CMFRI Training Manual Series No.24/2020

Course Manual on Marine Fisheries & Mariculture

Prepared for the Training programme for B.F.Sc students of Central Agricultural University (CAU), Tripura

ICAR - Central Marine Fisheries Research Institute
(Indian Council of Agricultural Research)
Mandapam Regional Centre in collaboration with
Agricultural Technology Information Centre (ATIC).

COURSE MANUAL

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February, 2020

Course conveners

Dr. R. Jayakumar.,

Principal Scientist & Scientist-in- Charge,

Dr. V.P. Vipinkumar.,

Principal Scientist & ATIC Manager,

Dr. Johnson, B., Scientist,

Shri. M. Sankar, Scientist.

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Director
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Contributors

Dr. R. Jayakumar,

Dr. G.Tamilmani,

Dr.M.Sakthivel,

Dr.P.Rameshkumar,

Dr.Johnson, B.,

Dr. R.Saravanan,

Dr.Anikuttan, K.K.,

Shri. Sankar, M.,

Smt.Remya, L.,

Shri.M.Rajkumar,

Shri.R. Vinothkumar,

Shri.S.Thirumalaiselvan.,

Manual Designed By

C.Samuel, *Captsee.com.*

PREFACE

The undergraduate Fisheries Science students are required to know about the inland and marine fisheries. The students from landlocked area are getting rare opportunity to understand the marine fisheries and mariculture activities. Hence, based on the request of the Central Agricultural University (CAU), Tripura as a part of their academic activities, the Director, ICAR-CMFRI, has kindly approved a 10 days in-plant training for B.F.Sc. final year students at the ICAR-Central Marine Fisheries Research Institute (CMFRI), Mandapam Regional Centre. The training was organized in collaboration with Agricultural Technology Information Centre (ATIC), ICAR-CMFRI, Kochi. A total of 27 B.F.Sc students and a faculty of Central Agricultural University (CAU), Tripura participated in the training programme during 17th to 26th January, 2020. The training emphasized on the skill development of students through hands on training on mariculture activities and field visits to fish landing centres. A course manual was prepared covering the major topics on marine capture fisheries and mariculture. I congratulate all those who have put in their sincere efforts to bring out this manual.

Dr. R. Jayakumar
Principal Scientist & Scientist-in- Charge
Mandapam Regional Centre

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MARINE FINFISH BREEDING AND SEED PRODUCTION WITH SPECIAL REFERENCE TO COBIA AND SILVER POMPANO

G. Tamilmani,A.K. Abdul Nazar, R. Jayakumar, M. Sakthivel, P. Rameshkumar, K.K. Anikuttan, and M. Sankar

Mariculture Division, MRC of CMFRI, Mandapam Camp, Tamil Nadu

Introduction

Since many centuries mankind grows almost all its food on cultivated farm land,as far as this food consists of terrestrial plants and animals.But, when it comes to sea water plants and animals, modern man still continues to collect them from natural sources like his forefathers. Marine animals have always been and still are an important source of protein and minerals in human food. With the rapid increase in human population the demand for sea food will certainly increase. However, production from capture fisheries has stagnated and fisheries cannot expand much further. Further increase in exploitation of the seas will lead to destruction of ecosystems and species extinction.

Mariculture - the farming and husbandry of marine plants and animals can augment the marine fish production and can supplement the capture fisheries. One of the major requirements for the establishment of a sustainable mariculture industry is the availability of quality seeds. Seed collection from the wild is unpredictable and cannot be relied upon. The ability to produce viable offspring from captive brood stock can ensure a steady supply of seeds. Countries like Australia, China, Indonesia, Japan, Malaysia, Philippines, Taiwan, Thailand and Vietnam have made substantial progress in the development of commercial level seed production technologies of high value finfish suitable for sea farming.

In India, the commercial mariculture industry for finfish is in its infancy and the primary technological bottleneck is the lack of commercial-scale hatchery technologies for targeted species.The ICAR –CMFRI is addressing the problem of seed availability through its research on breeding and seed production of several marine species. To date, technologies for breeding and seed production of cobia (*Rachycentroncanadum*), silver pompano (*Trachinotusblochii*), orange spotted grouper (*Epinepheluscoioides*), Indian pompano (*Trachinotusmookalee*) and pink ear emperor (*Lethrinuslentjan*) have been developed and are being refined continuously to make them commercially viable. With the expanding interest in aquaculture and the market demand for more diversified species hatchery technology is being developed for an increasing number of species.

This chapter aims at giving a general view of the major steps involved in marine finfish breeding and seed production with special emphasis on cobia and silver pompano.

Broodstock Collection and handling

It is not easy to obtain fully mature broodstock fish directly from the wild and hence broodstock development has to be done in captivity. Fish broodstock may be collected from the wild or captive stock.

It is advantageous to collect sub-adults for broodstock development. Larger fishes would have crossed the reproductive age and very small fishes will take longer time to sexually mature. In the case of cobia, fish weighing between 8 to 15 kg could be procured while silver pompano could be procured in weight range of 750 gm to 1.5 kg. Cobia and silver pompano does not have swim bladder as juveniles or adults, and there is no need to vent the fish after capture

Stress should be minimised during capture and handling of broodstock. It is best to collect broodstock fishes from hook & line and trap nets, as they cause minimum stress to the fishes. During transportation, dissolved Oxygen (DO) should be maintained at or above saturation. For handling and transfer, fish are anesthetized with 10-20 ppm clove oil (Aqui-S). Once anesthetized, the fish can be weighed, measured, tagged, sexed and sampled for assessment of sexual maturity.

On arrival at the hatchery prophylactic treatment is given to the fishes to reduce the risk of introducing ectoparasites or bacterial diseases into the hatchery facility. Formalin bath (100 ppm) for 2-5min followed by freshwater bath for 5-10 min is normally given. Silver pompano has a high tolerance for freshwater and freshwater bath can be given for more than 15 minutes also without any problem. During the treatment, fishes should be closely monitored. If the fishes suddenly become immobile or if their opercular movements become very slow or if the fishes are turning upside down, they should be immediately transferred to filtered seawater.

Broodstock Development

The basis for any hatchery operation is the maintenance of a healthy group of sexually mature fish (brooders) conditioned to spawn naturally or in response to hormonal induction. Broodstock development is the vital and time consuming procedure in marine finfish seed production.Good broodstock management involves providing close to natural, non-stressful environmental conditions as well as a nutritious and balanced diet.

Generally, broodstock development of marine finfish is being practiced either in sea cages or land-based cement concrete or fibre-reinforced plastic (FRP) tanks.Broodstock developed in sea cages are susceptible to changes in the water quality of the cage site, disease problems and impact of harmful algal blooms. Therefore, the broodstock developed in sea cages are not bio-secure. In land based broodstock development systems, continuous flow through is provided with 300-500 % of water exchange for maintaining the water quality. This involves huge expenditure. Further,this exposes the broodstock to varying water quality and parasites.

For practical and economic considerations, young adults are first reared in cages to reduce maintenance cost and later transferred to land-based facilities when the fish are ready for spawning.

Recirculation aquaculture systems (RAS) use land based units to pump water in a closed loop through fish rearing tanks and include a series of sub-systems for water treatment which include equipment for sterilization, heating or cooling, solid waste removal, water chemistry control, biological filtration and dissolved gas control. If the broodstock development is practiced in recirculation systems, it is possible to have control on the environment in which the broodstock are reared, the duration and intensity of light and water temperature can be manipulated to accelerate the gonadal maturation. Sustainable production of bio-secure marine finfish seed all through the year employing photo-thermal conditioning is possible only by recirculating systems and this can pave the way for the commercial level seed production.

FRP tanks of 10MT capacity are suitable for broodstock development of silver pompano. For cobia, tanks of 60 to 100 MT capacities with RAS were found suitable. The size of the tanks should be large enough for the species selected to reduce the stress of captivity and to enhance the chances of spawning. Circular tanks were found more suitable than rectangular or square tanks. The broodstock fishes should be given regular prophylactic treatments with freshwater with or without oxytetracycline (OTC) at least once in a month.

Broodstock Nutrition

The viability of the larvae is very much dependent on broodstock nutrition. The nutritional components in the diet can affect spawning, egg and larval quality. Yolk is the sole source of food for the developing embryo and the early larvae until feeding on live preys starts. Therefore, the yolk quality and quantity are key factors for a successful seed production. The process through which maturing oocytes in the ovary accumulate yolk is called as vitellogenesis. The yolk protein precursors, vitellogenins, are high molecular weight lipoproteins that are synthesized in the liver and secreted into the blood. The fatty acid composition of the vitellogenins can be affected by long term imbalances in the broodstock diet.

The broodstock fishes are to be fed with a highly nutritive diet. Diet rich in vitamins, poly-unsaturated fatty acids (n- 3 PUFA) and other micro-nutrients is essential for obtaining viable eggs and larvae. During gametogenesis, female fish require a food, richer than usual, in proteins and lipids to produce the vitellogenin.Both dry pellets and moist food can be employed during maturation. Dry pellets should include essential nutritional components like polyunsaturated fatty acids (n-3 PUFA), in particular EPA (20:5 ω 3) and DHA (20:6 ω 3), which cannot be produced by fish metabolism. Broodstock fishes are fed *ad libitum* once a day with squids, crabs, shrimps and chopped oil-sardines depending on the availability.The amount of the food given to the fish is about 3-5% of biomass day-1.

Induction of spawning

Spawning,the release of eggs and sperm can be obtained either naturally by placing the fish in an appropriate environment or by inducing with hormones. Induced breeding is commonly practiced in most commercial hatcheries. Hormones injected cannot produce the gametes; they can only trigger the release of fully developed gametes. In females the hormonal treatment is intended to trigger the last phases in egg maturation, i.e. a strong egg hydration followed by their release. Only females with oocytes in the late-vitellogenic stage, with a diameter around 750 microns in cobia and 500 microns in pompano, are selected. However, if eggs have not reached the late-vitellogenic (or post-vitellogenic) stage, the hormone treatment does not work; hence ovarian biopsy is essential for assessing the ovarian development. Flexible sterile catheters (1.2 mm internal diameter) can be used for gonadal biopsy.

The human chorionic gonadotropin (hCG) is used at a dosage of 500 IU per kg of body weight in cobia females and 250 IU per kg body weight for males, whereas, for pompano 350 IU per kg body weight is used for both male and female. This dosage can be administered as a single dose on the dorsal muscles. Being a large molecule, hCG may provoke immunization reaction, and as a result, fish treated with hCG may not respond when treated repeatedly with this hormone. However, hCG can be successfully replaced by an analogue of the luteinizing hormone-releasing hormone [LH-RHa des-Gly10 (D-Ala6) LH-RH ethylamide, acetate salt]. It is a small molecule with 10 peptides and acts on the pituitary gland to induce the release of gonadotropins which, in turn, act on the gonads.

Normally spawning could be noted within 36 -48 hours after hormonal induction. The spawning in cobia and pompano takes place usually between late night and early morning hours. The number of eggs spawned by cobia ranges from 0.4 to 2.5 million, whereas, pompano spawn 0.5 to 1.5 lakh eggs. The spawning unit should preferably be kept separated from the main hatchery building to avoid disturbance to the spawners.

Egg harvest

The fertilized eggs of cobia and pompano float and are scooped gently using 500 µm net. To minimise the presence of poor-quality eggs, which usually float deeper in the water, it is advisable to collect only the eggs which float at the water surface. Aeration can be switched off to allow the unfertilized / dead eggs to settle at the bottom of the tank. The floating layer of eggs thicker than one cm should be avoided. A thicker layer may reduce oxygen supply to the eggs, leading to possible anoxia after a short time. Then in the temporary container, eggs must be thoroughly examined to assess their quality, number and developmental stages. With a pipette eggs should be taken from the floating egg layer in the temporary container, and should be placed on a watch-glass or on a Petri dish for observation under microscope. Few dozens of eggs, which are placed under a microscope, have to be observed for the egg developmental stages.

As fertilized cobia/ pompano eggs float in the seawater, they can be collected using egg collectors. If well dimensioned and properly placed, these devices harvest only the floating eggs, while the dead or unfertilized ones sink to the bottom. The presence of eggs in the collectors should be checked rather frequently in the case of cobia, as its spawning releases a large amount of eggs in a very short time there is risk of clogging the collectors or of mechanical stress to the eggs.

Incubation of eggs

It is done in incubation tanks of 3-5 tonne capacity.As the fecundity is normally high in cobia, we may require more incubation tanks, whereas the pompano requires only one tank per female.

Aeration needs to be adjusted suitably, not too strong to avoid excessive physical collision among eggs, but not too weak either, to keep the eggs suspended in water column. The main purpose of aeration is to prevent clumping and settling down of eggs. Stocking density can be maintained at a moderate level of 200 to 500 eggs per litre. The development of embryo can be observed at frequent intervals under a microscope. The hatching of eggs takes place from 18 to 24 hours.

After hatching, only the hatched fish larvae/yolk sac fry have to be moved to the larval rearing tanks filled with filtered seawater. Prior to this, the aeration should be stopped briefly to enable the debris and exuviae to settle at the bottom which can be removed by siphoning.

Larviculture

Many of the marine fin fishes which are suitable for aquaculture are having altricial type of larvae. These larvae are having very less yolk reserves at hatching. Therefore, the larvae are are very small with a small mouth gape and are still in an under developed stage when the yolk sac is completely resorbed. The development of digestive system is also very primitive and the perceptive powers for searching and taking external feed is also very less in this type of larvae.

The time when the yolk reserves are fully exhausted and the larvae need to resort to exogenous feeding is a critical period in the larviculture of most marine fin fishes. Unless proper live feeds of required size is provided in sufficient densities in larviculture media and its nutritional requirements especially in terms of PUFA are met, large scale mortality is bound to happen at this stage. It is evident that the larviculture of marine finfish having altricial larvae is really challenging and proper management of live feed is the most vital pre-requisite for the success in terms of survival and growth of the larvae.

In addition, since most of the larvae are visual feeders providing the required light also affects the larval survival. During the critical period, the density of the live feed and its nutritional qualities determines the percentage of the survival of the larvae. The density of the larvae of the concerned species should also be regulated in the larviculture tanks for getting good survival. The marine fish larvae exhibit highly differential growth even from very early stages (in the case of cobia, starting from the first week) and hence size grading from an early stage is also very much needed for increasing the survival. In addition, a variety of other factors such as tank colour, size of the tank, water temperature, water quality, etc., also affects the larval survival and growth. Larviculture of marine finfish is highly complicated, unless each and every factor is taken care of, the survival and growth of the larvae will be very less.

The larvae hatched in the incubation tanks need to be distributed in larviculture tanks to have a stocking density of 5 to 10 larvae/ litre for cobia and 10-20 larvae per litre for pompano. Care should be taken to avoid any mechanical stress or damage.

Larviculture of cobia

Newly hatched larvae of cobia normally measures 3.4 mm size. Soon after hatching, the mouth is closed and the digestive tract is not fully developed. During this period the larvae survive on its reserves in the yolk sac.

Larval mouth opens at 3-5 days post hatch (dph). Metamorphosis starts from 18-21 dph. Newly hatched cobia larvae generally starts feeding at 3 dph and they can be fed with the rotifer (*Brachionusrotundiformis*) at the rate of 10-12 nos / ml, two times a day till 10 dph.Before being fed to fry, rotifers require enrichment to enhance their nutritional value. Six-hour enrichment with an INVE product called Protein Selco Plus is usually carried out.

As fry get larger, they quickly outgrow the prey size represented by rotifers (200 to 400 microns), and a larger live feed is required. From 8 dph, the larvae can be fed with enriched *Artemia*nauplii (500 to 900 microns) at the rate of 2-3 nos / ml, 2 times a day. During the rotifer and *Artemia* feeding stage, green water technique can be used in the larviculture system with the microalgae *Nannocloropsisocculata*at the cell density of $1x10^{7}$ cells / ml. The addition of this algae to the tank water gives the water a green hue, and is thus commonly referred to as the “greenwater” technique.

The weaning to artificial larval diets has to be started from 15- 18 dph. While weaning, formulated feed should be given 30 minutes prior to feeding with live feed. Size of the artificial feed has to be smaller than the mouth size of the fish. Continuous water exchange is required during weaning stage. While weaning the fish larvae from rotifers to *Artemia*nauplii, co-feeding with rotifers has to be continued due to the presence of different size groups of larvae.

Between 25-40 dph, the larvae are highly cannibalistic and hence size-grading has to be undertaken at every three days interval. During this stage, the fry could be weaned totally to formulated diets. Different sizes of formulated feeds need to be used as per the mouth size of the larvae. The fry are considered weaned once they are feeding solely on dry diets. At this point, they can be considered as fingerlings.

Larval rearing can be practised both intensively in tanks and extensively in ponds. The major factors affecting the growth and survival of larvae are nutrition, environmental conditions and handling stress. Since there is a high demand for essential fatty acids (EFAs), enrichment protocols are needed for live-feeds. The water exchange can be practically nil till 7dph and it can be gradually increased from 10-100 % from 8 to 12 dph. But, tank bottom siphoning should be carried out from day 1. The environmental conditions required during the larviculture period are DO_2: > 5mg / l, NH_3: < 0.1mg / l, pH: 7.8 – 8.4, Salinity: 25-35 ppt, water temperature: 27-33° C.

The juveniles measuring 10 cm length are ready for stocking in happas/ nursery tanks.

Nursery and grow-out rearing of cobia

Nursery phase of cobia can be carried out in happas or sea cages or indoor FRP / cement tanks. During nursery rearing, it is advisable to feed the juveniles with formulated feed of 1200 µ size which can be increased to 1800 µ size from 55 dph onwards. Once the juveniles reach a size of 15 gm, they are ready to stock in sea cages or land based ponds for grow-out farming.

Larviculture of Pompano

The newly hatched larvae were stocked at a density of 15000 larvae in FRP tanks of 2 m^3 capacity filled with 1.5 m^3 filtered seawater. The tanks were provided with mild aeration and green water at a cell density of 1 $x10^7$/ml. The mouth of the larvae opens on 3dph and the mouth size was around 230 µ.

The larvae were fed from 3dph to 14 dph with enriched rotifers at a density of 6-8 nos. per ml in the larviculture tanks. Co-feeding of rotifers with enriched *Artemia*nauplii has to be done during 12-14 dph, and thereafter upto 19 dph with enriched *Artemia*nauplii alone by maintaining a density of 3-5 nos. per ml in the larviculture tanks. Weaning to larval inert feeds has to be started from 15 dph and co-feeding with *Artemia* needs to be continued until 19 dph. From 20 dph feeding can be entirely on larval inert feeds. The metamorphosis of the larvae starts from 18 dph and all the larvae metamorphose into juveniles by 25 dph. Though cannibalism is less, grading has to be done during 20-25 dph to separate the shooters. Critical stage of mortality would occur during 3-5 dph and subsequent mortalities are negligible. The water exchange can be practically nil till 7dph and it can be gradually increased from 10-100 % from 8 to 14 dph.

Nursery Rearing of Pompano

Nursery rearing could be initiated from 25 to 30 dph. At this stage, artificial feed of 800 µ size could be provided. Thereafter, fingerlings were fed with progressively higher size range of floating extruded larval feeds. Daily water exchange of 100% is advisable. Water quality parameters like salinity, temperature, pH, Oxygen level and ammonia are closely monitored during the entire larviculture period. After 55dph, the fingerlings with size range from 1 to 1.5 inch size can be supplied to farmers for stocking in the happas / tanks for further nursery rearing and grow-out farming.

Conclusion

Research in the areas of broodstock nutrition, hormonal manipulations, live feed and larviculture technologies are required for the development of reliable techniques for mass production of fingerlings of many marine finfish species. This is necessary for establishing a sustainable mariculture industry in the region.

Fish Reproduction, Reproductive dysfunctions in captivity and Hormonal Induction of Spawning

G.Tamilmani, A.K.Abdul Nazar, R.Jayakumar, M.Sakthivel, P.Rameshkumar, K.K Anikuttan and M.Sankar

ICAR-CMFRI, Mandapam Regional Centre

Introduction

Since many centuries mankind grows almost all its food on cultivated farm land, as far as this food consists of terrestrial plants and animals. But, when it comes to sea water plants and animals, modern man still continues to collect them from natural sources like his forefathers.Marine animalshave always been and still are an important source of protein and minerals in human food. With the rapid increase in human population the demand for sea food will certainly increase. However, production from capture fisheries has stagnatedand fisheries cannot expand much further. Further increase in exploitation of the seas will lead to destruction of ecosystems and species extinction. When we consider that the amount of wild-captured fish has not increased in recent years, only one alternative remains: fish farming, or aquaculture.

Mariculture- the farming and husbandry of marine plants and animals can augment the marine fish production and can supplement the capture fisheries. One of the major requirements for the establishment of a sustainable mariculture industry is the availability of quality seeds. Seed collection from the wild is unpredictable and cannot be relied upon. The ability to produce viable offspring from captive brood stock can ensure a steady supply of seeds. However, most of the fishes exhibit reproductive dysfunctions when reared in captivity. The dysfunctions may be due to a combination of captivity induced stress and lack of appropriate natural spawning environments. Hormone-induced spawning is the only reliable method to induce reproduction in these fishes.

This chapter aims at giving a general view of fish reproduction, the reproductive dysfunctions found in captive-reared fishes and the hormonal interventions for the control of fish reproduction.

Reproduction of fish in nature

Like many other animals, fish also have evolved to reproduce during environmental conditions that are favourable to the survival of the off spring. Long before spawning (the release of eggs and sperm), seasonal cues begin the process of gametogenesis (gamete growth and differentiation) which leads to the formation of the female oocyte (oogenesis) or the male spermatozoon (spermatogenesis). In many fish, this can take up to a year.

The reproductive cycle of fish is separated into two major phases. The first phase (spermatogenesis and vitellogenesis) involves the proliferation, growth and differentiation of the gametes. The second phase (spermiation and oocyte maturation) involves the maturation and preparation of the oocytes and spermatozoa for release and insemination.

Maturation of the egg is a long process that involves complex physiological and biochemical changes. One important step, vitellogenesis, is a process in which yolk proteins are produced in the liver, transported to the ovary, and stored in the egg, resulting in tremendous egg enlargement. The yolk is important as a source of nutrition for the developing embryo. Also

critical is germinal vesicle migration and germinal vesicle breakdown (GVBD). Before it migrates, the germinal vesicle, or nucleus, is located at the center of the egg in an arrested stage of development. At this stage, the egg is physiologically and genetically incapable of being fertilized, even though it has the outward appearance of a fully mature egg. When conditions are appropriate for final maturation, nuclear development resumes, and the germinal vesicle migrates to one side. Finally, the walls of the germinal vesicle break down, releasing the chromosomes into the cell. In mature eggs, the migration of the germinal vesicle to the side of the cell will be complete. After the egg has matured; a class of compounds called prostaglandins are synthesized. These stimulate ovulation, which is the rupture of the follicle cells that hold the egg. The egg is then released into the body cavity or ovarian lumen, from where it may subsequently be released to the outside environment.

When the gametes have matured, an environmental stimulus may signal the arrival of optimal conditions for the fry, triggering ovulation and spawning. Examples of environmental stimuli are changes in photoperiod, temperature, rainfall, and food availability. A variety of sensory receptors including the eye, olfactory organs, taste buds, and thermo receptors detect these cues.

Control of reproduction

Marine fishes produce and release sex cells based on maturity of the individuals, their nutrition and overall health, triggered by cues from the environment that in turn influence their hormonal/endocrine systems. Along with endocrine control there is also a steady, intimate, more sudden interplay of the fishes' nervous system.

Reproduction in fishes is regulated by external environmental factors that trigger internal mechanisms. The external environmental factors that control reproduction (temperature, light/dark duration, tides, presence of conspecifics, mates, etc.), vary among species. However, the internal mechanisms (hormones) that regulate spawning are similar for most fishes. Therefore, more is known about the internal regulatory mechanism of fish reproduction than the specific environmental requirements for spawning each species.Knowledge of the normal sequence of endocrine changes taking place during oocyte maturation, ovulation, and egg release is necessary for understanding the endocrine framework that is manipulated by hormonal induction of spawning.

A cascade of hormones along the hypothalamus –pituitary–gonad (HPG) axis regulates the gametogenesis and final maturation.The hypothalamus, located at the base of the brain, is sensitive to signals from sensory receptors and releases hormones in response to environmental cues. Principal among these hormones is Gonadotrophin releasing hormone (GnRH), which travels from the hypothalamus to the pituitary gland. Certain cells of the pituitary receive GnRH and release gonadotrophic hormones, Follicle Stimuating Hormone (FSH) and Luteinizing Hormone (LH) into the bloodstream. The gonadotrophic hormones travel to the gonads, which synthesize steroids responsible for the final maturation of the gametes.Successful release of mature gametes could be achieved by providing proper environmental stimuli and /or administration of hormones acting at the level of the hypothalamus, pituitary, or gonads.

Reproductive dysfunctions in captive-reared fishes

Reproductive problems are usually more serious in female broodstocks, and can be classified into three types. The first and most severe reproductive dysfunction of captive fishes is the

complete absence of reproductive development observed in freshwater eel.The second type of reproductive dysfunction in cultured females is the absence of spawning at the end of the reproductive cycle. In species exhibiting this problem, the oocytes undergo normal vitellogenesis, FOM and ovulation in response to the appropriate physiological and environmental stimuli, but the ovulated eggs are not released into the water.This absence of only gamete release (i.e., spawning) is observed in cultured salmonids. In most other fishes, the reproductive dysfunction often observed in culture is that fish undergo vitellogenesis during the reproductive period, but fail to undergo FOM and, as a result, there is no ovulation and no spawning of eggs. The endocrine cause of the failure of female fish to undergo FOM has been identified to be a dysfunctional release of LH from the pituitary at the end of vitellogenesis. Comparison of plasma levels of reproductive hormones between cultured fish that fail to undergo FOM and wild fish captured on their spawning grounds showed that a plasma LH surge accompanied FOM and ovulation in wild females, but in females reared in captivity plasma LH levels remained low at the end of vitellogenesis.

The reproductive dysfunctions observed in male cultured broodstocks are limited to captivity-induced reductions in milt production or milt quality during the spermiation period. Reproductive problems often diminish over the years after domestication.

Assessing the ovarian development

Spawning, the release of eggs and sperm can be obtained either naturally by placing the fish in an appropriate environment or by inducing with hormones. Induced breeding is commonly practiced in most commercial hatcheries. Hormones injected cannot produce the gametes; they can only trigger the release of fully developed gametes. Effectiveness of hypophysation (injection with pituitary hormones) is dependent on the stage of reproductive development of recipients. Injection of hormones in an unripe adult will not generally induce gametogenesis or ripening of eggs. Care must be exercised in assessing sexual readiness in spawners. Sometimes generally adopted parameters have proven unreliable. An example of this is females with enlarged abdomens, reddish coloration and protrusion of the cloacal region may be due to engorgement of the intestine, or disease, even during the spawning season. Hence, ovarian biopsy is essential for assessing the ovarian development. Flexible sterile catheters (1.2 mm internal diameter) can be used for gonadal biopsy.

In females the hormonal treatment is intended to trigger the last phases in egg maturation, i.e. a strong egg hydration followed by their release. Only females with oocytes in the late-vitellogenic stage, with a diameter around 750 microns in cobia and 500 microns in pompano, are selected. However, if eggs have not reached the late-vitellogenic (or post-vitellogenic) stage, the hormone treatment does not work;

Hormonal therapies

Based on the evidence that the failure of cultured fishes to undergo full spermiation and FOM is the result of diminished LH release from the pituitary the hormonal therapies developed and applied for fish aquaculture can be grouped into two major types, “first generation” and “second generation” techniques. The first generation is the pituitary hormone based preparations, and includes the pituitary extracts and purified GTHs. The second generations are the brain hormone based treatments and includes the GnRH agonists (GnRHa) and dopamine (DA) antagonists. These two types of hormonal therapies act at different levels of the reproductive HPG axis. Exogenous LH preparations act directly at the level of the gonad.

GnRHa releases the endogenous LH stores from pituitary. Endogenous LH, in turn, acts at the level of the gonad to induce steroidogenesis and the process of FOM and spermiation.

Luteinizing hormone preparations include

(a) homogenates and purified extracts from the pituitary of mature fish during the reproductive season—most commonly of carp and salmonids—that contain high amounts of LH. Pituitary homogenates were the first type of exogenous hormonal treatments used by aquaculturists for the induction of maturation and spawning. Professor B.A.Houssay, an Argentinean, was the first to report (1930) on the ability of exogenous hormones to induce FOM and ovulation in fish. He injected female fish with ground pituitaries from another species and observed that the females underwent ovulation. However, use of ground pituitaries, is associated with various drawbacks, like the great variability in pituitary LH content, the administration of additional hormones present in the pituitary that may adversely affect the physiology of the treated fish, and the potential for transmission of diseases from donor fish to recipient broodstocks. Realizing the above drawbacks, efforts were later directed towards the acquisition of purified or partially purified preparations of LH.

(b) **GtH preparations of mammalian or human origin**

A wide range of GtH preparations of mammalian or human origin including mammalian FSH and LH, ovine LH, Pregnant Mare Serum Gonadotropin (PMSG) and human Chorionic Gonadotropin (hCG) have been tested in some fishes. However, only hCG – which is purified from the urine of pregnant women – has been used routinely in aquaculture. hCG has a very strong LH activity.

The human chorionic gonadotropin (hCG) is used at a dosage of 500 IU per kg of body weight in cobia females and 250 IU per kg body weight for males, whereas, for pompano 350 IU per kg body weight is used for both male and female. This dosage can be administered as a single dose on the dorsal muscles. Being a large molecule, hCG may provoke immunization reaction, and as a result, fish treated with hCG may not respond when treated repeatedly with this hormone. However, hCG can be successfully replaced by an **Analogue of the luteinizing hormone-releasing hormone** [LH-RHa des-Gly10 (D-Ala6) LH-RH ethylamide, acetate salt]. It is a small molecule with 10 peptides and acts on the pituitary gland to induce the release of gonadotropins which, in turn, act on the gonads. Almost 100% of injected fish spawn eggs whose quality usually matches that of natural spawning. The cost of LHRHa is very high compared to that of hCG. But, LHRHa is used in very low dosages, usually around 20 µg / kg of body weight.

Two sites of injection are in wide practice. Intramuscular, in the flank just below the dorsal fin and behind the gill cover. This method is safer but slower working. Intraperitoneal injections are faster acting but involve a greater chance of injury or death as the injections are made into the body cavity.

Normally spawning could be noted within 36 -48 hours after hormonal induction. The spawning in cobia and pompano takes place usually between late night and early morning hours. The number of eggs spawned by cobia ranges from 0.4 to 2.5 million, whereas, pompano spawn 0.5 to 1.5 lakh eggs. The spawning unit should preferably be kept separated from the main hatchery building to avoid disturbance to the spawners.

Larviculture of Cobia and Silver Pompano

Abdul Nazar, A.K*., Jayakumar, R., Tamilmani,G., Sakthivel, M., Rameshkumar, P., Anikuttan, K.K., Sankar,M., Hanumanta Rao, G and Krishnaveni, N.
Mandapam Regional Centre of CMFRI, Madapam Camp – 623520, Tamil Nadu, India
**Madras Research Centre, CMFRI, Chennai, Tamil Nadu, India*
aknazar77@gmail.com

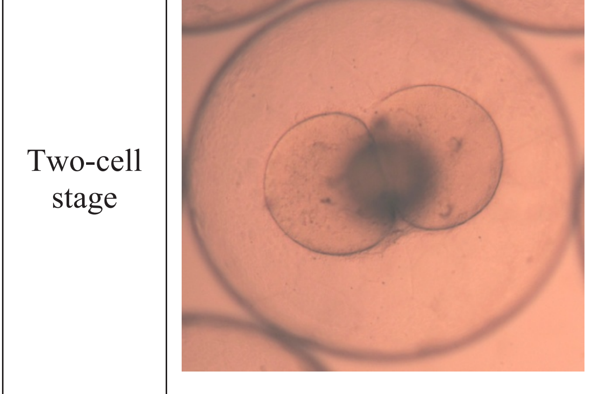
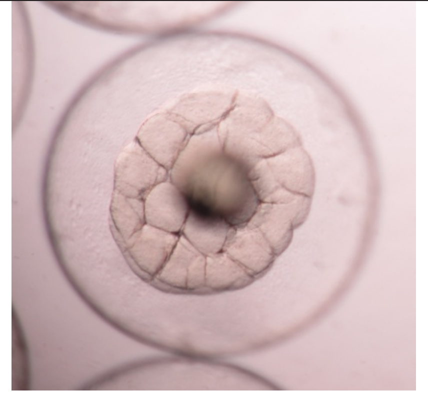
In India, technology for production of marine finfish seeds is in primitive stage except for sea bass. The Mandapam Regional Centre of the Central Marine Fisheries Research Institute (CMFRI) has developed hatchery technology for cobia, *Rachycentron canadum* during March 2010 for the first time in the country. Again the Centre has developed hatchery seed production technology for the silver pompano, *Trachinotus blochii* for the first time in the country. Both the technologies are standardised and hence the CMFRI has entered into agreements with interested entrepreneurs and farmers for dissemination of technologies for development of cobia and silver pompano aquaculture sector in our country.

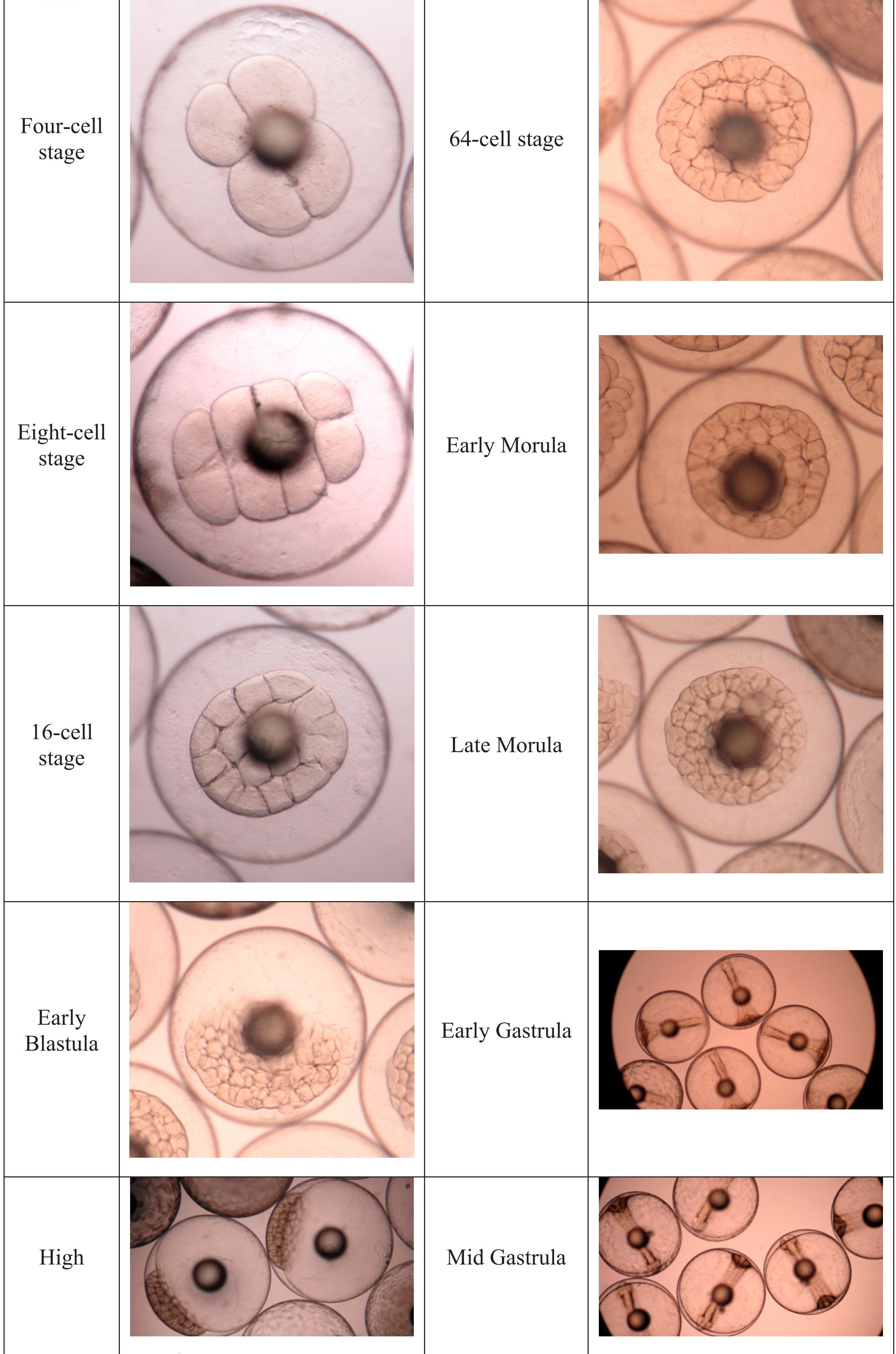
Cobia *Rachycentron canadum* is a marine finfish species with emerging global potential for mariculture and its positive culture attributes include capacity for natural and induced tank spawning, growth rates in excess of 7-9 kg/year. Further cobia has high disease resistance, survival rates (post larviculture stage) in tanks, pens and cages with adaptability to commercially available extruded diets. It also has high-quality meat for making fillets suitable for sashimi as well as white tablecloth restaurant markets. Similarly, among the many high value marine tropical finfish that could be farmed, the Silver pompano, *Trachinotus blochii* is one of the topmost, mainly owing to its fast growth rate, good meat quality and high market demand.

The larval rearing procedures of cobia and pompano are described below:-

As the fecundity is normally high in cobia, we may require more incubation tanks, whereas the pompano requires only one tank /female.

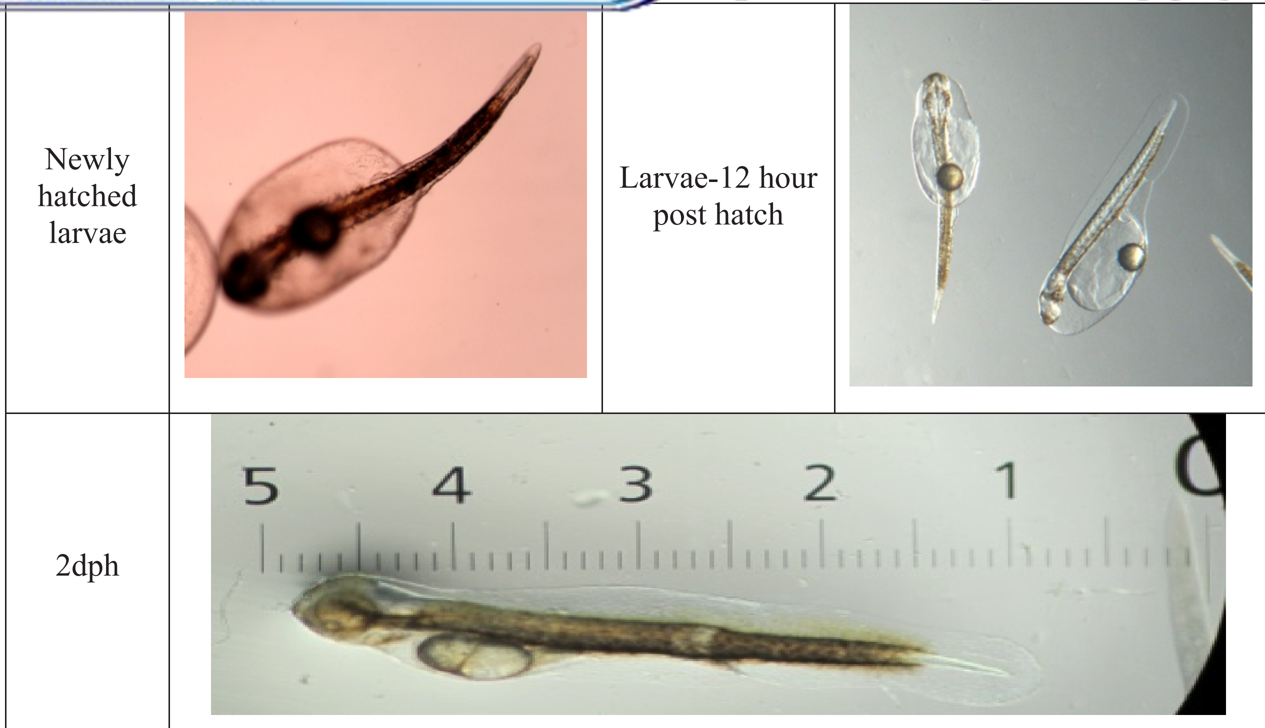
The embryonic developmental stages of cobia and pompano normally look alike except for the duration of development and size of the larvae. The photos of embryonic development and newly hatched larvae are provided below;

Two-cell stage		32-cell stage	



Stage	Stage
Four-cell stage	64-cell stage
Eight-cell stage	Early Morula
16-cell stage	Late Morula
Early Blastula	Early Gastrula
High	Mid Gastrula

Dome		Late Gastrula	
Oblong		Bud	
Epiboly		Segmentation	
High-pec		Hatching in progress	



Larviculture

The marine fish larvae are generally classified into altricial and precocial type. The altricial type of larvae is having very less yolk reserves at hatching and hence, the larvae are in an undeveloped stage when the yolk sac is completely resorbed. The development of digestive system is also very primitive in these types of larvae. Many of the marine fin fishes which are suitable for aquaculture are having the altricial type of larvae which poses challenges in their larviculture. When the yolk reserves are fully exhausted, the larval size and mouth gape are very small and the perceptive powers for searching and taking external feed is also very less. The period when the yolk reserves are fully exhausted and larvae need to resort to exogenous feeding is a critical period in the larviculture of most marine fin fishes. Unless proper live feeds of required size is provided in sufficient densities in larviculture media and its nutritional requirements especially in terms of PUFA are met, large scale mortality is bound to happen at this stage and hence it is evident that the larviculture of marine finfish having altricial larvae is really challenging and proper management of live feed is the most vital pre-requisite for the success in terms of survival and growth of the larvae.

In addition, since most of the larvae are visual feeders providing the required light also affect the larval survival. During the critical period, the density of the live feed and its nutritional qualities determines the percentage of the survival of the larvae. The density of the larvae of the concerned species should also be regulated in the larviculture tanks for getting good survival. The marine fish larvae exhibit highly differential growth even from very early stages (in the case of cobia, starting from the first week) and hence grading from an early stage is also very much needed for increasing the survival. In addition, variety of other factors such as tank colour, size of the tank, water temperature, water quality, etc., affect the larval survival and growth. From the foregoing, it is clear that the larviculture of marine finfish is highly complicated, unless each and every factor is taken care of, the survival and growth of the larvae will be very meagre.

The larvae hatched in the incubation tanks or larval rearing tanks need to be distributed in larviculture tanks to have minimal stocking density of 5 to 10 larvae/ litre for cobia and 10-20 larvae per litre for pompano. Care should be taken to avoid any mechanical stress or damage. Soon after hatching, the mouth is closed and the digestive tract is not fully developed. During this period the larvae survive on its reserves in the yolk sac.

Larviculture of Cobia

Newly hatched larvae of cobia normally measures 3.4 mm size. Larval mouth opens at 3-5 days post hatch (dph). Metamorphosis starts from 18-21 dph. Newly hatched cobia larvae generally start feeding at 3 dph and they can be fed with the enriched rotifer (*Brachionus rotundiformis*) at the rate of 10-12 nos / ml, two times a day till 10 dph. From 8 dph, the larvae can be fed with enriched *Artemia* nauplii at the rate of 2-3 nos / ml, 2 times a day. During the rotifer and *Artemia* feeding stage, green water technique can be used in the larviculture system with the microalgae *Nannocloropsis occulata* at the cell density of $1x10^{7}$ cells / ml. The weaning to artificial larval diets has to be started from 15- 18 dph. While weaning, formulated feed should be given 30 minutes prior to feeding with live feed. Size of the artificial feed has to be smaller than the mouth size of the fish. Continuous water exchange is required during weaning stage. Between 25-40 dph, the larvae are highly cannibalistic and hence size-grading has to be undertaken at every three days interval. During this stage, the fry could be weaned totally to artificial diets. Larval rearing can be practised both intensively in tanks and extensively in ponds. The major factors affecting the growth and survival of larvae are nutrition, environmental conditions and handling stress. Since there is high demand for essential fatty acids (EFAs), enrichment protocols are needed for live-feeds. The water exchange can be practically nil till 7dph and it can be gradually increased from 10-100 % from 8 to 12 dph. But, tank bottom siphoning should be carried out from day 1. The environmental conditions required during the larviculture period are DO_2: > 5mg / l , NH_3: < 0.1mg / l, pH: 7.8 – 8.4, Salinity: 25-35 ppt, water temperature : 27-33° C.

Green water has to be maintained in appropriate densities in the larval tanks. While weaning the fish larvae from rotifers to *Artemia* nauplii, co-feeding with rotifers has to be continued due to the presence of different size groups of larvae. The detail of weaning protocol is as follows.

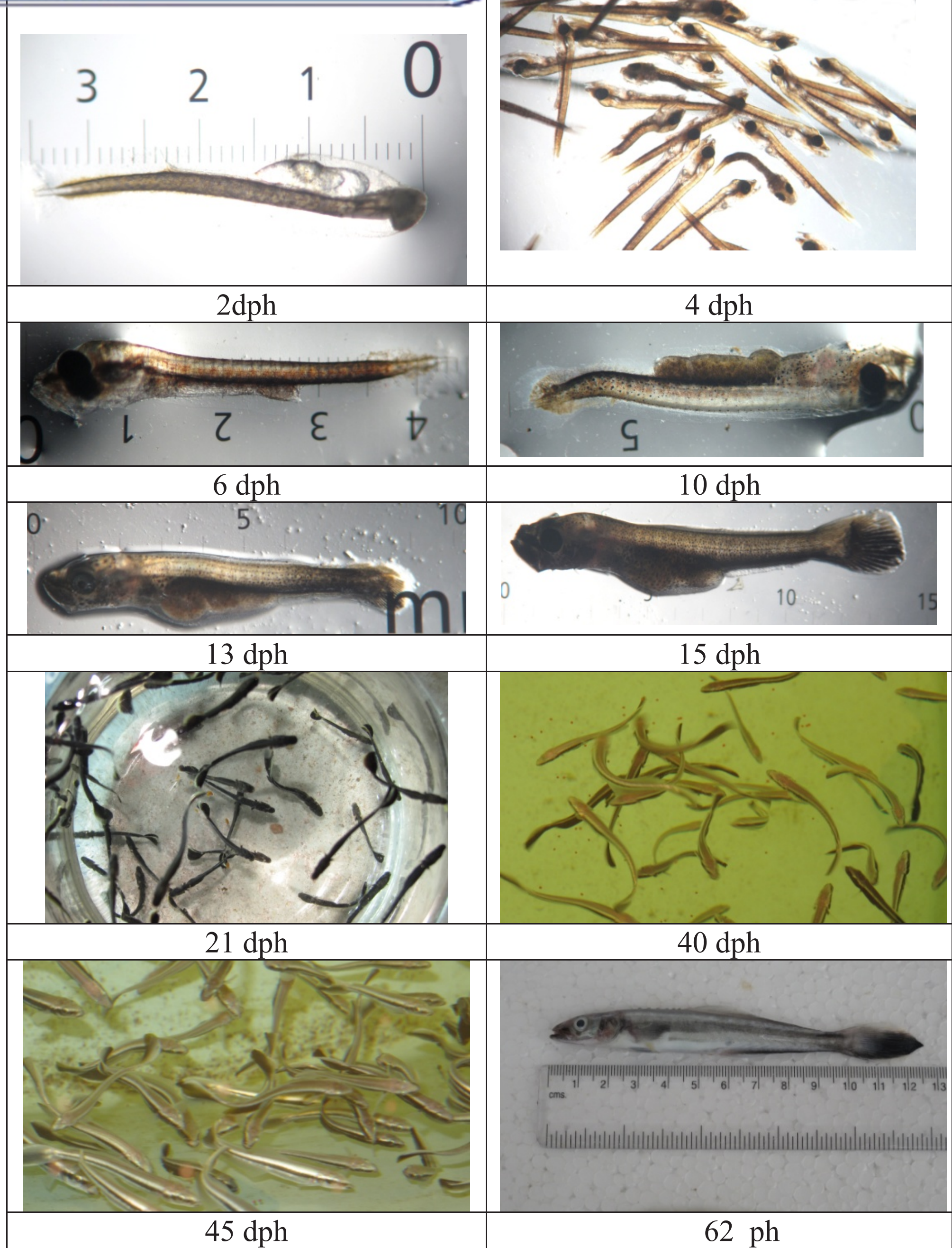
Stage of Larvae (dph)	**Size of Larvae (cm)**	**Size of Feed (µ)**
18 – 19	2.3 – 2.6	100-200
20 – 23	2.5 – 3.5	300-500
23 – 30	3.5 – 8.0	500-800
31 onwards	> 8.0	800-1200

The juveniles measuring 10 cm length are ready for stocking in happas/ nursery tanks.

Nursery and grow-out rearing of cobia

Nursery phase of cobia can be carried out in happas or sea cages or indoor FRP / cement tanks. During nursery rearing, it is advisable to feed the juveniles with formulated feed of 1200 µ size which can be increased to 1800 µ size from 55 dph onwards. Once the juveniles reach a size of 15 gm, they are ready to stock in sea cages or land based ponds for grow-out farming.

Few photos of larvae and fingerlings are provided below (*dph = day post hatch*)



2dph	4 dph
6 dph	10 dph
13 dph	15 dph
21 dph	40 dph
45 dph	62 ph

Larviculture of Silver Pompano

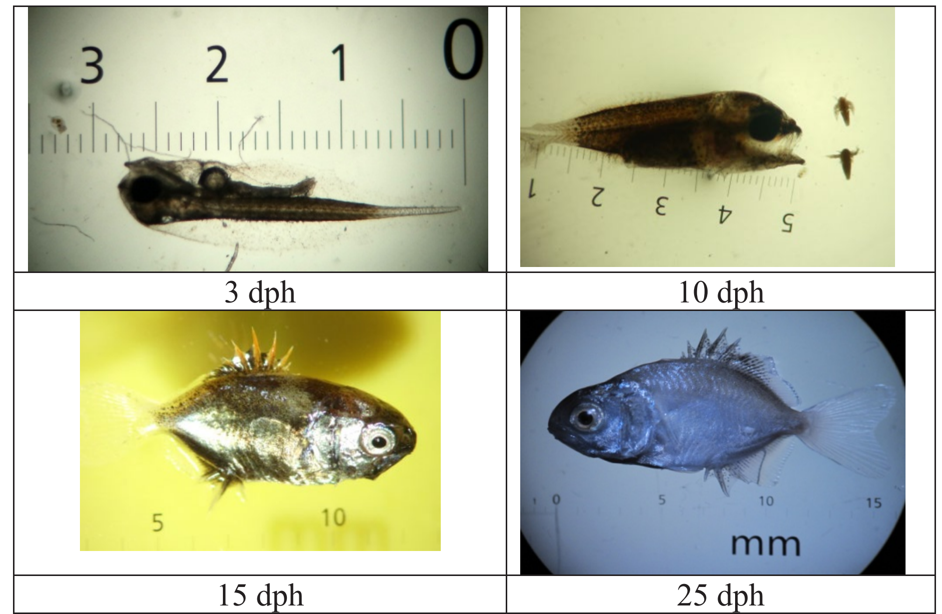
The newly hatched larvae were stocked at a density of 15000 larvae in FRP tanks of 2 m^3 capacity filled with 1.5 m^3 filtered seawater. The tanks were provided with mild aeration and green water at a cell density of 1 x10^7/ml. The mouth of the larvae opens on 3dph and the mouth size was around 230 µ. The larvae were fed from 3dph to 14 dph with enriched rotifers at a density of 6-8 nos. per ml in the larviculture tanks. Wherever possible, wild collected copepods could also be added as supplements. Co-feeding of rotifers with enriched *Artemia* nauplii has to be done during 12-14 dph, and thereafter upto 19 dph with enriched *Artemia* nauplii alone by maintaining a density of 3-5 nos. per ml in the larviculture tanks. Weaning to larval inert feeds has to be started from 15 dph and co-feeding with *Artemia* needs to be continued until 19 dph . From 20 dph feeding can be entirely on larval inert feeds. The metamorphosis of the larvae starts from 18 dph and all the larvae metamorphose into juveniles by 25 dph. Though cannibalism is not witnessed, grading has to be done during 20-25 dph to separate the shooters. Critical stage of mortality would occur during 3-5 dph and subsequent mortalities are negligible. The water exchange can be practically nil till 7dph and it can be gradually increased from 10-100 % from 8 to 14 dph.

Nursery Rearing of Silver Pompano

Nursery rearing could be initiated from 25 to 30 dph. At this stage, artificial feed of 800 µ size could be provided. Thereafter, fingerlings were fed with progressively higher size range of floating extruded larval feeds. Daily water exchange of 100% is advisable. Water quality parameters like salinity, temperature, pH, Oxygen level and ammonia are closely monitored during the entire larviculture period. After 55dph, the fingerlings with size range from 1 to 1.5 inch size can be supplied to farmers for stocking in the happas / tanks for further nursery rearing and grow-out farming thereafter.

The pompano fingerlings can be reared at salinities as low as 5 ppt. At lower salinities i.e. from 10 to 15 ppt, they grow faster than in pure seawater.

Some photos of larval stages of pompano are given below



3 dph | 10 dph

15 dph | 25 dph

Microalgae as live feed

K.K.Anikuttan, R.Jaykumar, A.K.Abdul Nazar, G.Tamilmani, M.Sakthivel,P.Ramesh Kumar, M.Sankar,G.H.Rao, Tinto Thomas, M.Jaysing, N.Krishnaveni and Aneesh.U

Introduction

The development of mariculture is fully dependent on the availability seed of the fish or shell fish to be cultured. For this, hatchery production of the seed is imperative owing to the uncertain nature of natural seed resources. At present the seed production technology of some species with mariculture potential have been developed in India, which includes Cobia, *Rachycentron canadum*, Silver/Snubnose pompano, *Trachinotus blochii,* Indian pompano, *T.mookalee*, Orange spotted grouper, *Epinephelus coioides*, Sea bream, *Lethrinus lentjan*, Asian sea bass, *Lates calcarifer* etc. The larviculture of all these fishes invariably comprises of a stage during which only live feeds can be fed to them since their internal organ system is not equipped to effectively digest any artificial feed /pellet feed. Hence live feeds form an integral part of the seed production of these fishes. Live feeds used in the hatchery can be broadly classified into two groups, viz; Phytoplankton and Zooplankton.

Phytoplankton, which forms the base of the food chain includes the microalgae. Microalgae are microscopic organisms, typically found in freshwater, estuarine, and marine environment. These are required for larval nutrition during a brief period, either for direct consumption, in the case of molluscs and Penaeid shrimp, or indirectly as food for the live prey fed to the larvae. They are typically photosynthetic autotrophic organisms, which can produce complex compounds using simple substances available in their surroundings. Besides chlorophyll, they also show various carotenoid pigments which impart different colours to them.

According to the nature of photosynthetic pigments, algae are further classified into three divisions such as Chlorophyta (green algae), Phaeophyta (brown algae) and Rhodophyta (red algae). Brown and red algae are mostly marine forms while green algae i.e. Chlorophyta is mostly freshwater and free floating type. Nearly 16 genera of microalgae are commonly employed for aquaculture purposes. They are generally free living, pelagic and in the nannoplankton range (2-20µm). All algal species may not be equally successful as a live feed. Suitable algal species have been selected on the basis of their mass-culture potential, cell size, digestibility, and overall food value for the feeding animal.

Today, micro algae is used as an essential food source for rearing all stages of marine larvae of fishes (cobia, pompano, cod, halibut, tilapia ect.,) bivalve mollusks (clams, oysters, scallops), gastropods (abalone, conch), and shrimps (*Penaeus* sp., *Macrobrachium* sp.,). Micro algae also constitute an important source of food for live food organisms (rotifers, copepods, cladocerans, brine shrimp etc.) used in aqua hatcheries.

Major classes and genera of micro-algae cultured in aquaculture (FAO, 1996).

Class	Genus	Examples of application
Bacillariophyceae	*Skeletonema**	PL,BL,BP
	Thalassiosira	PL,BL,BP
	Phaeodactylum	PL,BL,BP,ML,BS
	*Chaetoceros**	PL,BL,BP,BS
	Cylindrotheca	PL
	Bellerochea	BP
	Actinocyclus	BP
	Nitzchia	BS
	Cyclotella	BS
Haptophyceae	*Isochrysis**	PL,BL,BP,ML,BS
	Pseudoisochrysis	BL,BP,ML
	dicrateria	BP
Chrysophyceae	*Monochrysis (Pavlova)*	BL,BP,BS,MR
Prasinophyceae	*Tetraselmis (Platymonas)**	PL,BL,BP,AL,BS,MR
	Pyramimonas	BL,BP
	Micromonas	BP
Cryptophyceae	*Chroomonas*	BP
	Cryptomonas	BP
	Rhodomonas	BL,BP
Cryptophyceae	*Chlamydomonas*	BL,BP,FZ,MR,BS
	Chlorococcum	BP
Xanthophyceae	*Olisthodiscus*	BP
Chlorophyceae	*Carteria*	BP
	*Dunaliella**	BP,BS,MR
Cyanophyceae	*Spirulina**	PL,BP,BS,MR

PL- penaeid shrimp larvae; BL- bivalve mollusc larvae; ML- freshwater prawn larvae; BP- bivalve mollusc postlarvae; AL- abalone larvae; MR- marine rotifers (*Brachionus*); BS- brine shrimp (*Artemia*); SC- saltwater copepods; FZ- freshwater zooplankton.

Cellular dynamics and reproduction

Microalgae may have different types of cell organization: unicellular, colonial and filamentous. Most of the unicellular cyanobacteria are nonmotile, but gliding and swimming motility may occur. Baeocytes, cells arising from multiple fission of a parental cell, may have a gliding motility. Swimming motility occurs in a Synechococcus sp., even if flagella are not known. Unicellular microalgae may or may not be motile. In motile forms, motility is essentially

due to the presence of flagella. The movement by the secretion of mucilage is more unusual. Gametes and zoospores are generally flagellate and motile. Some pennate diatoms have a type of gliding motility, as well as the red alga Porphyridium and a few green algae.

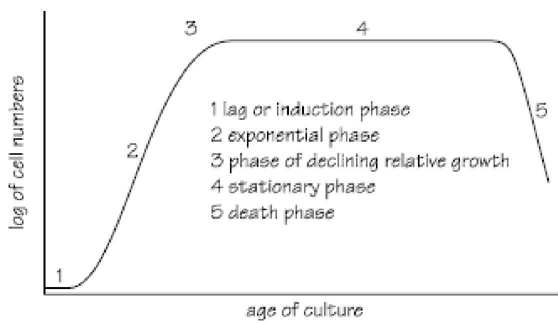
In unicellular microalgae the cell size generally doubles and then the cell divides into two daughter cells which will then increase in size. The cell cycle in eukaryotic algae involves two phases: mitosis and interphase. During the interphase the cell grows and all cellular constituents increase in number so that each daughter cell will receive a complete set of the replicated DNA molecule and sufficient copies of all other constituents and organelles. During the mitosis the nuclear division occurs. Vegetative reproduction by cell division is widespread in the algae and related, in many species, to an increase in cell or colony size. Other types of asexual reproduction occur by fragmentation and by production of spores, named zoospores if flagellate and aplanospores or hypnospores if nonflagellate. Autospores are also produced by various algae and are like aplanospores lacking the ontogenetic capacity for motility. Although sexual reproduction occurs in the life-history of most of the species, it is not a universal feature in algae. It involves the combination of gametes, often having different morphology and dimension, from two organisms of the same species (isogamy, anisogamy or oogamy). When the culture environment is favorable and all nutrients required for cell growth are present in a non-growth limiting quantity, i.e. at sufficiently high concentrations so that minor changes do not significantly affect the reaction rate, most unicellular algae reproduce asexually. The size and biomass of individual cells increase with time, resulting in biomass growth. Eventually, the DNA content is doubled in quantity and cell division ensues upon complete division of the cell into two progenies of equal genome and of more or less identical size. Population number is thereby increased, and population growth is therefore referred to as increase in population of the number of cells in a culture. The time required to achieve a doubling of the number of viable cells is termed doubling time. It is also termed generation time, as it is the time taken to grow and produce a generation of cells.

Growth cycle

A basic understanding of the growth cycle of micro algae would be helpful for successful operation of micro algae culture in a hatchery. The growth of micro algae passes through the following phases:

1. **Lag phase or induction phase**: This is the acclimation phase just after inoculation, where there is little or no growth is seen. The cells begin to absorb the nutrients and increase in size

2. **Log phase or exponential phase**: The cells reproduce very fast and population growth is exponential
3. **Transitional phase or phase of declining relative growth:** Here the cell division rate slows down as the light penetration through the culture reduces and nutrients becomes limiting.
4. **Stationary phase:**The number of cells remain constant as the growth and cell increase is compensated by cell death. This can last for many days in case of flagellates whereas for diatoms it can last for a short time.
5. **Decline phase or death phase:** The cell number decreases since the death rate exceeds growth.



Growth phase of micro algae

It is advisable to harvest the microalgae during their log phase, since in the new culture they will grow more rapidly and will yield a more viable population.

Micro algal culture Techniques

The micro algal culture in a hatchery can be broadly divided into two components:

- Stock culture of the pure strains
- Mass culture of the desired species

Stock culture

Stock culture of the pure strains of desired species of micro algae have to be maintained in a hatchery in a dedicated room under asceptic handling protocols to avoid any chances of contamination. This is essential to provide the desired species at any time of the hatchery operation. The stock culture forms the starter culture from which the quantity is scaled up to mass culture levels to meet the live feed requirements of the hatchery. Unlike natural seawater which contains many species of microalgae , in hatcheries, monoculture is to be maintained to scale up the desired species according to the nutritional requirements of the larvae or the zooplankton. Some of the common species of micro algae being used in mariculture seed production include

those belonging to the genus *Pavlova, Dicrateria, Thalassiosira, Isochrysis, Chaetoceros,Dunaliela, Nanochloropsis, Tetraselmis, Chlorella and Nannochloropsis.* The pure strains should be obtained from reputed, established and running hatcheries or repositories of R&D institutes. The traditional methods for obtaining pure culture involves isolating pure strains from raw sea water by various methods as listed below:

Enrichment: Twenty litres of water is collected from the water body and enriched with nutrients and left under low light (100 to 300 lux) until algal bloom occurs. The nutrient added for enrichment should be appropriate for the species to be isolated. The isolation of a single algal cell from the bloom can be accomplished by any one of the following methods:

2. Simple capillary pipette isolation Method: The mixed plankton sample is kept in a petridish under a binocular microscope. The desired species is isolated using a capillary pipette and transferred to culture tubes having suitable sterile culture medium.

3. Centrifuging method: By repeated centrifuging the water samples and then by inoculating the deposits, we can isolate several microalgae.

4. Serial dilution Method: This method is used mainly for the isolation of phytoflagellates (i.e. motile species). This involves systematic dilution of the inoculaum in five stages (1, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} or 4 steps 0.001, 0.01, 0.1 and 1ml) so that the subject species is well separated from any contaminant. The species thus isolated is transferred to the culture tubes.

5. Agar plating Method: Agar medium is prepared by adding 1.5 gm of agar to one litre of suitable culture medium. This agar medium is sterilized in an autoclave for fifteen minutes under 120 lbs pressure and 120ºC temperature. Now the medium is poured in sterilized 15 cm petri dishes and kept for 24 hrs. The required species can be picked by platinum needle or loop under microscope and streaked on the surface of agar plate. After inoculation, these petridishes are placed in an incubation chamber for 7-8 days providing light (1000 lux) and constant temperature (25ºC). Within this time, the required species, if it has grown into a colony is removed by platinum loop under microscope and transferred to culture tubes. Further from the culture tubes to small conical flasks and larger flasks, the algae can be grown on a mass scale.

The primary requirement for stock culture is the availability of pure cultures of the desired micro algae. The basic principle involved here is to culture and maintain the pure strains in sterilized sea water by adding nutrients and providing adequate light for the photosynthesis. The nutrients required for growth of micro algae is supplied by the culture media being used which is composed of chemicals, trace metals and vitamins. The common culture media used for marine micro algal culture includes Conway or Walne’s media (Walne,1974), F/2 medium (Guillard, R. R. and Ryther, J. H. 1962), Erd-Schreiber's medium and Miquel's media (Miquel, 1892). The

algal pure strains are kept under standard controlled environment in conditioned rooms or in especially designed incubators, in which routine work can be performed under strict hygienic control. The pure strain cultures are usually kept in small glass containers, such as 10 to 25 ml test tubes or 100 ml glass Erlenmeyer flasks, closed by a sterile stopper (screw cap or a folded aluminium foil). Pure-strains cultures should be maintained at a steady or resting stage, i.e. under environmental conditions which allow them to reproduce, but not to increase exponentially in number. In this way, their sexual reproduction is fostered, thus increasing their genetic variability, and the growth of unwanted organisms such as other algal species, bacteria and ciliate protozoa is prevented. Culture parameters are therefore kept below the values adopted for mass production. In particular, only half dose of nutrients is used, water temperature is kept at around 14-16°C, light intensity ranges from 300 (test tubes) to 1000 lux (flasks) and no aeration and carbon dioxide are provided. Under routine conditions, strain cultures are usually renewed every month. In the replication process, an inoculum of 0.1-0.2 ml (from test tubes) or 0.5 to 1 ml (from Erlenmeyer flasks) is taken from the best old culture which is free of contamination, to inoculate three new vessels of the same size to start a new strain. The old culture is then either utilised for upscaling, or is discarded. Strain culture vessels should be stirred at least once a day by hand, paying attention not to stir bottom debris up.

Inoculation of flasks from test tube

Follow the same procedure as previously described for pure strains. Each 0.1-l flask will receive 50 ml of enriched medium and 0.5 ml of inoculum. At this stage, no aeration is required. When mature, each small flask will inoculate a new 2-l flask.

Inoculation of 2 and 5-l flasks from another 2 or 5-l flask:

- Prepare the necessary amount of new flasks filled with sterilised seawater, as well as all equipment required for the operation (pipettes, nutrients, cotton stoppers, aluminium foil, glass tubing, etc.).
- Select the mature culture that will be used as inoculum, checking a sample under the microscope for contaminants.
- Remove its cap and flame its neck; then close with a flamed aluminium foil stopper and let it cool.
- In the meanwhile, add the fertilizing working solutions to each new flask, at a rate of 1 ml/l; using a new sterile pipette for each solution.
- Flame their necks and aluminium caps thoroughly.
- When cool, remove the stopper and pour some algal culture of the old flask into the new

vessels at a rate of about 10% of the receiving volume, avoiding to wet their neck with the inoculum, then gently shake flasks to mix the new culture.

- Flame thoroughly their necks, introduce the sterilized glass tubing for aeration and close tightly with sterilised cotton stopper (or any other type of sterile cap).
- Write date and algal species on the new flask.
- Place the flasks on the lighted shelf and connect to the air delivery system, adjusting its flow to a gentle bubbling.
- After an hour, check all new vessels for a proper air bubbling.

Note: Use only the upper layer of the old culture, leaving dead cells and debris in the flask used to inoculate the newones. The size of the inoculum for small volumes is only 10% of the new volume because of the high cell density. Remember to label the flask properly and get rid of every contaminated flask. The above mentioned procedure applies to the upscaling of the other medium size vessels.

A general set of conditions for micro algal culture		
Parameters	**Optimum values**	**Range**
Temperature (^{0}C)	18-24	16-27
Salinity (ppt)	20-24	12-40
Light intensity (Lux)	2500 -5000	1000 – 10,000 (depends on volume and density)
Photoperiod (L:D; hours)	16: 8 (minimum) 24:0 (Maximum)	
pH	8.2 -8.7	7-9

Micro algal Culture Media

Media for the culture of marine phytoplankton consist of a seawater base (natural or artificial) which may be supplemented by various substances essential for micro algal growth, including nutrients, trace metals and chelators, vitamins, soil extract and buffer compounds. The term ‘nutrient’ is colloquially applied to a chemical required in relatively large quantities, but can be used for any element or compound necessary for algal growth.

Seawater base

The quality of water used in media preparation is very important. Natural seawater can be collected near shore, but its salinity and quality is often quite variable and unpredictable, particularly in temperate and polar regions (due to anthropogenic pollution, toxic metabolites released by algal blooms in coastal waters). The quality of coastal water may be improved by ageing for a few months (allowing bacterial degradation of inhibitory substances), by autoclaving (heat may denature inhibitory substances), or by filtering through acid-washed

charcoal (which absorbs toxic organic compounds). Most coastal waters contain significant quantities of inorganic and organic particulate matter, and therefore must be filtered before use.

Media preparation

The salinity of the seawater base should first be checked (30-35‰ for marine phytoplankton), and any necessary adjustments (addition of fresh water/evaporation) made before addition of enrichments. Seawater, stock solutions of enrichments and the final media must be sterile in order to prevent (or more realistically minimize) biological contamination of unialgal cultures.

Sterile filtration is done to sterilizing seawater without altering the chemistry of the seawater, as long as care is taken not to contaminate the seawater with dirty filter apparatus. Sterilization efficiency is, however, to some extent reduced compared with heat sterilization methods. Membrane filters of 0.2µm porosity are generally considered to yield water free of bacteria, but not viruses. 0.1µm filters can be used, but the time required for filtration of large volumes of culture media may be excessively long. Most stock solutions of culture medium additions can be sterilized separately by autoclaving, although vitamin stock solutions are routinely filtered through 0.2µm single use filter units, since heat sterilization will denature these organic compounds. Filter sterilization of all additions may reduce uncertainties about stability of the chemical compounds and contamination from autoclave steam, but absolute sterilization is not guaranteed. Stock solutions were stored in ultra clean sterile glass bottles. In order to minimize effects of any microbial contaminations, all stock solutions should be stored in a refrigerator at 4°C, except vitamin stocks which are stored frozen at –20°C and thawed immediately prior to use.

Conway' or Walne's medium	
Solution A.	**In 1 litre Dist. water**
Potassium nitrate	100 gm
Sodium orthophosphate	20 gm
EDTA (Na)	45 gm
Boric acid	33.4 gm
Ferric chloride	1.3 gm
Manganese chloride	0.36 gm
Solution B.	**In 1 litre Dist. water**
Zinc chloride	4.2 gm
Cobalt chloride	4.0 gm
Copper sulphate	4.0 gm
Ammonium molybdata	1.8 gm
Solution C.	**In 100 ml Dist. water**
Vitamin B1 (Thiamin)	20 mg
Vitamin B12 (Cyanocobalamine)	10 mg

Solution D (for culture of diatoms-used in addition to solutions A and C, at 2 ml per liter of culture)	
Sodium metasilicate (Na2SiO3.5H2O)	40.0 g
Make up to 1 litre with distilled water; Shake to dissolve	
Prepare stock solution A, B and C (each) in different reagent bottles. Add 1 ml of solution A, 0.5 ml of solution B and 0.1 ml of solution C and 1ml of D (only for diatoms) to 1 litre of filtered and sterilized seawater.	

f/2-Si *(Guillard, 1975)*Medium			
To 996ml of sterile seawater aseptically add			
Quantity	**Compound**	**Stock solution** (sterile)	**Final conc. in medium**
1.0ml	$NaNO_3$	75.0g/litre H_2O	884µM
1.0ml	$NaH_2PO_4.H_2O$	5.0g/litre H_2O	36µM
1.0ml	f/2 trace metal solution	(see recipe below)	(see below)
1.0ml	f/2 vitamin solution	(see recipe below)	(see below)
f/2 trace metal solution			
To 950ml distilled H_2O add:			
Quantity	**Compound**	**Stock solution**	**Final conc. in medium**
3.15g	$FeCl_3.6H_2O$	-	11.7µM
4.36g	$Na_2EDTA.2H_2O$	-	12µM
1.0ml	$CuSO_4.5H_2O$	9.8g/litre H_2O	0.04µM
1.0ml	$Na_2MoO_4.2H_2O$	6.3g/litre H_2O	0.03µM
1.0ml	$ZnSO_4.7H_2O$	22.0g/litre H_2O	0.08µM
1.0ml	$CoCl_2.6H_2O$	11.9g/litre H_2O	0.05µM
1.0ml	$MnCl_2.4H_2O$	178.2g/litre H_2O	0.9µM
Make up to 1 litre with distilled H_2O, sterilize (autoclave or filter) and store in fridge.			
f/2 vitamin solution.			
To 950ml distilled H_2O add			
Quantity	**Compound**	**Stock solution**	**Final conc. in medium**
1.0ml	Vit.B_{12} (cyanocobalamin)	0.5g/litre H_2O	0.37nM
1.0ml	Biotin	5.0mg/litre H_2O	2.0nM
100.0mg	Thiamine HCl	-	0.3µM
Make up to 1 litre with distilled H_2O, filter sterilize into plastic vials and store in			

freezer
Silicate is specifically used for the growth of diatoms which utilize this compound for production of an external shell. **Silicate Solution** Working Stock: add 30 g Sodium silicate (Na_2SiO_3) to 1 liter distilled H_2O.

Mass culture of micro algae

The mass culture is carried out to supply the algae for use in the hatchery for culturing the zooplankton. It is also required for the green water technique being used for larval rearing of marine fin fishes. The technique involves in scaling up of the stock culture to mass scale sufficient to meet the requirements of the hatchery. This can be done in outdoor or indoor. In outdoor it can be carried out with FRP tanks, cement tanks, Acrylic tanks, Polythene bags etc. Indoor mass culture can be done by providing sufficient lighting apart from the nutrients. This is usually carried out in acrylic tanks, Polythene bags, plastic carbuoys etc.

Outdoor mass culture: The outdoor mass culture can be done using commercially available fertilizers as nutrient supply source to reduce the cost. The proportion of the same is given below:

Media for outdoor mass culture		
Ammonium sulphate	**Urea**	**Super phosphate**
100g/tonne of water	10g/tonne of water	10g/tonne of water

Mass culture facilities for microalgae

Algae are cultured in a dedicated sector of the live feeds production section, which is made of three working areas inside the hatchery building: a lab for duplicating small cultures, a conditioned room to maintain small culture vessels and pure strains and finally a large area for the mass cultures in FRP tanks. Small volume cultures are kept in vessels ranging from 20-ml test tubes up to 18 l carboys. They can be made of borosilicate glass, polycarbonate, PET or any other material able to stand a sterilization process. These vessels are placed on glass shelves lightened by fluorescent tubes and equipped with a CO_2 enriched air distribution system. The unit also stores the special equipment to process pre-treated seawater, such as fine filters and sterilizers, as well as a laboratory where nutrients and glassware are prepared and stored, and where the necessary monitoring operations are performed. Standard cleaning procedures have to be strictly followed to maintain proper hygienic conditions.

Maintaining monoalgal culture in outdoor tanks

The major difficulty in operating *Chlorella sp.* and *Nannochloropsis sp*. outdoors cultures completely open to air-borne contaminants is that cultures become, too often, contaminated thereby becoming useless. Open cultures may be operated, therefore (either batch wise or in continuous cultures), providing a working protocol has been developed to control growth of foreign organisms. The greatest damage to these cultures is caused by grazers; most serious of which is *Paraphysomonas imperferata*, a non-specific heterotrophic flagellate, 7–12 m, with very short generation time. Appearing as soon as temperatures become high, it may crash a dense culture of *Nannochloropsis sp.* in 24 h. Another grazer is *Euplotes sp*., a 50–90 µ ciliate, which may graze on *Nannochloroposis sp*. cells as well as other organic matter in the culture, including bacteria. The presence of this protozoon is always associated with aggregation of the host's cells and reduction of cell numbers. Species of algae, e.g. diatoms mostly *Amphora* sp., may also become established in an open *Nannochloropsis* culture. Although diatoms are not regarded as direct competitors to *Nannochloropsis*, their cells excrete polysaccharides which cause *Nannochloropsis sp*. cells to stick together. Relatively low numbers of diatoms can cause serious damage affecting such cell conglomerates, arresting thereby culture growth, making the culture useless. Other contaminating microorganisms are colorless microflagellates, bacteria, as well as *Uronema sp*., 20–25 µ ciliates grazing on bacteria.

Controlling contaminants

Different treatments may be applied to eliminate contamination, thereby facilitating cultivation of *Chlorella sp.* and *Nannochloropsis sp.* in open culture system:

(a) **Lowering the pH**: This represents a useful tactic by which to arrest development of some contaminants. pH 6 is low enough to eliminate diatoms in a *Nannochloropsis sp*. culture; the aggregates dissolve and within a few hours the culture regains its normal appearance. Elimination of *Paraphysomonas* cells and similar contaminants requires the pH to be lowered to 2.5 for a couple of hours. *Nannochloropsis sp*. cells lose their photosynthetic capacity at this pH, which should thus be soon raised to 5.5 or 6.0 at which pH cells regain their normal functioning and after a short period of photosynthesis, the pH rises to the 8.0–8.5 at which it is maintained.

(b) **Chlorination**: Chlorinating contaminated *Chlorella sp.* and *Nannochloropsis sp.* cultures using concentration of 4 to 10 ppm of active chlorine is quite effective in eliminating grazers. Density of the cultured cells, the organic load (dissolved and particles) and the temperature represent the parameters to be considered in applying an adequate chlorine concentration dose

for effective treatment. High doses of chlorine would be used for dense cultures of high organic loads and when culture temperatures are high, thus in summer, there is a need to chlorinate up to twice a week, whereas one application may be sufficient for two months during the cold season. Tank walls should be cleaned every five to six days in winter and after every successful culture in summer.

Harvesting

For both *Chlorella sp.* and *Nannochloropsis sp*. mass culture system, the desired volumes are pumped out every day either directly to the consumers or to an industrial centrifuge, creating a 40% solid paste, which is preserved for later use. After harvest, a mixture of fresh sea and tap water, including nutrients, is introduced in the tanks, bringing the culture back to its original volume.

Production quality

The harvested microalgae is used for culturing rotifers and for maintaining the rotifers' nourishing value in the larvae rearing tanks of marine fish (Green Water). It is essential to focus on the algae quality, particularly the total lipid content and polyunsaturated fatty acids (PUFAs). Cell density in an open culture of *Nannochloropsis sp*. may exceed 200 x 10^6 cells ml^{-1}. At this cell concentration, however, productivity would be much below maximal. For maximal sustainable yield, the optimal cell concentration of 100–120 x 10^6cells m^{-1}, depending on the season and cultures depth, should be maintained. The output rate (in dry weight) of cell mass varies greatly with the season. In summer, 25–30% of the culture is harvested per day, yielding an average of 6g (dw)/t. In winter, due to shorter daylight and lower temperatures, only 15% of the culture is harvested daily, averaging 4 g (dw)/t.

Methods of culturing micro algae:

The micro algal culture can be done as batch culture or continuous culture

Batch culture

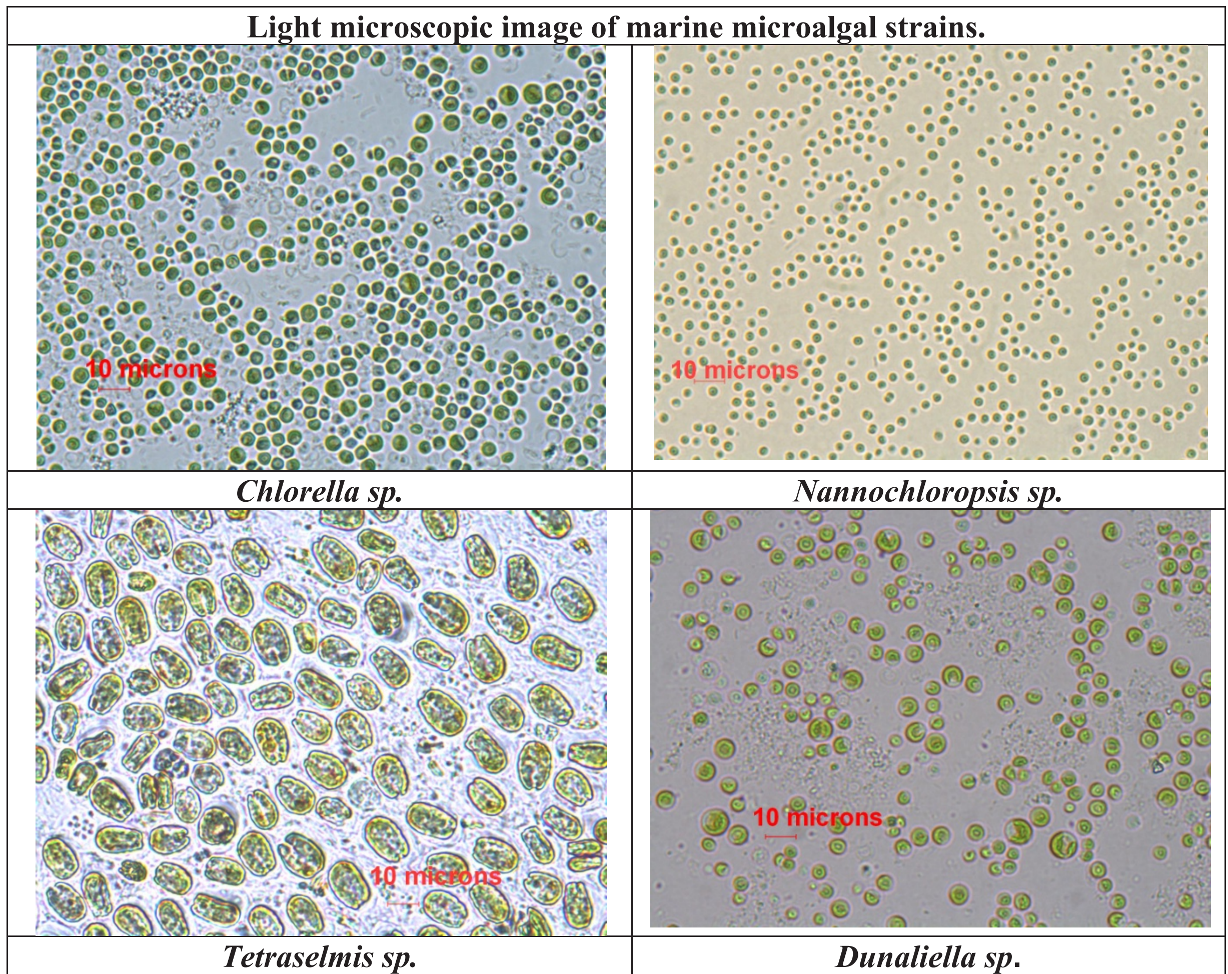
This is the most common method for cultivation of microalgal cells. In limited volume (batch) cultures, resources are finite. When the resources present in the culture medium are abundant, growth occurs according to a sigmoid curve, but once the resources have been utilised by the cells, the cultures die unless supplied with new medium. In practice this is done by sub culturing, i.e. transferring a small volume of existing culture to a large volume of fresh culture medium at regular intervals. In a simple batch culture system, a limited amount of complete culture medium and algal inoculum are placed in a culture vessel and incubated in a favorable environment for growth. Some form of agitation, such as shaking or impeller mixing, is necessary to ensure nutrient and gaseous exchange at the cell–water interface. The culture vessel can be a simple conical flask or an environment controlled fermentor. Batch culture is widely

used for commercial cultivation of algae for its ease of operation and simple culture system. Since the process is batch wise, there is low requirement for complete sterilization. For mass algal culture production, a portion of the culture could be retained as inoculum for the next culture batch. The different phases, which may occur in a batch culture, reflect changes in the biomass and in its environment.

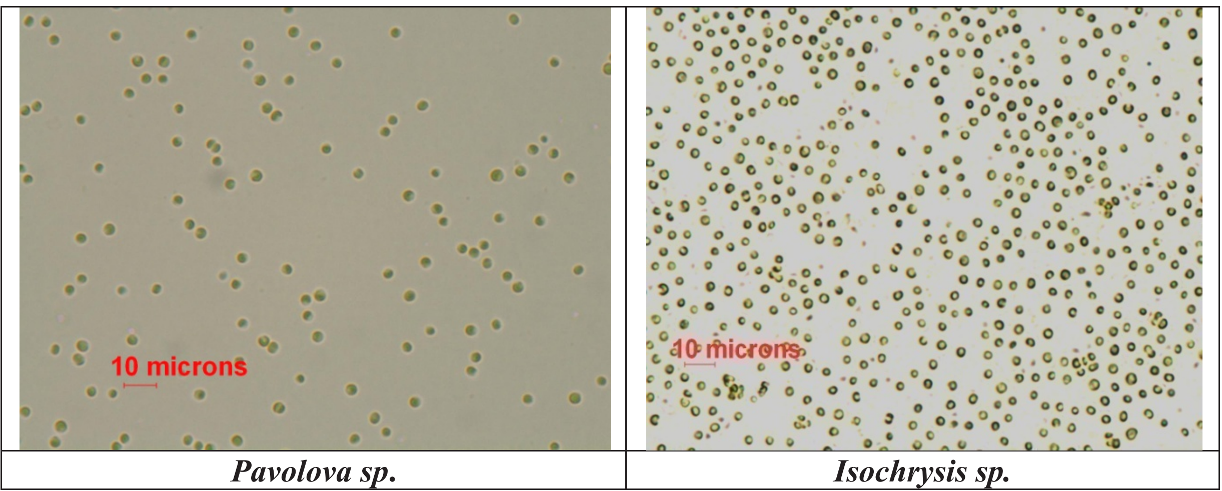
Continuous cultures

In continuous flow cultures, fresh culture medium is supplied to the homogeneously mixed culture and culture is removed continuously or intermittently. Here the resources are potentially infinite: cultures are maintained at a chosen point on the growth curve by the regulated addition of fresh culture medium. In practise, a volume of fresh culture medium is added automatically at a rate proportional to the growth rate of the algae, while an equal volume of culture is removed. The approach is based on the observations that substrates are depleted and products accumulate during growth. Eventually, culture growth ceases due to depletion of the growth limiting substrate or accumulation of a growth-inhibiting product. To sustain cell growth, the growth-limiting substrate needs to be replenished and the growth inhibitory product needs to be removed or diluted by adding fresh culture medium.

Light microscopic image of marine microalgal strains.



Chlorella sp.	*Nannochloropsis sp.*
Tetraselmis sp.	*Dunaliella sp.*



Pavolova sp.	*Isochrysis sp.*

Hygenic handling protocols:

The cardinal principles involved in micro algal culture section is to avoid any chances of contamination of the cultures, for which the personnel manning the section shall be aware of the hygenic handling protocols which are detailed below:

Work in clean place or preferably in a laminar flow cabinet (cabinet must be turned on at least 30 minutes before transfer; if equipped with UV lamps, leave on overnight prior to use). Clean the working surface with 70% alcohol (ethanol/isopropanol) prior to and after use. Clean hands with disinfecting soap and rinse with 70% alcohol prior to all operations. When not using a laminar flow cabinet (and to be safe even when using a cabinet), sterilise (flame) the neck of vessel of origin before and after transfer (not possible with some plastic vessels, which must, therefore, be opened in a laminar flow cabinet). Pipettes must be clean and sterile; use autoclavable tips for repeating pipettes, pre-wrapped sterile single use plastic/glass pipettes, or if using non-sterile glass pipettes (with cotton plugs), sterilise in the flame before use.

The preparation of glass wares and containers is a vital step in the microalgal culture: The step by step protocol is given below:

- wash with detergent
- rinse in hot water
- clean with 30% muriatic acid
- rinse again with hot water
- dry before use.

Alternatively, tubes, flasks and carboys can be sterilized by autoclaving and disposable culture vessels such as polyethylene bags can also be used.

Cleaning procedure

The culture flaks have to be scrubbed (abrasive brushes not appropriate for most plastics) and soaked with warm detergent (not domestic detergents, which leave a residual film on culture ware-use laboratory detergent such as phosphate-free Decon); rinse extensively with tap water; soak in 10% HCl for 1 day-1 week (not routinely necessary, but particularly important for new glass and polycarbonate material); rinse extensively with distilled and finally double distilled water; leave inverted to dry in a clean, dust-free place.

Sterilization

Sterilization can be defined as a process which ensures total inactivation of microbial life (not the same as disinfection, which is defined as an arbitrary reduction of bacterial numbers). The primary purpose of sterilization is to prevent contamination by unwanted organisms, but it may also serve to eliminate unwanted chemicals. There are several sterilization methods and the choice depends on the purpose and material used:

Moist heat

Autoclave or pressure cooker (1-2 Bar, 121°C, in pure saturated steam). For sterility the steam must penetrate the material (wrap in material that allows access of steam-kraft paper or aluminium). Autoclave steam may introduce chemical contaminants; glass and polycarbonate vessels should be autoclaved containing a small amount of double distilled water which is poured out (thus diluting contaminants) under sterile conditions immediately prior to use. Never close vessels (risk of implosion); use cotton wool bungs, or leave screw caps slightly open.

Mixing-Mixing of micro algal cultures may be necessary under certain circumstances: when cells must be kept in suspension in order to grow in concentrated cultures to prevent nutrient limitation effects due to stacking of cells and to increase gas diffusion. Mixing has to be given through gentle bubbling with air.

Microalgal indoor and outdoor culture systems.

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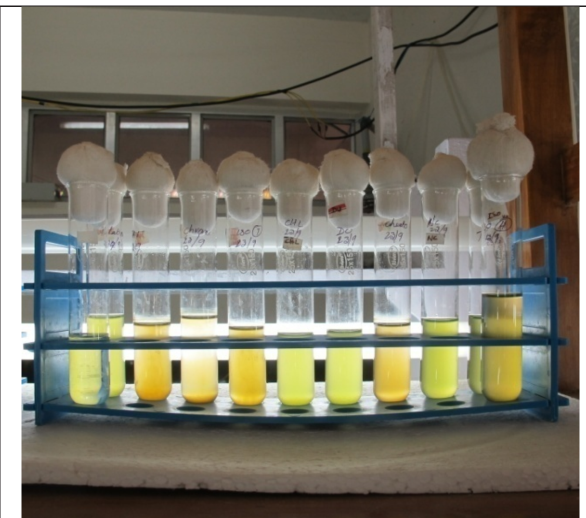
<tr><td></td><td> </td></tr>
<tr><td>Miacroalgal stock culture in test tubes</td><td>Miacroalgal stock culture in conical flasks</td></tr>
<tr><td> </td><td> </td></tr>
<tr><td>Nannochloropsis sp.in haffkine flask</td><td>Isochrysis sp. in haffkine flask</td></tr>
<tr><td> </td><td> </td></tr>
<tr><td>Nannochloropsis sp. in 20L carboys</td><td>Outdoor mass algal culture unit</td></tr>
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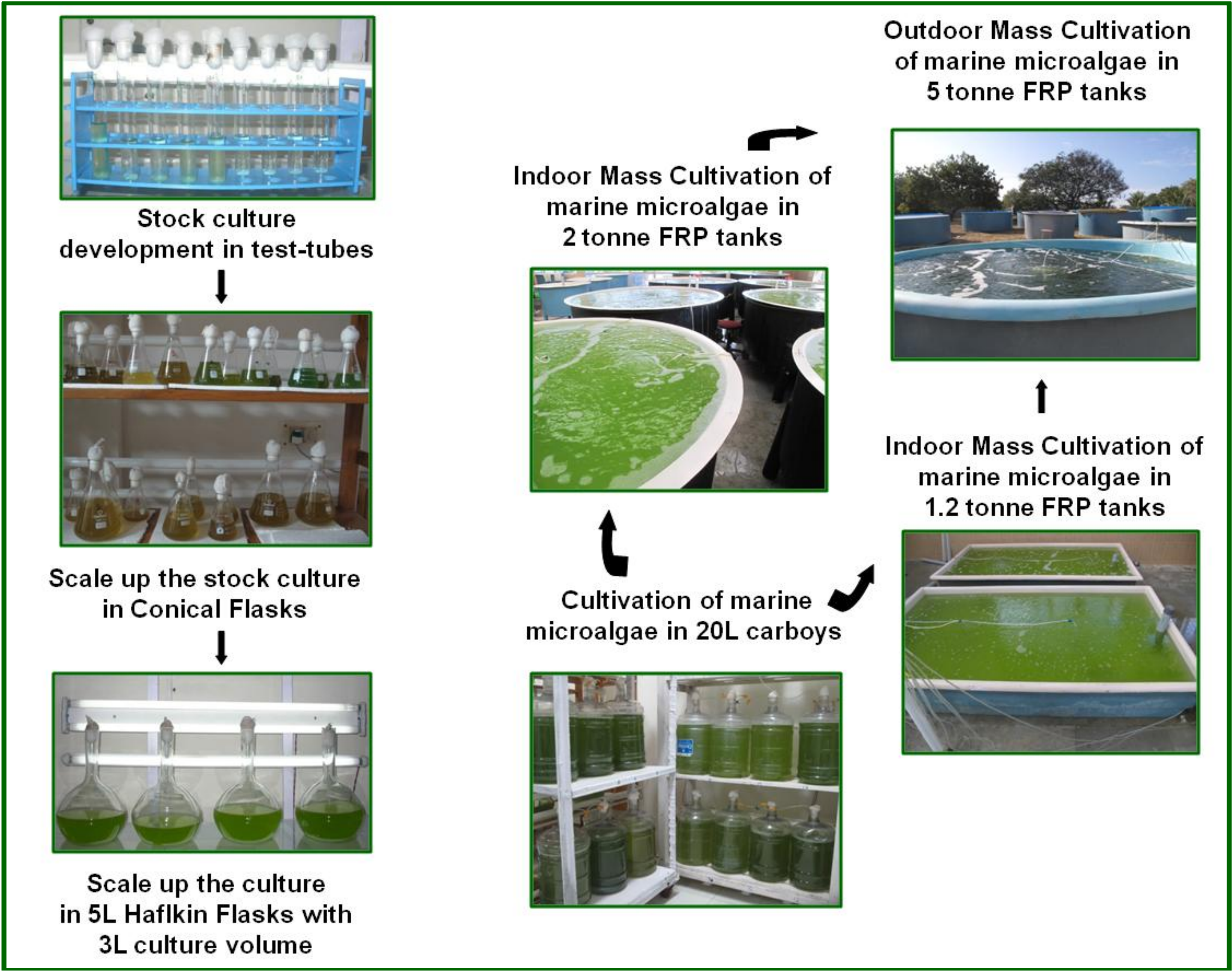
Figure 4. Marine microalgal scale up, indoor and outdoor mass cultivation process.



Measurement of cell growth and culture productivity

Net growth may be estimated quickly by measuring changes in the overall turbidity of the culture. This, however, provides only a rough estimation of growth and should be followed routinely with other measurements such as cell count, dry weight or total organic carbon (TOC). Cell chlorophyll and protein may be suitable for expression of growth in algae, but should be used with caution, particularly in outdoor cultures, being strongly affected by environmental conditions.

Turbidity

The turbidity can be measured at three different nanometers 540nm, 640nm, and 660nm respectively along with culture medium in sterile seawater as a blank by using a spectrophotometer.

Counting Cells in Cultures with the Light Microscope *using a Haemocytometer*

Haemocytometer

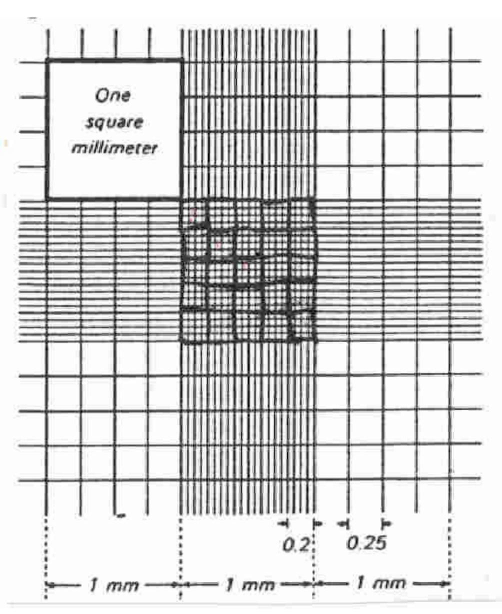
As the name suggests these counting chambers have been developed for counting blood cells but they can be used to calculate the cell density of an algal culture provided the cells are relatively small (~ 5-50µm) and either single cells or short chains. The size of these chambers can vary with manufacturer but we use a Neubauer brand which consists of two chambers, each with a volume of $0.1mm^3$, containing a marked counting grid $1mm^2$ in area. The haemocytometer can be used where cell densities are in the range 5 $x10^4$ - 10^7 cells / mL.

Method

1. Algal Sample –algae is non-motile cells which do not need fixing. Counting has to be done as soon as the sample is collected. Algal growth is measured by counting the cells in a 24hr interval.

2. To fill haemocytometer chambers, thick cover glass is placed over both grids and take a Pasteur pipette and fill its tip by capillary action with sample. Hold the pipette at an angle of 45^0 (higher or lower to control flow rate) and place the tip at the leading edge of the cover slip. With very gentle pressure, allow the sample to flow quickly and evenly into the chamber, exactly filling it. The chamber surface in the Neubauer brand is a flat mirror-like rectangle and the sample must cover this rectangle but not flow over its edges. It is useful to rest your hand on a bench and steady the pipette tip with a finger. If flooding occurs, rinse haemocytometer and cover slip with distilled water, and repeat procedure. Refill the pipette for each chamber. The time taken to fill the chamber should be short, to minimize setting of cells in the pipette.

3. Allow cells to settle (1min) and check the grid under the microscope for satisfactory distribution of cells, i.e. evenly spread.



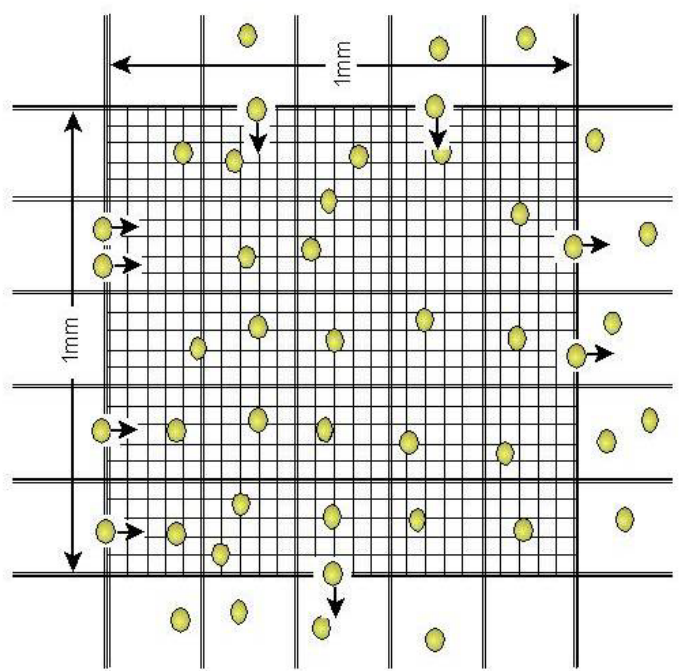
The Haemocytometer grid in detail

The grid is divided into 9 large squares, each 1mm x 1mm, by triple lines. Each large square is divided into 25 medium squares, each 0.2mm on a side, and each medium square is further divided into 16 small squares, each 0.05mm on a side.

Haemocytometer (Neubauer brand).

For all haemocytometers, the fundamental measurement is the average number of cells per $1mm^2$ so the centre large square is usually counted. To obtain the total number of cells in this large square, the number of cells in each of the 25 medium squares are counted, recorded then added.

Note: When counting cells bordering on triple rulings, the convention is to count only those cells touching the top and left-hand side rulings of each square



After counting each of the two haemocytometer chambers, the haemocytometer and coverslip are rinsed with distilled water. Usually the procedure is repeated twice more to give a total of 6 counts. The cell density was obtained by calculating the average cell count and multiply by the conversion factor (for Neubauer = No of cells $x10^4$ cells/ml).

Biomass estimation

Dry weight of algal cells can be determined by filtering and drying algae from aliquots of culture of known concentration

1. Filter an exact volume of culture on pretared glass-fiber filters (1 µm pore size) using a filtration setup connected to a vacuum pump.
2. Wash the filter with a solution of ammonium formate (0.5 M) to remove salts.
3. Follow the same procedure with control filters on which an equal volume of 0.22-µm filtered seawater is filtered.
4. Dry the filters at 100 °C for 4 h to volatilize the ammonium formate
5. Weigh on an analytical balance.
6. Calculate the dry weight per algal cell according to the formula:

$$DW\ (g.cell^{-1}) = (DWA - DWC) / (N \times V)$$

Where,

DWA = average dry weight retained on algal filter (g)

DWC = average dry weight retained on control filter (g)

N = algal concentration (cells/ml)

V = volume of algal culture and filtered seawater filtered on algal and control filter, respectively (ml)

In order to improve the correction for salt residues and the variation among samples, cellular dry weight can be determined in triplicate from regression analysis of DW retained on the filter versus number of algal cells filtered.

CONCLUSION

In mariculture seed production technology, microalgae culture is an inevitable component. It forms the food for growing the zooplankton apart from being used for the green water technique commonly employed in larviculture of marine finfishes. The microalgal section is the starting point of life in a hatchery. So it is the most important section with regard to observance of strict hygienic protocols to avoid any type of contamination, because any contamination at this stage would spread to all other sections. It is always advisable to assign different personnel for indoor stock culture maintenance and outdoor mass culture, so that cross contamination does not occur. The growth and quality of the algae has to be monitored regularly by well trained and qualified personnel. Periodically the stock cultures should be renewed with new stock, since over a period of time, the quality of the same strain/stock may deteriorate. The uncertain nature of weather conditions and risks of contamination is a major bottleneck in the successful mass production in outdoor tanks. This could be overcome to a great extend by establishing mass production facilities indoors by providing artificial lighting.

Rotifers as live feed

Anikuttan.K.K, G.Tamilmani, M.Sakthivel, P.RameshKumar, M.Sankar, R.Jayakumar, A.K.AbdulNazar, G.H.Rao, Tinto Thomas, N.Krishnaveni and T.T.Ravikumar.

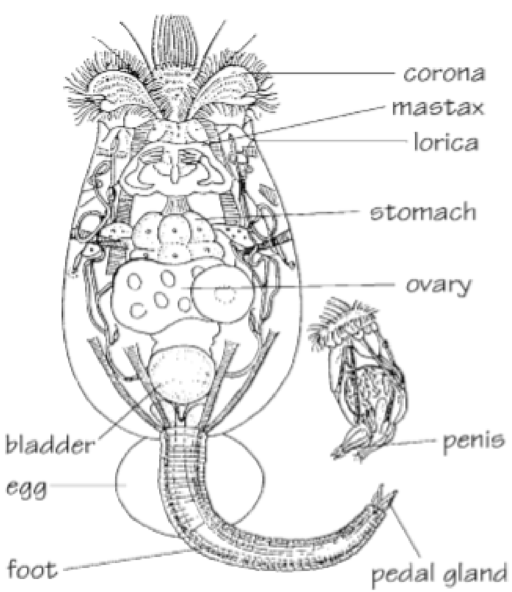
Introduction

The candidate species identified for mariculture includes Cobia, *Rachycentroncanadum*, Asian sea bass, *Latescalcarifer*Silver/Snubnose pompano, *Trachinotusblochii,* Indian pompano, *T.mookalee*, Orange spotted grouper, *Epinepheluscoioides*, Sea bream, *Lethrinuslentjan*, etc.The larvae of all these species is carnivorous and feeds on zooplankton such as rotifers or copepods. Hence their seed production technique invariably requires the production of the zooplankton such as rotifers, copepods and artemia among which rotifers are the most widely cultured live feed for the seed production of marine fin fishes. The phylum Rotifera (previously known as the Rotatoria) consists of a relatively small group of minute, unsegmented, pseudocoelomate, aquatic invertebrates with bilateral symmetry. Most rotifers are free-crawling or swimming, but sedentary and colonial forms are also known. Moe than 1000 species have been described, 90 % of which inhabit freshwater habitats. They seldom reach 2 mm in body length. Males have reduced sizes and are less developed than females; some measuring only 60 µm. The body of all species consists of a constant number of cells, the different Brachionus species containing approximately 1000 cells which should not be considered as single identities but as a plasma area. The growth of the animal is assured by plasma increase and not by cell division.

Morphology & Anatomy

The rotifer's body is differentiated into three distinct parts,viz; the head, trunk and foot. The head carries the rotatory organ or corona which is easily recognized by its annular ciliation. The retractable corona assures locomotion and a whirling water movement which facilitates the uptake of small food particles (mainly algae and detritus). The trunk contains the digestive tract, the excretory system and the genital organs. A characteristic organ for the rotifers is the mastax (i.e. a calcified apparatus in the mouth region), that is very effective in grinding ingested particles. The foot is a ring-type retractable structure without segmentation ending in one or four toes.The epidermis contains a densely packed layer of keratin-like proteins and is called the

lorica. The shape of the lorica and the profile of the spines and ornaments allow the determination of the different species and morphotypes.Rotifers possess an internal fluid-filled space known as a pseudocoelom that is boundexternally by the integument and internally by the epithelial cells of the various organs (digestive, protonephridial and reproductive). There are no respiratory or circulatory systems in rotifers, and the pseudocoelom internal fluid, bathing the internal organs, is equivalent to the circulatory system. Its composition is regulated by the protonephridia and it is replenished by the digestive tract. Rotifers exchange gases and dispose of nitrogenous wastes by diffusion through their body surface. Rotifers are also characterised by the syncytial structure of their body parts. Cell membranes in tissues disappear after embryonic development, forming multinucleated or syncytial tissues. All individuals of a species have a consistent number of nuclei in each organ. This situation, known as eutely, is also found in nematodes. The total number of nuclei, ranging from 900 to 1000, is fixed for life during embryonic development, indicating a limited capacity for repairing damage.



Brachionusplicatilis – Morphology & anatomy (FAO,1996)

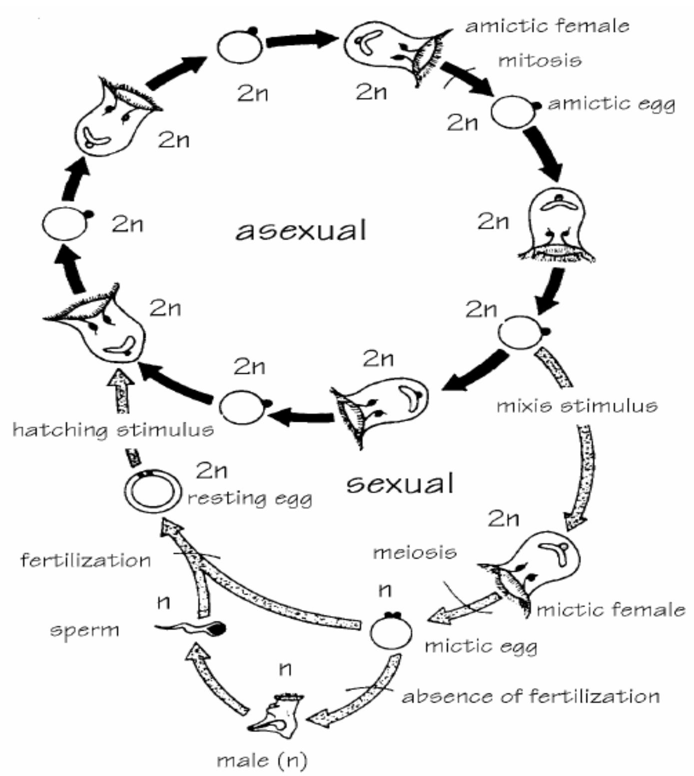
Food & feeding habits

Rotifers were described as mechanical grazers as they were found to graze non-selectively when offered two algal species with different cell size. The most common method of feeding in planktonic brachionid rotifers is by filter feeding. This type of feeding, also described as 'microphagus feeding', is found in rotifers having a developed ciliary corona and a crushing type of mastax. The type of food consumed, particularly its size, is directly dependent on the size and form of the ciliary apparatus and of the mastax. The rotational movement of the cilia directs a water current containing food particles towards the mouth and those that are suitable are swallowed, indicating a sensory mechanism regulating food selection. Food is captured by the corona, enters the mouth opening and passes through the buccal tube to the pharynx. Several studies indicate that food selectivity by suspension feeders such as rotifers is mainly based on prey size. The prey size spectrum for *B. plicatilis*ranges from approximately 1.4 to 4838μm^3, or 1.4 to 21 μm^3 of equivalent spherical diameter, with changes in efficiency with different particle size. The filtration or clearance rate is high at low food particle concentrations and declines in a curvilinear manner with the increase in food concentration.

Reproduction

The life span of rotifers has been estimated to be between 3.4 to 4.4 days at 25˚C. Generally, the larvae become adult after 0.5 to 1.5 days and females thereafter start to lay eggs approximately every four hours. Nearly all rotifers seen in nature are females. Males occur only for short periods and in many species have never been observed. Males are known from a limited number of monogonont species, but it is generally assumed that all members of this group are capable of producing males, including the brachionid species, *B. plicatilis*and *B. rotundiformis.* Males are much smaller than females and typically very fast moving. They have a rudimentary digestive gut and a sac-like testis containing free-swimming spermatozoa. A vas deferens leads from the testis to a penis and one or two prostate glands discharge into it. Males attempt copulation with amictic or mictic females and mating occurs at the region of the corona or cloaca. Successful fertilisation occurs in newly emerged females for a very limited period. The female reproductive organs is called Monogononts, as the name implies, composed of a single gonad. The gonad consists of the syncytial ovary that contains the ovocytes, the yolkproducing syncytial vitellarium and the follicular layer that surrounds the ovary and vitellarium and forms

an oviduct leading to the cloaca. The total number of ovocytes is present at birth. Rotifers are generally oviparous, with embryos developing outside the maternal body.



Types of reproduction in *Brachionusplicatilis*(FAO, 1996).

During favourable conditions, the population increases through diploid parthenogenesis (Asexual reproduction), whereby diploid females produce diploid eggs known as amictic eggs. Females are always diploid and males, when they appear, are haploid and very much reduced in size compared with females. Diploid females can either be amictic or mictic and morphologically they are indistinguishable. Amictic females produce parthenogenetically diploid eggs that develop mitotically into females, while mictic females produce, parthenogenetically, haploid eggs via meiosis. Thus, it is easy to obtain genetic clones from cultures originating from one amictic female. If a mictic female does not mate and is not fertilised, the haploid eggs form into males, but a mated mictic female that is fertilised (Sexual reproduction)will form diploid resting eggs (subitaneous eggs or cysts). Parthenogenetically formed eggs (diploid or haploid eggs) will develop immediately into embryos and hatch. Resting eggs will hatch under appropriate conditions into amictic females, after a dormant period. Thus, amictic females produce

parthenogenetically diploid amictic eggs and mictic females produce parthenogenetically haploid male eggs or sexually diploid resting eggs that hatch into diploid amictic females.

In the Brachionidae, the eggs are attached to the body of the female by a thin thread, and the embryos of amictic eggs hatch and are released from the maternal body leaving the egg shells still attached to their mother. Resting eggs at the initial stages of formation cannot be distinguished from those of amictic eggs. In *B. plicatilis*, where the resting eggs are formed outside the female's body, they are also attached by a thin thread to their mother until the end of their formation and later released and sink to the bottom of the culture vessel, pond or lake sediment. However, the resting eggs of *B. rotundiformis*(SM type rotifer strains) that develop within the maternal organism are not released from it. They will sink to the bottom of the culture vessel, pond or lake sediment with the death of their mother and are finally released only after the decomposition of her body. Resting eggs survive for long periods and have been hatched from sediment samples more than 60 years after their formation.

The fecundity of rotifers depends on whether asexual or sexual reproduction takes place. *B. plicatilis*amictic females produced 17–24 eggs during their lifetime, compared with 1–5 resting eggs produced by fertilised mictic females. In addition, the production of amictic eggs is, on average, 10 times faster (about 5 eggs/day) than that of the resting eggs during a similar oviposition period (approximately 108 h). The unfertilised mictic females produced 9–19 eggs, but these did not contribute to the increase in population as they form males. The number of eggs produced by a female is also dependent on the food algal species. The optimal temperature for culturing rotifers depends strongly on the species, as the optimal temperatures for *B. plicatilis*(10–30°C) are lower than those for *B. rotundiformis*(24–35°C). Within each species, differences have been found in the reproductive rates under the same culture conditions, indicating intraspecific variability.

Isolation of rotifer for stock culture

1. Rotifer samples can be collected from a marine or brackish water body with small plankton collecting net (150-300 µm mesh size).
2. The whole sample is then divided into sub samples, observed under microscope, from which healthy rotifer with eggs are collected.
3. The rotifers before being used in the production cycle it should first be disinfected.

4. The disinfection consists of killing the free-swimming rotifers but not the eggs by using a cocktail of antibiotics (*e.g.* Erythromycin- 10 mg/l, Chloramphenicol -10 mg/l, Sodium oxolinate- 10 mg/l, Penicillin- 100 mg/l, Streptomycin- 20 mg/l) or a disinfectant.
5. The eggs are then separated from the dead rotifers on a 50 µm sieve and incubated for hatching and the offsprings can be used for starting the stock cultures.
6. If the rotifers do not contain many eggs (as can be the case) the risk of loosing the complete initial stock is high and under such instances, the rotifer should be disinfected at sublethal doses.
7. The rearing water of the rotifers to be completely renewed and the rotifers to be treated with either antibiotics or disinfectants.
8. The treatment is repeated after 24 h in order to be sure that any pathogens which might have survived the passage of the intestinal tract of the rotifers are killed as well.
9. The concentration of the disinfection products differs according to their toxicity and the initial condition of the rotifers.
10. Orientating concentrations for this type of disinfection are furazolidone, 10mg/l, oxytetracycline, 30 mg/l,sarafloxacin, or linco-spectin-30 mg/l.

Maintenance of Stock culture

Small stock cultures are generally kept in closed vials in an isolated room to prevent contamination with bacteria and/or ciliates and further upscaling is essential for the regular mass production of rotifer.

1. The rotifers for stock cultures can be obtained from the wild, or from research institutes or commercial hatcheries.
2. Disinfection of the stock obtained has to be carried out as mentioned in the isolation procedure.
3. The culture water (seawater diluted with tap water to a salinity of 25 ppt) is aerated, pre-filtered over a 1 µm filter bag and disinfected overnight with 5 mg/l NaOCl.
4. The next day the excess of NaOCl is neutralized with $Na_2S_2O_3$ and the water is filtered over a 0.45 µm filter.
5. The vials (50 ml conical centrifuge tubes) which are previously autoclaved and filled with 20 ml of culture water are inoculated with an initial density of 2 rotifers/ml.

6. The vials are exposed to the light of two fluorescent light tubes at a distance of 20 cm (light intensity of 3000 lux on the tubes).
7. The food consists of marine *Chlorella or Nannochloropsis or Isochrysis*
8. Good cultures of 4 ml (2-4x10^6 cells/ml) fresh algae are to be added daily.
9. Shaking is needed mixed with enclosed air to provide enough oxygen for the rotifers.
10. The stock cultures for rotifers are to be kept in a temperature controlled room (28°C ±1°C).
11. After one week the rotifer density would increase from 2 to 200 individuals/ml.
12. The rotifers are rinsed, a small part is used for maintenance of the stock, and the remaining rotifers can be used for upscaling.
13. Furthermore, after some months of regular culture the stock cultures have to be disinfected as described earlier in order to keep healthy and clean stock material.

Mass production of rotifers

Growth phases

Rotifer growth under mass rearing conditions follow different phases, mimicking those of microalgae as described below:

- The lag-phase, when, just after the inoculum, rotifers begin to consume the phytoplankton of their culture medium and the number of both egg-bearing individuals as well as the quantity of amictic eggs increases.
- The log-phase (or exponential phase), where rotifers reproduce very fast and population growth is exponential.
- The transitional phase (or declining growth), where growth rate slows down and egg-bearing rotifers become rarer.
- The decline phase, where almost only old rotifers without eggs are found and their number decreases rapidly as death rate exceeds growth rate.

The quality of the rotifer population to start new cultures is even more important than in the case of microalgae. To be used as inoculum, the rotifer population must still be in the middle of

its log-phase with at least 20% fertility rate (measured as percentage of eggs over total rotifers, populations in their last declining phase, characterised by limited motility, scarce repletion and absence of egg-bearing animals, should always be discarded. With a proper inoculum and under optimal rearing conditions, a rotifer population should reach its harvesting density within 4 to 5 days. Under hatchery conditions, rotifer populations can reach the following densities:

in flasks after 5 to 7 days:

- S-type rotifers, 500 to 700 ind/ml
- L-type rotifers, 150 to 250 ind/ml

in tanks, after 4 to 6 days:

- S-type rotifers, 1000 and more ind/ml
- L-type rotifers, 400 ind/ml

Rotifers and eggs are counted at least in duplicate, alive or fixed in 4%formalin, in screened plastic petridishes (5 cm diam.).

As this microscopic animal is a filter feeder, its nutritional value strictly depends on its food. In hatcheries, the species is first cultured on microalgae, following the same scale-up protocol described for microalgae, then its final mass production is achieved in large tanks where artificial diets are fed to rapidly increasing numbers, improving at the same time their nutritional value. The rotifer *B.plicatilis*is a rather sturdy species able to tolerate a wide range of salinity, temperature and ammonia levels. Itcan also use several food sources, provided that particle size remains within a 2-20 µm range. Obviously, the highest growth rate is achieved under more restricted environmental parameters, and is closely related to the selected rotifer strain and feeding provided. In particular, good yields are obtained with high dissolved oxygen levels, temperature at 25°C, pH at 7.5-8.5, salinity in a 20-30 ppt range, less than 1 mg/l free ammonia (NH3), and moderate turbulence. Light is required only when rotifers are fed microalgae.

Preparation of the culture medium

The same procedures and precautions described in the algal production section apply to rotifer culture. The only enrichment added to the rotifer culture medium, be it either a log-phase algal culture or treated seawater plus artificial diet, is represented by the addition of vitamin B_{12} (cyanocobalamin) as a fertility booster for the rotifers. Its dosage is usually 100 ml of B_{12} stock

solution per m^3 of rotifer culture in tanks, whereas small vessels and bags are fertilized at the rate of 1 ml/litre. In both cases the vitamin is added together with the inoculum.

Mass culture

Rotifer mass culture is carried out in large tanks (1-2t). Because of the very high density achieved (up to 1000 individuals per ml or more), rearing procedures and protocols to maintain strict hygienic conditionshave to be applied. Such routine procedures are described below.

There are basically two main mass rearing methods for rotifers: (i)Using algae and baker's yeast as food for the rotifers, and (ii)using an artificial diet, the Culture Selco® produced by INVE SA of Belgium (or similar).

Standard Operating Protocols

Tank preparation

Before starting a new production cycle, normally after the harvest of the previous rotifer culture, prepare the tank as follows:

- Rinse with tap water to eliminate the bulk of organic debris.
- Wash it thoroughly with brush and detergent and rinse it again.
- Wash or spray the tank walls with 500 ppm active chlorine solution.
- After a couple of hours, drain the tank and rinse it well until the chlorine smell is gone.
- Let the tank dry and fill it with sterilized heated water only when needed.
- As an alternative possibility, fill the tank with seawater and sterilize with hypochlorite, then neutralize the residual chlorine with sodium thiosulphate.

Repeat the procedure for the equipment to be used in the tanks: aeration tubing, drain valves and suspended traps. A practical procedure is to assemble all small equipment in the new tank, fill with sea water and sterilize with hypochlorite: the equipment will be disinfected as a consequence.

Inoculation

To be used as inoculum, the rotifer population must still be in the middle of its log-phase with at least 20% fertility rate (measured as percentage of eggs over total rotifers). Never use rotifers which have already reached the last phase characterised by limited motility, scarce repletion and no egg presence. In algae/yeast fed tanks, the initial density of inoculum, one of the most important factors in rotifer culture, should be kept at least at 100 animals/ml, with an optimal density of 150-200 animals/ml. High density cultures with artificial feeding need up to 500 rotifers per ml. With an initial density of 200 animals/ml, rotifer density should reach its peak within four to six days at 25°C.

Feeding

As previously indicated two feeding methods are most widely adopted: 1) a combination of algae and baker's yeast and 2) a totally artificial diet.

Mass culture with algae/yeast as food

The initial method of mass culturing rotifers in hatcheries, makes use of a common and easily available food staple, the bakers' yeast *Saccaromyces cerevisiae*. It is a labour and cost sparing food, which has no nutritional value for rotifers that feed on bacteria associated with the yeast.

Compared to the artificial diets, this method has a lower yield and requires more time, typically one extra day. Density at harvest rarely exceeds 450 rotifers/ml with an average daily increase ranging from 19 to 33%. In addition, rotifers should be enriched with high levels of (n-3) HUFA and vitamins. Amajor constraint of this method is the absolute necessity to improve the otherwise very poor nutritional quality of yeast-fed rotifers before their distribution to fish larvae, by enriching them.

Procedure

- Fill the tank with sterilized sea water diluted with tap water to obtain a 20 ppt salinity; check the chlorine content of tap water and, if present, neutralize it with an excess of sodium. Take care to leave enough space for the algal cultures to be supplied as food (about 30% of the tank volume).
- Place the air diffusers and switch on the aeration.
- Place the traps for ciliates and impurities.

- Inoculate the tank to achieve an initial density of 150-200 rotifers/ml. This is considered as day 0.
- Add algal cultureas 20% of tank volume to provide rotifers with their initial food (as usual, the algae should be in their log-phase and from non contaminated cultures, even if of different species).
- The next day (day 1) fill the remaining 10% volume with algal culture.
- On the tank file, record all information on the culture growth, food distributed and environmental parameters monitored.
- From day 1 on, feed with bakers' yeast according to the recorded rotifer density.

Mass culture with Culture Selco® as food

A different technique based on a compound feed has been developed by the BelgianCompany INVE SA. The product, named Culture Selco® (CS) is a dry and complete rotifer diet thatdoes not require algae and is also effective as enrichment medium. Particle size (5 to 7 µm) andphysical characteristics ensure an optimal uptake by rotifers. The feed composition includes proteins(>35%), lipids (>15%, of which 23% are PUFA), carbohydrates (30%), carotenoids and othermicronutrients as minerals and vitamins A, D3, F and C.

To prepare CS suspend the amount required for a single meal in tap water, up to 50 g CS/l, and mix vigorously for 3 minutes (use a kitchen or better an industrial blender). Mixing or shaking by hand or using a magnetic stirrer is not sufficient to separate the CS cells. Remember that cell agglomerates left in the feed suspension cannot be ingested by rotifers because of their large size. Take care not to overfeed as uneaten feed can also quickly spoil water quality. Feed the daily amount in four to six meals evenly distributed over the 24-h period. In case it would be necessary, the feed suspension can be stored at temperatures below 8°C, and the amount needed for the whole day can be prepared at one time. The feeding ration can thus be distributed from the stored suspension at each meal.

Harvest

At harvest, rotifers are filtered and rinsed before being fed to fish or utilized as inoculum for newtanks. For this purpose, a double submerged filter is used. The inner filter has a mesh size of 300µ to retain larger particles, flocks of agglomerated food particles and ciliates which would rapidly clog the finer filter. The outer filter has a 50 mm filter mesh. Its capacity should be large enough to keep safely the whole rotifer population for the time needed to complete harvest and rinsing. Both filters are placed inside a large wheeled container full of water to avoid pressure build-up from the outgoing water that would smear rotifers against the net. A gentle air bubbling along the inner side of the filter helps to keep the filter free from clogging.

Harvesting and rinsing protocol for round-conical tanks with central drain:

- Prepare the harvesting device, always clean and disinfected with hypochlorite.
- Inject pure oxygen into the tank to be harvested for 10 to 15 minutes to have a supersaturated medium (at 10 ppm DO) in which rotifers could safely stand filtering operation.
- Fix a flex hose to the bottom drainage valve and place the other end into the harvesting Device.
- Open the valve and start filtering.
- Regulate the water flow so that the filter does not clog and the culture water does not overflow; do not exceed 100 l/minute.
- Stop water flow and clean the clogged pre-filter whenever necessary to avoid overflow of rotifer concentrated water.
- While harvesting, check for possible loss of rotifers through the net by sampling some filtered water with a beaker or a Petri dish.
- At the end of filtration, close the valve and rinse for 10 to 15 minutes the rotifers with filtered sterilized seawater at the same temperature as the tank of origin.

Harvesting of rotifers can also be done by the following method:

1. Switch off the aeration for some time (5-10 minutes)
2. Put a light source on the water surface, which will attract the rotifer on the water surface
3. Slowly collect the aggregated rotifers at the water surface along with the water using a bowl
4. Transfer the collected rotifers to a bucket which is prefilled with microalgae upto about 20% of its volume.
5. This can be fed to the larval rearing tanks after ascertaining the density.

Nutritional value and enrichment methods

Rotifers have a limited nutritional value for marine finfish larvae. Their nutritional value can be upgraded by an enriching process before their harvest through feeding them with microalgae rich in PUFA and vitamins such as *Chlorella, Nannochloropsis*and *Isochrysis*. It can also be done enriching with specially formulated artificial diets like the above mentioned Selco products. This oil emulsion gives excellent results in terms of high levels of EPA, DHA and vitamin C, which was not possible with the only use of algae. Moreover, labour, time, investment and running costs are spared. Rotifers can be enriched either in their mass culture tanks or after harvesting by placing them in dedicated enrichment tanks. The first method produces an enrichment of the tissues, as it is continuous along the entire culture period. The acquired fatty acids reserves are more stable and are less exposed to a rapid decrease in nutritional value during starvation. This method also saves time and reduces handling losses. The second system is a short term enrichment or, rather, a gut enrichment. It implies the harvesting and rinsing of the rotifers and the preparation of a separate enrichment tank.

Enrichment with algae:

- Use selected algae as specified above; enrich for about4-6 hrs.
- Maximum rotifer density: 500/ml.
- Microalgae density: *Isochrysis*5 million cells/ml; *Chlorella* sp. o *Nannochloropsis*sp. 12 million cells/ml.
- Resulting average total PUFA content of enriched rotifers: ± 7 mg/g dry weight.

Enrichment with Selco® products or other similar products:

- Follow the instructions provided by the manufacturer.
- Enrichment should take between 6 to 8 hours.
- Maintain the oxygen level at or above 4 ppm throughout the entire procedure.
- Dry products can be used directly in the production tank, whereas oily products are only fed in enrichment tanks.
- Use an antifoam product during enrichment to prevent rotifers losses by foam aggregation.

- During the enrichment process check frequently rotifer mortality and dissolved oxygen content; the latter should be kept above 80% saturation, with addition of pure oxygen if necessary.

The content of nutrients decreases rapidly in rotifers that are not immediately consumed by fish larvae. In starving rotifers the total PUFA loss reaches 60% after 6h at 18°C. Even in green water, i.e. with microalgae, this loss remains important (about 40% after 6 h). To prevent this degradation in nutritional quality, enriched rotifers not immediately fed to fish should be stored in containers at low temperature as follows:

- Storage time should not exceed 14 hours.
- Temperature should be kept between 5 and 10°C by means of insulated tanks and blue ice or ice bags.
- Rotifer density should not exceed 2500 to 3000 ind/ml.
- Oxygen level should be kept at or above 4 ppm.

Monitoring rotifer populations and health

Check all rotifer cultures daily for both quantitative and qualitative evaluations. From each vessel, flask and tank, take a 1 ml sample and observe under the stereo microscope.
Measure the following parameters:
The physiological and state of health of rotifer cultures can be assessed as follows.

1. Egg ratio
2. Swimming velocity
3. Ingestion rate
4. Viscosity
5. Enzyme activity
6. Diseases

Quantitative parameters:

- Total number of rotifers per ml
- Total number of eggs per ml
- Fertility as percent of total eggs over the total rotifers

Qualitative parameters:

- Average number of eggs per individual (estimate)
- Repletion (presence of food in the stomach, note 0 for empty, + for medium full, ++ when full)
- Motility (++ active, + slow, 0 absent)
- Filtration (activity of the ciliated corona)

In addition the following qualitative parameters of the culture should be checked:

- Presence of foam at the culture surface or sediment on the wall and bottom of the container.
- Presence of other micro-organisms, such as protozoa, fungi, bacterial flocks, etc. (Identification and frequency, note 0 when clean, + for medium contamination, ++ large contamination).

Production and use of resting eggs

Various methods of storing rotifers have been studied. Frozen rotifers are not usually adequate as feed because of leaching of nutrients. Amictic eggs of rotifers can be preserved by cryopreservation in liquid nitrogen after they have been impregnated with cryoprotective agents like dimethyl sulfoxide (DMSO).This method ensures full preservation of genetic traits of importance to aquaculture. Cryopreservation is not a suitable method for preservation of large numbers of rotifers for direct use as feed.

Artificially produced rotifer eggs have been tried as an alternative to daily production of rotifers. The production of these eggs can be manipulated by environmental factors, such as salinity, food quality and quantity, rotifer culture density, exchange of culture media and temperature and varies between *B. plicatilis*and *B. rotundiformis*. The cost of producing resting eggs is very high and therefore not yet been extensively adapted in hatcheries. Using preserved rotifers may eliminate the dependence on daily production of rotifers. Cheaper methods of resting egg production are another field which requires research attention in future.

For the mass rearing of rotifers as larval food the amictic way of reproduction is favoured. The resting eggs/ cystsare relatively large and are ideal for storage and transport and

can be used as inocula for mass cultures. Massproduction of rotifers for cyst production is performed in batch cultures in concrete tanks (Hagiwara *et al*., 1995; Dhert*et al*., 1995) or resting eggs are collected from sediments in earthen ponds. Resting egg production can be induced bylimiting the food supply or changing the temperature and/or salinity. Resting eggs will sink and need to be harvestedfrom the bottom. In case a lot of waste is trapped at the bottom it is advised to replace the water by brine so that restingeggs will float and can be collected from the water surface. Dry resting eggs can be stored for more than one year.When placed in seawater, rotifer cysts hatch in about 24 hours at 25°C under light conditions. Newly-hatched rotifersundergo asexual reproduction. The use of cysts is also highly recommended to prevent contamination. Cysts caneasily be treated before hatching in order to ensure that starter cultures are free from bacteria and ciliates. The resting eggscould be disinfected with heavy doses of antibiotics, so that the emerging rotifers are essentially bacteria free. Theresting eggs can also resist short exposure to disinfectants such as NaOCl or glutaraldehyde.

CONCLUSION

The significance of rotifer as alive feed for the larval rearing of various fish species with mariculture potential is widely understood. The basic criteria for selecting a live feed includes qualities such as nutritional value, size, ease of culture etc. Almost all these criteria suits well for rotifer to be qualified as a live feed. A new super small species of rotifer ,*Colurellaadriatica*is also now available for use in marine fish hatcheries (Madhu et al.2016) The size of this rotifer is much smaller than *B. rotundiformis*. Hence the mass culture of this species would be highly beneficial for the hatchery seed production of fishes with very small larval mouth size, such as groupers, damsel fishes, breams etc which are presently being fed on copepods during their early larval stages.

Suggestedreading

Madhu et al (2016). Isolation, Identification and culture of the marine rotifer, Colurellaadriatica Ehrenberg,1831(Family: Lepadellidae) from Andaman & Nicobar Islands: A promising live feed for larval rearing of high value shell fishes and fin fishes.JMBAI. 58 (1), pp 5-12

FAO (1996). FAO Fisheries Technical Paper no.361. Manual on the production and use of live food for aquaculture.

Artemia – the global live feed for aquaculture

Anikuttan.K.K, G.Tamilmani, M.Sakthivel, P.Rameshkumar, M.Sankar, R.Jayakumar, A.K.Abdul Nazar, Tinto Thomas, G.H.Rao, N.Krishnaveni, Munirasu, Suresh and Ganesan.

Introduction

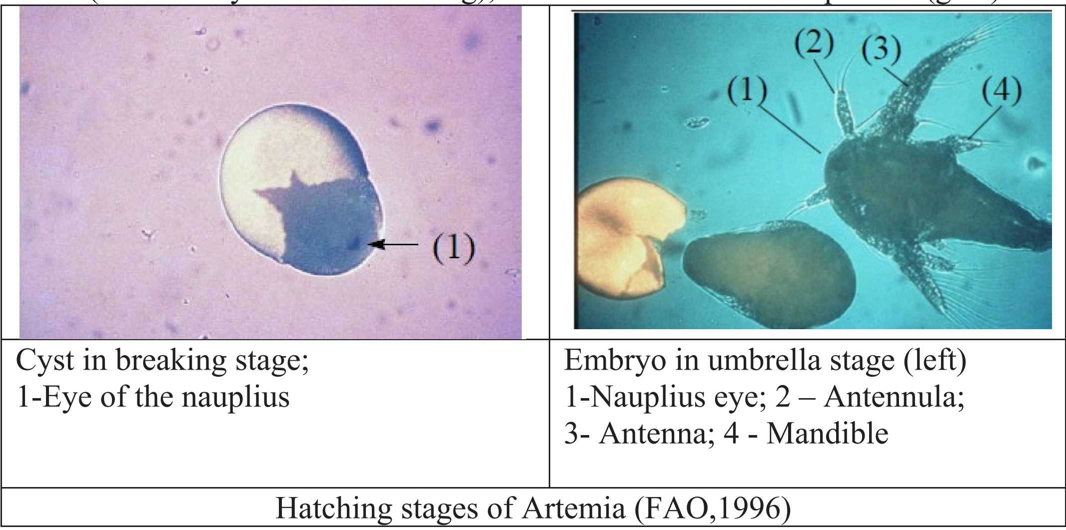
The most popular and globally accepted live feed and life line of aquaculture, *Artemia* is an inevitable component in the hatchery production of most of the species with mariculture potential. The advanced larval stages of these fishes/shell fishes are fed on artemia nauplii. *Artemia salina ,* popularly known as brineshrimp are found in salt pans, lakes or evaporation ponds at salinities from100 ppt onwards. Different geographical strains are available. Over 300 natural biotopes, spread over the 5 continents have been identified. It is characterizedby an elongated body with 2 stalked complex eyes in the head region,11 pairs of thoracic appendages and an abdomen that ends in a furca covered with spines. The unique property of Ar*temia* to form dormant embryos, called 'cysts', is the reason for making it the popular live feed. The cysts are available year round in large quantities along the shorelines of hypersaline lakes, coastal lagoons and solar salt works scattered over the five continents. After harvesting and processing, cysts are made available in cans as storable 'on demand' live feed. Upon some 24-h incubation in seawater, these cysts release free-swimming nauplii that can directly be fed as a nutritious live feed to the larvae of a variety of marine as well as freshwater organisms, which makesthem the most convenient, least labour-intensive live food available for aquaculture. Further, the adults of artemia can be frozen and stored which also can be fed to still bigger juveniles of fishes. So the naupliia s well as adults of artemia can be used to feed the larvae/juveniles of many fishes.

Average proximate composition of *Artemia* (Leger *et.al;* 1987)		
Components	**% composition**	
	Nauplii	**Adults**
Protein	52.2 ± 8.8	56.4±5.6
Lipid	18.9±4.5	11.8±5.0
Carbohydrate	14.8±4.8	12.1±4.4
Ash	9.7±4.6	17.4±6.3

Biology

In the natural environment, *Artemia* produces cysts during certain season of the year, that float at the water surface and that are thrown ashore by wind and waves. These cysts are metabolically inactive and do not further develop as long as they are kept dry. Upon immersion in seawater, the biconcave-shaped cysts hydrate, become spherical, and within the shell the embryo resumes its interrupted metabolism. After about 20 hrs the outer membrane of the cyst bursts and the embryo appears, surrounded by the hatching membrane The development of the nauplius is completed and within a short period of time the hatching membrane is ruptured and the free-swimming nauplius is born. The first larval stage (instar I; 400 to 500 µm in length) has a brownish-orange colour, a red nauplius eye in the head region and three pairs of appendages: *i.e.* the first antennae (sensorial

function), the second antennae (locomotory + filter-feeding function) and the mandibles (food uptake function). The larva grows and differentiates through about 15 molts. From the 10th instar stage on, important morphological as well as functional changes are taking place: *i.e*. the antennae have lost their locomotory function and undergo sexual differentiation. In males they develop into hooked graspers, while the female antennae degenerate into sensorial appendages The thoracopods are now differentiated into three functional parts namely the telopodites and endopodites (locomotory and filter-feeding), and the membranous exopodites (gills).



Cyst in breaking stage; 1-Eye of the nauplius	Embryo in umbrella stage (left) 1-Nauplius eye; 2 – Antennula; 3- Antenna; 4 - Mandible

Hatching stages of Artemia (FAO,1996)

Hatching of cysts:

Artemla nauplli is a widely accepted live feed for the advanced stages of fish and crustacean larvae and it can be made available by hatching of their cysts. *Artemia* cysts are commercially available in dried form and it can be stored in anaerobic condition for more than a year. When the cysts are transferred to filtered seawater under illumination and vigorous aeration, the biconcave cysts become spherical, after about 24 hours the cyst shell bursts and within a short period of time the free swimming nauplius will be developed.

When incubated in seawater the biconcave cyst swells up and becomes spherical within 1 to 2 hrs. After 12 to 20 hrs of hydration, the cyst shell (including the outer cuticular membrane) bursts, which is called the breaking stage and the embryo surrounded by the hatching membrane becomes visible. The embryo then leaves the shell completely and hangs underneath the empty shell (the hatching membrane may still be attached to the shell). Shortly thereafter the hatching membrane breaks open and the free-swimming larva (head first) is born. Dry cysts are very hygroscopic and take up water at a fast rate (*i.e*. within the first hours the volume of the hydrated embryo increases to a maximum of 40% water content; However, the active metabolism starts from a 60% water content onwards, provided environmental conditions are favourable.

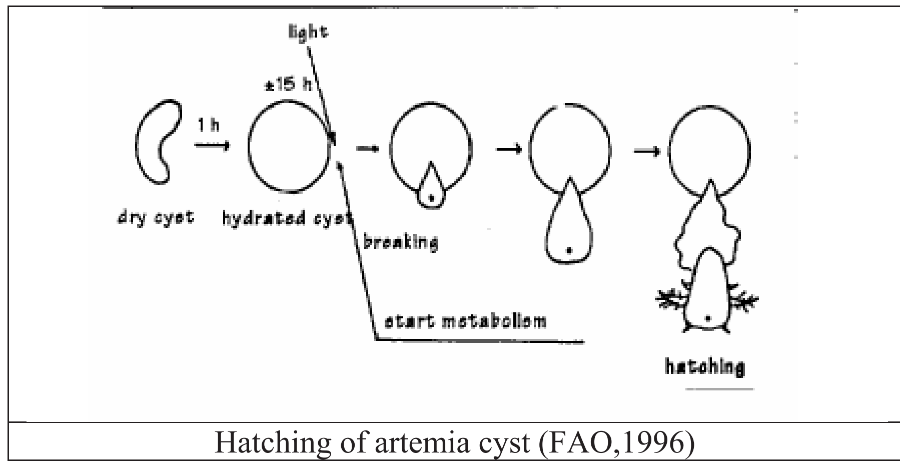
When hatching large quantities of cysts, an impressive bacterial load rapidly develops. Reducing bacterial development during hatching will improve the hygienic status of nauplii and may result in better hatching yields. It can be achieved through simple disinfection of the cysts using liquid bleach solution or through decapsulation.

Disinfection of *Artemia* cysts with liquid bleach

- Prepare 200 ppm hypochlorite solution
- Soak cysts for 30 min. at a density of ± 50 g cysts.l^{-1};
- Wash cysts thoroughly with tapwater on a 125 µm screen;
- Cysts are ready for hatching incubation.

***Artemia* hatching**

- Use a transparent or translucent cylindroconical tank
- supply air through open aeration line down to the tip of the conical part of the tank; oxygen level should be maintained above 2 g.l^{-1}, apply strong aeration
- use filtered natural seawater
- maintain the optimum hatching
- hydrate cysts prior to hatching incubation in tap water for 1-2 hours
- incubate cysts at density of 2 g.l^{-1}; for smaller volumes (<20l) a maximal cyst density of 5 g.l^{-1} can be applied. · Incubate for fixed time period (*e.g.* 20 hr).
- Remove the aeration prior to harvesting
- Wait to separate between nauplii and unhatched cysts. Nauplii will go down to bottom and unhatched cysts will float.
- concentrate nauplii using a light source
- Sieve nauplii, rinse well with tap water.



Hatching of artemia cyst (FAO,1996)

A simple hatching arrangement for artemia cyst

Decapsulation of the cysts:

A complete separation of *Artemia* nauplli from their empty cyst shells is a difficult task and always it may not be successful also. The presence of empty shells is harmful for the larvae too. This can be overcome by a method called Decapsulation of the cyst. The hard shell or chorion of cysts will be removed without affecting the viability of the embryos by short exposure of the hydrated cysts to a hypochlorite solution. This process is called cyst decapsulation. When the cysts are exposed to hypochlorite solution, the hard shell of the cyst dissolve, and a gradual colour change from dark brown to grey and then to orange can be observed. Decapsulated cysts offer a number of advantages compared to the non-decapsulated ones which are listed below:

- Cyst shells are not introduced into the culture tanks. When hatching normal cysts, the complete separation of *Artemia* nauplii from their shells is not always possible.
- Unhatched cysts and empty shells can cause deleterious effects in the larval tanks when they are ingested by the predator: they can not be digested and may obstruct the gut.
- Nauplii that are hatched out of decapsulated cysts have a higher energy content and individual weight (30-55 % depending on strain) than regular instar I nauplii, because they do not spend energy necessary to break out of the shell.
- In some caseswhere cysts have a relatively low energy content, the hatchability might be improved by decapsulation, because of the lower energy requirement to break out of a decapsulated cyst
- Decapsulation results in a disinfection of the cyst material
- Decapsulated cysts can be used as a direct energy-rich food source for fish and shrimp
- For decapsulated cysts, illumination requirements for hatching would be lower

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FARMING OF COBIA AND SILVER POMPANO

Jayakumar, R., Abdul Nazar, A.K*., Tamilmani,G., Sakthivel, M., Rameshkumar, P., Anikuttan, K.K., Sankar,M., Hanumanta Rao, G and Krishnaveni, N.
Mandapam Regional Centre of CMFRI, Madapam Camp – 623520, Tamil Nadu, India
**Madras Research Centre, CMFRI, Chennai, Tamil Nadu, India*
jayakumar.cmfri@gmail.com

INTRODUCTION

Mariculture can be defined as the controlled cultivation and harvest of aquatic organisms, including finfish, shellfish, and aquatic plants. Mariculture operations are conducted at both land and water facilities. Land-based mariculture systems include ponds, tanks, raceways, and water flow-through and recirculating systems. Water-based mariculture systems include net pens, cages, ocean ranching, longline culture, and bottom culture. Mariculture can provide a number of socio-economic benefits, including food provision, improved nutrition and health, generation of income and employment, diversification of primary products, and increased trade earnings through the export of high-value products. Aquaculture can also provide environmental benefits by supporting stocking and release of hatchery-reared organisms, countering nutrient and organic enrichment in eutrophic waters from the culture of some mollusk and seaweed species, and because aquaculture operations relies on good water quality, the prevention and control of aquatic pollution.

Cage farming

Commercially important marine fishes can be cultured in any of the four culture systems like ponds, raceways, recirculation systems or cages. In the simplest term, a cage is an enclosure in the water body whereby the juveniles of aquatic animals are kept, fed and grown to a marketable size. Cage culture uses existing water resources (ponds, rivers, estuaries, open ocean, etc.) but confines the fish inside some type of mesh enclosure. The mesh retains the fish, making it easier to feed, observe and harvest them. The mesh also allows the water to pass freely between the fish and surrounding water resource, thus maintaining good water quality by removing wastes. In recent years, cage culture has emerged as the most viable method of sea farming.

Cage culture probably originated with fishermen who used cages to accumulate fish for market. Over time, they learned to feed the fish in these cages to increase their size and improve their overall health. The first cages used for just holding fish were probably developed in Southeast Asia at the end of the 18th century. These cages were constructed of wood or bamboo and the confined fish were fed trash fish and food scraps. Modern cage culture in the U.S. began in the 1950s with the advent of synthetic materials suitable for cage construction. There has been little research on marine cage systems because of regulatory issues, a limited number of good quality sites and high cost of research. In freshwater sector, cage culture allows farmers to use existing water resources that may or may not be used for other purposes. The fish produced are usually sold to local niche markets. As wild-capture fisheries have declined and aquaculture has expanded, these niche markets have also grown. As a result to cater the demand more entrepreneurial opportunities have grown for cage farming. The cage culture was initiated in Norway during 70s and developed into an organised industry, particularly for salmon farming. Similarly the cage culture has spread in South East Asian countries for culture of a variety of

fishes. The major advantage in these countries is that they have large, calm and protected bays to accommodate the cages safely against natural bad weather conditions.

Advantages of Cage Culture

1. **Effective use of Resources**
   Cage culture can be established in any suitable body of water, including open seas, backwaters, lagoons or river mouths with proper water quality, seed, feeding strategies, access and permission from local authorities. This flexibility makes it possible to exploit underused water resources to produce fish.
2. **Low investment**
   The investment for pond construction and its associated infrastructure (electricity, roads, water wells, etc.) are much higher than the cage farming, which is practiced in an existing water body and can be less expensive. At low densities (when compared to pond water spread area) cages placed in open seas, backwater and lagoons do not require aeration. Cage materials are not much expensive and can be mended with little experience.
3. **Simple farming operations**
   In cage farming, observation of the growth and health status of the fish is easy and simple. The observation of fish behaviour, especially feeding behaviour, is critical in avoiding problems related to stress and disease outbreak.
4. **Easy harvesting methods**
   Cages are usually harvested by moving them into shallow water, crowding the fish into a corner of the net. Otherwise, the cage net can be lifted partially out of the water so that the fish are crowded into a smaller volume, and then it can be harvested. This makes it possible to partially harvest fish from cages as and when needed for local markets.
5. **Multi-use of water resources**
   The confinement of fish in cages will not affect other uses of the water resource, such as fishing, boating, swimming, irrigation or livestock watering.
   Cage farming requires low capital investment and the farmer can expand production with additional cages or intensify production by increasing the stocking density at an optimal level.

Species selection

Cage culture in open seas requires a fish variety with the basic characters like, suitability for marketing, commercial importance, consumer accepted fish, easy to culture, adaptability to the cage environment, acceptance to artificial diets, faster growth rate and resistant to common diseases.

A variety of commercially important marine fishes including, Cobia *Rachycentron canadum*), Seabass (*Lates calcarifer*), Snappers (*Lutjanus* sp.), Carangids (*Trachinotus* sp.) and Groupers (*Epinephelus* sp.) and lobsters are highly suitable for cage farming. Commercial level seed production technology for majority of these fishes has been developed in many of the South East Asian countries.

Cobia (*Rachycentron canadum*)

Cobia has gained popularity as a good candidate for mariculture due to its rapid growth and white meat of versatile use. It is considered as one of the most promising candidates for warm-water marine fish aquaculture in the world. Being the only member of the family Rachycentridae, it is found in the warm, temperate to tropical waters of the West and East Atlantic, throughout the Caribbean and in the Indo-Pacific off India, Australia and Japan. To date, research and development of cobia aquaculture has been initiated in over 23 countries and territories, half of them in the Asian-Pacific region. Statistics of FAO (2009) show that the

global aquaculture production of cobia has been increasing rapidly from only 9 tonnes in 1997 to nearly 30,000 tonnes in 2007. Since late 1990, cobia aquaculture production has been steadily expanding in Asia, primarily in Taiwan, Vietnam and China, but also in other Southeast and Indo-Pacific Asian countries including the Philippines, Indonesia, Iran and Reunion Island. Although cobia production is expanding rapidly, combined production of Asian countries is still rather lower.

Cobia farming techniques developed by CMFRI

India is late starter in cobia research and the seed production of cobia was achieved for first time in India by the Mandapam Regional Centre of Central Marine Fisheries Research Institute (CMFRI). Later the farming protocols in the High Density Polyethylene (HDPE) cages and Galvanized Iron (GI) cages with different feeding strategies were developed, tested and validated. Out of this farming trials an economically viable farming methods has been evolved. These farming methods have been executed in a participatory farming demonstration with M/s. Vitality Aquaculture Pvt. Ltd., Tuticorin and successful harvest of cobia was made during May 2013 in the presence of the Director General, ICAR, New Delhi.

The basic protocols followed for cage culture of cobia in different phases are narrated as below:-

Nursery Phase 1

The 4 weeks old fingerlings were reared for 6 weeks indoor (Nursery Phase 1) followed by 8 weeks outdoor (Nursery Phase 2) before stocking in grow-out cages. The nursery phase 1 can be carried out in FRP tanks of 7 ton capacity with 5 ton filtered sea water. The stocking density has to be kept as 8 nos. per litre. The fingerlings have to be fed with INVE (Thailand) formulated diet (assorted size from 400 µ to 1200 µ) thrice daily. The weaning to chopped low-value fishes can be practised during the last week of this phase. The water exchange has to be done 100% daily.

Nursery Phase 2

The nursery phase 2 has to be carried out in specially designed sea cages. These nursery cages should be made of HDPE pipes or GI Pipe (C - Class type) material. The dimension of the square sea cage has to be kept as 4x4 meter with the handrail fixed at one meter height from the base otherwise a circular cage of 6 meter dia can be used. The net cages fabricated with HDPE ropes of 2.5 mm thickness and the mesh size has to be used are 20 mm for inner net cage and 40 mm for outer net cage. The depth of the net cage shall be kept 3 meters from the base. The shape of the net cages has to be maintained with ballast. The buoyancy of the cages can be enabled by tying HDPE drums with the cage frame and has to be moored with two numbers of Galvanized Iron (GI) anchors of 70/100 kg each in opposite directions.

The fingerlings from nursery phase 1 have to be transferred to these floating nursery sea cages. The stocking density biomass at this phase can be maintained at 1.8-3.0 kg/m^3. The fingerlings have to be fed @ 5% total biomass of fish with chopped low-value fishes (Sardine, lesser sardine, rainbow sardine, etc.) twice daily. Net cages have to be changed based on the subjective assessment of clogging of the net in order to have sufficient water exchange. Random sampling has to be carried out weekly with the sample size of 30 nos. per cage. This phase can be continued for about 4 weeks.

Grow-out Phase

The grow-out culture has to be carried out in circular floating sea cages of 6 meter diameter. The cage frames should be made up of HDPE pipes or GI pipes. The handrail has to be fixed at half meter height form the base. The space between inner and outer rings of the cage has to be

kept as one meter. The net cages fabricated with HDPE ropes of 2.5 mm thickness and the mesh size of 40 mm for inner net cage and 60 mm for outer net cage has to be used. The depth of the net cages should be maintained at 4.0 meters from the base. The shape of the net cages can be maintained with circular ballast. The cages were floated and moored as mentioned in Nursery Phase 2.

The juveniles from nursery phase 2 have to be transferred to these grow-out sea cages. The stocking density at this phase has to be maintained at 3.0-5.0 kg/m^3 or 750 no.s of juvenile cobia per cage. The juveniles can be fed @ 5% total biomass of fish with chopped low-value fishes (sardine, lesser sardine, rainbow sardine, etc.) once daily. Net cages have to be changed based on the subjective assessment of fouling of the net in order to have sufficient water exchange. Random sampling has to be carried out at monthly intervals with the sample size of 30 nos. per cage. The entire grow-out culture can be carried out for a period of 6- 7 months.

Performance

The fingerlings stocked in indoor nursery at around 2 grams and will attain an average weight of 45 grams in 6 weeks, followed and about 70 grams in another 4 weeks of outdoor nursery rearing. The juveniles would reach an average weight of 1.0 kg in 4 months and 2.5 – 3.0 kg in 6- 7 months of grow-out culture in sea cages. The grow-out fishes would reach an average weight of 7.0 kg with a maximum weight of 8.0 kg within the culture period of one year which is almost 100 times the growth of the initial weight.

The unit cost estimate, performance of production and economics of operation gained through the farming trials and participatory demonstration were worked out and given below:-

ECONOMICS OF OPEN SEA CAGE FARMING IN A 6 METER DIA HDPE CAGE

Sl. No.	Head of expense	Cost in INR (in lakh)
Capital Expenditure		
	Cage and Net	
1	Cost of Cage (6 meter dia.) made of HDPE material	1.50
2	Cost of netting (4 m depth) for one outer net, two inner nets, one bird net cages and mooring materials ballast hose, anchor and anchor rope	1.00
	Sub Total	**2.50**
Operational Expenditure		
1	Cost of 900 Numbers of cobia seeds @ 8 fingerlings / m^3 @ INR 30/seed (Total volume of a cage: 113.04 m^3)	0.27
2	Transportation	0.10
3	Cost of 5.10 tonnes of Extruded pellet feed @ FCR 1:1.8 @ INR 0.75 lakh / tonne	3.83
4	Labour Charges @ INR 8000/ Person/month X 7 months	0.56
5	Boat Hire & Fuel Charges	0.50
6	Miscellaneous expenses	0.50
	Sub Total	**5.76**
	Grand Total	**8.26**

Sl. No	Production Estimate and Economics
1	Survival 90% = 810 fishes
2	Feed Conversion Ratio = 1 : 2
3	Average size of each fish at the time of harvest =3.5 kg
4	Total harvest = 2,835 kgs/cage (2.835 tonnes/cage)
5	Sale price of the produce @ INR 310/kg = INR 8.79 lakh
6	Gross Income from the harvest = INR 8.79 lakh
7	Gross income – Operational expenses = INR 3.03 lakh
8	Gross Profit = INR 3.03 lakh

Pond Farming of finfishes

Among the many high value marine tropical finfish that could be farmed in India, the silver pompano, *Trachinotus blochii* is one of the topmost, mainly due to its fast growth rate, good meat quality and high market demand. The silver pompano is caught only sporadically in the commercial fishery and hence its availability is rather scarce. It is a much sought after species and hence the demand can only be met through aquaculture. The aquaculture of pompano has been successfully established in many Asia-Pacific countries like Taiwan and Indonesia. The farming can be successfully carried out in ponds, tanks and floating sea cages. The species is pelagic, very active and is able to acclimatize and grow well even at a lower salinity of about 10 ppt and hence is suitable for farming in the vast low saline waters of our country besides its potential for sea cage farming. The shape, colouration and meat quality of this fish is comparable with silver pomfret. In the international market, the dockside price of Florida pompano averaged to $ 8 /kg and in India, the current price of silver pompano is about Rs.200/- per kg at the fish landing centres and around Rs. 250/- per kg in the retail markets.

The Central Marine Fisheries Research Institute has initiated aquaculture research on pompano from 2008 and the first successful broodstock development, induced breeding and larval production was achieved in 2011. Following the successful seed production of Silver Pompano, demonstration of farming in brackishwater ponds was initiated by the CMFRI to popularize among the farmers about its suitability for aquaculture. The first farming demonstration from the hatchery produced seed was carried out in a coastal aquaculture pond at Anthervedi Village, East Godavari District, Andhra Pradesh. It has been proven that Silver pompano can be cultured in the brackish water shrimp culture ponds as an alternative species with high survival rate, appreciable FCR and meat quality. These fishes have attained an average weight of 450 grams in 240 days (8 months).

Based on the experience gained on the brackishwater farming of silver pompano, the practices to be adopted for pompano farming are narrated as follows:-

Pond Preparation

The pond has to be dried properly until the cracks appear on the surface. The top layer of the soil containing waste accumulated through previous crop of fish or shrimp has to be removed. Ploughing has to be done to tilt the soil below 30 cm. Feeding areas, corners and side ditches in the pond has to be properly tiled and dried to avoid formation of black soil. The average water pH of 7.5-8.5 would be ideal for pompano farming. The level of lime application during pond

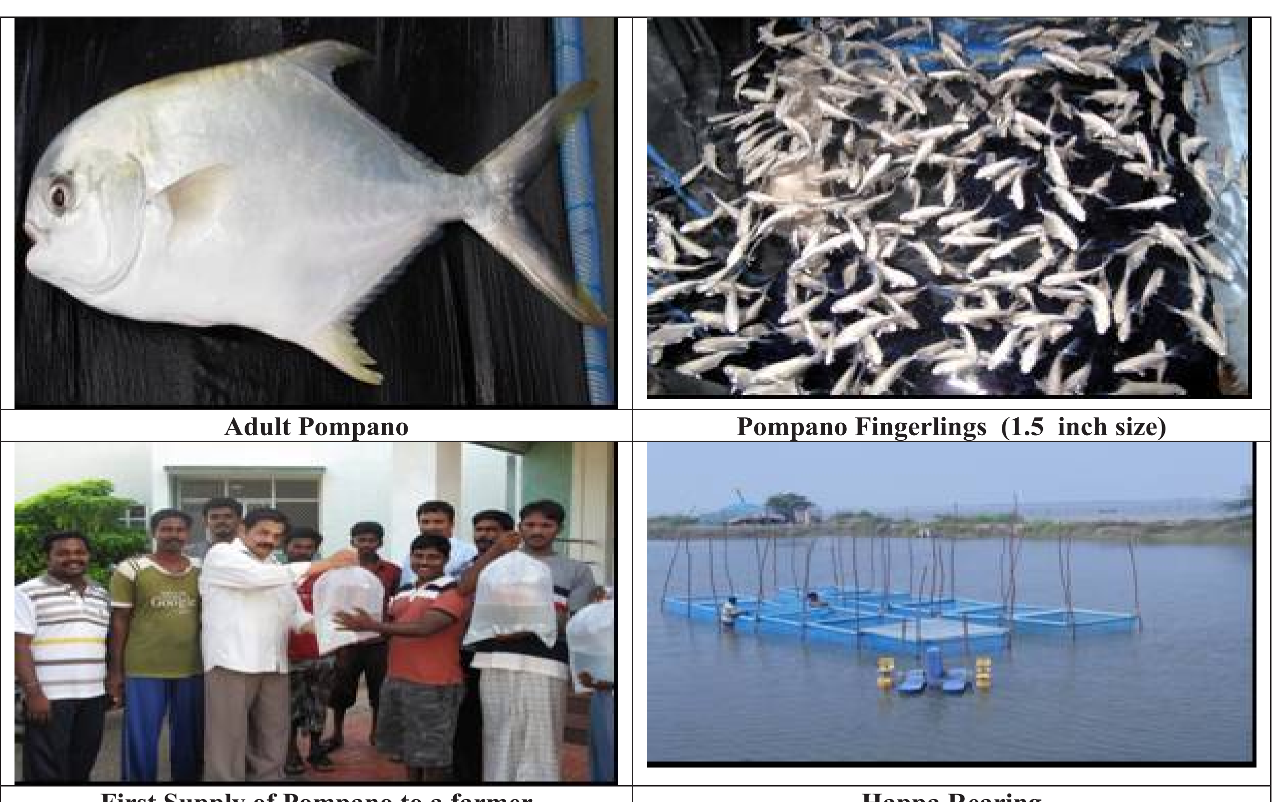
preparation depends on the pH of the soil. Hence, the dosage has to be calculated accordingly. Water filling has to be initiated by covering the inlet pipe by using 2 layers of fine nets (100 micron) to avoid introducing other fishes and predators. A week before stocking, the pond must be fertilized with either organic or inorganic fertilizers to stimulate the plankton bloom.

Salinity

Pompano can tolerate vide range of salinities from 5- 40 ppt. However, ideal salinity for farming would be between 15 – 25 ppt. Pond has to be filled with a minimum water level of 100 cm prior to stocking of fish seeds. During the entire culture period 1.5 meter water depth has to be maintained.

Nursery Rearing and Seed Stocking

Hatchery produced pompano fingerlings of 1 inch size can be stocked in happas/ pens of 2 meter length, 2.0 meter width and 1.5 meter depth. In each happa about 200 fingerlings can be stocked. While stocking care should be taken to avoid agitation of the pond bottom and too many persons getting into the pond may increase the suspended solid load in the water, which may cause gill chocking of the fish fingerlings leading to mortality. Initially the fishes have to be reared in happas for 60 days or until they attain 10 – 15 grams size and thereafter it can be released into the pond. The mesh size of the happa could be initially at 4 mm size and it can be changed with 8mm mesh size happas after 30 days. The stocking density in happa could be maintained as 200 nos/ happa. After attaining 30 grams size ideally 5,000 Nos. can be stocked in a one acre pond.



Adult Pompano | Pompano Fingerlings (1.5 inch size)

First Supply of Pompano to a farmer | Happa Rearing

Pond Culture of pompano

Feeding with Extruded Floating Pellet feed

Sampling of Pompano

Harvested Pompano

Nutritional Requirement & Feeding

Pompano is a fast moving marine fish and it requires highly nutritive feed to meet the energy requirements. During nursery rearing Pompano can be weaned to any type of feeds viz., extruded floating pellet, sinking pellet feed and chopped trash fishes. Ideally pompano can be weaned to extruded floating pellet feed to avoid feed wastage and spoilage of pond bottom. The CMFRI has conducted pompano farming demonstration by using the extruded floating pellet feed manufactured by M/s. Rudhra Techno Feeds, Bhimavaram, Andhra Pradesh. During the happa rearing phase, feeding has to be done 4 times a day and in pond culture phase it could be 3 times a day. The feed size should be lesser than the mouth size of the fish and hence, suitable sized feed has to be selected for feeding the fishes. The details of feed and feeding schedule of pompano are as follows:-

Weight of the fish	Feed Size	Crude Protein %	Crude Fat %	% to be fed as per the biomass	Feeding / day
> 1 Gram	800 - 1000 µ	50	12-15	30	4
1 – 10 gram	1.0 - 1.5mm	48	12	20	4
10 – 100 gram	1.8 mm	45	10	8	3
100 – 250 gram	3.5 mm	42	12	5	3
250 – 500 gram	4.5 mm	40	12	3	3

A mix of two sizes of feed pellet can be used if there is any size variation of the fishes found during the regular sampling. If sinking pellet feed is used, at least 4 – 8 feed trays (80 cm x 80 cm) per pond could be placed. Regular sampling of fishes once in 15 days has to be carried out to determine growth rate and to calculate the FCR. In the first farming demonstration, FCR was 1: 1.8 with the above formulations.

Water Quality Management

Plankton bloom is essential for early stages of pompano (until 100 grams) culture. If the color of the pond water is clear a mixture of organic (10-30 kg./ha.) and inorganic fertilizers (1-3 kg./ha.) can be applied to obtain algal bloom. Sufficient water level must be maintained in the ponds to reduce risks of the growth of benthic algae. The water depth in the shallowest part of the pond should be at least 100 cm. Water quality can be maintained by exchanging 10% of the water once in a week; 20% per week after 3 months and 30% per week after 6 months. If water colour is too dark, the quantum of water exchange can be proportionately increased. To maintain water pH within an optimum range of 7.5 - 8.5, agri-lime has to be applied regularly. Dissolved oxygen (D.O) level should be maintained above 5 ppm at all times. Paddle wheel aerators can be placed in the pond to create minor water current and to maintain the DO level. Aeration is a must during late evening to early morning period when the fishes attains 200 grams size and above.

Growth Pattern

DOC	Growth (mm)	Weight (g)
1	30.59 ± 0.24	2.00 ± 0.04
30	73.42 ± 0.53	15.08 ± 0.16
60	102.88 ± 1.91	34.60 ± 0.41
90	158.39 ± 2.42	72.54 ± 1.95
120	182.30 ± 2.03	101.82 ± 3.11
150	203.71 ± 3.73	172.39 ± 4.55
180	226.51 ± 2.90	258.31 ± 5.76
210	273.07 ± 3.62	375.32 ± 8.07
240	296.88 ± 6.27	464.65 ± 10.25

During the entire culture period the growth pattern of pompano was monitored through regular sampling of fishes at fortnightly intervals. The length and weight measurements taken is presented as below:-

Health Management

Pompano is a much hardier species and does not get much disease problems. When it is reared in high salinities parasitic infection of copepods may occur. Periodical application of commercially available pond management chemicals like Iodine solution would help to keep the fishes healthier. Feed supplements like LIV- 52 syrup can be given by mixing with the feed to improve the immunity levels.

Harvesting

Harvesting of pompano could be carried out by using drag net as in the case of fresh water fishes. To maintain the freshness and quality of harvested fish, washing in clean water and chill killing can be done. Harvested fishes can be stocked in plastic crates by adding layers of ice in equal quantities at the bottom and top of the fish. It is suggested that harvesting of fish can be carried out during the off season period of April to June to get a better price.

It is well recognized that for sustainable production in aquaculture, diversification of species is a vital requirement and from the lessons learnt from the shrimp farming scenario in India, it is very much needed to diversify the marine and brackish water aquaculture with high value fin

fish species. Generally, high value marine fishes are in good demand in the Indian market and often there is a scarcity of the same. In the domestic market, silver pompano has demand starting from 250 grams size onwards. Hence, it is felt that pompano aquaculture can prove to be much lucrative and can emerge as a major aquaculture enterprise in the coming years.

HDPE Cage (6 meter Dia)	**GI Pipe Cage (6 meter Dia)**
Cobia fingerlings (50 days old)	**Cobia juveniles (While feeding)**
Cobia Juveniles (3 Kg size)	**Harvested Cobia**

MARINE ORNAMENTAL FISH SEED PRODUCTION

K.K.Anikuttan, R Jayakumar, A.K.Abdul Nazar, G.Tamilmani, M.Sakthivel, P.Rameshkumar, M.Sankar, G. Hanumanta Rao, Tinto Thomas and N.Krishnaveni

INTRODUCTION

The ornamental fish trade is a multi- billion dollar industry and it is estimated that globally around 1.5 -2 million people keep aquaria. Over 46,000,000 organisms representing 2500 species are traded annually with a value exceeding US$ 300,000,000. Philippines and Indonesia supply the majority of livestock, with most specimens being consumed by the USA, Europe, and Japan (Calado *et al.,* 2017). Among the most commonly traded families of fish Pomacentridae dominate accounting for 43% of all fish traded. They are followed by species belonging to Pomacanthidae (8%), Acanthuridae (8%), Labridae (6%) Gobiidae (5%), Chaetodontidae (4%), Callionymidae(3%), Microdesmidae (2%), Serranidae (2%) and Blennidae (2%). The most traded species are the blue green damselfish (*Chromis viridis*), the clown anemone fish (*Amphiprion ocellaris*), the whitetail Dascyllus (*Dascyllus aruanus*), the sapphire devil (*Chrysiptera cyanea*) the three spot damsel (*Dascyllus trimaculatus*), *Amphiprion percula, Paracanthus lepturus, Dascyllus albisella,, Zebrasoma flavescens,* the cleaner wrass *Labroides dimidiatus,* the powder blue surgeon *Acanthurus leucosternon,* the sea goldie *Pseudanthias squamipinnis,* the fire goby *Nemeteleotris magnuifica,* and the dragonet *Synchiropus splendidus*.

The marine ornamental fish sector all over the world, is dominated by fishes/shell fishes/invertebrates caught from the wild and the share of hatchery produced fishes still continues to be at a very minimal level. Although no marine species collected for the aquarium trade have been driven to global extinction, studies carried out in Sri Lanka, Kenya, the Philippines, Indonesia, Hawaii and Australia have reported localized depletion of a number of target aquarium species of fish like butterflyfish and angelfish due to heavy collection pressure. Even on a global basis, the commercial level hatchery production technologies have been evolved only for a limited number of species. It is well accepted as an environmentally sound way to increase the supply of marine ornamentals by reducing the pressure on wild population and producing juvenile and market sized fish of wide variety of fish year round. Moreover, hatchery produced fish are hardier and fair better in captivity and survive longer than wild caught ones. The number of captive-bred marine aquarium fish species, comes to over 250 species (Sweet, 2014). However, to date, successful commercial rearing has been scientifically reported for only a few species and around 5% of marine aquarium fish traded are commercially produced from hatchery. The main families bred for aquarium purposes are Pomacentridae, Pseudochromidae, Gobiidae, Apogonidae, Pomacanthidae and Syngnathidae (Dominguez and Botella, 2014; Calado *et. al.*2017).

The marine ornamental fishes that are most commonly bred in captivity include clown fishes and damsel fishes. The absence of sexual dimorphism, the complex patterns of sex change in certain groups and the problems of larval rearing can be considered as the major reasons for the slow progress in the culture of marine ornamental fishes.

CLOWN FISHES

Clown fishes are distributed throughout the Indo-West Pacific Region. Clown fishes continue to be the most demanded marine tropical fish and the technologies available at present on marine ornamental fish breeding are mainly centred around clown fishes. They are distinguished and taxonomically separated from damselfish by their dependence on anemones for protection. They are further distinguished from damsels by their large capsule shaped eggs and large larvae at hatch.

Species of clownfish for which hatchery technologies are developed and standardized in India is given in Table.1. Recently seed production of designer clown fishes (such as Picasso, Platinum, snowflake etc) which are the most sought after variety among clown fishes, have also been achieved at Mandapam Regional Centre of ICAR CMFRI.

Table.1: Species of clownfish for which hatchery technologies have been developed

Sl.No.	Species name	Common name
1	*Amphiprion sebae*	Sebae clownfish
2	*A.clarkii*	Clark's anemonefish /yellowtail clownfish
3	*A.percula*	Orange clownfish
4	*A.ocellaris*	Ocellaris clownfish/false percula clownfish
5	*A.frenatus*	Tomato clownfish
6	*A.perideraion*	Pink skunk clownfish
7	*A.nigripes*	Maldive anemonefish /black finned anemonefish
8	*A.ephippeum*	Red saddle anemonefish/Fire clown
9	*A.akallopsisos*	Skunk clownfish
10	*Premnas biaculeatus*	Spine cheeked anemone fish/ maroon clownfish

SEED PRODUCTION OF CLOWN FISHES

The hatchery protocols for seed production of all clown fishes are more or less similar. The clown fishes are protandrous hermaphrodites. The major components in the seed production are Brood stock development, Pair formation, Egg laying, Larval rearing and Live feed culture.

The broodstock development and pair formation is a major component for successful hatchery operation. The brood stock of the fishes has to be maintained at optimum water quality conditions (Table 2) free from pathogenic organisms in order to condition them for breeding. This is possible by providing a Recirculatory Aquaculture System (RAS) in the hatchery. The basic components include a common sump, biofilter, UV filter, Chiller, Protein skimmer, Blower, water inlet and outlet assembly for the fish tanks and lighting arrangement for the fish tanks.

The clown fishes are monogamous in nature and pair formation is a tricky part of the captive breeding technique. The clown fishes are born as males and according to social conditions prevailing in the clownfish colony they reverse the sex. There will be only one functional pair (male and female) in a colony. All the other members of the colony remain as sub-adults. In a colony, one pair of fish will grow ahead of other fish – the larger of the two will become the female and the other one will be the male. Hence in a clownfish colony, the larger fish will be the female and the next large fish will be the male. Compatible pairs have to be identified by trial and error methods and once the pair formation is complete, they can be introduced into the brood stock holding tank, such that, only one pair is kept in a tank with a host anemone for better results. Age of the fish is the most important factor determining sexual maturity. Sexually matured adult clownfish are usually 9-18 months old. Conditioning the fish is a prerequisite for spawning any fish. Conditioning is a term used to describe the utilization and manipulation of a combination of environmental factors to induce gonadal maturation and spawning. The factors may include light intensity, light duration and possibly wave length, temperature, water current, water quality, nitrogen, phosphate, ammonia, p^H, type of food, tank size and shape, aeration and habitat. All fish do not respond to the same environmental cues which trigger spawning. Under natural conditions, wild clownfish spawn most of the year, but usually not more than one spawn per month. Under optimum conditions and proper feeding, they can be induced to spawn atleast twice a month.

The quality of broodstock diets greatly influence a successful spawning. Hence suitable diets at satiation levels must be fed to the broodstock fish. Boiled and chopped mussel/clam meat and fish roe can be fed *ad libitum* twice a day. Live feeds like *Artemia* nauplii, adult *Artemia* and *Moina micrura* can also be supplemented. If brood stock fish are not properly fed, the results are directly reflected in the number of eggs laid, pigmentation of the eggs, fertilization rate, hatch rate and the quality of hatched larvae. Poor quality eggs develop slowly, hatch late and often result in significant early larval mortalities.

Table.2:Optimum water quality parameters for marine ornamental fish brood stock development

Sl.No	Parameter	Values
1	Temperature	27-28 ºC
2	Salinity	30 – 35 ppt
3	pH	7.5 - 8.5
4	Dissolved Oxygen	> 5ppm
5	Ammonia	0 ppm
6	Nitrite	0 ppm
7	Nitrate	< 25 ppm

The clown fish spawns by attaching the eggs to any substratum available in the tank. Hence, suitable substratum such as tiles or earthen pots can be placed inside the brood stock tanks. The clownfish normally spawn during forenoon. Once spawning commences, females press their body towards the substrate and slowly move in a rowing fashion using their pectoral fins. She moves in a circular path depositing a continuous spiral of eggs from the

central outward. The male swims behind the female, releasing sperm over the newly deposited eggs. Spawning occurs during day time and it lasts for about one to one and half hours. Each female lays 300 to 1000 capsule shaped eggs. Generally the egg size ranges between 1.5 to 3mm in length and 0.8 to 1.8mm in width. Each egg is attached to the substratum by a stalk. During the incubation period both the parents carefully look after the eggs by fanning the eggs by their fins and removing the dead and infected eggs by mouth. After spawning the males assume a more dominant role. He intermittently fans the nest with his caudal or pectoral fins. He also cleans the eggs by gently mouthing them without removing them. Dead and fungal infected eggs are routinely removed and eaten. Substrate around the nest is also often cleaned. The male spends an average of 30-60% of its time during the day for tending the nest. Fanning the eggs is frequent on the day after spawning and diminishes considerably about mid way in the incubation period. On the day of hatch, fanning increases again. In captivity most pairs spawn a minimum of 11 months a year, regardless of the species.

The colour of the eggs will be bright orange initially which will change to black and finally to silvery colour with prominent eyes of the embryo on the 7th day. The eggs hatch on the seventh day shortly after sunset at a water temperature range of 27 – 29 ^{0}C . On the expected day of hatching, 2 hours before sunset the eggs along with the substratum are transferred to hatching tanks. The larvae break their capsules and hatchlings emerge soon after sunset and peak hatching takes place between 1900 – 2000 hrs in darkness.

Larval rearing

The newly hatched larvae measures 3-4 mm in length and each has transparent body, large eyes, visible mouth and a small yolk sac. Soon after hatching the larvae are free swimming. The initial nourishment to the developing fish larvae is obtained from the egg yolk. When the yolk reserves have been completely utilized, the larval feeding capabilities are developed and hence at this stage the larval survival is entirely dependent on the availability and quality of food in sufficient quantities. The phase when yolk has just been depleted and the larvae turn to exogenous feeding for further development is the most critical stage. At this stage, suitable live feed should be available in the larval rearing tanks. For this larval rearing has to be carried out in green water (using microalgae such as *Nannochlorposis oculata, N.salina etc*) and feeding with rotifers (*Broachionus plicatilis & B rotundiformis*) initially (upto 8th day after hatching) and then with *Artemia* nauplii from 9th day onwards. A minimum 8-10 nos of rotifers per ml is required during rotifer feeding period and 2-3 nos nauplii per ml during *Artemia* feeding stage. The larvae metamorphose between 15-20 days. After metamorphosis the larvae can be transferred to grow out tanks with sea anemone. Mild aeration can be provided during larval rearing. The larviculture period from 3-8 dph is critical due to the change in feeding from endogenous to exogenous. After 8 dph there will not be any further mortality if proper feeding and water quality parameters are maintained. The tank bottom should be cleaned daily with atleast 25% water exchange. Sufficient green water should be added daily. Weaning with pellet feed can be started after metamorphosis. Start with suitable sized pellets along with the live feeds and slowly wean them to the pellet feed. The ICAR CMFRI has developed a marine ornamental fish feed " **Cadalmin** ™

Varna" which gives very good colour enhancement for the fishes. Boiled and chopped mussel meat can also be used as feed. Two times feeding at satiation level is sufficient to rear them to marketable size.

DAMSEL FISHES

The damsel fishes which also belong to the Family Pomacentridae are very popular among aquarists due to their small size, bright colours, quick acclimation to captivity and interesting behaviour. The majority of species inhabit the Indo-Pacific region and about 100 species and 18 genera have been recorded from the Indian Ocean. More than 30 species belonging to the genera *Pomacentrus, Neopomacentrus, Chromis, Abudefduf* and *Chrysiptera* are commonly available from Indian coral seas. Broodstock development and larval rearing were achieved in India eight species of damselfishes (Table 3)

Table.3. Damselfishes for which hatchery production have been achieved.

Sl.no	Common name	Scientific name
1	Three spot damsel	*Dascyllus trimaculatus*
2	Striped damsel	*Dascyllus aruanus*
3	Blue damsel	*Pomacentrus caeruleus*
4	Peacock damsel	*P. pavo*
5	Bluegreen damsel	*Chromis viridis*
6	Filamentous tail damsel	*Neopomacentrus cyanomos*
7	Yellowtail damsel	*Neopomacentrus nemurus*
8	Sapphire devil damsel	*Chrysiptera cyanea*

(Gopakumar and Santhosi, 2009; Gopakumar *et al.,* 2009; Gopakumar *et al.,* 2002; Pananghat Vijayagopal *et al*., 2008)

SEED PRODUCTION OF DAMSEL FISHES

The damsel fishes exhibits protogynous hermaphroditism and they are group/harem spawners. One male can fertilise the eggs deposited by more than one female. The eggs are deposited on any hard substratum as in the case of clown fishes. Earthen pots or PVC pipes can be used as substratum for egg deposition in case of damsel fishes. The egg hatches out on 4th day. The egg size and larval size is comparatively smaller than clown fishes due to which the live feed also have to be smaller to suit the mouth size of the larvae. Hence copepod nauplii/copepodite of suitable size range and nutritional value has to be provided for first feeding of the damsel fish larvae. This is the major difference in seed production between clown fishes and damsel fishes. The mass production of suitable copepod species was a major bottleneck in the seed production of damsel fishes till very recently. However, techniques for culture of copepod species with suitable size and nutritional value have been developed by Vizhinjam Research Centre of ICAR CMFRI, which is being adopted for the seed production of damsel fishes. After the initial feeding stage with copepod nauplii or copepodites, rotifers can also be used as live feed along with green water technique. The rest of the larval rearing techniques are similar to that of clown fishes.

LIVE FEED CULTURE

Live feed culture is an integral part of marine ornamental fish seed production technique because the initial feeding of the larvae is purely on live feeds such as rotifers,copepods and artemia nauplii. To culture these zooplankton like rotifer and copepods, micro algae (phytoplankton) is necessary. Hence live feed comprises of micro algae and zooplanktons (such as rotifers, copepods and Artemia nauplii). Micro alage such as *Nannochlorposis occulata, N salina, Isochrysis galbana* etc are commonly cultured for rearing the rotifers or copepods. Rotifer species commonly used in hatcheries include *Brachionus rotundiformis* and *B plicatilis*. Copepods species widely used include *Parvocalanus, Pseudodiaptomus, Oithona, Temora turbinata* etc. Artemia cysts are readily available in the market, which can be hatched in the hatchery as per the requirement and given to the larvae when their mouth size is big enough to capture artemia nauplii.

Detailed description on all the live feed culture techniques are given in separate chapters in this book. The readers are advised to refer these chapters for more information.

Summary

The marine ornamental fish sector all over the world is dominated by fishes/shell fishes/invertebrates caught from the wild and the share of hatchery produced fishes still continues to be at a very minimal level. The ever increasing demand for ornamental fish has necessitated the development of captive breeding techniques of these fishes. The ICAR CMFRI has developed the seed production technologies for around twenty varieties of marine ornamental fishes/shell fishes. Promoting hatchery production of marine ornamental fishes would be the best option to ensure sustainable development of the sector. Eventhough collection from the wild continues to be the major contributor in the trade, it is imperative to shift gradually from the wild collection to captive breeding and seed production of marine ornamental fishes in order to ensure a sustainable development of the sector.

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STOCK ENHANCEMENT OF SHRIMP RESOURCES THROUGH SEA RANCHING

***Sankar M, Sakthivel M, Jayakumar R, Rameshkumar P, Tamilmani G, Anikuttan K.K, Ramakrishnan N, Krishnaveni N** and **Nazar A.K.A**

Mandapam Regional Centre of ICAR-CMFRI, Mandapam Camp, Tamil Nadu, PIN 623520, India, Email: sankar.biotech41@gmail.com

1. Introduction

Sea ranching is referred as method of stock enhancement. It involves mass release of juveniles of the selected species into the marine environment where, they can feed on natural prey and grows. The sea ranched stocks become recaptured and add biomass to the commercial fishery. Sea ranching was carried out mainly for stock improvement or enhancing the production or conservation of natural resources. The sea ranching programme was originated in USA as early as 1870's and sea ranching of red and Pacific salmon was carried out since 1964. In Japan, Sea ranching was started during 1975, for Kuruma Shrimp, *Penaeus japanicus* and also for other 45 species to supplement the natural stock. In India, ICAR-CMFRI, Mandapam was carried out sea ranching of green tiger shrimp *Penaeus semisulcatus* seeds PL 15-20 size 7.0 lakhs numbers per annum in the Pillaimadam lagoons of Palk Bay during 1985-92. Sea ranching of Pearl oyster *Pinctada fucata* spats of 1.02 lakhs was carried by CMFRI, Tuticorin during 1985-90. Sea ranching of clams *Meretrix casta*, *Anadara granosa*, *Paphia malabarica* was carried out and released 72,500 numbers of seeds in the back waters of Asthamudi Lake, Munambam during 1993.

Penaeid shrimps contributes a total of 24345 tonnes from TamilNadu (2018, CMFRI) with the value of about Rs.600 crores. Green tiger shrimp (*Penaeus semisulcatus*) is one of the major fishery all along the coast of Gulf of Mannar and Palk Bay. It contributes about 69% of total shrimp landings in Ramanathapuram district. *P. semisulcatus* is exploited mainly by the trawl net operated in mechanized boats. This species is globally distributed in the Indo west Pacific region, Red sea, East and South east Africa to Japan, Korea, the Malay Archipelago and Northern Australia. It is a bottom feeder inhabiting the intertidal sea grass beds as well as sub tidal algal beds at a depth of 7-13 m. The size at maturity is 23 mm carapace length and majority attains maturity at 31-32 mm carapace length. Mature female available throughout the year with maximum availability from June to October and January to March. Fecundity ranges from 2 lakhs to 6 lakhs for the size range of 40 to 65g respectively. Male shows a growth rate of 2 mm carapace length per month and female shows 3.5 mm carapace length per month.

The sea ranching involves different steps such as, Breeding, larval rearing, Nursery rearing, releasing of seeds in the suitable sites and monitoring the released stock for impact assessment on fishery.

2. Breeding and seed production

2.1. Brood stock collection and spawning

The healthy matured brooders for the breeding can be collected from shrimp trawls or Gill nets with full appendages and the brooder size varies from 165 to 200 mm in length and 37.0 to 64.5 g in weight. The brooders were given immersion bath in of 100 ppm potassium permanganate ($KMnO_4$) or liquid povidone (PVP) iodine for 30-60 seconds. The male to female ratio of 1:2 is optimum with feeding of protein rich diets, Polycheate worms and squid meat would be ideal for captive maturation and spawning. Gravid female can be identified by observing the gonadal development from the dorsal exoskeleton of the shrimp. Gravid female

is released into 150-500 litres circular FRP tank with 1 ppm EDTA and 0.1 ppm treflan as chelating agents for spawning. Spawning will takes place after 12-14 hours with a temperature range of 28-32ºC. After Spawning the incubation period would be 12-14 hours for hatching. The larval stages consist of six Nauplii (N-I to N-VI), three Protozoea (PZ-I to PZ-III) and three Mysis stages (M-I to M-III). The duration of nauplius to post larval production is about 12-14 days based on the water temperature.

2.2. Larval rearing

Nauplii can be stocked in larval rearing FRP tanks of 2-5 ton capacity @ 100 numbers per litre and feeding starts with protozoeal stage. Larvae from protozoea I to Mysis III are to be fed with *Chaetoceros* spp at the concentration of 50000 Nos. per ml and also supplemented with the micro encapsulated larval feeds @ 4 times per day. Post larvae 1 to post larvae 20 are to be fed with combination of rotifer, *Artemia* naupli and encapsulated larval feed. Larval survival of 32 to 52.5 per cent can be obtained during hatchery rearing. The water quality parameters such as salinity, temperature and pH were maintained at 34-36 ppt, 27-29ºC and 8.2-8.4, respectively. The growth and development were monitored by sampling of larvae and observed under microscope.

2.3. Health management during larval rearing

2.3.1. Swimming activity and Phototaxis

The healthy larve in zoeal stages swim rapidly and consistently usually in circles, Mysis swim backwards with intermittent flicks of their tails and post larvae swim rapidly and consistently. Zoea larvae swim strongly towards the light, Mysis and post larvae not showing attraction towards the light.

2.3.2. Condition of the hepatopancreas and gut contents

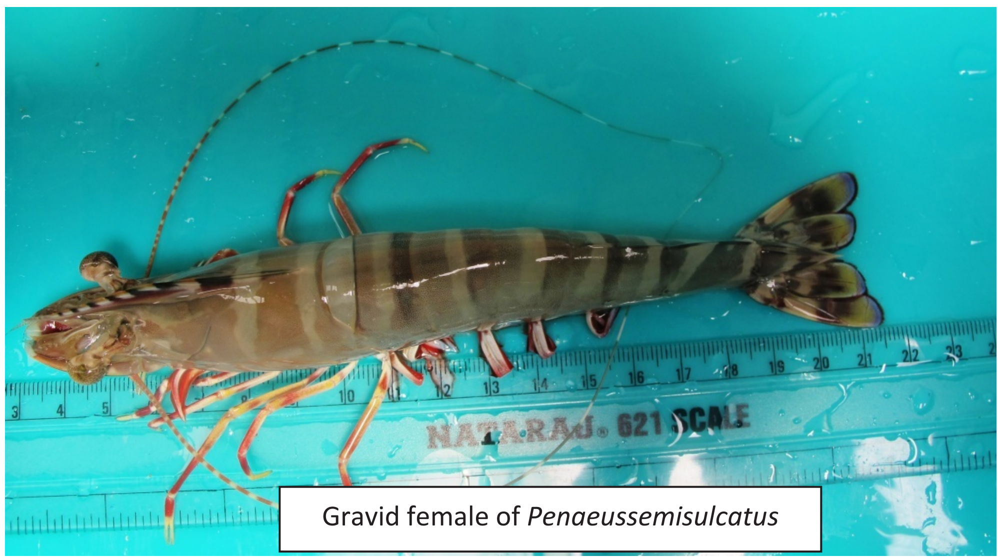
The larvae observed under microscope at a magnification of 100- 400X for the condition of hepatopancreas and presence of gut contents. The healthy larvae showing active feeding and digestion, mid gut with full of small digestive or lipid vacuoles and presence of strong peristalsis in the intestine. If the hepatopancreas appears empty or pale, without lipid vacuoles indicates that the larvae are either underfed or diseased. Proper diagnostic and treatment procedure can be adopted to treat the larval health problems.

2.3.3. Necrosis and Deformities

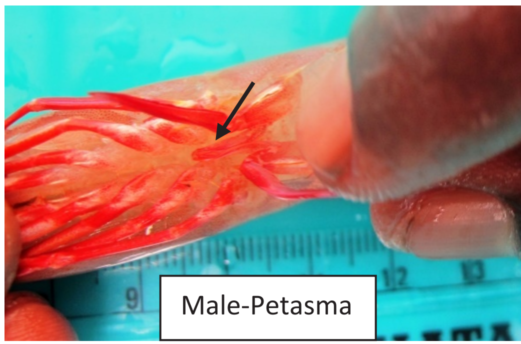
Necrosis of the larval body and appendages indicates that the larvae in severe cannibalism or bacterial infection. The poor nauplius and water quality results in occurrence of larval deformities such as, appendages, rostrums and antennal bending, broken or missing appendages, tail bending and termination of the gut before the anus.

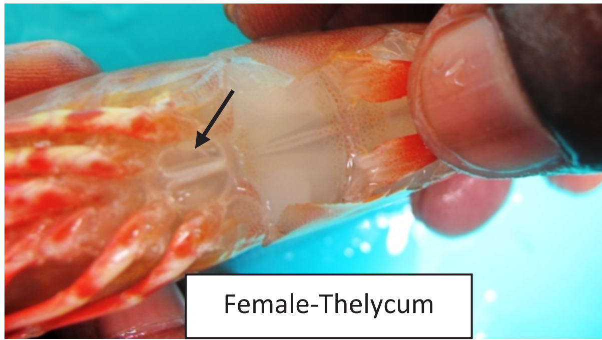
2.3.4. Epibiont fouling

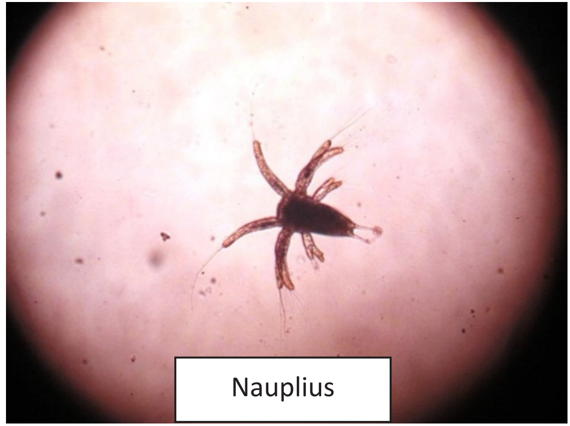
The larvae may become host for the range of fouling organisms such as, bacteria, fungi and protozoans. These organisms will attach to the appendages, exoskeleton and particularly around the gills. Where the infections are light, the next moult may remove the fouling without further problems. If severe fouling persists indicates the poor water quality and application of 20-30ppm of formalin with high aeration for one hour is recommended.

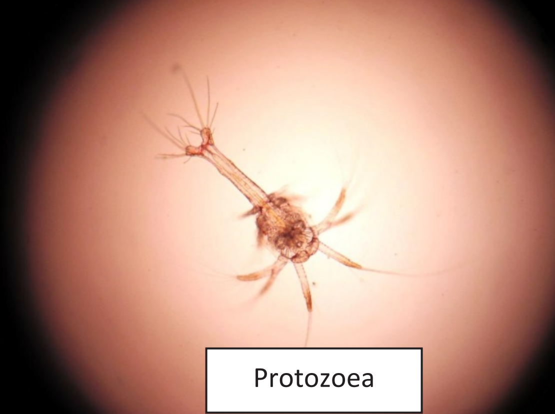


Gravid female of *Penaeussemisulcatus*


Male-Petasma


Female-Thelycum


Nauplius


Protozoea

4. Sea ranching of green tiger shrimp *P.semisulcatus*

Sea ranching programme is being reinitiated during 2016-17 by Mandapam Regional centre of CMFRI to enhance the brood stock population in the wild and for the replenishment of natural stocks. It is also useful to increase the shrimp production and promote the livelihood of fishermen in this region. Post larvae of 15-20 can be used for sea ranching after a larval rearing period of 30-35 days. During the period of 2017-19 a total of 8.725 million numbers of *P. semisulcatus* shrimp seeds of PL 15-35 were released in Gulf of Mannar and Palk Bay in the sea grass beds of the coastal waters. As a result the shrimp landings were increased during the year 2018-19 as reported by the fishers and data collected from landing centres. The fishermen associations also expressed that the sea ranching of *P. semisulcatus* is very useful in replenishing the shrimp resources of the region.

The details of successful sea ranching programmes carried out during 2017-19 in Gulf of Mannar and Palk Bay by Mandapam Regional Centre of CMFRI.

Date of Seeds released	Number of green tiger shrimp seeds released	Place of Sea ranching
11.05.2017	2.0 Lakhs (PL 35)	Thonithurai, Palk Bay
05.08.2017	10.0 Lakhs (PL 10)	Pamban, Palk Bay
13.03.2018	5.0 Lakhs (PL30)	Thonithurai, Gulf of Mannar
28.12.2018	5.0 Lakhs (PL 15-30)	Kunthukal, Gulf of Mannar
31.01.2019	9.0 lakhs (PL 15-35)	Sangumal, Olaikuda, Palk Bay
07.03.2019	11.0 lakhs (PL15-25)	VillundiTheertham, Palk Bay
29.03.2019	8.0 Lakhs (PL12-15)	Thonithurai, Palk Bay
04.05.2019	10.0 Lakhs (PL20-35)	Thonithurai, Palk Bay
03.08.2019	7.5 Lakhs (PL 15)	Thonithurai, Palk Bay
04.09.2019	2.75 Lakhs (PL 30)	Thonithurai, Palk Bay
08.12.2019	12.0 Lakhs (PL15)	Thonithurai, Palk Bay
08.12.2019	5.0 Lakhs (PL20-35)	Munaikadu, Palk Bay

Seeds of green tiger shrimp

Sea ranching of green tiger shrimp at Pamban, Palk Bay

Searanching of shrimp seeds by Shri. K. Veera Raghava Rao, District collector, Ramanathapuram at Villunditheertham, Palk Bay

Sea ranching at Kunthukal in Gulf of Mannar

Searanching at Sangumal, Olaikuda in Palk Bay

Sea ranching at Thonithurai in Palk Bay

Release of seeds at Thonithurai in Palk Bay

Release of seeds at Munaikadu in Palk Bay

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Seaweed Farming and Integrated Multi-Trophic Aquaculture (IMTA)

Johnson. B., Abdul Nazar, A. K.,[*] Jayakumar, R., Tamilmani, G., Sakthivel, M., Rameshkumar, P. Anikuttan, K. K. and Sankar, M.
ICAR – Central Marine Fisheries Research Institute
Mandapam Regional Centre, Mandapam Camp – 623 520, Tamil Nadu, India
Email: jsfaith@gmail.com
[*]Kovalam Field Laboratory, Madras Research Centre of ICAR-CMFRI, Chennai

Seaweed Farming Techniques

Kappaphycus farming is being widely adopted by floating bamboo raft method in Tamil Nadu coast (Plate 1). In few places tube net and monoline culture technique is also being practiced for seaweed cultivation. Floating raft is made of bamboo with 12' × 12' for mainframe and 4' x 4' for diagonals. In each raft, 20 polypropylene-twisted ropes are used for plantation. Around 150 – 200 grams of seaweed fragments are tied at a spacing of 15 cm along the length of the rope. A total of 20 seaweed fragments can be tied in single rope. The total seed requirement per raft is 60 – 80 kg. Fish net of 4m x 4m size is tied at the bottom of the raft to avoid grazing. In normal season, a cluster of 10 rafts are positioned in the near shore area of 1.0 to 1.5 m depth using a 15 kg anchor. Whereas during rough season the same cluster has to be installed using two or three anchors. Most of the seaweed farmers are using 25 to 45 rafts for their cultivation. Due to lack of space for the farming, in most of the villages a farmer is restricted to use maximum of 45 rafts only (Johnson *et al.,* 2017).

Plate 1 Floating raft method in *K. alvarezii* farming

Plate 2 Raft ready for harvest

Economics of seaweed farming

The total cost of production for making one bamboo raft for *K. alvarezii* farming worked out to be around Rs.1,600/- (Table 1). As the investment is comparatively less and farmers were also supported through subsidy scheme the spread of the technology was rapid.

Table 1. Unit cost for a bamboo raft

S.No	Particulars/Description	Quantity Required	Cost per Raft (Rs)
	FIXED COST		
1.	3-4" dia hallow bamboos of 12'x 12' for main frame + 4' x 4' for diagonals (without any natural holes, crakes etc.,) @ Rs.6.00 per ft of bamboo	64'	384.00
2.	Five-toothed iron anchor of 15 kg each (@ Rs.64 per kg) – one anchor can hold a cluster of 10 rafts	1.5 kg	96.00
3.	3mm PP twisted rope for plantation – 20bits of 4.5m each (@ Rs.230 per kg)	420 gm	97.00
4.	Cost of HDPE braider pieces (20 pcs x 20 ropes = 400 pcs of 25 cm each) (@ Rs.330 per kg)	165 gm	55.00
6.	Raft framing rope 6m x 12 ties per raft i.e., 36mts of 4mm rope(@Rs.230 per kg)	650 gm	150.00
7.	Used HDPE fishing net to protect the raft bottom (4m x 4m size) (@ 70 Rs/ kg)	1 kg	70.00
8.	2mm rope to tie the HDPE net (28 mts) (@ Rs.230 per kg)	100 gm	23.00
9.	Anchoring rope of 10 mm thickness (17m per cluster of 10 rafts) (@ Rs.220 per kg)	100 gm	22.00
10.	Raft linking ropes per cluster 10 rafts – 6mm thick – 2 ties x 3m x 9 pairs = 54m length (@ Rs.230 per kg)	100 gm	23.00
11.	Braider twining charges		80.00
	TOTAL		**1,000**

	OPERATING COST	**Quantity Required**	**Cost per Raft (Rs)**
1.	Seed material (150 gm x 400 ties = 60 kg) + 10 kg handling loss = 70kg@ Rs. 4.00 per Kg	70 kg	280.00
2.	Labour (Seeding, Raft / monoline laying & maintenance)		200.00
3.	Transportation		100.00
4.	Miscellaneous expenses		20.00
	TOTAL		**600.00**

Table 2. Economic Feasibility Analysis of Seaweed Farming from 45 rafts per person ***(Total 5 cycles in a year; each cycle is 45 days)***

1.	Annual seaweed production (260 kg/raft) *(Retaining 60 kg for next crop, total fresh seaweed production from 45 rafts; 5 cycles)*	45,000 kg
2.	Total seaweed production on dry weight basis (10 %) *(from 45 rafts; 5 cycles)*	4,500 kg
3.	Price of dried seaweed (Rs. per kg)	45
	Gross Revenue in Rs.	**2,02,500**
	Total cost of production (Rs.) (Rs.1,600 × 45 rafts)	**72,000**
	Net income (Rs.) (Gross revenue – Total cost of production)	**1,30,500**

The crop duration is for 45 days. In a year, four to six crops/cycle (6 to 9 months) can be harvested depending on the climatic condition. Planting of 150g grows up to 500 to 1000g in 45 days. In one raft of 12 x 12 ft size an average yield of 260 kg can be obtained. After retaining 60 kg as seed material for the next crop, remaining seaweed is sold either in fresh or dry weight basis. The average dry weight percentage of the harvested seaweed is 10 per cent. At present farmers receive Rs. 5 to Rs. 10 and Rs. 40 to 45 per kg for fresh and dried seaweed respectively. On an average a family earns Rs.10,000/- to Rs.15,000/ per month.

Constraints in K. alvarezii farming

i) Grazing

Nibbling of herbivores like siganid, acanthurid, sea urchin and starfish on tips of branches is the major problem faced by the seaweed farmers. During the month of May – June, the grazing intensity is more, which affects the yield up to 50-80 per cent.

ii) Epiphytism

It is the attachment of undesirable seaweeds to the cultured species, which is common among tropical seaweeds that usually occur at the onset of monsoon brought by change in water temperature, trade wind and water motion; drift seaweeds caused by limited substrate contribute also to epiphytism that compete for space, nutrient and sunlight. During the month of May – June, majority of the seaweed framers face this problem.

iii) High temperature / Disease

Diseases are caused by low salinity, high temperature, and light intensity. When the plant is under stress whitening of the branches occurs, which results in crop loss. Even few farmers discontinued the farming due to severe loss during high temperature period.

Apart from the above mentioned problems, natural calamities like heavy storm and cyclone cause complete damage to the *K. alvarezii* farming.

Environmental benefits

Seaweed farming is considered as one of the significant mitigating measures for the adverse impact of climate change. Seaweeds provide shelter to a variety of organisms and enhance biodiversity. They absorb carbon-di-oxide (CO_2) and reduce global warming (Israel *et al.,* 2010). They are also efficient in controlling organic pollution including heavy metals in the inshore waters and thereby ensuring ecological balances. Thus, seaweed cultivation is an

eco-friendly option with sustainable income to the coastal fishers. Experimental studies were conducted at Munaikadu, Ramanathapuram district, Tamil Nadu on assessment of carbon sequestration potential of seaweed (*Kappaphycus alvarezii*) and it was found that the specific rate of sequestration (per unit mass of seaweed per unit time) of CO_2 by the seaweed was estimated as 0.0187 $gday^{-1}$ (CMFRI Annual Report, 2015-16).

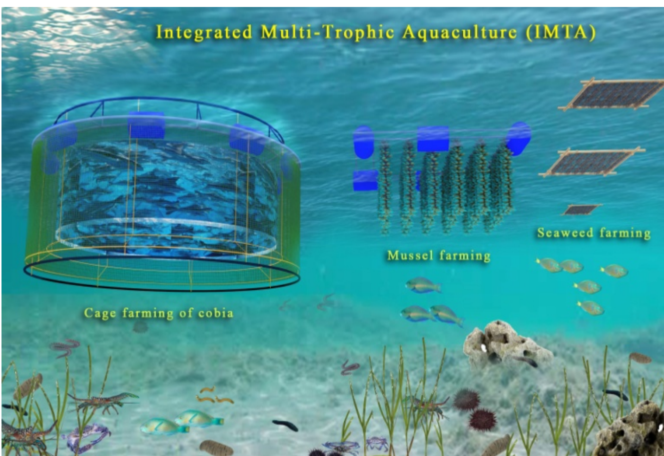
Integrated Multi-Trophic Aquaculture (IMTA)

One of the anticipated issues while expanding the sea cage farming is the environmental degradation and consequent disease problems. In this context, the idea of bio-mitigation along with increased biomass production can be achieved by integrating different groups of commercially important aquatic species which are having varied feeding habits.. This concept is known as Integrated Multi-Trophic Aquaculture (IMTA) which is getting importance at global level. The ICAR-CMFRI, Mandapam has successfully conducted demonstration of Integrated Multi Trophic Aquaculture (IMTA) under participatory mode with a fishermen group at Munaikadu (Palk Bay), Ramanathapuram district, Tamil Nadu by integrating seaweed *Kappaphycus alvarezii* with cage farming of Cobia (*Rachycentron canadum*). Since, seaweed farming is being widely adopted in Tamil Nadu coast; integration of seaweed with cage farming of cobia was initially attempted.

A total of 16 bamboo rafts (12× 12 feet) with 75 kgs of seaweed per raft can be integrated with one of the cobia cage. In one crop of 45 days the seaweed rafts integrated with cobia cage gave a better average yield of 260 kg per raft while the same was 150 kg per raft for the rafts which were not integrated. An addition of 110 kgs of seaweed/ raft was achieved due to the integration with cobia cage farming. Moreover there was increased number (average 90-100 nos.) of newly emerged apical portion/tips in a bunch of harvested seaweed from the rafts integrated with the cobia cages, whereas the same was less (average 30- 40 nos) from the rafts which were not integrated. The bunches having more numbers of newly emerged apical portion/tips, when used for replanting, will be ready for harvest within 40 days, whereas the seaweed with less numbers of newly emerged apical portion/tips, if used as seed, will be ready for harvest only after 54 days.

It was found that the organic waste mitigation of the integrated system of *Kappaphycus* farming is more efficient than the non-integrated system of farming. In addition to the revenue generated in cobia and seaweed sales by conventional method, an additional income of Rs.1,00,000/- could be realized due to increased seaweed and cobia fish yield from IMTA. The total amount of CO_2 sequestered into cultivated seaweed (*Kappaphycus alvarezii*) in the integrated and non-integrated rafts were estimated to be 223 kg and 100 kg respectively. Hence, there is an addition of 123 kg carbon credit due to integration of 16 seaweed rafts (4 cycles) with one cobia cage (one crop).

Integration of seaweed with cobia cages favourably generates additional revenue through increased yields of both cobia and seaweed. Hence fishermen are continuously adopting this technology with their own investment. IMTA is efficient in controlling both organic and inorganic pollution in the natural open waters and thereby ensuring ecological balances. Thus, IMTA is an eco-friendly option with sustainable income to the coastal fishers.



IMTA Cross-sectional view

Seaweed raft along with cobia cage

Staff along with the fishermen group during the harvest

Comparison of seaweed rafts which was integrated and not integrated with cobia cage

Comparison of a bunch of seaweed which was taken from the rafts which was integrated and not integrated with cobia cage

More numbers of newly emerged apical portion/tips from a bunch of harvested seaweed from the rafts integrated with the cobia cages

Conclusion

The seaweed farming has proved to be an economically viable alternate livelihood option in Gulf of Mannar and Palk bay region of Tamil Nadu. Seaweed farming is a low cost simple technology, provides substantial returns and had better adoption among the coastal fisherfolk. Adoption of sea cage farming of cobia is in take off stage and may pave a way to reduce the fishing pressure and contribute additional income to the fisher household. Integration of seaweed with cobia cages favourably generates additional revenue through increased yields of both cobia and seaweed. IMTA is efficient in controlling both organic and inorganic pollution and thereby ensuring ecological balances. Thus, IMTA is an eco-friendly option with sustainable income to the coastal fishers. It is also one of the significant mitigating measures for the adverse impact of climate change and earns carbon credit to our country.

Fish Population Genetics and Management of Captive Breeding Populations

M. Sakthivel, G. Tamilmani, M. Sankar, P. Rameshkumar,K.K. Anikuttanand R. Jayakumar
ICAR-CMFRI, Mandapam Regional Centre

The aim of genetic improvementis not to change individual fish, but rather the fish stock. Hence, the ***'Mendelian Genetics'*** extended at the level of population is the ***"Population Genetics"***. It is thus most essential to understand the meaning of a stock or population.

Definition of population

In general, a population refers to a group of individuals of a species assembled/living at a place at a time. ***Statistically,*** a population is referred to a group or collection of individual/items having the similar properties. ***Biologically,*** a population refers to a group of organisms/individuals of a species functioning together as a unit at a given place and time. ***Genetically,*** in population genetics, a population is defined as a group of interbreeding individuals. As a result of inter breeding there is gene exchange among the individuals of a population and hence they contribute to the gene pool of the offspring generation. The gene exchange is thus the main and important factor to define population. The population for a population geneticist means the ***"Mendelian Population"*** which is defined as a group of ***interbreeding individuals developed over both space and time*** sharing a common pool of genes, from which meaningful samples can be drawn and within which the characteristics under study follow the Mendelian rules of inheritance. The ***gene pool*** is taken as the sum total of genes in a population. In other words, the gene pool includes collectively all the genetic information distributed among all the individuals of a population.

Size of Population: The number of individuals constituting a population decides the size of the population. Therefore an individual is the unit of population. The number of individuals in a population should be large enough so that the sampling variation is as small as to be negligible. Thus, a population should not be affected by the sampling variation. Such a population which is not affected by the sampling variation is knows as large population and consists the number of individuals in hundreds or even in thousands rather than in tens.

The total number of individuals forming the population is important. The differential reproductive success of different individuals molds the genetic structure of a population in coming time. The counting of the number of individuals in a population is done for three purposes: to assure the existence of population, to determine the gene frequencies, and to estimate the role of chance factor played in the transmission of genes.

Genetic structure of a population

The organization of the genes into genotypes is the most essential part of population genetics. The genotypes are not transmitted to the offspring as such but they are broken during meiotic cell division as a requirement of genetic transmission from one generation to the next. Thus genotypes of the parents are broken into gametic pool and each offspring has its newly formed genotype different from its either parent. Therefore, the array of genotypes in a

population is re-determined in each generation. For example, the heterozygous parent (*Aa*) may not have any heterozygous offspring. The gametes produced by a heterozygous parent (*Aa*) contain either *A* or *a* allele which may unite with any allele of opposite sex and thus parental heterzygosity is not transmitted directly to the offspring. The relative genotypic frequencies in a population are determined by some biological forces *viz*. breeding behavior, genetic factor (mutation) and environmental factors (migration, selection, sampling process in small population) which act on individuals by affecting their survival and reproduction. Thus the genotype frequencies from one generation to the next are under the control of these forces.

Among the breeding behavior, the simplest and important one is the random breeding or random fertilization which followsno principle (criteria) for breeding of individuals of opposite sex and individual of one sex has equal chance to breed with any individual of the opposite sex. The main feature and consequence of random breeding in a large population is that the relative gene frequencies and genotype frequencies remain constant from generation to generation and thus there is no change in genetic structure of large population under random breeding. This is called as genetic equilibrium. The genetic equilibrium is disturbed if the breeding is non-random and if any of the genetic and environmental force is in action. Any force is capable to change the genetic equilibrium from one generation to the next. There are a number of situation and environmental factors (migration, selection and sampling) to disturb the genetic equilibrium.

Most of the captive populations are not ideal to maintain the genetic equilibrium in view of the man's activities and the role of nature. The ideal population is in which no disturbing force comes in action to upset the genetic equilibrium and the random breeding results in a genotypic distribution consistent with the frequencies of genes. However, the fish breeder is interested to change the existing genetic structure of the population in a desired direction to exploit the existing genetic variability to make the animals more productive and more useful. Therefore, breeders are not interested to maintain the existing genetic structure of the breeding population and they make their efforts to achieve high performance of their animals. This is achieved in a number of ways.

The ***first*** is the selection of the better production animals and to eliminate the low productive and inferior ones. This is called the artificial selection. ***Secondly***, the selected animals are bred following certain criteria rather than allowing random breeding. Thus breeding is non-random (assortative). ***Thirdly***, the breeders keep their captive populationin relatively small size. This results in small population size which upsets the genetic equilibrium in two ways. One is that inbreeding is inevitable in small population size which changes the genotype frequencies of next generation. The other effect of small population size is that the possibility of expected genotypic frequencies is low due to lesser number of animals according to the probability because higher number of events makes the expectation more close to reality. Thus smaller number of animals in the herd results in the occurrence of errors in sampling due to chance events. This leads to differentiation of gene frequencies in local populations resulting from sampling.The ***fourth activity*** of fish breeders is the trade breeding under which the animals

are sold or transferred to another breeder and thus migration of animals occurs which further increases the possibility of gene flow (migration) from one corner to other corner of the country or the world.

It is thus obvious that most of the conditions for genetic equilibrium are not fulfilled. The fish population size maintained under captive conditions in India is small, the breedings are not random but selective breeding is practiced andthe animals are migrated for a number of reasons. Therefore, the genetic equilibrium is not observed in hatchery and farm conditions in all practical situations and the change in genetic structure of populations is likely to occur. The change can be brought to favourable direction and magnitude after having the knowledge and being aware of the genetic effects. Therefore, it is most essential for a breeder to know the process of change in genetic structure of populations under the influence of various forces. There forces capable to change the genetic structure of a population can be classified in several ways as under-

Natural Vs. artificial factors: Both nature and manmade interventions disturb the existing genetic equilibrium and contribute to changes in the genetic structure of the population. The factors which are under the control of nature to upset the genetic equilibrium are the mutation (change in genetic structure of a gene) and the natural selection. The natural selection operates through differential fertility and viability.The manmade activities include artificial selection by way of selective breeding and preferential breeding (non-random breeding), migration of animals through purchase or transfer, and to keep small population size leading to inbreeding and sampling error (random drift).

Factors involved at individual and population level: The first is the change at gene level (mutation) whereby the genetic structure of gene is changed and produces a new gene. The second is the change at population level by gene flow (migration), selection, small population size, and by a change in breeding system from random breeding to preferential breeding (non-random breeding).

The second category of forces brings a change in gene frequencies between generations and consequently result a change in genotype frequencies. These forces include gene mutation, gene flow (migration), selection, small population size and disassortativebreeding. The genetic changes brought by the forces of second category arepermanent even after switching to random breeding. This is because these forces involve the change in gene frequencies.

Amount and direction of change: On this basis all the forces are divided into two groups. The first group is of the deterministic or systematic forces which are also called as the vectorial process. The second category is the stochastic process or random or dispersive forces. Among the systematic forces are the mutation, migration and selection which tend to change the gene frequencies predictable both in amount and direction. The dispersive process arises in small population. The change brought by dispersive process is predictable only in amount but not in direction.

Importance of recording the data

If the purpose of selection is to improve a production trait, the first step is to measure or record this trait on all animals in the population, and then estimate the average and standard deviation. Selection is then practised by selecting those animals, which have highest breeding values. A fishhatchery or farm is a dynamic and complicated enterprise having the objective of increasing the productivity and profit. In order to achieve the objective of increasing the productivity and changes, activities and requirements for which the fish breeder has to keep the records and get useful information for taking right decisions about selection of genetically superior animals on the basis of their breeding values.

The data or records are essentialin a hatchery or farm for the following purposes:

- To know the pedigree and history of each brooder pertaining to the production, reproduction and health performance.
- This helps to compare the between farm or between hatchery performance of brooders
- The breeding value for different economic traits can be estimated which help in culling and selection of animals for breeding purposes which in turn bring the genetic improvement of future generations.
- Based on the production performance the feeding requirement can be estimated.
- This also helps in research and development planning.
- To know the health status by keeping records of the daily treatment of animals.

Selection and its Response

Selection is applied to change the fish population for making genetic improvement in performance. The selection is a process of giving preference to certain individuals in a population to reproduce than other individuals which are denied the opportunity to produce next generation. Therefore, selection is the choice of individuals to produce the next generation. In genetic terms, the selection is a process of differential reproduction and survival of genotypes which may be natural or artificial or both.

The selection, without creating any new gene, changes the genetic structure of the population by changing the frequency of genes and genotypes. The frequency of desired genes is increased in the population through selection at the expenses of the frequency of undesirable or less desirable genes. This is the genetic effect of selection. The selection is more efficient for dominant genes at low frequency but it is relatively easy to select for a recessive gene.

The characters are controlled by genes. Therefore, with the increase or change in the frequency of desirable genes, the phenotypic mean of the character of offspring generation is also increased or changed. The change in performance of offspring generation due to artificial selection is known as **response to selection or genetic change or genetic gain**. Now the point of discussion here is that how the performance of the offspring generation of selected parent is changed.

Intensity of selection

The intensity of selection denoted by *"i"* is the mean deviation of the selected animals in units of σ_p of the trait *i.e.* it is the number of σ_p of the trait by which the mean of the selected group is above the population mean before selection. The intensity is expressed as:

$i = s/\sigma_p$

Factors affecting the response to selection

The change in performance due to artificial selection known as response to selection depends on the following factors:

1 Additive genetic variability in the trait (σ_A)
2 Intensity of selection (i)
3 Accuracy of selection (r_{ap})
4 Population size

Additive genetic variability in the trait ($_A$)

The selection acts on additive genetic variability. The variation in breeding values (BV) of the individuals within the population is the raw material to act for artificial selection. The selection will not be effective to bring change if there are no genetic differences among animals. Therefore if $V_A = 0$, the R = 0. The magnitude of R increases with the increase in differences in B.V. between animals. However, the genetic variability of a trait (B.V.) within a population is determined by the population and the characters and hence it is beyond the control of breeder. It is therefore better to exploit other factors like intensity and accuracy of selection.

Intensity of selection (i)

The intensity of selection depends on the proportion (p) of the population selected. When p is small, the selection is said to be more intense or rigorous. But when p is large (increase in proportion selected) then there is decrease in intensity of selection. The R will be more when p will be small. This is a straight forward way to improve the rate of genetic progress. If all fishes are selected, the S will be zero and no change in offspring mean will occur. The change occurs if some of the best fishes are selected. Therefore, the proportion of fishes selected should be less.

Accuracy of selection (r_{ap})

The selection is effective only when the animals with highest B.V. are selected. The true B.V. of fishes is not known. This requires one or more sources of information to know the estimate of B.V. of fish. The information to estimate the true B.V. of a fish may be collected on the performance of the fish itself and or of its any close relative. The estimate of true B.V. so estimated should be as much accurate as it can be to make the selection more accurate. The accuracy of selection is taken as the correlation between the true B.V. of a fish and the source of information (Selection criteria) which is denoted as r_{ap} where A is the true B.V. and P is the selection criteria. The selection criteria may be a single record of average of repeated records of the fishes itself or on any relative *viz.* dam or average performance of a group of relatives like full sibs, half sibs or offspring groups.

The r_{ap} is equal to square root of heritability (r_{ap}=h). Thus if h^2estimate is higher, ther_{ap} will also be higher. The h^2 is an indication of the reliability of phenotypic value as a guide to the breeding value (B.V.) because the h^2 shows the correspondence between B.V. and phenotypic value. In other words, the h^2 shows the extent to which the B.V. constitutes the phenotypic value. Thus is directly correlated to h^2as r_{ap}= h which is a measure of the accuracy of selection. It is, therefore, that for maximum accuracy of selection, the r_{ap}must be as high as possible to make the selection accurate. Thus selection will be more accurate when the h^2 of the trait is high.

The accuracy of selection can be increased by the following methods:

1. *Minimize the environmental variance:* It can be minimized by providing uniform environment, by use of multiple records to estimate h^2, by adjusting the data for environmental effects, by accurate measurement of data, and by analyzing the data based on contemporary group mean.

2. *Combined Selection:* When two or more criteria of selection are used to estimate an individuals' true breeding value (BV) it is called as the combined selection. This means to supplement the individuals' performance belonging records with those of its relatives. This gives more accurate estimate of B.V of the individual. The family selection may be used to support individual selection when h^2 of a trait is low. It is better to select an individual with better record belonging to a superior family compared to an individual with similar performance belonging to a mediocre or inferior family. The half sib family selection is better than FS family selection.

   The reason being that family selection is more effective when the environmental variations common to all the members of a family are as small as possible. This means that environmental similarities among family members should be low. The common environmental variance is less among half sibs than among full sibs. Thus, the environmental correlation among F.S. is more than among H.S. Secondly, the F.S. family selection reduces the genetic variability in the population and also results in a certain amount of inbreeding. The selected parents based on F.S. family averages are more closely related. Thirdly, the effective selection intensity is also reduced for a given testing facility for F.S. family selection.
3. *Selection based on future performance:* The selection should be based on the future performance (most probable producing ability or the expected producing ability) of the animal with more number of records.

Size of the population

The effect of population size on response to selection can be viewed in terms of inbreeding and genetic drift.The inbreeding is unavoidable in a population of small size. But inbreeding is less when the animals of both sexes are equal which is not possible in fish breeding for the reason that fewer individuals of either sex may be required according to species under selection. This needs to estimate the effective population size (Ne). The Ne is the number of

individuals that would give rise to the same rate of inbreeding, if they breed in the manner of an idealized population, in which the rate of inbreeding is $\Delta F = 1/2N$.

Criteria of selection

The performance records of ancestors and collateral relatives of the individual are also used as selection criteria to estimate the breeding value of an individual for the trait under selection.

Selection based on pedigree

The pedigree is a list or record of ancestors in the past few generations of the individual. The ancestors are the parents, grand parents, great grand parents etc. The pedigree having information on the economic characters of the ancestors is useful in selection of an individual. The B.V. of an individual is estimated on the basis of the performance of the ancestors. The selection criteria based on ancestors performance is called as the pedigree selection.

Basis of pedigree selection

An individual receives genes from his ancestors. The percentage of ancestral genes is halved in each generation. This decides the genetic relationship between an individual and his ancestor(s). This relationship is reduced to half in each generation. It is thus important to consider more recent ancestors (parents) rather than distant ancestors (great grandparents) for pedigree selection. This inclusion of more remote ancestors results only in marginal gain in accuracy of selection due to the halving process and sampling nature of inheritance. The pedigree selection adds very little to the accuracy of estimateing the B.V. of an individual if the information on individual are available. The significance of pedigree is decreased when information are available either on the individual or its family members (sibs and offspring).

Guides to pedigree selection

The following factors determine that how much attention is to be given to pedigree selection.

- The degree of genetic relationship of the individual with its ancestor – more closely related ancestors should be given more emphasis.
- Heritability of the character - the pedigree selection is more accurate for traits of high heritability.
- Environmental correlations among the individuals used in the prediction.
- Information available on ancestors.

Practical difficulties to use pedigree selection

- The ancestors' records are always not available.
- The pedigree records are destroyed with passage of time.
- Most of the characters have low heritability.

Merits of pedigree selection

- It is less costly as only compilation of pedigree is required.
- Allows selection at younger age and provides first-hand information.

- It is helpful in multistage selection.
- It is useful for sex limited traits and traits expressed in later life or after death of animal.
- It is helpful when two individuals have similar performance but one belongs to a better pedigree.

Demerits of pedigree selection

- There is a disadvantage of using pedigree selection that all animals of similar pedigree are culled out in spite of the fact that an individual may be of good merit and free from recessive allele causing defect.
- Some pedigree gets undue emphasis and favoured irrespective of the true merit of the individual. Better environment is provided to the offspring of favoured pedigree.
- It introduces non-random biases because pedigree records are for different environmental conditions.
- Pedigree selection provides no basis of selection among individuals which are descendants of the same ancestor.

Selection based on collateral relatives

The selection criteria to estimate the B.V. of an individual may be the information (Performance records) of its collateral relatives. An individual's collateral relatives are those which are not related with the individual on direct genes donor-recipient basis but receive common genes from their common ancestor(s) e.g. full sibs, half sibs, cousins, etc. The collateral relatives should be more closely related to the individual and these are full sibs and half sibs. The more closely related collaterals to the individual are likely to have more common genes possessed by the individual and can provide more accurate information about the individual. The more closely collaterals can be grouped as full sib families and half sib families. The family mean of these collateral relatives then form the basis to select the superior individual. The procedure to estimate the B.V. of an individual on the basis of family mean is called the family selection or sib selection depending upon the inclusion or exclusion of individual's own record in estimating the family mean. The selection criteria is called as family selection when the individual's own record is included to estimate the family mean but the selection criteria is called as the sib selection when the individual's own recorded is not included in estimating the family mean.

Sib Selection

It is the selection of an individual based on its sibs performance. The sibs may be full sibs or maternal half sibs or paternal half sibs. Thus sib selection is of two types *viz*. Full sib selection and half sib selection. The sib selection is practiced for the following traits for which the measurements on the individual are not available or recorded –

- Slaughter traits
- Sex limited traits

- Threshold traits like disease resistance
- Traits with low heritability in species with high reproductive rate so as many sibs are measured in short time
- The full sib (F.S) selection is more accurate than half sib (H.S.) selection. However, the H.S. selection is favoured for the following reasons:
  - Half sibs are easily available in more numbers than F.S.
  - The rate of inbreeding is more for F.S. selection than H.S. selection. The inbreeding counter balances the effect of selection.
  - The F.S. correlation is more likely to be increased by c-effects (common environment shared by F.S.). The intra class correlation (t) is rh^2 for H.S. and rh^2+c^2 for F.S. where c^2 is the added contribution of maternal or common environmental effects. This reduces the accuracy of F.S. selection.

Family Selection

The selection criteria are known as family selection when based on the performance of the sibs plus the individual's own record. The family selection like the sib selection is of two types of sibs*viz* Full sib family selection and Half sib family selection.The family selection is also taken in another way *viz*. as a unit of selection. Based on the family mean the whole of the family is selected or rejected as a unit of selection.

Common environment (c-effects)

The environmental effects which are different for different families but same for all members of one and the same family are known as common environmental effects denoted as c-effects. The family members share common environment during pre and post natal stage. The c-effects thus create resemblance within family members over and above the resemblance due to having common genes and this contributes to the variance between families. This increases the intra class correlation (t) among family members. The c-effects are more for F.S. than for H.S. To some extent the half sibs also share common environment *viz*. all the offspring of a sire being born almost at the same time and being reared together are likely to be subjected to similar environmental conditions like climatic conditions, feeding regime and management practices etc.

When environmental similarities (c-effects) are present among family members, the intra class correlation among the phenotypic values of family members (t) is increased equal to the amount of c-effects as ($t+c^2$) where C^2 is the portion of the total variation caused by differences in c-effects among families. This makes the denominator larger and hence the regression is decreased. Thus the c-effects decrease the accuracy of sib and family average.

Advantages of family selection

- The family selection can improve the characters of low heritability in species with high reproductive rates so as to get many sibs in a short time.
- The family selection does not allow the generation interval to increase

- Family selection is a support to individual selection because it is better to select an individual from a superior family.

Limitations of family selection

The family selection to be effective requires large family size and more number of families to avoid inbreeding as well as to increase the intensity of selection. In view of this it can be inferred that –

- Family selection is costly of space particularly when the breeding space and testing facilities are limited. The limited facilities reduce the intensity of selection.
- The family selection as a unit of selection results in inbreeding and thus limits the genetic diversity. This is because only few families represent the next generation.
- The F.S. family selection can only be applied in species with high reproductive rates to get large family size.

Within family selection

It is the selection criteria when individuals are selected on the basis of their performance expressed as deviation from their mean. The individuals that exceed their family mean by the greatest amount are selected. Thus it is opposite to family selection because family mean is given no weight. This selection criteria is preferred when c-effects are important i.e. when a large component of environmental variance is common to all members of a family. A large component of environmental variance common to members of a family arises when the different families differ in the environment to which they are exposed. Thus the families differ largely due to differences in environment and hence whole family get good or poor environment. The selection within family eliminates the environmental differences among families.

The within family selection economizes the breeding space and minimizes the rate of inbreeding. In within family selection, each family contributes equally to the parents of next generation, when single pair breedings are made and two individuals from each family are selected as replacement of the parents. The within family selection is better than individual selection when the sib correlation (t) is very high and caused largely by environmental effects. The within family selection operates on a large amount of additive variance within families.

Selection and breeding for disease resistance

There are three possible methods to identify and select the individuals for differences in resistance/susceptibility to a certain disease/parasite. These are as under:

- Occurrence of the disease under normal environmental conditions,
- Inoculation of animals with pathogen causing the disease, and
- Use of indicators of resistance.

The first method to identify the resistant animals on the basis of the incidence of the disease is not effective in the sense that under the changed environmental conditions the resistant

become susceptible. The second method of inoculation is not likely to be applied because of the objections to deliberate infection with pathogenic organisms. The third one requires very great efforts and research to find a better indicator of genetic resistance; which do not require deliberate exposure to pathogens.

The efficiency of individual selection and family selection on the basis of the incidence of the disease with two visible classes is discussed. The effectiveness of selection depends on the selection differential which, in turn, depends mainly on the proportion selected. But in case if threshold characters (all or none characters), the selection differential depends on the incidence rather than on the proportion selected. Selecting all the resistant individuals or only a portion of them will give their mean as the mean of the resistant group. Thus, selecting a smaller proportion than the incidence, does not give an added advantage. It is, thus, evident that maximum selection differential is achieved when the proportion selected is equal to the proportion of resistant individuals. The individual selection is less effective when the difference between the two (incidence of resistance and proportion selected) is more. Further, the individual selection is less effective when the proportion of resistant individual is very low, but the family selection is more effective. An individual's phenotype as resistant or susceptible is not precisely known whereas the family is more precisely known for the proportion of affected members of the family. However, the precision depends on the family size and on the incidence of a disease in the family.

Conclusion

Although aquaculture as a biological production system has a long history, systematicand efficient breeding programs to improve economically important traits in the farmedspecies have rarely been utilized until recently, except for salmonid species. This meansthat the majority of aquaculture production is based on genetically unimproved stocks. In farm animals the situation is vastly different: practically no terrestrial farm production is based on genetically unimproved and undomesticated populations. This difference between aquaculture and livestock production is in spite of the fact that the basic elements of breeding theory are the same for fish and shellfish as for farm animals. One possible reason for the difference is the complexity of reproductive biology in aquatic species, and special consideration needs to be taken in the design of breeding plans for these species.

In aquaculture, selection programs are not commonly used by the industry and for many species production still rely completely on catching wild broodstock and/or fry. There is no obvious reason for the lack of efficient breeding programs in aquaculture. The economic important traits in fish and shellfish appear to be little different from those in farm animals and plants. Selection response is usually higher in fish and shellfish than in farm animals. One reason for the scarcity of breeding programs in aquaculture species is that the reproductive cycle is often complex, and so is frequently not fully understood, and is therefore not able to be completed or controlled in captivity. This is the case particularly for marine species. A further factor contributing to the scarcity of breeding programs in aquaculture species may be the

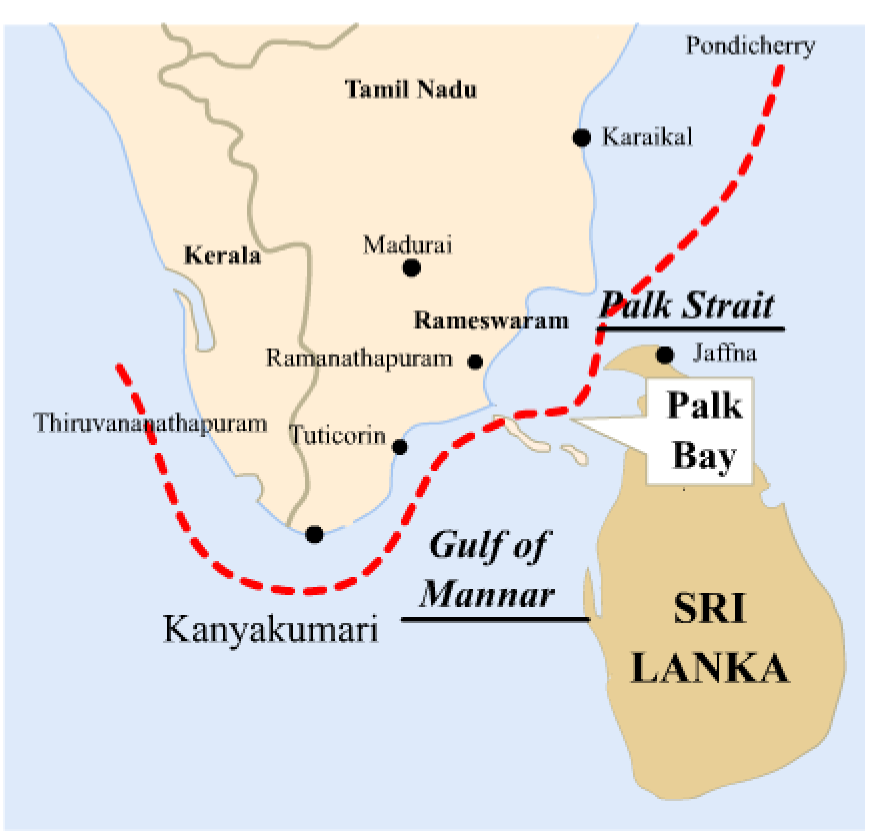
deterioration of the stock simply because of a rapid build-up of inbreeding as a result of using few broodstock each generation (and without identification to prevent re-use). This is a problem in all species with high fecundity. Because there has been little interest in developing breeding programs in aquaculture, the information about phenotypic and genetic parameters of economically important traits are quite limited for most of the species farmed. Before a breeding program can be established, breeding goal must be defined; estimates of genetic variance, heritability, phenotypic and genetic correlations among traits must be available. There is therefore a great need to run breeding experiments in order to get reliable estimates of genetic parameters for economically important traits in the most important farmed species.

The applications of molecular techniques in aquaculture are promising, but still somewhat uncertain. While high costs seem to be the only hindrance for widespread application of DNA markers for identification purposes and marker assisted selection, the situation regarding commercial use of genetically modified fish is more complex. Although the potential importance of gene transfer technology is large, a major concern relates to the possible impact, which release or accidental escapes of gene-modified individuals may have on natural ecosystems. Other technologies are also rapidly emerging which are either being used or are likely to be used in the future in the aquaculture species. For example micro-array technology has the potential to contribute very large amounts of information on the genes and pathways of genes, which affect the economic traits in aquaculture species.

Marine biodiversity and its conservation methods in Gulf of Mannar and Palk Bay

Dr.Raju Saravanan., Scientist , Marine Biodiversity Division
Mandapam Regional Centre of CMFRI

Introduction



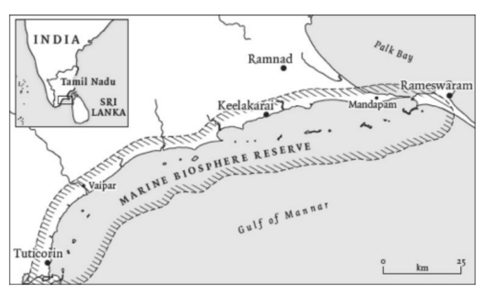
The coastline in Tamil Nadu can be broadly divided into three fishing zones. They are (1) Pulicat Lake to Point calimere that lies in the Coromandal coast; (2) Point calimere to Dhanushkodi that covers the Palk Bay and the Palk Strait; and (3) Dhanushkodi to Kanyakumari which covers the Gulf of Mannar.The Gulf of Mannar, the first Marine Biosphere Reserve in India as well in South East Asia covers a total area of 10,500km^2 from Dhanushkodi to Kanyakumari covering a coastline length of 365 km along the coast of Tamil Nadu between Longitudes 78^{0}08E to 79^{0}30E and Latitudes from 8^{0}35N to 9^{0}25N. This Marine Biosphere Reserveencompasses a chain of 21 islands with its fringing reefs from Ramanathapuram to Tuticorin district.

- Mandapam Group (7 islands): Shingle, Krusadai, Pullivasal, Poomarichan, Manoliputti, Manoli, Hare
- Keezhakkarai group (7 islands): Mulli, Valai, Thalaiyari, Appa, Poovarasanpatti (submerged), Valaimunai and Anaipar.
- Vembar Group (3 islands): Nallathanni, Pulivinichalli and Upputhanni.
- Tuticorin Group (4 islands): Kariyachalli, Vilanguchalli (submerged), Koswari and Vaan.

The 21 islands and the surrounding shallow coastal waters covering an area of 560 km^2 between Pamban and Tuticorin was declared as Marine National Park by the Government of Tamil Nadu in 1986 for the purpose of protecting marine wildlife and its environment. The Gulf of Mannar Biosphere Reserve (GoMMBR) covering an area of 10,500km^2 between Rameswaram and Kanyakumari was declared by the Government of India in 1989.Since time immemorial, the Gulf of Mannar is regarded for its pearl fishery and its rich fishery resources. There are about 4223 species of various flora and fauna has so far been found in this biosphere reserve.A unique endemic species of Balanoglossus - *Ptychodera fluva*, a living

fossil that links invertebrates and vertebrates, has been recorded only here at Kurusadai Island. Hence the Gulf of Mannar is famously called Biologist Paradise, however inthe past decades heavy exploitation has resulted in depletion of its resources.

Palk Bay, named after Sir Robert Palk (1717-1798) the then Governor of Madras Presidency (1755-1763), is situated in the southeast coast of India encompassing the sea between Point Calimere (Kodikkarai) near Vedaranyam in the north and the northern shores of Mandapam to Dhanushkodi in the south. It is situated between Latitude 9º 55' - 10º 45' N and Longitude 78º 58' - 79º 55' E. The Palk Bay itself is about 110 km long and is surrounded on the northern and western sides by the coastline of the State of Tamil Nadu in the mainland of India. Palk Bay and Gulf of Mannar to its south are connected by a narrow passage called Pamban Strait which is about 1.2 km wide and 3 to 5 m deep that separates the Island of Rameswaram from the mainland. The Palk Bay waters merge with those of the Bay of Bengal in the northeast and the Gulf of Mannar waters in the south. The Palk Strait is just 35 km of water that is narrower than the English Channel and separates the northerncoast of Sri Lanka from the southeast coast of India. Therefore the international boundary line is close to the shores of both the countries. The boundary is only 6.9 km away from Dhanushkodi, 11.5 km away from Rameswaram, 15.9 km away from Point Calimere, 23 km away from Vedaranyam and 24.5 km away from Thondi.



Gulf of Mannar Ecosystems

Gulf of Mannar covers a wide range of marine ecosystem that boasts marine life; they are

I. Coral Reef Ecosystem
II. Seaweed Ecosystem
III. Sea grass Ecosystem
IV. Mangrove Ecosystem
V. Lagoon and Wetland Ecosystem
VI. Other Resources

I. Coral Reef Ecosystem

Coral reef formation in the Gulf of Mannar is of Fringing type, which is bordered around the 21 uninhabited islands. Coral reef ecosystem is comparable with rain forest ecosystem on land and it support a wide variety of resources. The reefs of the Gulf of Mannar Biosphere Reserve and Palk Bay are the only major coral formations along the mainland coast of India. A discontinuous barrier termed Mannar Barrier extends over a distance of 140 Km from Tuticorin to Pamban in the Gulf of Mannar. The Mannar Barrier possesses a chain of 21 islands all along its length with fringing reefs around them. There are 117 coral species identified so far in Gulf of Mannar. They belong to 40 genera and 14 families. Of this, 106 8

species grouped in 30 genera are hermatypic and 11 species grouped in 10 genera are ahermatypic. Coral reef diversity of Gulf of Mannar and Palk Bay comprises of fourteen families, 40 genera and 117 species. Among the 89 genera recorded in India, only 40 are reported so far in this ecosystem. Species such as *Montipora monasteriata*, *M. informis*, *M. squamosa*,*M. turgescens*, *M. venosa*, *M. verrucosa*, *M. digitata*, *M. millepora*, *M. manauliensis*, *Acropora digitifera*, *A. secale*, *A. intermedia*, *Pocillopora verrucosa*, *Porites mannarensis*, *P. exserta* and *Goniopora stutchburyi* are common in these islands. Species such as *Montipora millepora*, *M. jonesi*, *M. manauliensis*, *M. edwardsi*, *M. exserta*, *Porites exserta* and *P. mannarensis* are reported only form Gulf of Mannar and Palk Bay. All coral species are protected under Schedule-I of the Indian Wildlife Protection Act (1972).

The following conservation and management strategies are outlined for Indian coral reef management.

- Identification of marine protected areas and their demarcation and protection.
- Coral Reef Monitoring Action Plans prepared and launched. Other significant international activities such as the Coral Reef Degradation in the Indian Ocean (CORDIP), India–Australia Training and capacity building programme (IATCB), initiated.
- National wide mapping of coastal areas by remote sensing techniques combined with land surveys to assess the rate of degradation initiated.
- Amendment and enactment of National policies (National Biodiversity strategy and Action Plan and National Biodiversity Bill) with relevance to the protection of respective ecosystem.
- Export trade control order.

II. Seaweed Ecosystem

About 90% of the species of marine plants are algae and about 50% of the global photosynthesisis algal-derived. The health of seaweeds is directly related to health of coastal and marine ecosystem. Based on their pigmentation, the seaweeds are broadly classified into Chlorophyceae (Green algae), Phaelphyceae (Brown algae) and Rhodophyceae (Red algae). In marine ecosytem the ecological services of seaweed is numerous viz., provide shelter for fish, invertebrates and food.

Seaweeds are used as food, feed, fodder and bio fertilizers, besides they form a source of iodine and bioactive substances. The important polysaccharides like agar-agar, alginic acid, and carrageenan are also obtained from the seaweeds. About 101 species of seaweeds are used for extraction of phycocolloids; out of them 33 are used for Agar, 27 are for Carrageenan and 41 for Alginates production.

Tamil Nadu has a 1076 km coastline. A recent survey encountered 282 species of which 146 were from Rhodophyta, 80 from Chlorophyta, and 56 from Ochrophyta. Gulf of Mannar supports around 181 species of seaweeds, comprising green algae, brown algae, red algae and blue-green algae. About 17 economically important species from agarophytes,carrageonophytes, alginophytes and edible seaweeds are recorded in this area. Commercial cultivation of *Kappaphycus alwarezii* is being carried out in the Palk Bay which is one of the important ingredients in the soft drink. The algal productive area along the coast line from Mandapam Camp to Kanyakumari is put at 17,125 ha. The stand crop estimate is about

22,050 tons within limited zones of intertidal area for the coastal stretch from Mandapam till Kanyakumari. *Gelidiella acerosa* is the most exploited species.

It is also interesting to note that most common red seaweeds of industrial importance *Gracilaria edulis* and *Gelidiella acerosa* (Rhodophyta)) have become locally extinct from some of the islands of the Gulf of Mannar due to the indiscriminate and unsustainable harvesting, whereas their natural resources existed in plentiful abundance a few decades ago. Although, it is very important to understand how species respond to anthropogenic activities, previous studies were only taxonomic or floristic account.

Seaweed Biodiversity Research Gaps and Challenges

- Filling Geographic Gaps to Comprehensively Document Species Diversity and Biomass
- Improving Data Access to the Scientific Community
- Expertise Short-Cut and the Need for Capacity-Building and Nationwide Expertise
- Developing Integrated Taxonomy

With only ~10% share in global seaweeddiversity and ~0.01% in farming, India still assume significant importance, as more than 20% of itscoastline is occupied by two island territories namely Andaman & Nicobar and Lakshadweep andlargely unexplored for its seaweed diversity. It may also be noted that, establishment of 100 marineprotected areas located in islands and costal union territories of India, coupled with identificationof nine critical habitats provide strong legal frameworks for protection and conservation of thiseconomically important resource.

III. Seagrass Ecosystem

Sea grasses are flowering plants from one of four plant families (Posidoniaceae, Zosteraceae, Hydrocharitaceae or Cymodoceaceae). They are productive near shore habitats that host many economically and ecologically important species. Sea grasses regulate water column dissolved oxygen, modify the physical and chemical environment, stabilize sediments,slow water movements and trap heavy metals and nutrient rich runoff, thus improving the water quality for coastal environment and associated communities.There are 15 species of sea grasses reported from the Gulf of Mannar and Palk Bay region.

1. *Enhalus acoroides*
2. *Halophila ovalis*
3. *H. beccari*
4. *H. ovata*
5. *H. stipulacea*
6. *Thalassia hemprichii*
7. *Cymodocea serrulata*
8. *C. rotundata*
9. *Halodule uninervis*
10. *H.pinifolia*
11. *H. wrightii*
12. *Halophila decipiens*
13. *H. ovalis var: Ramamurthiana*
14. *Ruppia maritima*
15. *Syringodium isoetifolium*

The seagrass species, *Halodule uninervis* is extensively distributed in Gulf of Mannar and is the dominant and primary species in the intertidal belt. It occurs both on sandy and muddy substratum with a thin layer of sand. It isalso observed on coral debris. *H. uninervis* plays an important role both as stabilizers and sediment accumulator and occurs either as a bed of monospecific community or a mixed vegetation with *Cymodocea rotundata*, *Cymodocea serrulata*, *Halophila ovalis* and *Enhalus acoroides*. *Cymodocea serrulata* occurs extensively in most of the islands of Gulf of Mannarand forms a significant browsing ground for the endangered dugong. *Thalassia hemprichii* and *H. uninervis* beds are the important habitat for Holothurids commonly known as sea cucumbers. The studies on seagrass in Gulf of Mannar are very limited and the baseline data has to be collected recently on the status, density, diversity and distribution.

Palk Bay is ashallow tropical marine water body wedged between Sri Lanka and India. It is connected to the Arabian Sea on its west through the Gulf of Mannar, and with the Bay of Bengal directly on its east. So far, 14 species of sea grasses are recorded from this area. The Palk Bay has more extensive sea grass growth compared to Gulf of Mannar because of its topography and sediment texture. The sea grass beds are present from the shore towards the sea up to 9 km distance. The area between Pamban and Athiramapattinam has approximately 254 km2 seagrass cover with dominant species, *Thalassia hemprichii*, *Syringodium isoetifolium* and *Cymodocea serrulata*.The luxuriant seagrass meadows in Gulf of Mannar and Palk Bay form a significant grazing ground for the sea cow, *Dugong dugon* and support the high number of dugong population presently in India.

The present major threat to sea grass meadows in Gulf of Mannar and Palk Bay is destructive fishing activities, deterioration of water quality and climate change.

IV. Mangrove Ecosystem

Mangroves are a group of trees and shrubs that live in the coastal intertidal zone.There are about 80 different species of mangrove trees. All of these trees grow in areas with low-oxygen soil, where slow-moving waters allow fine sediments to accumulate. Mangrove forests only grow at tropical and subtropical latitudes near the equator because they cannot withstand freezing temperatures.Mangrove forests stabilize the coastline, reducing erosion from storm surges, currents, waves, and tides. The intricate root system of mangroves also makes these forests attractive to fish and other organisms seeking food and shelter from predators. Mangroves, Sea grass and Salt marsh ecosystems are called Blue carbon ecosystems that can be up to 10 times more efficient than terrestrial ecosystems at absorbing and storing carbon long term, making them a critical solution in the fight against climate change.Overall, 17 species of mangroves and mangrove associate species were reported from 13 estuaries of the Palk Bay region.

Threats to Mangrove ecosystem

- Land reclamations for construction activity, aquaculture, agriculture, tourism
- Industrial and domestic pollution
- Port development
- Dumping of all kinds of waste and debris
- Deforestation for fuel wood
- Over harvesting of marine resources

Management issues in Mangrove ecology

- Reduction in freshwater: As the Muthupet mangrove wetlands are situated at the tail end of the Cauveryriverine system, fresh water reaching this region is very minimal due to the construction of many damsupstream of the Cauvery river. This affects agriculture and the nutrient and sediment transport to themangrove environment.
- Silt deposition in the mouth region of the lagoon in the last 20 years has caused shrinking of the lagoon, which ultimately caused the reduction in the migration of the fish, prawn and crabs and their juveniles into the mangrove wetlands.
- Silt deposition in the lagoon: In the eastern region of the lagoon siltation is severe where the depth ofthe water is not even 30 cm during high tide. Due to the shallowness marine fish that seasonally migrateinto the lagoon in large schools for breeding and feeding are no longer seen even near the mouth regionof the lagoon.
- Over-exploitation of the fishery resources in the nearby neritic water by trawlers.
- Restoration of large areas of degraded mangrove forests: It is widely accepted by the key stakeholders,Forest Department as management agency and local community as consumer of the mangrove resources,that restoration of mangrove forests will enhance the fishery potential of the region and also act as acyclone barrier.

V. Lagoon and Wetland Ecosystem

Wetlands are the ecotones or transitional zones between permanently aquatic and dry terrestrial ecosystems. Ramsar convention hasdefined wetlands as "areas of marsh, fen,peatland or water, whether natural or artificial,permanent or temporary with water that is static or flowing, fresh, brackish or salt, including areas of marine water the depth of which at low tide does not exceed six meters". A wide variety of wetlands like marshes, swamps, open water bodies, mangroves and tidal flats and salt marshes etc. exists in our country.

Lagoons form a particular type of natural capital which generates use values (fish, shrimp, fuelwood, salt, fodder, ecotourism, anchorage, recreation, etc.) and nonuse values (habitat preservation, biodiversity, ecosystem linkages, etc.) contributing positively towards improving the human wellbeing.

Gulf of Mannar and Palk Bay includes two lagoon and wet land ecosystems combined it is geographical range viz., Muthupettailagoon and Gulf of Mannar lagoon. Muthupet is the largest mangrove wetland in Tamil Nadu covering an area of 11,900 hectares. It constitutes the western limit of the Ramsar Site and is located 50 km to the west of Point Calimere Wildlife Sanctuary. The wetland comprises of mangroves, creeks, a lagoon and mudflats. *Avicennia marina* is the dominant mangrove species in Muthupet and accounts for about 95% of the vegetative cover.

Ecological services of Wetlands and lagoons

Ecosystem services offered by wetlandsinclude floodwater storage and control, rechargeof aquifers, treatment of waste water andpollution abatement, general water qualityimprovement, habitats for fish, wildlife andseveral other animals and plant species, andbiological productivity. In addition, wetlands areof high aesthetic and heritage value providingopportunities for recreation, research, andeducation.

VI. Other bio-resources

Sea turtle diversity

The Gulf of Mannar is the onlyecosystem in India where all 5 sea turtlespecies have been reported. These are the olive ridley (*Lepidochelysolivacea*), green (*Cheloniamydas*), hawksbill (*Eretmochelys imbricate*)and leatherback (*Dermochelys coriacea*).The Loggerhead turtle (*Caretta caretta*). All the sea turtles thatoccur in these coastal waters are protected under Schedule I of the Indian Wildlife ProtectionAct (1972), as well as listed in Appendix I of Convention of International Trade inEndangered Species of Wild Fauna and Flora (CITES) which prohibits trade in turtleproducts by signatory countries. At present there exists no commercial or international tradeof marine turtles or turtle products in India. However, incidental capture in trawls is a well-knowncause of mortality for sea turtle.

Sea cow Habitat

Dugong dugon are commonly known as sea cows and are the only species in thegenus Dugong which comes under the order Sirenia. In India, the dugong occurs in the Gulfof Mannar and Kutch, the Palk Bay and in the Andaman and Nicobar Islands. All these areashave sea grass beds, which are good foraging ground for the Dugongs. The most favoreddugong habitats are the Gulf of Mannar and Palk Bay.Dugongs are sea grass specialists,uprooting whole plants when they areaccessible, but feeding only on leaves when thewhole plant cannot be uprooted. Dugongsprefer sea grasses, the genera Halophila andHalodule, which are lowest in fibre and highestin available nitrogen and digestibility. Sea cow is the flagship species of Gulf of Mannar and Palk Bay ecosystems.

Sea horse diversity

Seahorses are fish belonging to the Syngnathidae family which also includes seadragons, sea moths, and pipe fish. Seahorses are a saltwater vertebrate fish belonging to theorder Perciformes, family Syngnathidae, meaning with jaw, genus Hippocampus, literallyhorse of the sea. 4 species of sea horses and 7species of pipefish are found to occur in Gulf ofMannar region. Most Seahorses are found incoastal waters, typically at depths of 1 - 15 meters, occurring in relatively sheltered environmentsamong seagrasses, kelp beds, rocky reefs,mangroves and coral reefs. Unfortunately these aresome of the most vulnerable of marineenvironments, highly susceptible to disturbancecaused by human activities. Seahorses feed onbrine shrimp, tiny fish and plankton.

Sea cucumbers diversity

Sea cucumbers are economically andecologically important echinoderms, which areexclusively marine and inhabit in habitats suchas rocky shores, sandy beaches, muddy flats,coral reefs, mangrove swamps, sea grass andsea weed beds. In Gulf of Mananr, 28 specieshave been reported and among this only seven are commercially important. The Ministry ofEnvironment and Forests, Government of India, imposed a total ban on both fishery and tradeof sea cucumbers and also listed all sea cucumber species under Schedule 1 of the Wild LifeProtection Act of 1972 since 2001.

Gastropod and Bivalve diversity

The Gulf of Mannar is rich in Molluscan diversity and mainly gastropods are being regularly exploited. Studies on the Gulf of Mannar pertaining to the Molluscan diversity revealed, 484 species of molluscs from this region, out of which 260 species are gastropods.Bivalves offer one ofthe important examplesof marine resourcemanagement along theIndian coast. However,apart from the restrictionon the pearl oyster fishery by the Government of Tamil Nadu, and the management measures on the short neck clam fishery of Ashtamudi Lake, Kerala, there are no regulations for effective utilizationand conservation of these sedentary marine resources.

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AN OVERVIEW ON COMMERCIALLY IMPORTANT PELAGIC FISHERY RESOURCES OF GULF OF MANNAR AND PALKBAY

Vinothkumar R., L.Remya, M.Rajkumar and S.Thirumalaiselvan
Mandapam Regional Centre of CMFRI

Introduction

Pelagic fishes are highly migratory and exhibit shoaling nature. They inhabited in the water column (not near the bottom of the shore) of the coast or open oceans. Based on the deepness of the water in which they exist, can be classified into coastal and oceanic fish. The "coastal resources" are called the groups like sardines, anchovies and shads which inhabit above the continental shelf,and oceanic resources are called Tuna, swordfish, and mackerel which inhabited below the continental shelf they are. There is no clear demarcation of the boundary between coastal and ocean waters. Some oceanic species become coastal inhabitant (some tunas) due to migration or lifecycle stages and vice versa. However, true oceanic species spend their entire life in the open ocean. The pelagic fishes form different taxon because of its significance in species richness and its abundance. The commercially important pelagic groups of Gulfs of Mannar and PalkBayare Lesser Sardine, Oil sardine, Other Sardines, Hilsa, Anchovies, Mullets, Wolf herring, Halfbeaks, Full beaks, Ribbonfish, Barracudas, Carangids, Mackerels, Seer fish, Tunas, Flying fish, Swordfish andBillfishes.

Capture Methods - Craft and Gear

The crafts used for exploiting the resources along this coast are Catamaran, Non-motorized boat (Vathai), Motorized Fibre Reinforced boat, Inboard/outboard engine fitted crafts (Vallam). Mechanised crafts like trawls, purse-seine, and gillnets used for exploit of the fishery. Along both coast, trawls are the most common method for exploiting the fishery resources. The single-day trawl or multiday trawl classified Based on a number of days' operation.The gill nets and trawls(Shrimp trawls/Fish trawls) are also commonly used gear and based on the depth of the gear, mesh size, a number of floaters and sinkers it can be varied according to the species usually they catch. The Paruvalai / Choodaivalai used all along the coast. The common traditional methods are traps, shore seine.

1. Belonidae (Needlefishes)

The fishes are Small to medium-sized (up to 2 m) with elongate bodies. Head with both upperand lower jaws extended into long beaks filled with sharp teethdorsal and anal fins posterior in position; pelvic fins located in an abdominal position; pectoral fins short.Lateral line running down from pectoral-fin origin and then along the ventral margin of the body.

Scales small, cycloid (smooth), easily detached. The fishery formed by eight species belonging to Four genera along the Indian waters. The commercially important species which support the local fishery namely *Ablenneshians, Platybeloneargalusplatyura, Strongyluraleiura, Strongylurastrongylura, Tylosurusacusmelanotus*and *Tylosurus crocodilus crocodilus*. The fishery mostly exploited by Modified gillnet and Mini trawl (ThalluMadi).

2. Carangidae (jacks, pompanos, jack mackerels, runners, and scads)

Carangids are called Jacks, and it is small to large and ranges up to 150 cm, and the body shapes extremely variable from elongate, fusiform to deep and strongly compressed. It is extremely variable in size, shape and colour during its developmental stages from juveniles to adult. More than 35 species formed the resource. However, few species support the commercial fishery. Scads and yellow stripe scads are the dominated resources along this coast. There is 16 commercially important genus under this family. The commercially important species and reasonable landings are *Parastromateus niger, Alectis indicus, Trachinotus blochii, Scomberoides commersonianus, Megalopsis cordyla, Elagatis bipinnulata, Decaptereus russeli, Alepes djedaba, Selaroides leptolepis, Atule mate, Selar crumenophthalmus, Seriola dumerili, Carangoides chrysophyrs, C. fulvoguttatus, C. bajad, C. Uii, C. headlensis, Caranx ignobilis, C. heberi C. sexfasciatus and Gnathanodon speciosus*. The trawls, gillnets and hook-and-lines are exploiting the carangids.

3. Chirocentridae (Wolf Herrings)

Dorabs are very elongate, highly compressed body lack of scutes along the belly and presence of large canine teeth in both jaws. Having single dorsal fin; pectoral fins set low on the body; pelvic fins about equidistant between the pectoral base and anal origin; caudal fin deeply forked. They are non-shoaling fishes, abundant along both east and west coast. There are two species, namely; *Chirocentrus dorab* and *C.nudus* supported the fishery. Around 50% of the total landing exploited from Palkbay and Gulf of Mannar coast by trawls.

4. Clupeidae(herrings, shads, sardines, hilsa, and menhadens)

Clupeidae is the most valuable family of food fishes in the world.The clupeids are small pelagic having highly migratory shoaling behaviour in nature. These small pelagic are more diverse. The Clupeidae are characteristically small (<50 cm), They have no spines in the fins, one short dorsal fin, deeply forked tails, ventral fins on their abdomens far behind the pectorals, deep bodies, and large scales that slip off at a touch. The clupeids further classified into five subfamilies they are:

- Dussumieriinae (*Dussumieria, Etrumeus)*
- Clupeinae (*Herklotsichthys, Sardinella, Amblygaster, Escualosa & Sardinops)*
- Dorosomatinae (*Nematalosa & Anodontostoma)*
- Alosinae (*Hilsa &Tenualosa)* andPristigasterinae (*Pellona, Ilisha & Opisthopterus)*

1.a. Indian Oil sardine

The oil sardine most important pelagic fishery resource of the country having high commercial importance and account first to the total landings. It is locally called "*Pei chala*" has been targeted and fully exploited by pair trawlers.The seasonal fishery starts from late September to April from PalkBay. The catch focused for fishmeal preparation, and some quantities sent to Kerala for fresh consumption.

1.b.Lesser sardines

Thirteen species formed fisheryall along the Indian waters and the dominant species alongthis water are *Sardinella gibbosa*, *S.albella*, and *S.fmbriata*. this fishery mostly exploited by trawls and gillnet

5. Coryphaenidae (Dolphin Fish)

Dolphin fish are moderately slender and laterally compressed, with slightly projecting lower jaw, a massive blunt head, a long, rather high dorsal fin without spines, extending from close behind the head to near the base of the caudal fin, and a widely forked tail. Coryphaena is monogeneric in the family and composed of two species, namely *C. hippurus* and *C. equiselis*. This species forms very rare landing along this water and exploited by gill net and hook and line

6. Engraulidae (White Baits)

Small silvery fishes, usually with fusiform, sub-cylindrical bodies, scutes present along the belly, strongly projecting, and lower jaw characteristically "under slung". No spiny rays in fins; with a single dorsal fin, anal fin short, moderate or very long. Anchovies range from 8 cm (*Stolephorus banganensis*) to 32 cm (*Setipinna brevifilis*) (FishBase, 2017).Five genera include *Encrasicholina*, *Stolephorus*, *Thryssa*, *Coilia* and *Setipinna* are recognisedand form the fisheries al along the Indian waters.

The whitebaits are constituted by two genera the *Encrasicholina*and *Stolephorus*only.The genus *Stolephorus* constitute nearly 70% of the catch. The *Stolephorous indicus* and *S. Commerson* are major landings from this coast. *Setipinna phasa and Setipinna taty*are also a commonly available resource from this group. In the Thryssa group, the commercially important species along this coast are *Thryssa dussumieri*, *T. malabarica*, *T. mystax*, *T. setrirositis* and *T. vitirostris*.

7. Hemiramphidae (Half Beaks)

The fishes are with an elongate body and prolonged lower jaw and short triangular upper jaw. Nostrils in pit anterior to eyes. No spines in fins; dorsal and anal fins posterior in position; pelvic fins abdominal in position, pectoral fins usually short. Lateral line running down from pectoral-fin origin and then back along the ventral margin of the body. Scales moderately large, cycloid (smooth), easily detached. The fishery formed by twenty-two species belonging to five genera along the Indian waters. But in this coast Hemiramphus far is dominant resources contribute more than 98% to the fishery

8. Rachycentriade(King Fish)

Rachycentron canadum is the only species in the family Rachycentridae. It is commonly called as cobia and sergeant fish and having large, moderately elongate fishes with a broad, flattened head, tiny scales, a long, low soft dorsal fin preceded by short spines unconnected by membranes, and a slightly shorter anal fin.This species forms very rare landing along this water and exploited by trawls and hook and line

9. Scombridae(Mackerels, Spanish mackerels, bonitos, Seer fish and tunas)

The Scombrids havean elongate, fusiform and compressed body with a pointed snout and Adipose eyelid sometimes present in some species. Two separated dorsal fins; finlets presentbehind dorsal and anal fins; caudal fin deeply forked,covering hypural plate and at least two small keels oneach side of caudal peduncle. The lateral line is simple. The bodyeither uniformly covered with small to moderate scales (e.g. *Rastrelliger, Scomber, Scomberomorous*) or acorselet developed and rest of body naked (*Auxis, Euthynnus, Katsuwonus*) or covered with small scales (*Thunnus*). There are 15 genera, and about 50 species forms the fishery all along the Indian water.Mackerels, Spanish mackerels, bonitos, and tunas form the basis of important commercial fisheries. All scombrids are excellent food fishes. They are the most relished fishes with very high market demand. The trawls, gillnets and hook-and-line exploit the Scombrids.

a.Seer Fishes

The Five major species supported the resource and fishery in Indian waters. The species *Scomberomorus commerson* one of the dominant and commercially important fishes among the seer fish along this coast. The other species,namely, *S.guttatus*, *Acanthocybiumsolandri,* are the least support to the fishery.

b.Mackerel

Three species stand for fishery namely*Rastrelliger kanagurta*, *R.brachisoma* and *R.faugni*in Indian waters. However,Indian mackerel *R. kanagurta* supported more than 98% landing, along this coast

c.Tunas

Tunas are fast swimming, and highly migratory pelagic fisheshave a cosmopolitan distribution. Nine species are belonging to the sixgenera.The *Katsuwonus pelamis*, *Thunnus albacore* and *Euthynnus affinis*are the three-dominantspecies along this coast.

***Scomberomorous commersonii* catch at Pamban Therkuvadi**

***Hemiramphus*far catch at Pamban lighthouse**

Seer fish catch from Shore seine Valinokkam

Mackerel catch from Pamban Therkuvadi

10. Sphyraenidae (Barracudas)

Body elongate, subcylindrical or slightly compressed (size to 170 cm);covered with small, cycloid scales. Headlong, pointed, scaly on sides and posteriorly on top. Mouth large with elongated Jaws having sharp, flattened orconical teeth with sharp canines near the tip

ofthe lower jaw. Two short dorsal fins, widely separated; Caudal fin forked; Lateral line welldeveloped, straight. This Carnivorous pike-like, fishes distribute both tropical and temperate regions. The Juvenile barracudas frequentlyoccur in small to large schools. The fishes are commercially important and edible among the pelagic resources. The fishery formed by more than five species, namely *Sphyraena barracuda*, *S. Putname*, *S. jello*, *S foresteri*, *S. obtusata*, and *S. flavicuda*.The fishery mostly exploited by trawls and hook and line.

***Istioptereus platypterus* catch at Pamban lighthouse**

***Caranx* sp. catch at Rameshwaram**

***Caranx* sp. catch at Pamban Therkuvadi**

Bycatch landings at Jegathapattianm

11. Trichuridae (Ribbon Fishes)

Ribbon fishes are extremely elongate and laterally compressed and ribbon-like fishes. Mouth large and lower jaw usually projecting a dermal process at the tip of each jaw; strong canine teeth in jaws, those at the front of upper jawfang-like; maxilla concealed by preorbital bone. Dorsal fin low and long, anal fin low or reduced to short spinules; pectoral fins short andlow on the body; pelvic fins reduced or completely absent (*Trichiurus* and

Lepturacanthus); caudal fin either small and forked or absent, the body tapering to apoint. Single lateral line with the absence of scales. The fishery was formed by six species and dominated by *Trichiurus lepturus* and *Lepturacathus savala* along this coast. The fishery mostly exploited by trawls

12. Miscellaneous

Other resources which contribute considerable fishery pelagic fishery areBarramundi, Sailfish, Swordfish, Mullets, Milkfish, Tarpons, Lady fishes, Fusiliers etc. They form commercial fishery at varying levels in certain areas.

Conclusion

Fishing pressure on marine ecosystems has increased significantly all over the world, particularly indeveloping countries. In this region increase in fisher population and demand, results shootup of overfishing marine fisheries resources. The enormous fishing pressure will lead to overexploitation. Along Gulf of Mannar and Palkbay management and conservation measures on marine fisheries need to ensure. It will enhance the resource through sustainable use of marine fisheries in future. And also, indigenous management measures willinstigate the community or regional enlargement of fisheries regulation for the sustainability of availed resources.

Demersal fishes of Gulf of Mannar and Palk Bay

Remya L., VinothkumarR., M. Rajkumar and S. Thirumaliselvan

Introduction

India has an extensive coastline length of 8,129 km, continental shelf area of about 0.5 million km^2 and the area available for fish production in the country is vast with 2.02 million km^{2}of EEZ. Gulf of Mannar (GoM) is situated in the Indian Ocean, between south east of India and West of Sri Lanka from Rameswaram (79° 14' East Longitude and 9°14' North Latitude) and Tuticorin (78° 9' East Longitude 8°48' North Latitude) on the south-eastern coast of the country (Tamil Nadu).The Gulf is 80–170 miles (130–275 km) wide and 100 miles (160 km) long with a chain of 21 islands stretching from Mandapam to Tuticorin. The Island system and coral reefs spread over this region offer shelter for a variety of marine fauna and flora. The Palk Bay (PB) region lies between 9° 17' N and 100° 18' N latitudes is practically calm except at the on set of northeast monsoon when turbulent condition prevails. The Palk Bay has landmarks between the Point Calimere and RameshwaramIsland as northern and southern borders, respectively. The eastern part of the bay is connected with Srilanka whereas the western part of the bay is the border of the Indian subcontinent (Fig.1). Both mechanised trawlers and non-mechanised vessels carry out the fishing throughout the year in both of these ecosystems. But the shore seine fishing is seasonal in certain areas particularly in the southern region, When the Gulf of Mannar covering its southern portion becomes rough during April to September, the shore seine operations shift to Palk Bay and when the Palk Bay become rough during October-March, the units migrate to Gulf of Mannar. The fish species that are distributed from the sea floor to 5 m depth above are called demersal fishes and those who are distributed from a depth around 5 m above the sea floor to the surface is called pelagic. The major commercially significant demersal fish groups in the GoM and PB is presented in the Figure 2.

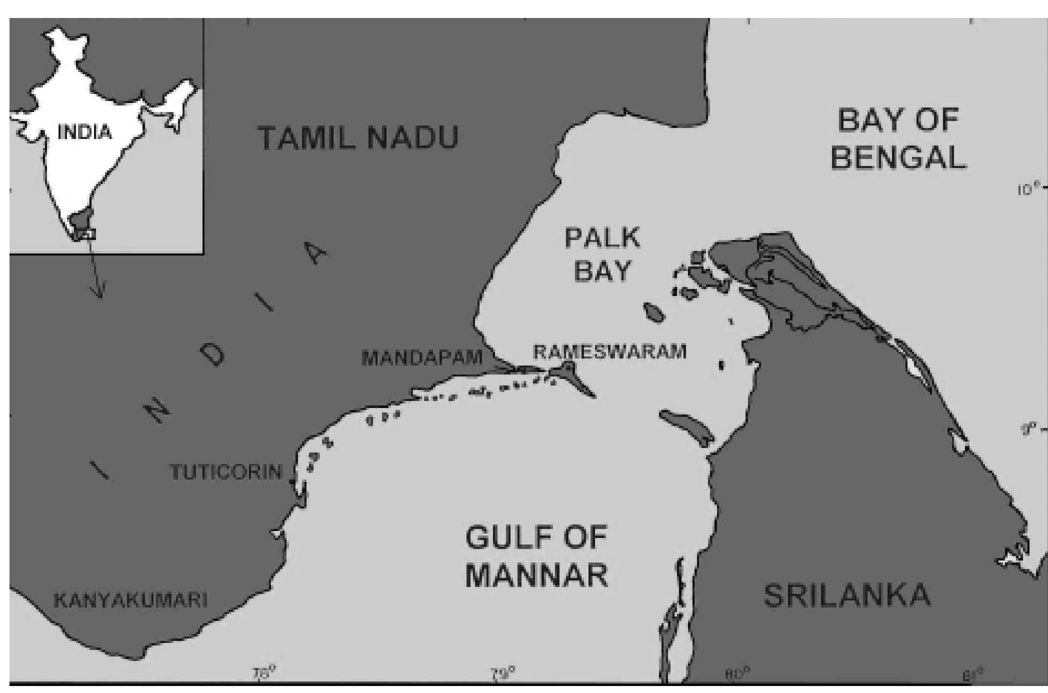


Fig 1. Schematic representation of Gulf of Mannar and Palk Bay

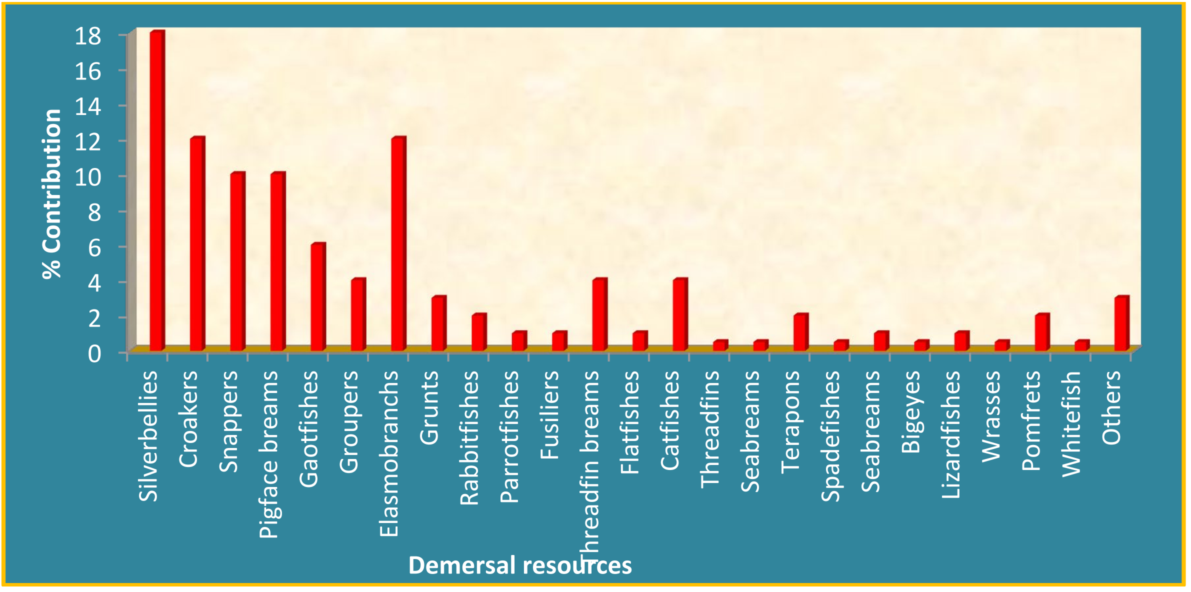


Fig.2. Different resources in the demersal finfish landing from Gulf of Mannar and Palk Bay (Mandapam, unpublished data ICAR-CMFRI)

The major teleost demersal fishes being caught from Mandapam vicinity of PB and GoM are Silverbellies, Croakers, Goatfishes, Emperors, Snappers and Groupers. Hence details of only these major resources are discussed here. In addition to it many other resources such as Threadfin breams, Flatfishes Rabbitfishes, Pomfrets, Whitefish, Terapons, Catfish as well make significant landing either throughout the year or seasonally in the Mandapam waters.

Silverbellies

The Silverbellies comes under the family Leiognathidae of order Perciformes are small to medium sized laterally compressed oblong or round bodied fishes with a bland silvery colouration. Silverbellies are also known as ponyfishes/slipmouths/ toothponies/ slimies due to the presence of highly extensible mouths, and the presence of a mechanism for locking the spines in the dorsal and anal fins. They also possess a luminous organ in their throats, which projects light through the animal's underside. These fishes harbour bioluminescent bacteria in their throats, which emits light through anterio-ventral surface of the fishes. They inhabit the bottom shallow coastal waters and brackish waters in the Indian, West Pacific and Western Central Atlantic oceans. They are exploited by bottom trawls, shore-seines, gillnets, ring-seines, bagnets, etc. These fishes are most commonly marketed as dried salted and in some place they utilized for fishmeal production to feed poultry, especially in Tamil Nadu.

Formerly the ponyfishes were classified into three genera viz., *Leiognathus,Secuter*and*Gazza* based on the direction of protrusibilty of mouth, which extend either upward, horizontal, or downward when open (James, 1983). Whereas the modern classification have 9 genera viz., *Aurigequula, Equulites, Eubleekeria, Gazza, Karalla, Leiognathus, Nuchequula, Photopectoralis* and *Secutor*. The genus *Leiognathus, Eubleekeria,Aurigequula, Equulites, Karalla, Nuchequula*and *Photopectoralis* is characterised with horizontal or pointing downward mouth when protracted (Fig.3) associated with absence of canniform teeth, consumes 59.75% plankton in their diet. Whereas the mouth bare of canine tooth is oblique, pointing upward when protracted and more number of gill rakers for genus *Secutor*, is associated with planktonic diet (81.46%). The presence of canniform teeth and horizontal extension of mouth when protracted are characteristics of the genus *Gazza,* which prefers equal percentages of plankton and benthos.

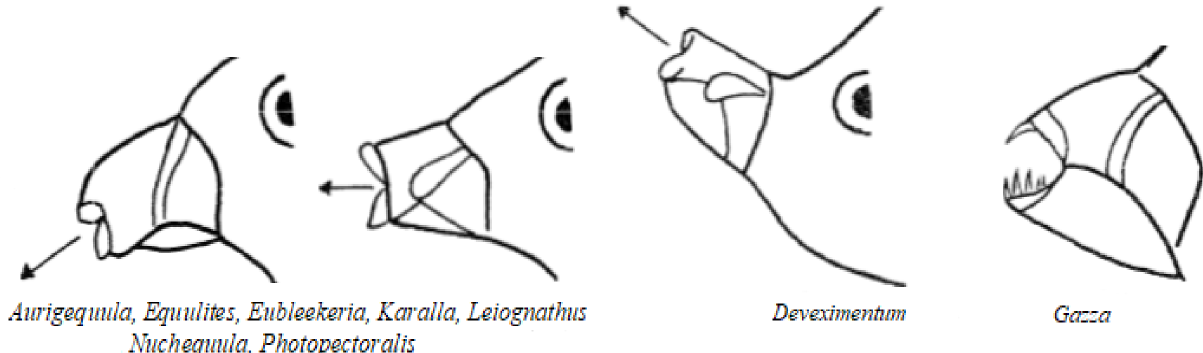


Fig.3 Protrucible snouts of various genera of silverbellies

Croakers

Croakers or drums are so called due to the repetitive throbbing or drumming sounds they make. They are identified externally based on the mouth position and pattern of pores on the snout (Fig. 4). They are primarily coastal fisheslive inshore over sandy or muddy bottoms. Sciaenids are benthic carnivores, feeding on invertebrates and smaller fish. Many croakers use estuarine environments seasonally as nursery grounds during their juvenile phase and as feeding grounds during young adult phase, others are year-round inhabitants of estuaries and coastal lagoons. Seasonally, some species occur in relatively limited geographic areas with large quantities, and move into estuaries or along shorelines; hence local artisanal and subsistence fisheries also exploit them. Croakers often represent a major component of near-shore bottom trawl catches and bycatches. Most croakers are valuable foodfishes, especially the larger species. Gas bladders of bigger sciaenids are used to produce isinglass for industrial use and as an esteemed oriental delicacy. Overfishing (including bycatch) and changing coastal environmental conditions have reduced many local stocks.

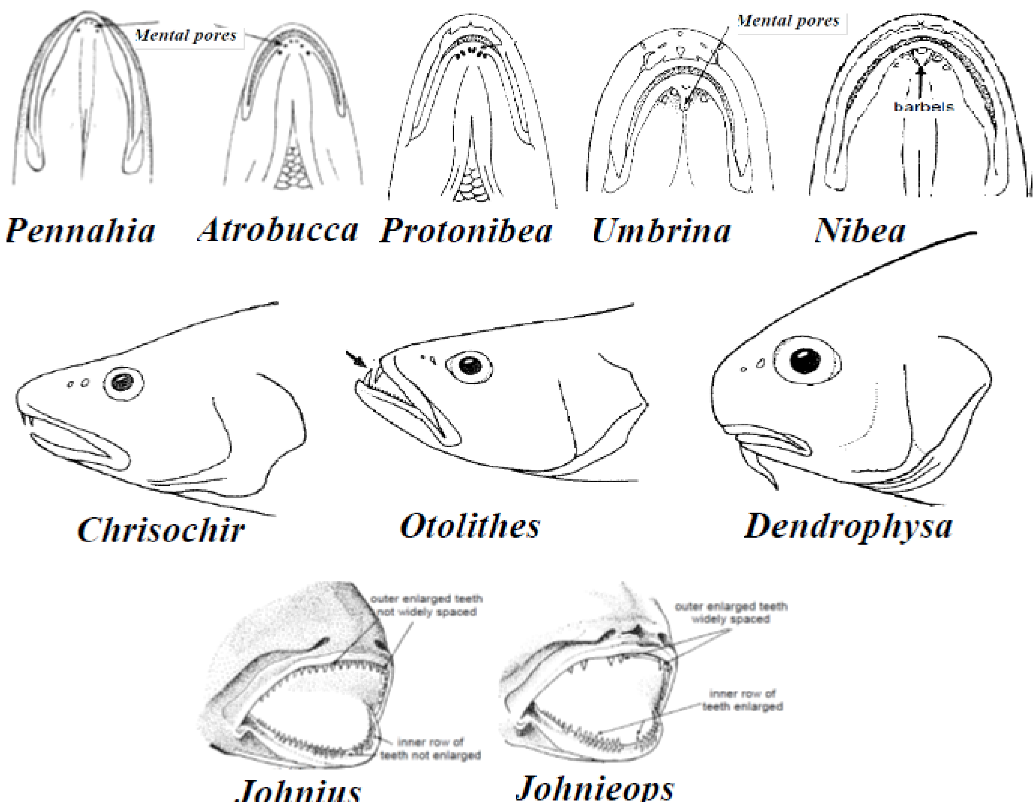


Fig.4 Cephalic and mouth variation in different genera of croakers

Goatfishes

Goatfishes are small to moderate-sized fishes of the family mullidae (order: Perciformes)with two long unbranchedbarbels on chin. Some species have distinctive dark, yellow, orange or brown bands or stripes or blotches on vertical fins and tail. Most goatfishes live in shallow water on open sand or mud bottoms, at least for feeding (though the species of *Parupeneus* and *Mulloides* are often seen on coral reefs). Their barbels, which have chemosensory receptors, are actively moved over or into the sediment to locate food organisms. These fishes often root with their snouts into the sediment for their food. They are carnivorous, feeding on a wide variety of small animals, particularly small crustaceans and worms. A few species prey on small fishes. TheMullidaeconsistsof6genera,distinguished primarily by dentition (Fig.5). Genus Mulloidicthys have very small teeth in 3 rows anteriorly and 2 on side of jaws and absence of vomer or palatines teeth.

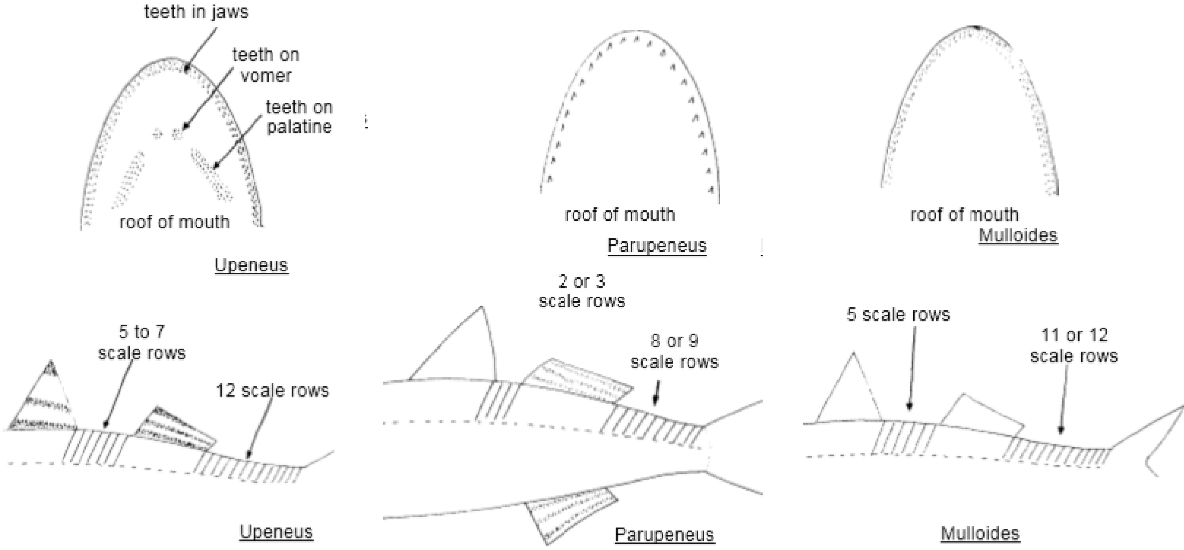


Fig. 5 Dentition, inter dorsal scale pattern and bands on fins of different genera of goatfishes

Snappers

Snappers occur worldwide in warm seas, juveniles of some species enter estuaries and the lower reaches of rivers and a few western Pacific species of *Lutjanus*are inhabitants of fresh waters.They occur from shallow inshore areas to depths of about 550 m, mainly over reefs or rocky outcrops. Snappers are mostly nocturnal predators feeding on fishes, crustaceans (especially crabs, shrimps, stomatopods, lobsters), molluscs (gastropods, cephalopods), and pelagic urochordates. Plankton is particularly important in the diets of those species with reduced dentition and numerous well-developed gill rakers. They are gonochoristic (sexes separate), reaching sexual maturity at about 40 to 50% of maximum length. Populations in continental waters have extended spawning throughout the summer, whereas those occurring around islands spawn throughout the year with peaks in spring and autumn. Snappers are batch spawners, with individual females usually spawning several times in a reproductive season.

The pelagic larvae avoid surface waters during the day, but display a more even vertical distribution at night. Long-living, slow-growing fishes with relatively low rates of natural mortality and with considerable vulnerability to overfishing.The species that reach large sizes are important recreational fishes in some areas.Some species have been reported to be occasionally ciguatoxic in certain areas. They are caught with bottom longlines, handlines,

traps, a variety of nets, and trawls. Distinct characters of various genera of snappers are illustrated in figure 6.

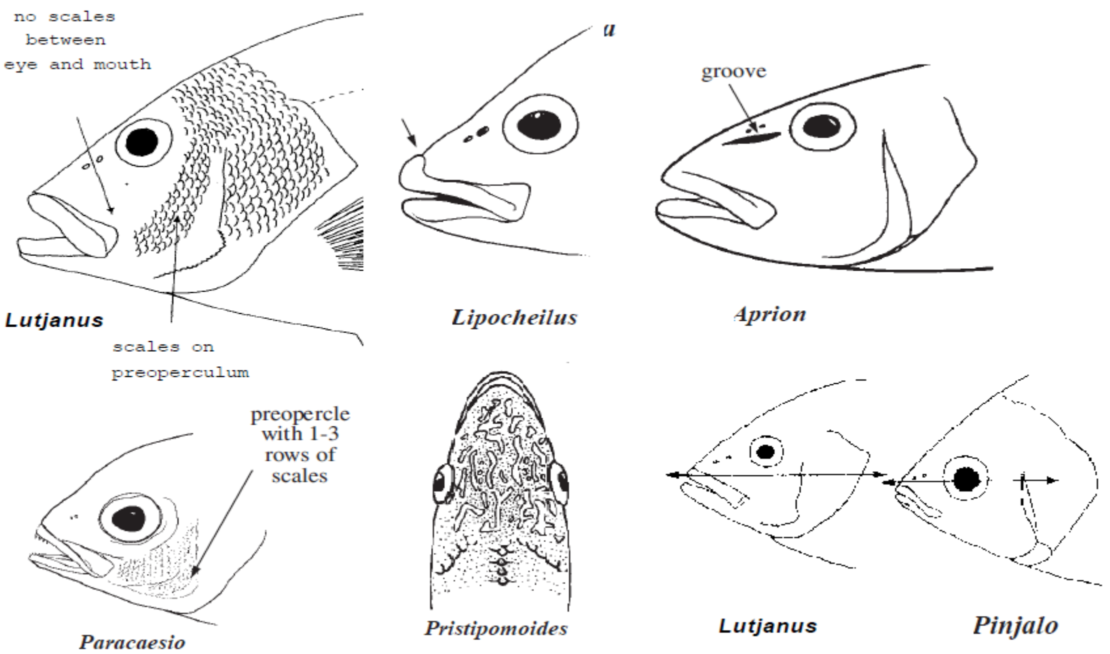


Fig.6 Different genera of snappers

Groupers

Groupers found in the tropical and subtropical waters of all oceans. Most species occur on coral reefs, but some live in estuaries or on rocky reefs. Groupers are generally associated with hard (rocky) bottoms, although juveniles are found in seagrass beds, and adults of a few species prefer sandy or silty areas. Some species occur in depths of 100 to 200 m (occasionally to 500 m) however, the majority inhabits depths less than 100 m, and juveniles are often found in tidepools. As the major predators of the coral-reef ecosystem, most groupers feed on a variety of fishes, larger crustaceans, and cephalopods. A few groupers (e.g., *Paranthias* spp. and*Epinephelusundulosus*) have long, numerous gill rakers and are thus adapted for plankton feeding. Most groupers are ambush predators, hiding amongst the coral and rocks until an unwary fish or crustacean goes by, then catching their prey with a quick rush and snap of their jaws. The distinguishing morphological differences of groupers is presented in the figure 7.

Except for occasional spawning aggregations, most species are solitary fishes. This site specificity and the relatively slow growth rate of groupers make them particularly vulnerable to over-fishing. Groupers are protogynous hermaphrodites and in a mature female, numerous oocytes are arrayed in lamellae surrounding a central lumen, with spermatogenic tissue in small dormant crypts on the periphery of the lamellae. After spawning as a female for one or more years, the grouper changes sex and thereafter functions as a male. At sexual transition, the oocytes degenerate, the spermatogonia proliferate, and the ovary is transformed into a functional testis. Evidence of the ovarian origin of the testes is the remnants of oocytes and the ovarian lumen, which can be seen in cross-sections of the testes.

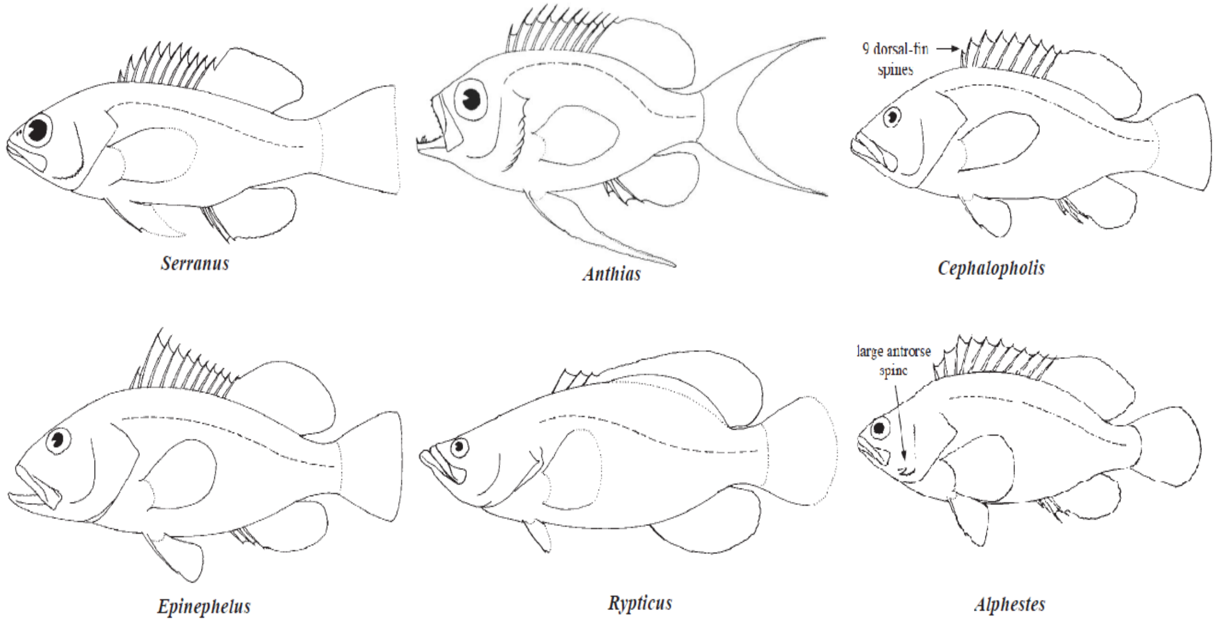


Fig. 7.Morphological differences in various genera of groupers

Pigface breams

Pigface breams or emperors are protogynousperchlike fishes with a large head and deep suborbital space (Fig. 8). These coastal fishes are abundant in the tropical Indo-West Pacific and West Africa caught primarily by handline, traps, and trawls.*Lethrinus* spp. exhibit protogynous hermaphroditism and predominantly inhabit back-reef seagrass beds at the juvenile life stage thereby utilizing the beds as a potential nursery habitat that produces relatively more adult recruits per unit area than other juvenile habitats. Afterwards, juveniles move to coral reef habitats at maturity, and adults often form large aggregations for pelagic spawning at outer reefs. The diet consists of echinoderms, crustaceans, molluscs, fishes, and polychaetes.

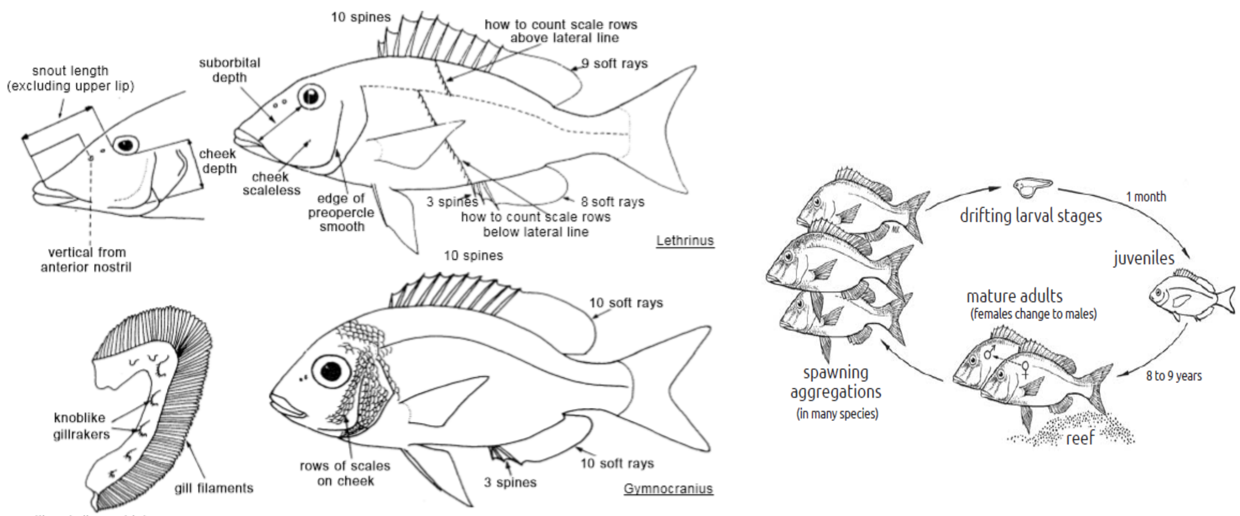


Fig. 8 Morphological characters of emperors

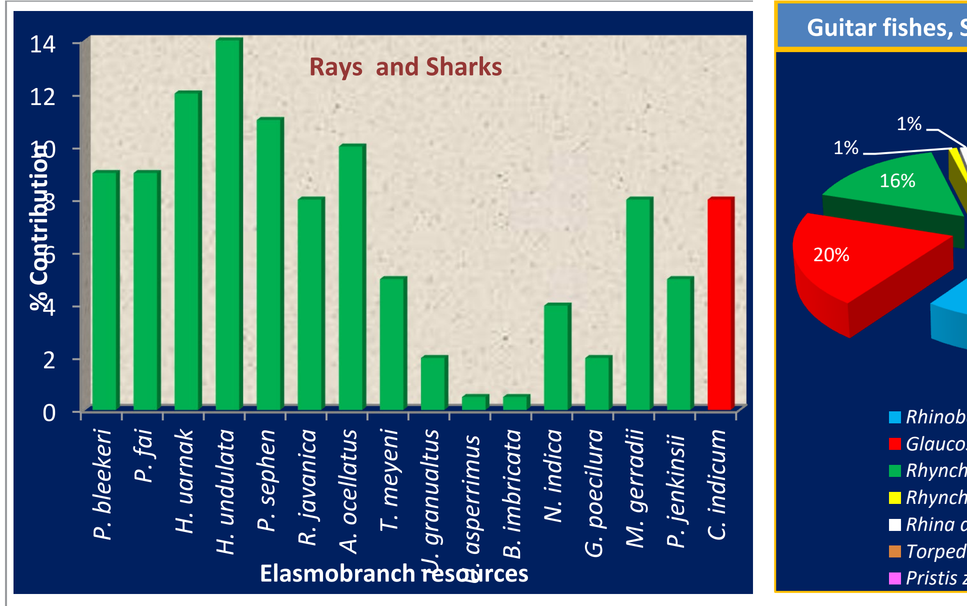
Life history of emperors

Elasmobranch

The name elasmobranch represents any of numerous cartilaginous of the Class Chondrichthyes, having 5 to 7 gill slits on each side, dermal denticles for scales and a small respiratory opening called a spiracle behind each eye (Fig.10 and 11). The class is divided into two subclasses;Elasmobranchii (sharks, rays, skates, and sawfish) and Holocephali (chimaeras). The pectoral fins of Elasmobranchs are often greatly enlarged. Their upper jaws are also fused with their skull and instead of the replaceable rows of teeth they have 3 pairs of large grinding tooth plates. Hearing is often the first sense to detect prey at long distances and it is used in conjunction with a special sensory organ called the lateral line which is a line of pressure detectors that runs down each side of their body. These detect changes in water pressure that might result from struggling prey. Around their snout and mouth are more special sense organs known as the 'Ampullae of Lorenzini' which detect electric fields given off by prey

The feeding habit ranges filter feeding of plankton to highly predatory carnivorous feeding. All Elasmobranchs exhibit internal fertilization when reproducing hence occurs as either male or female. Female sharks have no obvious external reproductive structures, whilst males have two extensions of the pelvic fin known as claspers. These claspers are used by males during reproduction to internally fertilize the female sharks. Some sharks and all skates lay egg cases on the sea bed or wrapped around seaweed called oviparity

The elasmobranch resources available in the PB and GoM are given in the Fig.9. Various ray species are *Rhinopterajavanica, Pateobatisjenkinsii, P. fai, P. bleekeri, Maculabatisgerrardi, Aetobatusocellatus, Brevitrygonimbricata, Himanturauarnak, H. undulata, Urogymnusgranulatus,U. asperrimus, Neotrygonindica, Pastinachussephen, Taeniuropsmeyeni*and*Gymnurapoecilura*. The only shark landing regularly in the PB water is *Chiloscylliumindicum.* Different guitar fishes being caught from the GoM and PB are *Rhinaancylostoma, Rhinobatosannandalei, Glaucostegus granulates, Rhynchobatusaustraliae, Rhynchobatusdjiddensis* and *Pristiszijsron.* One of the common electric rays landing in the PB waters is *Torpedo marmorata.*



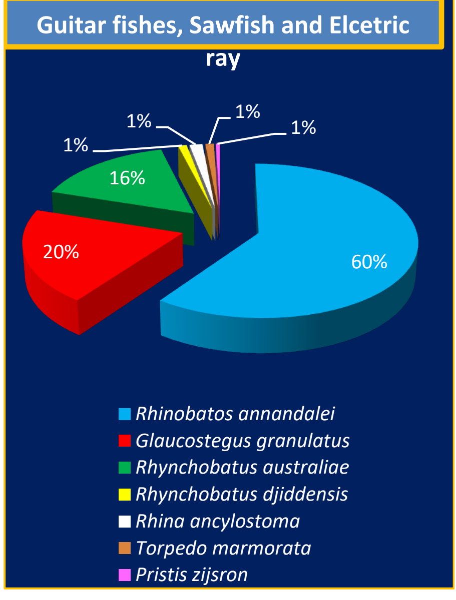


Fig.9. Various elasmobranch resources present in the GoM and PB (Mandapam, unpublished data ICAR-CMFRI)

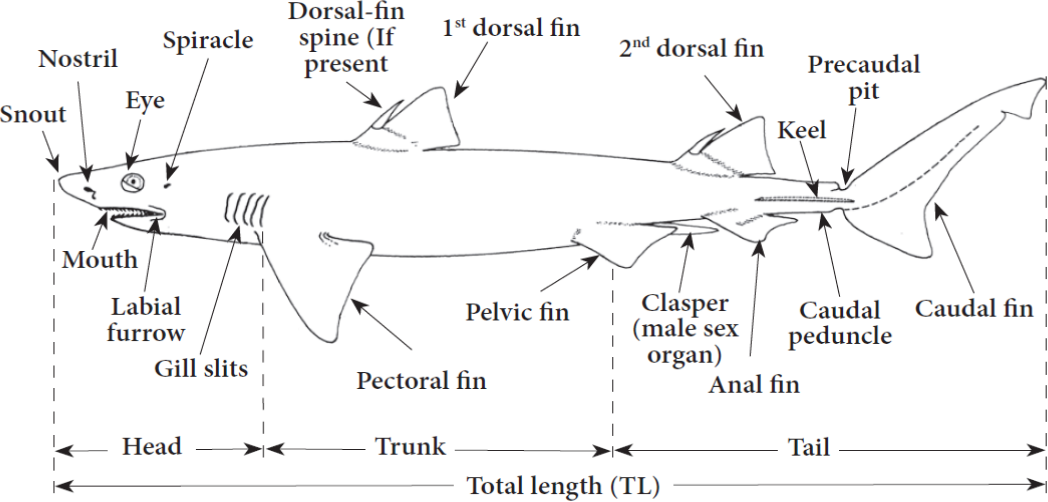


Fig.10 Technical terms and principal measurements used for shark

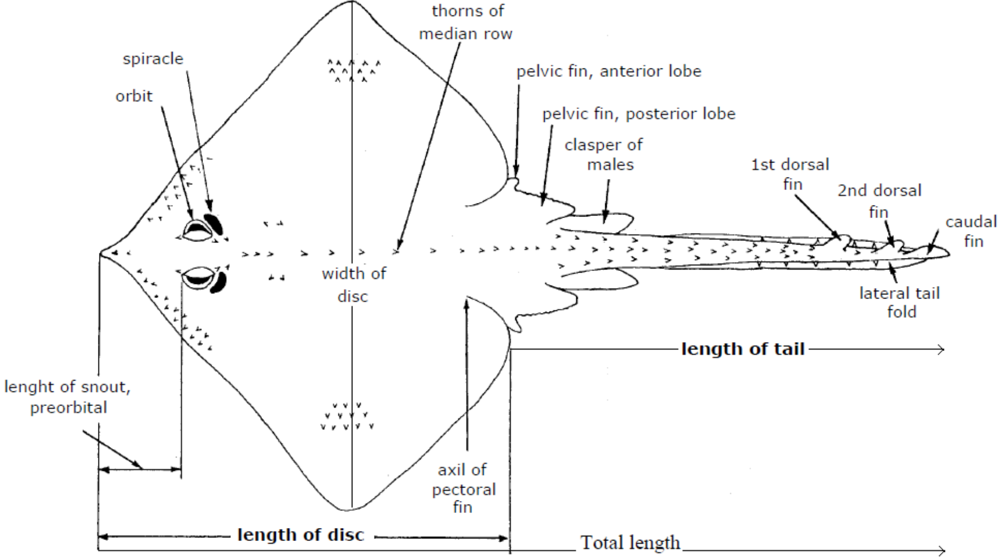


Fig.10 Technical terms and principal measurements used for rays, skates, sawfishes and guitarfishes

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Taxonomy and Biology of commercially important Species of Shrimps, Crabs and Lobsters

M.Rajkumar, L.Remya, R.Vinothkumar & S.Thirumalaiselvan

Introduction

The crustacean fisheries of India have considerable importance in the economy of the country, earning very valuable foreign exchange. Edible marine crustaceans consisting of prawns, lobsters and crabs form the most important constituents of the commercial fish landings of India. Shrimps and prawns constitute a large group of crustaceans varying in size and are widely distributed in marine, brackish, and freshwater regions from the equator to the Polar Regions. Although the majority of the commercial marine species occupy shallow or moderately deep water areas along the continental shelves at depths of less than 100 m, some are found at depths of nearly 5700 m. Many prawns are pelagic but the majority by far is benthic, living on a large variety of bottoms such as rock, mud, peat, and sand, fragments of shells or mixtures of these materials. At present, only slightly less than 300 species of shrimps and prawns are of economic interest worldwide, and out of these, only about 100 comprises the principal share of the annual world catch. The following species are commercially exploited for fishery in Palk Bay and Gulf of Mannar.

Family-Penaeidae

Penaeus monodon

Rostrum straight, toothed on both ventral (generally 3 teeth) and dorsal sides. Sub-hepatic ridge oblique. Petasma symmetrical and consists of two simple lobes united at the upper edge by hook-lets. Thelycum sub oval in shape, posterior process triangular. Pale yellow and dark brown bands on the abdomen. Uropods with pale yellow to pink median transverse bands.

Penaeus indicus

Rostral teeth on ventral (3 to 6) and dorsal side. Body white or cream in colour. Adrostral crest ending just before epigastric tooth. In males distal segment of third maxilliped as long as the second segment and bear long tufts of hair at the tip. Sub hepatic ridge in the branchial region lacking. Fifth pereiopod without exopod.

Penaeus merguiensis

The rostral crest is elevated and somewhat triangular in shape. Teeth on rostrum present both on ventral (3 to 5) and dorsal side. Adrostral carina not reaching as far as epigastric tooth. Body colour pale yellow or white. In males the distal segment of third maxilliped half as long as the second segment and bears hair at the tip.

Penaeus semisulcatus

Rostrum curved. Rostrum and abdomen are banded green or grey and white. Mostly the antennae are also banded white and brown. Rostral teeth on ventral (generally 3 teeth) and dorsal side. Adrostral grooves extending just beyond epigastric tooth. Sub hepatic ridge is horizontal. Small exopod present on fifth pereiopod (absent in *P. monodon*).

Penaeus latisulcatus

Rostrum with dorsal and one ventral teeth. Adrorstral crest extends almost to the posterior margin of carapace. Telson with three pairs of movable lateral spines. Vertical black bar on pleuron. Anterior process of thelycum horn like and strongly bifurcate.

Penaeus japonicas

Rostrum with dorsal and one ventral teeth. Adrostral crest extends up to near to the posterior margin of the carapace. Carapace with three continuous bands and the band on the last abdominal segment interrupted. Telson with three pairs of movable lateral spines. Thelycum closed infolding laterally forming anteriorly open pocket functioning as seminal receptacle. Distomedian projection of petasma curved forming hood.

Metapenaeus dobsoni

Rostrum extends little beyond the tip of the antennular peducncle. Distomedian projection of petasma with a short filament on ventral surface and another on dorsal surface. Thelycum is long grooved and tongue shaped and ensheathed in a horse-shoe like process formed by lateral plates. Merus of fifth pereiopod in adult males with one or two large triangular teeth.

Biology of penaeid shrimps

They have two phases in their life cycle – estuarine and marine. The post larvae migrate to the estuaries, where they grow to juveniles/adults and return to the sea. Here they mature and spawn and the cycle is repeated. The eggs, larvae and post larvae have pelagic existence and the juveniles/sub-adults and adults are benthic. Several species like *Penaeus monodon, Fenneropenaeus indicus, Metapenaeus dobsoni, Metapenaeus monoceros, Metapenaeus brevicornis* support important fishery in the estuarine systems in India - Hoogly-Matlah in WB, Mahanadi & Chilka Lake in Orissa, Godavari & Krishna in AP, Vellar & Killai backwaters and Pulicat Lake in TN, Cochin backwaters & Vembanad Lake in Kerala; Narmada-Tapthi and Little Rann of Kutch in Gujarat. Penaeid shrimps are carnivorous, females are usually larger than males and have high fecundity which depends on the species, size of the female and ovary weight. They spawn throughout the year, peak seasons varying between years. Their life span is usually 3+ years. The maturity stages in penaeid shrimps are classified as immature (IM), early maturing (EM), late maturing (LM), mature (M) and spent (SP). Stages of maturity can be ascertained externally through the exoskeleton. *Penaeus monodon* attains maximum length of 300 mm. In the backwaters and estuaries they grow to 120 to 130 mm. From inshore waters they are caught in various types of seine nets and from deeper waters in trawls. It is an important candidate species for culture because of its hardiness, fast growth, large size and high market price. *P. indicus* grow to 230 mm in total length and *P. merguiensis* up to 320 mm. *P. semisulcatus* grows to 250 mm total length. It is the most dominant penaeid shrimp species supporting commercial fishery along Gulf of Mannar and Palk Bay on the southeast coast. The maximum size of *M. dobsoni* recorded is 130 mm. *Litopenaeus vannamei* native of East Pacific coast is an introduced penaeid shrimp in India. It grows to a maximum length of 230 mm.

Family- Portunidae

Scylla serrata

Carapace smooth having strong transverse ridges; H shaped gastric ridges deep. Teeth on frontal margin sharp. Nine anterolateral carapace spines of same size projecting obliquely outwards. Carpus of chelipeds with two distinct spines on distal half of outer margin. Colour green to browny black depending on the habitat, outer surface of palm green and often with marbled pattern; last legs marbled both in males and females.

Scylla olivacea

Frontal margin usually with rounded teeth. Carpus of cheliped with only one reduced spine. Carapace smooth, more evenly convex with very low transverse ridges. H-shaped gastric groove shallow. The median pair of the frontal lobes more rounded and projecting slightly forwards of the lateral ones.

Biology of mud crabs

S. serrata is usually found in mangrove areas with high salinity, and also in offshore waters where they spawn, can tolerate reduced salinity also whereas *S. olivacea* prefer low saline water. They are found in low intertidal muddy bottom. The megalopa or postlarval stage migrates to the estuaries and backwaters attain maturity and go to the inshore waters for spawning. Immature and mature males have slender triangular abdominal flaps. Immature females have a broad and triangular abdominal flap and mature females a semicircular flap. They have five zoeal stages and one megalopa stage which metamorphose to the crab instar (seed). They are carnivorous and prefer small molluscs, trash fish and other crustaceans as food.

Portunus pelagicus

Carapace with reticulated markings. Front with four teeth. Inner margin of merus of cheliped with three spines. Nine teeth on anterolateral margin of carapace. Males with blue markings and females with dull green.

Portunus sanguinolentus

Carapace with three brown or purple spots on the posterior half of the carapace, having white border. Nine teeth on anterolateral margin of carapace.

Charybdis feriatus

Five teeth on each anterolateral margins. Longitudinal stripes of brown and white colour with distinct white cross mark on the median part of the gastric region, hence also called commonly as crucifix crabs. The pleopods or swimming appendages are banded white and brown. They grow to very large size.

Biology

They are marine crabs. *P. Pelagicus is* found at a depth of up to 50 m and is caught in trawl and gill nets. They show sexual dimorphism, males being bright blue in colour and females are dull

green. The males grow larger and their chelate legs are longer. They have five zoeal stages and metamorphose to the megalopa followed by the juveniles/seed stage. *Charybdis feriatus* are found at a depth of up to 60 m and are caught mostly in bottom trawl. They have six zoeal stages (stage I to stage VI) which metamorphose to the megalopa stage. They have good market in East Asia where it commands substantially higher premium prices than *Portunus* spp. *P. sanguinolentus* are caught at a depth of 30 m. All the three species prefer sandy to sandy muddy substrates.

Family Palinuridae

Panulirus homarus

Anterior margin of carapace with two frontal horns, Antennular plate bearing four equal well separated large spines, Each abdominal segment with a transverse groove, Body greenish in colour with numerous white spots, Transverse bands absent, Antennules banded white and green, Legs with white spots and stripes.

Panulirus ornatus

Antennular plate with one pair of principal spines anteriorly and a second pair half the size of first. Abdominal segment smooth without transverse grooves. Each abdominal segment with dark pale spot on the outer margin. Abdomen greenish or brownish grey. Legs with alternate bands of black and white bands.

Panulirus polyphagus

Broad antennular plate with one pair of principal spines. Abdominal segments without transverse grooves, having white transverse bands. Legs irregularly blotched creamy white.

Panulirus versicolor

Antennular plate with two unequal and separated spines. Abdominal segments without transverse grooves. Blue black patches and white lines on carapace and abdominal segments. Legs, antennules longitudinally striped. Bases of antennae bright pink.

Biology of spiny lobsters

Panulirus homarus is an important lobster fishery resource in India particularly around Kerala and Tamil Nadu. They are found up to a depth of 90 m and are caught in gill nets, trawls, trammel nets and traps. They use rocky reefs for shelter. *P. ornatus* is found at a depth of 10 to 50 m in sandy and muddy substrates. It is the largest of the *Panulirus* species and can attain a total body length of about 50 cm. The size of lobsters in the fishery ranges from 113 to 233mm TL in males and 128-452 mm TL in females with 41% falling in the size range of 181-190 mm TL, which are juveniles. *Panulirus versicolor* is also a coastal species found up to a depth of 15 m. *Panulirus polyphagus* inhabits coastal waters on muddy and rocky substrates to a depth of 40 m and occasionally seen at 90 m. This species is the most important commercial species contributing to nearly three-fourth of the total lobster catch of the country. Major fisheries are on the northwest coast of India. Size in the fishery range from 75 to 385 mm total length (TL) those between 160 and 230 mm TL forming the mainstay of the fishery in Maharashtra. *P. ornatus* and

P. polyphagus move to deeper waters for breeding. Phyllosoma larvae are planktonic and are carried away by currents. The last stage before becoming juveniles is the peurulus which swims towards the shore for settlement. Spiny lobsters are susceptible to diseases when held at high stocking density or due to stress or injury. Common diseases are white tail, tail fan necrosis and shell disease.

Family Scyllaridae

Thenus unimaculatus– Slipper lobster/sand lobster

Body dorsoventrally flattended, pale brown in colour. Three spines on the antero lateral border of carapace and a notch in middle of each segment. Fifth abdominal segment with a spine on the dorsal side. Tubercles present on the body. Variable purple to black pigmentation (blotch or large or narrow streak) on the inner surface of merus of second and sometimes third legs.

Biology

It forms a fishery in trawlers along the Saurashtra coast, Kollam and Chennai. They burrow in sand and generally feed on molluscs. The phyllosoma stages (I-IV) are completed in 7, 5, 7 and 7 days respectively and the nisto stage in 4 days. The lobsters are usually caught at a depth of 50 m. They form by catch in trawls and are also caught in gillnets.

SEAGRASS, SEAWEED AND MANGROVE ECOSYSTEM OF GULF OF MANNAR AND PALK BAY REGION

S.THIRUMALAISELVAN*, M. RAJKUMAR, R. VINOTHKUMAR, L. REMYA AND S.M. SIKKANNDER BATCHA

Mandapam Regional Centre of ICAR-CMFRI, Marine Fisheries Po, Mandapam camp, Ramanathapuram

GULF OF MANNAR

The Gulf of Mannar, a shallow bay, with a coastline of 364.9 km, is known for its coral reefs and sea grass beds which harbour several endangered species. The Gulf-of-Mannar Marine National Park (GOMMNP) declared in 1986, under the Wildlife (Protection) Act, 1972, covers an area of almost 560 sq. km andincludes 21 islands. The 21 islands are distributed in four groups – Mandapam, Keezhakarai, Vembar and Tuticorin groups and located in 3-6 miles distance from the coast and vary in area from 0.25 ha to 130 ha.The Gulf of Mannar, the first Marine Biosphere Reserve (GOMMBR) in the South and South East Asia, covers from Rameswaram to Kanyakumari in Tamil Nadu, India.The GOMMBR encompasses a chain of 21 islands and adjoining coral reefs off the coasts of the Ramanathapuram and the Tuticorin districts forming the core zone; the Marine National Park and the buffer zone includes the surrounding seascape and a 10 km strip of the coastal landscape covering a total area of 10,500 Km^2, in the Ramanathapuram, Tuticorin, Tirunelveli and Kanyakumari Districts with a long coastline of 364.9 Km.

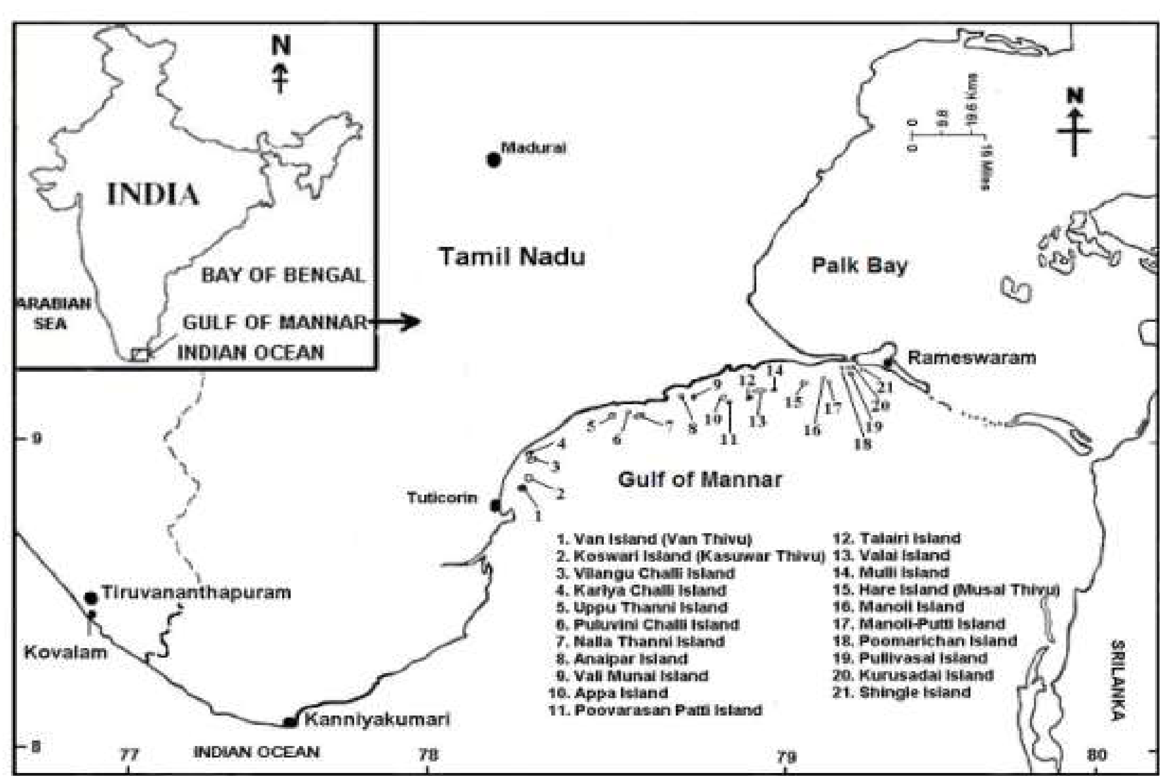


Fig 1: The map of Gulf of Mannar (with 21islands) and Palk bay region

PALK BAY

The Palk Bayis a shallow water area with biodiversity conglomeration nestled between the island nation of Sri Lanka and South East Peninsula India and a coastal length of 250 km on the Indian side. The bay is landlocked with three openings- One big eastern opening into the Bay of Bengal and two narrow openings into the Gulf of Mannar. It borders five coastal districts of Tamil Nadu between Kodiyakarai or Point Calimere in Nagapattinam district to Dhanushkodi in Ramanathapuram district. The width of the bay ranges from 64 – 137 km. The longshore currents from the Bay of Bengal and Gulf of Mannar transport sediments into the Palk Bay adding silt and clay to the shallow sea floor.

SEAWEED RESOURCES

Marine macroalgae or seaweeds are plant like that generally live attached to rocks or other hard substrata in coastal areas. They belong to three different groups based on thallus color, Chlorophyceae, Rhodophyceae and Phaeophyceae. Iyengar (1927) was the firstIndian phycologist to work on Indian marine algae, followed by Boergesen (1933-1938) whocarried out extensive work on the marine algae collected from southIndia, Bombay and Gujarat coast.

Further these resourceswere studied on the distribution, resource assessment, utilization and cultivation of seaweeds of the Indian coast by Krishnamurthy 1985; Krishnamurthy & Untawale 1985;Silas et al. 1986; Chauhan et al. 1990; Kaliaperumal, Kalimuthu 1993; Mairh 1994. Thediversified seaweed assemblages were present in the southeast coast of Tamil Nadu (Mandapamto Kanyakumari, including the islands in the Gulf of Mannar), Gujaratcoast (Okha, Dwarka, Porbandar, Veraval and Diu), Lakshadweep and Andaman – Nicobar Islands.

Table 1: Seaweed Diversity of the Indian Coast

Algae group	Order	Family	Genus	Species
Chlorophyceae	7	19	43	213
Rhodophyceae	16	36	135	431
Phaeophyceae	6	13	37	289
Total	**29**	**68**	**215**	**933**

Source: Kaliaperumal and Kalimuthu, 2004.

The intertidal and shallow subtidal waters, with rocky and coralline substrata of gulf of mannar harbour luxuriant growth of a diverse seaweed flora. The seaweed species of Sargassum, Dictyota, Gracilaria, Gelidium, Cystoseira and Codium mostly inhabit the midlittoral to lower littoral regions of the slope. Upper midlittoral regions are characterized by the occurrence of Ulva, Enteromorpha, Chaetomorpha, Cystoseira and Gelidiella sp.

The important seaweeds collected from the areas around Gulf of mannar are Brown algae-*Gracilaria edulis*(Kanchi pasi), *Gelidiella acerosa*(Marekozhudu), and Red algae - *Sargassamsp*(Kattakorai) and *Turbinaria sp* (Baagoda pasi).These are the native species found in Indian waters. Seaweeds contain different vitamins, minerals, trace elements, protein, iodine, and boiactive substances.The seaweed resources are exclusively utilized for the production of commercially and industrially important phycocolloids such as agar and alginates. While *Gracilaria edulis*and *Gelidiella acerosa*are used in the agar industry and *Gelidiella acerosa* (microbiological quality agar production), *Sargassam sp* and *Turbinaria sp* are used for agarose/alginate industries. The cultured seaweed *Kappaphycus alvarezii* have a quality raw material for carrageenan production.

Table 2: Seaweed Diversity of Gulf of Manner and Palk bay coast

Algae	Genera	Total number of species
Chlorophyta	23	80
Rhodophyta	60	146
Phaeophyta	18	56
Total	101	282

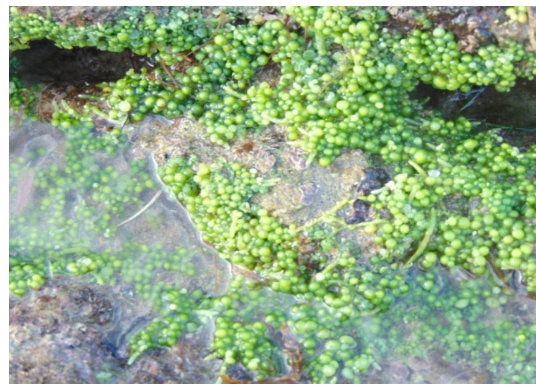
In Mandapam area 180 species of seaweeds are growing, of which about 40 species are economically important. They are the species of *Enteromorpha, Ulva, Caulerpa, Codium* (green algae); *Colpomenia, Hydroclathrus, Cystoseira, Hormophysa, Sargassum, Turbinaria* (brown algae);*Asparagopsis, Gelidiella, Gracilaria, Sarconema, Hypnea, Acanthophora* and *Laurencia* (red algae).

These seaweed resources are available in abundance only in shallow areas around the gulf of mannar islands. *G.edulis* produces edible quality agar and it has grown in any shallow waters on soft substratum such as clay. *Gelidiella acerosa* is available only around corals area of gulf of mannar islands.

Fig 2: Some important seaweed species are listed below.

Ulva lactuca ***Ulva reticulata***

Caulerpa sp



Caulerpa racemosa

Enteromorpha compressa

Halimedagracilis

Cladophora sp

Padina tetrastromatica

Sargassum wightii

Sargassum ilicifolium

Turbinaria conoides *Kappaphycus alvarezii*
Gracilaria verrucosa *Gelidiella acerosa*
Gracilaria edulis *Gracilaria salicornia*

The major threats faced by seaweeds resources are over-exploitation in and around coral reef areas by local fishers,washed ashore due to strong current & wave action, anthropogenic activities, diseases and climate change. Presently seaweed culture practices carried out by local fishers in Palk bay region mainly *Kappaphycus alvarezii*, some area in *Gracilaria edulis* culture in bamboo pole raft methods.

SEAGRASS RESOURCES

Seagrasses are a mixed group of flowering plants which grow submerged in shallowmarine and estuarine environments worldwide. Structurally, seagrasses are more closelyrelated to terrestrial plants, having specialized tissues that perform specific tasks within eachplant. Seagrasses possess true roots that not only hold plants in place, but also are

specializedfor extracting minerals and other nutrients from the sediment. However, they do not possessthe strong, supportive stems and trunks.

The seagrass beds provide food, habitat, and nursery areas for numerous finfish,shellfish and other marine organisms. The sea bottom areas that are devoid of seagrass are vulnerable to intense wave action from currents and storms. The extensive root system in seagrasses, which extends both vertically and horizontally, helps stabilize the sea bottom in a manner similar to the way land grasses prevent soil erosion.Detritus from bacterial decomposition of dead seagrass plants provides food for worms, sea cucumbers, crabs, and filter feeders such as anemones and ascidians.The seagrass meadows provide an ideal environment for juvenile fish and invertebrates to conceal themselves from predators. Seagrass leaves are also ideal for the attachment of larvae and eggs, including sea squirt and mollusc etc.

The vast seagrass beds were present in Palk bay and Gulf of Mannar between mainland and islands and towards seaward side from the islands. The seagrass species, *Haloduleuninervis*is extensively distributed in Gulf of Mannar and is one of the dominant and primary species in the intertidal belt. It occurs both on sandy and muddy substratum with a thin layer of sand. *H.uninversis*plays an important role both as stabilizers andsediment accumulator and occurs either as a bed of monospecific community or a mixedvegetation with *Cymodocearotundata*, *Cymodocea*serrulata, *Halophila ovalis* and *Enhalusacoroides*. *Cymodoceaserrulata*occurs extensively in most of the islands of Gulf of Mannarand forms a significant browsing ground for the endangered dugong. *Thalassiahemprichii*and *H. uninervis*beds are the important habitat for Holothurians commonly known as seacucumbers.

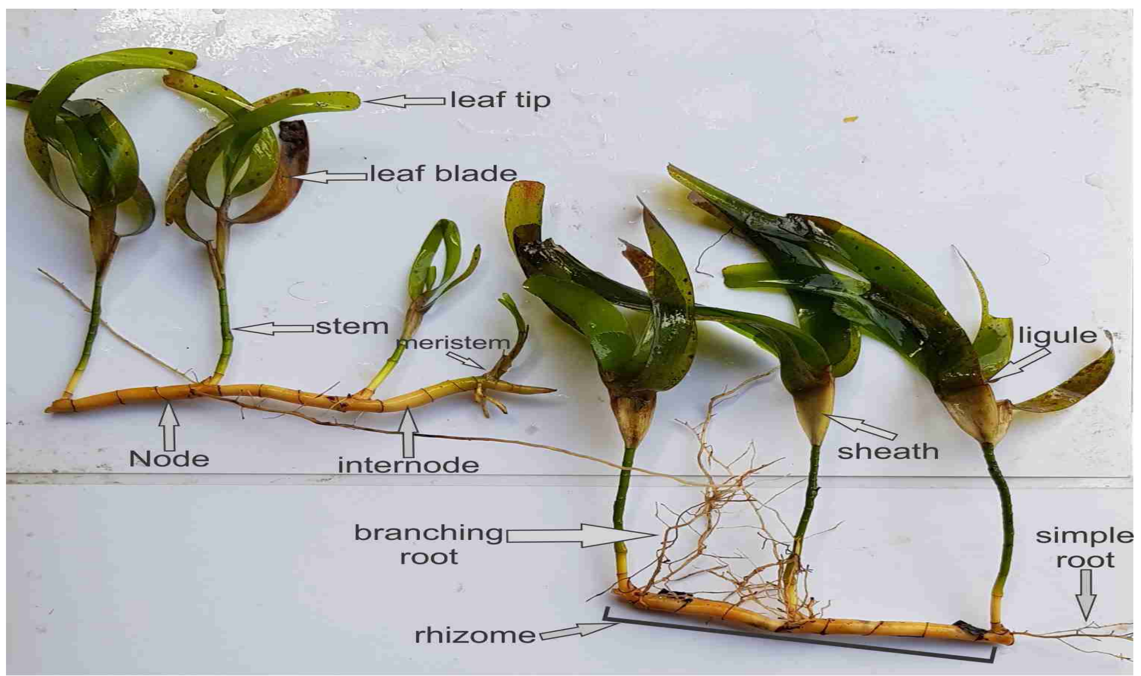


Fig 3: Schematic diagram of seagrass with different parts.

S.No.	Species	Gujarat	Maharashtra	Goa	Karnataka	Kerala	Tamil Nadu			Andhra Pradesh	Orissa	West Bengal	Lakshadweep	Andaman Islands	Nicobar Islands
							Gulf of Mannar	Palk Bay	Other sites						
1.	*Enhalus acoroides*	-	-	-	-	+	+	+	-	-	-	-	+	+	+
2.	*Halophila ovalis*	+	-	+	-	+	+	+	+	+	+	+	+	+	+
3.	*H. ovata*	+	-	-	-	-	+	+	-	+	+	+	+	+	+
4.	*H. decipiens*	-	+	-	-	-	+	+	-	-	-	-	+	+	-
5.	*H. stipulacea*	-	-	-	-	-	+	+	-	-	-	-	-	+	-
6.	*H. beccarii*	+	+	+	+	+	+	+	+	+	+	+	-	-	-
7.	*H. ovalis ramamurthiana*	-	-	-	-	-	-	+	+	+	+	-	-	-	-
8.	*H. minor*	-	-	-	-	-	-	-	-	-	+	-	-	+	-
9.	*Thalassia hemprichii*	+	-	-	-	-	+	+	-	-	-	-	+	+	+
10.	*Syringodium isoetifolium*	-	-	-	-	-	+	+	+	-	-	-	+	+	+
11.	*Cymodocea serrulata*	+	-	-	-	-	+	+	+	-	-	-	+	+	+
12.	*C. rotundata*	-	-	-	-	-	+	+	+	-	-	-	+	+	+
13.	*Halodule pinifolia*	+		-	-	-	+	+	+	+	+	+	+	+	+
14.	*H. uninervis*	+	-	-	-	-	+	+	+	+	+	+	+	+	+
15	*H. wrightii*	-	-	-	-	-	+	+	+	+	-	+	-	-	-
16.	*Rupia maritima*	+	-	-	-	-	+	-	-	-	+	-	-	-	-
Total		8	2	2	1	3	14	14	9	7	8	6	10	12	9

+: present; -: absent.

Table 3: Distribution of seagrasses in various sites of India

Source: Thangaradjou and Bhatt, 2017.

Fig 4: Some important seagrass species present in Gulf of Mannar and Palk bay waters

Enhalusacoroides

- It has dark green long linear grass like leaves, 1.0-1.5cm wide, 50-170cm long.
- Common in shallow intertidal areas with sandy and muddy substrata but can extend down upto the depth of 6m
- It has the only seagrass species which forms aerial surface pollination

Cymodoceaserrulata

- Cymodoceaserrulata has a smooth, herbaceous rhizome system, which produces short, erect shoots often with fibrous rootlets at each node, each shoot bearing 2-5 leave
- The leaf is narrowed at the base and the leaf tip is bluntly rounded and distinctly serrated.
- This species can quickly recover or return after a disturbance

Syringodiumisoetifolium

- Leaves of 5-10cm long, they can grow to 50cm long. The seagrass has tubular leaves much like a thick noodle &circular in cross-section.
- The leaves have a smooth pointed tip. The rhizomes are slender (1.5mm in dia).
- Shoots emerge from these rhizomes, each shoot with 2-3 leaves, the lower portions encased in a sheath.

Halophila ovalis

- It is a small herbaceous plant.
- The leaves are ovate in outline, appearing on stems that emerge from rhizome beneath the sand
- The roots get upto 80mm long and are covered in fine root hairs.
- It has connected by a series ofinterconnecting rhizomes.

Halodule pinifolia

Halodule uninervis

Halophila beccarii

Halophila decipiens

The present major threat to seagrass meadows in Gulf of Mannar and Palk bay is destructive fishing activitiesparticularly bottom trawling fishing operation, deterioration of water quality, boat anchoring, sedimentation, coastal pollution, dredging operation, Induatrial waste like thermal plant fly-ash waste, strong water current etc.

MANGROVES ECOSYSTEM

Mangroves are trees or large shrubs, including ferns, which normally grow in saline coastal habitats in the tropics and subtropics, in or adjacent to the intertidal zone and which have developed special adaptations in order to survive in this environment.Generally, mangroves are divided into two categories 'true mangrove species' (i.e. plants which are found only in tropical intertidal habitats) and 'mangrove associates' (i.e. plants which are not exclusive to these habitats)

Table 4: The list of true mangrove species reported by various authors in India.

Species names	Singh & Garge (1993)	Dagar *et al.* (1993)	Naskar (2004)	Selvam *et al.* (2004)	Kathiresan & Rajendran (2005)	Mandal and Naskar (2008)	Kathiresan (2008)
Acanthus ebracteatus	•	•	•		•		•
A. ilicifolius	•	•	•	•	•	•	•
A. volubilis	•	•	•				
Acrostichum aureum				•	•		•
Ac. speciosum					•		•
Aegialitis rotundifolia	•	•	•	•	•	•	•
Aegiceras corniculatum	•	•	•	•	•	•	•
Aglaia cucullata			•	•	•		
Avicennia alba	•	•	•	•	•	•	•
A. marina	•	•	•	•	•	•	•
A. marina var. *acutissima*			•				
A. officinalis	•	•	•	•	•	•	•
Atalantia correae			•				
Brownlowia tersa			•				
Bruguiera cylindrica	•	•	•	•	•	•	•
B. gymnorrhiza	•	•	•	•	•	•	•
B. parviflora	•	•	•	•	•	•	•
B. sexangula	•	•	•	•	•	•	•
Ceriops decandra	•	•	•	•	•	•	•
C. tagal	•	•	•	•	•	•	•
Cynometra iripa		•	•	•			•
Cy. ramiflora		•	•				•
Dalbergia spinosa			•				
Dolichandrone spathacea					•		•
Excoecaria agallocha	•	•	•	•	•	•	•
E. indica					•		•
Heritiera fomes	•	•	•	•	•	•	•
H. littoralis	•	•	•	•	•	•	•
H. kanikensis				•	•		
Kandelia candel	•	•	•	•	•	•	•
Lumnitzera littorea	•	•	•		•	•	•
L. racemosa	•	•	•	•	•	•	•
Nypa fruticans	•	•	•	•	•	•	•
Pemphis acidula				•	•		•
Phoenix paludosa	•	•	•			•	
Rhizophora × annamalayana					•		•
R. apiculata	•	•	•	•	•	•	•
R. mucronata	•	•	•	•	•	•	•
R. × lamarckii	•	•	•	•	•		•
R. stylosa	•	•	•	•	•	•	•
Sarcolobus carinatus			•				
S. globosus			•				
Scyphiphora hydrophyllacea	•	•	•	•	•	•	•
Sonneratia alba	•	•	•	•	•	•	•
S. apetala	•	•	•	•	•	•	•
S. caseolaris		•	•	•	•	•	•
S. griffithii		•	•	•	•	•	•
Xylocarpus granatum	•	•	•	•	•	•	•
X. moluccensis	•	•	•	•	•		
X. mekongensis	•	•	•	•		•	•
Total number of species reported	32	36	43	35	39	30	39

Source: Ragavan*et al*.,2016(• denotes occurrence.)

Indian mangroves consist of 46 true mangrove species belonging to 14 families and 22 genera, which includes 42 species and 4 natural hybrids. In other words, about 57% of the world's mangrove species are represented in India. The East coast has 40 mangrove species belonging to 14 families and 22 genera. The West coast has 27 species belonging to 11 families and 16 genera and the Andaman and Nicobar Islands (ANI) have 38 species belonging to 13 families and 19 genera. In India, the mangrove forest overall cover is estimated to be 4740 km^2, of which about 58% is along the east coast (Bay of Bengal), 29% along the west coast (Arabian Sea) and the remaining 13% in the Andaman and Nicobar Islands (Forest Survey of India, 2015).

In West Bengal, mangroves are present in the Sundarbans, the large deltaic complex of the riverGanges, shared by Bangladesh (62%) and India (38%). Totally 33 true mangrove species belonging to 21 genera and 14 families have been identified in Indian Sundarbans.

The mangrove cover can be attributed to two reasons in our region

I. The east coast has large estuaries with deltas formed due to runoff and deposition of sediments, whereas the west coast has funnel-shaped estuaries with an absence of deltas and
II. The east coast has gentle slopes with extensive flats for colonization by mangroves, whereas the west coast has steep slopes (Kathiresan, 2010).

The mangroves of Andaman and Nicobar Islands (ANI) are probably the best developed in India in terms of their density and growth. In Tamil Nadu mangroves are confined to Pichavaram, Muthupet, Palk bay and Gulf of Mannar. A total of 17 true mangrove species belonging to 12 genera and 8 families have been recognized from Tamil Nadu. The Gulf of Manner and Palk bay region harbours mangroves with a considerable diversity which supports a variety of biological organisms. It is believed that the region was once covered with thick mangrove forests.

Fig 5: Some of the important mangrove species present in Gulf of Mannar and Palk bay area

Avicenia marina

Pemphis acidula

Ceriops tagal

Rhizophora mucronata

In Gulf of Manner, a total of 11 mangrove species, 17 mangrove associate plants were identified. Mangroves species - *Avicennia marina, Bruguiera cylindrica, B. gymnorrhiza, Ceriops tagal, Excoecaria agallocha, Lumnitzera racemosa, Pemphis acidula, Rhizophora apiculata, R. mucronate and Acanthus ilicifolius*. *Avicennia marina* is the most abundant species followed by *Pemphis acidula*. This is followed in descending order by *Ceriops tagal, Rhizophora mucronata, Bruguiera cylindrica, Lumnitzera racemosa, Excoecaria agallocha and Rhizophora apiculata.*

The state forest department noted that there was overexploitation led to vanishing of mangroves species. As a result, themangrove species such as *Bruguiera gymnorrizha* and *Acanthus ilicifolius*collected earlier in Rameswaram have disappeared in recent years, and similar the cases of Pemphis acidula in Pamban and Acanthus ilicifolius inKrusadai Island. The increase in the extent of salt pans is yet another factor leading to the shrinkage of mangroves particularly around Tuticorin (Kathiresan, 2008)

Importance of mangrove ecosystem

Mangrove ecosystem are biodiversity hotspots extremely productive ecosystems, providing many critical services that benefit all of us. They provide nesting and breeding habitat for fish and shellfish, migratory birds etc. Mangroves are essential to maintaining water quality. With their dense network of roots and surrounding vegetation, they filter and trap sediments, heavy metals, and other pollutants. It has ability to retain sediments flowing from upstream prevents contamination of downstream waterways and protects sensitive habitat like coral reefs and seagrass beds below. Mangroves are the first line of defence for coastal communities. They stabilize shorelines by slowing erosion and provide natural barriers protecting coastal communities from increased storm surge, flooding, and hurricanes. It serves sequestering carbon at a rate of two to four times greater than mature tropical forests and store three to five times more carbon per equivalent area than tropical forests. It has the potential for sustainable revenue-generating initiatives including ecotourism, sport fishing, and other recreational activities.

The major threats faced by mangrove ecosystem are mangrove deforestation, Land reclamation, Industrial occupancy and waste dumping, coastal pollution, shortage/nil of river runoff, diseases etc. Adverse effects on mangroves could lead to serious consequences for the adjoining fragile and important ecosystems such as coral reefs and sea grass beds. Damage to mangroves affects the sediment budget and promotes the coastal erosion.

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பழனிச்சாமி
கோபிகா
ICAR - Central Marine Fisheries Research Institute
(Indian Council of Agricultural Research)
Mandapam Regional Centre, Marine Fisheries P.O.,
Mandapam Camp, Ramanathapuram District, Tamil Nadu, In