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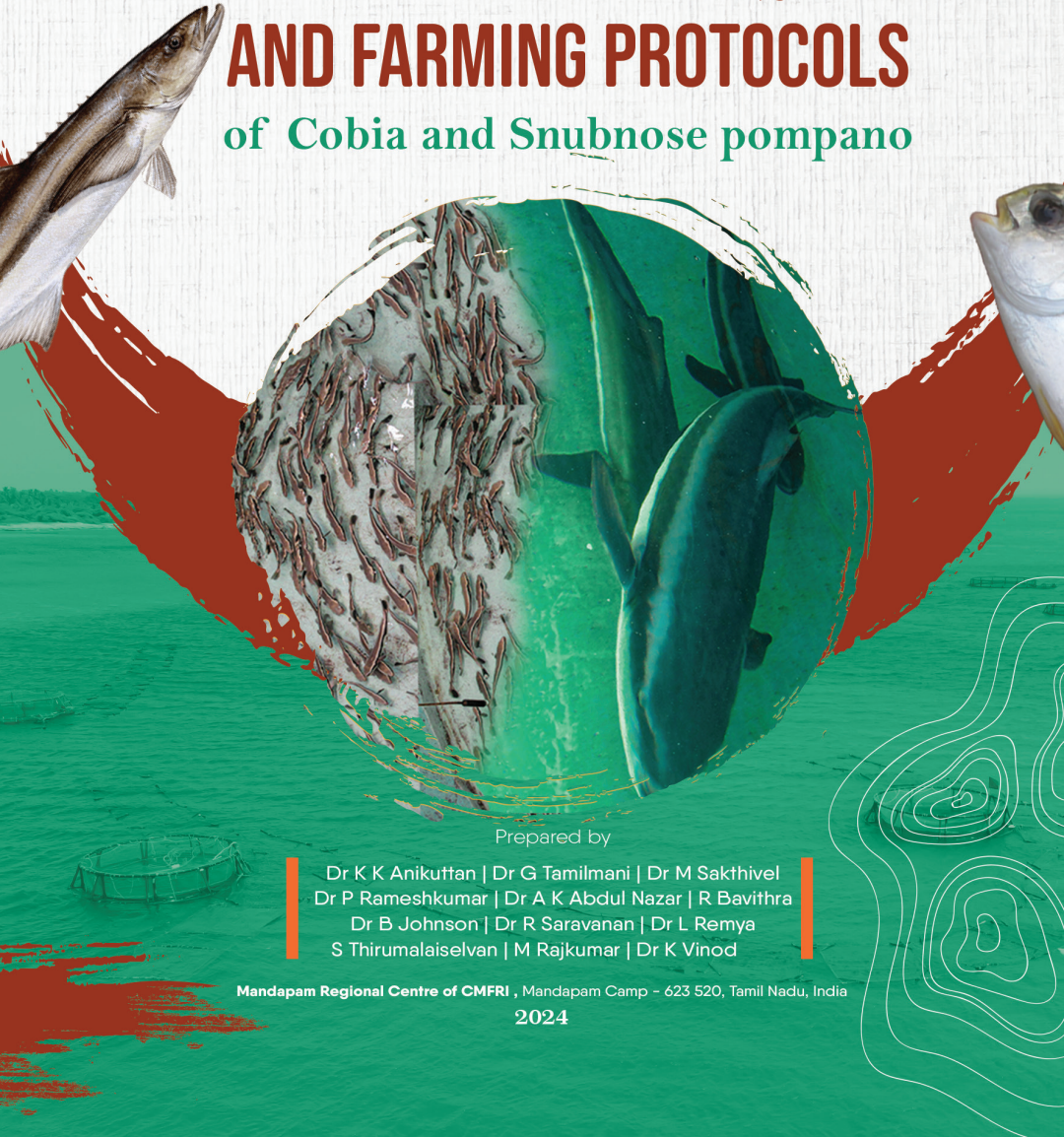
Central Marine Fisheries Research Institute



Handbook on



HATCHERY TECHNIQUES AND FARMING PROTOCOLS of Cobia and Snubnose pompano



Prepared by

Dr K K Anikuttan | Dr G Tamilmani | Dr M Sakthivel
Dr P Rameshkumar | Dr A K Abdul Nazar | R Bavithra
Dr B Johnson | Dr R Saravanan | Dr L Remya
S Thirumalaiselvan | M Rajkumar | Dr K Vinod

Mandapam Regional Centre of CMFRI, Mandapam Camp - 623 520, Tamil Nadu, India

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Published by

Dr. A. Gopalakrishnan

Director, ICAR-CMFRI

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M. Rajkumar, K. Vinod

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ICAR-Central Marine Fisheries Research Institute

Post Box No. 1603, Ernakulam North P.O., Kochi – 682 018.

Phone: +91 484 2394357, 2394867, Fax: +91 484 2394909

E-mail: contact@cmfri.org.in

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FOREWORD

The significance of mariculture in augmenting marine fish production is increasingly becoming evident in the present scenario of stagnating capture fisheries production.

As per the FAO figures (FAO-SOFIA 2022), the total fisheries and aquaculture production was 214 million tonnes in 2020, which is an all-time high, and comprised of 178 million tonnes of aquatic animals and 36 million tonnes of algae. This is mainly attributed to the growth of aquaculture which also includes mariculture.

Mariculture in India is in its initial stages of development, even though there is ample scope for its expansion, considering the vast coastline of the country and other resource potential. Selection of suitable sites, identification of candidate species, development of seed production and farming technologies are some of the cardinal requirements for mariculture development and are being addressed by the R & D sector of the country. Technologies for seed production and farming of around 10 finfish species with mariculture potential have been developed in India and are in different stages of standardization and commercialization.

The breeding and seed production of two high value marine fishes, Cobia (*Rachycentron canadum*) and Snub nose Pompano (*Trachinotus blochii*) was achieved for the first time in the country at the Mandapam Regional Centre of ICAR-Central Marine Fisheries Research Institute (CMFRI) in 2010 and 2011 respectively. This, along with the development of sea cage farming technologies by ICAR-CMFRI, has kick started the mariculture development in the country. The sea cage farming demonstrations of Cobia carried out by ICAR-CMFRI in collaboration with fishermen groups and farmers has generated interest among the fisherfolk to take up this activity as an alternate livelihood option. The farming demonstrations showed that the cobia can attain an average weight of 1.0 kg in 4 months, 2.5 - 3.0 kg in 6- 7 months and 7.0 kg to 8.0 kg within one year of farming. The snub nose pompano on the other hand is both suitable for sea cage farming as well as for farming in low saline coastal ponds. The farming trials of this fish were also conducted and fishermen groups and entrepreneurs are adopting the technology in different coastal states of the country.

One of the important requirements for the development of any sector is capacity building for the human resource and for this, training programmes have to be conducted for all stakeholders of the sector. In this context, the ICAR-CMFRI has been conducting various training/awareness programmes on breeding, seed production and farming of species with mariculture potential for all stakeholders of the sector such as fishermen, fish farmers, hatchery technicians and government officials.

I hope that, this hand book will be of immense use to all the stakeholders of the sector such as fisheries developmental agencies, researchers, hatchery technicians, entrepreneurs and fisherfolk. I congratulate all the personnel involved in preparation of this hand book.

Dr. A. Gopalakrishnan
Director, ICAR-CMFRI

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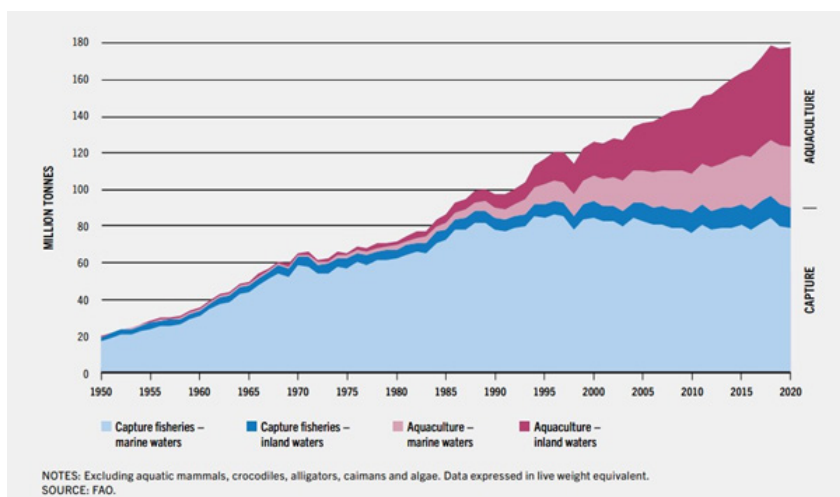
Chapter 01: Mariculture - An overview

KK Anikuttan, G Tamilmani, M Sakthivel, P Rameshkumar, A K Abdul Nazar & R Bavithra

The global aquaculture production reached a record 122.6 million tonnes in 2020, out of which around 54.4 million tonnes were farmed in inland waters and 68.1 million tonnes came from marine and coastal aquaculture. All regions, except Africa, experienced continued aquaculture growth in 2020, driven by expansion in Chile, China and Norway – the top producers in their respective regions. Asia continued to dominate world aquaculture, producing over 90 percent of the total. The contribution of aquaculture to the global production of aquatic animals reached a record 49.2 percent in 2020. Despite the great diversity

in farmed aquatic species, only a small number of “staple” species dominate aquaculture production, particularly grass carp for global inland aquaculture and Atlantic salmon for marine aquaculture (FAO,2022).

Mariculture, with the development and expansion of sea cage farming has been growing rapidly on a global basis which reached an all time high of 33.1 million tonnes during 2020. The major groups of fishes which are being cultured includes Salmonids, Amberjacks, Sea breams, Sea basses, Croakers, Groupers, Drums, Mulletts, Turbot, other flatfishes, snappers, cobia, pompano, cods, puffers and tunas.



WORLD CAPTURE FISHERIES AND AQUACULTURE PRODUCTION (FAO,2022)

One of the major reasons for the growth of sea cage farming is the availability of breeding techniques that can produce sufficient quantity of seeds of different high value marine finfish. Many countries in the Asia-Pacific Region like Australia, China, Japan, Taiwan, Philippines, Indonesia, Thailand, Malaysia and Vietnam have made substantial progress in the development of commercial level seed production technologies of high value finfish suitable for sea farming. But even in these countries, seed supply is one of the vital issues for further expansion of mariculture. Currently massive efforts are being taken by the CMFRI for demonstration of mariculture techniques at different parts of our country with promising results.

The main species farmed in brackish water are the Barramundi or Asian sea bass (*Lates calcarifer*) and the Milk fish (*Chanos chanos*). In inshore marine cage farming, the major farmed species include *Seriola* spp., snappers (*Lutjanus spp.*), groupers (*Epinephelus spp.*) and cobia (*Rachycentron canadum*).

The yellow tail, *Seriola quinqueradiata* contributes up to 17% of marine finfish production in Asia, with a production of about 160,000 tonnes annually.

Nearly all this production comes from Japan, where production levels have been relatively stable at 140,000 – 170,000 tonnes per annum since 1980s. Nevertheless, seabreams are the mainstay of Asian finfish mariculture production, and a range of species are currently cultured.

Grouper culture has been expanding rapidly in Asia, driven by high prices in live fish markets of Hong Kong and China. Since grouper farming is mainly dependent on wild collected seed, the decreasing availability of wild seeds due to overfishing is a major constraint for the expansion of grouper culture.

Southern blue fin tuna (*Thunnus maccoyii*) is cultured in Australia using wild caught juveniles. Although production of this species is relatively less (3500-4000 tonnes per annum), it fetches very high prices in the Japanese market and thus supports a highly lucrative local industry in South Australia.

Cobia (*Rachycentron canadum*) is a species of much interest for tropical marine finfish aquaculture. Most production currently comes from China and Taiwan Province of China. Production of this fast growing species is set to expand rapidly in Asia. Cobia has become a

global commodity, in the same way that salmon has become a global commodity in temperate aquaculture.

Milkfish (*Chanos chanos*) is traditionally cultured in Phillippines. Indonesia is a major producer of seed, much of this coming from small-scale hatcheries. Milkfish culture is also practised in some Pacific Islands viz. Kiribati, Nauru, Palau and the Cook Islands. Although most milkfish culture is undertaken in brackishwater ponds, there is increasing production from intensive mariculture cages.

Indian Scenario

In India research attention for developing seed production methods for high value finfishes suited for sea farming has started only in the recent past and the technologies developed has not reached a level of full fledged commercialisation so far. The vital issue for the expansion and commercialization of sea farming in India is the availability of suitable seeds of high value finfishes. Unless an intensified research and development effort is made to the development of commercial level seed production technologies, sea farming cannot emerge as a significant seafood production sector in the country.

The cage farming demonstrations conducted by CMFRI in the recent past have generated a lot of interest in the farming of marine fin fish among fish farmers and entrepreneurs. However, the lack of commercial scale availability of hatchery produced seed is the major bottleneck for any large scale venture of marine finfish farming. The availability of seeds from wild is often unpredictable and hence farming based on wild collection of seeds may not be a sustainable venture. Hence the development and standardization of seed production techniques of species with mariculture potential can address this issue. Further, the development of commercial hatcheries for ready supply of seeds is the primary step for the development and expansion of marine finfish farming in India.

Cobia and Pompano

Cobia (*Rachycentron canadum*) and silver pompano (*Trachinotus blochii*) are two marine finfish species with very high potential for aquaculture in India. Fast growth rate, adaptability for captive breeding, lowest cost of production, good meat quality and high market demand especially for sashimi industry are some of the attributes that makes cobia an excellent species for aquaculture. In

recent years the seed production and farming of cobia is rapidly gaining momentum in many Asian countries. Envisaging the prospects of cobia farming in India, broodstock development was initiated at the Mandapam Regional Centre of Central Marine Fisheries Research Institute in sea cages during 2008 and the first successful induced breeding and seed production was achieved in March – April 2010. Trials on sea cage farming carried out at Mandapam showed that the fishes attained an average weight of 2.5 kg in six months and 7.3 kg in twelve months. The species can be grown in low salinity and experiments revealed that upto 15ppt the growth and survival is comparable to that in seawater. These results point out the possibility of developing a lucrative cobia aquaculture enterprise in the country. However, standardization of technologies for seed production and farming of cobia to suit our environmental conditions have to be further pursued on a priority basis so that India can also emerge as a contributor for cobia production in the near future. Similarly among the many high value marine tropical finfish that could be farmed in India, the silver pompano is also one of the topmost, mainly due to its fast growth rate, good meat quality

and high market demand. The species 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. At Mandapam Regional Centre of CMFRI, successful broodstock development, induction of spawning and fingerling production of silver pompano was achieved during July 2011 for the first time in India and it has been later standardised for regular seed production and supply to the farmers of the country.

A pond farming demonstration of silver pompano at Anthervathi, East Godavari District, Andhra Pradesh from hatchery produced seeds showed that in eight months period fishes have grown to 450 - 550 gm with an average survival rate of 95%. This can be considered as a milestone towards the development of pompano aquaculture in the country. These achievements in cobia and pompano were the pioneering attempts in the development of aquaculture of these two species.

Chapter.02

Fishery and biology of cobia and snubnose pompano

Remya L., Anikuttan K. K., Rajkumar M. and Thirumalaiselvan S.

Introduction

Rachycentron canadum, cobia, a fast-growing pelagic fish belongs to the monotypic family Rachycentridae, is widely distributed in tropical, subtropical, and warm temperate waters of $>20^{\circ}\text{C}$ temperature and totally absent in central and eastern Pacific Ocean (Fig 1.). It makes seasonal migrations in connection with changes in water temperature and also for spawning. In the Chesapeake Bay, cobia was found to enter the bay in early June, and leave by mid-October (Richards 1977). Arracott (1977) indicated that cobia from southern Indian Ocean waters may move north to spawn off the coast of Arabia. Smith and Heemstra (1986) reported that

cobia migrate to South African waters during the austral summer, occasionally reaching False Bay. Cobia associate with larger fish, such as rays and sharks, and sea turtles (Baughman 1950). It is so well known that fishermen often consider schools of large rays to be indicators of cobia (McNally 1985, Moe 1970). Cobia is considered to have low abundance throughout its range. It has relatively higher abundance in the Arabian Sea and in the Gulf of Mexico. The rate of recruitment for cobia is considered to be low. The fish is highly preferred for its table value and fetches very good price in fresh condition. Fast growth rate and high market value both in domestic and export market

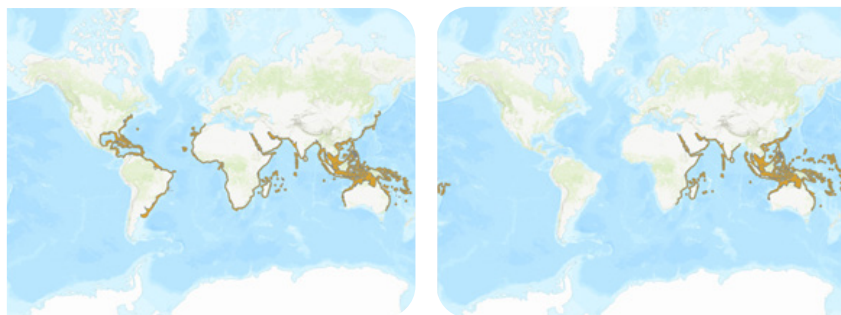


Fig. 1. Global distribution of cobia (left) and snubnose pompano (right) (Source: Collette et al., 2015; Smith-Vaniz, W.F. and Williams, I. 2016)

has made *R. candum* an ideal candidate species for mariculture (Liao, 2003).

Trachinotus blochii, snubnose pompano is an Indo-Pacific carangid has distribution from Red Sea and East Africa to the Marshall Islands and Samoa, north to southern Japan and southern extend up to Australia (Fig 1.). In India, this species is distributed all along the Indian coast from Gujarat to West Bengal including Andaman and Nicobar Island. It inhabits along shallow coastal waters over coral and

rocky reefs (Smith-Vaniz 1984). It spends more time in the pelagic water with in 7 to 55m depths. Juveniles inhabit sandy shorelines and shallow sandy or muddy bays near river mouths while adults move out in schools to clear seaward coral and rock reefs (Fischer et al., 1990). Juveniles are in small schools, while adults are usually solitary (Kuiter and Tonozuka, 2001). This pompano has only minor commercial importance in India and elsewhere in the world. There are no species-specific conservation measures for this species.

***Rachycentron canadum* (Linnaeus, 1766), cobia**

Phylum: Chordata

Order: Perciformes

Subphylum: Vertebrata

Suborder: Percoidei

Superclass: Gnathostomata

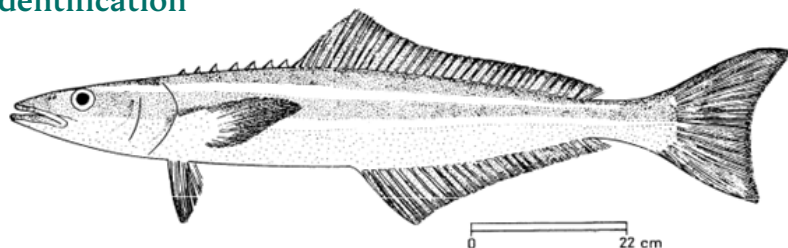
Family: Rachycentridae

Class: Osteichthyes

Genus: *Rachycentron*

Superorder: Acanthopterygii

Identification



Body elongate, subcylindrical; head broad and depressed. Mouth large, terminal, with projecting lower jaw; villiform teeth in jaws and on roof of mouth and tongue. First dorsal fin with 7-9 (usually 8) short but strong isolated spines, not connected by a membrane; second dorsal fin long, anterior rays somewhat elevated in adults; pectoral fins pointed, becoming more falcate with age; anal fin similar to dorsal, but shorter; caudal fin lunate in adults, upper lobe longer than lower (caudal fin rounded in young, the central rays much prolonged). Scales small, embedded in thick skin; lateral line slightly wavy anteriorly (Collette 1978; FAO, Rachycentridae, 1983).

Fishery in India

In India the fish occurs along both the coasts forming an incidental catch in trawls, gillnets, trolls and handlines (Pillai, 1982). An estimated 302t of cobia landed annually along Mangalore coast which formed 0.1% of the total fish catch of the region (Rohit and Bhat, 2012). Peak landings in the area recorded during October followed by April. Gillnets landed bulk of the catch (53%) followed by trawls (45%). The fishery was comprised of fishes of length range 26 - 125 cm TL with the mean at 58 cm.

Diets

Cobia is an apex predator preying actively on fishes, crustaceans and molluscs. Cobia popularly known as 'crab eaters' globally due to their assumed fondness for crabs as its major diet, moves to areas of high food abundance, particularly crustacean abundance (Daracott, 1977). The diet of cobia collected from Karnataka predominantly consisted of finfishes *Decapterus russelli*, *Encrasicholina devisi*, *Nemipterus* spp. etc. followed by crustacean such as *Acetes* spp., crab, squid, octopus and other (Rohit and Bhat, 2012). In India cobia feeds on a wide variety of food items and are nonselective feeders foraging on whatever pelagic or benthic organisms that are locally available. Franks et al. (1996) found that juvenile cobia captured by trawl in the northern Gulf of Mexico were carnivores and fed exclusively on small fish, crustaceans and squid. It exhibits opportunistic feeding behaviour and is strong swimmers capable of capturing fast moving fishes, squids and decapod crustaceans which are open nektonic, benthic and demersal.

Reproduction

Cobia is a gonochoristic fish without any noticeable external sexual dimorphism. It forms spawning aggregations. Fertilization is probably external, with both eggs and sperm released simultaneously. Male cobia in Chesapeake Bay matures after 2

years at 51.8 cm FL and 1.14 kg. Females mature at a larger size than males in their third year, at 69.6 cm FL and 3.27 kg (Richards 1967). In Indian waters, Rajan et al. (1968) collected a 42.6-cm TL female with ovaries in the third stage of maturity. Female-to-male ratio of 1.54: 1

Trachinotus blochii (Lacepede, 1801), snubnose pompano

Phylum: Chordata

Superorder: Acanthopterygii

Subphylum: Vertebrata

Order: Carangiformes

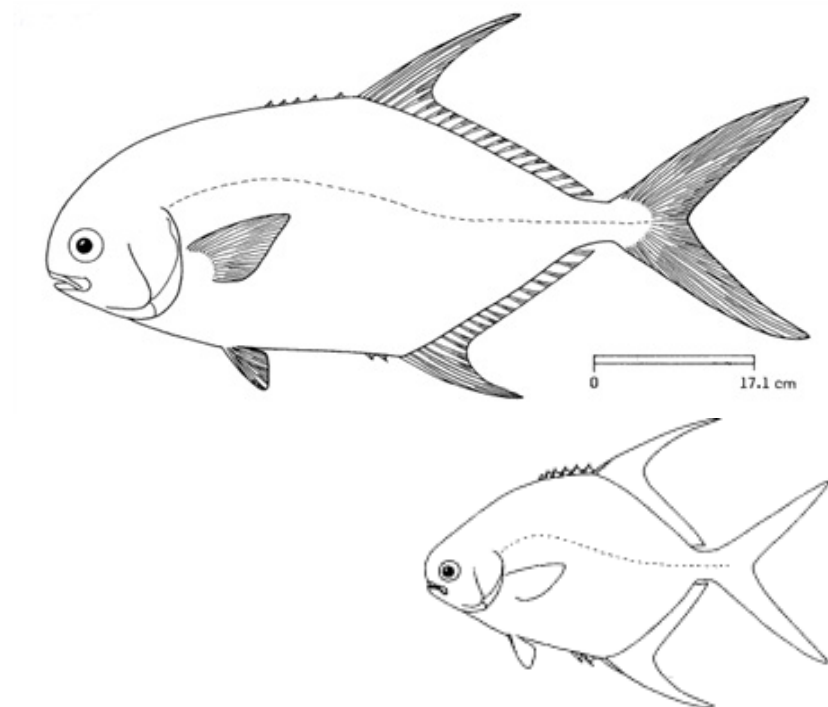
Superclass: Gnathostomata

Family: Carangidae

Class: Osteichthyes

Genus: *Trachinotus*

Identification



Juvenile

Body ovate in juveniles, subovate and compressed in adults and; profile of snout broadly rounded, in adults becoming nearly straight to interorbital region. Both jaws with bands of small villiform teeth; tongue toothless (except 2 or 3 slender teeth rarely on small specimens); gillrakers (including rudiments) 5 to 8 upper and 8 to 10 lower on first gill arch; 2 separate dorsal fins, the first with 6 short spines (the anterior spines often becoming completely embedded in large adults), followed by 1 spine and 18 to 20 soft rays; anal fin with 2 detached spines (becoming embedded in large adults), followed by 1 spine and 16 to 18 soft rays; height of second dorsal fin lobe 35 to 60% of fork length in specimens of 10 to 40 cm fork length; pelvic fins shorter than pectoral fins. Lateral line only slightly irregular, weakly convex above pectoral fin, becoming straight posteriorly. No scutes or caudal peduncle grooves. First predorsal bone shaped like an inverted tear-drop or oval-shaped, this character is easily observed by a simple dissection. along midline of nape; supraoccipital bone of skull thin and bladelike in adults. Vertebrae 10+14 (FAO, Carangidae, 1983).

Fishery in India

In India snubnose pompano do not form an established fishery and hence treated as a minor commercially important fish as it lands along with other pompanos in a smaller percentage. The major gears employed for pompano catches are traps, trawls, gillnets and handlines etc.

Diets

The adults of snubnose pompano prefer molluscs and other hard-shelled invertebrates especially gastropods, crab, acetes, bivalves etc.

Reproduction

Snubnose pompano is a gonochoristic fish without any noticeable external sexual dimorphism. Fertilization is external, with both eggs and sperm released simultaneously. Length at maturity is probably at 58cm TL. Female-to-male ratio of similar species *T. mookalee* from Vizag waters is 1.46:1 and running male and female observed only during February to April.

Chapter.03

Broodstock development of Cobia and Snubnose Pompano

G Tamilmani, M Sakthivel, P Ramesh Kumar, KK Anikuffan, R Bavithra, A K Abdul Nazar & Tinto Thomas

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



Cobia

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



Silver Pompano

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.

Quarantine

Upon arrival at the hatchery, broodstock fishes are released into the quarantine tanks for prophylactic treatment. Fish anaesthetics like MS 222 (50-100 ppm) and Aqui-S (4 ml / 100 L), can be used for broodstock handling. The prophylactic treatment is given to limit the risk of introducing parasites or bacterial diseases into the hatchery facility. Short time

exposure of brooders (maximum 5 minutes) in freshwater will help to remove the external parasites. The prophylactic treatment in hatcheries includes a sequence of medicated baths in formalin, malachite green and Oxytetracycline (OTC). Prophylactic treatment can be repeated three to four times within a week.

It is preferable to have a flow-through water circulation in quarantine tanks when treatments are not underway. Smooth inner surface in tanks allow easy and complete cleaning.

Following sequence of treatments can be followed :

Day 1 :

Fresh water bath for 10 minutes and then Oxytetracycline treatment (50 ppm) in seawater for 30 minutes.

Day 2 to Day 7 :

Treatment with a mix of 200 ppm formalin and 0.2 ppm malachite green for 1-2 minutes, followed by a freshwater dip for 5 minutes. Before returning the

fishes to quarantine tanks with filtered seawater, they can be given an Oxytetracycline treatment at 50 ppm for 30 minutes.

The fishes should be closely observed during treatments. If the fishes suddenly become immobile or are found with very less opercular movements or are turning upside down, they should be immediately transferred to filtered seawater. During the quarantine, fish should be closely



Cobia brooders in sea cage

monitored and fed during the day time when it is not undergoing treatment. Over feeding should be avoided and the fishes can be transferred to maturation tanks after the treatments are over.

Apart from quarantine treatment, the broodstock fishes should be given regular prophylactic treatment with



Cobia broodstock fishes



Silver pompano Brooder tanks

freshwater with or without OTC at least once in a month.

Maturation

After quarantine, broodstock fishes are moved into bigger capacity tanks for maturation and long-term holding in the hatchery. During gonadal maturation, water salinity needs to be 31-35 ppt. Water quality parameters like salinity, temperature, dissolved oxygen, pH, ammonia, and fish stock condition viz., general behaviour, feeding activity, diseases symptoms, prophylactic treatments, etc. are monitored regularly. Normally sex ratio of 1 female: 2 males are maintained for cobia while it is 1: 3 for pompano.

Broodstock development is the vital and time consuming procedure in marine finfish seed production. It is not easy to obtain broodstock fish

directly from the wild and hence broodstock development is to be done in captivity. Pre-adults can be collected from the wild and maintained in sea cages or recirculation facility. Broodstock development in sea cages was successfully done for cobia at Mandapam Regional centre of CMFRI.

Circular cages of 6m diameter and 3.5m depth with HDPE frame were

employed for the purpose. The major risk in the development and maintenance of the broodstock in sea cages is the risk of contracting diseases and subsequent loss of broodstock. The sudden loss of broodstock will affect the seed production, since, the loss of broodstock cannot be resolved by collecting brooders from the wild immediately. Hence, on shore facilities like recirculation aquaculture systems is advised for development and maintenance of biosecured broodstock. The vital aspects which affect development of broodstock are the photoperiod, temperature and broodstock nutrition. In a shore based facility, photo thermal conditioning can be practiced which will accelerate the gonadal maturation. In addition, it is also possible to obtain year round spawning in such a controlled system.

Broodstock Nutrition

The quality of the larvae is very much dependent on broodstock nutrition. The nutritional components in the diet, the feed intake rate or the feeding period can all affect spawning, egg and larval quality. In the case of tropical fishes, ovarian development is often asynchronous – oocytes in all stages of development are present at the same time and sometimes

independent of season. The ovarian development starts with the formation of primary oocytes. During the primary growth phase, the surrounding granulosa and theca cells envelop the oocyte to form the ovarian follicle. In the early stages of secondary growth, cortical alveoli appear and accumulate in the periphery of the oocyte. Even though the oocyte may increase in size several fold during primary and early secondary growth, the most conspicuous size increase occurs during the last part of secondary growth, vitellogenesis. Vitellogenesis is the process of yolk formation and incorporation in the growing oocytes. 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. It has been well established that feeding broodstock fish with squid, cuttlefish or meals made from cephalopods have beneficial effects. These feed ingredients make the diet more attractive and

therefore increase feed intake. Squid and cuttlefish also contain high levels of essential fatty acids.

For quicker maturation, the broodstock fishes are to be fed with 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. As the sole source of food for the developing embryo and the early larval stage until feeding on live preys starts, yolk quality and quantity are key factors for a successful reproduction. Both dry pellets and moist food are also 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, cuttlefish, crabs, shrimps and chopped oil-sardines depending on the availability.

Tagging of Brooders

Tagging or physical marking of broodstock fishes through easily detectable methods is very much essential for selection of broodstock for identification,



PIT tagging equipment

selective breeding and segregation. The most popular method is PIT Tagging.

PIT tag

Passive Integrated Transponder (PIT) tag, is a radio frequency device to permanently mark fishes internally. The tag is designed to last throughout the life of the fishes providing a reliable, long term identification method.

Principle of PIT tag

The PIT tag contains a microprocessor chip and antenna. It has no internal battery, hence the term “passive”, so the microchip

remains inactive until read with a reader. The reader sends a low frequency signal to the microchip of the tag providing the power needed to send its unique code back to the reader and therefore fish is positively identified.

Read range and frequency of the tag

The distance from which a tag can be read is the read range. Most read ranges using hand-held readers are 3 to 9 inches depending on the reader. There are currently three basic tag frequencies. The 400-kHz tag was one of the first developed but it has limited read range. As microchip technology evolved, the 125-kHz and 134.2-kHz tags became available. Compared to the older 400-kHz tags, they have a much better read range and reduced read time. The 134.2-kHz tag was developed to meet international standards for code format. It is very much important that the tag type and reader unit should be compatible. Most readers are capable of detecting both 125-kHz and 134.2-kHz frequencies. Tags can be read through materials such as soil, wood and water. Ferrous metals and noisy environments can cause interference between the electromagnetic communication of the reader and the tag.



Tagging of pompano

Durability of the tag

Design engineers' calculations suggest that PIT tags can last as long as 75 years or more. There is no battery to fail and the glass encapsulation is impervious to almost everything. PIT tags can be removed or recovered from a primary location and reused indefinitely.

Safety to animal

Reducing stress to the fish is the prime factor in ensuring the success of the tagging and safety of the fish. Therefore, the fish should be anesthetized during the implantation of PIT tags. Species, size and age should be considered when making a decision about anesthetization and restraint. Sterile implants are advised but many field conditions do not allow for sterile implants. Equipment

can be disinfected prior to use with alcohol and iodine-based solutions. The tag is encased in glass that protects the electronic components and prevents tissue irritation, thereby very much safe to the fish.

Advantages of PIT tag over other tags

- Highly reliable individual identification
- Permanent identification marker
- Small size and no interference with the behaviour of fish
- No error in recording data
- Rapid data collection

Disadvantages

- Initial cost is high
- Low detection distance

PROCEDURE OF TAGGING

Site of implantation

The implant site depends upon the species, size of the fish and the size of the tag. It is preferable to implant the tag on the dorsal musculature of the fish which will be convenient for the brood fishes to be read.

Stepwise protocol

- Use sterile needle or implanter to tag the fish. In field condition, disinfect all the components prior to use with alcohol and iodine-based solutions.
- Read the tag before inserting into the fish and record the identification code or number.
- Catch the fish and anaesthetize it with suitable anaesthetic. In sea cages, it is easier to restrain the fish inside the catching net.
- Disinfect the site of implantation with alcohol or iodine-based solution.
- It is a better practice to keep a standard site of implantation so that the reading will be easier and quicker.
- The tag loaded inside the implanter has to be inserted into the muscle tissues. It is advisable to insert the tag parallel to the muscle fibres to avoid much damage to the tissues.
- The tag should be released slowly and steadily from the implanter while removing the implanter from the tissue in such a way that the tag fills the space created by the implanter needle.
- Once implanter needle is taken out, the site should be disinfected again with alcohol or iodine-based solutions to avoid secondary infection.
- Release the fish as soon as the tagging is over or once it has recovered from anaesthesia.

Chapter.04

Induced breeding of Cobia and Snubnose Pompano

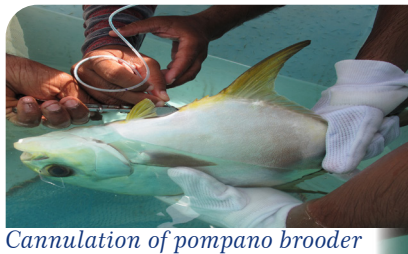
M Sakthivel, G Tamilmani, P Rameshkumar, KK Anikuttan, AK Abdul Nazar, R Bavithra & Tinto Thomas

Maturation and spawning

The natural process of sexual maturation of the broodstock fishes can be accelerated by altering the photo-thermal period and it is also possible to obtain viable larvae almost throughout the year. At the onset of the spawning season, it is necessary to move selected broodstock fishes from maturation tank to spawning tank after assessing the ovarian development through cannulation. Only females with oocytes in the late-vitellogenic stage, with a diameter around 700 μm in cobia and 500 μm in pompano, are selected.

Ovarian biopsy can be carried out as follows :

- Female brooders have to be transferred to a small tank containing anaesthesia in sufficient quantity.
- Flexible sterile catheters (1.2 mm internal diameter) can be used for cannulation biopsy.



Cannulation of pompano brooder

Introduce the sterile catheter into the oviduct, up to the ovary for a few cm; then suck carefully a small sample of oocytes up into the catheter and place the sample on a glass slide.

- After sampling, release the animal into the spawning tank, where recovery from sedation will take place.
- Put few drops of filtered sea water on the biopsy sample and examine under the microscope and measure the diameter of the oocytes and record the measurements.



Cannulation of cobia brooder

Spawning can be obtained either naturally or by inducing with hormones. Induced breeding is commonly practiced in most commercial hatcheries. The hormonal treatment is intended to trigger the last phases in egg maturation, i.e. a strong egg hydration followed by their release. However, if eggs have not reached the late-vitellogenic (or post-vitellogenic) stage, the treatment does not work; hence ovarian biopsy is essential for assessing the ovarian development. 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. Use of hCG treatment sometimes gives serious setbacks like not all

females respond to it, egg quality may be below acceptable standards with hatching rate lower than 80%, being a large molecule it 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.



Hormonal administration to pompano brooder

Spawning tanks

The spawning unit should preferably be kept separated from the main hatchery building to avoid disturbance to the spawners and possible risk of disease contamination. However, for economic reasons, it is usual to keep the brooders inside the hatchery in a specific dedicated area. Though we use only rectangular tanks based on availability, it is preferable to use circular tanks with at least 1.20 m depth. Shape and depth of tanks count for easy and free movement of brooders. Normally the spawning could be noted within 36 -48 hours after hormonal induction. The spawning in cobia and pompano takes place normally between late night and early morning hours. The number of eggs spawned by cobia ranges from 0.4 to 4 million, whereas, the pompano brooders spawn 0.5 to 1.5 lakh eggs.

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. Therefore, aeration can be switched off allowing the unfertilized or 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 or a transmitted-light stereomicroscope have to be observed for the egg developmental stages.

Check for the following egg characteristics:

- presence of opaque, whitish eggs which are unfertilised. Similarly, eggs in the sample with transparent, but without evidence of cell divisions
- regular rounded shape and size (diameter 900-1000 mm in cobia; 800 -900 mm in pompano), regular cell division that can be observed only in the first blastomers; regular shape of yolk (it should occupy the egg volume entirely, without perivitelline space),
- absence of parasites or associated micro-organisms on the chorion surface.

Incubation of eggs

Incubation of eggs is done in tanks of 3-5 tonne capacity. After hatching, the larvae 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. 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. Air bubbles should not be too small as seen while using air diffusers instead of stones, as it results in clumped eggs and damage of the eggs. It is suggested to limit as much the number of air stones as possible. 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 stereo / compound binocular microscope. The hatching of eggs takes place from 18 to 24 hours.

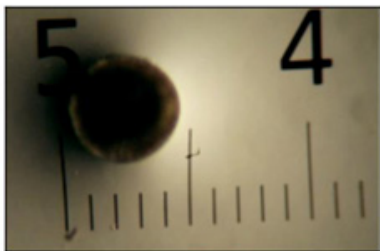
As fertilised cobia/ pompano eggs float in the seawater, they can be collected using egg collectors. If well dimensioned and properly placed, these devices harvest only the floating eggs, while the dead or unfertilised ones sink to the bottom.

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 leading to mechanical stress to the eggs.

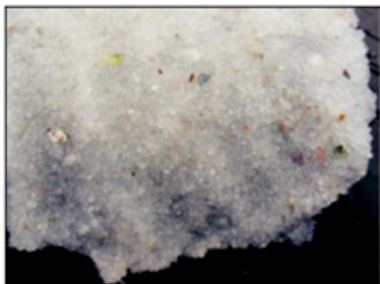
As the fecundity is normally high in cobia, we may require more incubation tanks, whereas the pompano requires



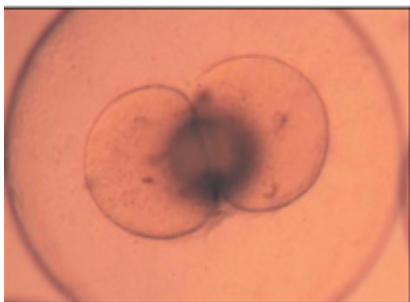
Good quality egg



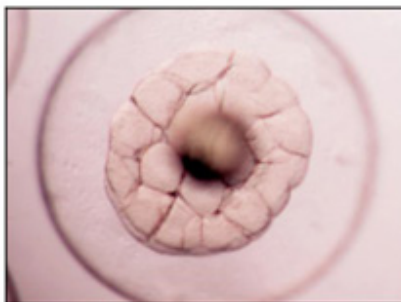
Under developed eggs



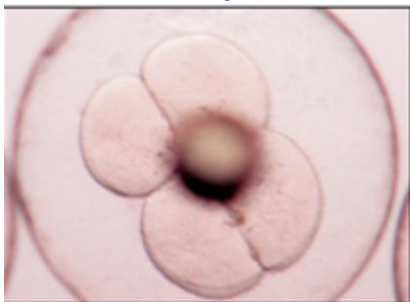
Unfertilized eggs



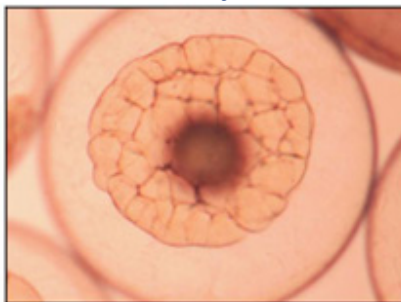
Two-cell stage



32 - cell stage



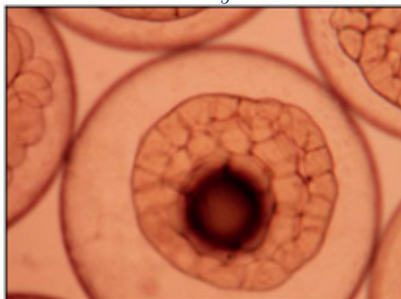
Four - cell stage



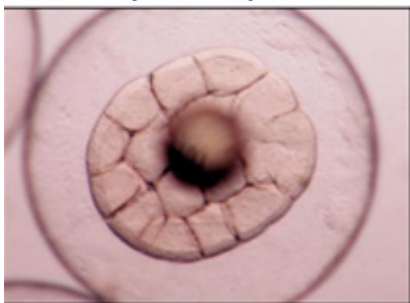
64 - cell stage



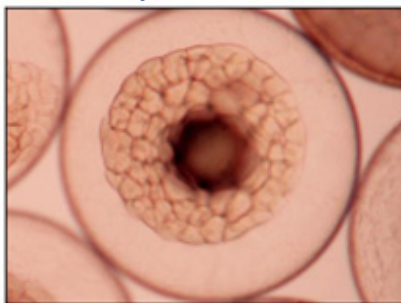
Eight - cell stage



Early Morula



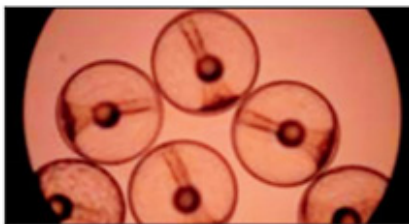
16 - cell stage



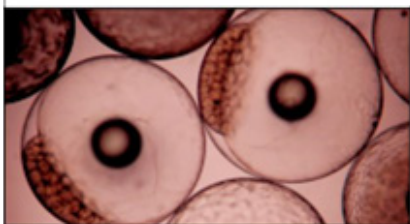
Late Morula



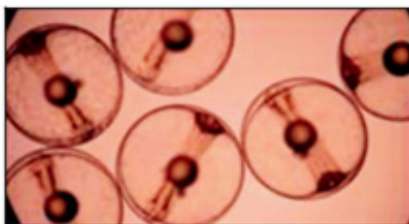
Early Blastula



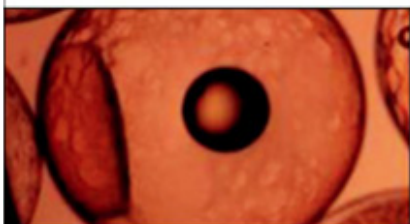
Early Gastrula



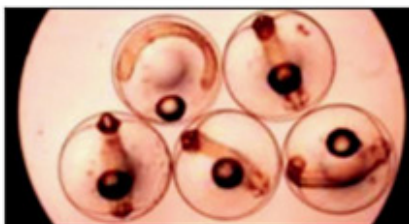
High



Mid Gastrula



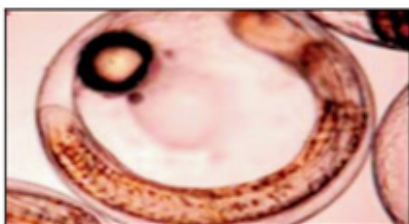
Dome



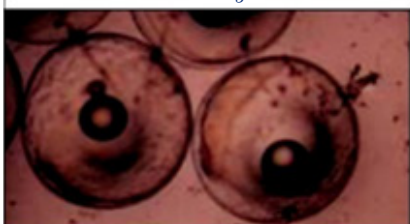
Late Gastrula



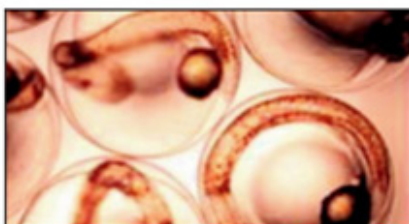
Oblong



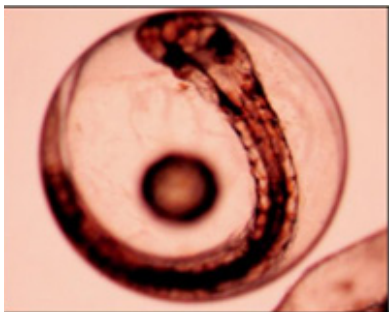
Bud



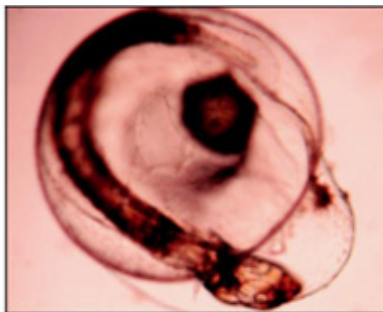
Epiboly 30%



Segmentation



High-pec



Hatching in progress



Newly hatched larva



Larvae-12 hour post hatch



2dph

Chapter.05

Larval rearing and Nursery rearing of Cobia and Pompano

R Bavithra, G Tamilmani, M Sakthivel, P Ramesh Kumar, KK Anikuttan, A K Abdul Nazar & Tinto Thomas

The marine fish larvae are generally classified into altricial and precocial type. The altricial type of larvae are having very less yolk reserves at hatching and hence, the larvae are in a 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 marinefish species suitable for aquaculture are characterized by having the altricial type of larvae which pose 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. Hence, larviculture of marine finfish is highly complicated and unless each and every factor is taken care of, the

survival and growth of the larvae will be very meager.

Newly hatched larvae have to be checked to assess their viability and condition prior to stocking in the larviculture tanks. At least 10 to 20 fish larvae have to be observed under the microscope for the following:-

- shape and dimensions
- deformities, erosions and abnormalities
- appearance of internal organs
- pigmentation

- absence of external parasites

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.

Larval rearing 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 9-11 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 oculata* at the

cell density of 1×10^7 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 DO₂: > 5mg/L, NH₃: < 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.

Age 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 ideal for stocking in happas/ nursery tanks.

Nursery and grow-out rearing of cobia

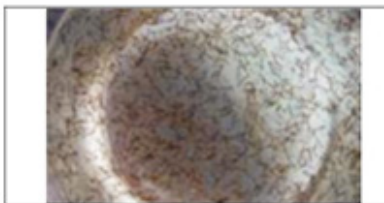
Nursery phase of cobia can be carried out in happas or sea cages or indoor FRP / cement tanks. During nursery rearing, it is advisable to feed the juveniles with formulated feed of size ranging from 800 to 1200 μ size

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.

Larval stages of cobia



3 dph



4 dph



6 dph



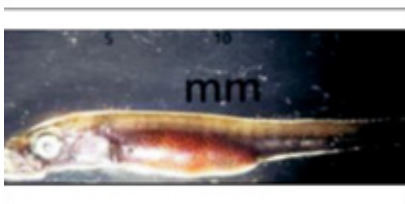
10 dph



13 dph



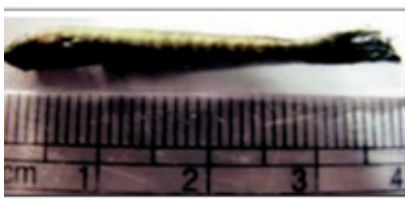
15 dph



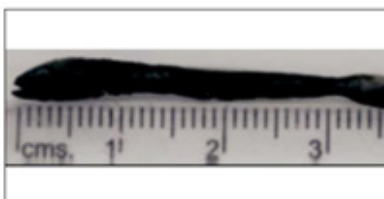
20 dph



21 dph



25 dph



30 dph

Larval rearing of Pompano

The newly hatched larvae can be stocked at a density of 15000 larvae in FRP tanks of 2 m³ capacity filled with 1.5 m³ filtered seawater. The tanks shall be provided with mild aeration and green water at a cell density of 1 x10⁷/ml. The mouth of the larvae opens on 3dph and the mouth size would be around 230 μ .

The larvae have to be fed from 3dph to 14 dph with enriched rotifers at a density of 6-8 nos. per ml in the larviculture tanks.

Wherever possible, copepods nauplii can 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. However, regular bottom siphoning to remove settled matter is also practised in some hatcheries.

Nursery Rearing of Pomapano

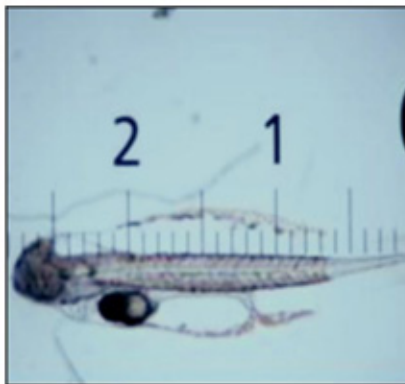
Nursery rearing could be initiated from 25 to 30 dph. At this stage, artificial feed of 800 μ size could be provided. Thereafter, fingerlings can be fed with progressively higher size range of floating extruded larval feeds. Daily water exchange of 100% is advisable. Water quality parameters like salinity, 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 from 10 to 15 ppt, they grow faster than in pure seawater.

Larval stages of pompono



Newly hatched larva



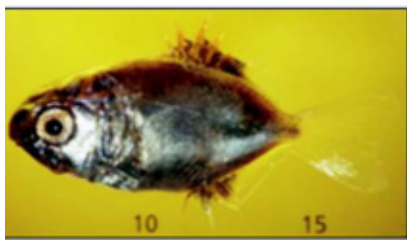
Larva on 2 dph



3 dph



13 dph



18 dph



28 dph

Size grading during nursery rearing

Size grading is advisable during the nursery rearing period to avoid cannibalism and increase the survival of fingerlings and also helps in stocking of uniform size seeds in grow-out culture. Grading of fish should be initiated once larvae reach 18-25 DPH and

to be continued for every three days with an automatic grader and grouped into different sizes. The mechanical grader also available in the market which can be used for grading the fry and fingerlings.

After egg hatching, fish larvae go through important changes to reach the juvenile stage, the most evident being

a dramatic biomass increase. Feeding success in fish larvae is critical for obtaining the nutrients and the energy necessary for healthy growth and development that allows them to survive to the end of the larval period. Feeding behavior is the result of interaction of complex processes viz. searching, detection, attack, capture, ingestion, digestion and



Grading of seeds in hatchery

excretion. Each of them has a specific pattern that changes throughout development. The feeding strategy is related to the specific characteristics of each species. Availability of suitable prey is one of the most determinant biotic factors, but feeding mode and amount of food intake are also influenced by prevailing environmental conditions.

Searching and detecting food

Searching for prey and detecting them depend on the appropriate functioning of some organs and tissues that become progressively available throughout development. From hatching, larvae are progressively aware of different external stimuli that indicate the presence of

potential food items. Searching depends basically on swimming capacity, while detection depends largely on sensory organs. Food detection occurs by means of visual, chemical and mechanical stimuli. Olfaction allows detection of distant stimuli, sight allows the identification of objects at medium and relatively short distance, while touch needs very close or direct contact with the source of stimulus. Most marine fish hatch with immature anatomical features. The sensory organs develop quickly during the first days after hatching. Sight allows the larvae to perceive objects that are relatively close. Altricial marine teleosts hatch with undeveloped eyes although the pigment in the retina appears in a few hours or days. This early retina has only one type of photoreceptor that allows vision only under bright light. Double and mosaic cone structures and rod photoreceptors appear later and enable vision at low light intensity. Olfaction allows for more remote detection of a stimulus. The olfactory organ appears early during embryonic development. Olfactory placodes and pits are already present at the onset of feeding and develop further by the late larval stage. The intra and extra oral taste buds develop or proliferate some days or weeks after the first feeding.

Mechanical stimuli such as touching or water movements are detected by neuromasts and the

lateral line system. In larval fish some few free neuromasts are already present at hatching and progressively proliferate during their growth and development. The progressive development and completion of all these sensory organs increase the capacity for detection and recognition of potential prey.

Locomotor capacity

Basically fish larvae exhibit alternating periods of swimming ability and inactivity. Swimming speed, pause duration, reactive distance, perception angles and duration of predation cycle define the changes in behavior during searching and attack throughout development.

At first feeding, even the smallest larvae have some primordial hunting habits, but the efficacy increases with development and growth, changing from passive feeding to an active prey searching capacity.

Capture and ingestion

Capture success relies not only on development stage and concomitant hunting capacity but also on the availability and accessibility of prey. Once the prey is perceived, the foraging has three possible results: unsuccessful attacks, aborted attacks and successful attacks. After mouth opening, fish larvae need to learn hunting and have to

do it quickly. High prey availability and accessibility are crucial for successfully initiating feeding. Prey size and swimming ability are primary factors determining the efficacy with which the prey is caught. The ability to start feeding after mouth opening is typically affected by prey size. During the very early stages with low swimming capacity, encounter opportunity depends on prey density.

Mouth gape limits the dimensions of the prey that can be ingested. Prey/gape ratio determined in different species usually ranges between 25 and 60%. Searching for appropriate prey of adequate size has been a priority for rearing fish larvae. The established prey sequencing is based on rotifers of different sizes and *Artemia* nauplii and meta nauplii. However, there is a need to search for live feeds below 100 micron size for rearing of very small marine fish larvae. Eventhough copepod nauplii can be employed for this purpose, mass scale production of copepod nauplii for large scale larval rearing is a major constraint. Overall, the current commonly used live feeds, *Brachionus* spp. and *Artemia* spp. meet well the feeding behavior of larvae except very small larvae at mouth opening.

Chapter.06

Live feed culture- Phytoplanktons

KK Anikuffan, G Tamilmani, M Sakthivel, P Rameshkumar, R Bavithra, A K Abdul Nazar & GH Rao

Live feeds are the main items in the diet of cultured fish larvae and they are of particular importance when rearing marine fish larvae of the altricial type. Altricial larvae are those that remain in a relatively undeveloped state until the yolk sac is exhausted. At first feeding the digestive system is still rudimentary, lacking a stomach, and much of the protein digestion takes place in the hind gut epithelial cells. Such a digestive system is in most cases incapable of processing formulated diets in a manner that allows survival and growth of the larvae comparable to those of live feeds. Despite the recent progress in the development of inert diets for fish larvae, feeding of most species of interest for aquaculture still relies on live feeds during the early life stages.

The low digestive capacity of the altricial larvae might not be the only aspect responsible for them requiring live feeds. Live preys are able to swim in the water column and are thus constantly available to the larvae. Most formulated diets tend to aggregate on the water surface or sink within a few minutes to the

bottom and are thus normally less available to the larvae than live feeds.

In addition, since larvae are visual feeders adapted to attack moving prey in nature, the movement of live feed in the water is likely to stimulate larval feeding responses. Live prey with a thin exoskeleton and high water content may be more palatable to the larvae once taken into mouth, compared with the hard, dry formulated diet.

Microalgae

Microalgae constitute the first link in the oceanic food chain. In aquaculture, microalgae are produced as a direct food source for various filter feeding larval stages of organisms. They are also used as an indirect food source in the production of rotifers, *Artemia* and copepods which in turn are used as food for the carnivorous larvae of many of the marine fish species. For rearing marine fish larvae according to the 'green water technique' microalgae are used directly in the larval tanks. This technique is nowadays a normal procedure in marine larviculture and is reported to improve fish

larval growth, survival and feed ingestion. The role of microalgae in the rearing water is attributed to (i) providing of nutrients directly to the larvae (ii) contributing to the preservation of live prey nutritional quality (iii) promoting changes in the visual contrast of the medium and its chemical composition and (iv) playing an important role in the microflora diversification of larval gut.

Whenever microalgae are used as a direct food source or as an indirect food source in the production of rotifers, *Artemia* or copepods, growth of the animals is usually superior when a mixture of several microalgal species is used. This probably occurs as different species compensate one another for eventual deficiencies in given nutrients. Special care is needed when selecting microalgae for on growing live feeds for marine fish larvae, in order to avoid the nutritional deficiencies of the latter especially in terms of n-3 highly unsaturated fatty acids. Deficiencies in the n-3 PUFA contents of microalgae may cause severe mortalities and quality problems in marine fish larvae. Such deficiencies may also cause reduced fecundity of rotifer and copepod cultures.

Recently commercial microalgal products are developed which can also be effectively

employed for larviculture. These include microalgae concentrates, frozen and freeze dried microalgae and microalgal pastes. Results of these products are generally good.

Microalgae like *Chlorella sp.*, *Nannochloropsis sp.*, *Tetraselmis sp.*, *Dunaliella sp.*, and *Pavlova sp.*, *Isochrysis sp.* can be used as algal diet for growing the rotifers. The size, nutritive value, proliferation rate and digestibility of the algae are the critical factors for selecting the algae for the use in marine hatchery use.

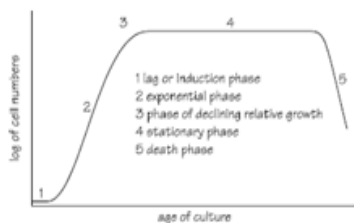
Nutrition required by Microalgae

- Macro elements include nitrate and phosphate as prominent nutrients
- Micro elements are iron, molybdenum, copper, zinc, cobalt, B1 (thiamine), B12 (cyanocobalamin) and biotin.

Growth phase of Microalgae

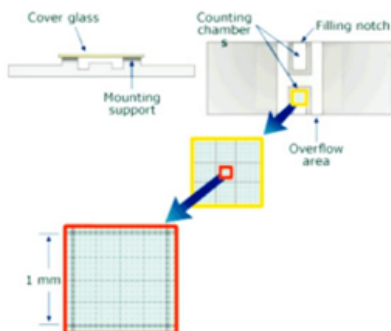
Microalgae grow by normal cell division and cultures supplied with excess resources will normally exhibit an exponential increase in cell numbers. After inoculation, the cultures need to acclimate to the new culture condition and the microalgae culture will have a lag or induction

phase. When the cells have become acclimatised to the conditions of excess resources, they grow and divide faster and the culture will follow an exponential growth phase, the cell density increases, while the nutrient in the growth medium will be exhausted and the increased density of the algal cells will increase self-spreading of the culture. The culture will then come into a phase of declining growth rate before it reaches the stationary phase. In the stationary phase, the net increase in cell numbers is zero. After some time depending on the species, the culture will continue into the death phase, where mortality rate exceeds growth rate.



Measurement of Algal Density

The algal cell density is measured using haemocytometer. The haemocytometer is a specialized microscope slide on which 2 grids have been engraved, in a central region that is 0.1 mm lower than the rest of the slide. Each grid comprises 25 large squares, each containing 16 smaller squares of area $1/400 \text{ mm}^2$. This creates a region of known volume (0.1 mm^3) when a special coverslip is correctly placed over the central region (get someone to show you how to do this - and take care as the cover slips are easily broken). $10 \mu\text{l}$ of culture are then pipetted under the coverslip and cells counted in a proportion of the grid squares (count as many as is convenient). Multiplying the total number of cells in the entire grid by 10^4 gives the number of cells per ml.



Algal Culture Management

STAGE I -

(Agar culture, test tube and
Erlenmeyer 100 - 250 ml, 500 -
1000 ml)

- The turbidity of media is approximately zero/nil which can be achieved by filtering water with 5 μm and 1 μm cartridge filters and UV filter.
- Salinity can be decreased (28-30 ppt) with the addition of 10 % distilled water ($V_1N_1 = V_2N_2$).
- Use only laboratory grade media.
- Autoclaving is mandatory
- Incubation has to be done at 19°C-23 °C and 1 or 2 neon lamp with 40 watt each has to be provided above the culture tank.
- One or two drops starter algal culture in a ratio 1 to 5 or 1 to 10 has to be added.

STAGE II

(bottle culture 1000 ml)

- The turbidity of media is approximately zero/nil which can be achieved by filtering water with 5 μm and 1 μm cartridge filters and UV filter.
- Salinity has to be maintained at 30 - 32 ppt
- Laboratory grade fertilizer can be used in a ratio of 1:0.5:0.1
- Sterilization with chlorine 10 ppm has to be done and it can be neutralized with < 5 ppm of sodium thiosulfate.
- Incubation at 24 °C with two neon lamps with 40 watt each is required.
- Starter algae can be added in a ratio 1 to 2 or 1 to 5
- Incubation of 21 to 25 days is required.
- Frequent mixing of algal culture by thorough shaking of the flask is required.

Laboratory Scale media (for stock culture upto 2000 ml flask)

STAGE III (Carboy culture 20 liter)

- Salinity of media can be 31-32 ppt

Conway' or Walne's medium

Solution A.	In 1 litre Dist. water	Solution C.	In 100ml Dist. water
Potassium nitrate	100 gm	Vitamin B1 (Thiamin)	20 mg
Sodium orthophosphate	20 gm	Vitamin B12 (Cyanocobalamine)	10 mg
EDTA (Na)	45 gm	Solution D (for culture of diatoms)	
Boric acid	33.4 gm	Sodium metasilicate ($\text{Na}_2\text{SiO}_3 \cdot 5\text{H}_2\text{O}$)	40.0 g
Ferric chloride	1.3 gm	Make up to 1 litre with distilled water; Shake to dissolve	
Manganese chloride	0.36 gm	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.	
Solution B.	In 1 litre Dist. water		
Zinc chloride	4.2 gm		
Cobalt chloride	4.0 gm		
Copper sulphate	4.0 gm		
Ammonium molybdate	1.8 gm		

- Use laboratory grade fertilizer (ration 1:0.5:1)
- Sterilization with chlorine 10 ppm and neutralization with sodium thiosulfate (5 ppm)
- Temperature has to be maintained at 24 °C and lighting with two neon lamps (40 watt each)
- Add algae starter with ratio 1 to 7
- Supply of CO_2 using aeration
- Incubate for 5 to 7 days

STAGE IV

(Intermediate Culture in an aquarium with volume of 100 liter)

In outdoor

- Salinity 32-33 ppt
- Use commercial grade fertilizer
- Chlorine sterilization 5 -10 ppm
- Use filter 1 micron Filter bag to filter the culture water
- Vigorous aeration is required
- Temperature 29-30° C
- Expose in direct sun light
- Incubate for 5 to 7 days

STAGE V

(Intermediate fiber glass culture in 1 m3 volume)

In outdoor

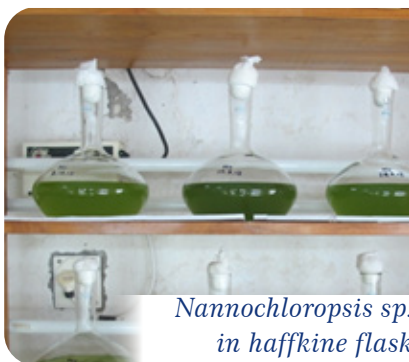
- Salinity media 32 - 33 ppt
- Use commercial grade fertilizer
- Sterilized with chlorine with dosage of 10 ppm
- Use filter 1 micron Filter bag to filter the culture water
- Vigorous aeration is required
- Expose in direct sun light
- Incubate for 5 to 7 days



Microalgal stock culture in test tubes



*Miacroalgal stock culture in
conical flasks*



*Nannochloropsis sp.
in haffkine flask*

*Isochrysis sp.
in haffkine flask*



*Nannochloropsis sp.
in 20L carboys*

*Outdoor mass
algal culture unit*



MASS CULTURE OF ALGAE

Production Systems

Common micro algal production strategies use either batch cultures or continuous cultures. A batch culture follows a typical growth curve, starting with an inoculation phase, an exponential growth phase and further into a stationary phase, where the carrying capacity of the culture is reached. Batch cultures are started at a low density and are harvested when desired density is achieved.

Continuous culture means that the cultures are harvested more or less continuously. There are several types of continuous cultures-the chemostat and semi continuous culture. In both a specific volume is harvested each day and the harvested volume is replaced by new growth media. A chemostat culture is continuously harvested and will have stable conditions of light, nutritional state etc., over time and the produced biomass and composition of algae can be practiced. Recently, hatcheries with requirements for microalgal produce it by photo bio reactors built up by horizontal or vertical tubes. The benefit of such reactors is that they have very large surface in relation to the volume, with good supply of light energy to the culture.

- The objective of mass plankton culture is to fulfil the necessity of zooplankton feed (rotifer) and to supply into the larva tank as the enrichment of zooplankton feed, to support the water quality and shading effect (Green water culture).
- The tank culture of algae has to be equipped with aeration on some points for fertilizer turbulence.
- The initial culture of microalgae (inoculums) has to be taken from the intermediate laboratory culture and then cultured in all the tanks gradually until the sufficient algal mass is achieved.
- It is necessary to sterilize the water using chlorine 10 ppm for 24 hours, then to neutralize by using Sodium Thiosulfate 5 ppm. To enrich fertilizers, several agricultural fertilizers can be used as shown in the Table below

Fertilizer	Concentration (ppm) / tonns
Urea	40-60
A m m o n i u m sulphate	20-40
A m m o n i u m phosphate	20-30
EDTA	1-5
FeCl ₃	1

(OR)

A m m o n i u m sulphate	100 g
A m m o n i u m phosphate	10 g
Urea	10g
Ratio - (1:1:1)	

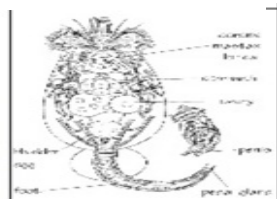
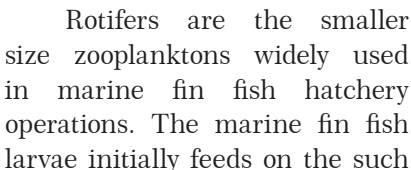
For the mass culture, an early density
usually have 2-3 million cells/ml.

- If the sunlight is normal, the algae will grow and will be ready for use after 4-5 days, with the density of 12-15 million cells/ml.
- Microalgae should be harvested before it reaches the peak of growth or when the algae are on stationary phase.
- Algae can be directly pumped to the destination tank such as algae seed tank, rotifer culture tank, copepod tank and larval rearing tank.
- The rest of the microalgae in a mass culture tank can be used for re-culture by adding filtered seawater and fertilizer. This method can be employed for several repeated times (2 – 3 times).
- After that microalgae in the culture tank is totally harvested and the tank needs to be cleaned to prepare for a new culture.
- For a medium size fish hatchery, several tanks are required to culture phytoplankton so that it can be harvested every day by turns.

No.	Parameters	Range
1.	Salinity	30 – 33 ppt
2.	Temperature	26 – 31 °C
3.	Light	12 L : 12D
4.	pH	8.1 – 8.3
5.	Dissolved Oxygen	4.5 – 6.5 ml / lit

Rotifer culture

smaller size zooplanktons and hence suitable size of rotifers need to be cultured in mass to feed the fish larvae. The important criteria for selecting the rotifer depends on the mouth size of the fish larvae, digestibility, nutritive value of the rotifer and easy for culture and proliferation. Marine and brackish water rotifer species can be artificially propagated in seawater and more popular rotifer species used for marine fin fish hatcheries are



Brachionus plicatilis and
Brachionus rotundiformis.

Based on the length of lorica,
Brachionus is separated into 3
strains,

B. plicatilis as L type (large) with
long of lorica 200 – 360 μm

B. rotundiformis as S type (small)
with long of lorica 150 – 220 μm

B. rotundiformis as SS type (super
small) with long of lorica 70 – 160
 μm .

Advantages of rotifers as live feed

- Small body size and round shape.
- Slow swimming speed and habit of staying suspended in the water column.
- Easily enriched with external nutrients resources.
- High reproduction rate and high density cultures.
- Very rich In low molecular weight water soluble proteins.
- Contain a broad spectrum of digestive enzymes such as proteases, peptidases, amylases, lipases and even celluloses.

Pure culture of Rotifer

- Incubated in 500 ml erlenmeyer containing sterile sea water. The culture condition should be equipped with fluorescent lamp and aeration to supply oxygen.
- Microalgae such as *Chlorella* sp, *Tetraselmis* sp, *Dunaliella* and *Isocrysis* sp can be used as feed for rotifer.

Mass culture of Rotifer

- Outdoor mass culture can be carried out in volume range of 5 – 12 m³ (1 tonne, 2 tonne, 5 tonne, 10 tonne).
- Phytoplankton has to be added to rotifer culture tank with density 3-4 million cell/ml for starting the rotifer culture. Rotifers have to be inoculated into tank with initial density 50-100 rotifers/ml.
- The next day, algae has to be added into rotifer culture tank.
- Practically, growth of rotifer can be identified by change of water colour into transparent.

No.	Parameters	Range
1.	Salinity	27 – 33 ppt
2.	Temperature	26 – 31 °C
3.	Light	2,000 – 3,000 lux
4.	pH	7.5 – 8.3
5.	Dissolved Oxygen	3 – 6.5 ppm

Continuous Culture

- Rotifers can be harvested by flowing out culture water through a plastic hosepipe into a rotifer sieving bag with mesh size of 60micron at the end point of those hosepipe
- Collected rotifers can be transferred into bucket for enrichment with different types of enrichment media.
- Every day, around 30% volume of rotifer culture tank has to be harvested from the total volume of tank
- After repeated use of 3 weeks, the rotifer culture tank should be totally cleaned for fresh culture

Rotifer enrichment

- Rotifers can be enriched with Highly Unsaturated Fatty Acids (HUFA's) which contains high levels of the essential Omega-3 fatty acids, Eicosapentaenoic acid (EPA) Docosahexaenoic acid (DHA).
- HUFA's are important to maintain fluidity of blood in blood vessel under low temperature condition for the fish larvae
- Especially DHA, an essential fatty acid that accumulates in the brain of fish during early development and it increases neural function
- Feeding with DHA-enriched diet at an early stage of fish larvae has been successful in improving pigmentation
- EFA deficiency creates syndromes like poor vitality, poor growth, low survival and death to simple stress (shock).

Enrichment methods

- Indirect method through feed medium like Nannochloropsis and
- ω – yeast. Rotifers in each culture tank can be fed with concentrated microalgae of Nannochloropsis (1x10⁴) every 8 hrs and yeast once in a day at morning.
- Direct method with emulsified oil (fish oil, etc) for 100 million rotifer with fish oil $\pm$ 5 ml, raw egg yolk $\pm$ 2 ml and tap water 100– 200 ml

Enrichment with scot emulsion

- Harvested rotifer has to be transferred into a 35 lit tub containing 3/4 part of Nannochloropsis medium and 1/4 part of rotifer (600 to 800 nos / ml)
- 1-5 ml scots emulsion oil can be mixed with freshwater and stirred well.
- This emulsion can be added into the tub containing rotifer and sea water.
- Strong aeration has to be provided for 2 hours.

Harvest rotifers and can be fed to the fish larvae

INVE A1 DHA and SELCO INVE- SELCO

(Self Emulsifying Liquid Concentrates)

- Enrichment is done in buckets containing a volume of 40 L with a
- density of 800-1000 rotifers/ml. Approximately 10 lit of N.
- oculata are added.
- Buckets receive aeration to maintain DO levels above saturation.
- Enrichment media (INVE DHA selco) is added and rotifers are allowed to feed for 4-6hrs.
- Once enrichment is completed, rotifers are filtered to remove residual enrichment diet and placed into a clean bucket containing a final volume of 35 L.
- Enriched rotifers can be fish larvae.

Copepod culture

Copepods are basically classified under the Phylum Arthropoda, Subphylum Crustacea, Class Hexanauplia and Subclass Copepoda. Basically there are nine orders in the Subclass Copepoda: Calanoida, Cyclopoida, Harpacticoida, Platycopioidea, Mormonilloidea, Misophrioida, Siphonostomatoida, Monstrilloidea and Gelyelloidea. Among the marine pelagic copepods, calanoid copepods dominate (79.2%) the others. The name copepod is basically derived from the Greek words meaning “animals with oar shaped foot” - *ie, kope* means ‘oar’ *podos* means ‘foot’ (Stotttrup, 2003).

The basic body structure of Copepods comprises of a large cephalothorax (cephalosome) formed by the fusion of head and thoracic segments and a small segmented abdomen (urosome). The thorax has basically six segments. All segments possess a pair of legs or pleopods which are used for swimming. The 5th and 6th legs are considered to be taxonomically very important and often these are modified or reduced. Cephalic region has a rostrum, a pair of median eyes, a pair of antennule, antennae, mandible, maxilla and maxilliped. Most of the appendages except the antennule are generally biramous.

The abdominal segments are reduced and without any limbs except for the caudal furca which form a tail fan with long setae. The sixth thoracic segment unites with first abdominal segment to form a genital double somite. The genital double somite together with abdominal somites form a slender tail like portion called urosome.

Reproduction

Most copepods reproduce sexually. The male deposits a sac containing viable sperm called a spermatophore near the genital aperture of the female. Most calanoids are broadcasters, shedding eggs singly into the water. The number of eggs spawned in a single event may vary from a few eggs to 50 or more eggs, and each spawning event may occur once every 24 hour for extended periods. Free spawning species such as various *Acartia* species may produce between 11 and 50 eggs per female per day, producing a total of more than 1200 from one single spawning and *Calanus* species between 15 and 230 eggs per female per day to a total of upto 3800 eggs per female. In most cases, a new mating is necessary for a female to produce eggs again. Other copepods including cyclopoids and harpacticoids, have their eggs contained within one or two egg sacs (ovisac),

which remain attached to the female genital segment until they hatch. In calanoids, the eggs are not contained in a membrane but adhere to each other as an egg mass and remain attached to the female. Each egg sac or egg mass may contain a few to 50 or more eggs and may be produced at frequent intervals of a few days. Egg production is measured as number of eggs per female per day. Resting eggs are produced by several species of copepod and are the primary mode of dormant state in calanoids. These copepod eggs are laid during development, whereby development is arrested, and possess an additional external envelope of variable thickness. Resting eggs are able to withstand long periods of desiccation, heat or cold. Resting eggs have only been reported for calanoids. Day length and temperature were the principal environmental cues that induce females to switch from active reproductive to a resting reproductive state.

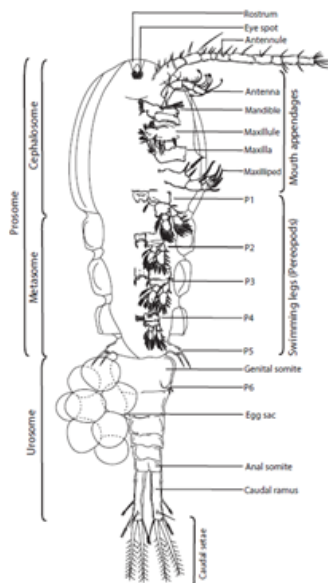
Life Cycle

Once fertilised the eggs pass into the water or into an egg sac. The egg is spherical and protected by a chitinous envelope. The larva that hatches from copepod eggs, the nauplius (NI), develops through five or six moults before passing onto the copepodite stage where they display the general

adult features. Most species commence feeding during the third or fourth naupliar stages, although a few species commence feeding during the second and even fewer during the first naupliar stage.

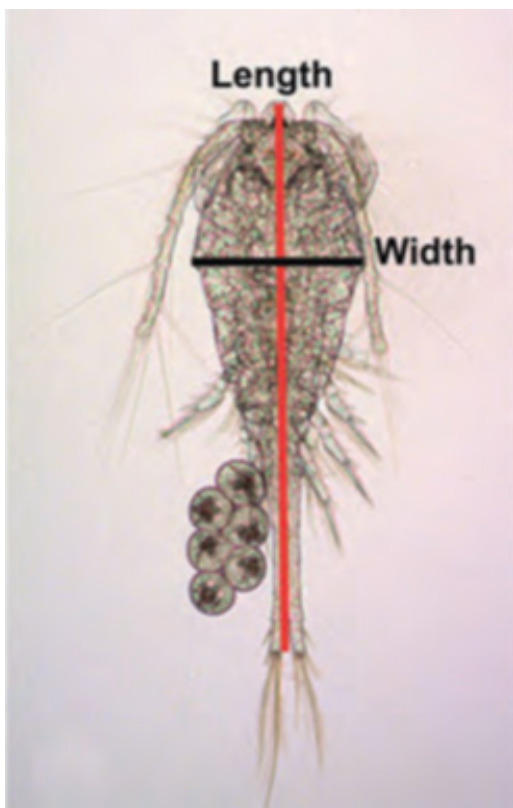
Size

Calanoid eggs produced in egg sacs range in diameter from 70 to around $800\mu\text{m}$ and are generally larger than freely spawned eggs, which measure around $200\mu\text{m}$. Size ranges of newly hatched nauplii also vary. Newly hatched nauplii such as *A. tonsa* measure less than $100\mu\text{m}$ in body length whereas as the larger calanoid nauplii measure around $220\mu\text{m}$.

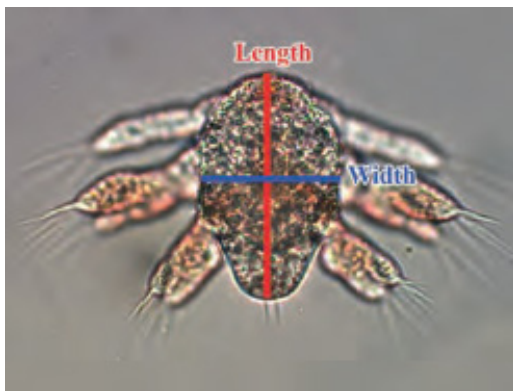


*Basic body structure of
Copepods (Santhosh et al, 2018)*

Size measurement of adult copepod (Santhosh et al, 2018)



Measurement of copepod nauplii (Santhosh et al, 2018)



Generation time

The generation time defined as the time interval between hatching of an individual and the hatching of its progeny which differs from species to species and is positively correlated with increasing temperature. In calanoids reared at different temperatures, generation times varied from around one week in *Acartia* spp to months. Food supply and salinity may also influence development rates.

Feeding, Food quality and Food availability

Calanoids are generally herbivorous filter feeders, able to distinguish between particles and selecting between different food particles based on size or taste. The rate of consumption of algal particles is influenced by the size, quantity and quality of the food. However, few species of calanoids have a non-visual, active raptorial mode of feeding, capturing and ingesting a variety of animal prey. Copepod species may also create feeding currents that entrap non-evasive prey. The food concentrations at which egg production commences and at which it attains a maximum level differ between species. Egg production per female per day increases with increasing food concentrations to an asymptotic

level. Food quality also influences growth and reproduction.

Harpacticoids are primarily detritivorous, benthic grazers, efficiently utilizing various food sources such as bacteria, microalgae, marsh grass, algal biofilm, diatoms, polychaete meat, etc. Although harpacticoids eat practically anything, their offspring production is not independent of the food quality. Food supply in terms of quality and quantity affects feeding, development and reproduction in harpacticoids.

The use of copepods in marine fish hatcheries for larviculture has started gaining importance recently, due to the development of culture techniques of many species of copepods. The nauplii or copepodite stages of copepods are an ideal feed for fish larvae owing to its small size and rich nutritional profile when compared to rotifers. The most commonly used copepods species in aquaculture belong to the orders – Calanoida, Harpacticoida and Cyclopoida.

Culture of Copepods

Outdoor production in ponds and tanks can be carried out. Filtered seawater is generally used in this system. By using filters of around 20-40 μm , natural phytoplankton can be

transferred to ponds without the accompanying zooplankton or potential predators. The phytoplankton can be fertilized to induce blooms. Filtering devices for selective sieving are used to collect primarily nauplii (80-250 μm), copepodite stages (80-350 μm) or primarily adult stages (250–600 μm) to inoculate the rearing tanks. In Asian countries, copepodites and adult stages of copepods were added in tanks 3 days before stocking of the newly hatched fish larvae. In this system, using wild harvested copepods (*Acartia* spp, *Pseudodiaptomus* spp., *Oithona* spp., and a few harpacticoids) an average survival of 3.4% at harvest of *Epinephelus coioides* was obtained. Regular monitoring of densities of the live prey in these outdoor systems is important for the successful rearing of marine fish larvae.

Calanoids

Most frequently cultured calanoids are coastal species such as *Acartia*, *Centropages*, *Eutemora* and *Temora*. They are small, relatively of short generation times and a wide thermal and salinity tolerance and are easily adaptable to laboratory condition. Most calanoids require phytoplankton. In many cases the copepods are reared on monoalgal diets, which may not comply with all the requirements for maximum egg

production. Somatic growth ceases in adult copepods and growth rate is more or less equivalent to the rate of egg production. The rate of egg production in copepods is dependent on the size, quantity and quality of the algae provided. As a general rule to reach food saturation, high ingestion rates and high egg production rates, cell concentrations of around 103 cells/ml would be sufficient using larger cells and around 104 cells or 105 cells per ml using smaller cells. A combination of at least two algal species with high n-3 polyunsaturated lipid content, and of a size that can be utilized by both the feeding naupliar stages and the copepodite and adult stages comprises an adequate diet for culture.

In one of the culture systems, the eggs are sedimented to the bottom from where they are siphoned daily, simultaneously siphoning out debris, faecal matter and associated ciliates. During the siphoning, the eggs are concentrated on 45 μm sieve, allowing most of the debris and ciliates to pass through and get removed from culture. The daily removal of eggs eliminates the potential loss of nauplii through cannibalism by adult population. The presence of ciliates (*Euplotes* sp.) cause deterioration of water quality and a thorough water

exchange is necessary. Failing to do this will result in a culture crash. The culture is filtered through a 180 μ m sieve submerged in sea water to retain the adult population and wash out the ciliates. The adults were then used to inoculate a new tank filled with filtered (1 μ m) sea water.

Light

A photoperiod of 12 hour of light is sufficient. High solar radiation is harmful to copepods; hence adults show negative phototaxis during the day and positive phototaxis during the night.

Aeration and Oxygen

Aeration is required to maintain phytoplankton in suspension and create small turbulence, which helps to distribute the copepods. Too vigorous aeration should be avoided and unnecessary.

Culture tank size and shape

Most calanids require large volumes and the adult density rarely exceeds 100 per litre. Cylindrical tanks with flat bottom (200 litres) are suitable for culture of copepod, *Acartia tonsa*.

Temperature and Salinity

Temperature has a vital role for copepods. But their adaptability to temperature

is remarkable. Coastal species have wider thermal and salinity tolerances than oceanic species.

Contamination

Contamination of copepod cultures by bacterial blooms, ciliate infections, other copepods or rotifers may pose a problem. Use of same siphon/sampling devices, etc. for all copepod tanks should be avoided. In commercial facilities, contamination by rotifers is the most likely cause of the collapse of copepod culture, since the rotifers with their higher reproductive rate would quickly outcompete the copepods. It is therefore important to keep these cultures strictly apart.

Ciliates are utilized by copepods and may in periods of low phytoplankton concentrations constitute the major dietary source. In intensive cultures, the presence of certain ciliates such as *Euplotes* sp. is often an indication of overfeeding and should be avoided.

For eliminating ciliates it is advisable to empty the culture using a 60 or 80 μ m mesh which retains the adult copepods, but allows the ciliates to be washed out. Cultures may succumb to uncontrolled proliferation of bacteria. Eventhough bacteria often constitute a part of the diet of copepods. Calanoids are sensitive to high ammonia concentrations.

Harvest and Storage

Copepods can survive for short periods in gauze as they are transferred from one tank to another. They can also survive for an extended time at very high densities, provided that there is sufficient oxygen. Freely spawned calanoid eggs sink to the bottom and can be harvested by siphoning the bottom once daily. The day's production can be transferred to individual hatching tanks to be used as live feed.

Harpacticoids

Harpacticoids have been cultured in batch and continuous systems to provide food for marine fish larvae

Advantages

- i. High tolerance to a wide range of environmental conditions
- ii. Ability to feed on a wide range of live or inert diets
- iii. High reproductive capacity
- iv. Relatively short life cycles (7-29 days)
- v. Ability to be cultured in high densities
- vi. Requirement for surface area rather than volume
- vii. Planktonic naupliar stages

- viii. Can be used as tank cleaners in rotifer cultures, other copepod cultures or larval tanks.
- ix. The culture conditions for harpacticoids are less demanding than those for calanoids. Filtered seawater may be used and a whole range of inert food is acceptable to many harpacticoid species. This simplifies the culture method and eliminates the need for culture of phytoplankton.

Food and feeding:

If algae are readily available, a mixture of two algal species would be the preferred choice. Algae which quickly sediment are very appropriate for benthic copepods, possibly because bacteria colonise these cells, and the mixture of algae and bacteria may be a superior combination for harpacticoids.

Light: A photoperiod of 12L/12D was shown to be most favourable for offspring production.

Aeration: Aeration may be applied to maintain an even distribution of food

Culture tank size and shape:

The mass culture of benthic harpacticoids is dependent on the availability of surface area rather than culture volume.

Temperature and Salinity:

Most harpacticoids have wide thermal and salinity tolerances.

Contaminants: Rotifers and ciliates are the major contaminants

Harvest, storage and Transport

Since harpacticoids are not free spawners, harvest methods for collecting nauplii need to be developed. Concentrating nauplii by light can be practised for harvesting. Harpacticoids are relatively tolerant to high stocking densities and can be transported for a period of upto 2-3 days, kept cool in blood transfusion bags (2 litres) at densities of 200,000 individuals per litre. Excess nauplii can also be stored at 4°C for upto one week and used on days when production output is below the required amount.

Isolation and Identification

Collection from the wild – filter with particular mesh size (100micro to 500 micron)

100 nos individual (1:1 M/ F) isolate to 50 ml test tube Add microalgae as feed (5 ml / 2 days once)

Provide light period 12L / 12 D; Salinity: 27 to 35; Temperature: 28 to 31°C

Generation time – 7 days

Harvest nauplii / copepodite (harvest continuously for 3 months)

Intermediate culture

Nauplii (1000 nos) transfer to 1000 ml beaker Add microalgae as feed (25 ml / 2 days)

Provide light period 12L / 12 D; Salinity: 27 to 35; Temperature: 28 to 31°C

Generation time – 7 days

Collect nauplii / copepodite

Mass culture

Nauplii transfer to 1 to 2 ton tank

Add microalgae as feed (500 lit algae / week)

Provide light period 12L / 12 D; Salinity: 27 to 35; Temperature: 28 to 31°C

Generation time – 7 days

Harvest nauplii / copepodite (3-5 nos / ml) Total drain and harvest the tank after 1 month.

Culture Protocol

- Population counts should be done weekly for feeding adjustments.
- Count algae concentrations and compute required feed volumes to be added.

- Siphon detritus from tank bottom daily.
- Remove sufficient water volume to allow addition of new feed volume(s), while maintaining 200 L total volume. As the population matures this volume can be increased to 300 L. Adjust feed computations accordingly.
- Use 40 micron will retain faeces, nauplii, and eggs. Rinse sieve gently into separate collection bucket. Buckets are allowed to stand for 10 minutes to allow settling. Adults and nauplii are then attracted to the upper layer of the bucket with light and decanted for return to the tank.

Advantages of Copepods

- i. The three main copepod orders viz., Calanoida, Harpacticoida and Cyclopoida have been investigated for their suitability as feeds for larval and juvenile fish. While each copepod order has its advantages and disadvantages, it is generally agreed that the following are the benefits of using copepods for larviculture.
 - ii. Copepods have a larger size range from first nauplii to adult copepodites and offer good size ranges for the entire hatchery phases for certain species of finfish
 - iii. They have superior nutritive value in comparison to rotifers and Artemia
 - iv. Copepod nauplii may be more easily and completely digested than either rotifers or Artemia
 - v. Copepods are natural sources of antioxidant astaxanthin and Vitamins C and E
 - vi. The movement of copepods and their nauplii triggers the feeding responses in fish larvae. The 'jerking' swimming action of most copepod nauplii and adults is an important stimulus for initiating feeding by fish larvae
 - vii. Use of copepods in larval fish diets have been associated with a decrease in fish malpigmentation and deformity rates

Artemia nauplii

It is used in marine aquaculture worldwide. Although *Artemia* is not a natural part of marine larvae, it has been favoured due to its convenience for use and high nutritional value. One of its most interesting features of this organisms is its ability to form dormant cysts that are highly resistant to adverse environmental conditions and can be kept viable for years. They are normally stored under dry and cool conditions. The ease and simplicity of hatching brine shrimp nauplii makes them the most convenient and least labour intensive live food available for aquaculture. However, the only negative aspect is its high cost, hence now a days the practical strategy adopted in larviculture of marine species has been to attempt early weaning in conjunction with a prolonged rotifer feeding period to eliminate the use of *Artemia*. However, this is not always possible and in some species whose larvae are relatively larger at hatching, *Artemia* nauplii might even be the only live prey used in larviculture.

Artemia cyst strains

Having a larger size than rotifers, the brine shrimp *Artemia salina* are used as the second (after rotifers), live feed organisms to feed fish larvae. *Artemia* is not cultivated in the

hatchery as in the case for algae and rotifers, but their larval stages are obtained by incubating and hatching their resting eggs, which are available commercially as dry storable cysts. The first *Artemia* larval form is the nauplii, which are smaller in size and richest in yolk, and followed by a larger size metanauplius, whose nutritional value has to be boosted by feeding them with special enrichment diets 12 to 24 hours before feeding to the fish larvae.

Cysts of different strains can be used as per the requirement:-

- i. Cysts giving small Instar I-nauplii (with a length of around 430 μm at yolk- sac stage) with high levels of the essential highly unsaturated fatty acids (n-3 HUFA). An example is the AF strain of the Belgian producer INVE. Such nauplii allow an early switch from rotifers to *Artemia* in larval feeding.
- ii. Cysts producing medium-sized Instar I-nauplii (around 480 μm) with high levels of n-3 HUFA, such as the AF 480 strain of INVE. These nauplii are useful to switch from the smaller nauplii to the bigger metanauplii.
- iii. Cysts hatching large Instar I-nauplii (around 520 μm)

with low levels of n-3 HUFA, such as the EG strain of INVE. These cysts, which are the commonest and cheaper, are widely used to produce metanauplii which represent the bulk of live feeds in larval fish rearing.

Their enrichment is mandatory to increase the essential fatty acids content to meet the nutritional requirements of young fish. Two additional parameters characterize the *Artemia* batches: the number of cyst per gram and their hatching rate (the number of nauplii produced per gram of cysts). The best strains can give about 290 000 - 300 000 nauplii per gram of cyst hatched, with a hatching rate close to 95%. In a hatchery the use of good quality cysts allows a synchronization of the production cycle on a 24-h period, with the harvest of freshly hatched nauplii coinciding with the start of the incubation of new batches.

Disinfection and decapsulation of brine shrimp cysts

Artemia cyst shells are usually contaminated with bacteria, spores of fungi and other microorganisms. Fish larvae can be infected when untreated empty shells, unhatched cysts or cyst hatching medium residues are

introduced into the larval rearing tank. Before incubation, cysts should therefore be disinfected. This process also improves hatching by reducing the bacterial load of the hatching medium. Disinfection is done by keeping the cysts for a few minutes in a hypochlorite solution at a maximum density of 50 g/litre. This product is easily available as commercial grade bleach. The duration of the treatment varies according to the active chlorine concentration of the disinfecting solution.

Typical duration is :

- 1 minute in a 10 000 ppm solution,
- 20 minutes in a 200 ppm solution.

As in commercial bleach, the chlorine content may range from 5 to 15%, it is mandatory to check the actual chlorine concentration in the bleach that is going to be used. This can be done either by titration or by determination of the refractive index. The following example shows how to disinfect one kg of cysts in a 200 ppm hypochlorite solution obtained from a household bleach with 5% active chlorine:

- One kg of cysts needs 20 l of fresh water for the disinfecting solution.

- If this solution is going to be used for a 20 minutes bath you will need $20 \text{ l} \times 200 \text{ mg/l} = 4000 \text{ mg} = 4 \text{ g}$ active chlorine.
- The quantity of 5% bleach required to give 4 g active chlorine is: $(1000/50) \times 4 = 80 \text{ ml}$ pour 80 ml of 5% bleach in 20 l of fresh water.
- Add one kg of cysts; place an airstone for continuous aeration to keep cysts in suspension, and keep the cyst in the solution for 20 minutes.
- Harvest cysts on a sieve (125 μm mesh size) and rinse thoroughly with plenty of tap water.
- Transfer the rinsed cysts to the incubation tank.

A more effective way to obtain completely contaminant-free cysts is decapsulation, which implies the elimination of the cysts thick external layer, the chorion, by chemical oxidation. This process, which requires greater attention, has additional advantages. As they spend less energy to hatch after the removal of the chorion, the hatching nauplii have better nutritional value. Moreover, fish do not risk suffocating by gulping empty or unhatched cysts offered together with the nauplii, as it may happen when using disinfected cysts. The decapsulation process consists in

four steps: hydration, treatment in a chlorine solution, washing and deactivation of the residual chlorine. The example described below refers to the decapsulation procedure of one kg of cysts. The hydration, a necessary step as the complete removal of the chlorine may only happen when cysts are spherical, proceeds as follows:

- Water volume required: around 6 l per kg (maximum amount: 200 g/l); both fresh and sea water can be used; water temperature should be between 20-25°C; duration: 45 minutes;
- aeration: sufficiently strong to keep cysts in constant suspension; use an open end pipe in a 10 l bucket.
- Collect the hydrated cysts on a sieve and treat them immediately with the decapsulation solution.

The decapsulation solution requires a source of hypochlorite, usually liquid bleach (NaOCl), and an alkaline product to increase pH level of the decapsulation solution above pH10. Usually technical grade caustic soda (sodium hydroxide NaOH) is used. The first product is added at 0.5 g active chlorine per gram of cysts, and the second as 0.15 g of sodium hydroxide per gram of cysts. For hydrated cysts the procedure is as

follows (figures refers to one kg of cysts):

- Prepare 0.5 g Cl x 1 000 g cysts = 500 g of active chlorine, equal to 3.33 l of a 15% bleach.
- Prepare 0.15 g NaOH x 1 000 g cysts = 150 g of NaOH, equal to 0.375 l of a 40% NaOH solution.
- Put the bleach and NaOH in a suitable container (e.g.: a 20 l plastic bucket) and fill with seawater to 14 litres (14 – 3.33 – 0.375 = about 10.3 l of seawater) provide a strong aeration and eventually if available add antifoam.
- Place the hydrated cysts in the bucket; control the temperature: it should remain within 25°-30°C. In case of higher temperatures, add ice to prevent that it reaches 40°C which are lethal for the cysts.
- Verify cyst colour changes. The change in cyst colour confirms that decapsulation

is in progress.

- The cyst colour shifts from dark brown to grey and finally to orange, which is the colour of the nauplius body seen by transparency through its outer cuticular membrane, left exposed by the dissolution of the chorion. The process usually lasts 5 to 15 minutes.
- Using a pipette or a graduated cylinder, check floatability: non decapsulated cysts will float and decapsulated cysts will sink; as soon as all cysts have turned orange, stop the process by harvesting them on a sieve and rinse thoroughly with plenty of tap water and rinse well until no more chlorine smell is noticed
- The residual hypochlorite adsorbed by the decapsulated cysts has to be neutralised by dipping them in a 0.1% solution of sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$) for 5 minutes; then, after a final rinsing, they were transferred to the incubation tank.



Figure A. Small scale Artemia
cyst hatching system and B.
Microscopic observation
of hatched Artemia nauplii

Counting and evaluating *Artemia nauplii*

To assess the hatching results and to feed the larval rearing tanks at the established densities you have to count the *Artemia nauplii*. Three methods are described below, first for high nauplii densities, such as after harvesting and in a cold storage tank, second for counting the nauplii when they are in the incubation tank and the third for low nauplii densities, similar to those which can be found in fish tanks.

Counting high density nauplii samples

- Take a 10-ml sample of the population to be estimated.
- Dilute the nauplii concentration by adding 90 ml of sea water to obtain a total sample volume of 100 ml.
- Take three sub-samples with a 1-ml pipette, avoiding sucking air bubbles.
- Transfer each sub-sample to a Petri dish.
- Add a few drops of the fixative staining solution Lugol to each of
- the Petri dishes and wait until all nauplii are immobile and deeply stained.

- Add water so as to distribute the nauplii over the whole surface of the three Petri dishes.
- Put each Petri dish on a grid and count the nauplii present in each 1 ml sub-sample.
- Calculate the average number of nauplii per ml by dividing the sum of the three counts by three.
- The total nauplii density per litre is given by the average number of nauplii per ml multiplied by 10.
- To give the number per undiluted ml and finally by 1 000 to get the final density per liter.

Counting nauplii samples from the incubation tank

- Take with a test tube a 50-ml sample of the population to be estimated.
- Take three sub-samples with an automatic 0.1-ml pipette.
- Transfer each sub-sample to a 3 cm-wide Petri dish, whose bottom has been subdivided in a 5mm-grid.
- Add a few drops of the fixative staining solution Lugol to each of the Petri dishes and wait until all nauplii are immobile and deeply stained.

- Add water so as to evenly distribute the nauplii over the whole surface of the three Petri dishes.
- Count the nauplii present in each 0.1 ml sample.
- Calculate the average number of nauplii per ml by dividing the sum of the three counts by three.
- The total nauplii density per litre is given by the average number of nauplii per ml multiplied by 10 to give the number per ml and finally by 1000 to get the final density per liter.
- Calculate the nauplii density per litre by multiplying this count either by 20 (50 ml sample) or by 10 (100 ml sample).
- Hatching rate: the number of nauplii hatched per 100 cysts; good batches have a hatching rate around 90-95%.
- Hatching efficiency: the number of nauplii produced per gram of cysts; top quality cysts yield about 300 000 nauplii/g.

Counting low densities nauplii samples

- Take three 50 or 100 ml samples, using a graduated cylinder which has been cut at the 50 or 100 ml mark.
- Transfer each sample to a large Petri dish.
- Add several drops of Lugol to each of them and wait until all nauplii are immobile and deeply stained.
- Put each Petri dish on a grid and count the nauplii present in each sample.
- Calculate the average number of nauplii present in each sample.
- Prepare culture vessel for the enrichment process using fresh seawater.
- Salinity 35 - 45 (ppt); temperature: 27 -28 C and vigorous aeration.
- Harvest the newly hatched nauplii.
- Gently rinse with fresh water with suitable filter.
- Transfer to a clean culture vessel.
- Stock at the rate of 8,000 -10,000 newly hatched brine shrimp per liter
- Do not feed nauplii at this time - they are absorbing their attached yolk sac.

Artemia enrichment

The two main criteria to evaluate hatching results are:

- Approximately 8 to 10 hours from time of transfer to clean culture vessel, the nauplii will have molted into the Instar II feeding stage.
- Add **SELCO 0.2 g per 1,00,000** Artemia nauplii per liter.
- Ensure that the aeration is vigorously mixing the water column.
- After approximately 12 hours, the intestinal tract of the nauplii should be fully enriched with SELCO.
- Harvest enriched nauplii and feed immediately maintaining a nauplii concentration of 2-3 nos. per ml.
- Unfed enriched nauplii can be stored in the refrigerator for later feedings .

Enrichment using Instar-II-nauplii :

Add **0.6g (in a minimum of 2 rations (0 hrs – 12 hrs)** of A1 DHA SELCO per liter of seawater containing up to 3,00,000 Artemia nauplii, in the enrichment tank. Enrich for a period of 24 hours while maintaining min, 4 ppm DO and p H of 7.5-8.5.

Optimal enrichment conditions

- For water quality and container design, see previous section; initial nauplii density: between 150,000 and 300,000 nauplii/l.
- Vigorous water agitation to keep the nauplii in suspension and pure oxygen to keep dissolved oxygen above 4 ppm throughout the enrichment period: use one open PVC pipe for air and a micro-bubbles diffuser for oxygen.
- Lighting: not required.

Prepare the enrichment meal as specified by the producer, and make sure to prepare a new enrichment emulsion for each meal. At the end of the enrichment time harvest the metanauplii as usual, rinsing them thoroughly with seawater until no oily emulsion is noticed in the outflowing water.

Chapter.08

Farming of Cobia and Snubnose Pompano

M Sakthivel, G Tamilmani, P Rameshkumar, KK Anikuttan, R Bavithra, B Johnson & K Abdul Nazar

Cobia fish is popularly known by the common names- Black kingfish, Black salmon, runner or sergeant fish, crab eater and Sea Murrel. It is considered as a promising candidate species for farming due to its fast growth rate, excellent meat attributes and easy adaptability to farming conditions. 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 (2018) shows that the global aquaculture production of

cobia has been increasing rapidly from only 9 tonnes in 1997 to 43,100 tonnes in 2016. Since the late 1990s, cobia aquaculture production has been steadily expanding in Asia, primarily in Taiwan, Vietnam and China and also in other Southeast and Indo-Pacific Asian countries including the Philippines, Indonesia, Iran and Reunion Island. Although the majority of cobia aquaculture production currently comes from China, most of the detailed information about culture and grow-out methods is reported from Taiwan Province of China. The fast growth rate, adaptability to captive breeding are the major attributes which makes cobia an excellent candidate species for mariculture. The meat of cobia is served raw, called as *Sashimi*, at the restaurants in the Southeast

Duration	Length (cm)	Weight (g)
Week – 0	7.1 ± 0.1	2.2 ± 0.1
Week – 1	10.0 ± 0.2	4.2 ± 0.1
Week – 2	12.0 ± 0.1	5.5 ± 0.2
Week – 3	13.5 ± 0.2	13.6 ± 0.6
Week – 4	15.2 ± 0.4	23.3 ± 0.6

The growth recorded during nursery rearing is provided below for reference

Asian countries. India is a late starter in cobia research and the fingerling production of cobia was achieved for first time in India by the Mandapam Regional Centre of Central Marine Fisheries Research Institute (CMFRI) and later the farming protocols in the High Density Polyethylene (HDPE) cages and Galvanized Iron (GI) cages with different stocking densities, feeding strategies were developed, tested and validated. Out of these farming trials an economically viable farming method has been evolved. Farming of Cobia in sea cages was also successfully demonstrated by CMFRI all along the coasts of India. Subsequently, the Rajiv Gandhi Centre of Aquaculture (RGCA) under the Marine Product Export Development Authority of India (MPEDA) also contributed to the cobia fingerling production and farming in India.

Nursery rearing should be carried out for a period of one month to make the fingerlings into a cage stockable size of 20

Farming in cages

The cobia can be farmed in sea cages made of High density Polyethylene (HDPE) or galvanized iron (GI) pipes. The HDPE cages will last for 10 - 15 years, while the GI cages will last only for a maximum of three years

grams. The nursery rearing of juveniles could be carried out in 10 tonnes capacity tanks or sea cages (6 meter diameter) fitted with smaller mesh sized nets. If the nursery is carried out in cages, it is pertinent to clean or brush the nets daily to maintain the free flow of seawater. If nursery is carried out indoor, adequate water quality and optimum aeration should be maintained in the tanks. Recommended stocking density in indoor nursery tanks is 1 number per 10 litre with 200% water exchange and for nursery cages it is 1.8-3.0 kg/m³. Suitable sized artificial feeds (800 – 1800 micron diameter) should be provided during nursery rearing. Floating or slow sinking pellet feed with 50 % crude protein and 10% crude fat composition would be more suitable for successful nursery rearing. Such high protein and fat containing nursery feeds are available in India at an affordable price. Storage of such feeds plays a vital role in maintaining the quality during their shelf life period.

even after good maintenance. But, the initial investment would be much lesser, if the GI cages are used.

as over density, over feeding or improper disposal of dead fish will increase load of organic matter in the water body and will cause problems like turbidity, anoxia, death of benthic species and increase in bacterial growth. The cage farming site should have minimum depth of 6 meters depth during low tide, when a cage net depth is maintained at 3.5 meters. Dissolved oxygen level is comparatively lower at the sea floor and fishes cultured too close to the sea bed may suffer from anoxia.

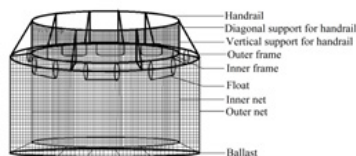
Optimal Stocking density

As fishes grow, they need more space for movement however, the space in net cage is limited. Therefore, it is necessary to stock optimum number of fish fingerlings. Overstocking will weaken the fish resulting in higher risk of bacterial, viral or parasitic infection. Higher stocking densities may also lead to insufficient levels of dissolved oxygen to sustain the entire stock. During low tide and poor water current, non-availability of required level of dissolved oxygen leads to anoxia and death. A cage with 6 meter diameter having a net depth of 3.5 meters can be stocked with 900 numbers of cobia fingerlings.

Acclimatization and stocking of fish fingerlings

Cobia fingerlings procured from the hatchery needs to be acclimatized to the new environment to get better survival and growth. If any abnormal behaviour or symptoms of infection are noticed, such fingerlings have to be isolated and reared separately. To avoid spreading of diseases, fingerlings infected with pathogens should be given proper treatment. Approved disinfectants/antibiotics can be used for treating the diseases in consultation with the fisheries officials/ CMFRI scientists. Excessive use of disinfectants or therapeutic drugs will lead to problems like increasing organic matter in the water, excessive drug residues in the fish, drug resistance in bacteria. Further, it is a wastage and may also have adverse impact on both the environment and the health of fish.

Schematic diagram of GI cage



Schematic diagram of GI cage
with nets and floats

Management of Bio-fouling in net cages

Cage farming activities enrich the sea with nutrients, this coupled with warm water temperature, forms an ideal habitat for fouling organisms like barnacles, mussels, sea weeds and algae to grow on the net and cage surfaces. Proliferating fouling organisms not only consume a great deal of dissolved oxygen, but also block the meshes of nets and impede effective replenishment of dissolved oxygen in sea water inside the cage area. Fouling organisms may also add weight to the cage nets and cause damage to nets and sinking of the cages. To avoid this situation, cage nets have to be cleaned regularly to prevent the colonization of fouling organisms. The cage nets have to be inspected regularly and repairing of torn or damage parts and exchange of nets having more fouling needs to be undertaken. Net cages can be changed based on the subjective assessment of fouling of the net in order to have sufficient water exchange. Normally net cages have to be changed once in 45 to 60 days depending on the intensity of bio-fouling. Nets having high level of fouling needs to be dried under sunlight through prolonged exposure and all the barnacles and algal attachments on the nets need to be cleaned. Repairing of

any damage found in the cage unit including mooring system would help to maintain the buoyancy as well as healthy cage frame.

Feed Management

Fish feed forms a major part of the operational expenditure for cage farming of cobia. Proper feed management strategies would help to reduce overall production cost. Optimal use of feed also helps to improve the farming environment and ensures the health of fish stock. Fish feed management includes choosing the right feed, following correct feeding methods, feeding the optimum quantity and cost effectiveness. Feeding of cage farmed cobia fishes with appropriate quantity and quality feeds will prevent the presence of excessive organic matter and mitigate problems like low dissolved oxygen and bacterial growth. Use of extruded formulated pellet feed instead of low value fish/trash fish will help to reduce organic matters in water. If the formulated feeds are not readily available at affordable price, farming can be taken up by feeding with low value fishes and by-catch. The juveniles of cobia have to be fed @ 5 to 10% of total biomass of fish with chopped low-value fishes (sardine, lesser sardine, rainbow sardine, etc.) twice daily up to two months of culture. As a thumb rule, the feed

can be provided initially at the rate of 10 % of the biomass which can be slowly reduced to 8% and then to 5% as the fish grow. The timing of feeding should also be maintained, feed them at about the same time of the day, preferably early in the morning or late in the afternoon as the fishes will automatically get used to the timing and will also come near the surface when they hear the sound of the boat. Feed quantity has to be reduced when the fishes are under stress or during rough weather or during low water temperature. Feeding has to be done slowly to give enough opportunity to all the

fishes to feed. In general, marine fishes require high level of protein (35 to 40%) and fat (8 to 10%) for their metabolic activities and growth.

Growth assessment

Random sampling can be carried out at the time of net exchange with the sample size of at least 30 cobia fishes per cage. The entire grow-out culture is carried out for a period of 6-8 months. The growth details of cobia as recorded in sea cages at a stocking density of 8 Nos./m³ are given below for reference:

Duration	Length (cm)	Weight (g)
Month – 1	21.5 ± 0.3	70.8 ± 2.4
Month – 2	22.4 ± 0.6	94.1 ± 1.3
Month – 3	26.0 ± 0.8	125.3 ± 2.5
Month – 4	32.9 ± 1.1	468.5 ± 27.8
Month – 5	46.3 v 1.0	1109.3 ± 87.7
Month – 6	56.4 ± 1.1	1985.5 ± 92.3
Month – 7	73.5 ± 1.0	3316.2 ± 57.6
Month – 8	77.9 ± 1.1	4015.4 ± 74.0
Month – 9	85.7 ± 0.9	4851.1 ± 88.8
Month – 10	90.8 ± 1.2	5622.4 ± 146.5
Month – 11	96.6 ± 1.6	6291.8 ± 138.9
Month – 12	103.0 ± 1.7	7276.6 ± 148.6

Better Management Practices in sea cage farming of cobia

The better management practices need to be adopted to satisfy public demand and expand the market by offering quality aquaculture products that meet food safety standards. Adoption of BMP's also helps fish farmers to achieve greater economic returns. Some of the key factors in BMP includes:-

- Proper acclimatization of cobia fingerlings prior to stocking.
- Avoiding over-stocking of cobia fingerlings
- Care feeding of cobia fingerlings using of dry pellets to allow all the fishes to get equal ration of feed.
- Monitoring the growth rate and sub-dividing cobia fingerlings/juveniles in different cages at appropriate time intervals.
- Cleaning and regular exchange of cage nets for effective water exchange.
- Close observation of fish behaviour while feeding them to assess the health status.
- Proper removal and disposal of dead fishes.
- Usage of approved feed supplements and additives as recommended by the

fisheries officials and CMFRI scientists/technical staff.

- Regular prophylactic treatment of fishes with disinfectants and dipping in fresh water.
- Periodic monitoring of dissolved oxygen level, pH value, water temperature, etc.
- Observing the weather conditions, changes in seawater quality, and emergence of red tide etc.

Farming of Pompano

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. This much sought after species silver pompao is caught only sporadically in the commercial fishery and hence its availability is rather scarce 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 it is found suitable for farming in the vast low saline waters of our country besides its potential for sea cage farming. This species readily accept artificial feeds and has a rapid and uniform growth rate (Chavez et al., 2011). In India, this fish is known in various vernacular names in various parts of the country like paarai or seevani paarai in Tamil Nadu. And sandhuva paara in Andhra Pradesh. The maximum recorded length for pompano from wild catch is 110 cm FL and weight is 3.4 kg. In culture conditions, the silver pompano gains a weight of around 450-500 gm in eight months, from the initial stocking weight of around 2 gm.

Farming practices

The silver pompano can be farmed either in sea cages or in ponds. The various aspects related to cage design, fabrication and cage management practices are given under the section on Cobia farming which is similar in case of pompano farming also. A stocking density up to 20 nos./ m³ is possible in sea cages. During cage farming, important aspects are the net cage maintenance and changing of nets. The farmers involved in cage farming should practice changing of nets in the sea at the farming site instead of dragging the cages to near shore areas for such exchanges. This might stress the fishes and weaken them. The timing of feeding should also be maintained as the fishes will automatically get used to the timing and will also come near the surface when they hear the sound of the boat. In India, suitable pellet feed for grow-out farming of this species is commercially available now.

Details of feeds and feeding of silver pompano *T. blochii*

Weight of the fish (g)	Feed size (mm)	Crude* protein (%)	Crude fat* (%)	% biomass feed/day	No. of feeding / day
< 1	0.8 - 1.0	50	12	20	4
1 – 10	1.0 - 1.5	45	10	10	4
10 – 100	1.8	45	10	5	3
100 – 250	3.5	40	10	4	3
250 – 500	4.5	40	10	3	3

**Other ingredients of feed includes: Crude Fibre: 2.5-5.0 % Max; Crude Ash: 15.0 % Max; Calcium: 2.0 % Min; Phosphorus: 1.5 % Min; Moisture: 5.0 - 8.0 % Max; Mineral and Vitamin Premix*

Pond farming

In pond farming, while it is advisable to stock only 1.5-2 nos./ m³ in ponds. water quality parameters like optimal algal growth, pH and dissolved oxygen content could be maintained by exchanging 10% of the water once a week for the initial period of three months; 20% per week after 3 months and 30% per week after 6 months. If water colour is too dark due to algal bloom, the quantum of water exchange could be proportionately increased. Other

water quality parameters can be maintained similar to shrimp culture. Use of paddle wheel aerators is advised when the fishes reach a weight of 150 gm size, whenever it is necessary. Details on feeds, feeding frequencies and growth characteristics, as observed in pond condition, are provided below for ready reference. However, for sea cage farming of pompano, feed with higher percentage of crude protein and crude fat is required to achieve good growth.

Growth of pompano in terms of length and weight during the pond farming (mean $\pm$ SE)

Days of Culture (DOC)	Growth (mm)	Weight (g)
1	30.59 $\pm$ 0.24	2.00 $\pm$ 0.04
15	49.84 $\pm$ 0.36	9.05 $\pm$ 0.08
30	73.42 $\pm$ 0.53	15.08 $\pm$ 0.16
45	85.02 $\pm$ 0.80	22.59 $\pm$ 0.23
60	102.88 $\pm$ 1.91	34.60 $\pm$ 0.41

75	137.78 ± 1.81	54.72 ± 1.62
90	158.39 ± 2.42	72.54 ± 1.95
105	168.80 ± 1.73	80.02 ± 2.67
120	182.30 ± 2.03	101.82 ± 3.11
135	186.02 ± 2.82	138.78 ± 4.49
150	203.71 ± 3.73	172.39 ± 4.55
165	224.17 ± 3.16	220.05 ± 3.54
180	226.51 ± 2.90	258.31 ± 5.76
195	248.13 ± 3.21	303.72 ± 4.49
210	273.07 ± 3.62	375.32 ± 8.07
225	288.36 ± 5.19	416.60 ± 7.72
240	296.88 ± 6.27	464.65 ± 10.25

Chapter.09

Recirculation Aquaculture System (RAS)

R Bavithra, G Tamilmani, M Sakthivel, P Rameshkumar, KK Anikuttan & A K Abdul Nazar

A Recirculation Aquaculture System can be defined as an aquaculture system that incorporates the treatment and reuse of water with less than 10% of total water volume replaced per day. The concept of RAS in to reuse of water through conditional treatment and delivery to the organisms being cultured. The Virginia Tech, USA, has conducted industry-oriented research with cobia based on the application of recirculation based life support systems. Further, researchers at the University of Texas at Austin Marine Science Institute's Fisheries and Mariculture Laboratory also spawned cobia in recirculating systems under photo-thermal controlled conditions. Besides a successful recirculating aquaculture system for maturation of cobia in captivity is also reported by researchers.

The Aquaculture Group of Virginia Tech (VTAG) initiated the development of an international initiative for sustainable and bio-secure aquafarming (IISBA) in 2005 and cobia was the first species that IISBA chose to address. The IISBA identified that the most significant and immediate bottleneck for cobia

commercialization was fingerling production in required quantity.

The recirculation system proposed is with following minimum standards for design, construction and management.

1. Mechanical filtration: It is to remove the solids. It is usually designed a) to incorporate treatment streams for settleable (more than 100 micron) and suspended solids (less than 100 microns), b) to treat all recirculated water to a minimum of 100 micron and if possible 20 micron, and c) to incorporate facilities of cleaning and back washing with minimal water usage.
2. Biological Filtration: It is proposed to use biological filter of appropriate dimension. This system normally employs plastic biological filter media to provide adequate surface area for nitrifying bacteria. The water flow rates expected through the biological filters should exceed 200 % of recirculation system volume per hour.
3. Disease control: Disease control systems are proposed to be incorporated in all

- RAS as an essential water treatment element for management of fish health. Ultraviolet irradiation and ozone are used for disease control in RAS.
4. Oxygen management: The cobia fish require high dissolved oxygen (DO) content and hence DO below 5 mg/l will result in stress. Therefore, the required DO level should be maintained in the culture water by employing air blower / oxygen cylinders.
 5. Carbon dioxide management: A degassing device is also proposed to be added for management of carbon dioxide.
 6. Culture tanks and pumps: The RAS incorporates suitable RCC / FRP culture tanks. The RAS incorporates industrial pumps constructed of corrosion resistant materials.
 7. Foam fractionation: It is required to incorporate a foam fractionation system to remove fine solids and dissolved organic materials.
 8. Thermal control: The RAS should have thermal control mechanisms to maintain appropriate temperature for broodstock maturation and spawning.
 9. Supporting infrastructure: Should have supporting infrastructure for photo-period control to facilitate controlled maturation of cobia / pompano.

Chapter.10

Integrated Multi-Trophic Aquaculture (IMTA)

B Johnson, KK Anikuttan, G Tamilmani, M Sakthivel, P Rameshkumar, A K Abdul Nazar & R Bavithra

Intense fishing pressure along the coastal waters, coupled with negative impacts of climate change has lately started impacting the livelihoods of fishers. While harvests are dwindling, the demand for marine fish is increasing steadily owing to its crucial role in ensuring food and nutritional security of the population. This necessitates augmenting marine fish production through farming of promising commercial species of fish in the sea. Realizing this important priority, the ICAR-CMFRI has developed and standardized the technologies for seed production and farming of

marine finfishes and shellfishes in open sea cages. One of the anticipated issues while expanding the sea cage farming is the increased organic and inorganic load in the water 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 gaining global importance in recent times. The ICAR-CMFRI has successfully conducted trials



and demonstrated the IMTA by integrating seaweed with sea cage farming of marine finfishes/shellfishes in Tamil Nadu, Gujarat and Andhra Pradesh. This has resulted in increased production of seaweeds which has improved the livelihood of farmers and has also contributed to the carbon credit of the country.

The ICAR-CMFRI is promoting cage farming of cobia, a high value marine fish since 2010. To achieve environmental sustainability and economic stability, an innovative idea of

integration of seaweed with sea cage farming of cobia was demonstrated during 2014-17 at Munaikadu, Palk Bay, Tamil Nadu. A total of 16 bamboo rafts (12× 12 feet) with 60 kg of seaweed per raft were integrated for a span of 4 cycles (45 days/cycle) along with one of the cobia farming cages. The rafts were placed 15 feet away from the cage in a semi-circular manner, so as to enable the seaweed to absorb the dissolved inorganic and organic nutrient wastes which moves along the water current from the cage.

Particulars	With IMTA	Without IMTA	Gain
Fresh seaweed production excluding the seed material (for 4 cycles, 16 rafts)	21,120 kg (330 X 16 X 4)	11,136 kg (174 X 16 X 4)	+ 9,984 kg
Dried seaweed production (for 4 cycles, 16 rafts)	2,112 kg (33 X 16 X 4)	1,114 kg (17.4 X 16 X 4)	+ 998 kg
Price of dried seaweed (Rs. per kg)	55.00	55.00	-
Revenue (Rs.)	1,16,160	61,270	+ 54,890
Costs (Rs.2,000 /raft)	32,000	32,000	-
Net Profit (Rs.)	84,160	29,270	+ 54,890

Currently through IMTA, seaweed rafts integrated with cobia farming cage had a better average yield of 390 kg per raft, while in the non-integrated raft the yield was 234 kg per raft.

An additional yield of 156 kg of seaweed per raft (67% additional yield) was achieved through the integration with the cage farming of cobia. An additional net income of Rs. 54,890/- was realized through integration of seaweed rafts with cobia cage.

Sl.No	Particulars	With IMTA	Without IMTA
1	Fresh seaweed production (for 4 cycles, 16 rafts)	24,960 kg (390 X 16 X 4)	14,976 kg (234 X 16 X 4)
2	Average dry weight percentage of the harvested sea-weed (%)	10.00	10.00
3	Average carbon content (%)	19.92	19.92
4	Total amount of carbon sequestered/year (1) × (2) × (3)	497 kg	298 kg

The carbon sequestered into the cultivated seaweed in the integrated and non-integrated rafts was estimated to be 435 kg and 261 kg, respectively. Hence an **additional 199 kg carbon credit was achieved through the integration of 16 seaweed rafts (4 cycles) with one cobia farming cage (per crop). In one hectare of area, a total**

of 20 cages of 6 m diameter can be integrated with 320 bamboo rafts (12× 12 feet) @ 16 bamboo rafts per cage. IMTA is an eco - friendly option ensuring sustainable income to the coastal fishers. It is also one of the significant mitigating measures for reducing the adverse impact of climate change and also earns carbon credit to our country.

Chapter.11

Health management in marine fin fish hatchery and farms

P Rameshkumar, G Tamilmani, M Sakthivel, KK Anikuffan, R Bavithra & A K Abdul Nazar

Introduction:

Sustainable aquaculture production can only occur when fish are healthy and free from disease. Fish disease management is a combination of preventing the onset of disease and measures to reduce losses from disease when it occurs. Fish cultured in floating cages become particularly susceptible to disease when various environmental parameters such as temperature, salinity, dissolved oxygen and suspended particles fluctuate suddenly or widely, or following rough , although often unavoidable, handling operation. Once conditions suitable for pathological changes develop, progress to disease in the warm water environment is rapid. Early detection of behavioural changes and clinical signs in the cultured animals are critical for proper diagnosis of the disease.

Disease rarely results from simple contact between the fish and a potential pathogen. Environmental problems, such as poor water quality, or other stressors often contribute to the outbreak of disease.

Fish Health Management

Fish health management is a term used in aquaculture to describe management practices which are designed to prevent fish disease. Once fish get sick it can be difficult to salvage them. Successful fish health management begins with prevention of disease rather than treatment. Prevention of fish disease is accomplished through good water quality management, nutrition, and sanitation. Without this foundation it is impossible to prevent outbreaks of opportunistic diseases. The fish is constantly bathed in potential pathogens, including bacteria, fungi, and parasites. Even use of sterilization technology (i.e., ultraviolet sterilizers, ozonation) does not eliminate all potential pathogens from the environment. Suboptimal water quality, poor nutrition, or immune system suppression generally associated with stressful conditions allow these potential pathogens to cause disease.

Predisposing factors

- Fish stocks living under stressful conditions become less able to defend against a pathogen and hence will become sick more readily. Fish that are well cared for generally do not become sick even in the presence of a pathogen. The most common error in fish husbandry is overstocking. This leads to problems such as:
- Fish to fish aggression
- Increased fish and feed wastes
- Ease of disease spread,
- Increased concentration of pathogens
- Resultant poor water quality

High fish density, stress, and ease of transmission increase susceptibility of the fish population to diseases and parasites. In marine aquaculture, diseases present in wild fish can infect cultured fish and spread rapidly through the population

Types of Fish Diseases

There are two broad categories of disease that affect fish, infectious and non-infectious diseases. Infectious diseases are caused by pathogenic organisms present in the environment or carried by other fish. In

contrast, non-infectious diseases are caused by environmental problems, nutritional deficiencies, or genetic anomalies; they are not contagious and usually cannot be cured by medications.

Infectious diseases.

Infectious diseases are broadly categorized as parasitic, bacterial, viral, or fungal diseases.

- **Vibriosis** is a bacterial disease causing significant losses of fish in marine fish farms. Cobia, Grouper, seabream, snapper and pompano species are affected. Vibriosis results in severe skin, muscle, fin, eye and internal organ damage of fish. Diagnosis of the disease requires bacteriological culture of kidney, spleen, skin or eye lesions.
- **Non-infectious diseases:** Non-infectious diseases can be broadly categorized as environmental, nutritional, or genetic.
- A hygienic fish culture environment is essential to the health and productivity of farming operations. The reasons for this include:
 - Disease risks are increased in poor and polluted environments.
 - Quality of the product depends on clean and healthy environments.

Common Diseases of Cobia (*Rachycentron canadum*)

S.No	Bacterial disease	Causative organism
1	Pasteurellosis	<i>Photobacterium damsella</i> sub <i>sp piscida</i>
2	Streptococcosis	<i>S. iniae</i>
3	Vibriosis	<i>V.alginolyticus</i> , <i>V.anguillarum</i>
4	Mycobacterium infection	MY. Sp 2 nd <i>Aeromonas hydrophila</i>
5	Viral disease	• Lymphocystis- Irido virus • Viral nervous necrosis (VNN) - Betanoda virus
6	Amyloodiniosis	<i>Amyloodinium ocellatum</i>

Common Diseases of Pompano (*Trachinotus blochii*)

S.No	Bacterial disease	Causative agent
1	White spot disease	<i>Ciliate protozoan</i> , <i>Cryptocaryon irritans</i>
2	Cardiac myxosporidiosis	<i>Myxosporidian protozoan</i> , <i>Henneguya sp</i>
3	Monogenetic trematode infestation	<i>Bicotylophora trachinoti</i> - gills <i>Benedenia sp</i> - body
4	Fatty degeneration	Dietary deficiency - protein
5	Parasitic dermatitis (infestation)	Sea lice (<i>Calligus elongatus</i>)
6	Amyloodiniosis	<i>Amyloodinium ocellatum</i>

Development and standardization of Vaccine against vibriosis

- The three vibrio organisms (*V. alginolyticus*, *V. parahaemolyticus* and *V. harveyi*) were selected for multivalent vaccine preparation based on the repeated outbreaks of the vibriosis in cobia cage culture. The whole cell inactivated multivalent vibrio vaccine against vibriosis was developed standardized and evaluated in laboratory and in field condition.
- After completion of the nursery phase and before stocking in the cage, mass immunization was done in cobia fingerlings (300no) against vibriosis by intraperitoneal method. Booster dose also injected after 35th day post vaccination. Serum was collected on 14th, 21st, 35th, 42nd and 63rd DPV and kept in -20 C for further antibody titre evaluation.
- The OD (Optical Density) values of antibody to multivalent vaccine differed significantly ($P < 0.05$) in the laboratory and in the field trial. There was a significant ($P < 0.05$) increase in the OD values of antibodies from 7th to 21st day and dropped significantly ($P < 0.05$) at 28

DPV. So it was decided to give one booster on 28th DPV. The 35th day OD values were higher than 21st and 28th day which indicates serum antibody OD levels were in increasing trends after the booster dose of the vaccine and the immunity was extended upto further 35 days.

- Thus, the regular epizootics observed in cage culture of cobia, every year during the month of July to September (pre-monsoon season) could be prevented by vaccination and proper sea cage farming management.

Parasitic infestations in Cobia and Pompano

The ciliates *Trichodina* sp infestation was recorded in cobia and pompano fingerlings in the brood tank and hatchery. Sudden mortality of 30 to 40 fingerlings were observed with the sluggish swimming, surfacing and off feed symptoms. Wet mount examination of the gill revealed moderate infestation of *Trichodina* spp. Immediately the fingerlings were treated with Bioline plus (a combination of Formaldehyde, Gluteraldehyde and, Benzalkonium chloride) followed by RO water dip and shifted to separate tank. The fish health status was improved without any mortality further. At the same time a herbal drug neem

oil also was applied @ 1ml /100L sea water and it was maintained in the same water for one whole day. Significant improvement was noted in the herbal treatment also. No ciliates could be seen neither in the tank bottom nor in the gills after treatment.

As per the FDA-approved recommendation a formalin bath of 200-250 ppm for 60 minutes 3 times a day at 3 hrs interval was also given. The very next day all the fingerlings recovered and no mortality were observed.

Incidence of neoplasms in cobia -Dermal Fibro Chondroma

A hard solid growth structure was observed on the caudal fin of cobia which was maintained for brood stock development in the sea cage. Grossly, a visible small growth was observed and the size was increased after a month. A biopsy was carried out and bleeding was noticed after Biopsy. Cytology of the smear showed only

the collagen and the basophilic mucus cells. Histologically, the section revealed replacement of the entire stratum compactum and the dermis layer with the chondrocytes. Below the epidermis and mucus cells layer there was a growth of cartilaginous structure. The chondrocytes were hyperchromatic with vacuoles formation and the area of cartilaginous structure replaced the entire stratum compactum structure.

A cauliflower like growth was observed in the gill rakers of cobia grown in cages. Grossly, it was a small tiny mass initially which later increased its size. A biopsy was taken from the gill surface and no bleeding was observed. Cytology of the smear revealed grouping of spindle shaped basophilic cells. The fish did not showed any symptoms, and it was sacrificed and samples were collected for histology and rest of the tissue has kept in -20°C.

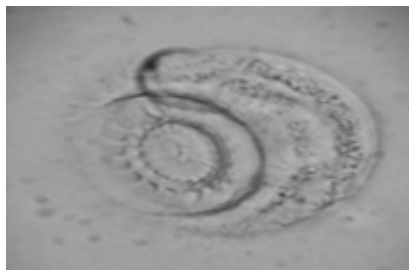
Some tips for fish health management

- Preventing the introduction of pathogens
- Maintenance of good water quality
- Avoidance or reduction of environmental stressors
- Adequate nutrition
- Isolation of cultured animals from feral stocks
- Immunization
- Identifying the cause of the disease or source of the distress
- Successfully curing the fish and eliminating the disease or cause of distress

PARASITIC DISEASES OF MARINE FINFISH



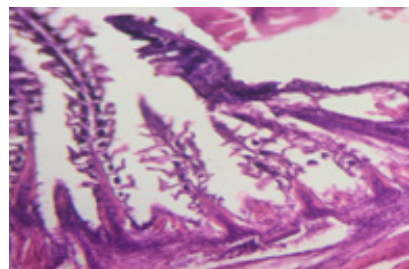
Pompano fingerlings haemorrhage at the operculum



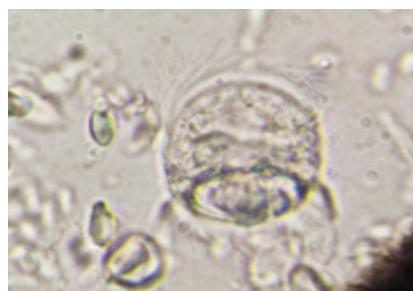
Wet smear: Trichodina sp.



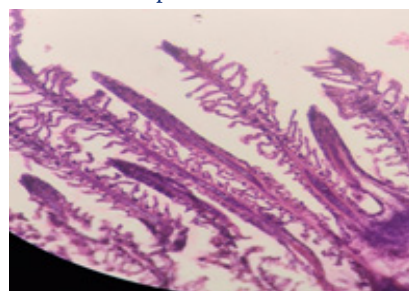
Gill infested with Trichodina sp.



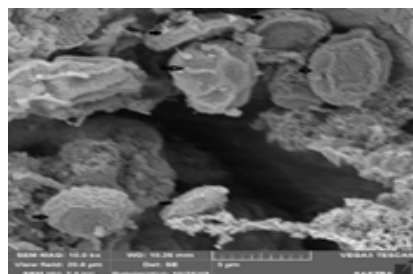
Histology; Gill atrophy and Trichodina sp



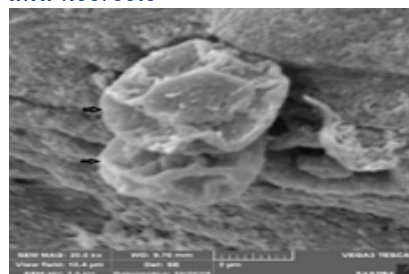
Wet smear: Trichodina sp.



Gill secondary lamellar atrophy and necrosis

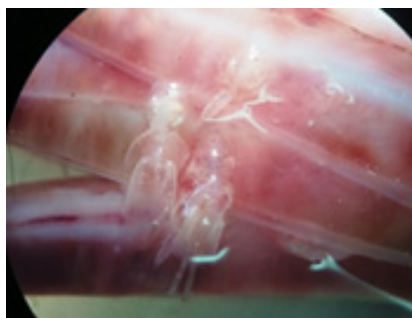


SEM: Trichodina sp.



SEM: Trichodina sp.

Parasitic infestation of marine finfish



Cobia gill: Parapetalus occidentalis



Parapetalus occidentalis



Male and female: Parapetalus occidentalis



Cobia ;Philometra sp in ovary



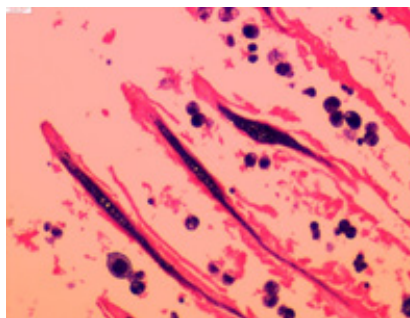
Cobia ;Philometra sp in ovary



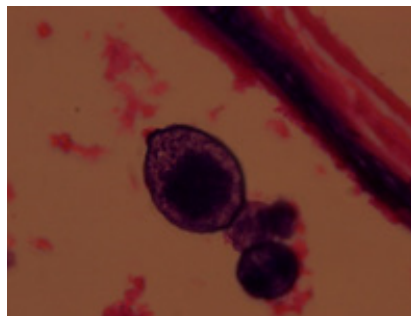
Philometra sp and larvae



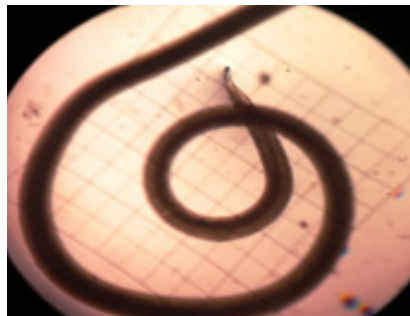
Cobia gill Amyloodinium ocellatum



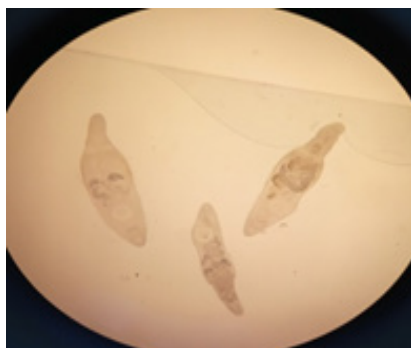
Cobia gill trophont attachment to lamillae



A. ocellatum trophont



Cobia stomach; Anisakis sp



Cobia stomach : Digenea infestation



Pompano; Henneguya sp spores

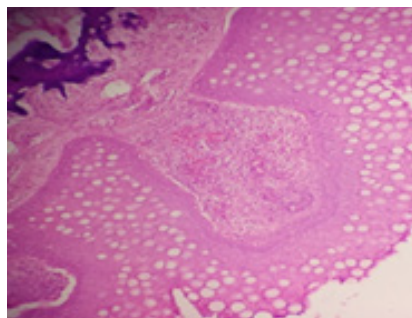
NEOPLASM RECORDED IN COBIA



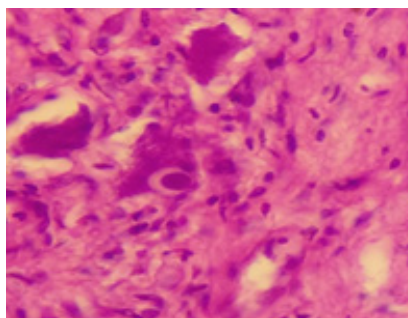
Gill chondroma



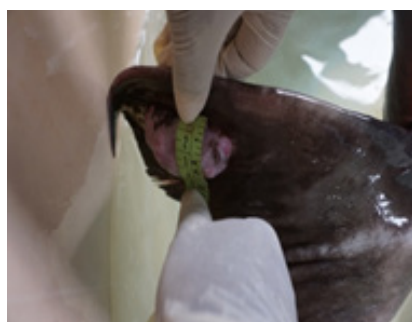
Chondroma in gill arch



*Chondroma steatosis and fibrous
tissue proliferation*



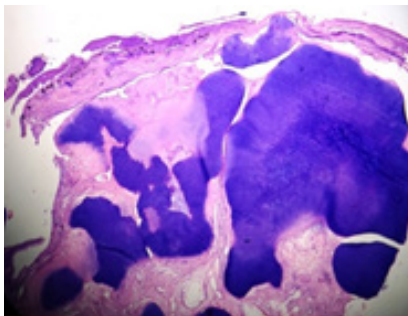
*Chondroma; Intracytoplasmic
inclusion bodies*



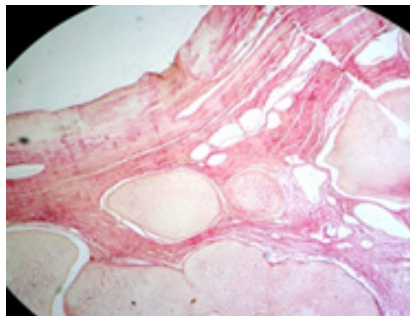
Chondroma in caudal fin



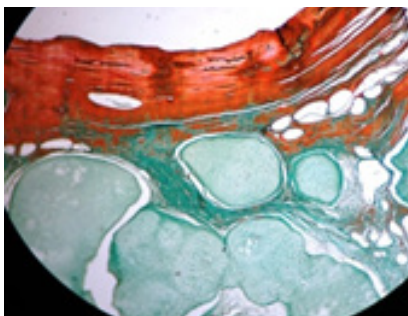
Chondroma mass in caudal fin



Histology of chondroma



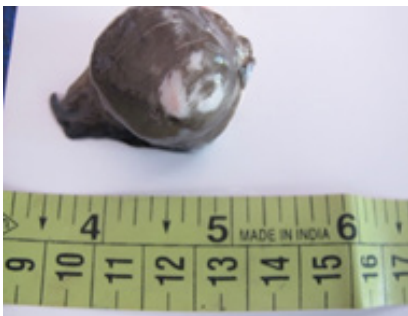
Picrocerous red.- Epidermis with Red collagen bundles and dermis with chondrocytes



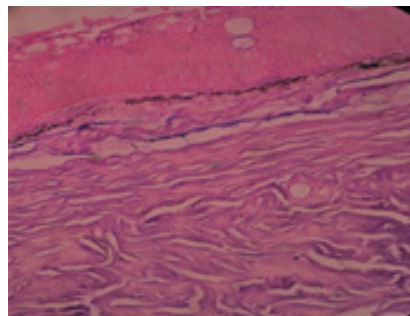
Masson's trichrome- Epidermis with Red collagen bundles and dermal layer with chondrocytes



Tumour on the pelvic fin

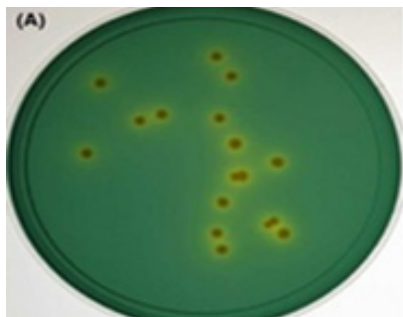


Dissected tumour mass

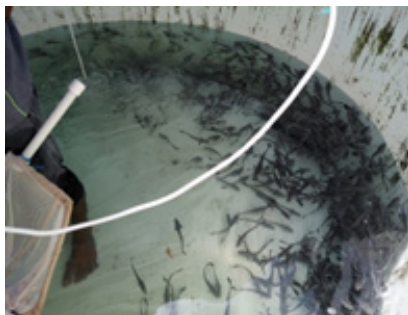


Histology : Fibroma

VACCINATION OF COBIA AGAINST VIBRIOSIS AND ITS IMMUNE RESPONSE



*Vibrio candidate species for
antigen preparation*



Fingerlings ready for vaccination



Vaccination by immersion route



*Vaccination by intraperitoneal
route*



Fingerlings ready for stocking



Stocking in the cage



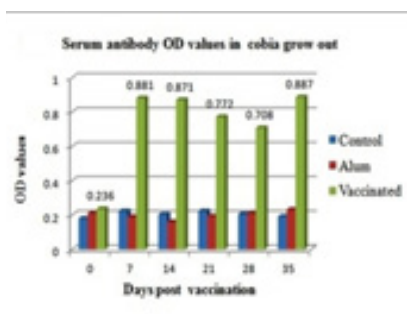
Vaccination to cobia subadults



Vaccination to cobia brood stock



Serum collected in different interval



Immune response of Serum OD values by ELISA



Conventional vibri-vac vaccine against vibriosis



Harvested healthy cobia

Chapter.12

WATER QUALITY MANAGEMENT IN MARICULTURE

S. THIRUMALAISELVAN*, RAJU SARAVANAN, K. VINOD, M. RAJKUMAR,
L. REMYA, S. M. SIKKANDER BATCHA AND SYED SADIQ

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

Introduction:

Marine aquaculture has now become a leading sector globally, as the wild harvest has depleted the natural fishery resources to much extent. Of the total fish production of 154 million tonnes (FAO 2011), marine capture fisheries stands at 78.9 million tonnes with only 19.3 million tonnes by aquaculture and it is much less compared to that of inland aquaculture production of 44.3 million tonnes. Preliminary estimate for 2010 points to a further increase in fish consumption to 18.6 kg and it will further increase in the coming years. As a cheap protein source, fish is regarded as a high nutritional component which is to be ensured to feed the increasing world population. Aquaculture is the remedy not only for the increased marine fish production and also for the conservation of the nature. The factors involved for a successful culture of marine fishes are therefore important to adhere to. In a confined system of aquaculture where fishes are cultured in captivity,

the environmental conditions (*ie.* clean hygienic water) are to be ensured at par with that of natural seawater for increased fish production. The fundamental water quality parameters are changed by various factors, while stocking the biomass for culture. The fish metabolic wastes like faecal matter, carbon dioxide (CO_2), un-ionized ammonia (NH_3), nitrite and nitrate, depletion of oxygen by respiration, activity of microbes, etc. are the variants and their stability is necessary for a successful aquaculture practice

Disease rarely results from simple contact between the fish and a potential pathogen. Environmental problems, such as poor water quality, or other stressors often contribute to the outbreak of disease.

Critical parameters

In the order of importance, the parameters can be listed as the dissolved oxygen (DO), temperature, pH, un-ionized ammonia (NH_3), salinity, carbon-di-oxide (CO_2), nitrite (NO_2), nitrate (NO_3), suspended solids, hydrogen sulphide (H_2S), methane (CH_4) and alkalinity. Degradation of surrounding water quality will be faster unless proper water quality management techniques are implemented. NH_3 , H_2S , CH_4 and CO_2 are toxic gases detrimental to fish health.

Reasons for water quality degradation

Fish stocks excrete metabolic wastes of urine, fecal matter, etc. The dead algae, feed residues are decomposed by bacterial action with high depletion of DO of water. Temperature increases results in series of changes in DO, CO_2 , etc. As water temperature increases, fish become more active and consume more dissolved oxygen, while simultaneously producing more carbon dioxide and other excretory products, such as ammonia. These increasing rates of consumption of necessary elements and production of detrimental elements can have a direct effect on overall fish health and survival. With depletion of oxygen, the biochemical oxygen

demand (B.O.D.) is not fulfilled for the fish. The disease occurrence in fish is mostly attributed to stress. Increased fish stocking density is also the reason for increased organic load.

Water quality parameters

Water quality parameters are invoked with greater care, as the success of a good hatchery practices solely depend on them. For a typical hatchery, the approximate water quality parameters are given, though they are not warranted and the conditions are to be standardised depending on the specific cases of candidate species. If these parameters are allowed to exceed nominal values, fish will show symptoms of stress.

They can be broadly classified into different categories as a. Physical parameters, b. Biological parameters, c. Heavy metals, d. Pesticides, e. Bacteria

a. Physical parameters

They are attributed to physical condition of seawater with respect to DO, pH, Salinity, Temperature, Water depth etc.

1. Dissolved Oxygen (DO)

DO is formed by direct diffusion from the atmosphere and as a by-product of photosynthesis. It is also obtained

by mechanical means. e.g. paddle wheels, agitators, vertical sprayers, impellers, airlift pumps, air diffusers, liquid oxygen injection. Considerable wind and wave action and presence of aquatic plants and algae also give oxygen in the aqueous medium. The oxygen concentration is measured in terms of parts per million (ppm) or mg/L. Marine fish requires minimum level of > 5.0mg/L. As the temperature and salinity increase, the solubility of oxygen in the water decreases. It is for this reason that aeration is used as an option during summer months. Aeration and exchange of water are done for good living conditions to the fish culture stock. High absorption of heat increases the temperature which results in lower oxygen level, clog fish gills, prevent development of egg and larva.

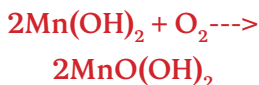
Other organisms such as bacteria, phytoplankton and zooplankton also need oxygen, thus compete for dissolved oxygen with fishes. Decomposition of organic materials is the greatest consumer of oxygen in the system. Food wastage and feed quality should be monitored as both significantly affect the DO level.

DO estimation by Winkler method

This method, popularly known as Winkler method, depends upon the oxidation of manganous dioxide (bivalent manganese) by the oxygen dissolved in the sample resulting in the formation of a tetravalent compound. When the water containing the tetravalent compound is acidified free iodine is liberated from the oxidation of potassium iodide. The free iodine is chemically equivalent to the amount of dissolved oxygen present in the sample and is determined by titration with a standard solution of sodium thiosulphate.



If the precipitate is white there is very little dissolved oxygen in the sample. A brown precipitate indicates that oxygen was dissolved in it and reacted with the manganous hydroxide to form manganic oxide.



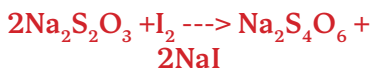
On addition of acid the precipitate is dissolved forming manganic sulphate.



Due to an immediate reaction between this compound and the potassium iodide added previously, iodine is liberated resulting in the typical iodine colouration of the sample.



The number of molecules of iodine liberated by the reaction is equivalent to the number of molecules of oxygen dissolved in the sample and this can be determined by titrating against standard solution of sodium thiosulphate using starch as indicator.



2. pH:

It is the measure of negative logarithm of hydrogen ion concentration with the scale from 1 to 14 ie. Acidic to alkaline. pH is interdependent with other water quality parameters, such as carbon dioxide, alkalinity, and hardness, known to influence the toxicity as well of hydrogen sulfide, cyanides, heavy metals, and ammonia. Optimum pH is usually between pH 7.5 and 8.5, for a seawater hatchery. At lower pH, the organism's ability to maintain its salt balance is affected and reproduction ceases. Below pH 6.5, some species experience slow growth. The suitable pH for mariculture is from 7.8 to 8.4.

3. Alkalinity

It is the measure of the capacity of water to neutralize or buffer acids using carbonate, bicarbonate ions present in the sea water. Recommended alkaline level for seawater is 116 (mg/L).

4. Temperature

Fish and other farmed organisms have no means of controlling body temperature, which changes with that of environment. A rise in temperature increases metabolic rate and causes a concomitant increase in oxygen consumption and activity as well as production of ammonia and carbon dioxide. Rapidly fluctuating conditions of temperature and salinities are harmful for marine life culture. Considerable seasonal changes also to be taken care of during the culture period. For most tropical marine life aquaculture, a temperature of 26-28°C with no abrupt changes is considered as suitable.

5. Salinity

It refers to the total concentration of dissolved ions of calcium, sodium, potassium, bicarbonate, chloride and sulfate in water. It is expressed in parts per thousand with the symbol (‰). Its relevance to mariculture lies principally in its control of

osmotic pressure, which greatly affects the ionic balance of aquatic animals. Preferred salinity range is within 28 – 38 ppt, evading abrupt changes

Seawater Ionic Composition (in 35 ppt)

Cation	Concentration (‰)	Anion	Concentration (‰)
Sodium Na ⁺	10.56	Chloride Cl ⁻	18.98
Magnesium Mg ⁺²	1.38	Sulfate SO ₄ ⁻²	2.65
Potassium K ⁺	0.27	Bicarbonate HCO ₃ ⁻	0.14
Calcium Ca ⁺²	0.40	Bromide Br ⁻	0.07
Strontium Sr ⁺²	0.01	Borate BO ₃ ⁻	0.03

6. Water depth

The depth of water column of a location determines the type of culture method to be adopted. For mussel culture method, it can be in the range from 1-15 m at average mean low tide. For culture in the estuarine conditions, even 1 m depth is suitable for horizontal culture of mussels in lesser muddy bottom conditions. In HDPD cage mariculture of cobia and silver pompano, the suitable range of water depth is within 5.0 to 7.5m which ensure sufficient gap between cage bottom net and sea bottom floor. Cages should be in sufficient depth to maximize the exchange of water, yet keep the cage bottom well above the substrate (sea floor) in order to

avoid interaction between the cage bottom and sea floor. Shallow bays with limited depth of water under cages are not favorable for water renewal. It can cause chemical and bacterial interactions, net damage and predation of the fish by crab and bottom organisms.

7. Wind

The wind can determine the suitability of a particular site or area for cage fish culture through its influence on cage structures and caged stock. Areas of violent storms are to be avoided. But, effects due to moderate winds can be profitable since it helps the mixing of water. Maximum permissible wind velocity limit is 10 knots for floating cage.

b. Biological Parameters

Nitrogen cycle

Nitrogen enters into the aquaculture system through rainfall, *in-situ* N_2 fixation, river run-off, and diffusion from sediments and uneaten feeds. The fish creates and expels various nitrogenous waste products through gill diffusion, gill cation exchange, and urine and feces excretion. Two primary water pollutants that need to be removed are (1) fish waste (toxic ammonia compounds) excreted into the water and (2) uneaten fish feed particles.

In addition to the urea, uric acid, and amino acid excreted by the fish, nitrogenous wastes accumulate from the organic debris of dead and dying organisms and from nitrogen gas in the atmosphere. Nitrogen is largely controlled by redox reactions mediated by phytoplankton and bacteria. The processes include remineralization, ammonification, nitrification, denitrification and fixation.

1. Ammonia and ammonium nitrogen (NH_3 & NH_4^+-N)

Ammonia is the initial product of the decomposition of nitrogenous organic wastes and respiration. Nitrogenous organic wastes come from uneaten

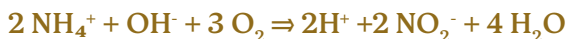
feeds and excretion of fishes. Total Ammonia Nitrogen (TAN) represents un-ionized free form and ionized ammonium radical (NH_4^+) present in the aquaculture system. Un-ionized ammonia (NH_3-N) is the most toxic because of its ability to move across cell membranes.

The relative concentration of ammonia is primarily a function of pH, salinity and temperature. NH_3 level increases as the temperature and pH increases. Concentration levels below 0.02 ppm are considered safe. High concentrations of ammonia cause an increase in pH and its concentration in the blood of the fish can damage the gills, the red blood cells, affect osmoregulation, reduce the oxygen-carrying capacity of blood and increase the oxygen demand of tissues. NH_4^+ is harmless. NH_3 can be converted into harmless nitrates through biological processes by nitrification bacteria (*Nitrosomonas* and *Nitrobacter*), as it is utilized as energy source and oxidized it to nitrite and nitrate. This process is nitrification which is done by the highly aerobic, gram-negative, chemoautotrophic bacteria found naturally in the system. Ammonia, nitrite, and nitrate are all highly soluble in water. 70% of NH_3 / NH_4^+ in water is associated with organic solids and not excreted nitrogen compounds.

Safe concentration of ammonia: Marine fish < 0.02mg/L

Nitrite/Nitrate formation by bacterial action

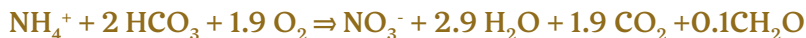
Nitrosomonas bacteria



Nitrobacter bacteria



Nitrifying bacteria



2. Nitrite-Nitrogen (NO₂-N)

Nitrite is a byproduct of oxidized NH₃ or NH₄⁺, an intermediary in the conversion of NH₃ or NH₄⁺ into NO₃⁻. High levels of nitrite do occur. It can cause hypoxia, due to deactivation of hemoglobin in fish blood, a condition known as the “**brown blood disease**”. Nitrate (NO₃-N) and nitrite (NO₂-N) also contribute to the inorganic nitrogen. The total inorganic nitrogen desirable for culture is < 0.1 mg l-l.

Optimum level of 0.03-0.06 mg/L

High nitrite concentrations plus low chloride levels can result to reduced feeding activities, poor feed conversions, lower resistance to diseases and susceptibility to mortality. Water is treated with sodium chloride or calcium chloride to reduce molar ratio of

nitrite to chloride. The suggested treatment is to add 3 mg/L of chloride (usually in the form of NaCl = 62% Cl) for every 1 mg/L of nitrite.

3. Nitrate-Nitrogen (NO₃-N)

Nitrate is formed through nitrification process, ie. oxidation of NO₂ into NO₃ by the action of aerobic bacteria. Nitrate not taken up directly by aquatic plants is denitrified in anaerobic sediments and microzones. It is stable over a wide range of environmental conditions and least toxic. High levels can affect osmoregulation, oxygen transport, eutrophication and algal bloom.

Optimum level < 1.00 mg/L

4. Phosphorous (P)

It found in the form of inorganic and organic phosphates (PO₄). Inorganic phosphates

include orthophosphate and polyphosphate while organic forms are those organically-bound phosphates with glycerol base. Phosphates are not toxic to people or animals, unless they are present in very high levels. It is a limiting nutrient needed for the growth of all aquatic plants. Excess concentrations can result to algal blooms.

Concentration of < 0.010 mg/L - *Oligotrophic*

Concentrations between 0.010 and 0.020 mg/L - *Mesotrophic*

Concentrations > 0.020 mg/L - *Eutrophic*

Sources of phosphorous are wastewater, septic effluents, detergents, fertilizers, soil runoff, phosphate mining, industrial discharges, and synthetic materials which contain organophosphates, such as insecticides. However, excess concentrations of P can result to algal blooms. The total inorganic phosphorus for marine life culture is < 0.015 mg/l.

Acceptable levels of phosphorus - nil - 0.015 mg/L

5. COD (Chemical Oxygen Demand)

The COD of water represents the amount of oxygen required to oxidize all the organic matter,

both biodegradable and non-biodegradable by a strong chemical oxidant. Preferred Chemical Oxygen Demand for mariculture is < 1 mg/l.

6. Chlorine

Both free and combined, residual available chlorine is extremely toxic to fish. The measurable concentrations of chlorine in water for mariculture is < 0.02 mg/l.

c. Total Solids

Any matter either suspended or dissolved, usually 0.45μ in size for filter determinations is regarded as total solids. Total Suspended Solids (TSS) come from silt, decaying plant and animals, industrial wastes, sewage, etc. High concentrations decrease the amount of light thereby slowing photosynthetic processes which in turn can lower the production of dissolved oxygen.

Total Dissolved Solid (TDS) includes organic & inorganic ions and molecules in solution like bicarbonate, sulphate, phosphate, nitrate, calcium, magnesium, sodium, organic ions, etc. Most settleable is in the size of > 10μ m. Particles passing through a 1.2μ m filter are termed “dissolved”. Colloids are the particles in the size range from 1 nm to 1μ m in size. High concentrations damage organism's cell, water turbidity,

reduce photosynthetic activity and increase the water temperature. TDS results from soil erosion, decaying plants and animals, and geological features.

Acceptable levels 180 to 500 mg/L.

However, both TDS and TSS are location specific depending on the environment. In confined hatchery the threshold level should be standardized for successful culture of fish stocks. Uneaten feeds, fish' fecal matters will not be spread out evenly in the water body.

d. Heavy Metals

They have potential toxic effects and ability bioaccumulate in fish tissues thus resulting to lower product quality and human health risk. Sources are industries, smelters, tanneries and textile industries, ore processing, plating industries, etc. They are determined by chemical analysis of the water, sediments and fish tissue and spectrally by atomic absorption spectroscopy (AAS).

1. Mercury (Hg)

Most common sources are caustic soda, fossil fuel combustion, paint, pulp and paper, batteries, dental amalgam and bactericides. The derivative methyl mercury tends to accumulate in the fish tissue, thus making the fishes unsafe to

eat. Low pH, low dissolved oxygen, and high organic matter are favourable environmental factors for Hg accumulation.

Lethal levels on fish range from 1 mg/L for tilapia to 30 mg/L for guppies

2. Lead (Pb)

It comes from deposition of exhaust from vehicles in the atmosphere, batteries, waste from lead ore industries, lead smelters and sewage discharge. Toxic effect on fish is increased at lower pH level, low alkalinity and low solubility in hard water. It results in nervous damage which can be determined by the blackening of the fins, gill damage and suffocation.

Maximum Pb concentrations < 5.6 ppb

3. Cadmium (Cd)

It is highly toxic.

Sources: electroplating, nickel plating, smelting, engraving, batteries, sewage sludge, fertilizers and zinc mines. In fishes, damage of the central nervous system get affected with adverse effects on the reproductive organs, maturation, hatchability and larval development as well as mortality. Toxic level is reduced by high concentrations of calcium and carbon dioxide,

since these two elements compete with cadmium for binding sites. In humans it is accumulated in kidneys through oysters, clams and crustaceans.

**Maximum Cd concentrations < 3.0
ppb**

Cadium has the most stringent criteria, ranging from less than 0.2-5.0 $\mu\text{g/L}$, followed by mercury, lead and then lastly by nickel.

4. Nickel (Ni)

It is moderately toxic to fish and in humans it is carcinogenic and teratogenic (malformation

of embryo). Its sources are by disposal of batteries and effluents from metal plating and ore processing industries.

**Maximum Ni concentrations < 100
 $\mu\text{g/L}$**

e. Pesticides

Insecticides, acaricides, herbicides, fungicides, algicides, chemicals are designed to be toxic and persistent. Pesticide can be split into seven main categories namely, inorganic, organophosphorous, carbamates, derivatives of phenoxyacetic acid, urea, pyridinium, and derivatives of triazine.

Safe level of some chlorinated hydrocarbons insecticides to aquatic species

Safe levels of some pesticides (ppb)

Pesticide	Concentration
Aldrin	0.003
BHC	0.08
Chlordane	0.004 (marine)
DDT	0.001
Endrin	0.0023
Heptachlor	0.0036 (marine)

The chlorinated form is of particular concern due to its persistence and tendency to bioaccumulate in fish and shellfish.eg. DDT, aldrin, dieldrin, heptachlor, and chlordane.

f. Coliform bacteria

The coliform bacteria come under the family Enterobacteriaceae and found mostly in feces and intestinal tracts of humans. High levels of fecal coliform in the water may cause typhoid fever, hepatitis, gastroenteritis, dysentery and eat infection. Sources are presence of wastewater, septic system, animal wastes, run-off, high temperature and nutrient-rich water.

Marine water: 30 to 50 count per 100 ml

g. Remedial measures

Aeration: It is the lifeline of a hatchery. It increases the DO level, facilitates the volatilization of undesirable gases such as N₂, NH₃, CH₄ and H₂S, reduces the daily fluctuation range of pH value and accelerates the decomposition and mineralization of organic matter in water. Periodic segregation of cultured fish stock is to be done for admissible biomass level.

The standards for good quality of mariculture practices are to be ensured by strict quality control measures, quarantine system, law enforcement and information campaign for water quality.

h. Gadgets - Devices useful in maintaining water quality

External Reservoir: It is essential for storage of required seawater source. It has pollution and salinity control. Various filtration units are provided for in-line water inlets to get rid of debris present in the water. The range of filtration sizes are 20–50 µm for Pressurized Sand Filtration and 5–0.5 µm for Cartridge Filtration. Ozone contacts results in disinfection and degassing.

Sand Filtration: It is done mechanically using pressure to get rid of particles (fecal, feed residues) with sizes of 20µm \ 5 µm \ 0.5-1.0 µm. Rapid pressurized sand filtration is done for micro filtration at 40 psi. Water from sand filters flows to a battery of cartridge filters housed in a common canister to capture particles of sizes of 5 µm and 0.5-1.0 µm.

Flow-meters are provided for the quantification of water in-flow.

Biofilters: Biological filter is composed of a media (corrugated plastic sheets or beads or sand grains) upon which a film of aerobic beneficial bacteria (probiotics) grows, with pressurized filter system to convert toxic ammonia of fish excretory products, into N₂ present in sea water.

Oxygenation is done for required DO and subsequent removal of CO₂.

In-line UV Irradiation: UV light is emitted by low-pressure mercury discharge to disinfect water. Disinfection of pathogenic organisms present in sea water by UV sterilization is ~ 20,000 times more efficient than boiling the water. In-line heating of 'Flow-Through Tanks' is provided for the manipulation of water temperature to a desired manner. Ozone is used to sterilize water and disinfect surfaces. It oxidizes most organic matter, bacteria and viruses.

Recirculating Aquaculture System (RAS)

It has benefits of saving the stocks from diseases, parasites, predation, pollutants, stress, and seasonally suboptimal growing conditions. RAS conserve both water and land. Using a RAS it is possible to produce over 100,000 pounds of fish in a 5,000 square-foot building, whereas

20 acres of outdoor ponds would be necessary to produce an equal number of fish with traditional open pond culture. Since water is reused, the water volume requirements in RAS are only about 20% of what conventional open pond culture requires. The gadgets are integrated with the system and desired water quality parameters are ensured. They offer a promising solution to water use conflicts, water quality, and safe waste disposal. These concerns will continue to intensify in the future as water demand for a variety of uses escalates. So, RAS is the remedy for safe culture of marine fish stocks.

Harmful algal blooms

Another criterion of deciding the suitability of potential culture site is eliminating the threat of Harmful Algal Blooms. Some coastal waters are known for the appearance of sudden blooms of certain phytoplankton capable of producing highly potent toxins that are harmful to marine fauna and any other animal that feed on them. Unfortunately, it is often difficult to predict if any area is prone to be affected by these toxic blooms, however, during the site selection process, an enquiry of the past history of the HAB in the area is necessary.

Conclusion

With the incorporation of more sophisticated electronic controlled gadgets, the water quality parameters are monitored round-the-clock to avoid any malfunction of units of a big hatchery. Since the successful running of a hatchery or cage culture, solely depends on these parameters, great care is taken into consideration in the breeding technology of a marine fish.

Chapter.13

CONSERVATION AQUACULTURE FOR AUGMENTING MARINE BIODIVERSITY AND RESOURCE MANAGEMENT

R.Saravanan*, M.Rajkumar, S. Thirumalaiselvan and K.Vinod

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

Introduction:

Globally, marine biodiversity and the ecosystem services that marine species offer are declining, but there is still time to stop these trends. The structure and resilience of coastal ecosystems are dependent on marine foundation species, such as seaweeds, mangroves, corals, and mollusc, which also provide important ecosystem services to human communities globally. A number of marine foundation species have seen significant population losses as a result of human activities, such as habitat loss, overfishing, and climate change. Conservation aquaculture offer hope to correct these imbalances.

The general definition of conservation aquaculture, also known as aquaculture for supporting conservation aims, is “the use of human cultivation of an aquatic organism for the planned management and protection of a natural resource.” (Halley, 2017). The conventional understanding

of aquaculture, which was limited to food production, has changed to include the promotion of biodiversity and resource management through a variety of action frameworks. Aquaculture has expanded from the cultivation of aquatic species to include habitat restoration and the preservation of important species and environments. Aquaculture for conservation is not only a theoretical concept. Around the world, aquaculture is already being used in almost every way to enhance the status and condition of species and ecosystems; usually on a local level, but occasionally on a larger one.

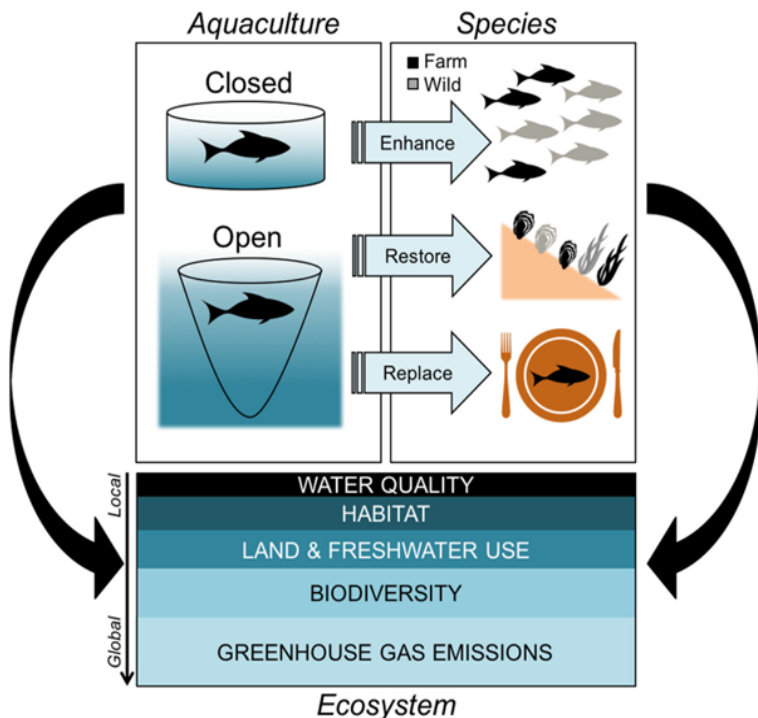
Every usage of the environment by humans has some effect, at least on certain species and/or habitats. Aquaculture is not an exception, therefore depending on the original goals, every aquaculture practise will have some environmental implications to varied degrees. Therefore, conservation aquaculture

cannot be considered a net-zero environmental endeavour; rather, it can be used to investigate ways to employ aquaculture to support conservation goals while admitting and allowing for a certain amount of (preferably minimal) environmental damage.

In the end, conservation aquaculture must promote the recovery or sustainable use of natural resources, whether by means of preventative, remediation, or mitigation strategies. The best way to accomplish this goal necessitates

taking the magnitude of conservation aquaculture into explicit account. Specifically, we investigate the ways in which conservation aquaculture can preserve and enhance: (1) particular wild species/stocks via commodity production and direct (e.g., hatcheries) and indirect (e.g., habitat restoration) wild population enhancement; and (2) larger system-level implications through aquaculture-based ecosystem services and a decrease in the overall environmental footprint (e.g., greenhouse gas – emissions).

Conceptual framework of how aquaculture is or can be used for conservation at a species and ecosystem scale



Large arrows indicate conservation links. At the species level, enhance refers to supplementing a wild population (hatchery), restore indicates biogenic restoration, and replace signifies farmed species replacing wild species – particularly overexploited or threatened – on the market (food or ornamental)

Species-level conservation

For a single species, conservation aquaculture can mean protecting wild stocks from overexploitation, bolstering populations of animals that are vulnerable, or re-establishing vital

habitat. The primary cause of the concurrent rise in the exploitation and harvest of wild and farmed species for food has been the expansion in human population, wealth, and seafood consumption (FAO, 2016).

Conceptual diagram of steps to take in evaluating conservation aquaculture for a new species or region.



Aquaculture is becoming more and more popular as a means of relieving the strain on aquarium species, especially those taken from areas where coral reefs are fragile (Domínguez and Botella, 2014, Holt, 2003, Rhyne et al., 2012, Rhyne et al., 2017). Approximately 90% of freshwater ornamental species are farmed. Most marine species that are auctioned for millions of dollars (USD) come from coral reef systems, which are among the planet's most biodiverse but endangered ecosystems (Monticini, 2010, Thlusty, 2002, Wood, 2001). Crucially, if a sustainable trade market that takes into account trade-offs between the environment and socioeconomics were to support in situ farming of ornamental marine species, it might be more feasible for conservation (Rhyne et al., 2014).

Stock enhancement, are among the most prevalent instances of potential conservation aquaculture (Costa-Pierce, 2008). Many species have been raised in hatcheries, with the “success” of those species relying on the goals of their initial management. There is undoubtedly a limit to population supplementation, one that may even cause unfavourable management effects like overcompensation (Foss-Grant et

al., 2016). Indeed, if management of fishing effort and habitat for wild populations is disregarded, hatcheries alone will not suffice. In fact, recent studies indicate that hatcheries may be crucial to improving stocks when paired with habitat restoration (Taylor et al., 2017). Fundamentally, biogenic habitat restoration in aquatic environments aims to either directly or indirectly improve other species and/or ecosystem features that depend on that habitat. It is also, thus, a sort of conservation aquaculture. Anthropogenic pressures, such as overfishing, coastal development, and commodity farming, have resulted in the degradation of many biogenic habitats, such as corals (Kennedy et al., 2013), oyster beds (Baggett et al., 2015), seagrass meadows (Waycott et al., 2009), mangrove forests (Spalding, 2010).

Ecosystem-level conservation

The restoration of native biogenic habitat through conservation-focused farming is becoming more popular as a means of achieving advantages at the ecosystem level. Even yet, it might be difficult to quantify the ecosystem benefits that restoration brings. The enhancement of water quality (Humphries et al., 2016), coastal defence (Narayan et

al., 2016), carbon sequestration (Greiner et al., 2013), and vital habitat for wild species (Seitz et al., 2014) can all be achieved by the cultivation and growth of specific oyster, seaweed, seagrass, and mangrove species. Furthermore, the possible advantages of planting and cultivating specific species are supported by basic ecological and biological principles. Increasing the abundance of farmed species leads to different ecosystem outcomes based on the system and metric of interest (Fig. 2b) (Beck et al., 2011). For example, improvements in water quality of a body of water from filter-feeders (e.g., oysters) is, in part, a function of nutrient inputs (rate and concentration) and has a maximum filtration-extraction limit or equilibrium (Ermgassen et al., 2013).

The basic goal of habitat restoration is frequently conservation, but a crucial next step in conservation aquaculture is to take into account the benefits to the environment and the production of commodities at the same time. Bivalve farms and seaweed farms can both absorb carbon dioxide from the ocean (Jiang et al., 2015), and it is expected that the capacity to do so will rise as the amount of biomass grown increases.

Conclusion

Aquaculture, like fisheries, seems to be moving from single-species to ecosystem-level, encouraging a more comprehensive strategy that could lead to greater conservation alignment. Aquaculture is undoubtedly not a panacea for all environmental problems, but it can contribute more to conservation in the future with careful planning and sustainable policies.

A constant focus on sound management is required for conservation aquaculture in order to optimise the potential advantages to the economy, society, and environment from development while averting needless dangers from harmful methods. Aquaculture can be used to support conservation through cooperation, thoughtful planning, and monitoring procedures, allowing us to preserve our aquatic resources while also safeguarding the environment.

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