750	900	725
Items						
Bamboo	6400	9600	7980	9000	11437	7800
Nylon rope	9954	17500	12000	15000	18000	14500
Coir rope	1100	1500	1200	1587	2000	1450
Cloth	3000	3250	1700	3338	3600	2250
Seed	6500	10000	8700	9000	10800	9770
Labour						
Construction	1600	2400	2170	2250	2700	2200
Seeding	1500	2565	1500	1875	2500	1800
Harvesting	1300	2000	1500	2000	2750	1875
Miscellaneous	1000	1600	1200	1500	1800	1450
Total Cost	32,354	50,415	37,950	45,550	55,587	43,095
Returns	40,000	64,000	48,000	60,000	72,000	58,000
Net Operating Profit	7,646	13,585	10,050	14,450	16,413	14,905
B : C Ratio	1.236	1.269	1.265	1.317	1.295	1.346
GDE Index	52.78	54.33	53.91	57.32	55.68	59.14

Experiences and observations already indicated that for a group to be developed as an SHG it requires a period of at least 36 months and it is a hectic process. It has to pass through various phases such as Formation phase, Stabilisation phase and Self Helping phase. These Self Help Groups promote a cooperative and participative culture among the members, which ensures the empowerment culture of the Self Helping phase.

The loan sanctioning, utilisation, accounts maintenance and timely repayment of loans etc. are all perfectly accomplished with proper maintenance of the documented records by the group members. This ascertains the fulfillment of norms and standards of the SHG leading to economic empowerment of the members.

Case Study 2

Self Help Groups (SHGs') of fisherfolk were mobilised in *Karwar* and *Bhatkal* locations of Karnataka coastal belts. Three SHG's of 15 members each comprising a total of 45 were mobilised in Majali (Open Sea) of Dhandebag and three SHG's of 15 members each comprising a total of 45 were mobilised in Sunkeri of Kali estuary in Karwar coastal belts in Uttar Kannada district of Karnataka state. Training and demonstration on mussel farming was undertaken in these SHGs.'. Initially, two training and demonstration programmes in these two sites in Karwar were undertaken, one for *raft culture* in open sea in *Majali* of *Dandebag* and one for *rack culture* in *Sunkeri* of *Kali* estuary. The training was imparted to 45 members of three Self Help Groups, each possessing 15 members in 2 sites separately comprising a total of 90 participants. At *Majali* in open sea, a 5 x

5 metre raft and at *Sunkeri* of Kali esturay, a 5 x 5 metre rack were constructed for mussel farming.

Similarly In *Mundalli* river of *Bhatkal* estuary in Karnataka, 4 Self Help Groups of 15 members each exclusively of women fisherfolk mobilised under the NGO, ' Snehakunja ' comprising a total of 60 participants were trained on mussel farming. They initiated a trial in 5 x 6 metre rack mussel culture by long line method.

The sample design for observation including the number of SHGs' trained, beneficiaries and method of culture is given in Table 2.

Table 2: Mussel culture interventions in Karnataka state

Site	No.Of SHG's Trained	No. of beneficiaries	Method of culture	Size of the rack / raft
Sunkeri of Kali estuary	3	45	Rack culture	5 x 5 m
Majali of Dhandebag	3	45	Raft culture	5 x 5 m
Bhatkal of Mundalli estuary	4	60	Raft culture	5 x 6 m

Data were gathered from these 10 Self Help Groups through personal interviews of the respondents. For the study, the Group Dynamics of members of Self Help Groups was again measured by developing an index called Group Dynamics Effectiveness Index (GDEI). The growth parameters were monitored every week in all the sites and the yield particulars of mussel during harvesting in each SHG was also noted.

Results and Discussion

The major expenditure required for mussel farming is for the materials such as bamboo, nylon rope, coir, cloth, seed, etc. and labour costs essentially for construction, seeding, harvesting etc. The SHGs' of *Majali* and *Sunkeri* were mobilized by the project team of CMFRI and the SHG's of *Bhatkal* were mobilized by a NGO namely *Snehakunja*. The first two trials and demonstrations were under the funding of CMFRI and for the last one, only the technical helps during the training and demonstration were offered by CMFRI. The Yield particulars in all the ten SHG's was noted and found as substantially good which proves the profitability of mussel farming in the subsequent trials because the material costs such as those of bamboo, rope, cloth and labour cost in construction etc. are negligible, this ensures reasonable profit as a major consequence of adoption of Mussel farming enterprise bringing about economic empowerment of rural women through organised Self Help Groups.

The yield in Kg per metre length of the rope recorded in all SHGs' as Average Yield showed a positive relationship with GDEI score. The correlation ($r = 0.958139$) was found significant owing to the 't' value 9.465624 at 1% level of significance. (Table 3.)

Experiences and observations already indicated that for a group to be developed as an SHG it requires a period of at least 36 months and it is a hectic

process. It has to pass through various phases such as Formation phase, Stabilisation phase and Self Helping phase. These Self Help Groups promote a cooperative and participative culture among the members, which ensures the empowerment culture of the Self Helping phase.

The utilization of fund sources, accounts maintenance etc. are all perfectly accomplished with proper maintenance of the documented records by the group members. This ascertains the fulfillment of norms and standards of the SHG leading to economic empowerment of the members.

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Table 3. Relationship of Yield and GDEI of SHGs'

SHG	Yield in Kg / m	GDEI score	Correlation Coefficient (r)	't' value
SHG 1	9.2	53.71	0.958139	9.4656248**
SHG 2	9.1	52.31		
SHG 3	8.9	51.91		
SHG 4	12.6	57.32		
SHG 5	12.7	56.68		
SHG 6	12.5	57.14		
SHG 7	13.6	60.01		
SHG 8	13.1	59.98		
SHG 9	13.8	61.29		
SHG 10	13.2	60.02		

Constraints Faced by the Fisherfolk in Mussel Farming

Mussel farming faces a number of impediments like water salinity, seed availability, selection of location / site, climatic vagaries, identification of proper beneficiaries and proper monitoring opportunities. The major problems and constraints faced by the fisherfolk in mussel cultivation are as follows

- Unpredictable seed availability.
- Mortality of seeds during transportation.
- Reduced growth during certain years.
- Meat shucking problems.
- Marketing of mussels.

- Social constraints like caste splits, conflicts, politics etc.to a limited extent.

The open sea mussel culture in this particular case met with the impediment of unfortunate sabotage of the seeded mussel by some miscreants. It was rectified by reseeding, but the yield was not that much conspicuous compared to the trials undertaken in estuaries. All the SHG members are of unanimous opinion that the government agencies should come forward with improved marketing facilities, as marketing of the mussel was perceived as one of the biggest constraints. Provision of loans with reduced interest rates and freezer facility for storage of harvested mussels can bring about a breakthrough in this sector in the near future.

Conclusions and Remarks

An attempt has been made to assess the socio economic impact of mussel farming by mobilizing Self Help Groups in Kerala and Karnataka coastal belts. Mussel farming is slowly achieving considerable significance because of its profitability. But it is inevitable to take care of the selection of suitable sites fulfilling the essential parameters for undertaking mussel culture trials. It would be pertinent to have study on the effect of coir retting zones on growth and attachment of mussel seeds to the strings, which often found to be not suitable by experiences and observations. Laboratory experiments should be widened to study the effect of coir retting zones on growth of mussel.

Similarly, export potential of mussel can be promoted through value addition experiments on depuration plants in filtered seawater. Organised fishermen's cooperatives can play a vital in various stages of seeding, harvesting, sorting, grading, packing, and marketing with an intention of export potential.

The study emphatically disclosed the deep rooted influence of Group Dynamics network among the farmer folk as influenced by their participation, influence & styles of influence, decision making procedures, task function, maintenance function, group atmosphere, membership, feelings, norms, empathy, interpersonal trust and achievements of SHG.

Irrespective of the location specific problem oriented resource based alternative programmes for income generation, this study emphasises on the economic empowerment of rural women through mussel farming as a means of poverty eradication through Self Help Groups because, poverty can only be alleviated by mobilising the poor to solve their actual problems in the form of organised SHGs'. In the impact assessment, the correlation analysis revealed, a proportional relationship between the Group Dynamics Effectiveness and Average Yield obtained for each SHG, which ensures reasonable profit as a major consequence of adoption of Mussel farming enterprise bringing about economic empowerment of fisherfolk through organised Self Help Groups.

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Seed Production of Whelk

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Babylonia sp., commonly known as 'Whelk,' 'Spiral Babylon' and 'Puramuttai chank' (Dove egg shell) in local parlance and 'Baigai' in the trade sector, is a marine edible gastropod. It is widely distributed in the Indo Pacific region. In India, it is well represented in the Indian Peninsula at places such as Gulf of Mannar, Poompuhar, Nagapattinam, Madras and the waters around Andaman and Nicobar islands (Ayyakannu, 1994). *Babylonia* is a much sought after marine gastropod and it fetch a good foreign exchange. This edible gastropod is an important food species in the Indo-pacific region (Ayyakannu, 1994). The total quantity of whelk trade during 1993-94 was 300 tonnes and it increased to 500-600 tonnes during 1995-96.

Global trade in the whelk meat and by-products, although presently meager, has considerable potential. Whelk or "Baigai" is exported frozen shell-on to Australia, France, Hong Kong, Japan, Singapore and Thailand. Export of shell-on frozen Baigai from India was 703 t in 2001 and fetched revenue of Rs 386 lakhs. Japan is the leading importer of frozen Baigai from India (689 t in 2001) followed by Hong Kong (11.3t in 2001). Live whelk is exported to Hong Kong and Thailand (Statistics of Marine Products Exporters 2001).

Economic Importance of *Babylonia* spp

Baigai (*Babylonia)* have received considerable attention due to their economic importance and the increased demand for meat of these snails in the western countries. The boiled meat of the snail was Rs 40/kg (Ayyakannu, 1994), though it is not available in the market nowadays. Presently it is exported mainly to Japan. *Babylonia zeylanica* is sold at Rs. 30/kg shell on where as *Babylonia spirata* fetches Rs. 10/kg in the local. The shell has ornamental value and the operculum has medicinal importance and therefore no part of the whelk is wasted.

The shells of *Babylonia* are used for interior decorations after cleaning, processing and polishing. A well polished whelk shell fetches Rs 3 / shell. Beautiful items such as curtains, pen stands, mementos, key chains and other novelties are made out of small shells. There is a good market for them not only in India but also in western countries. Handicraft items made from *Babylonia* shells are widely sold in almost all cities and tourists centres in India.

The operculum popularly known as 'fish nail' is an important by- product for export and is valued at Rs.400 / kg. 100 kg gastropod shells yield 1 kg of operculum. The total export of operculum for 1992-93 is 2 t worth Rs. 4.14 lakhs (Statistics of Marine Products Export, 2001).

Two species of Babylonia occur in the Indian waters; *Babylonia spirata* and *Babylonia zeylanica*. These together form nearly 56% of the shrimp by catch. From the by-catch, *Babylonia spirata* and *Babylonia zeylanica* are segregated and separately auctioned due to their market preferences and considered as an emerging resource. In view of its high economic value and the increasing fishing pressure on the present stock, efforts were initiated for the seed production and farming of the whelks. The breeding, spawning and larval development of *Babylonia spirata* has been successfully carried out in the Central Marine Fisheries research Institute and is detailed below.

Breeding, Spawnimg and Larval Rearing of *Babylonia spirata*

i) Collection and Transportation of the Brood Stock

The brood stock of *Babylonia spirata* and *B. zeylanica* were collected from landing centers of Neendakara and Sakthikulangara and transported to molluscan hatchery at CMFRI, Cochin for breeding and spawning studies. The best method to minimize the transportation stress and ensure complete survival the whelks were kept in cotton moistened with seawater or gunny sacs presoaked in seawater.

ii) Management of Brood Stock in the Hatchery for Spawning

Live and healthy specimens were transferred to 1 t FRP tanks with pure filtered seawater and provided with aeration. 70% of the fresh seawater was exchanged daily. The snails were allowed to acclimatize in the hatchery for two days after transportation. During this period no food was given to them. After acclimatization, they were fed with clam, squid, prawn, annelids, etc. From the observations, it was found that within five minutes they were able to locate the food and extended the proboscis to take the food. After two days of acclimatization, they were transferred to the tanks with sandy substratum for providing them a natural environment. The FRP tanks were provided with sand as the substrate bottom. The tanks were fitted with two biological filters to maintain water quality. The environmental parameters like salinity, temperature and pH were regularly monitored and kept with in the range of 32-35ppt, 26-29°C and 8-8.3 respectively. *Babylonia spirata* had a distinct preference for clay (51.9%) as their substrate. The order of preference for other substrate was coarse silt (24.7%), coarse sand (17.3%), and gravel (6.1%).

iii) Spawning

The acclimatized brooders took average 15 days to spawn in the hatchery though some took nearly two months to show the spawning activities. The average size of the spawners was 36mm. Spawning occurred during night and continued up to the early morning hours. An erect position of spawners by pressing its foot in the substratum indicated spawning and any slight disturbance halted the spawning activity. The average number of capsules per spawner was 35-40 with 350-800 eggs per capsule.

Egg capsules: The eggs were laid in transparent vasiform capsules. Due to the transparent nature of the egg capsules, the eggs were visible and could be counted

externally. The apical portion of the egg capsule was concave in appearance and the membrane in this region was thinner than the walls. The stalk of the egg capsule was firmly attached to the substratum to hold it in an erect position till the larvae hatch out. The average total length of the egg capsule was 27.8 ± 2.5mm and the capsular length excluding the stalk showed variation. The average width of the capsule at the apical region was 8.4 ± 1.5 mm. The average diameter of the fertilized egg was 275µm, irrespective of the size of the capsule and number of eggs in the egg capsule. There was positive linear correlation (r= 0.8764) between the average length of the egg capsule and average number of eggs in the capsule. The average size of the fertilized eggs in the egg capsule was 260-280 µm.

Hatching, Larval Rearing and Larval Development

The capsules attached to the substratum with the help of the hold fast were transferred from the spawning tanks to the hatching tanks of 50 lit capacities with fresh filtered seawater and provided with gentle aeration. The salinity was maintained at 32 ppt, pH 8±0.2 and temperature 28±2°C.

i) **Fertilized Eggs to Planktonic Larvae:**

First polar body was released within 60 minutes after the release of fertilized egg capsule. The release of second polar body commenced at 90th minute. The first cleavage occurred 30 minutes after the release of the second polar body, which was followed by the second cleavage after one hour. The divisions were clearly visible up to 16-cell stage. Subsequently, it becomes an opaque mass due to large quantity of yolk in the egg. After 24 hours of spawning, the divisions completed and the embryo transformed into the morula stage with marginal cells at the anterior region. Further development resulted in the rotation of the morula and this stage lasted for about 48 hours.

On the 3rd day, the cilia were visible at the top and transformed to trochophore larva. On 4th day the larval size increased to 380 µm. Subsequently the larval size increased to 420 µm on 5th day and developed velum boarded by two rows of fast beating cilia along its margin. On 6th day, the velar lobes become enlarged and a thin, transparent larval shell was clearly visible. From this day onwards veliger larvae were fully developed and concentrated at the tip of the egg capsule. Though the exact mechanism of the releasing of the larvae is not known, the apical part splits and releases the larvae from the egg capsule. The average hatching percentage of larvae from each capsule was 90 and all of them were released by 7th and 8th day after spawning. The larvae are plank tonic, swim towards the surface of the water and exhibit photo tactism. The larvae are transparent; possess bi-lobed velum fringed with cilia. The larval shell is fully developed. Eyespot is also developed.

ii) **Larval Rearing**

On the 7th day, the larvae were transferred from the hatching tanks to the rearing tanks (Perspex/glass tanks) by filtering through a sieve of 400µm and stocked seawater in the rearing tank at a density of 150 larvae/liter. The rearing

conditions were salinity 32±1ppt, pH 8±2 and temp 28±2°C. Prior to stocking, the water for rearing was treated with hypochlorite and potassium permanganate solution to eliminate the unwanted microorganisms. Different algal feeding regimes were tried. Poor growth and heavy larval mortality occurred when fed with *Tetraselmis* sp. and *Nannochloropsis* sp. Pure cultures of *Isochrysis* and *Chaetoceros* were provided to the larvae up to the 17th day and larvae settled as juvenile. The larvae were fed at the rate of 7000 cells/ml/hr. Almost 95% of the larvae hatched out from the egg capsule and the survival rate was 60% from the hatching to settlement.

***iii)* Metamorphosis and Settlement**

The larvae feed and swim actively with the fast movement of cilia along the rim of the velum. Eyespot becomes clearer. This stage lasts up to the 13th day. The larval shell is fully developed and the foot protrudes out Operculum is seen as a scar; pair of tentacles develop at the base of the tentacles. Velum is 4-lobed, as a folding along the horizontal position. Active feeding of phytoplankton continues up to the 17th day. The plank tonic lifestyle begins to change and the larvae begin creeping and crawling along the bottom, actively searching for food and become carnivorous in nature, feeding on shrimp, clam squilla meat etc. Settlement begins when they attain the average size of 895 µm. At this stage the velum is shed, radula and digestive tract is developed and the juveniles secrete mucus along their path.

iv) Rearing of Juveniles

Metamorphosis of the larvae completed 17-19 days after the release of the capsule. The settled juveniles were transferred to 5 liter beakers provided with filtered seawater and gentle aeration. After settlement, the feeding habit changes and they become carnivorous and the juveniles begin to creep and crawl along the bottom. Algae settled on glass slides, shrimp feed, agar based feed (composition agar 0.25gms, shrimp 1.5 gms, soyabean 0.25 gms and boiled egg albumin 0.25gms,in 100ml sea water) egg yolk; egg albumin, tubifex and rotifer were tried as food for the juveniles. However, only shrimp feed found as better for the growth and survival of the young ones. The survival rate after settlement was 70%. During the settlement stage, they attained 800-1000µ shell lengths. The juveniles had well developed radula and digestive tract suitable for carnivorous life and fully developed shell and operculum for protection.

The growth of Juveniles was recorded from day1 to 18 months of growth. The average total length on the 1st day was 1.5 mm. On the 15th day, the average total growth was 2.218 mm. After 1 month, an average total length of 2.3 mm was attained. After 45 days of growth, the average total length was 2.82 mm. At 2 months 3.84 mm of average total length was recorded. And after 75 days, 4.06 mm was attained.

After 6 months, the average total length was 14.41mm, average width was 9.87mm and average weight gain was 0.92g. After 10 months the average total length was 23.33 mm average width 15.06 mm and average weight gain was 3.2 g. After 14 months, the average total length was 28.7mm average 29.15 mm average

width 20.07 mm and average weight gain was 8.8 g. After 18 months, the average total length was 30.98 mm average width 21.29 mm and average weight gain was 9.99 g.

Preference of Microalgal Feed by Larvae of *B. spirata*

Although the larvae showed the general acceptance of Isochrysis galbana, Chaetoceros calcitrans and Tetraselmis gracilis as feed, Chaetoceros calcitrans proved to the most preferred micro algal feed by the larvae followed by Isochrysis galbana. Tetraselmis gracilis found poor acceptance due to the fact that algae was not available to the larvae, since it remained at the surface of the water column. Nannochloropsis salina did not find acceptance at all, since there was no settlement and complete mortality occurred on the 2nd day it self.

Larval Stocking Density

Larval survival, settlement and growth at different stocking densities was studied. Babylonia spirata larvae were maintained in 4 lit containers in pure, filtered sea water 0f 32 ppt, at 8 different stocking densities 75/lit, 100/lit, 125 /lit, 150/lit, 200/lit, 225/lit, 300/lit, 325/lit. They were provided with Chaetoceros calcitrans at the rate of 10,000 cells/ml/hr and gentle aeration. The growth and percentage of settlement were recorded till 17 days when complete settlement was observed.

The optimum stocking density was found to be 150 nos/lit, giving very high settlement rate and good growth compared to other stocking densities. Lower stocking densities viz, 75/lit, 100/lit and 125/lit showed of better growth compared to that of 150 /lit, however the settlement was very poor in these stocking densities 49.5%, 44%and 52% respectively. Higher stocking densities 250 nos/lit, 225 nos/lit 300 nos /lit and 325 nos/lit resulted poor growth and very low settlement.

Elimination of Vorticella Infestation on the Larvae

Vorticella infestation occurred in the veliger and juvenile stages of growth and lead to extensive mortality. Vorticella was found on the velar lobes and shell of the larvae cover the opercular opening of the shell, causing mortality. Experiments were conducted to eliminate the infestations on the larvae. It was found that 20ppm formalin was effective in eliminating nearly 85% of Vorticella.

Effect of Salinity on Hatching of Larvae

The egg capsules of Babylonia spirata were maintained in different 20, 30, 35 and 40‰ salinities in 2 lit pure filtered seawater in 3 lit capacity plastic containers and provided with gentle aeration. Complete water exchange was done on alternate days. Three egg capsules were introduced into each container of average size 26 ± 2mm total length; 16 ± 2mm capsule length and 10 ± 2 mm capsule width.

From the experiment it was observed that no hatching occurred at lower salinities of 20 and 25 ppt. 50-70% hatching occurred on the 7th day in the other

salinity ranges, 30, 35 and 40 ppt and 25-30% hatching occurred on the 8th day. Thus the ideal salinity range for the hatching of the eggs is 30-40 ppt.

The larvae were reared in different salinities ranging from 5 to 50‰ to study the effect of salinity on settlement % and growth of juvenile on the settlement stage. The larvae were stocked at the density of 150 larvae/ lit in different salinities in duplicates and growth were recorded till the day of settlement on 17th day. In the 5, 10, 15 20 and 50‰ salinities, the larvae did not survive and there was total mortality. The settlement percentage was very low at 40‰ salinity (14%) and no settlement was observed in 45‰-till17th day. Good growth and maximum percentage of settlement obtained at 30‰ salinity (56%). So the ideal salinity required for the larval growth was confirmed as 30‰ with a pH ranging between 8.1-8.3 and the temperature 26-28^0 C.

Effect of Water Change

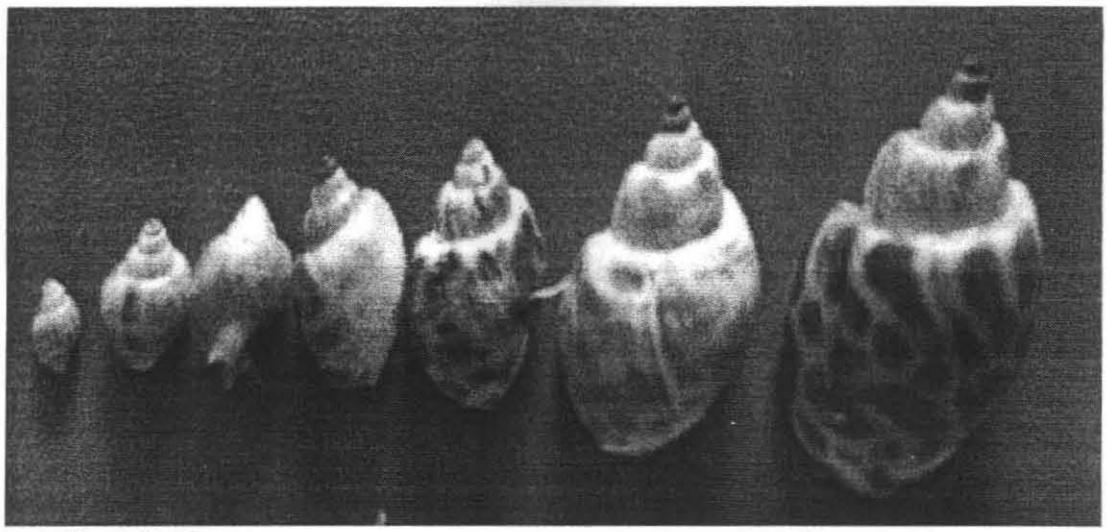
Maximum settlement of 57.3% and growth was recorded when complete water exchange was carried out on alternate days of the experiments. Daily change of 25% of water also provided good settlement (50.5%). Fifty percentage of water exchange on alternate days resulted in poor settlement (35.5%) while no exchange of water through out the experimental period did not facilitate settlement and growth. Thus for achieving maximum settlement of B.spirata larvae, complete water exchange on alternate days is ideal.

Feed Preference of *Babylonia spirata* Juvenile in the Hatchery

The survival of juveniles highest among those fed with shrimp feed (92.5%), followed by those fed with squilla (85%). The survival when fed with squid was 65% and those fed on clam was only 50%. Egg custard was found to be unsuitable as feed for juvenile as the total mortality observed after 10 days. Growth was highest among those fed with shrimp feed. However squid was more acceptable than squilla in terms of growth although there was better survival when fed with squilla.

The life cycle of Babylonia spirata is appended.

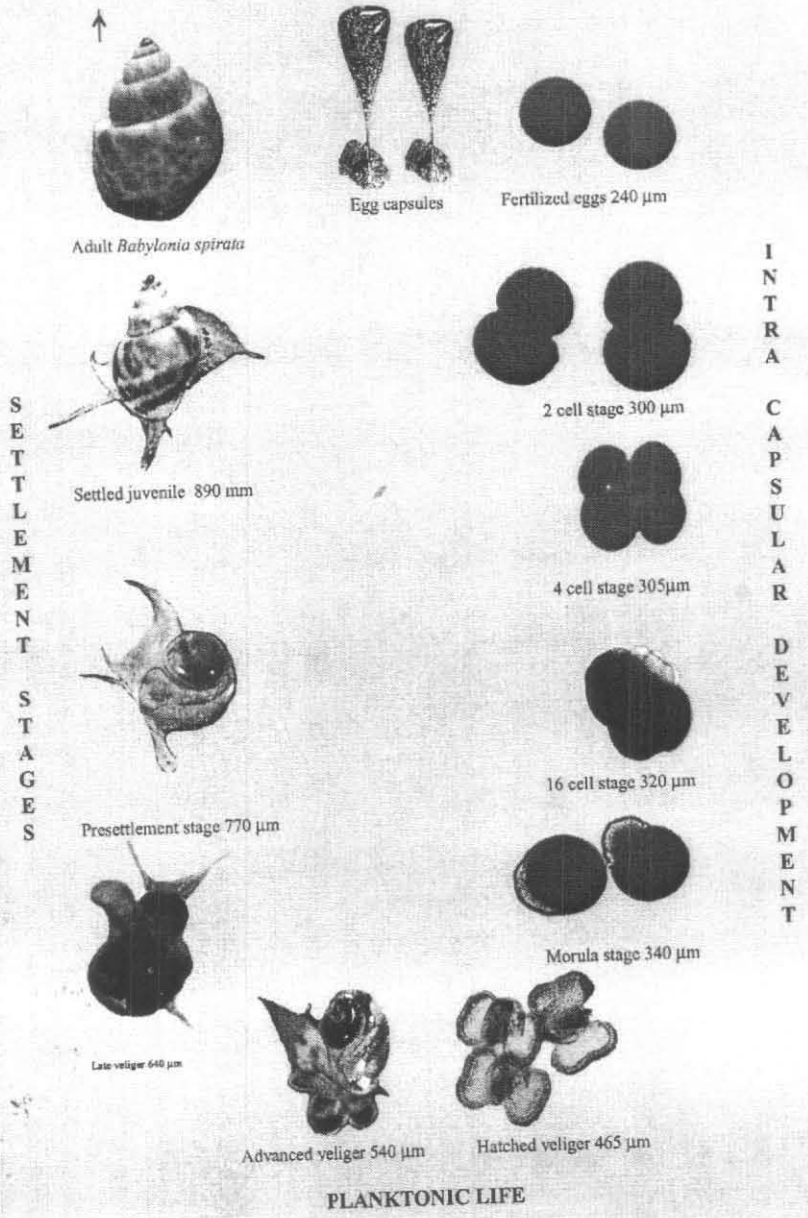
The up gradation of the present larval rearing and hatchery technique will help to develop whelk farming on commercial basis in India, which will ultimately help in increasing production besides reducing the fishing pressure in the natural whelk stocks.



Juvenile *B. spirata*

Spawning of *Babylonia spirata* in the hatchery

Figure 34: Life cycle of *Babylonia spirata*



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Phytoplankton Culture

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Introduction

The floating microscopic plant components of the seawater are the phytoplankton or the micro algae, which forms the basic food of almost all the larval organisms either crustaceans, molluscs or fishes. They are the primary producers of the sea, constituting various classes of Algae. The important components of micro algae are the Diatoms, Dinoflagellates, Silicoflagellates (Phytoflagellates), Coccolithophores, blue-green algae and the 'hidden flora', the nannoplankters. Among these, the diatoms and phytoflagellates are significant organisms since they forms the primary link in the food chain of the sea. It is known that the hatchery operations depend mainly on the availability of the basic food, the phytoplankton.

Mass Culture of phytoplankton has been in prevalence in many research Institutions, universities and hatcheries the world war, since the past 50 years. As is well known, the success of any hatchery system, prawns, oysters, fishes or sea cucumbers, entirely depend or the availability of the suitable live feed, the phytoplankton. In the natural environment, the larvae feed on any minute plant components, which are readily available to them. But in a hatchery, the organisms which are acceptable to the larvae for their growth and further development have to be identified and isolated. In the early critical stages of the rearing larvae of finfishes and shellfishes, the phytoflagellates (species of *Isochrysis*, *Dicrateria*, *Chromulina* and *Tetraselmis*) and other nannoplankters (species of *Chlorella, Nannochloropsis* etc) forms the basic food. But in the post-larval stages of crustaceans and post Juvenile stages of molluscs, the diatoms (species of *Chaetoceros*, *Skeletolema* and *Thalassiosiara*) forms the primary food. Hence the culture of phytoplankton is an essential pre-requisite for the rearing operations of economically important cultivable organisms in a hatchery system.

Methodology – Isolation

Isolation of the required species of phytoplankton can be done by the following methods.

1. Pipette Method: Larger organisms can be pipetted out using a micro- pipette under microscope and transferred to culture tubes, having suitable culture media.

2. Centrifuge or Washing Method: By repeated centrifuging of the samples in different revolutions and by inoculating the deposits, we may get different

organisms. Transferring the deposits in various culture media, different organisms can be isolated.

3. By exploiting the Phototactic Movements: By this method, most of the phytoflagellates can be isolated. Make a dark chamber with a small hole on one side and keep the sample in a beaker nearer to the hole. Place a candle near to the hole outside. Since the phytoflagellates have a tendency to move towards the light, it is visible after some time that these organisms crowded near to the candlelight. By pipetting we can separate these organisms, and by tube culture method, can be raised to a pure culture.

4. By Agar Plating Method: For preparing the agar medium, 1.5gm of agar is added to one litre of suitable culture medium or even natural seawater. This agar solution is sterilized in an autoclave for 15 minutes under 120 lbs pressure and 100°C temperature. Now this medium is poured in sterilized 15cm petridishes and keep for 24 hours. For the isolation, the required species can be picked up by pipette or needle or loop under microscope and streaked on the surface of agar plate. After inoculation, these petridishs are placed in an incubation chamber for 7-8 days providing light (1000 lux) and constant temperature (25°C). Within this time, the required species, if it has grown into a colony removed by platinum loop under microscope and transferred to culture tubes. Further, from the culture tube to small conical flasks and larger containers, the algae can be grown and keep as stock culture and later for mass culture.

5. Serial dilution culture method: This method is used mainly for the isolation of nannoplankters and phytoflagellates (Sournia, 1971). In this method mainly 5 dilution steps (the inocula corresponding to 1, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} or 4 steps 0.001, 0.01,0.1 and 1 ml) are involved for the isolation of the required species. For the serial dilution technique, nearly 25 culture tubes (15ml 'Borosil') are required. After filtering the seawater through 10 – 40 μ seive, the filterate has to be inoculated to 5 series of culture tubes in various concentrations. This has to be kept under sufficient light (1000 lux) and uniform temperature (25^0C) conditions. After 8 to 10 days some discoloration can be seen in the culture tubes, due to the growth of micro algae. Further purification of this culture can be done by sub culturing it in 500ml and 1 litre conical flasks. Once the culture is fully purified, it can be transferred to 3 or 4 litre Hafkin culture flasks and maintained as stock culture.

Culture Media

For the successful culturing of the micro algae, either diatoms or flagellates, various chemical culture media have been used depending on the type of organisms cultured and their growth phases. Though Schreiber's and Miquel's media (Miquel, 1892) were found to be very effective for culturing the diatoms and nannoplankters, several other media also came into existence with the addition of trace metals, vitamins and other organic and inorganic salts. Usually for culturing the flagellates, Conway or Walne's medium (Walne, 1974) is used in the laboratory for the maintenance of the stock culture as well as mass culture. The important culture media used for the phyto plankton culture are:

1. Schreiber's medium

Potassium nitrate	..... 0.1gm
Sodium ortho phosphate	.....0.02gm
Soil extract	.....50 ml
Filtered and sterilized sea water	.....1 litre

Soil extract is prepared by boiling 1kg of garden soil in 1 litre of fresh water for one hour. After 24 hours, decant the clear water and keep in a bottle. This is the soil extract. 50ml can be added to each litre of sterilized seawater.

2. Miquel's medium

A.	Potassium Nitrate	.....20.2 gm
	Distilled Water	.....100 ml
B.	Sodium Ortho phosphate	.....4 gm.
	Calcium Chloride	.....2gm
	Ferric Chloride	..... 2 gm
	Hydrochloric acid	..... 2ml
	Distilled Water	.....100ml

Add 0.55ml of A and 0.50ml of B to one litre of filtered and sterilized seawater.

3. Conway or Walne's medium

A.	Potassium nitrate	.....100gms.
	Sodium Orthophosphate	.....20gm
	EDTA (Na)	.....45 gm
	Boric Acid	.....33.4gm
	Ferric chloride	.....1.3gm
	Manganese chloride	.....0.36gm
	Distilled Water	.....1 litre
B.	Zinc chloride	.....4.2gm
	Cobalt chloride	.....4.0gm
	Copper Sulphate	.....4.0gm
	Ammonium Molybdate	.....1.8gm
	Distilled water	.....1 litre
C.	Thiamine(B_1)	.....200 mg in 100 ml DW
	Cyanocobalamine (B_{12})	.....25 mg in 100ml

Prepare A, B and C (each) in different reagent bottles. Add 1ml of A 0.5ml of B and 0.1ml of C to 1 litre of filtered and sterilized seawater.
For the preparation of mixture of various phytoplankton in the open tanks, using direct sun light, the following medium can be used:

4. Mixture Culture Medium

Potassium Nitrate	.....1.2 gm
Sodium Orthophosphate	.....0.66 gm
EDTA (Na)	.....0.66 gm
Sodium Nitrate	..... 0.66 gm

Besides the above mentioned laboratory prepared chemicals which serve as nutrients, commercial feelings can be used for the mass culture of diatoms and nanoplankters, in open tanks for economy purposes. The media used for the open culture are:

5. Fertilizing Medium

Urea 46	..... 10 mg/l
16.20.0 (NPK)	.....10 mg/l
20.0.0	.....100 mg/l

Growth Phases of the Algal Culture

The usual way of the laboratory culture of phytoplankton is one in which a limited volume of medium containing the necessary inorganic and organic nutrients is inoculated with a relatively small number of cells and these exposed to suitable conditions of light, temperature and aeration. Increase in cell numbers in such a culture follows a characteristic pattern in which the following phases of growth may usually be recognized:

1. Lag or induction Phase: The cells taken from the stock culture room are inoculated to a new flask have to acclimatise the surroundings or in the new medium. Hence there will be no cell division for a few hours and this stage is known as lag phase.

2. Exponential Phase: Once the cells are acclimatized to the surroundings it starts multiplication and grows rapidly. This growing phase is known as exponential phase.

3. Declining Phase: Once the cells reached the maximum concentration, the growth and multiplication will be arrested and slowly show the symptom of declining. This arrested growth of the cells in the culture is known as declining phase.

4. Stationary Phase: After the arrested growth, the culture will be stationary without any further cell division for a few days. In the stationary phase, if the cells get a new environment, they may start further growth and reproduction.

5. Death Phase: After a long period in the stationary phase, the cells may lose its viability and started to die and thus the culture will become useless, either for reculturing or for feeding.

Harvest of the Culture

The fully grown culture should be harvested during the exponential phase of the phytoplankton after determining the cell concentration. If the culture has entered the declining or stationary phase, the metabolite will be very high and the cells may not be in healthy condition. The rearing larval organisms may not show the required growth if fed with this feed.

Preservation of the Culture

The maintenance of the culture and constant supply of the same whenever required is a problem in the hatchery especially during adverse weather conditions. In this case preservation of the algae either by freezing or by sun drying could be done in the sense that during scarcity of the feed, the rearing operations may be successfully controlled. For the method of freezing, the culture has to be flocculated either by adding lime or by adjustment of pH using Sodium Hydroxide. After knowing the quantity of the culture to be flocculated, measure the volume of Sodium Hydroxide solution needed to flocculate to get one degree raise in pH. Suppose the pH of the culture is 8.4, rise to 9.4 by adding sufficient quantity of Sodium Hydroxide solution. After vigorous stirring, leave the culture for one hour to settle the algal mass at the bottom of the tank. Decant slowly the clear water and collect the mass in a plastic bucket. Then bring the pH of the mass to the original level of pH by slowly adding dilute HCl. Now the algae are ready for freezing or sun drying. Drying of the algae can be done by pouring the mass in white enamel trays and keep it in glass bottles. Before freezing the algal mass, some protective reagents like Dimethyl Sulphoxide or Glycerol (few drops) can be added. Then pour the concentrate into polythene bags after measuring. Label the polythene bags and keep the same in deep-freezer. The frozen algae may not have the same protein content as in the live condition. Whenever adverse conditions arise the frozen algae can be used for rearing the larval organisms.

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26 Health Management in Bivalve Larval Rearing Systems

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Suitable hatchery techniques for producing pearl oyster *Pinctada fucata* spat were perfected in India dring 1981 at C.M.F.R.I (Alagarswami *et al.*, 1987). In general, the larvae grow through the straight – hinge, umbo, eyespot and pediveliger stages in the pelagic phase, before metamorphosing into plantigrade and setting on a suitable substratum as spat. Large scale differences in survival and larval growth within and between different rearing conditions were noticed and reflected in several research reports. This could be attributed to health conditions of the bivalve young ones.

The available space, feed and microbial pathogens are critical factors, which determine the survival of bivalve larvae, as their defense mechanisms are age - related. For example, the adult oysters can tolerate exposure to much higher populations of bacteria. *Crassostrea virginia* exposed to high densities of *Aeromoas* and *Vibrio* may be unaffected while the lower concentrations in larvae produce disease and mortality. Though bacteria and protozoa infect the weak and dying larvae, systematic investigations on diseases are scanty. The environmental and other factors responsible for the lower survival rate of larvae have to be monitored so as to devise suitable disease management strategies. Observations also indicated that twenty to thirty percentage of spat production compared to the initial stock at veliger stage could be only obtained in mass production in one-ton capacity tank.

Ex. 1: Vibriosis or bacillary necrosis of bivalve's larvae in culture environments.
Seasonal changes in the abundance of vibrios and other related genera.
The exotoxin produced by Vibrio, kills the developing oyster larvae.
Bacterial swarming around mantle margins of larvae is a reliable indicator of epizootics. Mortality may reach up to 100%.
Out break of vibriosis in oyster larvae, with associated mortalities were correlated with peaks in abundance of Vibrio in inflow water and in culture water.

Ex. 2: *Pseudomonas* has also been implicated in disease outbreaks.
Larvae of *Ostrea edulis*, *Crassossstrea virginica* and *C. gigas* were susceptible. Juveniles were less susceptible.
Effects on larvae vary with the particular strain of isolate of bacteria.
Ex: abnormal embryonic development, leading to incomplete shell formation or velum protrusion, decreased growth or death of larvae in veliger stage.

Three types of pathogenesis could such as:
1. Progressive mantle disruption,
2. Severe velar deformation and damage, and
4. Progressive visceral lesions and atrophy.

Physiological stress during spawning also predisposes them to bacterial infections.

Management by Manipulating the Available Space/stocking Density

The stocking density in the rearing system played a key role in larval development (Krishnan and Alagarswami, 1993). Oyster larvae are generally reared in static water in dense numbers and fed with the required density of unicellular algae (Alagarswami *et al.*, 1987). These conditions are also favorable for the proliferation of heterotrophic bacteria such as *Acinetobacter*, *Aeromonas*, *Pseudomonas* and *Vibrio* (Colwell and Sparks, 1967). All these bacteria are reported as opportunistic pathogens, which induce epizootics in hatcheries. According to Skjermo (1999), the combination of high larval densities, debris from dead larvae and high load of organic matter and bacteria due to addition of live food stimulates selection and growth of such opportunistic bacteria in larval tanks.

In an investigation into the cause of high mortality of the pearl oyster, *P. maxima* in the northwest of Western Australia revealed that the majority of diseased oysters were infected with marine *Vibrio* bacteria. Among them the common isolate *Vibrio harveyi*, induced the disease similar to that seen in the field (Pass *et al.*, 1987). Apart from causing mortalities, bacteria have long been reported to be associated with decreased growth of bivalve larvae. The findings of Lipton *et al.*, (2003) indicated that hatchery production of *Pinctada fucata* was seriously affected by massive larval mortalities caused by *Vibrio* sp.

After a series of research investigations, it is noted that the higher survival of 41.02 % of pearl oyster *Pinctada fucata* larvae could be achieved in low stocking density of 200/L compared to 10.26 and 0.82 % in the increased density of 1000 and 5000 larvae/L respectively at an ambient mean temperature of 28.6 °C. Although the larvae were fed with *Isochrysis galbana* at the recommended cell densities and the rearing pH, salinity, dissolved oxygen contents were similar in the three rearing densities the microbial load was high with 5.8 $x10^3$ cfu/ml in the high stocking density. In the low-density culture system, the microbial load fluctuated between 6.0 x 10^1 and 4.0 x 10^2 cfu/ml. The total number of spat produced in 200 and 1000 larvae/L stocking density was more or less similar. Considering the management methods and cost, the lower stocking density is advantageous as it reduces microbial load and possible water exchange thereby augmenting higher survival as well as spat settlement. It is also probable that the lower growth and lower survival in high stocking densities could be attributed to frequent collisions among larvae and increased metabolites. Thus the lower stocking of larvae reduces the mechanical stress.

Antibiotic Exposure to Minimise Microbial Load in Live Feed

High microbial load noted in the rearing water, tissue samples and in the micro algal feed, resulted in poor spat production of less than 3.0% in *Pinctada fucata* hatchery. In general, the pathogenic microbes invade the hatcheries, by three principal routes viz., the seawater, brood stock and algal food. Prophylactic antibiotic usage has been suggested to reduce bacterial load in live feed. However, the exposure time and the minimum dose of antibiotic agent required to reduce the proliferation of bacteria in the mass culture of micro algae has to be evaluated.

Example:

Experiments were conducted in the Marine Biotechnology Laboratory of Central Marine Fisheries Research Institute, Vizhinjam (South India). *Isochrysis galbana* was inoculated in one litre flask and maintained under constant illumination for growth. Log phase culture (100 ml) was aseptically dispensed in four 250 ml conical flasks. Chloramphenicol (Hi Media) was added at 10, 100 and 1000 mg/L to each 250 ml conical flask respectively and one flask was kept as control along with replicates.

The bacterial load in the *I. galbana* culture was determined by the plate count method. The algal samples were collected aseptically at four different time intervals viz., immediately after the application of antibiotic, after fixed hours. Each sample was serially diluted, plated in nutrient agar and incubated at 37°C for 24 h. The viability of *I. galbana* was examined using hemocytometer at the respective time intervals.

The use of chloramphenicol in *I. galbana* culture resulted in decreased bacterial load with the increase in time (Fig. 1). After three hours of exposure, 88.7, 90.6 and 94.3 % reduction was noted at 10, 100 and 1000 mg/L respectively. The algal cells in the three experimental groups were active. After six hours of exposure, the reduction in bacterial load was 75, 42 and 93 % at 10, 100 and 1000 mg/L respectively. The algal cells at 10 and 100 mg/L were actively moving while at 1000 mg/L 25 % of algae were inactive or dead, indicating the adverse effect of antibiotic. In the control group, bacterial population increased with the advancing culture period. The load almost doubled at the end of 12 h as could be seen from Fig. 1.

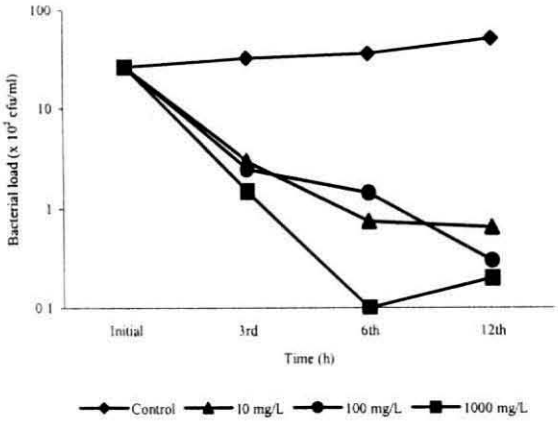

Fig. 1 Effect of antibiotic on the reduction of bacterial load in *Isochrysis galbana* at different time interval

Management by Incorporating Probiotic Microbes

Experiments indicated that hatchery reared *Crassostrea gigas* larvae are susceptible to attack by three strains of sucrose fermenting *Vibrio* initially isolated from diseased larvae. Administration of 10^5 cells/ml of any of these strains led to the collapse of culture within 48 hours. Larval oysters (*Crassostria virginica*) could be experimentally infected with isolates of pure cultures of marine Vibrio species. All the inoculated groups demonstrated decreased growth and/or high mortality.

These research findings indicated that the artificial production of bivalve seed has been seriously affected by the occurrence of massive larval mortalities of which one probable cause has been infection by Vibrio species. One of the usual methods of controlling proliferation of pathogens in hatcheries has been by using antibiotics. Though these antibiotics have been used as feed additives, the associated toxicity, allergy, residues in food and resistance obtained after long-term administration of low doses makes their use worthy of second thought. Also the indiscriminate use of broad – spectrum antibiotics may alter the normal gut flora by suppressing its growth and cause an over growth of pathogenic bacteria. Hence, the use of probiotics such as food additives is preferred over the use of antibiotics.

Jory (1998) defined probiotics from aquaculture point of view as culture (single or mixed) of selected strains of bacteria that are used in culture and production systems (tanks, ponds and others) to modify or manipulate the microbial communities in water and sediment, reduce or eliminate selected pathogenic species of microorganisms, and generally improve growth and survival of the targeted species.

The mechanism of action of probiotic, which include depletion of nutrients, production of acids and antimicrobial substances as well as competition for adhesion receptors in the intestine and immunostimulation create an environment incompatible to the growth of pathogens. Apart from this, very little work has been carried out on the effects of potential probiotic strains on bivalves. The research work by (Riquelme *et al.*, 1997) revealed that among a total of 506 bacterial isolates, obtained from laboratory and hatchery sources, one strain (*Vibrio* species), when used as a pre-treatment, protected the scallop larvae against subsequent experimental infections with the *Vibrio anguillarum* – related (VAR) larval pathogens. Preliminary investigations in CMFRI indicated that the probiotic bacteria *Lactobacillus* offered good health and survival rate to the pearl oyster larvae even in adverse/unfavorable conditions

Therefore further research is needed towards standardization of this beneficial practice of addition of probiotics. The first question unanswered in many cases, is the fate of the probiotics in the rearing medium and in gastrointestinal tract. In this context, immunological and molecular probes will be useful tools to trace the probiotics cells. It is essential to investigate the best way of introduction and the optimal dose of the probiotics. Technological solutions are required, especially to keep the probiotics alive in dry pellets. The potential strains of probiotics species of microorganisms have to be identified and evaluated through extensive trials.

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27 High Density Phytoplankton Cultures and use of Probiotics in Bivalve Larval Rearing

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Introduction

Unicellular marine algae are widely used as food in the hatchery production of commercially valuable fish and shellfish. Bivalves and their larvae feed by filtering them from seawater. Rotifers and brine shrimps also ingest microalgae, which are then used as food for larval fish and prawns. In some systems algae are added to the water containing fish or prawns to improve quality.

Microalgae can be cultured using closely controlled methods on laboratory bench top, with a few litres of algae, to less predictable methods in outdoor tanks, containing thousands of litres. Coutteau (1996) described three basic types of phytoplankton culture systems. a) **Batch culture** is a system where the total culture is harvested and used as food. b) **Semi-continuous culture** is a system where part of the culture is harvested and used as food and the amount taken is replaced with fresh culture medium. After allowing 2-3 days for the remaining cells to grow and divide, the process is repeated. c) **Continuous culture** in which the number of algal cells in the culture is monitored and as the cells divide and grow an automatic system keeps the culture density at a pre-set level by diluting the culture with fresh medium.

Although batch culture is relatively easy to carryout, its efficiency is very poor and the cultures are prone to crashes. Considering the advantages of continuous and semi-continuous culture systems over the traditional batch culture systems, a number of workers (Persoone and Sorgeloos, 1975; Boussiba et al., 1988; James and Al-Khars, 1990; Fabregas et al., 1996) have reported on several designs for continuous production of algae in high densities. Published works on microalgal culture in India are sparse. Gopinathan (1982) has described the batch culture method for culturing marine phytoplankton for use in shellfish hatcheries. In batch culture technique adopted presently in various Indian hatcheries and laboratories, production of microalgae is highly inconsistent, with frequent collapse of cultures due to ciliate infestation and consistent high production are never achieved.

Using 2.5 times the normal nutrient concentration and limited supply of CO_2 the culture density could be increased from an average 1.5 million cells/ml concentration in traditional batch culture systems to an average of 13.7 million cells/ml in continuous systems (Lambade and Mohamed, 2002). Besides the increased biomass production, the duration of culture was enhanced to 30 days without crashing. The semi-continuous system was up-scaled (to 60 l capacity) with an internal illumination system to yield 528 litres of average 3.2 million cells/ml within 36 days. The velocity of cell growth during the logarithmic phase in

doublings/day ranged from 0.21 in control (batch culture) to 2.51 in the up-scaled semi-continuous system. Furthermore, the costs of production, even with additional inputs, are comparable or even less than the traditional batch cultures. The economic efficiency was highest in continuous systems as compared to semi-continuous systems (Rs. 0.03 and 0.017 /billion cells as against Rs. 0.082 and 0.037 /billion cells).

The results of this study reveal that algal cultures in the laboratory can be more efficiently carried out using continuous and semi-continuous systems. The advantage of sustained higher biomass production and use of limited laboratory space are evident. Moreover, the costs of production, even with additional inputs, are comparable or even less than the traditional batch cultures.

Table 1. Details of production, input costs and cost of production per billion cells of *Chaetoceros* in different treatments. Assuming charges are same for facilities like container, treated seawater, aeration, illumination, etc. (From Lambade & Mohamed, 2002)

Experiment/ Particulars	**Batch culture (Control)**	**Outdoor batch culture (Control)**	**Cont. with CO_2**	**Cont.**	**Semi-cont with CO_2**	**Semi-cont.**	**Semi-cont culture Outdoor (60 litres)**
Duration (Days)	30 (5 batches)	36 (6 batches)	27	21	30	26	36
Total volume harvested (litres)	20	360	11	8	26.2	17.5	530
Average cell density (million cells/ml)	1.5	1.0	13.7	8.5	4.2	4.0	3.2
Total cells harvested (Billion cells)	30	360	150	68	110	70	1696
Cost (Rs.)of production							
1) Chemicals	1.1824	21.78	1.6258	1.1824	3.8724	2.5865	78.334
2) Carbon dioxide	-	-	2.916	-	5.832	-	12.000
Total	1.1824	21.78	4.5418	1.1824	9.7056	2.5865	90.334
Cost (Rs.) of production per Billion cells	**0.039**	**0.059**	**0.030**	**0.017**	**0.082**	**0.037**	**0.053**

Use of Probiotics

The origin of the term probiotic is attributed to Parker (1974) who defined them as organisms and substances, which contribute to intestinal microbial balance. However, the concept of microbial manipulation was first appreciated by Metchnikoff during the early 1900s when he viewed the consumption of yoghurt by Bulgarian peasants as conferring a long span of life. Although evidence for a link between longevity and ingestion of fermented milk products has not been proven yet, some workers have claimed that its therapeutic value is related to viable bacteria, in particular *Lactobacillus* sp. Although a strict definition of probiotics is difficult to come by, Tannock (1997) proposed it as "*living microbial cells administered as dietary supplements with the aim of improving health*". Gatesoupe (1999) reviewed the state of probiotic usage in aquaculture and stated that the first application of probiotics in aquaculture is relatively recent, but the interest in such environmentally friendly treatments is increasing rapidly.

There now exist a growing number of scientific papers, which deal specifically with use of probiotics in aquatic animals. Yet, more questions have been raised as to whether probiotics have any relevance in the aquatic environment (Gatesoupe, 1999). Aquatic animals are quite different from land animals for which the probiotic concept was developed. Live-bearing endotherms undergo embryonic development within an amnion, whereas the larval forms of most fish and shellfish are released into the external medium at an early ontogenetic stage. Thus the latter are exposed to all types of microflora available in the medium, while the former develop a particular type (obligate or facultative anaerobes) of gastrointestinal microbiota. Most identified probiotics belong to the dominant or sub-dominant genera of *Bifidobacterium*, *Lactobacillus* and *Streptococcus*. On the other hand environmental microbes like *Vibrio* and *Pseudomonas* are the most common genera in crustaceans (Moriarty, 1990), marine fish (Sakata, 1990) and bivalves (Prieur et al., 1990).

Although the use of probiotic bacterial strains in microalgal cultures does not come within the strict definition of probiotic usage, recent work by Avendano and Riquelme (1999) and Gomez-Gil et. al., (2002) has shown the significance of such probiotic addition in marine larviculture. One of the obvious advantages of such treatments is that microalgal cultures can be used as vectors for the delivery of bacterial antagonists to bacterial pathogens in marine larviculture.

Avendano and Riquelme (1999) established the feasibility of incorporating bacteria with the ability to produce inhibitory substances (BPI) into axenic cultures of *Isochrysis galbana* with the object of using this microalga as a vector for transmitting BPI into cultures of larval bivalves as antagonists of pathogenic bacteria in these cultures. As a first step, the ability of seven strains of BPI to grow in extracellular products of *I. galbana* was evaluated, with positive results with four of these (334,C33, 11, and 77). Subsequently, the effect of the addition of these strains on the growth of I. galbana was evaluated. Comparison of growth rates of I. *galbana* with and without the addition of BPI showed no significant differences ($P>0.05$). A stable and persistent inhibitory capacity of strain C33 on the pathogen *Vibrio anguillarum* was also observed. Finally studies were made on the ingestion of BPI by larvae of *Argopecten purpuratus* (Lamarck 1819). Results demonstrated significant ingestion of strain 11 ($p>0.05$), when it was inoculated directly into the water, and bacterium C33, when delivered in conjunction with the microalga. Upon evaluating incorporation and maintenance of BPI strains 11 and C33 after 5 days of larval culture, we observed the major presence of strain C33 ($3x10^2$ CFU/larva) compared with strain 11 (90 CFU/larva). The results obtained suggested that it was feasible to use microalgal cultures as vectors for the introduction of bacterial antagonists to bacterial pathogens in molluscan larval culture.

Gomez-Gil et. al., (2002) studies made to evaluate the performance of the microalga *Chaetoceros muelleri* then cultured with a potential probiotic bacterium *Vibrio alginolyticus* strain C7b as compared when both are cultured alone in medium f/2. Strain C7b grew significantly better and lasted longer when grown with the microalga than when grown alone. The microalgal density was not affected by the presence of the bacteria compared when grown alone. *C. muelleri*

and the bacterial strain C7b can be cultured together for up to 9 days to achieve a high density ($5.15x10^6$ and $6.63x10^4$ cell/ml, respectively) and then fed to the protozoeal and mysis stages of penaeid shrimp.

A recent study by Rajiv (2003) showed that generally, more number of bacterial colonies was observed in *Isochrysis galbana* than in *Chaetoceros* cultures. In both these cultures, the mean total aerobic count was less (10^3-10^5 CFU/ ml) during the exponential and stationary phases than in the declining phase (10^6-10^7 CFU/ ml) when the cultures were either senescent or dying. This study therefore shows that if microalgal cultures are used when they are in the log and stationary phase rather than in the declining phase, the amount of bacterial added to the larval culture medium can be reduced by 3-4 orders of magnitude.

In both the algal species tested the Simpson Diversity index was at the maximum at peak log phase, indicating that one or two species were dominating the bacterial community in the algal culture medium when the growth rate was high. The Margalef's species richness index showed an inverse proportionality with Simpson diversity in *Chaetoceros* culture. This relationship was not evident in *I. galbana* culture. The hierarchical cluster analysis clearly established the dissimilarity in bacterial taxa occurring during the different phases of growth of *Chaetoceros* and *I. galbana*. There was marked clustering of bacterial taxa during the initial lag phase, early log phase and peak log phase and death phase.

The addition of the probiotic yeast *S. boulardii* as a single addition to *Chaetoceros* culture resulted in significantly ($P < 0.01$) improved (162% increase in maximum algal density) algal growth rates with prolonged stationary period when compared to the control. The daily addition of the same yeast yielded very poor algal density.

The addition of the probiotic yeast *S. boulardii* as a single addition helped in keeping low the total aerobic bacterial count in the medium to between 10^4 and 10^5 CFU/ml as compared to control, which had counts of $10^7$and 10^8 CFU/ml. *S. boulardii* treatment as a single addition also helped to keep the vibrios in TCBS at lower level than control (10^2 vs 10^4 CFU/ml on Day 21 and nil on Day 28). The mean total aerobic flora showed a steeply increasing trend in control and DA treatments, while the trend in SA treatment was that of slow increase.

In hierarchical cluster analysis there was a marked increase in the similarity percentage of clusters indicating a much better discrimination of bacterial taxa in treatment SA as compared to control. It is likely that such heightened discrimination helped in prolonging the culture in SA treatment.

28 Rearing of Baby Chanks and Mark – Recovery Studies

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The sacred chank, *Xancus pyrum* is a gregarious, large, marine gastropod and its dwelling places form distinct chank beds (Nayar and Mahadevan, 1974, Lipton *et al.* 1996a). In addition to the ornamental purposes, the recent demand for chank shells, flesh and operculum led to the increased exploitation. Chank flesh is rich in protein and minerals (Chari, 1966) and the values compared favorably with those of fishes.

In the live condition, the shell of the chank is covered by a surface skin, called periostracum, which protects the shell from several environmental factors including corrosive effects. The periostracum in live animals is brown in colour, soft and velvety, which peels off after the animal dies. Upon removal of the periostracum, the shell shows its characteristic milky white appearance.

Although, there are restrictions by the respective state Fisheries Departments, specific exploitation of chanks by long-lines in Kerala (Appukuttan *et al.* 1980) and by modified trawl nets along Rameswaram coasts in Tamil Nadu (Lipton *et al.* 1996 b) have been reported. Such intense bottom trawl activities also led to depletion of population of chanks in the traditional chank bed areas.

Chank Bed Areas

In Gulf of Mannar and Palk Bay, the depth ranging from 5 to 6.5 m with substratum such as dead coral reefs, sand mixed with mud and algae supported the chank settlement and the resulting chank bed area. In addition to the traditional practice of chank diving, chanks were also exploited using a modified trawl net (=chanku madi). The details are presented in a paper (Lipton *et al.* 1996 b). With the operation of such modified trawl nets (which comprises large number of sinkers), the bottom biota is disturbed. Discussions with the traditional chank divers revealed that in Rameswaram area six traditional chank beds (`paars') adjacent to the coral reefs are totally destroyed by the operation of the `chnku madi'. During the chank diving season, which extends from January to March, they find almost barren seabed, which was earlier flourishing with chanks, holothurians, corals and other mollusks. In addition, the size of chanks obtained from these chank bed areas are also decreased and thus fetch lesser rates. This information is very important in the conservation aspects of chanks.

Morphometric Characteristics of the Sacred Chank

The morphometric measurements revealed two well distinguished sub-species of the chank viz., *Xancus pyrum* var. *acuta and Xancus pyrum* var. *obtusa.* In the *Xancus pyrum* var. *acuta*, the profile of whorls in the spires is convex. In the

case of *Xancus pyrum* var. *obtusa*, the profile of whorls in the spires in very short and the shell appears as a `top'. In addition to these two well marked sub-species, which are also recorded earlier in literature, there are two more sub-species could be distinguished viz., *Xancus pyrum* var. *comorinensis* and *Xancus pyrum* var. *irupiravi.* However, it could be inferred from the data on the collection of chanks that these two latter sub-species formed less than 5.0% of the total chanks either landed or collected by diving.

Breeding of Chanks

The laboratory-reared (maintained) adult chanks exhibited breeding behaviour during the different months. Upon close observation on their breeding behaviour, the males and females can be marked individually and subsequently transferred and reared in the `brood stock' tanks. The `brood tanks' are made of FRP with a water holding capacity of 500 lit. Washed sand was provided at the bottom of the tanks up to 20 cm as substratum. Seawater flow rate was adjusted at a rate of 500 ml/ min. They were fed *ad-libitum* with live clams (*Donax cuneatus* and *D. faba*). The sand substratum was changed every month. During the breeding time, the mating behaviour was recorded carefully. After their mating, the females start releasing the characteristic `ram-horn' shaped egg capsules. The release of egg capsules by the female chank takes a few hours to almost 3 days in some cases. Initially, they secrete a holdfast and paste it to the bottom surface of the tank. Then the female (mother) secretes and makes individual chamber and carefully lays the eggs in to the chamber, which is sealed and this process is repeated till the eggs last. Subsequent to the complete release of the egg capsule the egg capsule stands erect.

In general, the mean length of egg capsules of *Xancus pyrum* was about 224 mm, depending on the size of the mother chank. The width of the egg chamber ranged from 9.64±0.81 (minimum) to 33.0±4.79 mm (maximum). Examination of the total number of chambers in each capsule indicates that they vary between 20 and 33 per capsule. From each egg capsule, 99 to 275 (average 222) babies hatch out.

Release of Baby Chanks from the Egg Capsules

Depending on the hydrological conditions of the water and after 30 to 35 days of release of egg capsules, babies hatch out from the egg capsules. Regarding the hatching mechanism, the juveniles of *Xancus pyrum* rasp the wall of egg chamber with their radula and then come out from their respective chamber. The juveniles of *Xancus pyrum* are benthic and very active in creeping movement. At the time of their release, the baby chanks actively move on the surface of the egg capsule and subsequently on the substrata of the rearing tanks.

Rearing of Baby Chanks

The babies of the *Xancus pyrum* are carnivores. They feed on very small/young ones of polychaete worms up to 2 months. After two months, according to the size of baby chanks they prey on live earthworm and Neries. After

eight months, the baby chanks feed live clams. The growth obtained in experimental studies is given below:

Growth of Baby Chanks, *Xancus pyrum*

The baby chanks, which hatch out from the egg capsule, are of about 09.09 mm in length. After 120 days, they attain an average length of 42.88 mm and after eight months they reach 53.66 mm in length. They attain about 62.0mm after one year of their release from the egg capsule.

Tagging and Recapture

In order to detect the natural growth rate and the migratory behaviour the chanks were tagged using Letro labels with araldite and sea ranched in the Gulf of Mannar and Palk Bay. The results indicated that:

- With the relaxations of chank fishing restrictions, the sacred chank is over-exploited (using modified trawls).
- It is a non-migratory species, which lives in restricted chank beds.
- The sacred chank, *Xancus pyrum* is a slow growing species with an MSD-wise growth is about 8.0 mm/year.
- Its fecundity is also not very high and it breeds once in a year.

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29

Oyster Farming Methods

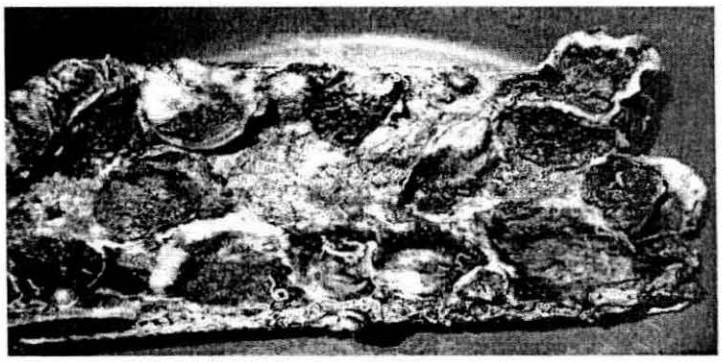
P. Muthiah,
CMFRI, Tuticorin.

Edible oyster is one of the most widely cultivated bivalves. As early as the first century BC, the Romans practiced simple method of oyster culture by collection oyster seeds and growing them for food. The important oyster producing countries are Japan, Korea, France and China and together they contribute 78.7% of the total oyster production by culture. In India, Hornell (1910) initiated experiments on spat collections of oyster *Crassostrea madrasensis* at Pulicate Lake. Realising the resources potential, nutritive and commercial values of edible oysters the Central Marine Fisheries Research Institute made attempts to evolve suitable farming techniques for edible oysters from 1970.

The technique of oyster farming involves two important phases namely 1. Oyster seed collection/production, and 2. Rearing seed oysters to marketable size.

1. Seed Collection from Wild

The seed required for culture is met either from natural spat collection or through hatchery system. For collection of spat from nature suitable spat collectors or cultch materials are provided at appropriate time. The spat collectors should be able to retain the oysters till they reach marketable size or upto the size at which they could be scrapped for further rearing. The choice of spat collectors depends on the culture method adopted, local availability, and economic and practical consideration. In culture experiments at Tuticorin, cultch materials viz. semi-cylindrical roofing tiles, oyster, mussel and coconut shells, asbestos sheet, netlon and automobile tyre pieces were used. The tiles are given lime coating for roughness. The oyster shells are made into strings on a GI wire or synthetic rope. The collectors are laid on the racks. Of these collectors, lime coated shell (with an average of 34 spat/tile) (Fig.1) and oyster shell (with an average 7 spat/shell) were found suitable for large scale spat collection from wild.



Spat settled on lime coated tiles (average 35 per tile)

2. Spat Fall Prediction

The prediction of spat fall is essential for collecting seed oysters in the appropriate time with minimum foulers interference. This time is called as cultching time. The prediction of spat fall is based on the study of maturation and spawning of ripen gonads in the oyster population or by the appearance of oyster larvae in the plankton samples of the area. The collectors are exposed just a week before spawning period. Large scale spat collection experiments showed the abundance of seed oysters in intertidal areas, creeks and bays. The method, season of spat collection and the type of spat collectors to be used vary from place to place, depending on the local conditions.

3. Seed Production through Hatchery System

On the establishments of a shellfish hatchery in 1980, the Central Marine Fisheries Research Institute succeeded in mass production of both clutched and cultch free spat.

4. Site Selection

- The following requirements are essential in the selection of farm site.
- Sheltered areas offering protection from strong wave with a depth ranging from 2-5 m.
- Salinity range of 22 to 35 ppt.
- Temperature range is 21-31°C
- Area with pollution free water.

5. Methods of Culture

The farming methods are broadly divided into i) on-bottom and ii) off-bottom culture; the seed oysters are sown on the ground. This method is substrata specific and the area sown is free from silting and predators. When oysters are grown by off-bottom methods, the advantages lie in better growth and good condition of the meat. The methods involved in off-bottom culture are 1) rack and tray 2) rack and string 3) stake and 4) raft

On-bottom Culture

This method is practiced in France. The seed oysters are sown on the ground. This method is substrata-specific and the area should be free from silting and predators. In France oyster seed are stocked at the rate of 20-50 oysters/m^2 in the intertidal culture areas called 'parks' of 0.5 – 1 ha. The production rate is 0.9 kg/m^2. In America, oyster seed are sown in the sub-tidal areas with a depth of 5-18 feet.

Off-bottom Method

The methods involved in off-bottom culture are (1) rack and tray (2) rack and string (3) stake and (4) raft.

Rack and Tray Method

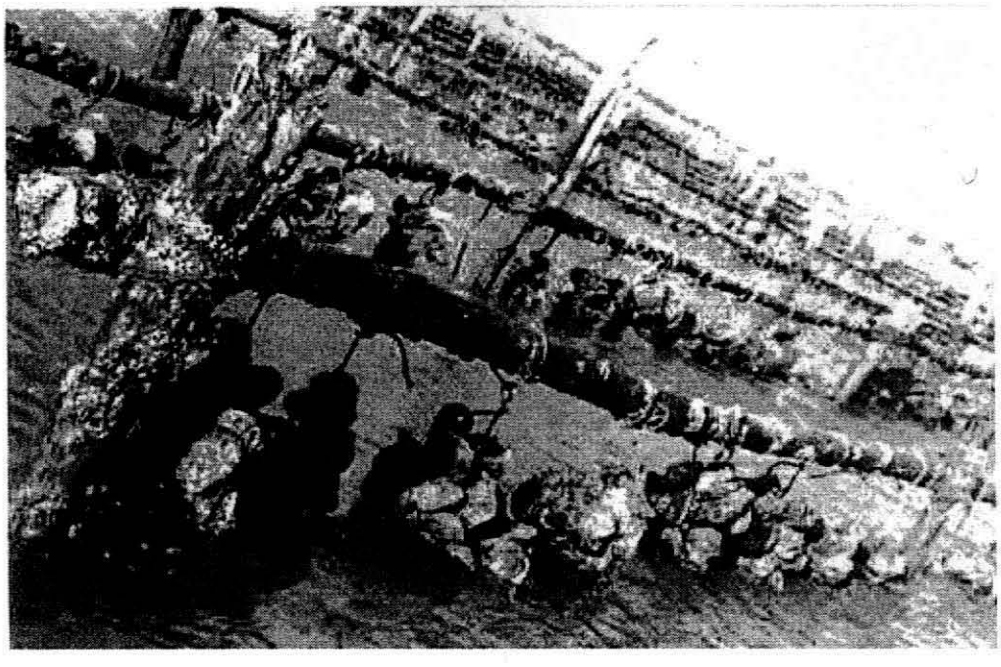
The spat attached on lime coated tiles on attaining 25 mm were scrapped or cultchless seeds produced in the hatchery are stocked in box cages. The cages are of 40 x 40 x 10 cm size made of 6 mm mild steel rod and webbed with 2.5 mm synthetic twine. For nursery rearing of hatchery produced cultch free seed (of 5-10 mm) the cages are covered with velon screens. The cages are suspended from single rack system. After 2-3 months rearing, oysters of 50 mm and above are transferred to reactangular trays. Each tray is of 90 x 60 x 15 cm size accommodating 150 – 200 oysters. Twenty such trays are placed on a rack. Rack, a wooden platform for placing the rearing trays is constructed using eucalyptus poles (Fig.2). Each rack occupies an area of 25 sq.m and holds 3000 – 4000 oysters. The estimated production rate is 110 t/ha (Fig.2).

View of Oyster farm by Rack & Tray method Production 110 t/ha (Meat 9 ton)

Rack and String Method

The oyster spat collected on shells are made into strings having six shells using 5-6 mm synthetic rope. These strings 2-3 are kept inside a velon screen bag and suspended from racks for nursery rearing. Racks for this purpose are a series of vertical poles driven into the bottom in rows and horizontal poles are connected on

top of the poles. The oyster shell strings are suspended from the horizontal poles of the rack with a space of 10 cm between two strings. The production rate is 80 t/ha. Total racks is one ha is 125. No. of strings/rack = 90 No. of oysters/string = 40 Fig.3



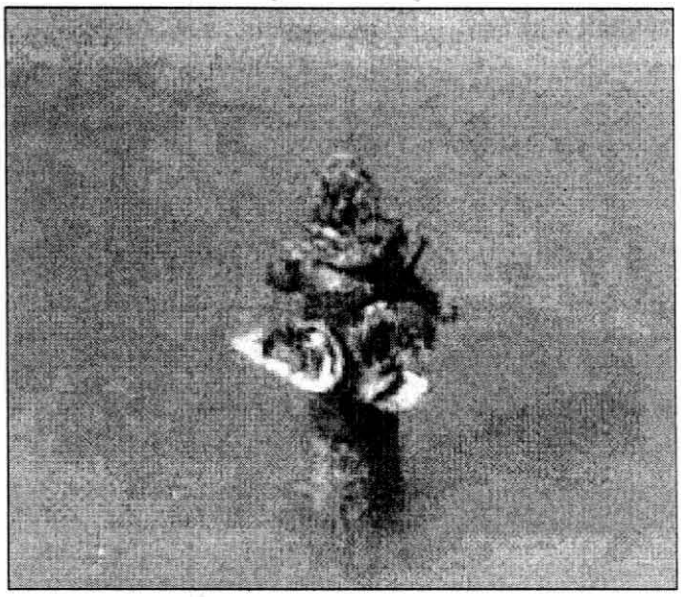
View of rack and string farm production rate 80 t/ha

Stake Method

Stake method is adopted if the culture site is soft and muddy. Each stake, casuarina or eucalyptus poles of 1½ m length with a nail on top and two nails on the sides is driven into the ground. The nail holds a shell with spat. To protect the spat against crab predation, initially the top of the stake is covered with a piece of velon screen. Once oysters attain 25 – 30 mm the velon screen is removed and oysters are grown on stakes upto harvestable size of 70 – 80 mm in length.

No of spat / shell	= 16
No of oysters / stake	= 14
No of stakes / ha	= 17500 – 18000
Production rate	= 20 t / ha

Fig. 4



3 to 4 Shells nailed on a stake and erected in muddy bay

Raft Method

In this method, oysters are suspended from floating rafts. Rafts are constructed using bamboo or wooden poles and are floated with empty oil drums or wooden barrels. Once raft is positioned by anchors, shell strings with attached spat are hung from raft. Where wave action is more, series of small floats are joined by synthetic ropes. The line is anchored at both ends. From the ropes, the strings are suspended. These methods are also called as floatation methods. The production rate is 12-15 kg/ m^2.

6. Growth of oysters

Stage	Growth rate (mm/month)
Initial growth Upto 4 months	10.2 – 10.6
4 – 8 months	6.7
8-12 months	5.5
Overall growth rate	6.6 mm/month

7. Foulers, Predators and Diseases

Fouling organisms such as barnacles, ascidians, sponges and algae settle on rearing trays and oyster and compete for food and space. They are periodically cleaned. Woodborers like *Martesia* sp. and *Teredo* spp. Damage the wooden farm structures. Crabs, fishes, starfishes and gastropods are the oyster predators. Predatory gastropods Cymatium spp. Causes 13% mortality of oyster in the farm. Apart from these, diseases caused by Haplosporidians such as Perkinsus *marinus, Minchinia spp.* Cause considerable large scale mortalities of oysters in temperature waters. Some of the termatodes notably Bucephalids cause castration of gonads.

8. Harvesting

Oysters reach harvestable size (above 80 mm) within 10-12 months. They are harvested when the condition of meat reaches high value. The condition factor of *C. madrasensis* at Tuticorin reaches maximum of 170 during pre-spawning periods (February-March and July-August) Harvesting is done manually.

9. Depuration and Shucking

Harvested oysters are kept for 10 – 12 hours in the tanks under a flow of filtered seawater. As a result the bacterial load of the shellfish is reduced. The depurated oysters are taken for shucking. Shucking is the removal of meat from the oyster. Depurated oysters are kept in a gunny bag and held for 3 minutes in boiling seawater. This treatment makes the meat removed easy with a shucking knife. Shucked meat is washed and dipped for 10 minutes in salt solution containing citric acid. The meat is weighed and packed in polythene bags as 2 kg units. These are quick frozen at -30°C, using horizontal contact plate freezer. The frozen meat is transported to canning factory and marketing. Live oysters could also be transported them in wet gunny bags.

10. Utilisation of oyster shell

Since oyster shells on ignition contains 52% Calcium oxide are used in manufacturing calcium carbide & lime. The shells crushed to suitable size are used as grit along with the poultry feed.

30 Remote Setting and Upwelling Systems

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Introduction

The first report of successful artificial oyster spawn was in 1879 when Dr. Brooks produced "free-swimming" oyster larvae by stripping eggs and sperm from ripe adult oysters. Following this several other researchers attempted to rear larvae in their laboratories; however, it was not until 1920 that W.F. Wells successfully reared and set oyster larvae. The development of modern shellfish hatchery methods has evolved over the last hundred years. To solve the problems related to transportation of huge quatities of shell clutches, the modern method of remote setting was developed. Remote setting is the method of transporting eyed larvae of bivalves in cool and moist condition to distant areas where ambient conditions are provided to setting. The rapid growth of remote setting in the last few years has now almost replaced the unreliable and costly collection of natural spat. Currently, over 40 remote setting operations are in production within British Columbia, more than any other region of the world.

Bivalve Larval Settlement

Oyster larval settlement involves two phases:(1) attachment to substrate and (2) metamorphosis. Settlement is a general term to describe the transition from larva to juvenile. During transition larvae cease feeding, search for a suitable substrate and finally attach to their chosen location. At the time of attachment metamorphosis is initiated.

After the larva has attached to the substratum, the velum and foot are no longer needed. Their disappearance marks the transition from free-swimming to a sedentary mode of life. During the metamorphosis the larval organs disappear and there is an anatomical reorganization of the permanent organs. At this time the relative size of the organs and their orientation are changed. The recent practice of remote setting of larvae a considerable distance from hatcheries or natural setting areas has caused dramatic changes in the oyster industry. Since many growers shuck their own oysters, large amounts of discarded shell are on hand for cultch. In the past this material had to be hauled to and from the natural setting locations or hatcheries. Now, instead of hauling cultch long distances, cultch can be artificially set with spat close to the grow-out site.

Setting Methods

Ready-to-set larvae are now available to remote setting operations from several hatcheries. The basic method for producing setting larvae is as follows though the basic procedure of oyster setting is frequently fine-tuned by hatcheries and setting operations to suit. Prior to shipment larvae are sieved out of the tank, wrapped in nylon cloth and moist paper towels to prevent dehydration and transported in an insulated container at 50 C. Two and a half million eyed-larvae are the approximate volume of a golf ball. The study by Carlson (1981) showed that holding eyed larvae in this way at 50 C for five to eight days before setting appears to have no effect on the viability of the larvae, However, this length of storage is not recommended since this stress may contribute to post-set mortality problems.

Larvae should be put into the setting tank of seawater within 24 hours of leaving the hatchery. Setting normally starts as soon as the larvae are put into the setting tank with cultch, which can be either oyster shell or plastic collectors. For best success, cultch should be aged for one year in the ocean and be thoroughly cleaned. Some growers add algae to their setting water, although the feeding of setting larvae hasn't yet been proven to be beneficial. When metamorphosed larvae are fed there is greater initial survival. After the setting process is complete, the cultch is placed on the beach in the inter-tidal zone (covered to protect from the effects of the sun), or hung subtidally.

Seawater used for setting should be filtered with a 50 or 100 micron filter bag or sand filter while the larvae are swimming. After the set has occurred, raw or filtered seawater is pumped into the tank to feed the larvae. If algae are being cultured, it is usually added at a concentration that the larvae can consume in less than a day. It has not yet been proven whether feeding with algae improves the setting density but some growers consider it to be a good idea and is not harmful if done properly. Aeration can be provided by any compressor, air pump or blower that does not produce an oil mist. Oyster larvae do not require aeration for extra oxygen, aeration is provided to create water movement to evenly distribute larvae throughout the tank and to prevent uneven heating of water. The air pump must be positioned above the setting tank so that it will not fill with water when shut off. Tank water should be only aerated gently, not at a boil and only while adding larvae or algae or intermittently during setting. Excessive aeration can cause the larvae to separate out of the water into the foam.

Cultch (whatever is used for a setting substratum) must be clean and leached for one year or longer by pre-exposing it to seawater or by placing it high in the intertidal.zone. During the conditioning process the cultch becomes covered with a thin film of bacteria or micro fouling that seems to attract the larvae at time of settlement.

Set Inducers

Once the environmental conditions are satisfactory for larvae to set, competent larvae appear to respond to a chemical cue to settle and attach themselves. In nature this cue is reported to be a pigmented bacterium called LST,

which adheres strongly to surfaces like oyster shell. Simple chemical compounds trigger attachment and metamorphosis of many larval marine invertebrates. DOPA (L-3, 4-dihydroxyphenylalanine), an amino acid, has been used to induce the setting of oysters. The use of chemical cues appears to be most applicable to cultchless setting systems where there is no natural attractant.

Cultch Material

Different materials are used as cultch material. The most common cultch used by the oyster industry is given below.

- Shells: Vexar bagged shells are used as the cultch material, shells must be clean and aged for at least two year s before being used.
- French plastic pipe: These are 2 meter pipes with 25mm diameter (hollow tubes) with longitudinal groves 2mm deep on the outer surface. With the older pipes (ones that have been used before) it is possible to get sets of 2,000 to 3,000 larvae per pipe. New pipes leach for about two or three months and the maximum setting have been found to be 400 to 500.
- Shell chips: The shell chips are spread over the tank bottom and the larvae added to the tank. This is a simple and easy method of producing single seed.
- 'Chinaman hats" (to produce single seed oysters): These plastic spat collectors are dipped in a slurry of cement at least six months prior to setting. The mixture of the slurry is 1/3 Portland cement to 2/3 sand. Water is added till it has a consistency that will leave a 1/8 inch coating on a stick.

Metamorphosis is a critical stage for oyster larvae because this is the point at which mobility is lost and the internal organs are modified to adapt to a sedentary existence. This process of change in morphology reduces the ability to filter feed for 24 to 48 hours and as a direct response to the reduced feeding activity growth rates are also reduced.

Recommended Algal Feeding Rates

A recent development in the feeding of bivalve larvae is the use of centrifuged algae subsequently made into a paste. Algae paste is made by passing dense algae cultures through a continuous flow centrifuge called a separator or clarifier. This spins the algae cells out of the culture water and deposits them on the rotating bowls of the clarifier. These packed algae cells have the consistency of toothpaste and can be refrigerated for several weeks without any loss of nutritional value. *Thalassiosira pseudonana* clone 3H), a diatom, is an excellent food for large larvae and spat. One liter of 3H will spin down to 0.2 grams of paste. One gram of paste contains roughly 1X10, or 10,000,000,000 cells.

Larvae Density

The number of larvae added to the tank depends on the density of set required. Since most growers seem to prefer 10 to 20 spat per shell, we recommend a larval density of 150 larvae per shell. A tank containing 300 cultch bags would

take 5 million larvae. Underestimating the amount of larvae needed for a set is a common mistake made by beginning setters. The optimum density on unbroken seed was determined to be between 20 and 25 spat per shell. These survival studies also found that initial density must be at least 6 spat per shell to be economically viable.

Post-Settlement Handling and Survival

Postset survival is the largest problem facing the remote setter. The main facters affecting the spat are heat and drying during transfer from the set tank to the nursery area. Freshly set spat can be killed in less than an hour at room temperature by drying. Some cultch types hold moisture better than others. Freshly set spat are very delicate and severe mortalities can be caused by exposure to heat and sun when the cultch is removed from the setting tank. The transfer should be done in cool temperatures, (early mornings or rainy weather) and as quickly as possible.

In the absence of silt, seed mortalities could be substantially reduced. It appears that during times of poor seawater quality, survival is better if the spat are kept in the intertidal zone rather than hanging subtidally.

Remote setting method has helped to revitalize the oyster industry. Larvae users are apparently learning from their past mistakes and now the mortality problems that occur are usually post set problems. The most common setting problems are still: (1) toxicity of the tank surface, (2) cultch condition (too dirty or not conditioned) and (3) water temperature (too hot or too cold). Several years ago the future of oyster farming seemed dependent on a "reliable source of seed". Now that there is an adequate supply of larvae and the methods and procedures for its use are established and results consistent, the new problem appears to be water quality degeneration

Floating Upweller Shellfish Nursery System

Land-based upweller nurseries for shellfish culture have been around since the 1960's.Upwelling is an efficient way to pass water vertically through a three dimensional mass of shellfish seed resting on a mesh in order to culture them from a hatchery size to a field nursery orgrowout size during the first season of growth. Land-based systems use valuable waterfront real estate, operate at relatively high heads, which require expensive to operate centrifugal pumps and are limited in the number of seed the shallow "silos" of the majority of such systems can hold. A floating upweller system (FLUPSY) takes the silos and places them floating just above the surface of a water body or floating tank, thereby reducing the "head" that makes pumping water so expensive in land-based systems. (FLUPSY)'s move water through the use of tidal flow, airlifts, paddlewheels or pumps.

31 Bivalves and Harmful Algal Blooms

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Introduction

An algal bloom is the rapid growth of one or more species which lead to an increase in biomass of the species. Its defined as 'those which are noticeable, particularly to the general public, directly or indirectly through their effects such as visible discoloration of the water, foam production, fish or invertebrate mortality or toxicity to humans' (ICES, 1984). It is estimated that globally approximately 300 people die due to consumption of shellfish contaminated with toxin produced by phytoplankton.

Of the 4000 marine planktonic microalgae described to date, approximately 80 toxic species and 200 noxious species have been implicated in the formation HAB's. Sournia (1995) has remarked that the major toxin producing algae are dinoflagellates followed by diatoms. Though toxic blooms have been known to occur for centuries, there has been a phenomenal increase in the HAB records in coastal waters in the recent years. With the advancement of scientific research and enhancement of human activities in the coastal zone more information about the HAB has been documented. The increase in biomass is specific for each species and may vary considerably in space and time depending on the environmental conditions.

Most harmful species become hazardous only when their concentration exceeds a threshold level, which varies with species. The diatom *Chaetoceros concavicornis* and *C.convolutus* which have long siliceous spine become a cause for fish mortality due to lesions produced on the gill tissues even at low concentration of 5 cells ml^{-} while some like the *Phaeocystics* become noxious only when they reach very high concentration. This micro algae which is a normal component in the temperate areas, at high concentrations affects the fisheries, confer bad taste to fish, deviate the herring migration patterns and in some cases produce slime and foam. Similarly very dense concentration of non toxic diatoms like *Cosinidiscus* spp, *Thalassiosira mala* have caused discoloration or fish gill clogging.

Types of Algal Bloom

There are 3 different types of algal blooms.

1. Blooms which are basically harmless water discolorations (under exceptional condition they may cause mortality of aquatic organism)
   Gonyaulax polygramma, Noctiluca scintillans, Scrippsiella trochoidea, Trichodesmium erythreum.

2. Blooms which produce potent toxins

   Alexandrium spp., and *Pyrodinium bahamense* – PSP; *Dinophysis acuminata* – DSP *Nizschia pungens* – ASP; *Gambierdiscus toxicus* - Ciguatera fish poisoning
   Gymnodinium breve - NSP

3. Blooms which are non toxic to humans but toxic to fish and invertebrates by damaging or clogging gills and they are problematic especially in intensive aquaculture systems/mariculture system.

   Chaetoceros convolutus, Gymnodinium mikimotoi, Chrysochromulina polylepsis, prymnesium parvum, heterosigma carterae.

Under favorable conditions following algal groups produce bloom

- Diatoms (Bacillariophyceae)
- Dinoflagellates (Dinophyceae)
- Members of Green algae (Chlorophyceae)
- Blue green algae (Cyanophyceae).

Dinoflagellate bloom

Dinoflagellates occur in both salt and freshwater and can be both planktonic and benthic. These are the protist group with the largest number of harmful species. Dinoflagellates show a great range of forms, they can be grouped into 5 basic types as bloom causing agents.

1. **Gymnodiniods And Noctilucoids:** *Gymnodium breve, G. stein, G. catenatum, G.mikimotoi, G. pulchellum, G. veneficum. Gyrodinium Noctiluca scintillans* (*N. miliaris*)
2. **Peridinoids** : *Peridinium polonicum*
3. **Gonyaulacoids**: *Alexandrium/Gonyaulax catenella, A. angustitabulatum, A. cohorticula, A. hiranoi, A. minutum. Pyrodinium bahamense, Gambierdiscus toxicus, Ostreopsis lenticulatris, Ceratium fusus*
4. **Dinophysoids**: *Dinophysis acuta, D. acuminata.D. sacculus*
5. **Prorocentroids**: *Prorocentrum concavum, P. emarginatum, P. lima etc*

Diatom bloom.

Diatoms are one of the largest algal groups known.The diatoms are found in all types of aquatic habitats and in marine plankton at all latitudes and through out all season. Harmful events observed when blooms of *Cosinodiscus concinnus and*

C. centralis, Thallassiosira mara, Rhizosolania chunii and Cheatoceros spp. occurred.

Most algal bloom represent useful contributions to plankton production but some periodically produce harmful results.

Physical damage: dense concentration of tide may suffocate fish by clogging or irritating their gills. In 1962, mortality of more than 100 tons of fish in False Bay was attributed to gill clogging by the Dinoflagellate Gonyaulax *polygramma*. Oxygen depletion can kill indirectly by depleting the oxygen dissolved in the water.

Direct poisoning: toxins of dinoflagellates are more potent, which disrupt normal nerve functions. This has caused numerous marine fish and shellfish mortalities. In 1980 entire mussel population of Elands Bay was destroyed by *Gonyaux catenella* and in 1989 in Japan, 30 tons of abalone were washed up due to *G. nagasakiense.*

Indirect poisoning: Mussel, clams and oysters which are filter feeders-accumulate toxins in the digestive system-cause illness or death to consumers such as birds, marine mammals and man. Four different types of indirect poisoning have been identified as harmful to man.

Paralytic Shellfish Poisoning (PSP)

PSP was discovered in 1700. Most serious of the shellfish poisoning. Several hundreds human deaths have been recorded worldwide during past 300 years. Wide spread in U. S., west coast, Maine to NewYork. Mussels, clams, oysters, scallops, herring, sardines, marine mammals, birds are directly affected. Humans are affected by eating contaminated shellfish which contains the toxin-Saxitoxin which disrupts normal nerve function.The toxic molecule inhibits the passage of sodium ions causing numbness, paralysis, respiratory failure, death.*Alexandrium spp.*, and *Pyrodinium bahamense* are some PSP producing alage

Diarrhetic Shellfish Poisoning (DSP)

The causative organism: *Dinophysis acuminata*. Which produces the toxin Okadaic acid. It is reported from South African waters, no report in US. The chemicalaffects proteins that control the sodium secretion by intestinal cells, causing nausea, vomiting, abdominal pain and diarrhea in humans.

Neurotoxin Shellfish Poisoning (NSP)

It is common in Gulf of Maxico coast, Florida, North caorolina, South Carolina and South African coast. Mantaees, bottlenose dolphins, oysters, fish, clams and birds are affected. Humans may be affected by breathing sea foam or eating contaminated shellfish. Toxin molecule induces greater flux of sodium ions causing diarrhea vomiting, tingling in lips, dizziness.

Amnesic shellfish Poisoning (ASP)

ASP was recorded first time off the coast of Canada in 1987. 3 deaths and over 100 confirmed cases of acute intoxications followed the consumption of cultured mussels. The main causing organism is *Nizschia pungens*. The toxin produced is domoic acid. Razor clams, dungeness crabs, scallops, mussels, anchovies, sea lions, brown pelicans and cormorants are affected. Humans may be affected by eating contaminated shellfish. Toxin molecule attacks human central nervous system causing vomiting, abdominal cramps, diarrhea, short-term memory loss etc.

Ciguatera fish poisoning (CFP)

Gambierdiscus toxicus is the main phytoplankton and it affects reef fish and their predater like Barracuda, snapper, amberjack, grouper, and kingfish. Humans may experience vomiting, cramps, diarrhea, headache, weakness, numbness.

Blooms and Bivalve Utilisation

It is essential to avoid areas, which have previous records of blooms, or if such a bloom occurs, the harvest should be postponed. The retention level for diffrernt toxins varies between species and for the same toxin clearance rates are different (Table 1). The sample from the bloom site should be analyzed for toxin and only if it is within the safe levels it should be harvested and marketed. The levels set by different countries for PSP and DSP is given in Table.2. The total ASP content should not exceed 20µg of domoic acid per gram using the HPLC method.

Table 1. The toxic retention time for different bivalves

Species	Toxin sources	Retention time
Anadara maculosa	*Pyrodinium bahamense*	6weeks
Arctica islandica	*Protogonyaulax tamarensis*	2 months *in vivo*
Choromytilus meridionalis	*Gonyaulax catenella*	3 months
Clinocardium nuttalli	*Gonyaulax acatenella*	9 weeks
Crassostrea cucullata	Not specified, probably *Pyrodinium bahamense*	2 months
Crassostrea echinata	*Pyrodinium bahamense*	3 weeks in closed system; longer period *in vivo*
Crassostrea gigas	*Gonyaulax acantenella*	1-9 weeks
Crassostrea iridescens	*Gymnodinium catenatum*	> 1 month
Crassostrea virginica	*Gymnodinium breve*	2-6 weeks
Modiolus auriculatus	*Pyrodinium bahamense*	6 weeks
Modiolus modiolus	*Gonyaulax tamarensis*	Up to 60 days
Mya arenaria	*Gonyaulax acatenella*	5 weeks
Mytilus californianus	*Gonyaulax catenella*	Up to 45 days
Mytilus edulis	*Protogonyaulax tamarensis* *Gonyaulax acatenella* *Gonyaulax excavata*	10days-7 weeks up to 50 days 11 weeks 2-3 weeks
Patinopecten yessoensis	*Protogonyaulax tamarensis*	6 weeks to 5 months

Protothaca staminea	*Protogonyaulax acatenella*	5 weeks
Saxidimus solidissima	*Gonyaulax catenella*	< 1 month
Spisula solidissima	*Protogonyaulax tamarensis*	Up to 1 year
Spondylus sp.	*Pyrodinium bahamense*	Still highly toxic after months
Tresus capax	*Gonyaulax acatenella*	11 weeks
Venerupis japonica	*Gonyaulax acatenella*	5 weeks

Table 2. Regulations of paralytic shellfish poisons in various countries

Country	Product	Toxins	Tolerable level	Responsible Authority
Australia	Shellfish	Saxitoxin	80 μg100g $^{-1}$	State Authority under supervision of the Australian Quarantine and Inspection Service
Austria	Shellfish	Saxitoxin	40μg100g $^{-1}$	Ministry of Public Health and provincial authorities
Canada	Molluscs	PSP	<80μg100g $^{-1}$	Dept of Fisheries and Oceans; Dept of Health and welfare
		DSP	0.2μgg $^{-1}$	
European Union	Bivalve molluscs	PSP	80μg100g $^{-1}$	Various
Hong Kong	Shellfish	PSP	400MU100g $^{-1}$	Dept of Health: Agriculture and Fisheries Dept
Japan	Bivalve	PSP	400 MU100g $^{-1}$	Ministry of Health &welfare, Bureau of Environmental Health
		DSP	5 MU100g $^{-1}$	
Korea	Bivalve	Gonyautoxin	400MU100g $^{-1}$	Ministry of Health & social services
		DSP	5 MU100g $^{-1}$	
Singapore	Bivalve	Saxitoxin	80 μg100g $^{-1}$	Ministry of National Development
Norway	All types of mussel	PSP	40-80 μg100g $^{-1}$	Food Council Authority
		DSP	5 –7 MU100g $^{-1}$	

Economic Impact

1. Economic loss of due to closure and mortalities.
2. Consumer fear of purchasing seafood
3. Fear of investing in aquaculture business
4. Discoloration of water - aesthetically unpleasant
5. Loss of marine recreational opportunities including tourism, fishing, swimming and sunbathing.

Monitoring and Managemnet

1. A surveillance programme should be established-to monitor bloom and physicochemical parameters to predict bloom.
2. A research design should be setup systematically collect and analyze information on variables (contributors) associated with increased kills of fish.
3. Development of antibody and DNA "probes" that are being used to detect HAB and toxins in natural waters.
4. Development of methods to utilize satellite imagery of costal waters to follow HABs and water masses with which they are associated.
5. Educational strategy to the general population should receive special attention.

32 Post Harvest Processing and Depuration of Farmed Bivalves

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The edible bivalves like the mussel and the oysters are harvested when the condition index is high i.e., when the gonad is ripe and the meat occupies the entire shell cavity. In temperate countries, mussel and oyster harvest is mechanised while in India the `mussel ropes' and oyster strings are collected manually and brought to the shore. Mussels are normally marketed shell-on. Harvested oysters, which are kept under moist and cool conditions, survive for several days. However, it is desirable that they reach the consumer within three days of harvest. Studies indicate that oysters packed in wet gunny bags can be safely transported for 25-30 hours without mortality and in good condition. Oysters are eaten in fresh condition in the half shell in many countries. Removal of meat from the shell is termed shucking. Live mussels and oysters are shucked easily using stainless steel knives or by gently heating to open the shell. Remnants of mussel byssus thread if any are removed before marketing the meat. The post-harvest procedures for mussel and oysters are shown in Fig.

By-products and Value Addition

A variety of products have been developed in India from mussels and oysters (Table). These products have been developed by the R & D activities of the Central Institute of Fisheries Technology (CIFT), Cochin.

- **Icing:** Fresh oysters and mussels can be preserved in ice in organoleptically acceptable condition up to 9 days. Fresh frozen oyster and mussel meat remains acceptable for 40 weeks.

- **Canning:** Cleaned meat after blanching in 5% brine for 5 minutes can be canned. The blanched meat with medium is canned by heat processing in steam at 115°C for 20 minutes.

- **Smoking:** Smoking improves flavour of the meat. The blanched bivalve meat after drying to a moisture level of 40-45% is smoked at 80-90 °C for 30 minutes. It is then dried further to bring the moisture level to 10%. The shelf life of smoked oyster and mussel in room temperature is six months.

- **Drying:** Blanched meat can be sun-dried or dried in an electrical dryer to bring down the moisture content to 10-15%. Shelf life of the dried meat is six months in room condition.

Table. Value added mussel and oyster products from India

Mussel products	*Pearl Oyster Products*	*Oyster products*
▪ Iced and frozen mussel ▪ Canned mussels ▪ Smoked mussels ▪ Dried mussels ▪ Marinated mussels ▪ Mussel pickle ▪ Mussel chutney powder	▪ Pearl powder ▪ Pearl liquid ▪ Seed pearls ▪ Mother-of-pearl shell	▪ Frozen oysters ▪ Canned oysters ▪ Smoked oysters ▪ Oyster stew

For further economic utilization, value added products of mussels like seafood cocktails are also prepared and marketed by many seafood export firms from India. The export of these items from India has by and large, been showing an increasing trend.

The two shell valves constitute about 85% of the total weight of oyster and contain about 52-55% calcium oxide. They are used in the manufacture of calcium carbide, lime, fertilisers and cement. Larger oyster shells are useful spat collectors in oyster culture. The shells are broken to pieces and also used as poultry grit. The mussel shell finds use as a liming agent in coconut plantations. Another important economic use of bivalve shell is in the making of curios, a small-scale industry which is rapidly developing along the east coast of India, and in the Andaman and Nicobar Islands.

Marketing

India is presently a net importer of cultured pearls and the pearl trade is centred in the city of Hyderabad. Export of Indian cultured marine pearls is of recent origin (Fig. 3) and the main country to which it exports is Hong Kong (@ US $ 8.5 per gram). Besides, there is a growing internal market for cultured marine pearls.

The edible bivalve market channel is a relatively straightforward and fresh and frozen farmed mussel and oysters have a healthy and growing domestic demand in maritime regions of the country. There is now increasing appreciation of the fine texture and taste of mussel and oyster meat and these comparative new products look set to captivate the urban connoisseurs. New strategies need to be developed to fully exploit the domestic markets. On the export front, in the case of mussels, Indian products have found a place in the markets of UAE, Germany and Republic of South Africa, and the list is growing. Although live oysters are an expensive gourmet food in Europe and America, they have not found such a niche in India. Markets are limited to few isolated pockets like the Parsi community in Mumbai, who have a specific preference to smoked oysters marketed in cans.

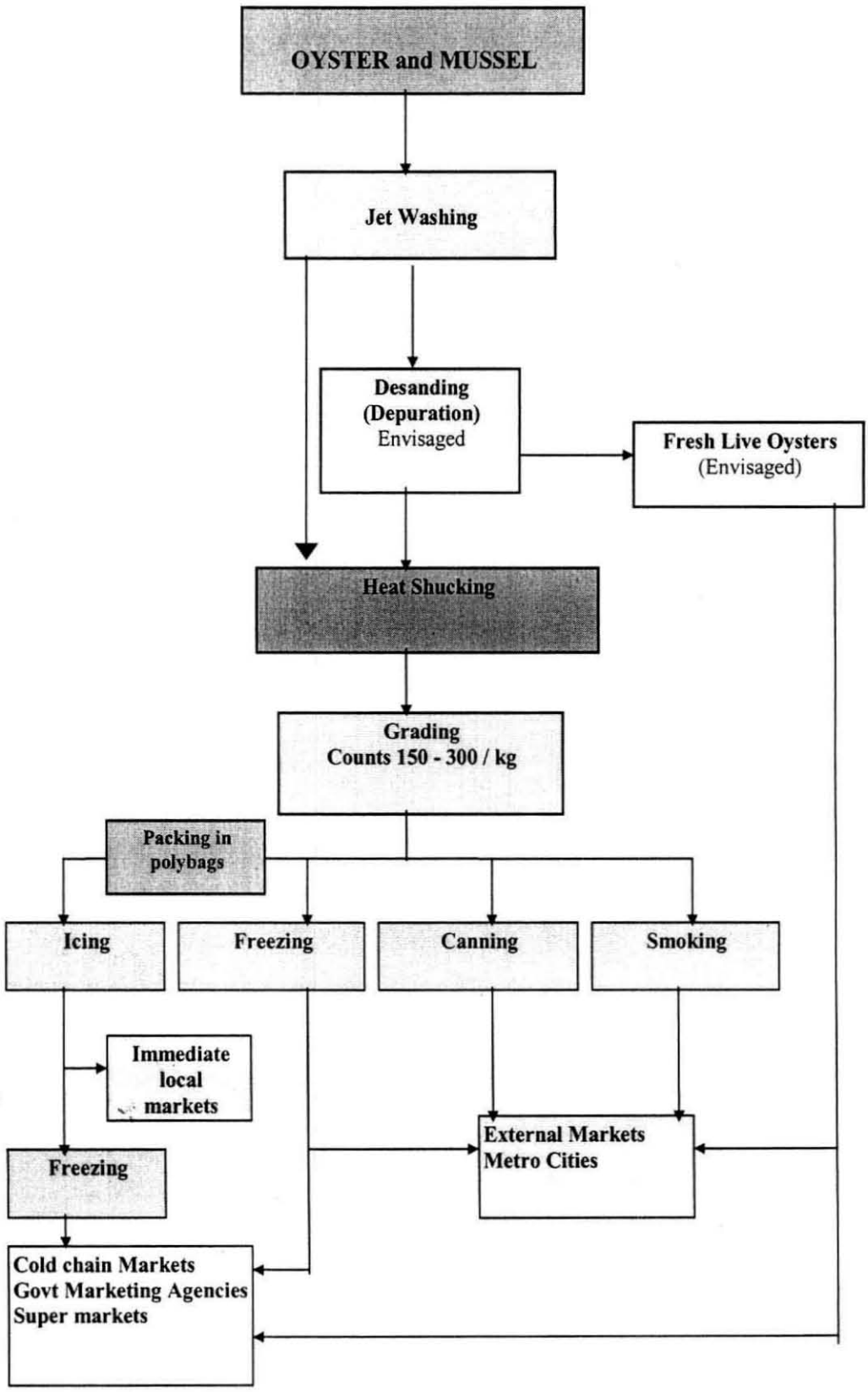


Fig. Bivalve Post-Harvest procedures/ processing

The product is exportable if it meets basic quality and sanitation standards. Through export to western markets the farmers can get better price for their produce, thereby, increasing their profit margins. However, European Union markets are very stringent about the quality of bivalve products that they import from Asian markets. To ascertain and maintain the quality of bivalve products depuration is essential.

What is Depuration?

Bivalves are filter feeders in their feeding habit. During this process they accumulate all suspended biological materials including harmful microorganisms. Before the product reaches the market, these materials have to be removed from their gut. The process of such purification is called depuration.

Simple depuration can be achieved by starving the bivalves in clean and filtered seawater/ brackishwater for a certain period of time. More effective depuration can be achieved by using disinfected water in the depuration process.

Even a simple and small depuration unit will be beyond the capabilities of the small-scale farmers, and hence, it is proposed to have depuration plants where bivalve farmers are concentrated, thus enabling the farmers to use it as a common facility for a price to be determined later.

Depuration Process

1. Requirements

(a) The basic principle for controlled purification or depuration of bivalve involves providing clean and purified seawater in tanks, whereby the bivalve filter and pump such water for a period of 24 hours or more if required.

(b) Ideally a depuration plant should be located near the least polluted source of water in the vicinity of bivalve farms. Also the physical characteristics (salinity, temperature, dissolved oxygen etc.) of the seawater used in the depuration plant should not be radically different from that of the bivalve farming areas. Care should be taken such that the level of dissolved oxygen should not be allowed to drop below 2 mg/l.

(c) A concrete seawater storage tank of the dimension 20 x 8 x 8 m (capacity 160 tonnes) should be constructed at a level above that of the depuration tank to facilitate gravity flow into the depuration tank (see figure). The water to be used will be first pumped into a rapid sand filter (preferably 2, arranged serially) to remove all suspended material.

(d) The choices for disinfection of seawater are chlorination, ozonation and UV light irradiation. The latter two are expensive, and hence chlorination (@ 3 ppm) is the method chosen for this project. After chlorinating, the water will be dechlorinated using vigorous aeration and / or neutralization with Sodium thiosulphate.

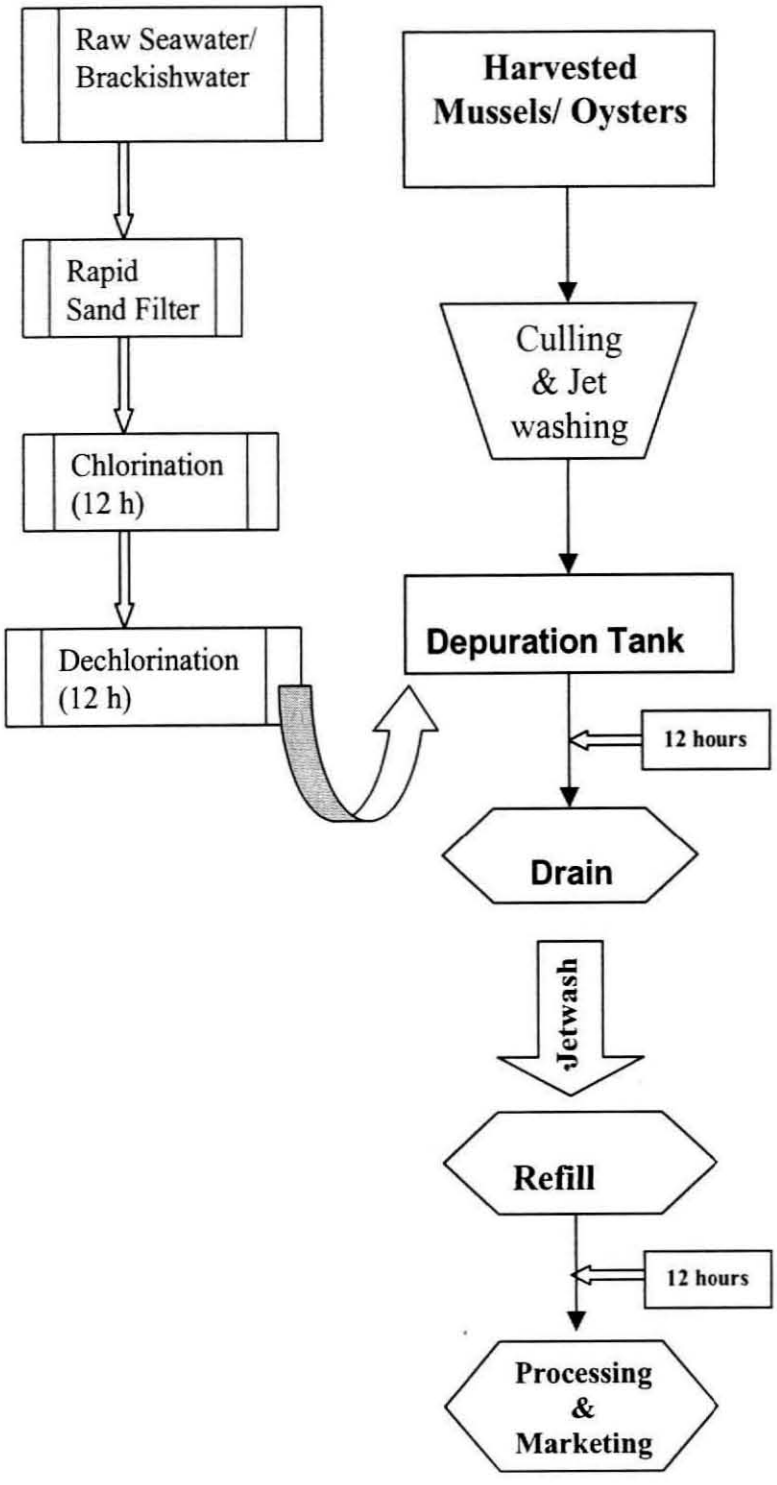
(e) Most depuration plants use flow through, once through or fill and draw principles. It is proposed here to use the batch process (fill and draw), wherein seawater is drawn from the supply treated with predetermined amount of disinfectant to reduce bacterial levels, stored for a time, then pumped to the tank containing bivalves. The process will be repeated once to ensure complete depuration (see flow chart).

(f) Each depuration unit will consist of two concrete tanks of the size 15 x 4 x 1 m with a gradient of 3% to hold bivalves. Bivalves will be placed in perforated plastic trays of standard size. The trays in a single tier will be raised from the tank bottom with the help of PVC pipe runners. The tank will have a drain plugs at the lower end to facilitate cleaning and flushing.

2. Run Duration and Capacity

(a) The duration of the run will be 24 h, in two cycles with one complete flushing for both mussels and oysters (see flow chart). The unit has the capacity to hold 1.0 tonnes of mussels and 0.62 tonnes of oysters per run. The water requirement per run will be 144 tonnes.

Depuration Protocol



33 Environmental Impact Assessment of Suspended Bivalve Culture

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Bivalves are sedentary organisms that require substrate for spat settlement and subsequent growth during which time they filter feed on phytoplankton, detritus, protozoans and bacteria. It is well known that large scale aquaculture can pose complex ecological socio-economic and management problems. As commented by Hastings and Helnte in the introduction to the dedicated issue on "Effects of Aquaculture in the Estuarine Environment (Estuaries Vol. 18 (1)-1995)- *the potential for increased farming of coastal marine waters is considerable but the potential for significant environmental degradation associated with such activities is also large*". Considerable work has been done on the variations in the hydrological, sediment and benthic faunal composition due to mussel and oyster farming by off bottom methods. The main impacts of suspended bivalve farming are given below.

Effect on primary productivity: Commercial large scale bivalves farming will consume substantial quantities of phytoplankton particularly when there is a high density of culture units over a large area, resulting in reduction in primary productivity of the area. In Japan, the culture of 50,000 to 60,000 oysters reduced the amount of seston (predominantly phytoplankton) by 76.95%. Suspended culture of green mussels in New Zealand has been found to remove upto 60% of the available food as the water flows through the farm. A mussel raft in Spain has been found to remove 35-40% of plankton and detritus, whereby 30% of the carbon,42% of the nitrogen and 60% of the chlorophyll *a* of the particulate organic matter is retained. However it has also been suggested that primary productivity may be stimulated by an increase in nutrient cycling although field evidence of increased primary production in the farm vicinity is still lacking. Bivalve culture competes with other planktonic herbivores which has been shown for the Spanish Ria de Arousa where suspended mussel culture replaced copepods as the main pelagic grazing organism.

Effects on current velocity and water movements: Bivalve farm structures modify current velocity and direction of water movements. In turn, these movements may alter patterns of erosion and sedimentation of particulate matter. Reduced water flow may result in decrease in natural erosion by wave action, which in turn is followed by siltation and accumulation of suspended matter in cultured areas.

Effects on sedimentation: Bivalves produce pseudofaeces (mucous-bound particles expelled without passing through the gut) in addition to the normal faeces (biodeposition) which constitutes organic –rich particulate waste. For example in Hiroshima Bay a raft holding 420 000 oysters generate 16 m tons of faeces and pseudo faeces in about a 9 month period and several such farms have been found to have a major impact on the sediment deposition in the bay. Studies have shown that

for a farm covering an area of 1500 m^2 the sedimentation of dry matter would amount to about 10 t, and sediment under the raft would accumulate to 10 cm per farming season.

Table 1. Faecal waste production and from bivalve farming

Species	System	Faecal production
Mytilus galloprovincialis		14.3 –149.3 mgDW/individual/24h
Mytilus edulis	Natural shore population	1.76gDW/gDW/mussel/yr 0.13gC/gDW/mussel/yr 0.0017 g N/ gDW mussel/yr 0.00026 g P/ gDW mussel/yr
Mytilus edulis	Rafts	9.5 kg carbon /sqm/yr 1.1 kgnitrogen/sqm/yr

Sediment accumulation below bivalve farms				
Species	System	Depth	Current velocity	Sediment accumulation
M.edulis	longline	8-13 m	App 3cm/sec	10-15 cm
M.edulis		11-13 m	Very weak	7-30 cm
M.edulis	Rafts	>15m	Upto 200cm/sec	No sig biodeposition, shells present

Effect on benthic productivity: The deposition of particulate organic wastes can result in physico-chemical changes of the substrate, particularly in the immediate vicinity of the culture site. The enrichment of sediment with organic material stimulates microbial activity resulting in deoxygenation of the substrate and bottom waters due to reduced interstitial oxygen concentration and increased oxygen consumption, increased sulphate reduction, increased denitrification and increased release of inorganic nutrients such as nitrate, nitrite, ammonium, silicate and phosphate from mussel beds. The regeneration of potentially limiting nutrients may increase primary productivity.

Effect on benthic community structures: Benthic communities beneath suspended farms may be affected. Macro fauna may be lacking entirely in the area directly under the culture site. Species richness is reduced and opportunistic enrichment tolerant species become predominant. A relatively large number of detailed studies of fine sediment deposition have been carried out. A range of responses of the sea-floor biota have been identified, from little or no community modification after low levels of nutrient enrichment, through to major alterations and the dominance of small polychaetes and absence of larger animals such as molluscs and urchins after high levels.

Introduction of predaters: Introductions of bivalves have negative ecological effects, particularly when parasites and diseases are also introduced. A typical

example is the introduction of the Japanese oyster drill and flatworm to North America from Japan

Impact on birds: The structures could have several impacts on birds. The rope system could impede diving and the pursuit of prey and possibly cause injury to birds. However, there is no evidence that this is a problem, the ropes being coarse and very visible, and birds have been observed feeding within farms on occasions.

Creation of new habitat: The mussel lines can be considered to be new temporary habitats created in the water column for a range of animals in addition to mussels. The epifaunal community on mussels has been recorded to consist of over 100 different species. There is also deposition of live shells, mussel shell litter, and the remains of other associated biota below a farm. 'Shell drop', the deposition of shells, live mussels and associated biota, largely affects the area directly below the farm, typically to 20 m from its boundaries. The value of shell drop in creating a reef-like substrate seems very variable; under some farms the litter is barren, whereas under others there can be a rich biota, including sponges, ascidians, anemones, tube worms together with starfish, sea cucumbers and crabs

Effects on water column: The column is frequently stratified due to the separation of water layers with different densities associated with changes in salinity or temperature. The impacts of a farm can be considered in terms of nitrogen alone, which can at times be at such low levels as to limit plankton growth. The harvesting of mussels will periodically remove nitrogen from the aquatic system. It has been calculated that, based on an average turnover of mussels of two years, denitrification was 68% higher at the farm study site compared to a nearby reference site. Further research is in progress on the role of nitrogen in limiting phytoplankton growth and thus in turn mussel growth. A positive response was seen from adding nitrogen to the water in summer. 'Fertilising' the sea in this way could become a management practice and flow-on effects on zooplankton and fish

Increase in pelagic resources: Farms exclude trawlers from areas, and this has resulted in enhanced numbers of scallops and horse mussels at sites. There is debate about the extent to which the mussel lines and their attached.

Though the impacts are not as large as shrimp and cage culture, the intensity of negative impact cannot be neglected. Bivalve farming practices are simple and are known to provide employment opportunities and promote development of ancillary industries in coastal areas. Hence it is essential that proper management practices be stipulated for sustainable development of bivalve farming industry.

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Selective Breeding of Bivalves

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Introduction

Recently a variety of approaches have been introduced in the field of genetics of molluscs, including, Mendelian genetics, cytogenesis, quantitative genetics, cytogenesis, quantitative genetics, biochemical genetics and hybridization. Wada (1975a, b, 1985) has estimated the response to selection for several attributes of Pinctada fucata for variance of full siblings. He analysed the genetic variability and genefrequencis at three loci in two strains selected for four to five generations. The change of frequencies of colour of nacre in the selected lines of pearl oyster to yellow prismatic layer for five generations has been studied (Wada, 1985), Wada (1976, 1985) and Wada and Komaru (1985) have studied the chromosome morphology of different species of bivalves.

Research Achievements in India

Variations in Indian pearl oyster, edible oyster etc has been noticed as in the case of European, American, and Japanese oyster. Alagarswami et.al, (1983) and (1986) artificially produced Pinctada fucata and P.margaritifera pearl oysters from wild brood. Velayudhan et.al (1996) produced 4 generations of pearl oysters and studied the heritability and noticed inbreeding depression in the hatchery produced pearl oysters at the same time noticed the increase in the percentage of yellow nacre bearing oysters which produced the Golden yellow pearls. He again noticed the nacre thickness in the second generation stock from the hatchery produced stock. The triploid edible oysters were produced in India. Recently it was proved that the pearl oysters from Gujarat had more thickness than from all other regions, Tuticorin, Vizhinjam and Mandapam. The oysters from Vizhinjam had more shell cavity, grow faster and produced larger pearls of 8 mm in diameter. The faster growth and more shell thickness will be suitable for producing larger good quality pearls. Production of triploids, transgenic oysters also will enhance the pearl production in India. The bio-technological approach in Molluscan research will increase the production and quality of molluscs produced in India.

Haley (1977) as reported by Newkirk (1980) has been following the frequency of changes in the 5 full sib families of *Crassostrea virginica*. Matsui (1958), in Pinctada martensii the right shell is slightly convex, where as the left shell is more strongly so. The degree of convexity of shell is very important from the practical point, because oysters with more strongly convex shells harbour larger pearls. Singh and Green (1984) have reported that the relative mortality of the heterozygote (faster growers) of *Macoma bathica* during the larval period is expected to vary from year to year depending on the environmental conditions particularly then relative abundance of the phytoplankton blooms and faster

particularly then relative abundance of the phytoplankton blooms and faster growing heterozygotes with higher food requirements have relatively higher mortality. Triploids in *Macoma bathica*, transgenic forms in Manila clams are also some of the good achievements in Molluscan genetics.

Selection of animals is important in the selective breeding programmes

The more phenotypic variation in a trait the more intense the selection from the natural stock/ populations. The selected oysters are then mated according to a prearranged programme. Unless there are sufficient numbers of spawners, at least 50 numbers, significant inbreeding may occur and a number of stocks can be taken and performance evaluation could be done during the first generation.

Second, one can take a number of stocks and do performance evaluation during the first generation

The third is to cross males and females from different populations to form mixed population. This can be done if parents from a number of stocks spawned together. If we keep each generation (50 males and 50 females) of each stock or line, the inbreeding rate will be 0.5 % per generation and total accumulation of inbreeding after 5, 10 and 20 generations is 1, 3 and 5 % respectively (Newkirk, 1983). More control can be kept exercised and consequently less inbreeding will occur if separate lines are maintained at least in the first generation.

- Inbreeding rate after the first generation with the magnitude of over estimation decreasing as the effective population size increases
- Inbreeding coefficient of matings:
- Half-sib fx =1/2 $(1/2)^2$ or ½(0.25) Inbreeding coefficient
- of individual x %12.50
- Full -sib =1/2(0.5) 25.00
- Sire X daughter=1/2(0.5) 25.00
- Sire X daughter
  with Sire inbred = 1/2[0.50(1.25)]=1/2(0.625) 31.25

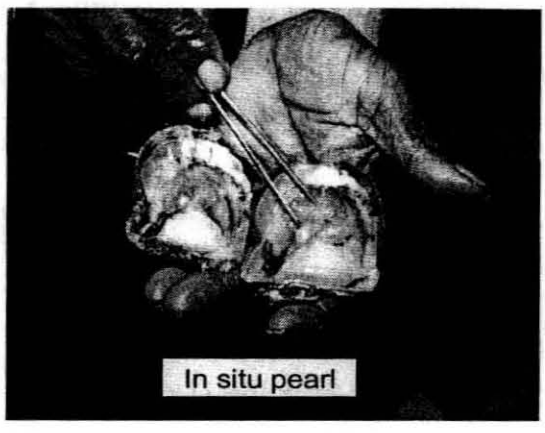
1/8 Nm +1/8 Nf = Inbreeding coefficient				
	Number of male parfents			
Number of female parents	1	10	20	∞
1	0.2500	0.1375	0.1312	0.1262
3	0.1667	0.0541	0.0478	0.0425
5	0.1333	0.0375	0.0312	0.0262
10	0.1250	0.0250	0.0187	0.0137
20	0.1312	0.0187	0.0125	0.0075
25	0.1300	0.0175	0.0113	0.0063
50	0.1275	0.0150	0.0088	0.0038
75	0.1267	0.0141	0.0079	0.0029
100	0.1262	0.0137	0.0075	0.0025
150	0.1258	0.0133	0.0071	0.0021
200	0.1256	0.0131	0.0069	0.0019
250	0.1255	0.0130	0.0068	0.0018

BROODSTOCK MANAGEMENT

- Useful to keep the identity of the progeny groups and for providing evaluation of their performance

Needs much care to maintain adults without inbreeding , interbreeding and loss of genetic variation

Tagged oysters



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Ploidy Manipulation in Bivalves

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Introduction

The number of chromosomes is generally specified for each species. Irregularities during meiosis, mitosis or fertilization may produce cells with variations in the number of chromosomes generally specified for the species. Such variations in chromosome number generally specified for the species is called ploidy. Ploidy may occur through duplication or loss of complete set of chromosome **(euploidy)** or a part of the chromosome complement **(aneuploidy)**..

Euploidy: The term euploidy designates genomes containing whole sets of chromosomes. The euploids are those organisms, which contain whole set or sets of chromosomes (genomes) in any number, in their body cells. The euploidy is of following types:

Monoploidy: The monoploid organisms have one-full set of cghromosome in their body cells and is represented by the notation (**n**). When monoploidy occurs in gametes (sperms and eggs) it is termed as ***haploidy***. Most micro-organisms (*e.g.,* bacteria, fungi and algae); gametophytic generation of plants (*e.g.,* bryophytes and other plants); sporophytic generation of some higher angiospermic plants (*e.g., Sorghum, Triticum, Hordeum, Datura,* etc.) and certain hymenopteran male insects (*e.g.,* wasps, bees, etc.) have one genome in their body cells, hence are monoploids. They are usually smaller and less vigorous than their diploid prototypes. Characteristically, monoploid plants are sterile. The reason of sterility is that the chromosomes have no regular pairing partners (homologous chromosomes) during meiosis and meiotic products are deficient in one or more chromosomes. For instance a monoploid maize will have 10 chromosomes and in a gamete it can range from 0 - 10. Consequently, considerable sterility will be found in monoploid maize.

Diploidy: The diploidy is characterized by two genomes (2n) in each somatic cell of the diploid organisms. Most animals and plants are diploids. The diploidy is related with fertility, balanced growth, great vigorsity, adaptability and survivability of the diploid organisms.

Polyploidy: The organisms with more than two genomes are called polyploids. Among plants and animals, the polyploidy occurs in a multiple series of 3,4,5,6,7,8, etc., of the basic chromosomes or genome number and thus causing triploidy, tetraploidy, pentaploidy, hexaploidy, heptaploidy, octaploidy, respectively. Ploidy levels higher than tetraploid are not commonly encountered in natural populations, but our most important crops and ornamental flowers are polyploid, e.g., wheat (hexaploid, 6n), strawberries (octaploid, 8n), may commercial fruit and ornamental plants, liver cells of man, etc. Polyploidy is rare in animals, however has been

reported in *Ascaris, Daphina, Cypris, Drosophila, Bombyx mori, Hbrobracon,* bees, wasps, *Artemia, Echinus, Rana esculenta, Rana pipiens, Ambystoms, Jeffersoniarium, Cricetus cricetus* (hamaster) *etc.*

Polyploidy occurs naturally in somespecies and it can be induced artificially also. Fish like Mahseers, Common carp, Gold fish and some mullets are considered as natural tetraploids. First extensive studies on artificially produced triploid fish were done on three-spine stickleback, *Gasterosteus aculeatus* by Swarup (1959a,b). He reared triploid fishes to adulthood and compared growth rate and fertility with diploids. Mass production of tetraploids was achieved for the first time in Rainbow trout by using pressure shock. To produce polyploid seed on a commercial scale standard protocol for the ploidy induction and efficient screening method for their identification is necessary. Amenability of some fish species (*M. anguillicaudatus*) to chromosome multiplication is amazing! Experimentally produced penta, hexa and heptaploids are reported in in Japanese loach *Misgurnus anguillicaudatus*; and rainbow trout (*O.mykiss*).

Kinds of polyploidy: Following two main kinds of polyploidy, auto and allopolyploidies have been distinguished on the basis of the source of chromosomes.

(1) **Autopolyploidy** - The prefix "auto" indicates that the ploidy involves only homologous chromosome set and

(2) **Allopolyploidy** - The prefix "allo" indicates that non-homologous sets of chromosomes are involved. The union of unreduced or diploid or polyploid gametes from different diploid or polyploid species could produce in one step, an amphipolyploid or allopolyploid.

For example:

Species A x Species B

Diploid chromosome (AA) ↓

AB
(Diploid sterile)

↓ (Induction polyploidy) with cytochalacin B/ colchicine

AABB
(Allotetraploid)

Allopolyploids behave like new species.

Aneuploidy

An aneuploid is an individual whose chromosome numbers differs from the wild type (diploid number) by part of a chromosome set. Generally the aneuploid chromosome set differs from that of wild type only by one (or) a small number of

chromosomes. Aneuploids can have a chromosome number either greater or smaller than that of the wild type. Non-disjunction during mitosis (or) meiosis is the cause of most of the aneuploidies.

Type of aneuploidy

1. Monosomy – The diploid organisms which lack only one chromosome from a single homologous pair is called monosomic.
   (General formula = 2n-1)
   A monosomic produce two types of gametes i.e. (n) and (n-1)
   Turner syndrome in human is a monosomic condition involving" X "chromosome i.e. 46-1, the absent one being an X chromosome.

2. Nullisomic – The diploid organisms that have lost a pair of homologous chromosome are called nullisomic.
   (General formula = 2n-2)
   Nullisomy is mostly lethal. If they survive, the individual will generally exhibit reduced vigour, fertility, survivability etc.

3. Trisomy - Diploid organism having an extra chromosome, i.e. 2n+1. It can produce two types of gametes i.e. (n) and (n+1).
   Klinefelter syndrome is a condition in males which has trisomy involving an X chromosome, i.e. 44Autosomes + XXY. A male has an extra X chromosome.
   Down syndrome: is due to trisomy of chromosome 21.

4. Tetrasomy – One homologous pair is duplicated i.e. 2n+2. Gametes only one type (n+1).

5. Double trisomy – In a diploid organism two different chromosomes have an extra i.e. 2n+1+1

Chromosome Manipulation for Induction of Polyploidy

Chromosome sets can be manipulated by meticulous application of temperature (cold and heat) shock, pressure shock or chemical shock in dividing cells. Shock treatment disrupts the normal cycles of mitosis and meiosis. In freshly fertilized embryos it suppresses the extrusion of second polar body or arrests the first cleavage division and can lead to induction of triploidy and tetraploidy.

Shock Treatment

(a) **Thermal Shock**: Cold shocks are usually applied near 0^0C for cold water species and somewhat at higher temperature (5-12 ^{0}C) for warm water species. Heat shock is applied at a lower temperature (around 26-28^{0}C) in the case of cold water species than in warm water species (37-42^{0}C).

(b) **Pressure Shock**: This method is simple to administer. The pressure range varies between 7000 to 9000 pascals (Psi). The hydrostatic pressure is applied by a specialized instrument designed by mechanical engineering method. Pressure shock

is supposed to have fewer side effects than the thermal shock. But given in a sub-optimal intensity, pressure shock produces aneuploid offspring and

(c) **Chemical Shock**: Colchicine and cytochalasin-B have the potential to disrupt cell division and induce ploidy induction. But the results are inconsistent and unsatisfactory. Anesthetics such as nitrous oxide and Freon 22 have also been tried to induce triploidy. Of late 6-Dimethyl aminopurine (6-DMAP) has been proved to be an ideal chemical for induction of ploidy.

Significance of Polyploidy

Polyploidy has great genetic, taxonomical, evolutionary and economical significance. The genetic significance is that it helps in understanding dosage effect. The taxonomical and evolutionary significance is that it forms new species. Autopolyploidy is less significant in this respect than allopolyploidy, because it adds no new alleles to the genome. Allopolyploidy on the other hand offers great opportunities for production of new adaptive gene combinations and since it accumulates diverse genomes, it provides better adaptability to the species for a wider variety of habitats, which consequently increases the chance of being successful in natural selection. In plants polyploidy at least causes gigantism and accumulation of greater quantities of vitamins etc in the cells and therefore polyploidy provides various economically important food, fruits and ornamental plants.

Triploids are generally infertile and it has many advantages. Triplody therefore results in better growth rate in animals as no energy is wasted for reproduction. In plants triploidy is used to produce seedless fruits.

Procedure optimized for Inducing Triploidy in Edible Oysters

Triploid oysters have been successfully produced at CMFRI. Protocol for induction of triploidy by pplication of heat & cold shock and chemical (cytochalasin-B & DMAP) shock have been optimized. This leads to the retention of the I or II polar body resulting in the production of Meiosis I (M I) triploids or Meiosis II (M II) triploids respectively. Procedure optimized for inducing triploidy in edible oysters at CMFRI is presented below.

Collection and Conditioning of Oysters

Oysters in the size range 60-90 mm of which about thirty percent belong to zero year class or just one year old are recommended. Mature oysters in the size range 60-90 mm are collected and samples of 10 oysters opened for checking the gonad maturity stage. If gonad is ripe the oysters are induced for spawning. If the gonadal condition is in maturing stage, the oysters are kept in the conditioning room, where the oysters are intensively fed with mixed algal culture at 22 ± 1°C (Nayar *et al.*, 1987). After 10-15 days on assessing the gonadal condition the oysters are used for induced spawning experiments.

Brood stock conditioning plays an essential role in successful production of triploids. Ripe eggs uniformly conditioned for fertilization will go through meiosis synchronously and arrive together at the time of treatment. Unripe eggs will produce asynchronous finish. Synchrony is important in the eggs collected from different females. Brood stocks of fully mature males are selected so that the sperms are not only active but will fertilize the egg instantaneously to promote synchronous development. All the females should be at the same stage of maturity so that development in all the eggs from a single female and all the eggs pooled from all the females are synchronous.

Induction of Spawning

Gametes are collected by natural spawning or by stripping the mature oysters. The mature oysters are thoroughly washed and transported to a 100 litre perspex spawning tank containing about 50 litre of sea water at a temperature of 2-4°C above the ambient water temperature with proper aeration. If spawning did not occur within an hour, fresh sperms stripped from a sexually ripe male are introduced in the tank containing the brood stock to induce sympathetic spawning. Once an oyster starts spawning it is transferred to a glass tray containing filtered seawater. One oyster is placed in each tray. Each individual oyster is allowed to complete its spawning in the tray. The gametes in the trays are filtered through a 100μ sieve into 10 litre glass beakers. At this stage mild aeration is given to ensure sufficient supply of oxygen. Care is taken to prevent inadvertent contamination (fertilisation). The eggs are fertilized by adding the sperm. Diluted sperms suspension is used as the dilute suspension will facilitate the dispersion of the sperm throughout the egg and promote synchronous development.

Triploidy Induction

The method generally suggested for the induction of triploidy is by blocking the extrusion of the second polar body through the interference with the second meiotic division of the freshly fertilized eggs. Freshly fertilized eggs were exposed to all the above agents to arrest II PB extrusion and induce triploids. Trials were carried out with different concentrations of DMAP and CB and different temperatures with varying durations of exposure to optimize the protocol for inducing triploidy with each of them. Triploids were identified by the larval chromosome count in the metaphase plates.

The ideal time suggested for treatment of the freshly fertilized egg for arresting second polar body and inducing triploidy is when 50% of first polar bodies have been extruded. In the present study the extrusion of 50% first polar body was achieved 16minutes post fertilization at 28-29°C (normal room temperature). Hence treatments were initiated 17 minutes post fertilization to arrest II PB extrusion using both physical and chemical agents. General scheme of the protocol optimized in the present study for induction of triploidy in edible oyster is shown in (Fig.1). The optimum dosage and duration of treatment standardized for each of the inducing agents are presented in table 1.

Table 1. Dosage and duration of treatment for optimum triploidy in *C.madrasensis* (Thomas *et al.* 2004)

Inducing agent	Concentration / Temperature	Duration
Heat	37°C	5 minutes
Cold	5°C	10 minutes
Cytochalascin- B	0.05mg/l	3 minutes
6-DMAP	100µM	8 minutes

Among the various physical and chemical treatments tried for producing II meiotic triploids, 6-DMAP yielded the highest percentage of 66.6% at larval stage and 61.82% at "D" stage. The results indicate that 6-DMAP is the ideal for inducing triploidy in *C.madrasensis*.

Detection of Polyploid Individuals

The production frequency of polyploidy individuals varies if the shock treatment is not given in proper dosage at appropriate time. It may so happen that a mixture of diploid, triploid, tetraploid and mosaic offspring may be produced. The diploid and polyploid fishes are difficult to differentiate on the basis of external morphology but can be distinguished by following methods:

i) Chromosome analysis is the simplest and appropriate method for determining the ploidy level. But it is very much labour intensive and time-consuming.

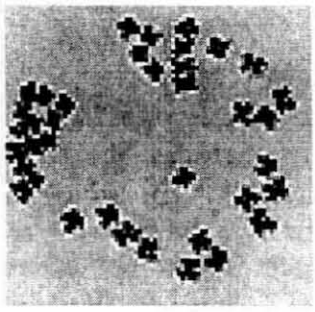
ii) By the measurement and comparison of the nuclear volume and cell volume of the erythrocytes, the ploidy level can be distinguished. This can be done with the help of light microscopy. The electronic instruments like Coulter counter can be used to measure the cell size rapidly.

iii) Flow cytometer is an instrument that helps to detremine the DNA content and cell size. Flow cytometry involves staining the cells with a DNA specific fluorescent dye, propdium iodide, followed by quantification of fluorescence upon laser excitation.

iv) Counting of the number of nucleoli after silver staining method that can be applied in those species having a constant number of nuclear organizer regions (NORs) per cell. However, it is not a suitable methods if the number of NOR varies per cell.

v) Isozyme analysis can be applied insome cases. For example, electrophoretic examination of Phospho Gluco Isomerase (PGI). Esterase and other allozymes in Brown trout (*Salmo trutta fario)* and in some Cyprinids could distinguish the diploid and triploid individuals

Plate. 1 Metaphase plate of triploid edible oyster (3n=30)



The duration and dose of thermal or pressure shock and the optimum conditions for each species have to be found out by trial and error process. Table 2 summarizes the conditions applied for manipulating the chromosome sets in some species of fishes.

Table 2. Conditions applied for manipulating chromosome sets in some species of fishes

Species	Method (shock/duration/time after fertilization)	Reference
A. Triploidy: Retention of second polar body		
Common carp	40 or 41^{0}C/2 or 1.5 min/6 min	Recoubratsky *et al.*, 1992
Grass carp	5-7^{0}C/25-30 min/2.0 - 4.5 min	Cassani & Caton, 1985
Laboe rohita	42^{0}C/1-2 min/ 7 min	Reddy *et al.*, 1990
O.mossambicus	42^{0}C/3 min/2.5-4.5 min	Varadaraj & Pandian, 1990
Ictalrus punctatus	7500 psi/2-5min/2.5 min 0^{0}C/1 hr/5min	Wolters *et al.*, 1981
Heteropneustes fossillis	4^{0}C/30 min/ 2min	Tiwary *et al.*, 1997
B Tetraploidy: Prevention of first cleavage		
Rainbow trout	490 Kg-cm^2 4 min/ 5.8 hr	Chourrout, 1984
O.aureus	11.0^{0}C/ 60 min /80-104 min	Don & Avtalion, 1988
l. Punctatus	40^{0}C/80-90 min/ 3 min	Bidwell *et al.*, 1985
Plecoglossus altivelis	650 kg-cm^{-2}/ 6 min/80 min	Taniguchi *et al.*, 1990

Figure. 1. Scheme for triploid induction in Edible oyster

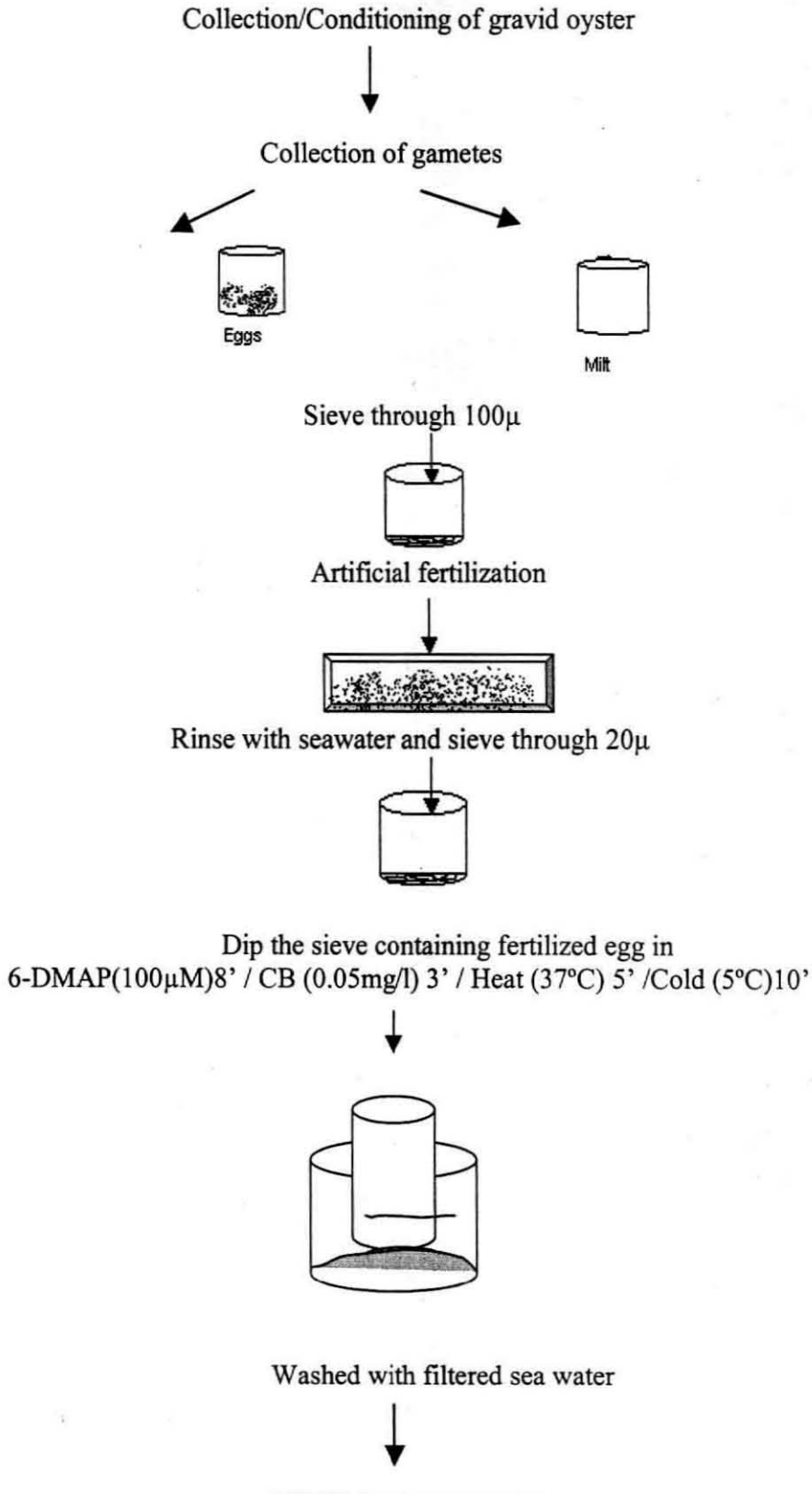
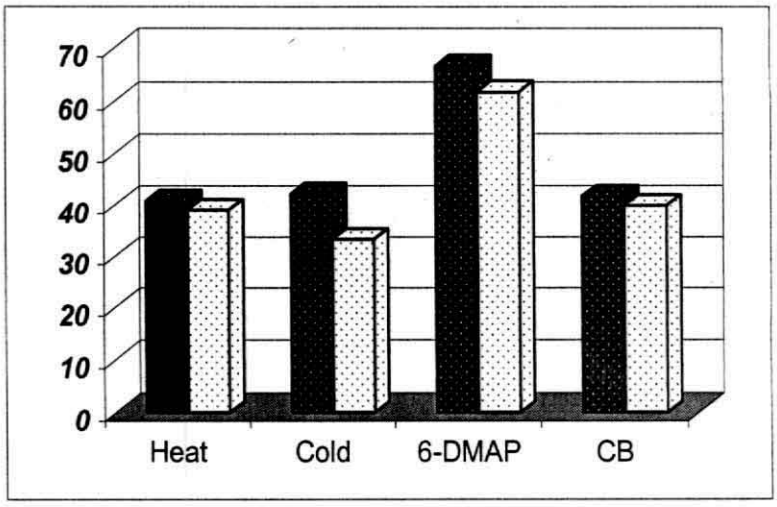


Figure 2. Relative efficiency of triploidy inducing agents in *C.madrasensis*



Applications of polyploidy in aquaculture

Polyploidization may enable an animal to adapt to varying environmental conditions. Common carp and the catostomid fishes are naturally polyploid species. It is believed that the polyploid genome enables these species to live in diverse ecological conditions. Triploid hybrids are more viable than diploid hybrids. Triploids are also more heterozygous and heterozygosity helps in maintaining their developmental stability.

Triploidy led to sterility in some fishes like Grass carp and Channel catfish. Triploid Grass carp cannot breed in natural waters and thus does not pose any environmental problem if transplants into larger water bodies for weed control. The culture of triploid Channel catfish is more profitable.

Sterile triploid progeny can be produced by crossing tetraploids with diploid ones. Triploid Rainbow trout produced by mating of tetraploid female with diploid male exhibited higher growth rate and survival than artificial triploids. Diploid spermatozoa of tetraploid males are useful for producing androgenetic offspring.

In India research on induction of triploidy is mainly undertaken at the Central Institute of Freshwater Aquaculture (CIFA), Bhubaneshwar, where triploids of most of the major carps and their hybrids have been produced. In the Central Marine Fisheries Research Institute (CMFRI), Cochin, the technology has been perfected for the production of triploid edible oysters (*Crassostrea madrasensis*) by arresting the release of both first and second polar bodies by chemical (6-DMAP) treatment They observed 30% increase in growth and 2 fold increase in glycogen and lipid content in triploid oysters.

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Concept of Integrated farming - Bivalves as Bio-filters

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Background

Environmental concerns have surfaced in the recent years and it has been proved that for long term growth of aquaculture industry both ecologically sound practices and sustainable resource management are a pre requisite. Among the aquaculture practices, semi intensive and intensive farming of shrimp and finfish have done considerable damage to the environment. The excessive use of supplementary feeds and the metabolic wastes from high-density farms have made the effluent pond water quality detrimental. Very high levels of suspended solids, organic carbon and frequent algal blooms are all indicative of the ecological imbalance – signs of negative impacts. Suspended solids are essential for growth of shrimps and absence of suspended solids may also adversely affect shrimp.

Intensive fish culture systems also have produced detrimental impacts. The feed required to produce 1 tonne of fish contains 110 to 130 kg N, of this 20-25% is retained in the fish, and remainder is either not ingested or converted to waste products. Similarly high concentration of Chlorophyll *a* has been reported within 500 m of the salmon farms. To utilise excess algae and suspended solids, farming bivalves and seaweeds has been suggested. Bivalves low in the food chain are filter feeders and seaweeds are autotrophic utilising the dissloved nutrients.

Bivalves as Biological Filters

Bivalves subsist mainly on particles filtered from the surrounding water, which they pump through the lamellae of their gills. Filtration rate can be termed as the volume of water from which all particles are removed in a given period of time. Filtering rate is equivalent to pumping rate if all the suspended particles are removed from the water passing through the filtering mechanism. The filtration rate of a bivalve depend on a) size of the species b) environmental conditions like temperature, salinity, pH etc c) water movement and d) particle size and their concentration/ density. Some of the particles are utilized while others are rejected as pseudofaeces. Studies have also shown that bivalves remove more cells from flowing water than from stagnant water.

Phytoplankton has been identified as the main component of bivalve feed. Apart from phytoplankton suspended solids are also observed to play a positive role in bivalve growth. Several studies have indicated that considerable weight gain is observed in oysters when a small quantity of suspended solids is added to the oyster diet. Wyban *et al* (1988) has found that diatoms, which are excellent food,

dominated the algal blooms in shrimp ponds. Considering these it is suggested that the solution to shrimp pond water effluent control may well in the utilization of the effluent instead of the current practice of discharging it into the open waters

Bivalves like mussels, oysters and clams are considered a delicacy in the temperate countries and the possibilties of utilising the nutrient rich water from the shrimp pond for farming oysters was researched since the 70s (see Table 1).

1. Hhigher growth rate and survival of bivalves grown with fishes and prawns.
2. Low fouling and good shape for the farmed oysters indicating their suitability for half shell trade
3. Sustainable production of shrimp.
4. Possible to reduce the input of fresh water by 50% (through reduction in phytoplankton concentration) in fishfarms when *oysters* were stocked with the fish.
5. Bivalve farmed in effluent drainage canals can reduce the concentration of organic matter by approximately 50%. Can significantly reduce the concentration of Ammonia –nitrogen, nitrite -nitrogen, phosphate and total suspended and bacterial numbers solids per tonne of shrimp pond water.

Model Systems

Reduction of particulate organic matter by sedimentation and microseiving has been found to be relatively expensive requiring regular maintenance. The biological treatment of sewage by algae and bivalves has proved to be efficient and expensive but the questionable quality of the cultured organisms as food has lead to the discontinuation of this method in many areas. Such objections do not arise for biofilter organisms cultured in fish/ shrimp pond effluents as long as the fish/ shrimp consume commercial feed and the water source is clean.

The concept of developing an environmentally clean aquaculture practice based on an integrated fish –mollusk- seaweed system has been tried at the National Center for Maiculture in Israel. In the model the water from the fishponds drains through an earthern sedimentation pond, a bivalve filtration unit and a seaweed filtration /production unit and is finally discharged back into the sea. An additional loop recirculates water from the sedimentation pond through a bivalve production unit. The performance of each of the component in terms of total nitrogen budget is: fish yield, 26% of N introduced in the feed; bivalve yield , 14.5%; seaweed yield,22.4%; settled feces,32.8% suspended and dissolved discharge back into the sea, 4.25%. Folke and Kautsky (1992) have also proposed a model for integrated coastal aquaculture linking species from different tropic level such as salmon, mussels and seaweeds. Building on this model Newkrik (1992) has suggested that environmental impact can be further reduced by including a benthic species such detritus consuming bivalves, bait worms etc.

Feasibility of Integrated Shrimp Aquaculture with Bivalve Farming in India

India has a diverse range of cultivable bivalve species among the Indian backwater oyster like *Crassostrea madrasensis* and the green mussel *Perna virides*

is commercially farmed. Though these are euryhaline, tolerance levels, the lower limit of salinity tolerance varies widely. Integration of shrimp culture with green mussel was done in a shrimp farm along the Gujarat coast with technical guidance from Central Marine Fisheries Research Institute, Cochin. The farm had a waterspread area of 9.36 ha with 9 ponds of 0.5 ha and 5 ponds of 0.25 ha independently fed through a feeder canal and dained into a drain canal. The drain water was collected in a waste settling pond of 0.5ha before disposal. The pond was provided with paddle wheel aerators. About 306 kg of mussel was obtained from $48m^2$ area (32.6% meat percentage). With a stocking density of 60,000 per ha it was possible to obtain 330 kg Penaeus *monodon* in 150 days (Subramanyan and Gopalakrishnan 2000). Similar results were obtained in Goan shrimp farms also.

Growth and survival of bivalves are location specific and information on their tolerance ranges and filtration rates is essential before stocking them with shrimp. Rajesh *et al* 2000 has found that the filtration rates of these commercially important species vary with the salinity of the environment, the concentration of the algal species and the size of the species. Oysters were found to be having higher filtration rates than mussels and clams. Experiments conducted at the demonstration farm of CMFRI at Ashtamudi Lake, Kerala has shown that the brown mussel, *Perna indica* is not suitable for farming in the estuaries. Though the salinity range falls within the tolerance limit, their growth and survival was very low. Location testing studies have shown that the oyster *Crassostrea madrasensis* can survive in low salinities even below 10 ppt. while for the mussels it is on the higher side; between 15 and 18ppt. Based on these it is possible to work out a model for different coastal regions of India.

Feasibility of integration of shrimp and finfish in a bivalve farm was experimentally tried in Ashtamudi Lake, Kerala with the main objective of increasing the profit obtained from the bivalve farm. In the estuarine systems the usual grow out system is the wooden rack. In this farm where mussels and oysters are the main crops, shrimp seed, *Peneus monodon* and *Etroplus suratensis* were stocked in separate closed cages in the space between the vertical poles. High growth rates and survival were observed for both the species. Though only very few studies have been conducted in this line, the preliminary results have indicated the scope for developing an integrated approach in the aquaculture practices of India.

Though bivalves can be considered as natural clearing agents of blooms, they filter other substances like calcium from the system. Since calcium carbonate is the major component of clam and oyster shells it would undoudtely be depleted from the water faster than other salt. Galtsoff (1964) found that *Crassostrea virginica* (size unspecified) held in flowing seawater deposited a median of 1.4 mg of shell material $/cm^2$ of shell surface per day during peak growing season. Excessive pseudofeces production coupled with low water movement can damage the benthic habitat structure and ecological web. Before taking up integrated farming it is essential that the information be gathered.

1. The rate of consumption of food, oxygen and dissolved chemicals by the animals
2. The rate of production of wastes by the animals

3. The tolerance of the animals to various water qualities conditions and particularly those resulting from accumulation of their own wastes.

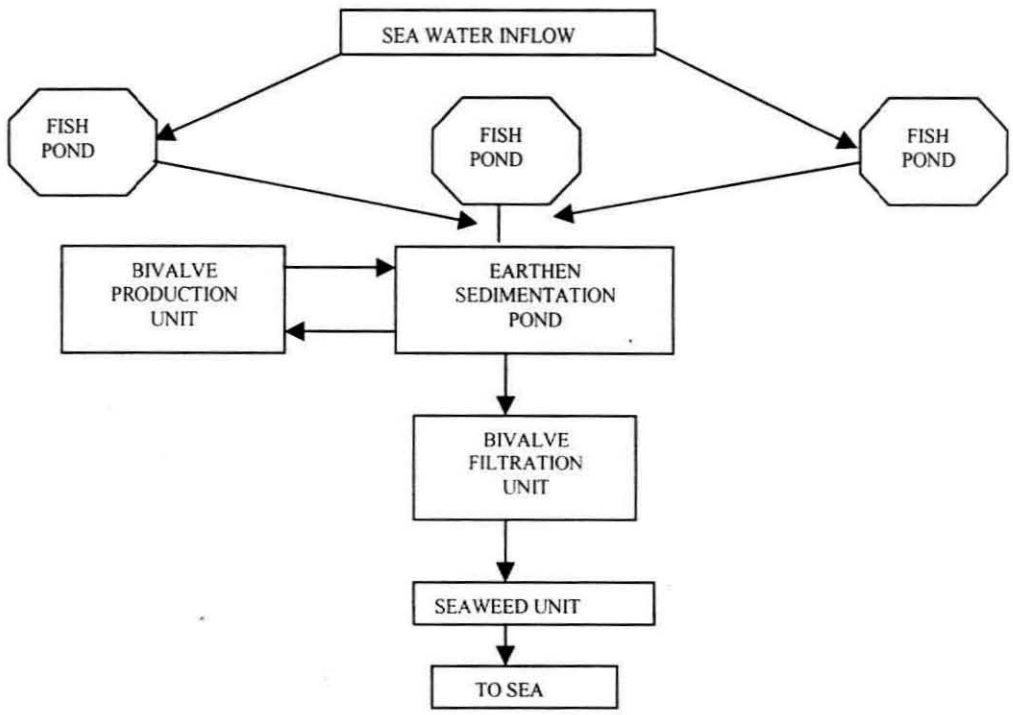
Among the bivalves, oysters and mussels will be better than clams. Studies have shown that placing bivalves on bottom will effect their survival (Hopkins *et al*, 1993). Most clams are infaunal and their growth and survival depends on the nature of the substratum in habit. Moreover shrimps are mostly benthic and their feeding habits also do not recommend the use of clams in shrimp ponds. Suspended culture of oysters and mussels will be beneficial to both bivalves and shrimps.

Table.1. Details regarding the integrated farming experiments

Candidate groups	Observations
Crassostrea gigas with fishes and prawns	Higher growth and survival of oysters but very little gonad development, oyster shape was good for half shell trade Oysters free from fouling.
Oyster with shrimp, *Penaeus vannamei*	Growth and survival of both species high, Oysters free from Dermo, *Perkinsus marinus*
Crassostrea virginica in commercial shrimp pond water	Capable of producing excellent half-shell oysters in Hawai. High growth rate (0.1 g to 54.2g in 198 days) survival 96%, meat to shell ratio 16.3 and condition facter 14.9%
Ulva lactuca grown in fish pond effluent in Israel	10 m^3 of Ulva can remove 90% of the ammonia produced by approximately 75 kg of fish
Seaweed in shrimp farm effluent water	In 24 hrs ammonia –nitrogen was absorbed by seaweed at 100% efficiency and BOD_5^{20} reduced by 39%
Abalone,(*Haliotis rufescens*), mussels , (*M. californianus*), and spot prawns, (*Pandalus platyceros*)	Growth of abalones and prawns were significantly higher in Polyculture systems
Crassostrea gigas with chinook salmon, *Oncorhynchus tshawytcha*	Growth and condition indices of oysters near the fish farms three times higher, growth increments were dependent on POM and Chlorophyll a
Sparus aurata (gilthead seabream) with *Crassostrea gigas*	Reduction in phytoplankton level possible to reduce the input of fresh water by 50%
Artemia and green mussel	In 24 hrs could reduce ammonia nitrogen, chlorophlll a, total suspended soloids and BOD in effluents with an efficiency of 67,87,13 and 77% respectively
Peneaus monodon and mussel	Good production of mussel and shrimp, mussel culture component played played a significant role in sustainable shrimp production
Prawns and clams	Net profit increased by 169.53% compared with monoculture

Penaeus vannamei, Clams, *Mercenaria mercinaria*, and oyster *Crassostrea madrasensis*	Growth and survival of shrimp not affected by the bivalves Low survival of clams and oysters placed on bottom,, high,95% survival of oysters placed in trays
Crassostrea virginica grown in effluent water from shrimp pond in Hawaii	High growth rate of oysters – hydrographic parameters not studied.
Crassostrea gigas grown in fish farm effluent	Better growth rate, condition indices in oysters grown in the effluent water Reasons attributed are Higher algal diversity, additional nutritious food consisting of benthic diatoms and stable algal concentrations
Green mussel in Shrimp effluent drainage canals	1kg of mussel for an effluent load of 4 tons per day reducing the concentration of of organic matter by approximately 50%
Penaeus japonicus and *Ostrea rivularis*	Shrimp yield increased by 30%, survival rate of oysters raised by 17%, meat percentage increased by20.3%, High economic benefit
Perna virides grown in effluent water from shrimp ponds.	One kilogram of mussel significantly decreased the concentration of Ammonia – nitrogen, nitrite – nitrogen, dissolved oxygen phosphate and total suspended solids per ton of effluent.
Saccostrea cucullata and *Penaeus japonicus*	Reduced the effluent total suspended solids to 49%, Bacterial numbers to 58%, Total nitrogen to 80%, Total phosphorous to 67%, Chlorophyll a to 8%
Crassostrea madrasenis in shrimp farm effluent	Higher level of reduction of suspended solids and chlrophyll concentration possible with larger oysters

Fig.1. Schematic diagram of the integrated system proposed by Shpigel et al (1993)



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37 Concept of Organic bivalve farming

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Organic farming is based on holistic production management systems, which promote and enhance agro-ecosystem health, including biodiversity, biological cycles and biological activity. Complete lack of application of fertilizers and feed including antibiotics, vaccines and growth hormones during the farming period either to promote growth or to increase survival is one of the advantages of molluscan shellfish farming. Molluscan shellfish are biofilters, which have the beneficial secondary effect of taking up nutrients and purifying the water column, thereby "enhancing ecosystem health." In addition to this the farm structures, provide cover and forage for other species - enhancing biodiversity, biological cycles and biological activities through the creation of critical habitat.

Organic certification addresses the processes involved in production rather than the qualities of the product itself. The molluscan shellfish farming processes adhere to the principles of organic farming by default. However, bivalves being grazers or filter feeders can accumulate pollutants, pathogens and algal biotoxins, which pose a threat to the health of the consumer. Hence the public health safety standards on which the shellfish industry is based will be applied to the concept of organic farming in molluscan mariculture. Monitoring of the growing areas, its classification, certification of farm sites in terms of water quality are essential to evaluate the quality / safety of farmed molluscs. Periodic residue testing is also permitted.

Fundamental Organic Certification

Water quality is an essential part of Organic farming. The main pollutants, which hamper the quality of bivalves, are microbial pollutants, biotoxins and trace metals. Simultaneous observations of the level of these pollutants in the environment and the farmed animal are essential for organic farm certification. Targeted research on this aspect for a period of three years is essential for certification.

Protocols of bivalve farming, which are conventionally followed, have been critically evaluated and modifications at various levels such as method of farming, source of seed, method of seed collection from natural bed, type of cultch material used, pest control, waste management stocking density, method of harvest and processing have been suggested.

Impact on the Environment should be minimal and it has to be proved that the farming has not hampered the normal ecological and biological processes taking place in the environment. Its impact on the naturally occurring species should be documented. This is vital for certification.

The general guidelines currently available are intended for bivalve farming in temperate countries. Comparison of bivalve farming under the Indian conditions and the temperate waters show significant variation *viz.* short grow out period of 5 to 7 months in India compared to 36 to 48 months in temperate regions, occurrence of non toxic blooms in nearshore areas rather than toxic blooms as in temperate waters, dependence on natural seed by Indian farmers rather than hatchery produced spat or genetically modified or triploid / tetraploid spat etc.

Status of Organic Farming

Molluscan shellfish industry has been subjected to considerable threat and public criticisms in the beginning of last century due to human fatalities resulting from consumption of shellfish. To safeguard the interests of this industry, the Government and the industry jointly initiated several programs in several parts of the world. One of the main programs started in 1920 in the United States is the National Shellfish Sanitation Program (NSSP) a cooperative program among the Food and Drug Administration (FDA), State and the foreign Shellfish Regulatory Authorities. The public health regulations governing the shellfish farming and harvesting are among the strictest imposed upon any food producer in the U.S., and pose an excellent foundation that aligns well with the organic standards. Under the NSSP, each growing area must be tested for pathogens for several months before they are classified. Growing areas are classified based on the water quality Quayle and Newkirk (1989) and harvesters to use tags on each container the company name, harvest location and date and it is illegal to sell shellfish to the public without proper commercial certification.

With the development of global interest in organic farming, the question of developing standards for molluscan farming arose before the Certifying agencies. Unlike shrimp farming, the open nature of the bivalve farming, without closed boundaries posed several problems.

In the US, a report by Goldberg and Triplett (1997) titled "Murky waters: Environmental Effects of Aquaculture in the United States" documented the adverse impacts some types of aquaculture are having on the environment. A strong recommendation which came out of this is the need to develop organic or ecocertification programs that empower consumers to chose aquaculture products grown in an environmentally friendly sound manner and that give aquaculturists incentives to produce products which can bring higher prices.

One of the guidance documents which has critically considered the various points regarding the certification of bivalve farming is the: "White paper : Developing Organics standards for Mollusk Shellfish" by Dr.Robin Downey, Executive Director, Pacific Coast Shellfish Growers Association, USA (Reference: wed site http://www.pcsga.org/). This document considers the principles of Organic Agriculture as it applies to Shellfish farming and lists some points for consideration while formulating standards that can be further developed based on the shellfish industry and organics experts to assure equitable and implementable standards. One of the major points suggested in the document is reproduced below in the box.

Areas for consideration in establishing criteria and management plans for organic molluscan shellfish standards: To obtain organic molluscan shellfish certification:

1 a) Require shellfish growing areas to be in the "Approved" classification status for three consecutive years and require periodic lot testing at intervals of 6 months.

OR

b) For Conditionally Approved growing areas, and only during the "open" status, require lot testing once a month during "dry" weather periods or once a week during periods of intermittent rainfall. Following periods of closure due to rainfall, require testing on the first lot harvested once the areas achieves "open" status again.

2. Require shellfish growers to adopt Environmental Codes of Practice that include farm plans that explicitly describe their sustainable and conservation management farming and processing systems.

Source: "White paper: Developing Organics standards for Mollusk Shellfish" by Dr.Robin Downey, Executive Director, Pacific Coast Shellfish Growers Association, USA (Ref wed site http://www.pcsga.org/).

One of the certifying agencies for Organic Aquaculture is the " ***Naturland"***, a member of the international umbrella organization- International Federation of Organic Agriculture Movement (IFOAM), which issues binding standards in the fields of both production and processing. They have elucidated standards for culture of marine mussels (Ref: website www.naturland.de - Section I: general guidelines and Section III C). Importance has been given to site selection, type and origin of stock and culture systems. Site is required be Class IA, wherein the faecal *Eschererichia coli in* the farmed mussel should be ≤ 3 counts /g tissue), the origin of the seed be traceable to the area of collection and on bottom faring is prohibited. Another organization the BIO-GRO has set standards for New Zealand (Ref website: www.bio-gro.co.nz) and as per the document revised in April 2001 (version 1:30 under Module 4.7.) importance has been given to site and presence of biotoxin.

The organics movement in New Zealand incorporated the marine environment and there are several BIO-GRO oyster farmers substantiating the fact that demand for organic products is reaching aquaculturists (Ref :Organics and Aquaculture , Paper by Dr.Sean Handly). In support of the organic aquaculture production, the NZ has in place some of the most stringent export testing regimes facilitating the farmers to sell their products in the most challenging overseas market like the U.S. and Japan. The NZ Mussel Industry Council has also produced its own Environmental Code of Practice- the first marine farmers to do so in the world.

In London a charity has launched world's first seafood products under the " Marine Stewardship Council " standards (MSC) – an initiative with links to the

Dutch organic certifier "Skal" and the World Wide Fund for Nature WWF in the Netherlands (MSC-2000, Agro- Eco Consultancy 1999). One of the first seafoods to be launched under the MSC standards is the mussel and cockle fishery in the Waddensea and this is under pre certification process (MSC 2000, agro-Eco Consultancy 1999)

Table.1. Guidelines set for Oyster Farming (in British Columbia) and Mussel (for details refer COABC site)

Bivalve Farming Feature	**Required**	**Prohibited**
Grow out method	Off bottom like rack, long line, stake	On bottom
Material for mussel seeding	Net /rope should be appropriate for reuse. They should be decomposed or recycled after use	
Location of farm site	Maximum possible turn over from open sea	Mussel culture in immediate proximity to shore or close to nutrient inflows is not permitted
Water quality	• Should meet the criteria of sites classified as "Approved ' in terms of general water quality, trace metal content, biotoxin levels and microbial load • Should be monitored monthly and recorded for reference	Polluted water bodies, areas with history of toxic algal blooms and high loads of enteric bacteria
Seed	From natural bed or hatchery	Triploid or Genetically Modified
Setting of larvae in the hatchery		Use of epinephrine
Collection of wild spat	Collecting activity must be documented and traceable to respective collecting area, quantity of seed collected, name of seed collector,	Collecting activity should not cause lasting damage to the natural ecosystem
Type of cultch material	Shellfish shell, food grade plastic, cement and French tubes made of allowable material	Tires, plastics that are not food grade quality, plastics that have previously contained toxic or harmful material, new PVC French tubes
Pest control	Should have only minimal impact on the fish and wildlife habitat	Fungicides, traps etc
Waste management	Shells and other wastes must be disposed of and should not attract vermin &insects	
Shellfish stocking density	Must reflect health of the organism	Should not exceed the sustainable yield of the ecosystem in which the farm is situated
Harvest	Producers must only harvest shellfish within the boundary of their production site	Dredging
Visual quality	Tidy and uniformly laid out site Floatation devices must be of uniform shape and colour	

Important Web Sites

Agro-Eco Conultancy.1999. MSC pre-certification of mussel and cockle fisheries-the Netherlands. http://antenna.nl/agrroeco/welkom.htm

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38

Cephalopod Culture

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Introduction

Cephalopods are the largest and most active invertebrates. About 1, 17, 278 tonnes of cephalopods are exploited during 2003 in India (Annam *et al.*, 2004). During 2002-2003 India has exported 41,381 tonnes of frozen cuttlefish and 37838 tonnes of frozen squid valued at US$ 166.2 million to countries such as Japan, USA and the European Union (Anon, 2003). Cephalopods are unique because they are 85% protein by dry weight (16-21% by wet weight) (Lakshmanan and Balachandran, 2000) and are considered a delicacy in seafood restaurants.

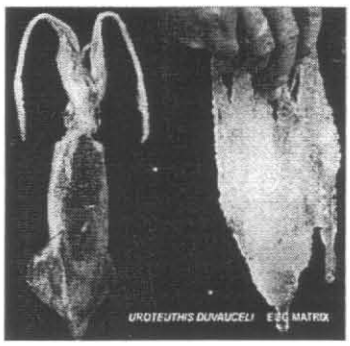
Recent years have witnessed a significant amount of research interest in cephalopod culture, in order to develop technology for commercial farming as well as to produce multiple laboratory generations for research in neurobiology (Minton *et al.* 2001). They are highly promising biomedical models because of their giant axons and are of interest to neurobiologists. Squids 4 months old have giant axons larger than 450µm in diameter. Studies have shown that the ultrastructure and physiology of these systems rival the sophistication of their vertebrate counterparts, the vestibular end organs and the vestibulo-oculomotor system. In detail, many parallels exist, e.g., the dynamic response characteristics (gain and phase lag values) of the cephalopod angular acceleration receptor systems are similar to those of the vertebrate semicircular canals, the putative transmitters in the afferent and efferent fiber systems are similar, and the cephalopod brain pathways involved in oculomotor control have vertebrate-like organizations. Thus, these systems are interesting invertebrate models that can substantially contribute to our understanding of the basic principles of morphology, physiology and pathology of these systems in higher vertebrates, including man.

Choe and Oshima (1963) and Choe (1966) reared three species of the genus *Sepia*, the squid *Sepioteuthis lessoniana* and the sepiolid *Euprymna berryi* from egg to adult size. Nabhitabhata and co-workers of Rayong Brackish water Fisheries Station have conducted pioneering research work on the culture of several species of commercially important cephalopods in Thailand (Nabhitabhata, 1978a, b, Nabhitabhata *et al.*, 1984 and Nabhitabhata and Nilaphat, 1999). *Sepia pharaonis* was successfully bred under laboratory conditions in Thailand as well as the USA using sophisticated, temperature controlled recirculation systems (Nabhitabhata, 1994, Minton *et al.*, 2001).

In India our first major success in Cephalopod Mariculture was realized in 1999 with the cuttlefish *Sepiella inermis* (Sivalingam, 1999) at Tuticiorin Research Centre of CMFRI. Since that time we have worked on squids *Uroteutis duvauceli*, *Sepioteuthis lessoniana*, *Doryteuthis singhalensis*, cuttlefish *Sepia pharaonis*,

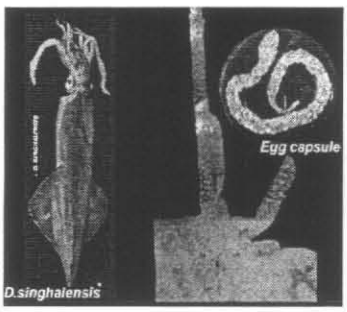
Sepiella inermis and Octopus *Octopus dollffusi*. However, for the past three years we have focused our efforts on developing the potential of the cuttlefish *Sepia pharaonis* and squid, *Sepioteuthis lessoniana*.

Egg Masses of Different Species of Cephalopod

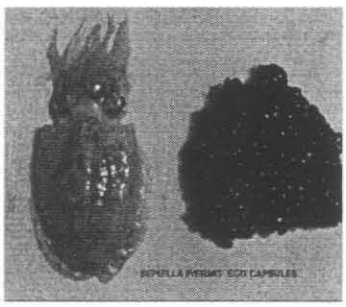


Egg mass of *Uroteutis duvauceli*

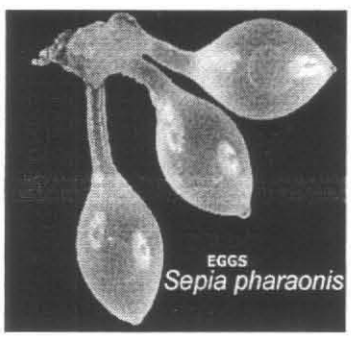
Egg mass of *S.lessoniana*



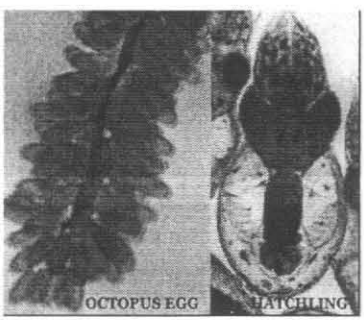
Egg mass of *D. singhalensis*



Egg mass of *S. inermis*



Egg mass of *S. pharaonis*



Egg mass of *O. dollffusi*

Rearing of Cephalopods

Cephalopods require high standards of water quality while feeding at high rates and producing copious quantities of ammonia and ink.

Water Quality

The water quality criteria for cephalopod culture in both nursing and grow-out phases regardless of species, are as follows: -

Dissolved oxygen:	>5 mg/l
Salinity	: 30-35 ppt
Temperature	: 27-32^{0}c
P^H	: 7.0-8.5
Ammonia	: <0.005 mg/lit
Nitrate	: <25 mg/ml.

Food and Feeding

The limitation is that the cephalopods are carnivorous and selective feeders; they require live feed with a specific size, shape and movement. Feed without these characteristics will be ignored and the cephalopods will starve to death. The degree of selectivity is higher in the early stages compared to the adults. After a stage they can be trained to accept low value fish.

Brine shrimp nauplii, which is used as live feed for most of the cultivated marime fishes and shellfishes, is unfortunately not suitable for cephalopods. But adult brine shrimp can be used as a feed supplement. Mysid shrimp collected from natural waters is used world over to rear cephalopod hatchlings. Experiments conducted in Thailand and India have shown that live prawn postlarvae can be used as feed for Cephalopod but will substantially increase the production cost. In USA the first successful defined diet formulated specifically for cephalopods.

At Karwar Research Centre of CMFRI, spineless cuttlefish *Sepiella inermis* was successfully reared from the egg mass collected from wild. They mated under captivity and spawed on 86th day at a size of 60 mm mantle length producing 214 viable eggs. Only live food organisms, consisting of mysids, shrimp post larvae, and juvenile fishes formed the diet of these animals in different stages. The initial average size of hatchling was 4mm ML (0.02g) that increased to 69 mm (54.67g) on 110th day. Average survival was 43, 37 and 28% at the end of first, second and third months (Anil, 2003).

At Vizhinjam Research Centre of CMFRI, Pharaoh cuttlefish (*Sepia pharaonis*) was successfully reared from egg to an average size of 168 mm mantle length (ML) and weight of 521 g in 226 days in the laboratory, using simple biological filtration systems. The period of egg incubation was 15 days at a temperature range of 27-31 C. Food items given were live mysids, *Artemia salina*, juveniles of fishes and prawns. Subsequently, the juveniles were slowly acquainted with food items such as dead caridian prawns and small fishes. Hatchlings were reared at a stocking density of one animal/litre during the first month, and subsequently stocking density was reduced as the growth proceeded. The study shows that the pharaoh cuttlefish can be reared under captivity with a survival rate

of 40% with the use of live feed limited to the initial phase of 50 days. (Anil *et al.*2004).

At Vizhinjam the PallkBay squid *Sepioteuthis lessoniana* was also successfully bred under captivity. The squids reared from egg masses collected from wild, in rearing systems containing biological filtration units, successfully mated and spawned on 105th day of rearing.

With the use of cage type of rearing systems in open waters and with better feeding schedules, commercial culture systems with good survival rate and growth can be developed. The future of cephalopod culture depends on the development of mass culture techniques of mysids for feeding hatchlings with *Artemia* as supplement and artificial feed for the adults. The recent success achieved in feeding the young ones with *Artemia* as supplement and acquainting the cuttlefish to food items other than live feed such as anchovies and sardines which can be obtained in large quantities are steps in this direction.

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