SEED PRODUCTION TECHNOLOGY FOR EDIBLE MARINE MOLLUSCS

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INTRODUCTION

In recent times, noticeable increase is observed in the mariculture activities in many parts of the world in order to augument the fish production. One of the major constraints in the mariculture industry is the shortage of seed supply for the farming activities. This fact is applicable to molluscan farming also, because availablity of seed in the wild is severely restricted to few patches and to certain seasons in the natural beds which cannot meet the demand. Therefore, need was felt to enhance the seed supply either by increasing effort for seed collection from the natural beds by identifying areas of abundance or production of seed by hatchery techniques.

Growth of seed production technologies for molluscan species was slow, but steady. Until recently, rearing of larvae and juveniles of marine molluscs, on a basis where repeatable results could be expected, was virtually impossible. The failure in the rearing experiments were usually due to poor culture methods and want of good and specific food for the larvae, especially when they were grown in higher concentrations. Diseases, including those caused by fungi, were also responsible for the persistent failures. Limited knowledge on the effects of various environmental factors, singly and in combination, upon the growth of larvae was another drawback. But with increased mariculture activities of molluscs, which induced greater demand for seed, extensive experiments were conducted to understand the intricacies of these factors and techniques were developed to overcome many of them.

SPECIES USED

Hatchery production of seed was restricted to commercially important species of molluscs of which main consideration always being given to economic viablity. Pioneering experiments were conducted at Milford Laboratory, United States of America, based on the results of which many hatcheries were established along the Atlantic Coast. Most of them used only the bivalve species such as the clam, *Arca transversa*, mussel, *Mytilus edulis*, the scallop, *Pecten irradiens*, European oyster, *Ostrea edulis*, the native Pacific coast oyster *Ostrea iurida*, the American oyster *Crassostrea virginica*, the Japanese oyster *Crassostrea gigas*, the hardshell clams, *Mercenaria mercenaria* and *Mercenaria campechiensis* and soft shell clam, *Mya arenaria*. Presently,

China is the country which carry out large scale mariculture of molluscs, specifically the clams. Seed of the clam *Argopecten irradiens* (which is an exotic species brought from eastern coast of Pacific), are produced in very large quantity in the hatcheries and the entire demand of the mariculture activities are met by hatchery produced seed.

In India, technologies are available for large scale seed production of the oyster *Crassostrea madrasensis,* pearl oyster *Pinctada fucata,* green mussel *Perna viridis,* brown mussel, *Perna indica,* clams *Meretrix casta, Meretrix meretrix, Anadara granosa, Villorita cyprinoides* and *Paphia malabarica.*

Life-history

Life-history of a typical bivalve involves a floating free-swimming veliger stage. Since most of the bivalves are sedentary in the adulthood, this larval phase enables them to attain wider distribution and settlement in a favourable environment.

Sexes are separate among the bivalves. Spawning normally takes place during that part of the year, when environmental conditions and availability of food are conducive. During spawning female releases millions of eggs into the surrounding water, where they are fertilised by the sperms released by the male. After fertilization, the egg divides continuously to develop into blastular, gastrular and trochophore stages and finally D-shaped floating larva named "veliger". The larva can swim with the help of the swimming organs called velum. After passing through Umbo and Eyed stages, the larva enters the Pediveliger stage when it loses the swimming capacity. At this stage, the larva possesses a foot, which help the larvae move actively on the bottom. Subsequently, the foot of the larvae gets reduced and it settles as "spat". With further growth, juvenile phase is attained and changes take place in the shell thickness, colour and in the internal organs. Stages of development of green mussel *P. viridis* and the clam *Mererix casta* are shown in Figs. 1 and 2, which are typical of other bivalves also.

The floating larval phase varies from 7 to 20 days among different species of the bivalves. For example, floating larval life of the clam *M. casta* is 7 to 9 days, the edible oyster *C. Madrasensis* is 20 days and the green mussel *P. viridis* is 18 to 20 days.

Hatchery Techniques for Seed Production

Infrastructure requried

Establishment of bivalve hatchery requires the following facilities :

1. ***Space and lay-out :*** The primary requirement of an operational hatchery is uninterrupted supply of good quality seawater. The seawater should be free from pollutants and from suspended solid particles and silt and hence site of drawal of water should be rocky, coralline or sand mixed. It should be far away from industrial areas, areas of discharge of domestic sewages and from river mouths. Proximity to natural beds and farm site and availablity of transportation, are other ideal requisites.

   The hatchery building should be designed and constructed in such a way as to get maximum light and air inside the hatchery. The roof should be partly provided with

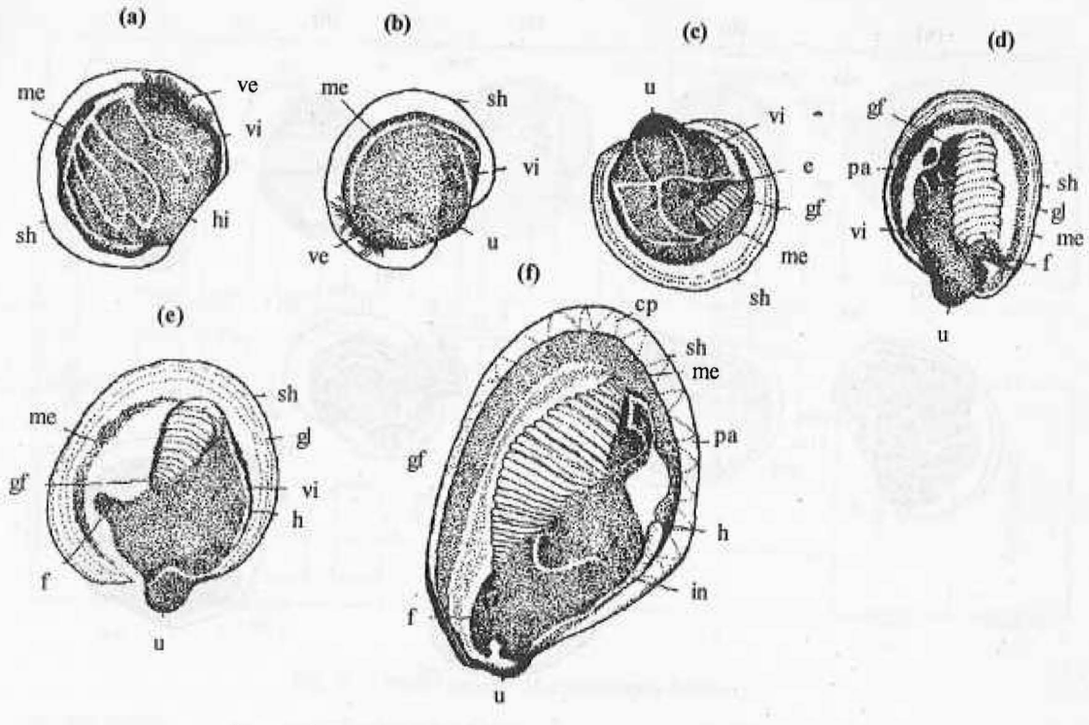

Fig. 1. ***Stages of life-history of the green mussel Perna viridis***

a. D-shaped veliger (4days old)
b. Early umbo staged veliger (6days old)
c. Eyed stage (15 days old)
d. Plantigrade (19 days old)
e. Spat (27 days old)
f. 45 days old spat

translucent fiberglass sheets, which will enable the provision of light to mass culture of algal food. Free passage of light and air should be ensured by providing glass panelled windows, air vents and exhaust fans. Concrete flooring with sufficient gradient and gutters for easy drainage of water should be provided . An ideal hatchery for producing about five million seed per annum requires not less than 20 m x 10 m of built-up area. A typical lay-out of a molluscan hatchery is given in Fig. 3.

2. ***Seawater supply :*** Seawater supply system consists of an intake point, a draw well, sedimentation tank, filter bed, a water sump, overhead tank and PVC delivery lines. The filter bed, normally consists of river sand at the top, charcoal, pebbles and granite stones at the bottom. The seawater passed through the filter bed is further purified by passing through 15 μm, 10 μm and 5 μm cartridge filters and sterlised in UV chamber prior to use in the hatchery tanks. The daily water requirement of the seawater is around 10 tonnes. Capacity of the storage tank is around 20 tonnes and that of the overhead tank is 10 tonnes. Necessary electric pumps are to be provided for pumping the seawater at various points. Stand-by motors and generator are also required to meet any contingencies.

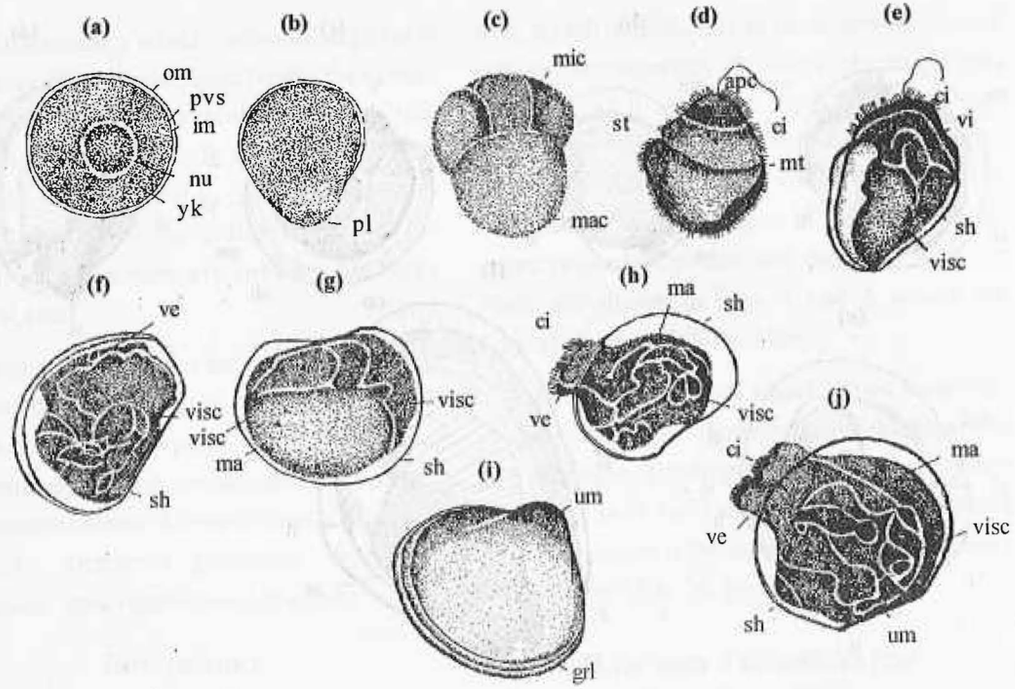

Fig. 2. *Stages of life -history of the clam, Meretrix casta*

a. Fertilised ovum
b. First polar lobe formation
c. 8-celled stage
d. Trochophore larva
e. Shell formation
f. D-shaped larva
g. 1-day old veliger
h. 2-days old veliger
i. 5-days old veliger
j. 20 days old spat

apc : apical cilia, asp : anterior siphon, ci : cilia, cp : chromatophore pigments, e : eye, f : foot, gf : gill filaments, gl & grl : growth line, h : heart, hi : hinge, in : intestine, inm : inner membrane, ma : mantle, me : mantle edge, mac : macromeres, mic : micromeres, mk : markings on the shell, mt : metatroch, nu : nucleus, om : outer membrane, pa & pam : posterior adductor muscle, pl : polar lobe, psp : perivitelline space, sh : shell, st : stomadaeum, u : umbo, um : umbonal end, ve : velum, vi : visceral mass, visc : visceral organ, yk : yolk

3. ***Aeration :*** Air circulation to the tanks is being carried out by using air compressors which can be either of piston or rotary vane type. The air is passed through a series of filters to remove oil and moisture and supplied to the hatchery through PVC pipes. Air can be drawn at the required places from these pipes running the entire length of the hatchery at a height of 3 m through the nozzles. The air is supplied to the culture tanks through diffuser stones. Electrical air blowers are also used which can supply oil-free air.

4. ***Microalgal culture :*** Flagellates measuring less than 10 μm are the main food of bivalve larvae while mixed algal culture are used for feeding the spat and seed. The important species used in the bivalve culture system are *Isochrysis galbana*, species of *Pavlova*, *Chromulina* and *Dicretaria*. Usually for culturing flagellates, Conway or

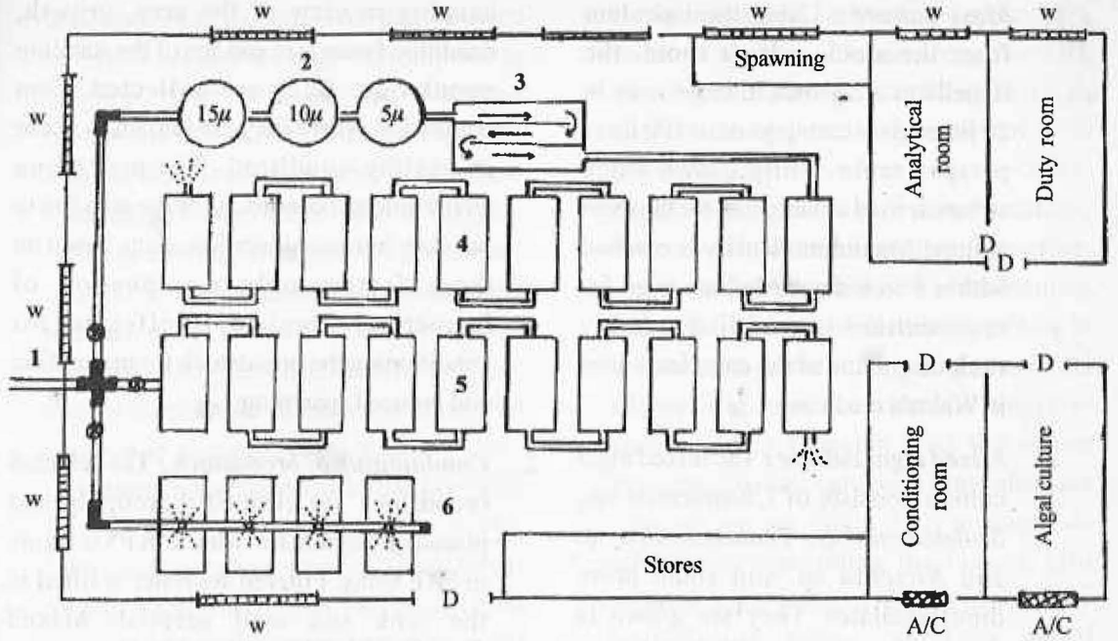

Fig. 3. ***A model lay-out of a molluscan hatchery***

1. Seawater supply
2. Cartridge filters
3. UV Vhambers
4. Larval rearing tankss
5. Spat rearing tankss
6. Algal culture tanks

(W : Window, D: Door and A/C : Airconditioner)

Walne's medium is used for the maintenance of stock culture as well as for mass culture. Prior to mass production of the flagellates, isolation of required species is undertaken by serial dilution. Normal room temperature is not ideal for the maintenance and culture of flagellates. Hence air-conditioned rooms are used which have 23-25° C during daytime when all the tube lights are burning. One of the most important factors determing the successful culture of the microalgae is the type and quantum of illumination. Too much of light will cause the culture to decline earlier. For the growing phase of mass culture 1,000-1,5000 lux is optimum upto 5-6 days and for maintaining the stock culture, 400-500 lux is enough. Twelve hours of light and 12 hours darkness is ideal for maintaining the stock and the mass culture, and this can be done by control switch clocks.

a. ***Stock culture :*** Haufkin flasks with Walne's medidum are inoculated with the required species. The flasks are placed under tube lights (800 lux). When their maximum exponential phase is reached, light intensity is reduced to 400 lux to enable further growth. Normally the flagellates will enter the stationary phase of growth after 12-15 days. In this phase, the culture can be kept for a period of 2 months without aeration.

b. ***Mass culture :*** Using the inoculum from the stock culture room, the flagellates are grown in large scale in 20 litres glass carbuoys or in 100 litres perspex tanks. Fully grown stock culture is used as inoculum for the mass culture. Maximum density is reached within 5 to 6 days. Medium used for mass culture is modified suitably excluding some of the chemicals used in Walne's medium.

c. ***Mixed algal culture :*** The mixed algal culture consists of *Cheatoceros* sp., *Skeletonema* sp., *Thalasssossira* sp. and *Nitzchia* sp. and some more dinoflagellates. They are grown in fertilized medium in outdoor tanks.

5. ***Broodstock maintanence chamber :*** The broodstock maintenance chamber need be about 20 sq.m. in size. The room is fitted with an airconditioner to maintain a temperature of about 23° C. One tonne FRP tanks filled with seawater are used for keeping the broodstocks.

6. ***Larval and spat rearing :*** Rearing component consists of about 50 sq. m. area. FRP tanks of one tonne capacity are used for rearing the larvae and spat. Few FRP white coloured tanks of one tonne capacity are used for mass culture of dinoflagellates used to feed the larvae and spat. Sieves of different mesh sizes made of Nylobolt material ranged from 30 μm to 250 μm were used for filtering the larvae, while changing the water.

Seed production technology

1. ***Collection of broodstock :*** Broodstock required for induced spawning are selected keeping in view of the area, growth, condition factor size and age of the standing population. They are collected from population where they are known to occur in healthy condition. The prevailing environmental conditions of the area has to be taken into consideration, since based on these factors only manipuation of temperature regime is effected for conditioning the broodstock for maturation and induced spawning.

2. ***Conditioning of broodstock :*** The selected broodstock are cleaned thoroughly and placed on a synthetic twine knit PVC frame in FRP tanks. Filtered seawater is filled in the tank and well aerated. Mixed phytoplankton cultured in outdoor tanks using sterilised seawater are added twice a day. The broodstocks are conditioned about 5° C below the ambient temperature. Periodical examination of the gonads is made to assess the maturity of the gamets. On observing suitable maturity, the broodstocks are transferred to spawning tanks.

3. ***Induced spawning :*** Thermal manipulation by raising the water temperature few degrees above the ambient temperature is found to be effective to induce spawning in most of the bivalves. Chemicals such as tris, hydrogen peroxide and sodium hydroxide were also found to induce spawning.

4. ***Larval rearing and spat production :*** Soon after spawning, the parents are removed from the spawning tank. The water in the tank is kept without disturbance for the fertilization to take place. After fertilization, the seawater in the spawning

tank containing the fertilized eggs is diluted several times and the eggs were allowed to develop. After 24 hours, the D-shaped larvae are transferred to one tonne FRP tanks at the rate of 2 larvae/ml of seawater. Feeding with microalgal food is initiated from the first day after spawning. Quantity of algal cells supplied is dependent on the number of larvae and is also increased gradually with growth of the larvae. Water change is undertaken once in two days. Mild aeration is also resorted. Utmost hygenic conditions is maintained in the hatchery with proper cleaning of the containers, seives, tubes and aeration stones. Since larval growth is influenced by larval density, food supply, water quality, water temperature and other factors, regular monitoring is done on the water quality and conditions of the larvae. Records are maintained on initial larval density, growth and number of spat settled.

5. ***Nursery rearing :*** Spat settled in the hatchery tanks were transferred to the nursery either in open sea or in enclosed bay systems for further growth. After attaining suitable size for transplantation, they are transferred to the farms.

6. ***Diseases and their control :*** The field of diseases and parasites of larval and juvenile molluscs is entirely new, because studies of this nature is yet to be developed. No information was available, therefore, as to whether the bacteria, protozoans, etc. observed in weak and dying larvae, were the primary cause of their death of merely secondary invaders or only scavengers.

Species of epizootic fungus such as *Sirolpidium* sp. was observed to cause heavy mortality of molluscan larvae in the culture system. Fungi are generally introduced into larval cultures through untreated seawater or more often with phytoplankton grown in outdoor tanks. Control of fungus can be done by chemicals, but they affect the larvae also. Therefore, precautionary measures such as maintaining general cleanliness of all utensils coming in contact with larvae and in using germicidal ultraviolet rays to treat all water is recommended. Plankton food, if it comes from impure mass cultures, should also pass over ultraviolet tubes. This measure, in addition to controlling the fungus, also protects larval cultures against invasion by undesirable forms, such as small crustaceans, larvae of different worms, rotifers and protozoa, which compete with bivalve larvae for space and food. In some instances raising the water temperature to approximately 32.5° C for several hours may kill fungus without causing serious injury to the young molluscs. Colonies of bacteria belong to *Vibrio* and *Pseudomonas* were also found to affect the larvae by their toxins. They can be controlled by use of antibiotics such as Penicillin, Streptomycin, Kanamycin, Aureomycin, Combistrep and Sulmet. Use of antibiotics in higher doses beyond certain limits is likely to retard the growth or kill the larvae. Epizootic, such as bryozoans, ciliates and ascidians also can be controlled by use of Pentachlorophenal at 1 ppm, formalin at 40 ppm and dichlorophene, in the hatchery system.

7. ***Selective breeding and hybridization :*** Development of reliable and standard

hatchery techniques opened fields of studies on the heredity of molluscs and their selective breeding. Using these methods it is now possible to cross individuals showing specific characteristics and attempt to develop strains or races of oysters, clams, mussels, scallops, etc. with desirable characteristics such as rapid growth, high glycogen content in their bodies, resistance to certain diseases and finally, ability to propagate and to survive under less favourable conditions. Cross breeding was successfully done among different strains of the Japanese oyster *C. gigas*, where the hybrids displayed a greater adaptability to environmental conditions than the inbred strains. Similarly, two closely related species of clams *M. mercenaria* and *M. campechiensis* were also successfully cross bred. Larvae resulting from these crosses were observed to metamorphosis at a faster rate than normal, due to hybrid vigor.

Remarks

The development of hatchery technology for production of seed has been timely in respect of marine bivalve resources. Because of this, farming of bivalves took off successfully, without total dependance on the supply from natural sources, which fluctuated unpredictably. The technologies developed and practised are simple for adoption and is of low cost in terms of equipments and operational expenditure. Hatchery technology of certain species of bivalves such as pearl oysters enabled research development in another direction *i.e.* to attempt to repopulate the natural beds themselves by sea-ranching.

Hatchery technology is multidisciplinary in nature and there are several aspects which need further directed research. These relate to broodstock management for continuous supply of quality mature animals for the breeding programmes, genetic improvement, water quality management, larval physiology, nutritional requirements at different stages, further identification of diseases and their control measures, improvement in larval survival, synchronisation of growth and metamorphosis, spat setting requirements, spat nutrition and juvenile rearing.

Economics of hatchery production of seed is another aspect which has to be duly considered. The break-even point of production can be minimised and profitability enlarged only through achieving scheduled production rates, high survival, fast growth, early spat setting and high amount of success in juvenile rearing. These aspects need careful consideration and would decide the future of shellfish hatchery programmes.