प्रशिक्षण / Training manual on

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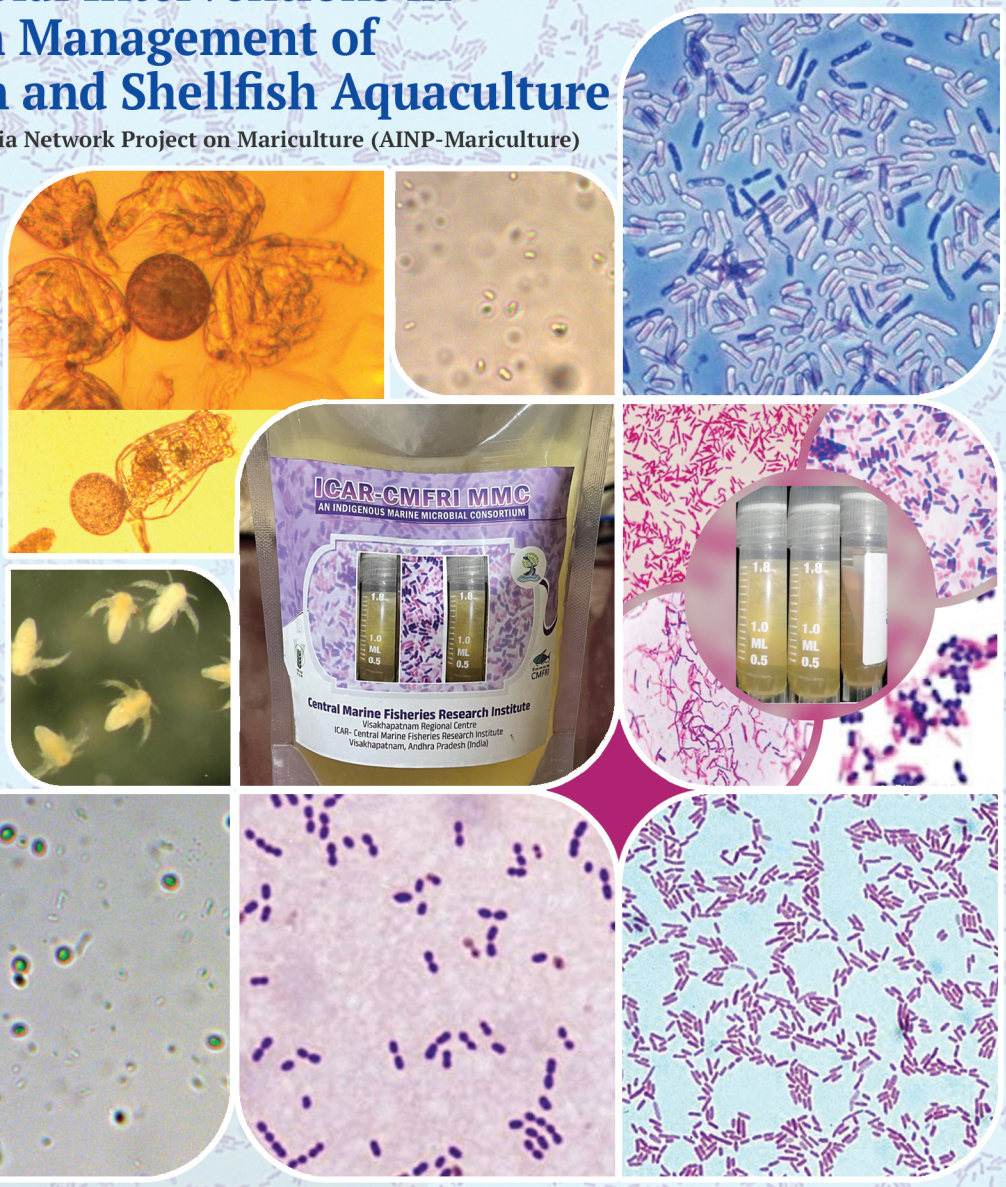
Microbial Interventions in Health Management of Finfish and Shellfish Aquaculture

Under All India Network Project on Mariculture (AINP-Mariculture)



विशाखपट्टणम क्षेत्रीय केंद्र
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विशाखपट्टणम, आंध्र प्रदेश, भारत

Visakhapatnam Regional Centre of
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Visakhapatnam, Andhra Pradesh, India

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Microbial Interventions in Health Management of marine finfish and shellfish aquaculture

Under

All India Network Project on Mariculture (AINP-Mariculture)

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VISAKHAPATNAM REGIONAL CENTRE OF

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Visakhapatnam, Andhra Pradesh, India - 530003

About the Manual

This training manual on "Microbial Interventions in Health Management of marine finfish and shellfish aquaculture" is published by Visakhapatnam Regional Centre, ICAR-Central Marine Fisheries Research Institute, Visakhapatnam, Andhra Pradesh with the financial assistance from the ongoing research project on All India Network Project on Mariculture (AINP-M). This manual is published as a resource book for the trainees, who attend the training programme held during the financial year 2023-24. The participants in the training programme include academicians, researchers, students, hatchery technicians, farmers and entrepreneurs who are associated with various mariculture activities. This manual explains different aspects related to the tools and techniques in the manipulation of microbial flora for health management of fish and water quality and development of indigenous microbial consortium and its applications in mariculture. I am sure that the knowledge gained from this training programme will significantly elevate the skills of the participants in health management of mariculture systems. This will instill in them an increase sense of poise in achieving success in their diverse mariculture endaevours. I express my sincere gratitude to all my colleagues who have contributed to the preparation of this training manual and also those who have helped me in the organization of the training programme.

JAYASREE LOKA

Preface

Aquaculture is a fast-growing food producing sector and plays a vital role in enhancing food and nutrition security, and providing a valuable income and employment prospects globally. Mariculture in India has gained significant importance in Aquaculture industry and currently focus is on the expansion in the direction of intensification, diversification and commercialization of marine finfish production, through the sustainable mariculture technologies developed at ICAR - Central Marine Fisheries Research Institute. In marine ecosystems the intimate relationship between bacteria and their hosts, and the relatively open production systems, adds to the complexity of mariculture. Intensive aquaculture provides an excellent medium for proliferation and growth of opportunistic bacteria in a large scale, leading to disease outbreaks, under stress and variation in other environmental factors, causing serious economic losses. In aquatic systems bacteria travel easily between habitats ,hosts and during intense operations in hatchery, nursery and growout and high density hold outs. Thsu the risk of transfection and epizootics is high.Early host -microflora and microbe interactions could lead into a positive community build up or an infectious development. Several pathogens, such as bacteria, virus and parasites, are considered as cause for serious disease outbreaks in aquaculture industry. In addition to the the microbial infections, nutritional and environmental factors associated with feed and seed quality form a major contribution for outbreak of diseases, either through horizontal or vertical mode of transmission. Records of few bacterial and viral pathogens in marine finfish culture indicate a signal to the mariculture industry, for the preparedness of scientific and farming community, to develop and standardize fish health management and biosecurity protocols, through microbial interventions at different stages of marine finfish culture and production (from brood stock to growout fish). A better understanding of these developents and management is thus imperative for successful mass -production systems. Health management of cultivable marine finfish and the water quality management of hatchery and other mariculture systems through microbial interventions is an emerging researchable field in mariculture. Successful aquaculture will rely on better insight into complex interactions between cultured organisms and the complex bacterial communities that develop in the rearing systems. It is understood that the fish contains a specific

intestinal microflora -aerobic.facultative anaerobic and obligate anaerobic bacteria. And the proportion of these forms change with age, nutritional status and environmental conditions. The epithelial and gut microflora (indigenous/normal) are typical to each species and even may outnumber the somatic and germ cells by a ratio of 10:1but later on several epifloral build up on the surfaces and compete. Although several beneficial microbial technologies are developed globally in different sectors, as microbial interventions in management of diseases, their application in aquaculture industry is gaining importance in only recent years. It is now well understood that the use of antibiotics does not constitute a sustainable solution,and may result in microflora imbalance. Several commercially available beneficial microbes (addition/manipulation) may be antagonistic or probiotic in nature to the pathogens, are being used in aquaculture industry as disease resistant and growth promoting agents. With the complexity of vaccine development and cost efficiency and the restriction in use of antibiotics, several therapeutic agents the industry is turning to, for prophylaxis and control, like nutritional and elemental supplementation, immunostimulants and immunomodulators, by improving the cost efficiency and promote species diversification at a time when the fisheries production is stagnant .

Significant contributions made by Central Marine Fisheries Research Institute, on disease surveillance programmes, molecular diagnosis of parasites and pathogens of cultivable fish, fish microbiome, beneficial microbial consortium, bioremediation and biosecurity protocols in marine culture systems, at Visakhapatnam Regional Centre of ICAR - CMFRI which has an expertise in isolation and culture of pathogenic and beneficial microbes from marine finfish and also in the development of marine microbial consortia, which can be used for application in mariculture, is now initiating to conduct awareness and training programmes on the health management and biosecurity protocols for hatcheries and culture systems. The present training program aims to provide professionals with vitally important knowledge in Health Management of marine and coastal aquaculture effectively and sustainably, in aquaculture industry. The present training manual helps the aqua/mariculture industry to make necessary preventive and control measures to develop a healthy sustainable mariculture industry in India. Enormous expansion of hatcheries, ponds and cages for fish and shellfish aquaculture along east coast of India, needs attention to have a preparedness programme for farmers and entrepreneurs regarding the health management of aquatic organisms and environment.

(JOE K. KIZHAKUDAN)

Head, Visakhapatnam RC, ICAR-CMFRI

Details of the Training Programme

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Microbial Interventions in Health Management of marine finfish and shellfish aquaculture

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AN OVERVIEW OF FISH AND SHELLFISH DISEASES IN MARICULTURE: AN INDIAN SCENARIO

Krupesha Sharma S.R.

Introduction to significance of fish health monitoring and management

Globally, aquaculture expansion and growth are targeted for increased intensification and commercialization of fish production with an intention to maximise profits in low volume-high density mode. Global food fish production from aquaculture reached 52.5 million tons in 2008, compared to 32.4 million tons in 2000. Like any other farming sectors, the likelihood of occurrence of diseases increases as culture operations intensify and expand involving diverse species and eco-system. Thus, the aquaculture industry has been overwhelmed with its share of diseases caused by viruses, bacteria, parasites, etc. Along with feed and seed, diseases are also being considered as one of the factors influencing the profitability of the farming since diseases are now a primary constraint to the development and expansion of culture of many aquatic species. Several factors can be attributed to the present situation of disease problems in aquaculture: 1. increased globalization of trade of live aquatic animals and their products; 2. the intensification of aquaculture mediated through the translocation of brood stock, larvae, fry and fingerlings; 3. the development and intensification of ornamental fish trade; 4. interactions between cultured and wild fish populations, especially in cage farming in natural waters; 5. inadequate biosecurity measures; 6. Absence of vaccines for major bacterial and viral diseases.

A unique feature of farming in natural aquatic environment is that once a pathogen is introduced into the farming system and becomes, treatment of the infected animals is not possible due to the limitation in using drugs to avoid environmental contamination. Since large numbers of animals are kept at high density in close proximity, aquaculture systems can create a conducive environment for infectious disease to establish and spread. Compared to terrestrial farming system, aquaculture poses a continuous threat for the emergence of new diseases due to new fish species being cultivated.

The nature and severity of diseases are mostly influenced by the species of fish being cultured, conditions in which the fish are reared, and farm management. Fish cultured in floating cages in natural waters are particularly susceptible to diseases when environmental variables such as temperature, salinity, dissolved oxygen, and suspended particles suddenly or widely alter. Generally, compared to temperate conditions,

progression to disease once the pathogens are established in warm water environments is rapid.

Similar to diseases of terrestrial animals, aquatic animal diseases are also classified into infectious and non-infectious diseases. Infectious diseases of are caused by various infectious agents like viruses, bacteria, parasites, or fungi. Non-infectious diseases are generally due to environmental stress, chemical contaminants, or nutritional deficiencies.

Economic importance of aquatic animal diseases

Aquatic animal diseases have profound social and economic impacts on the public, businesses, and economies that depend directly or indirectly on aquatic animal production. Considerable importance is being given for the study of economic importance of aquatic animal diseases at the farm and national level, while information on many of the diseases of maricultured species is currently not available. Understanding the economic importance of aquatic animal diseases at the national level necessitates its understanding at the farm level, so that measures in can be taken up for the effective allocation of resources to develop required control and prevention strategies for sustaining the aquatic production and enhancing profitability of farming.

Monitoring, surveillance and management of bacterial diseases of maricultured finfish caused by bacteria

Diseases of marine finfish caused by pathogenic bacteria have been one of the limiting factors for the sustainability of aquaculture and among infectious diseases, those caused by bacterial pathogens remain to be the largest single cause of economic losses in aquaculture world over (Colorni et al., 1981). It is noteworthy to mention that a large number of aquatic marine bacteria are opportunistic as they are normally present in seawater, sediments and fish gut. They generally do not cause infection in fish under normal environmental conditions. It is evident that pathogenic bacteria ubiquitous to marine and estuarine ecosystems are the significant drivers of mortality due to bacterial diseases in marine fish culture (Pruzzo et al., 2005).

Vibriosis: The most common Gram negative bacteria associated with marine fish belong to the family Vibrionaceae. Vibriosis, disease caused by the bacteria of the species *Vibrio*, is among the most common diseases leading to considerable mortality and economic loss to cultured fish and shell fish in the tropics. Juveniles are more susceptible to Vibriosis than adult fish, hence mortality may reach upto 80% in case of juveniles. It has been reported that a few species of *Vibrio* including *V. parahaemolyticus, V. alginolyticus, V. harveyi, V. owensii*and *V. campbelli* are recognized as the most common vibrio species causing vibriosis farmed aquatic animals.

In case of vibriosis, the pathogen may enter the host orally, through skin lesion and gill surface consequent to wound caused by ectoparasites and protozoa. Fish affected by classical vibriosis show typical signs of a generalized haemorrhagic septicaemia with the presence of haemorrhagic lesions at the base of fins, ulcerations on the body surface, especially in chronic cases, exophthalmia and corneal opacity. Ailing fish are often anorexic with pale gills due to anaemia arising from haemorrhages. Microscopic lesions in case of vibriosis also reflect the haemorragic nature of the disease. Histologically, bacteria invading the dermis, subcutaneous adipose tissue, and the underlying musculature are evident. Affected tissues are necrotic and heavily infiltrated by granulocytes. Gill filaments and lamellae are also infiltrated by neutrophills with haemorrhage. Liver shows hypertrophy of the bile ducts, necrosis, haemorrhage and congestion. In myocardium, loss of cross striations and infiltration of polymorphoneuclear cells in to the endocardium is noticed. Kidneys reveal characteristic lesions of acute glomerulonephritis with increased expression of melano-macrophage centres. Gastric mucosa contains engorged capillaries and loss of tubular glands. Extensive tissue lesions in vibriosis are primarily due to the release of proteinases and other extra-cellular enzymes produced by the bacteria.

*Vibrio*s are gram negative rods characteristically curved or comma shaped. This morphological appearance may not be always observed when organisms are selected for gram staining from solid media. Specific media like Thiosulfate-citrate-bilesalts-sucrose agar (TCBS) agar may be used for selective growth of Vibrios. Species level identification can be done by biochemical tests, PCR using specific primers and 16S rDNA amplification using universal primers and sequencing.

Even though *vibrio*s are susceptible to majority of broad-spectrum antibiotics, limitations exist based on the farming system. Since vibrios are opportunistic pathogens, vibriosis can be best managed by proper husbandry practices. Handling, transportation, overcrowding, low dissolved oxygen and increased water temperature make the farmed fish susceptible to vibriosis. Periodical enumeration of the bacterial load of water and sediment would help in preventing outbreaks. Although species -specific vaccines have shown effective protection against the specific bacterial strain, the antigenic diversity of *Vibrio* strains and their serotypes have made the vaccines unable to elicit protection against multiple *Vibrio* infection s resulting in slow progress of vaccine development. Due to diversity of V*ibrios* and their serovars, the advancement in vaccine development against vibriosis has been dawdling, and commercial vaccine is not currently available. However, attempts have been made to vaccinate fish against different *Vibrio* spp. using oral, killed and sub-unit vaccines. In case of oral vaccination, the vaccine is either mixed with the feed, top dressed on the feed, or bio-encapsulated. Bio-encapsulation is used when fish

fry are to be vaccinated. In case of bio-encapsulation, live feed, such as artemia nauplii, copepods or rotifers incubated in a suspension of vaccine are fed to the fish fry. Oral vaccination causes no stress to the fish. However, they have a very short term stability once mixed with the feed. More recently, the outer membrane proteins molecules are used for development of subunit vaccines due to the exposed epitopes on the bacterial surface and conserved nature in different serovars. It has also been demonstrated that outer membrane proteins molecules like OMP -K acts as protective antigen against fish vibriosis caused by *V. alginolyticus*.

Photobacteriosis: Photobacteriosis, also known as fish pasteurellosis, is caused by the halophilic bacteria, *Photobacterium damselae subsp. piscicida*. Gauthier et al. (1995) included the fish pathogen Pasteurella piscicida in the species *P. damselae* according to phylogenetic analyses of 16S rDNA sequences and DNAD DNA relatedness, and named as *Photobacterium damselae subsp. piscicida.* Pasteurellosis has been a serious disease in Japan affecting the aquaculture production considerably. The disease is characterized by the presence of whitish nodules on liver, spleen and kidney. Severe mortalities occur in pasteurellosis when water temperature is above 18– 20 °C. Below this temperature, fish can harbour the pathogen for prolonged periods without causing clinical infection. The disease affects various species of fishes like yellow tail juveniles (*Seriola quinqueradiata*), ayu (*Plecoglossus altivelis*), black seabream (*Mylio macrocephalus*), red seabream (*Acanthopagrus schlegeli*), oval file fish (*Navodan modestus*) and red grouper (*Epinephelus okaara*).

Fish pasteurellosis is a septicaemic disease and manifests as an acute or chronic form. Pale gills, dark pigmentation and presence of petechial haemorrhages on the body surface and fin base are normally observed in acute form. Enlarged spleen and mottled liver are seen internally. In case of chronic form, nodules resembling tubercles are seen in spleen and kidney.

Another disease caused by *Photobactrium damselae subsp. damselae* is also responsible for mortality in many cultured marine fish species. This disease has been reported from India in cage farmed cobia. The lesions are haemorrhagic in nature resembling the lesions found in vibriosis. The pathological manifestations in both the infections are primarily due to the extra cellular products (ECP) secreted by the bacteria. Both pathogens are normal inhabitants of marine environment. Disease transmission in case of photobacteriosis occurs through direct contact and ingestion. The bacteria are unable to survive in fresh or brackish water. Predisposing factors for the outbreak normally include rise in water temperature. The pathogen can be isolated and cultured on marine agar and ordinary media supplemented with sodium chloride. The organism can be confirmed by biochemical tests, 16S rDNA sequencing and slide agglutination. *Photobacterium damselae*

subsp. piscicida has to be differentiated from *Photobactrium damselae subsp. damselae* using a multiplex PCR that combines specific primers for 16S rRNA and urease genes.

Several commercial vaccines against *P.damselae subsp. piscicida* are available, wherever the disease is more prevalent. Efficacy of these vaccines depends on the species of fish, fish size, etc. Since outbreak of pasteurellosis normally occurs during larval stages to fingerling stage, a vaccination programme involving dip immunisation during the larval stage with a booster dose when fish reaches a size of 1–2 g is advocated.

Streptococcosis: This is a re-emerging disease of both freshwater and marine fish caused by gram positive bacteria characterized by central nervous system damage followed by exophthalmia and meningoencephalitis. Streptococcosis is also known as "pop-eye", since one of the most characteristic clinical sign found in this disease is the accumulation of muco-purulent exudates around the eyes. Streptococcosis, a problem in both farmed and wild marine fish, has been reported from USA, Japan, and Spain. In Japan and Spain, this disease forms a major limiting factor for the marine fish production of yellowtail turbot. Fish of all the size are susceptible. The sea water and sediment harbours the pathogen which can be isolated from these sources round the year. It has been reported that this pathogen can survive in frozen products for at least 6 months. Warm water streptococcosis which occurs when water temperatures are above 15 °C is normally caused by *L. garvieae*, *S. iniae*, *S. agalactiae* and *S. parauberis*. Cold water streptococcosis seen when water temperatures are below 15°C is generally caused by *L. piscium* and *V. salmoninarum*. Pathogens responsible for warm water streptococcosis are of zoonotic importance since they can cause disease in humans. Horizontal transmission can occur through injuries and abrasions.

Streptococci capable of causing disease in marine fish falls into three categories: alpha-haemolytic, beta-haemolytic, and non-haemolytic. The majority of disease epizootics are generally caused by streptococci belonging to alpha-haemolytic group. Among these fish streptococci, *L. garvieae, S. iniae and S. parauberis* can be regarded as the main aetiological agents causing diseases in marine aquaculture. *L. garvieae* infects marine fish like yellowtail in Japan. *S. Iniae* is an important fish pathogen causing disease and mortality in many cultured fish species in both tropical and sub- tropical environments. *S. iniae* is the main aetiological agent of streptococcosis in tilapia in USA and rainbow trout in Israel. However, *S. iniae* was also isolated from marine fish including yellowtail, flounder, European seabass, and Asian seabass. There have been no reports from India. *S. parauberis* is reported to be endemic to cultured turbot.

The lesions caused by different streptococcal species in diverse host species are similar. The lesions are of general septecemic in nature affecting liver, spleen, eye, brain and kidney. The eyes show severe exophthalmia with granulametous inflammation. Granulomas with the presence of bacteria may also be seen in pericardium, alimentary tract, peritoneum, brain, ovary, testes and spleen. The most significant clinical signs are exophthalmia, distended abdomen, haemorrhages in the eyes, opercula, fin base, ulceration of the body surface and darkening of the skin. Internally, the abdominal cavity is filled with variable amounts of purulent exudate. A yellowish exudate often covering the peritoneum and the pericardium may be seen. Yellowish exudates may also be seen in cranial cavity. Haemorrhages are found on all visceral organs.

Fish *streptococci* can be generally isolated from internal like spleen, liver and brain using brain heart infusion agar or tryptone soya agar supplemented with 1% yeast extract or 0.5% glucose, and growth is enhanced on blood agar. The incubation period is 24 days at 25-30°C. The isolates are then characterized either biochemically or serologically. Significant characteristics are spherical or ovoid colony morphology and formation of pairs or chains. All strains of streptococci are Gram positive, oxidase and catalase negative, non-motile and non-sporulating. Slide agglutination and immunofluorescent techniques are widely used for diagnosis of streptococcosis. PCR based diagnosis including 16S rRNA amplification and sequencing can also be employed.

Good husbandry practises including avoiding over-crowding, excess feeding, handling and the timely removal of diseased or dead fish would help to minimise the economic losses due to streptococcosis. Apart from this, vaccination, use of chemotherapeutics and immunostimulants has also been tried. Many of the isolated strains were sensitive to antibiotics like erythromycin, tetracycline, ampicillin and doxycycline. Attempts have been made to develop vaccines against streptococcosis in fish. Intra-peritoneal route was found to be most effective. β-1-3-glucans used as immunostimulants is also found to be effective.

Monitoring, surveillance and management of viral diseases of maricultured finfish and shellfish

Viruses are microorganisms that can replicate only inside the living cells of other organisms. Viruses can infect all types of life forms including animals, plants and microorganisms, including bacteria and archaea. Virus particles or virions normally consist of three components: i) genetic material made up of either DNA or RNA which carries the genetic information; ii) a coat protein that protects these genetic materials; iii) a lipid

envelope that surrounds the protein coat which may be absent in some viruses. The shape of viruses may be helical or icosahedral or more complex structures.

Fish viruses have been the subject of research interest in the past two decades. Compared to diseases caused by fresh water fish viruses, there have not been extensive studies on marine fish viruses. Establishment of various fish cell lines lead to path breaking research in fish virology in the recent years. Major viral groups under which fish viruses can be classified include herpes virus, iridovirus, rhabdo virus, reo virus, noda virus and calci virus. However, the most lethal viral disease causing enormous loss to finfish farming is the disease caused by betanodavirus.

Viral nervous necrosis: Betanodavirus is one of the genera making up the family Nodaviridae which is the etiological agent of viral nervous necrosis (VNN) also known as encephalomyelitis and vacuolating encephalopathy and retinopathy. This virus has remained as a major threat for the establishment and expansion of Asian seabass (*Lates calcarifer*) and striped jack (*Pseudocaranx dentex*). The disease was first documented in 1990 in hatchery-reared Japanese parrotfish (*Oplegnathus fasciatus*) in Japan and Asian sea bass in Australia. Later, it was reported in turbot (*Scophthalmus maximus*), European sea bass (*Dicentrarchus labrax*), redspotted grouper (*Epinephelus akaara*), striped jack (*Pseudocaranx dentex*) and more recently in cultured warm-water and cold-water marine fish species throughout the world. An Indian strain of betanodavirus belonging to RGNNV group was isolated from Asian seabass juveniles reared in a brackish water farm in Bhimavaram in Andhra Pradesh in 2012. Outbreak of mortality due to nodavirus infection in Asian seabass juveniles cultured in fresh water cages in the south west coast of India has also been reported. Mortality of Asian seabass juveniles cultured in indoor cement tanks as well as open sea cages and in cobia cultured in cages in India associated with RGNNV was also recorded.

Betanodaviruses can infect fish species belonging to tropical, sub-tropical, or temperate waters. These viruses can multiply at an optimum temperature depending on the strain of the virus. For RGNNV, the optimum temperature requirement is 25–30°C while for SJNNV, it is 20–25°C. Mostly, betanodaviruses are a concern in marine fish species. The species susceptible cobia, sea bass, seabream, bluefin tuna, grouper, halibut, surgeonfish, lined surgeonfish and tiger puffer. The freshwater fish species susceptible to betanodaviruses include tilapia and the guppy.

In farming system stress factors like high density, transportation, and high temperature can act as predisposing factors making the fish susceptible to VNN. Although young fishes are more susceptible, older fishes may also get infected especially when water temperature is high.

During the acute stage of the disease, when the mortality is very high, especially in juveniles, there would be no gross lesions on the body surface or gills. However, affected juveniles and older fish show an abnormal swimming behaviour such as spiral, whirling, floating with inflation of swim bladder, or lying down at rest, circling on their own axis. This erratic swimming behaviour may not be noticed in infected fish larvae. Grossly, the brain is oedematous and in many cases severely congested.

Microscopically, lesions are characterized by severe vacuolation and necrosis of the central nervous system. In general, the anterior brain is more severely affected when compared to the posterior part of the brain and spinal cord. Larvae of the fish are more severely affected by betanodaviruses than juveniles. The most characteristic lesion in the fish larvae is the presence of vacuoles in the grey matter of the brain which are intracytoplasmic. Basophilic, intra cytoplasmic inclusions have been reported in brain cells of Asian seabass. Lesions in the retina of the infected fish have also been described in all Species. The lesions include vacuolation of the cellular components of the retina especially the bipolar and ganglionic nuclear layers. Under transmission electron microscopy, fish betanodaviruses appear icosahedral, non-enveloped with a mean diameter of about 25 nm. The virions may be membrane bound by endoplasmic reticulum or are free in the cytoplasm and may present as paracrystalline arrays. Cells containing virions normally include neurones, astrocytes, oligodendrocytes and microglia cells.

VNN can be diagnosed by:1. Demonstration of characteristic vacuolar lesions in the brain or retina by light microscopy; 2. Detection of virions and viral antigens by electron microscopy and serology; 3. Detection of viral nucleotides by molecular techniques including RT PCR, RT Nested PCR, cloning and sequencing by designing the primers for the strain of interest; 4. Tissue culture of virus in a suitable cell line.

Betanodaviruses are highly resistant to various environmental conditions and they can survive for a long time in sea water. The disease can also be reproduced by simple co-habitation of the healthy fish with infected fish. Control measures are including imposing strict bio- security to exclude the virus from the farm premises. The broodstocks should be tested for the presence of viruses in the gonadal tissues and VNN specific antibodies in serum and any positive fish should be culled at first.

Red sea bream Irodivirus Infection: Red sea bream iridovirus disease is caused by Red sea bream iridovirus (RSIV) of Megalocytivirus . This disease is an emerging epizootic disease of fish having a significant impact on marine and brackish aquaculture systems. It is caused severe mortality in many cultured and wild fish species of several countries. So far, Red sea bream iridovirus disease has been reported from more than 30 cultured

marine fish species of many east and Southeast Asian countries. Recognizing its potential impact on the fisheries sector, World Organisation for Animal Health has listed this disease as a reportable disease.

The iridovirus infected fish are lethargic, swim aimlessly and exhibit anaemia, petechiae of the gills and enlargement of the spleen. The mortality ranges from 20–60%. Histopathological findings of the disease include eosinophilic degenerated cells and basophilic enlarged cells in the spleen, heart, kidney, liver and gills. Appearance of inclusion body-bearing cells is reported to be the pathognomonic lesion in the infected fish. The presence of the virus can also be confirmed by transmission electron microscopy examinations which show viral particles in all the vital organs like spleen, liver, kidney and brain. The viral particles are found to have a central spherical highly electron-dense nucleoid, surrounded by an electron translucent peripheral zone.

Although the disease can be diagnosed based on histopathology and electron microscopy to some extent, these tools do not provide sufficient and precise information for identification of different Megalocytivirus species. The recommended method by World Organization of Animal Health is the use of molecular diagnostics to differentiate RSIV and ISKNV employing two primer sets as a confirmation assay.

Megalocytivirus has a wide host range with mostly similar clinical signs. The steps towards disease prevention include achieving alkaline pH around of the system by disinfecting the reservoirs such as ponds and tanks with sodium hypochlorite or potassium permanganate at 100–200 mg/l or higher.

***Whitespot disease of shrimp*:** White spot disease (WSD) is an economically signficant disease of farmed shrimp. The disease is caused by a virus called as White Spot Syndrome Virus (WSSV), which is a WSSV is a rod shaped double stranded DNA virus. The disease is transmitted vertically from infected brood stock to larvae and horizontally through ingestion. Most crustaceans including crabs can be affected by WSD and acts as potential carriers. All the life stages of shrimp may get infected by this virus. Affected shrimp exhibit anorexia, lethargy, reddish discoloration and presence of circular white spots on the carapace and other exoskeletal parts. In *vannamei* shrimp, white spots may not be clearly visible. Mortality of shrimp may start 2-3 days after infection and reaches 80-90 per cent within 5-7 days of onset of mortality. WSSV can persist in wet soil for a long time.

The disease can be prevented by some management measures like pond preparation properly by drying, applying lime etc., stocking only healthy and PCR tested and WSSV negative post larvae (PL) of at least PL15 stage, and adopting strict biosecurity measures by providing reservoir ponds, bird and crab fencing.

Monitoring, surveillance and management of parasitic diseases of maricultured finfish

There is less scope for control over water quality and flow as well as the presence of other marine life, including planktonic or net fouling organisms in marine cage farming. The advancement in cage aquaculture has been associated with emergence of parasitic diseases. Parasitic diseases can have a serious economic impact on marine cage culture because anti-parasitic treatment of fish in cages is often practically not possible or highly expensive. Many parasitic diseases affecting cage cultured fishes are caused by ectoparasites. An overall reduction in parasite diversity and a shift from endoparasites to ectoparasites is at least partly due to the use of manufactured feed since trophic transmission is likely when fishes are fed with trash fish.

***Monogeneans*:** Monogeneans or monogenetic trematodes are flatworms attached to body, fin & gills of fish. Monogeneans infecting marine fish have a direct life cycle, and are considered dangerous due to their high rate of transmission among fish in culture systems. Innate susceptibility and stressful environmental conditions can cause massive infections. Capsalid monogeneans have a flattened, leaf-like body with attachment organs, inhabit the skin and occasionally on the gills and even nostrils. They feed on epithelial cells and mucus causing excessive mucus production, skin lesions, opaque eyes, anorexia and mortality. Many of them have low host- specificity making them dangerous pathogens in fish culture facilities. Benedenia, Neobenedenia and Megalocotyloides are some of the genera that have high pathogenic potential. Dactylogyrids are common gill parasites of teleost fishes and are characterized by the presence of two pairs of large hooks or hamuli that helps parasite attachment. The attachment and feeding cause severe irritation, excess mucous production, epithelial hyperplasia, erosions, ulcerations, haemorrhage and hyperemia with leukocytic infiltrations. Heavily affected fish may die due to asphyxia as a result of gill pathology and interference with gaseous and ionic exchange mechanisms. Easy multiplication & dispersion coupled with its direct life-cycle make them more dangerous in culture systems. Other important monogenean parasites of cultured fish include Diclidophorids which include Heterobothrium and Neoheterobothrium and Microcotylids, capable of causing serious mortalities in cultured fish.

***Copepods*:** Copepods are considered the most important disease-causing parasites in both wild and cultured fish populations. They are small, dioecious crustaceans, with female carrying eggs in egg sacs attached to the genital segment. The egg hatches and develops into nauplius that further develops into copepodids and eventually adults in a rather shortened life-cycle. Typical examples include the sea lice (*Lepeophtheirus salmonis*), and

the cymothoid isopods, which are notorious parasites of cage reared fish. Caligids are generally known as sea lice and are common in tropical and warm temperate waters. They are dorso-ventrally flattened and attach to their hosts by a combination of claws and suction. Two important genera parasitizing fishes are *Caligus* and *Lepeophtheirus*. Sea lice infestation is the biggest problem faced by salmonid farming industry, where the cost of treatments alone could be around 6% of the fish production. The presence of sea lice on non-salmonid production systems has also been associated with mortality and diseases. Caligids are considered the most important parasites in marine fish aquaculture.

Isopods: Isopods are marine/brackish parasitic crustaceans inhabiting warm waters. Majority of the isopods are cymothoids parasitizing marine teleosts. Massive attack by the juveniles of the parasite can kill fry and fingerlings. The parasites that enter the gill chamber cause anemia and loss of gill filaments due to their movements and feeding habits. The genus Nerocila has been found in groupers, seabass and snappers in southeast Asian countries. Isopod menace is considered an emerging problem in Mediterranean sea cages causing reduced growth and mortality. The first record of serious mortalities in cage cultured fishes in India was caused by the isopod *Cirolana fluviatilis* in cage cultured seabass in Cochin backwaters. Mortalities appeared one month after stocking and fish were found dead in cages with their flesh eaten away, leaving the remnants of skeleton. *C. fuviatilis* is a voracious, carrion-feeding bottom dwelling isopod, but sometimes colonize the fouled net surrounding the cage and attack the stressed fish causing heavy mortalities. This is an example where parasites/pests that have not been previously considered pathogenic can cause serious mortalities under certain circumstances.

Monitoring, surveillance and management of co-infection in aquaculture practices

Coinfections or concurrent infections are infections of aquatic animals caused by two or more genetically different pathogens, where each pathogen causes damage to the host in concurrence with other pathogens. Coinfections may involve bacteria, fungi or viruses or their combination. Coinfections caused by homologous bacterial pathogens have been reported in many fish species. During co-infection, interactions between the infectious agents may lead to increase of the load of one or both pathogens or one or both may be suppressed or one may be increased and the other suppressed. Such interactions during coinfection can have a significant impact on the pathogenesis and severity of the diseases and should be considered during the planning of treatment using therapeutics and vaccination. Moreover, concurrent infections in marine cage culture should gain greater emphasis as the farming is done in natural water bodies which harbour many infectious organisms

Monitoring, surveillance and management of nutritional disorders of cultured marine fishes

Nutritional diseases of cultured marine fish may develop consequent to deficiency, excess, or imbalance of nutrients like protein, lipid and fat present in the feed. Nutritional diseases do not generally develop all of a sudden instead they develop gradually since the fish have body reserves of nutrients that make up for nutritional deficiency up to a certain extent. Clinical signs of nutritional deficiency develop only when supply of any diet component falls below critical level. Further, when fish consumes excess food, whatever in excess would be converted to fat which will be deposited in body tissues and organs. The deposited fat affects the physiological functions of the fish.

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ROLE OF CRUSTACEAN PARASITES IN MARINE FINFISH CULTURE SYSTEMS

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Introduction

In India, marine fish farming is gaining popularity in recent years. One of the most important bottlenecks in farming is the incidence of diseases. Diseases are mostly due to environmental, nutritional, genetic, pathogenic and parasitic. The disease problems are very common in marine fish farming. In some cases, those parasites which are generally considered harmless in the wild, can cause serious loss in marine fish culture. Parasites which have a direct life cycle can easily flare up and cause mortalities in fish culture. Stocking fishes in higher densities in the culture systems can provide a suitable environment for the emergence, establishment and transmission of new parasites and diseases (Murray and Peeler, 2005).

The development of intensive fish farming has been associated with the emergence of several serious parasitic diseases (Kent, 2000). As treatment for parasites in cage culture is often impractical and expensive, parasitic diseases can cause serious economic losses in marine fish farming. In some cases, parasitic infection may not cause mortality but can increase the production cost. Most of the serious problems reported in fish farming were due to parasitic infections, especially due to ectoparasites. Parasites with indirect life cycles are generally transmitted through food (trophic transmission) and are comparatively lesser in fish farming because of the use of parasite-free artificial diets.

In comparison to wild fishes, cultured fishes have lesser parasite diversity and a clear shift from the dominance of ectoparasites to endoparasites. Monogeneans and crustaceans infections are more common in farmed fishes than in the wild (Thoney and Hargis, 1991). Some of the free-living organisms become parasitic and can cause serious problems in culture conditions like infection of *Neoparamoeba* spp. (Tan *et al*., 2002; Dykova *et al*., 2005) *Uronema* (Jee *et al*., 2001). Higher parasitic infection and susceptibility can be due to low genetic diversity, repeated introduction of wild fishes or the presence of similar hosts near the culture sites.

Parasitic crustaceans can cause serious injuries to farmed fish, causing destruction of skin and gill tissues and causing secondary infection, diseases, and massive mortality worldwide. There are around 60,000 species of crustaceans, that are significant to the

aquaculture and fisheries sector, including parasitic species affecting other crustaceans, molluscs, and fishes. Parasitic crustaceans are mainly copepods, brachyurans and isopods that are important in the fish farming sector. Compared to copepods and isopods, brachyurans are a serious concern in fresh waters and in the marine sector, it has only limited importance in fish farming. These parasites can cause mortalities in all types of systems including hatcheries, nurseries and farms. Most of the reports of copepod parasitic infections in cage culture are from temperate waters. The risk associated with cage culture is that most often the wild fishes are also there near the culture sites. Sea lice infection in the cage culture is a serious problem in salmon farming. Parasite treatment alone contributes 30-40% of the total expenditure in salmon farming. Once the disease has occurred, the fish become very weak and the treatment trial will make the fish weaker. Prevention is often related to control of the environment and management of the culture system including important aspects like maintaining ideal water quality conditions, adequate nutrition, appropriate stocking ratio and density.

Strict monitoring of preventive measures such as maintenance of disease-resistant stock, ideal environmental conditions, reduction in stress, chemical and immunological prophylaxis, proper hygiene, and prevention of introduction and spread of parasites through water, feed and accessories need to be taken care.

Parasitic copepods are silent killers. The presence of one or two copepods in a fish may not be recognised as a serious problem. In the wild, it has developed an equilibrium and both fish and parasites survive for longer periods. In farming conditions, confinement and increased population density of the host give a favourable environment for the parasite. In such conditions, various stress factors can also reduce the immunity of the host. This gives a most favourable chance for the survival and multiplication of copepods and within a short period, the infection becomes heavy and affects the growth, fecundity, and survival of the farmed fish. This is true in the hatcheries also, even accidental introduction of a single copepod can cause big havoc and cause mortalities in the broodstock and larval rearing.

Caligid copepods (sea lice) are commonly reported from brackish and marine waters. These are the most common reasons for disease outbreaks in marine fish farming. The outbreaks and impacts of sea lice on salmonid farming are well documented, with disastrous losses reported in almost all places where salmon farming is undertaken. The second major group which causes mortality is the isopods. Isopod infection is largely seasonal and in the daytime, these parasites hide in cages and nearby vesitations. During the night, swarms of isopods attack the farmed fishes and create mortality. Unlike that of caligids, isopod infection is not common in indoor hatcheries and larval-rearing units.

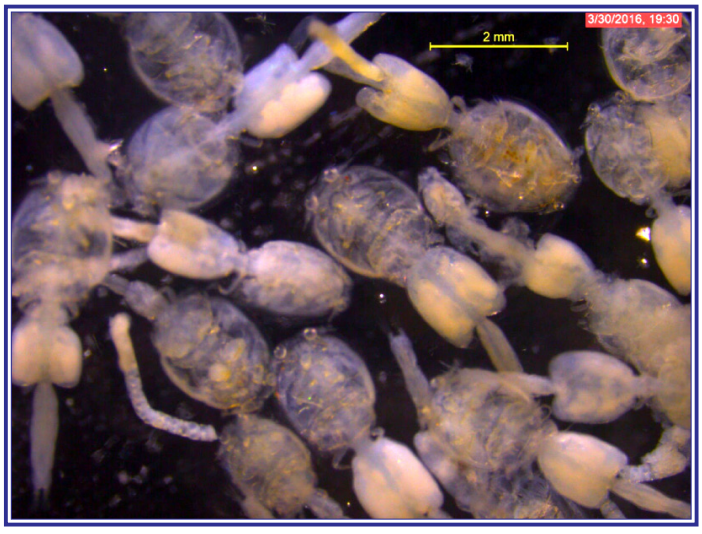
Isopods and caligids infections were reported from nursery and farming units of pompanos, cobia, grouper, seabass and pearl spot from Indian waters.

Due to the recent developments in the control and management of sea lice, mortalities in salmon farming have been reduced significantly. However, there is a heavy economic loss due to treatment and management practices. Many times, the treatment alone comes 30-40% of the production cost. Similarly, sea lice infection has been reported in the farming of Atlantic halibut, Atlantic cod, turbot, and haddock (Johnson *et al.*, 2004).

Parasitic crustaceans feed on host mucous, tissues and blood. Their attachment and feeding activities cause injuries to the host and can cause diseases and mortality directly and indirectly by secondary infections. The severity of the infection depends on the size, age and health conditions of the fish. Severity also depends on the nature of the infection and the severity of the damage. Basically, the losses are due to direct mortality, mortalities due to secondary infections, reduction in growth, loss of market value due to deformity and damages and increased cost of production due to treatment and management. Parasitic crustaceans have a direct life cycle and can flare up within a short time. The important factors affecting the infection are temperature, salinity, size and age of the host.

Caligids

Caligid copepods have 2 free-living planktonic naupliar stages, 1 infectious copepodid stage, 4-6 attached chalimus stages, 1-2 pre-adult stages and 1 adult stage (Lin et al., 1997, Pike and Wadsworth, 1999). The family Caligidae includes 31 genera and 487 species and



Caligid copepods collected from carangid fishes

the dominant and commonly reported causing mortalities among farmed fishes are from the genera *Caligus* (250) and *Lepeophtheirus* (121) (Morales-Serna et al., 2016).

Severe mortalities due to caligid infections have been reported in almost all farmed fishes from marine waters. In India, this is frequently reported from pompano and pearl spots. Caligid infection is more common in cage-farmed fishes. The infections can be noticed due to the damage in the skin, fins, eyes and gills. Parasites can be recovered easily from a moribund fish.

Isopods

Parasitic isopods are mostly opportunistic and some are obligatory. These are more frequently reported from tropical waters. Mortalities due to isopod infection is mostly repotrted from marine waters. According to WoRMS, (2024), the order Isopoda has 37 families, 379 genera and 3154 reported marine species.

There are mainly two groups of isopods that attack farmed fishes; cymothoids and gnathiids. Cymothoids are mostly parasites of fish, both adult and immature forms. In gnathiids only the larval forms are parasitic and the adults are free-living and non-feeding. Isopods generally attack the gills, gill chamber, mouth and the external body surface of fish. The life cycle and larval forms are different among each group.

Like caligids, isopods also can cause similar damages and mortalities in farmed fishes. Damages have been reported in the gills and mouth due to feeding and erosion. Some isopods can cause damage and lesions on the skin and body surface. These infections can lead to secondary infections. Many isopods are also carriers of several microbial infections.

Pearl spot fish devoured by isopod parasites

Enlarged view of isopods collected from pearl spots

Control of parasites

Control of parasites can be by physical, chemical and biological management methods. All these methods are difficult and labour-intensive and need constant attention from fish farmers.

Manual removal of parasites

Visible parasites can be removed manually using forceps. Bigger copepods and isopods can be removed manually by the use of fine forceps. This is a very effective method, especially for larger isopods. But fishes will be subjected to physical and physiological stress and the process is very labour intensive. Some of the copepod parasites will protrude out from the host fishes and can be cut and removed using fine scissors and subjected to a dip treatment with some antibiotics. Caligids especially the genus *Lepeophtheirus* is a very common external parasite in many fishes and can be removed by using fine forceps in ornamental fishes. This method is more difficult if the parasites are very small and hide under the operculum, scales or in the fin folds. Since most of these larger parasites have a direct life cycle, there is enough chance of recurrence of infection after a few days.

Isopods are a very common parasites and mostly are larger in size, especially in the brooder fishes caught from the wild. These are mostly found on fins, skin, gills, mouth and operculum. In some cases, this parasite will be like a tongue in the mouth. Isopods should be removed mechanically using forceps. After the removal of the parasite, the fish will become extremely sensitive and should be treated with a suitable antibiotic and kept under very good care for at least one month to regenerate the lost tissue.

Using cleaner fishes

Keeping cleaner fishes along with larger fishes is an ideal method to remove the parasites from the larger fishes. Cleaner wrasse, bluehead wrasse, neon goby, and cleaning goby are very ideal fishes (Cowell *et al.,* 1993). Cleaner wrasse is an important and beautiful fish to be kept along with cultured fishes and this species goes well with almost all groups including larger groupers to smaller damselfishes. Salmon farmers have used this treatment method for decades. The fishes like lumpfish (*Cyclopterus lumpus*), ballan wrasse (*Labrus bergylta*) many species of Labridae including, blu streak wrasse, goldsinny wrasse (*Ctenolabrus rupestris*), corkwing wrasse (*Symphodus melops*), rock cook (*Centrolabrus exoletus*) and cuckoo wrasse (*Labrus mixtus*) are being used (Philis, 2021).

Physical methods

Caligids are temperature-sensitive. Optilicer and thermolicer are the systems used in salmon farms for raising the temperature for 30 seconds upto 34 degrees which can dislodge most of the parasites. In salmon farms, vacuum turbulence and spraying are commonly used for dislodging the parasites from the body surface. Hydrolicer, is used for low negative pressure, creating vertical turbulences dislodging sea-lice from the salmon.

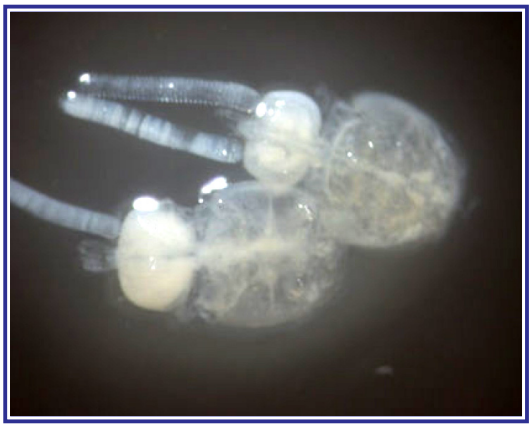
Chemotherapeutics

A wide range of chemicals are used in treating most of the parasites. However not much is known about toxicity and pharmacokinetics of these chemicals. But in cage fishes, we have a little more freedom to use these chemicals. Whenever the chemicals are used, they should be used in a controlled volume of water. It is very difficult to go for the treatment in ponds and cages and doses cannot be so accurate. A tarpaulin bag or a polythene bag can be used to completely cover the net hanging from the float and then treatment can be implemented. Further, the required quantity of chemicals added should be ensured and properly mixed.

In salmonids, there are several reports of increasing resistance of sea lice to various chemotherapeutics. Still, chemical treatment of crustacean parasites is common in salmon farming. Chemical treatments are mostly through baths or medicated feeds. Baths are made using crowded net pens covered with tarpaulin. Vigorous aeration was given during the entire treatment time. Use of medicated feed is comparatively easy but the medicine should not go beyond a limit which affects the physiology of the host. Organophosphates like Azamethiphos and synthetic pyrethroids such as Deltamethrin affect the nervous system of sea lice. Hydrogen peroxide can make gas formation in the lice which causes

paralysis of the parasite and detachment from hosts. Emamectin and Ivermectin can disrupt nerve impulses and can cause the death of parasites. Emamectin and Ivermectin are commonly used for the treatment of isopods.

Sea lice infection on the skin and fins of a live fish



Lepeophtheirus sp. (females)

Isopod attached on the body of a live fish

Isopod parasite (live)

Parasitic infection in the wild is not considered a serious problem but in fish culture, this can cause serious losses. Often the treatment and prophylactic measures cost a significant portion of the operational expenditure. In the case of yellow tail culture, this alone sometimes costs more than 50% of the total expenditure. A confined environment makes the fish more vulnerable to parasitic infection than the wild. Copepods and isopods can flare up very easily and can cause serious damage in marine fish farming. Common methods of removal of parasites are not efficient and cost-effective. Further research is essential to formulate safe, cost-effective treatment and preventive measures for

parasitic infections. Nowadays the main focus is to develop and use of safe natural substances through the feed to control crustacean parasites.

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PROBIOTICS AS BENEFICIAL MEDIATORS IN HEALTH MANAGEMENT OF MARICULTURE SYSTEMS

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Narasimhulu Sadhu, Suresh, R.D., Damodararao, P

Introduction

The mariculture industry is experiencing a rise in global demand, as it serves as an alternative source of income and plays a crucial role in providing protein and export opportunities for coastal communities. In the last two decades, mariculture has seen substantial expansion globally, representing 25.5% of the total aquaculture production (FAO 2017). In India, the Central Marine Fisheries Research Institute has played a significant role in standardizing breeding and cage culture technologies for various marine finfish species such as Cobia, Siver Pompano, orange-spotted grouper, Indian pompano, john's snapper, rabbit fish and seabreams and achieved successful production in different maritime states. It is essential to establish a health protocol for both marine finfishes and the environment across various culture systems, in order to secure sustainable growth and production. Hence, it is crucial to adapt efficient health management strategies to ensure the long-term viability of mariculture. The occurrence of disease outbreaks presents a major obstacle in intensive culture systems, resulting in decreased profitability within the aquaculture sector. An increase in vulnerability to several diseases of aquatic organisms will be established through their surrounding environment, over-crowding, improper feeding practices etc.

In recent years, the prevalence and spread of diseases has been increasing enormously in marine fish farming, which are caused by a wide range of infections including bacteria, viruses, fungi, protozoan and metazoan parasites; nutritional and environmental problems etc. Many of the marine finfish and shellfish are encountered with many viral, bacterial and parasitic infections during the culture period due to several environmental stress conditions and also through horizontal transmission. Hence, a thorough knowledge on diseases, parasites and pathogens profiling, surveillance and monitoring programmes and also development and implementation of preventive protocols as better management practices of mariculture systems is the need of the hour.

Many bacterial diseases of cultured fish are reported worldwide, most of which are found to be opportunistic in nature. Vibriosis is a common disease outbreak in both hatchery and cage cultured fish. Although a number of bacteria are reported to be associated with diseases in fish, only a few are responsible for large-scale mortalities. Bacteria such as *Vibrio anguillarum, V. alginolyticus, V. vulnificus, V. damsela, V. harveyi,*

Photobacterium damselae are the major pathogens recorded in marine finfishes. Among the *Vibrios, V.harveyi and V.alginolyticus* and *Photobacterium damselae* are the most pathogenic bacteria of cultured fish, especially Asian Seabass, Cobia, Snapper, Grouper and Pompano which cause haemorrhagic septicaemia.

The transmission of *Vibrio* spp. in marine fish remains unclear due to the ubiquitous nature of Vibrios, and the complex interaction with the host and environment. The use of antibiotics is a widespread practice in the aquaculture/mariculture sector for pathogen elimination. Various nations have enforced limitations on the utilization of designated antibiotics in aquaculture sectors. Consequently, the adoption of probiotics and dietary supplements has emerged as an immensely efficient strategy to confront disease-causing agents. This alternate approach provides a variety of mechanisms to counteract these agents, thereby serving as a feasible replacement for antibiotic treatment.

Probiotics are a type of microbial supplement which contain living microorganisms. These microorganisms have beneficial effects on the host by modifying the microbial community associated with the host or its surrounding environment. Probiotics can be incorporated into feed or water to modify the microbial balance in the host and the culture environment. Furthermore, probiotics play a crucial role in optimizing the utilization of synthetic feed and elevating the nutritional content of the feed. Moreover, they contribute to strengthen the host's immune system, enabling it to effectively combat diseases and to enhance its overall vitality. Probiotics have the ability to enhance various nutrient-specific digestive enzymes such as amylase, protease, chitinase, and lipase in the intestines. This process not only improves digestion and absorption in fish but also helps in the elimination of toxicity. Probiotics enhance the digestive process by increasing the population of beneficial bacteria, improving microbial enzymatic activity, and thus maintaining microbial balance. This ultimately leads to better digestibility and absorption of nutrients, resulting in higher growth rates and improved diet digestibility. The effectiveness of probiotics can differ depending on various factors such as the concentration of probiotics, whether a single strain or a combination of strains is used, the species and hygiene of the host, the developmental stage of the host (larva, juvenile, or adult), and the environmental conditions including physical, chemical, and biological aspects.

Management of water quality and enhancement of biomass of fish in culture systems can be attained by the application of effective probiotics. Using water and feed probiotics in hatchery tanks, as well as feed probiotics in the nursery and grow-out culture systems, is considered the best preventive measure against microbial infections. It is recommended

to use probiotics in Mariculture systems, both open and closed, along with other water quality management practices, to control bacterial infections.

Advantages of probiotic application in Aquaculture

Probiotic research is gaining momentum in the aquaculture sector as a means to address the potential risks associated with disease enhancement, thereby promoting the development of sustainable practices in the industry.

Advantages of application of probiotics in aqua / mariculture

1. Eenhances growth, survival rates and immunity of fish
2. Enhances nutrient utilization
3. Exhibits bacteriostatic and bactericidal activity against pathogens
4. Prevents colonization of fish pathogens
5. Stress tolerance

Major probiotic bacteria used in Mariculture

The use of probiotics in mariculture has become increasingly popular, particularly when sourced from the gut of fish. Lactic acid bacteria (LAB), *Bacillus*, and *Streptococcus* are among the most favoured bacterial candidates. Although the application of probiotics is a relatively new approach, it has gained attention for its potential to regulate various physiological activities in aquatic organisms.

Commercial propbiotics

Currently, there are several probiotics that have been identified as highly effective in aquaculture, including *Lactobacillus spp., Lactococcus spp., Leuconostoc spp., Enterococcus spp., Carnobacterium spp., Shewanella spp., Bacillus spp., Enterobacter spp., Pseudomonas spp.*, *Pseudoalteromonas spp.* which have shown significant benefits. Additionally, there are reports suggesting that Gram-negative facultative symbiotic

anaerobes like *Vibrio, Pseudomonas, Plesiomonas, and Aeromonas* could also be potential probiotic candidates found in the gastrointestinal tract (GIT) of fish and shellfish. Apart from laboratory-based probiotics, various experimentally approved commercial probiotics available in the market are also found effective in aquaculture.

Some of the commercially available Probiotic products for use in aquaculture (Source: Shoaibe H T Shefat (2018)

S.No	Microorganisms	Target Species
1.	*Bacillus sp.*	Catfish, Penaeids
2.	*Carnobacterium divergens*	*Gadus morhua*
3.	*Alteromonas sp.*	*Crassostrea gigas*
4.	*Lactobacillus helveticus*	*Scophthalmus maximus*
5.	*Lactobacillus lactis*	*Brachionus plicatilis*
6.	*Streptococcus thermophilus*	*Scophthalmus maximus*
7.	*Lactobacillus casei*	*Poeciliopsis gracilis*
8.	*Enterococcus faecium*	*Anguilla anguilla*
9.	*Lactobacillus rhamnosus*	*Oncorhynchus mykiss*
10.	*Pseudomonas fluorescens*	*Oncorhynchus mykiss*
11.	*Roseobacter sp.*	Scallop larvae
12.	*Saccharomyces cerevisiae*	*Litopenaeus vannamei*
13.	*Vibrio alginolyticus*	Salmonids
14.	*Lactobacillus acidophilus*	*Clarias gariepinus*
15.	*Bacillus spp*	*Farfantepenaeus brasiliensis*
16.	*Enterococcus sp.*	*Farfantepenaeus brasiliensis*
17.	*Lactococcus lactis*	*Epinephelus coioides*
18.	*Lactococcus helveticus*	*Scophthalmus maximus*
19.	*Shewanella putrefaciens*	*Solea senegalensis*
20.	*Lactobacillus delbrueckii*	*Dicentrarchus labrax*
21.	*Bacillus subtilis*	*Poecilia reticulata*
22.	*Lactobacillus rhamnosus*	*Danio rerio*
23.	*Lactobacillus acidophilus*	*Xiphophorus helleri*
24.	*Lactobacillus casei*	*Xiphophorus helleri*

Specific probiotic species in controlling pathogens and diseases of fish and shrimp

S.No	Microorganism	Benefits	Fish/Shrimp
1	*Bacillus subtilis*	Inhibits vibriosis outbreak	Shrimp
2.	*Bacillus megaterium*	Improves the resistance to viral infections	Shrimp
3.	*Enterococcus faecium*	Control *Vibrio parahaemolyticus*	Shrimp
4.	*Bacillus pumilis*	Improves larval survival and control *Aeromonas hydrophila*	Fish
5.	*Lactobacillus acidophilus*	Controls disease outbreak with *Pseudomonas fluorecens*	Fish
6.	*Lactobacillus rhamnosus*	Improves disease resistance to *Salmonella*	Fish
7	*Rhodococcus sp*	Controls the disease of *Vibrio anguillarum*	Fish
8	*Saccharomyces cerevisiae*	Control Vibrio disease	Shrimp/Fish

Screening of probiotics

The utilization of probiotics in aquaculture has been widespread due to their diverse range of biological activities. The initial and essential step in this process is the screening of probiotics, which requires a systematic and scientific approach.

The criteria for selection of probiotics consist of the following:

(1) It must not cause harm to the host

(2) It should provide resistance to the acid stomach environment, bile and pancreatic enzymes

(3) It should not invade into the tissues and cause tumors

(4) it should effectively reach the intended site within the host

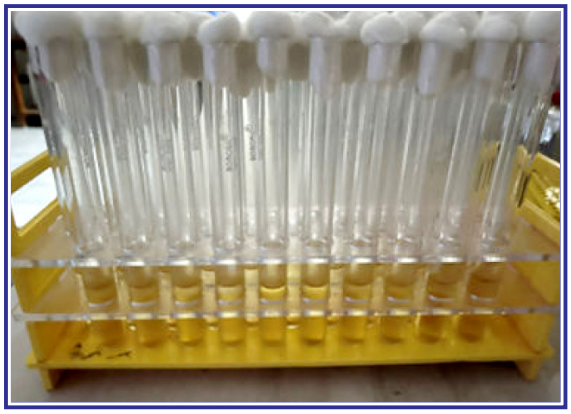
(5) it should contain plasmids without antibiotic and virulence resistance genes

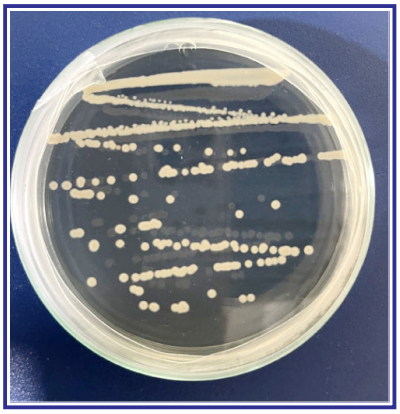
(6) it should demonstrate efficacy in host model systems rather than just in vitro studies

(7) It should remain alive for a long period of time, for effective colonization

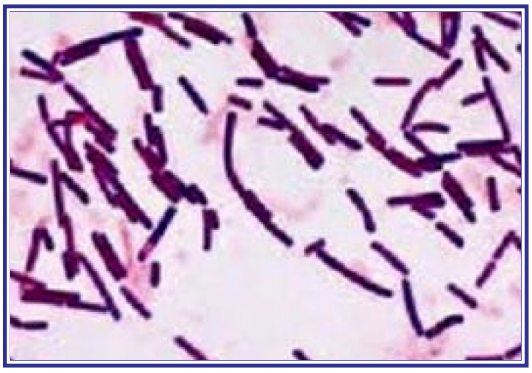
(8) it should be able to replicate in the intestine of the host for a sustained period

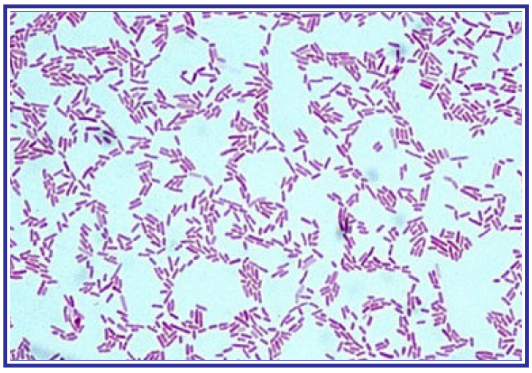
(9) It should be able to produce antimicrobial substances against fish pathogens

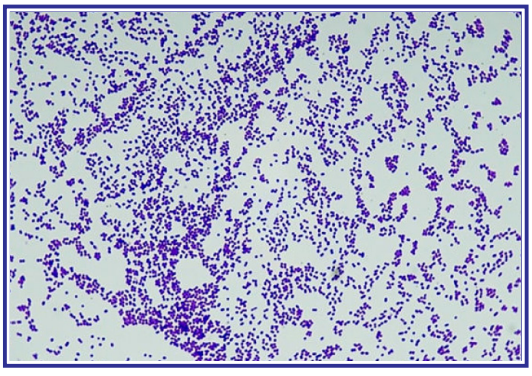


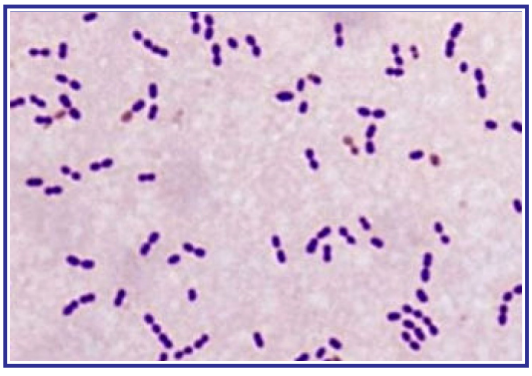


Isolation and identification of probiotic bacteria from fish gut and marine sediment

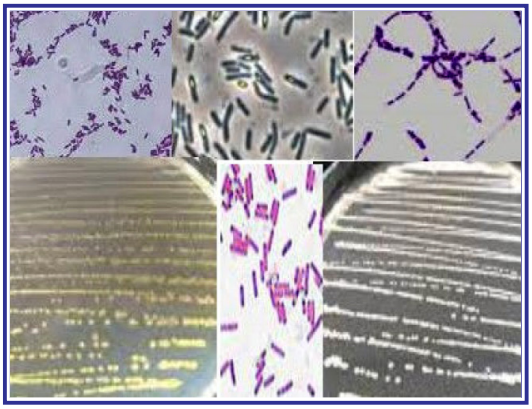


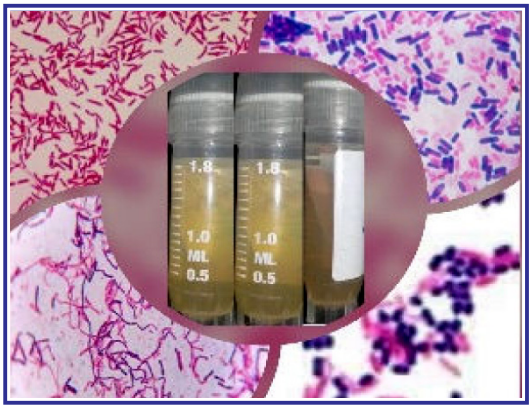






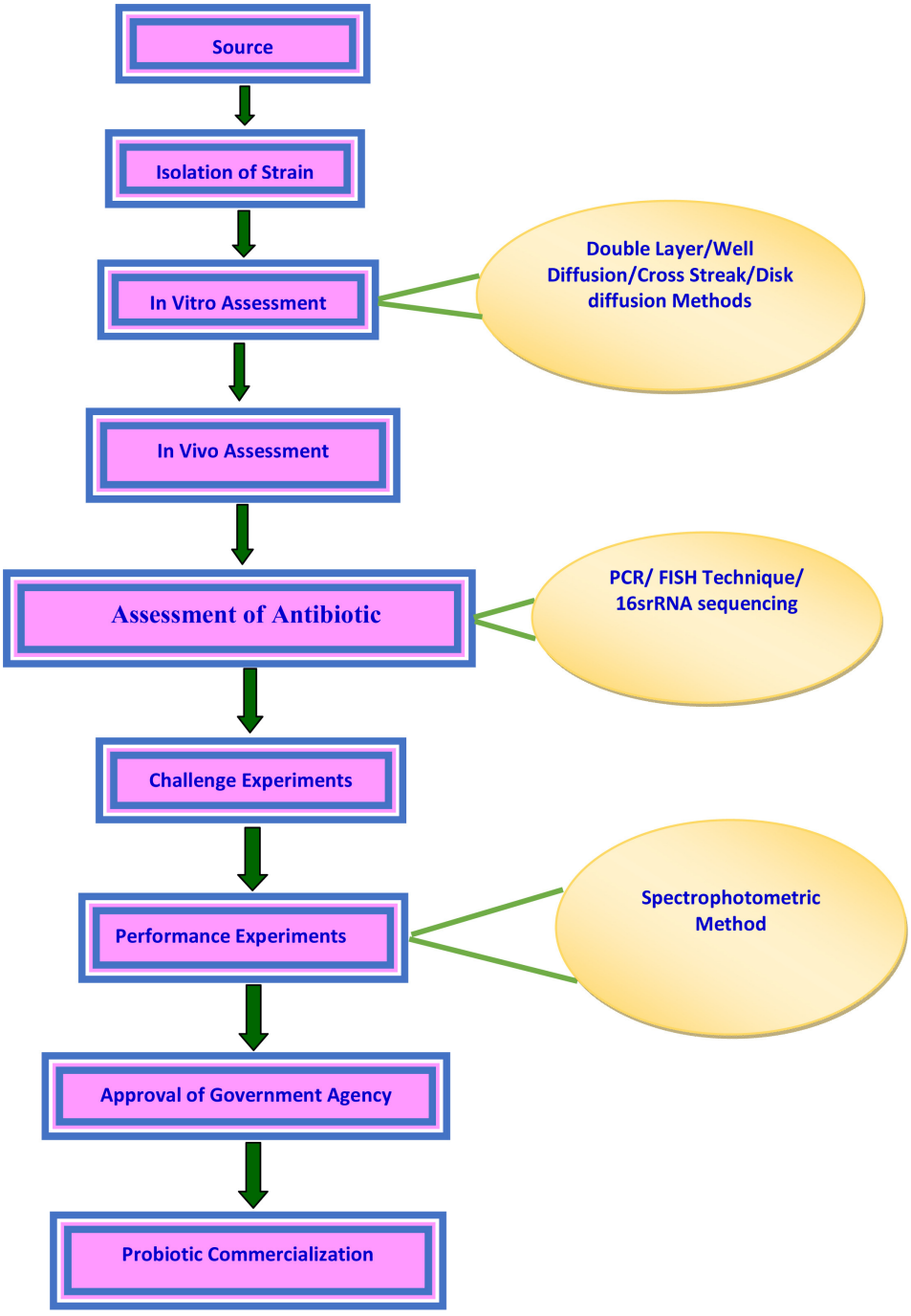
Bacterial isolates (Gram negative and Gram Positive) showing probiotic properties





Laboratory Developed probiotic consortium

In invitro screening for potential probiotics, inhibitory or antagonistic activity of bacteria need to be checked. In vitro screening for inhibitory substances commonly employs four methods: the double layer method, the well diffusion method, the cross-streak method, and the disc diffusion method. All these methods are based on the same fundamental principle, where a bacterium generates an extracellular substance which has the ability to inhibit either itself or another bacterial strain.



Schematic diagram for Selection of probiotics

Application of probiotics in Aquaculture requires several key procedures:

(1) acquiring a comprehensive understanding of probiotic application

(2) conducting both in vivo and in vitro assessments to determine their pathogenicity

(3) conducting a long-term practical evaluation of the treated probiotics

Additionally, modern techniques such as ERIC-PCR, PCR-DGGE/TGGE, FISH, and 16S rRNA gene sequencing are the best molecular tools for the selection and evaluation of probiotics.

Application of Probiotics for Enhancement of growth and survival of fish

The utilization of probiotics extends beyond their role in promoting the growth of various cultivated species in aquaculture. For instance, the addition of *Bacillus* spp., *Enterococcus* spp. and *Pseudomonas* spp. at concentrations of 10^7 and 10^9 cfu g^{-1} in the diet results in notable weight gain in fish.

Probiotic supplementation enhances feed utilization and weight gain in cultured fish. Probiotics can stimulate feed palatability by breaking down indigestible components, producing vitamins, and detoxifying poisonous compounds in the diet. Probiotics increase fish resistance to stress caused by environmental and technological hazards. The application of beneficial bacteria provides micronutrients such as vitamins, fatty acids, and essential amino acids to support the healthy growth of cultured fish.

Microorganisms have the ability to establish themselves in the gastrointestinal tract (GIT), because they reproduce at a faster rate than they are expelled after administered for an extended period of time. Probiotics are continuously introduced into fish cultures to promote health by boosting the expression of various immunological factors and by occupying physical space in the gut mucus layer, thereby reducing the presence of pathogens. Probiotic candidates also play a vital role in nutrient enhancement in host. Fishes fed with probiotics, *Lactobacillus* sp. *Bacillus* spp. and *Streptococcus* spp. exhibit enhancement of body weight crude lipid, total protein.

Efficacy of probiotics on fish digestive and immune systems

1. Increase in the activities of lipase, protease and amylase enzymes in the digestive tract
2. Help to improve digestion and nutrient absorption, resulting in better use of food and growth

3. Increase phagocytic activity of leucocytes
4. Stimulate the respiratory burst activity which further enhances the ability to fight against pathogens and disease attack

Methods of Probiotic Application

The application of probiotics plays a crucial role in attaining goals like disease prevention and treatment. The administration can be done through feed or water, depending on several factors such as the specific probiotics utilized, the form of supplementation, the mode of administration, the dosage level, and the duration of application. These variables influence the choice of method for probiotic application.

Probiotics can be categorized into two main groups, according to their mode of action. The first group is gut probiotics, which are taken orally with food to enhance the beneficial microbial flora in the gut. The second group is water probiotics, which thrive in water environments and effectively eliminate pathogenic bacteria by consuming all the nutrients available in the specific medium, ultimately starving the harmful bacteria and eliminating them.

Oral Administration Via Diet

The most commonly used method involves incorporation of probiotics into the feed. Probiotics can also be introduced into the tank or pond water to provide protection against infections. Parabiotics, on the other hand, are inactive microbial cells derived from probiotics. They contain cell components like peptidoglycans and surface proteins, offering advantages such as being available in a pure form, easy to produce and store, and having a higher likelihood of triggering specific responses through ligand-receptor interactions. Probiotics can be administered continuously or at regular intervals. Many studies have focused on continuous feeding of the host fish for varying durations, ranging from 15 to 90 days.

Application of multi-strain Probiotics

The use of multiple-strain products offers the benefit of being effective against a wider range of conditions and species. It is also common to combine probiotics with prebiotics and/or plant products. Research on the application of multistrain probiotics suggests that probiotics containing *Bacillus* spp. ($1x10^9$ CFU/mL) and *Lactobacillus* spp. ($1x10^{11}$ CFU/mL), with concentrations of 0, 0.5, and 1.0 mL/L in water for 8 weeks, can

enhance the growth of fish by improving the health of their gut, liver, and muscles. Numerous reports have demonstrated the positive impact of probiotics in aquaculture/ mariculture, including improved fish growth performance, immune response, and resistance against certain pathogenic bacteria.

Indian pompano, *Trachinotus mookalee* fed with Laboratory developed probiotic consortium

Probiotics in improving water quality

Bacillus sp. is particularly linked to the use of probiotics in enhancing the quality of culture water. This is due to the fact that Gram-positive bacteria, such as *Bacillus* sp., have a superior ability to convert organic matter into CO_2 compared to Gram-negative bacteria. The presence of high levels of Gram-positive bacteria, facilitated by the use of *Bacillus* sp., can effectively reduce the accumulation of dissolved and particulate organic carbon. Consequently, the utilization of *Bacillus* sp. in aquaculture systems leads to improved water quality, enhanced survival and growth rates, as well as better health conditions for cultured shrimp and fish. The utilization of commercially available water and feed probiotics in Marine culture systems is highly recommended as the most effective preventive measure against bacterial infections.

Probiotics have gained attention in the aquaculture/mariculture industry, prompting research efforts to investigate their utilization and potential advantages. This is due to the increasing demand for probiotics in the aquaculture of animals. Investigations should focus on screening host-specific probiotic strains from aquaculture-rearing systems to effectively manage their quality and functional properties. Although probiotic bacteria provide numerous advantages to the host, there exist certain restrictions that need to be addressed. For instance, the antimicrobial compounds or bacteriocins produced by probiotic candidates against pathogenic bacteria are not specific to particular species. To

increase the efficiency of probiotic bacteria, it is imperative to prioritize strain improvement. In conclusion, the application of single or multistrain probiotic bacteria play a great role in the sustainable production of disease-resistant fish in cage culture or any kind of other mariculture systems.

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MANAGEMENT OF MARINE MICROALGAL PRODUCTION QUALITY IN MASS PRODUCTION OF LIVE FEED

Biji Xavier

Vamsi, B., Padmaja Rani, Lingaraju

Introduction

Phytoplankton species play a crucial role in the food chain as a key nutrient in aquatic environments. These species use ammonia, urea, nitrate and phosphate nutrients, vitamins, trace elements and using some form of organic matter formed by chemical breakdown of dead cells (Gökpýnar 1990). The marine algal cultures have a great importance both in the laboratory and in the natural environment with reference to economic value. Phytoplankton or zooplankton is the first step in the food chain for the most marine fish farming as feedstock. For this reason, proper feeding of marine fish larvae, production of phytoplankton and zooplankton has a great importance. Problems in marine fish farming, as well as the culture of oysters and shrimp solved with the development of single-celled algae production methods.

Microalgae, belonging to various taxonomic groups, are microscopic single celled, multi-celled, planktonic or benthic algae. Microalgae are the first staple of shellfish and fish larvae of aquatic animals. In addition, it has a great importance for feeding other fish staples like rotifers, *Cladocera*, or copepods (Timur 1992). Microalgae can produce not only biomass but also complex molecules necessary for life activities with the assistance of any light source (sun or artificial light); by combining various sources such as nutrient salts and CO_2.

Species selection for culture

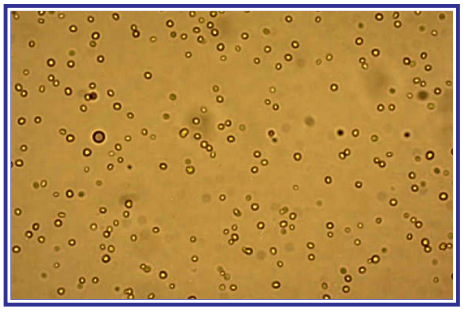
All microalgal species are not equally suitable for culture in laboratory or hatchery. A suitable species or strain of microalgae should be selected depending upon the following criteria:

1. Species of finfish or shellfish which will feed on the microalgae
2. Mass culture potential
3. Cell size

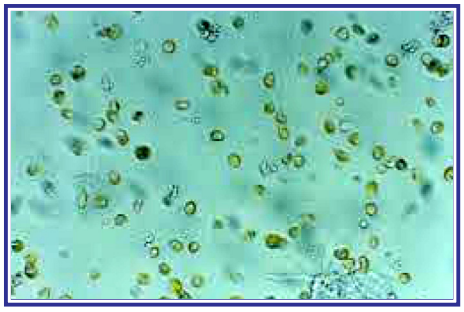
4. Digestibility
5. Overall nutritional value

Common Micro algae and applications in Aquaculture

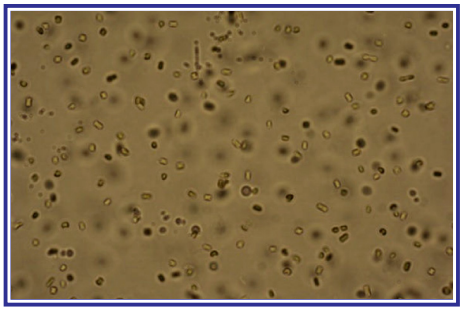
Sl No	Scientific Name	Size (µm)	Nutritional value	Practical application
1	*Nannochloropsis oculata* Eustigmatophyte (Yellow-green)	2-4	EPA	Rotifer culture, water conditioner in finfish hatcheries, reef tanks for feeding corals and other filter feeders
2	*Isochrysis galbana* Prymesiophyceae (golden brown flagellate)	3-5	DHA	Rotifers, copepods, brine shrimp, oysters, clams, mussels and scallops; enrichment of zooplankton ;
3	*Chaetoceros calcitrans* Bacillariophyceae(diatom)	*Elliptical* 3-6	EPA and Vitamins (B_2 &C)	Shrimp hatcheries to increase the vitamin levels.
4	*Chlorella salina*	2-10	EPA	Used for feeding rotifers in finfish larval rearing.
5	*Pavlova lutheri*	3-10	DHA/EPA	oysters, clams, mussels and scallops to increase the DHA/EPA levels in their broodstock.
6	*Tetraselmis suecica* (Prasinophyceae)green flagellates	4-5	amino acids	Feeding stimulant for oysters, clams, mussels and scallops. excellent feed for shrimp larvae and *Artemia*
7	*Thalassiosira weissflogii* Bacillariophyceae(diatom)	4-32	EPA and Vitamins (B2 &C)	single best algae for shrimp larvae and also good feed for copepods, brine shrimp, and broodstock conditioning of oysters (post set), clams and mussels.
8	*Skeletonema costatum* Bacillariophyceae(diatom)	2-21	EPA	extensive and intensive shrimphatchery systems



Nannochloropsis oculata

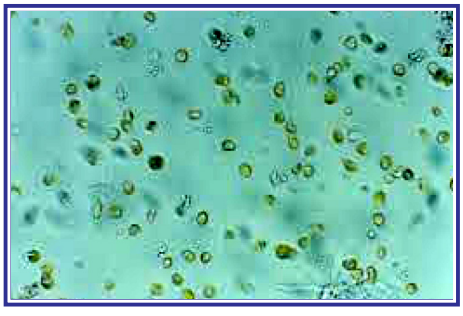


Isochrysis galbana



Chaetoceros calcitrans

Chlorella salina; (source: google)



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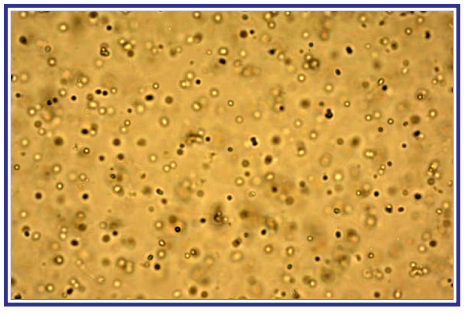

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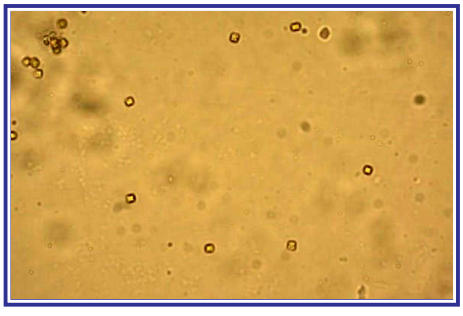
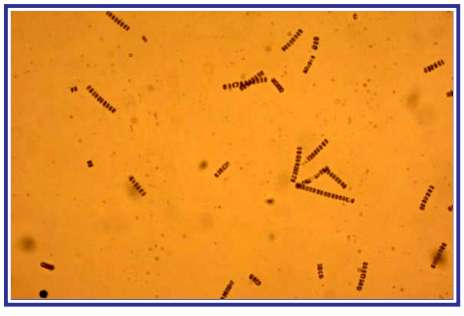
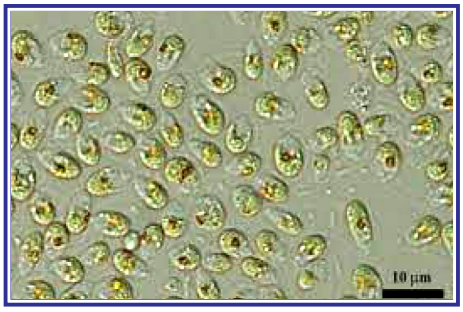


Pavlova lutheri (source: google)



Tetraselmis suecica



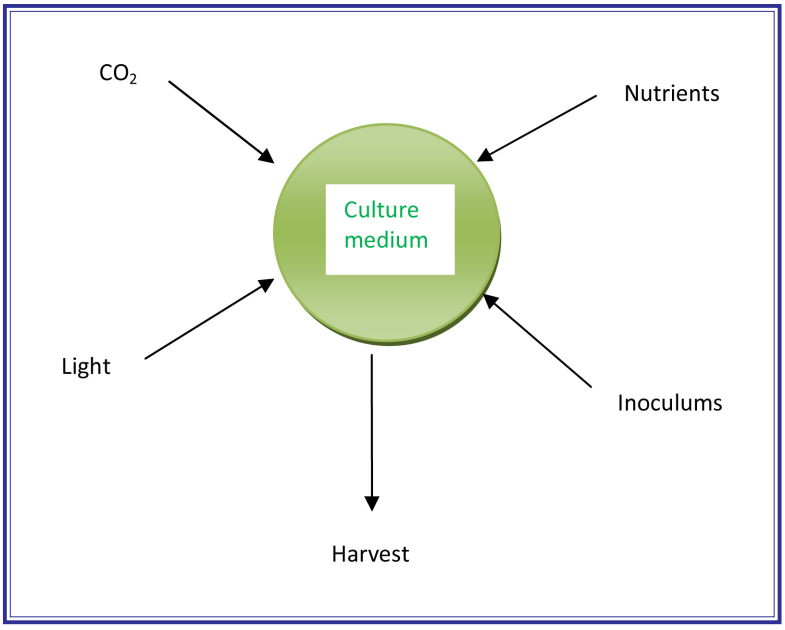
Dunaliella salina (source: google)

Temperature, light, and salinity range for culturing selected microalgae species.

Species	Temperature (°C)	Light (Lux)	Salinity (ppt –)
Chaetoceros calcitrans	25 - 30	2,000-10,000	20 – 35
Isochrysis galbana	25 -30	2,000-10,000	10 – 30
Skeletonema costatum	10 - 27	2,500-5,000	15 – 30
Nannochloropsis oculata	20 - 30	2,500-8,000	12 – 30
Pavlova sp	15 -30	4,000-8,000	10 – 40
Tetraselmis sp	20 -28	5,000-10,000	20 – 40
Chlorella sp	10 -28	2,500-5,000	26 – 30
Thalassiosira sp	25 - 30	2,000-10,000	20 – 35

Microalgae culture

Microalgae culture is a form of aquaculture involving farming of different species of microalgae in a confined environment. The culture has three components namely, culture medium contained in a suitable vessel; the algal cells growing in the medium and air, to allow exchange of carbon dioxide between medium and atmosphere. An autotrophic alga need light, CO_2, water, nutrients and trace elements for their growth. Some auxotrophic algae, which cannot prepare biochemical compounds like vitamins through photosynthesis, need additional biochemical compounds to be added in the culture medium.



Process of micro algal culture with various inputs

Growth dynamics of microalgae

The knowledge of microalgae growth dynamics is important for aquaculturist to know when to harvest the microalgae, to estimate growth rate and population doubling time. Generally, the microalgal culture follows a characteristic pattern of growth and follows five reasonably well defined phase of algal growth in batch culture (Fogg and Thake, 1987). (i) Lag or Induction phase (ii) Exponential phase (iii) Declining phase (iv) Stationary Phase (v) Death phase

i. Lag or induction phase: The Inoculation of culture into a new medium have to acclimatize with the surroundings or to the new physico-chemical conditions, so there will be no cell division for slight time thus the stage is known as lag or induction phase.

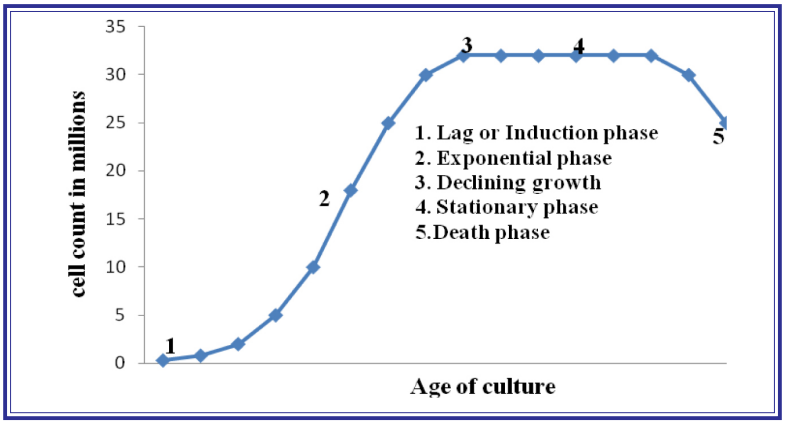
ii. Exponential phase: The inoculum once gets acclimatized to the new physico-chemical conditions, it starts multiplication and grows rapidly, thus this phase is known as exponential phase. The duration of exponential phase in cultures depends upon the volume of the inoculum, the growth rate and the capacity of the medium and culturing conditions to support algal growth.

iii. Declining phase: When the death of the cells will exceed the multiplication of the cells in a culture, then the phase is known as declining phase. This phase generally occurs in culture when either a specific requirement for cell division is limiting or something else is

inhibiting reproduction. The biomass is often very high and exhaustion of a nutrient salt, limiting carbon dioxide or light limitation becomes the primary cause of declining growth. At low cell densities too much CO_2 may lower the pH and depress growth. CO_2 limitation at high cell densities control any further biomass increase to be linear rather than exponential (with respect to time) and proportional to the input of CO_2. Light limitation at high biomass occurs when the cells absorb most of the incoming irradiation and individual cells shade each other. This is known as self-shading.

iv. Stationary phase: After the arrested growth, the culture will be stationary without any further cell division. The net growth of the culture becomes zero during this phase and within an hour's culture cells may undergo dramatic biochemical changes. The nature of the changes depends upon the growth limiting factor. Nitrogen limitation may result in the reduction in protein content and relative or absolute changes in lipid and carbohydrate content. Light limitation might affect the pigment content of most species and changes in fatty acid composition. The longer the cells are held in this condition the longer the lag phase will be when cells are returned to good growth conditions for sub culture.

v. Death phase: This is last phase of growth dynamics, when the cells may lose its viability and start to die. The death phase of culture is generally very rapid, thus the term 'culture crash" is often used for this phase. The cultures of some species will loose their pigmentation and appear washed out or cloudy, whereas cells of other species may lyse (no recognizable cells) but the culture colour will be maintained.



Growth curve of micro algae (*Nannochloropsis* sp.)

Algal culture techniques

The following are the different algal culture techniques used for microalgae culture.

Batch culture: In this culture the resources present in the culture medium are abundant; the micro algae grow according to sigmoid curve. Once the resources are utilized by the cells, sub culturing is followed by transferring a small volume of the existing culture to the large volume of fresh culturing medium.

Continuous culture: In this culture, the resources are potentially infinite. Culture is maintained on chosen point on the growth curve by the regulated addition of fresh medium.

Indoor/outdoor: Indoor culture allows the control over illumination, temperature, nutrient level, contamination with predator and other competing algae; however, outdoor culture will not have any control on the above said parameters.

Open/closed: Open cultures in uncovered tanks and ponds are more contaminated than closed cultures in test tube, conical flask etc.

Axenic (sterile) / xenic: Axenic cultures are pure cultures free from all foreign contaminants and this type of culture requires strict sterilization of glass wares, culture media and vessels to avoid contamination, where as xenic culture might be a contaminated one.

The production of marine finfish and shellfish larvae in hatchery requires different types of phytoplankton and zooplankton. The quality of these live feeds directly affects the production cycle of finfish and shellfish larvae since these life feeds are used either directly or indirectly in larval rearing system. Thus proper sanitary measures for better management need to be taken at each level of culture operation of live feeds to maintain the quality and quantity of these live feeds, right from stock culture to mass culture.

Sanitary measures are routine biosecurity measures that should be taken before, during and after production runs to maximize the health of the stock by minimizing the impact of contamination. These measures are taken to control the movement of different contaminants into and through the live feed stocks. This may begin with facility design, water intake and passes through the entire range of possible sources of contamination throughout the production cycle. Optimum level of sanitization in the system will result in good quality stock, which will reduce the failure risk of the larval production cycle. Hence, it reduces the cost of the production and helps in maximizing the profit from the production cycle.

Maintanance of algae culture

Algal multiplication is normally dependent of various steps like culture water sterilization, nutrient enrichment, inoculation of new culture with the pure algal strain, microscopic observations for growth of the algal cells and finally mass culture of algae in larger containers.

Stock culture

It provides reservoir of algae cells from which large cultures can be initiated. Stock cultures are kept in small volume containers.

Subculture: Subculture involves inoculating some cells from old stock culture to fresh culture medium, so that the cells can continue to grow, devide and healthy. If sub-culturing is not done, cells in the stock culture will die eventually. Precaution must be taken while transferring the stock culture, so that contaminants from air should not enter into the stock culture.

Contaminants in phytoplankton

The major contaminants in phytoplankton stock and mass culture are as follows:

i. Microbes such as bacteria, virus etc

ii. Ciliates

iii. Other microalgal species

iv. Zooplankton in outdoor culture

Source of contamination

The following are the source of contamination in phytoplankton stock culture:

i. Glass ware

ii. Culture media

iii. Water

iv. Air

v. Personnel

Precautions/ sanitary measures

i. Phytoplankton stock culture

It is necessary to practice aseptic techniques to protect the sterile culture media, glassware and finally the cultures from different contaminants.

- Personnel hygiene is the first important step to maintain sterile conditions in stock room.
- The person involved in maintaining stock room should first complete the work related to stock culture and then only carry out any other assigned work.
- He should always use sanitizer whenever he is handling the stock.
- The microalgae stock culture room needs to be kept clean and the door should be kept closed at all times to maintain desired temperature.
- The stock of the algae should be sub cultured under the laminar flow so that contamination in the stock can be avoided.
- The laminar flow UV light should be kept on at least half an hour before doing inoculation work.
- The equipment such as weighing balance, microscope and laminar flow need to be cleaned regularly.
- All glasswares such as culture containers, pipettes etc used in indoor culture are to be cleaned, washed with chromic acid and sterilized in hot air oven to avoid contamination.
- The culture media except heat labile media need to be autoclaved after preparation to avoid contamination. The heat labile chemicals need to be sterilized by 0.22 µm filtration techniques.
- Water is a major source of contamination. It needs to be treated with different filtration processes before sterilization.
- The culture container with water needs to be autoclaved for maintaining stock culture.
- The air supplied to the culture container must be passed through filters so that contamination can be avoided.

- Each and every step must be monitored carefully so the contamination can be stopped early on.
- The working area needs to be cleaned and wiped with alcohol to limit contamination.
- The air stone, pipes and air control need to be sterilized after each cycle by boiling in water for 15 minutes.
- Finally only authorized personnel should be allowed entry into algal stock culture room.

The ciliates are the major source of contamination in algal culture.

It can enter either though culture solution or air. The autoclaving of culture solution and use of filter before passing of air to the culture container can help in controlling ciliates in stock culture.

The contamination of one species of microalgae with another species of microalgae:

- It is common in a stock culture room if it is housing more than one species in the same room.
- This contamination can be controlled by the personnel involved in stock culture room.
- They should sub culture one species at a time and at least give half an hour time interval before starting sub culture of another species.
- In addition, they need to follow personal hygiene before starting sub culture.
- The pipette or the inoculum loop used for the sub cultures need to be sterilized each time before use.

ii. Phytoplankton mass culture

- Design of the water pumping system, water storage facility and culture tanks have effect on the contamination of phytoplankton culture.
- Proper design of the tanks is required to facilitate easy cleaning and drying so the contamination can be avoided.
- The tank inner surface needs to be smooth and the corners should be curved in such a way that cleaning should be easy.
- The bottom of the tank should have some slope so when the water is getting drained it should dry easily.

- An epoxy or fiber glass or plastic coating inside the tank will help in these things. Among these, epoxy coating is the best solution for making inner wall of tank smooth.
- The pumping facilities need to be treated every alternate month with chlorinated water (30-40 ppm) to avoid any contamination.
- The aeration system needs to be sanitized every alternate month by using formalin.
- After the harvest of live food organisms proper management measures for wastewater treatment is required for proper hatchery production.
- Drainage pipes carrying wastewater need to be of suitable diameter for water draining and for avoiding backflow.
- In outdoor culture system, carboys must be cleaned using common salt, rinsed with tap water and keep upside down for sun drying.
- All tanks should be cleaned, disinfected and dried before use.
- The water used for the mass culture of phytoplankton needs to be filtered before stocking in the tank.
- The chemicals required for the mass culture need to be prepared in filtered fresh water.

Contaminants in zooplankton

The major contaminants in zooplankton culture are as follows:

i. Microbes such as bacteria, virus etc

ii. Ciliates

iii. Other zooplankton

Source of contamination

The following are the source of contamination in zooplankton culture:

i. Culture container

ii. Water

iii. Phytoplankton

iv. Personnel

Precautions/ sanitary measures

- All tanks should be cleaned, disinfected and dried before use.
- The water used for mass culture of zooplankton needs to be filtered before stocking in the tank.
- The use of the same siphon pipe for the all tanks should be avoided. Each tank should have a separate siphon pipe.
- Each tank should have a separate set of items such as filters, mugs, and buckets etc required for day to day activity.
- Generally, in commercial facilities, contamination of one zooplankton species culture with another zooplankton species is the most likely cause of the crash of culture. Therefore, it is important to keep these cultures strictly apart.
- The presence of other zooplankton species may pose a problem, so care should be taken while adding fresh seawater and feed to the culture tank.
- The water used for the phytoplankton culture as well as copepod culture should be filtered with 10 µm filter bag.
- Generally ciliate proliferation is more in over-fed culture tanks. Sometimes these ciliates act as a feed for some zooplankton during the periods of low phytoplankton concentrations. However ciliates also compete for the same feed with the zooplankton, thus care should be taken to avoid contamination with ciliates.
- It is advisable to empty the culture tank using 40-60 µm mesh size gauze if ciliate contamination is more, which retains the zooplankton, but allows the ciliates to be washed out. Then, culture can be started afresh.
- Generally, bacteria constitute a part of the diet of zooplankton. Sometimes, cultures may succumb to uncontrolled proliferation of bacteria. Some bacteria, such as *Vibrio* sp., are known to infect zooplankton in eutrophic coastal waters, resulting in low survival rates with further contamination of the fish larval rearing system which leads to mass mortality of the fish larvae also.
- Sea water is one of the main routes that causes contamination in phytoplankton culture. Sanitation protocols need to be followed to maintain water quality which has been provided in the next chapter on water quality for live feed in aquaculture

In order to bridge the gap between demand and supply of marine aquatic resources it is necessary to bring significant changes in live feed production technology. Appropriate sanitary measures are required to combat contamination and maintain hygiene. Technological developments for improving the sanitary conditions of marine hatcheries are critical. Further, awareness for sanitary cleanliness and personal hygiene among the workers is highly essential for improving the quality and quantity in the supply of live feed.

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POTENTIAL INDIGENOUS MARINE COPEPODS IN QUALITY SEED PRODUCTION IN MARINE FINFISH HATCHERY

Santhosh B.

Muhammed Anzeer F., Aneesh K. S. and Dispin Das

Introduction

Copepods are the most abundant metazoans on earth. These are small planktonic crustaceans that occur in almost all kinds of water bodies on the earth's surface. Copepods are the primary consumers and secondary producers in the aquatic ecosystem. Most of the free-living planktonic copepods feed on phytoplankton. Many benthic copepods feed on other animals and many are parasitic also. Some of them live in association with many invertebrates, especially molluscs and echinoderms. Many prefer to graze on living and nonliving substratum. Almost all of them form ideal prey for fishes and invertebrates. Paradoxically many species are notorious parasites of these fishes and invertebrates (Razouls *et al.,* 2005-2024). Copepods are more abundant and diverse in marine and brackish waters in India. Copepods are mostly well-studied and documented in Indian marine waters.

There are more than 210 families 2400 genera and 24,000 species identified in this group. These form an important component in the aquatic ecosystems and ultimately contribute maximum to the food chains as primary consumers. Almost all types of marine organisms, directly or indirectly depend on these small organisms for their food. Copepods form important food for many marine fishes and invertebrates. Certain fishes and fish larvae are evolutionarily adapted for feeding copepods. Copepods are nutritionally superior to all live feeds. Certain fishes, especially those with weak larvae depend on copepod nauplii for their survival at least for the initial few days. Due to their smaller naupliar stages and nutritional superiority, copepod cultures become an integral component in a marine fin fish hatchery (Santhosh *et al.,* 2018).

Basically, there are nine orders in the Subclass Copepoda: Calanoida, Cyclopoida, Hapacticoida, Platycopioida, Mormonilloida, Misophrioida, Siphonostomatoida, Monstrilloida and Gelyelloida. The Subclass Poecilostomatoida is mostly considered as a group under Cyclopoida but phylogenetically separated from it. Members of the Subclass Calanoida, Cyclopoida and Hapacticoida form important components in marine plankton. Among marine pelagic copepods, calanoid copepods dominate over more than 79.2%. The

Indian Ocean has the maximum species composition and the Arctic Ocean the minimum (Boxshall and Halsey, 2004). Marine copepods of India are more well-studied and documented than most of the other sea coasts. So far there are 992 species of marine planktonic copepods reported from the Indian Ocean (Razouls *et al.,* 2005-2024). The maximum number of endemic species (172) is also recorded from India. (Razouls *et al.,* 2005-2024). Copepod resources of the Indian coast are rich and abundant.

Copepods and their importance

Copepods of aquaculture importance mainly belong to the orders Calanoida, Cyclopoida and Harpacticoida. Calanoids can be easily distinguished by their long (20-27 segments) antennules. These are mostly pelagic and filter feeding. Rarely these can be carnivores or predatory. Cyclopoids have shorter antennules than calanoids with six to 17 segments. They have a variety of feeding habits from filter feeding to parasitic. Antennules are much reduced in parasitic forms. These are distributed in all depths and more abundant in freshwaters. Harpacticoids are more numerous in species and occupy more than 50% of the total species of copepods. They have a short antennule having less than 10 segments. Generally, harpacticoids are benthic with a wide variety of food habits from filter feeding to detritus feeding. There are many predatory and parasitic forms also in this group (Huys and Boxshall, 1991; Dussart and Defaye, 2001).

Initial feeding of marine finfish larvae has been one of the major problems in fish seed production. The newly hatched fish larvae have an undeveloped digestive system, poor vision, very small mouth gape and very high nutritional demand, especially in terms of highly unsaturated fatty acids. Only live feeds can meet all these requirements. The conventional live feeds used for the initial feeding of fish larvae are *Artemia* spp. and rotifers. But both these have limitations for feeding smaller fish larvae, in terms of size range, nutritional composition, digestive efficiency and feed preference (Cahu and Infante, 2001). Gut content examination of wild-caught fish larvae proved that the larvae feed mainly on copepods. Live feed size should be much smaller than the mouth size of fish larvae for successful initial feeding. In terms of size, copepod nauplii are highly suitable when compared to *Artemia* and rotifer. Moreover, copepods do not require any sort of enrichment. They have a highly relevant and rich biochemical profile needed for the proper development of most marine fish larvae. Copepods are rich in essential fatty acids which are important for larval fish survival and growth. Highly unsaturated fatty acids such as DHA and EPA are present in the most appropriate ratios in copepods suitable for fish larval development (Sun and Fleeger, 1995; Stottrup and Norsker, 1997; Sargent *et al.,* 1997, 1999; Olivotto *et al.,* 2008). The characteristic moving pattern 'pause and move' of

copepod nauplii makes them more vulnerable prey for initial feeding. Copepods can improve health, reduce abnormalities in growth, increase stress tolerance, enhance development and improve the pigmentation and growth of fish larvae (Bell *et al.,* 2003; Copeman *et al.,* 2002; Olivotto *et al.,* 2006, 2008; Vagelli, 2004).

Copepods are the best option as initial feed for larvae of a variety of marine food fishes and ornamental fishes. However, due to the difficulties in rearing, these are being used as feed for a critical period when larvae are unable to feed or survive with *Artemia* and rotifers. This may be in terms of size, nutritional factors, digestibility, vision or movement. In general, feeding protocols are developed in such a way that the use of copepods/ naupliar stages is limited to the critical stages above and a combination of *Artemia* and rotifers are used till the weaning of fish larvae to artificial feeds. The practice of using wild copepods from natural ponds is not advisable as it increases the risk of parasitic infections (Ajiboye, *et al.,* 2010).

The main demerit of copepods as a commercial larval feed is that, these cannot be cultured in high densities. Rotifers are routinely cultured in numbers exceeding 2000 nos per ml and *Artemia* can also be hatched and provided at a higher density. But copepod cultures rarely exceed densities of 2-5 nos per ml for adults and 10 nos per ml for nauplii. Some harpacticoid copepods have been reported to reach densities of more than 100 nos per ml but due to their epibenthic nature, they may not be available to pelagic fish larvae (Stottrup, 2006). So an efficient feeding protocol needs to be developed for each fish species by using combinations of cultured copepods, rotifer, *Artemia* and artificial feed for fish larval production.

Use of copepods in larval rearing

Copepods are widely used in larval rearing of many ornamental fishes in CMFRI. Trials on seed production of damselfishes were successful only after using copepods as initial feed. Initial trials were all done using a combination of a calanoid copepod *Pseudodiaptomus serricaudatus* and a harpacticoid copepod *Euterpina acutifrons*. Mostly co-culture method was used initially. Damsel fishes like *Dascyllus trimaculatus* (Three spot damsel), *Dascyllus aruanus* (Humbug damsel) *Pomacentrus caeruleus* (Caerulean damsel) *Chromis viridis* (Blue green damsel) *Neopomacentrus nemurus* (Yellowtail damsel) and *Chrysiptera cyanea* (Sapphire devil damselfish), were successfully bred and reared here (Gopakumar and Santhosi, 2009; Gopakumar *et al.*, 2009 a, b.). Recently, *Neopomacentrus cyanomos* (Regal demoiselle) (Rohini Krishna *et al.,* 2016) and *Dascyllus carneus* (Cloudy damsel) were also successfully reared here using nauplii of *Parvocalanus crassirostris, Dioithona* sp. and

Acartia southwelli. Recently larval rearing was successful in few species of anthias and few more species of damsel fishes using copepod as initial feed.

It is also confirmed that in turbot *Scophtalmus maximus* and Atlantic herring *Clupea harengus* larvae, copepods not only donate their digestive enzymes (protease and trypsin) but also activate zymogens in the larval gut (Pedersen and Hjelmeland, 1988; Munilla-Moran *et al.,* 1990; Sun *et al.,* 2013; Rasdi and Qin., 2016). Use of copepods as live feed helps to decrease malpigmentation and deformities in fishes (Stottrup, 2000). Hamre *et al.,* (2005) reported significant improvement of both eye migration and pigmentation by using copepods in Atlantic halibut, *Hippoglossus hippoglossus*.

Copepods are being used in larval rearing of many species of food fishes including Turbot *Scophthalmus maximus* (Kuhlmann *et al.,*1981*), Psetta maxima* (Stottrup *et al.*,1997) Herring *Clupea harengus* (Hjelmeland *et al.,*1988), Red seabream *Pagrus major* (Ohno, 1992), Mahi mahi *Coryphaena hippurus* (Kraul, 1993; Schipp, 2006) Grouper *Epinephelus coioides* (Toledo *et al.,*1999; 2005) Flatfish *Scophthalmus maximus* (Bell *et al.,* 2003) Barramundi *Lates calcarifer,* Almaco jack *Seriola rivoliana,* Giant Trevally *Caranx ignobilis* (Schipp, 2006), Japanese Flounder *Paralichthys olivaceus* (Liu and Xu, 2009), Florida Pompano *Trachinotus carolinus* (Cassiano *et al*., 2011) and Atlantic Cod *Gadus morhua* (Karlsen *et al.,* 2015).

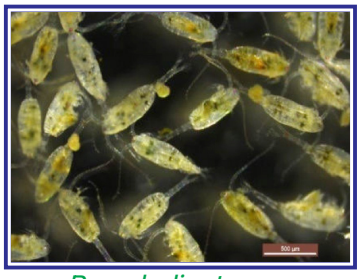
Copepods developed by CMFRI gave promising results in all trials conducted in larviculture**.** Orange spotted grouper, *Epinephelus coioides*, Indian Pompano, *Trachinotus mookalee* and John's snapper (*Lutjanus johnii*) were successfully bred and reared at Visakhapatnam Regional Centre of CMFRI (Ranjan *et al.,* 2018). A combination of nauplii *Parvocalanus crassirostris, Dioithona* sp. and *Acartia southwelli* are being used as the first larval feed. Using *P. crassirostris,* Karwar Centre of CMFRI could develop a larval rearing protocol for vermiculated spine foot *Siganus vermiculatus* and goldsilk seabream *Acanthopagrus berda.* Copepods are successfully used in larval rearing of silver pompano (*Trachinotus blochii)*, spangled emperor (*Lethrinus nebulosus)*, pink ear emperor fish (*Lethrinus lentjan*) and banded grunter (*Pomadasys furcatus*) at Vizhinjam Centre of CMFRI.

The stock culture and mass culture technologies developed by CMFRI are simple, effective and economical when compared to the technologies existing in the rest of the world. This is one of the main reasons why we could go for a successful culture of many suitable species, whereas the other pioneers from the rest of the world are continuing with one or two species. The techniques are simple and do not require any sophisticated facilities but need specialised training to understand the life stages, production parameters, harvesting specific stages, health status and long-term management of stock and mass culture. Training and skill development are essential for the hatchery production

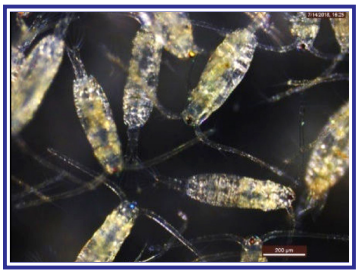
of copepods. Based on practices developed and adopted by CMFRI, nine species of copepods can be successfully produced in the hatchery for utilization in larval rearing. Hatcheries which have already developed algal production units can go for copepod culture without much investment. In our methods, space and feed requirements are comparatively less. All the 14 species of copepods for which culture technologies have been developed by CMFRI are being successfully used at many centres of CMFRI and a few hatcheries in government and private sectors where larval rearing is being practised.

Indigenous species cultured and popularized by Vizhinjam Regional Centre of CMFRI

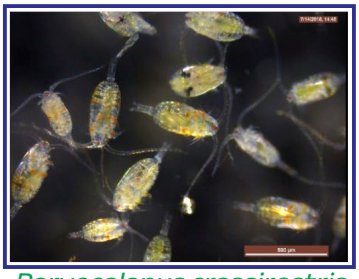
Temora turbinata



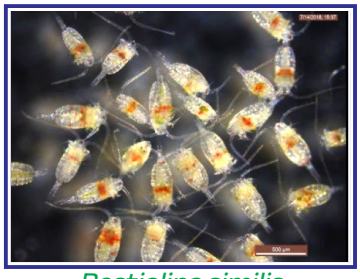
Pseudodiaptomus serricaudatus



Acartia southwelli

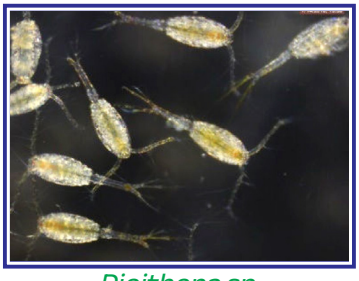


Parvocalanus crassirostris

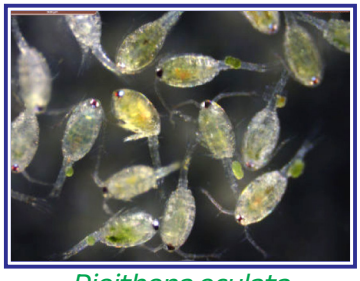


Bestiolina similis

Apocyclops cmfri



Dioithona sp.



Dioithona oculata

Euterpina acutifrons

Economic analyses of copepod culture carried out Santhosh *et al.,* (2018) indicated that the methods developed by CMFRI has been found profitable than the present cost of *Artemia*. The analysis also proved that the production system can withstand risk to the

tune of 30 per cent reduction in production (Santhosh *et al.* 2018). One of the main problems in shrimp and fish farming has been the poor seed quality. If copepods are used as live feed, the production and survival rates can be increased upto 20-70%. If the larvae were fed with copepods, seeds were found to be more healthy, resistant and brightly coloured without any abnormalities. All these can contribute to 20-30% increase in hatchery production of prawn and fish seeds. This can reduce the cost of production and at the same time the impact of this technology shall help to increase the farmed fish and prawn production in our country.

Copepods are small planktonic crustaceans which are the most abundant metazoans on earth. Copepods are present in almost all aquatic environments and from an integral component in the ecosystems. Copepods are generally filter feeders and many are parasitic also. Copepods form ideal food for fishes and invertebrates. Copepods are more abundant and diverse in marine and brackish waters. Members of the Subclass Calanoida, Cyclopoida and Hapacticoida form important components in marine plankton. Among marine pelagic copepods, calanoid copepods dominate over more than 79.2%. The Indian Ocean has the maximum species composition and the Arctic Ocean has the minimum. In India, copepods are mostly well-studied and documented in marine waters. Mostly the species reported from the Indian Ocean were also reported from the Arabian Sea coast of Kerala. So far there are 992 species of marine planktonic copepods reported from the Indian Ocean. The maximum number of endemic species (172) is also recorded from India. All these are well-studied and documented. Certain fishes and fish larvae are evolutionarily adapted for feeding copepods. Copepods are nutritionally superior to all live feeds. copepods are rich in proteins, lipids, essential amino acids (EAA) and essential fatty acids (EFA). Copepods contain higher DHA than almost all other live feeds. Due to their smaller naupliar stages and nutritional superiority, copepod cultures become an integral component in a marine finfish hatchery. Vizhinjam Regional Centre of CMFRI has developed culture technologies of 9 important species of copepods which are being utilized for larval rearing of many ornamental and food fishes. The economic analyses of a medium-scale copepod production system developed by CMFRI indicated that the system is profitable for a medium-level hatchery unit where we can reduce the use of *Artemia* significantly. With the use of copepods, CMFRI has developed breeding and larval rearing protocols for many species of damselfishes, clowns fishes and anthias. Among food fishes using copepods, CMFRI could standardize the larval rearing of food fishes like Orange spotted grouper, Indian pompano, silver pompano, John's snapper, pink ear emperor fish, vermiculates spine foot, John's snapper, banded grunter and goldsilk seabream.

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MICROBIAL INTERVENTIONS IN LIVE FEED CULTURE

Jayasree Loka
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Introduction

Probiotics serve a crucial role in aquaculture, whether it is to establish the ideal microbial communities in live feed cultures or to directly supplement the diet of larvae of aquatic organisms. Over time, the inclusion of probiotics in the water used to raise live feed, can greatly benefit the larvae, by improving both their quantity and quality. Various commercial probiotic products have emerged within the aquaculture industry, exhibiting diverse levels of performance and efficacy. Probiotics have gained significant recognition as a valuable tool in managing microbial populations in mariculture. They play a vital role in mariculture by promoting the establishment of favorable microbial communities in live feed cultures and by directly enhancing the diet of fish larvae. The cultivation of the majority of marine fish larvae relies heavily on the utilization of live feeds. The bacteria present in and around these live feeds play a critical role in shaping the microbiota of the larvae. Given the growing environmental and health concerns, there has been a gradual decrease in the use of chemotherapeutics in larviculture, prompting a shift towards exploring alternatives that involve the diagnosis of bacteria as integral components of rearing ecosystems. The long-term inclusion of probiotics in the water, used for cultivating live feed, can have positive impact of enhancing the quality and quantity of the larvae. Through the process of bioencapsulating various live feeds like copepods, rotifers, and Artemia, the nutritional quality and overall health of fish will be enhanced. Enrichment of live feed with the application of probiotics as microbial adjuncts, showed promising effects on the growth, health and culture environment of aquatic organisms (FAO & WHO, 2002), and this resulted in a growing interest to learn about the advantages of probiotics in live feed cultures. There has been a considerable amount of focus on altering the microbial community's composition, to enhance culture's stability and reduce the growth of harmful bacteria. The incorporation of probiotics into live feed cultures boosts the populations, movements, and feeding efficiency of phytoplankton and zooplankton, intended as food for fish and shrimp larvae. Aquaculture encounters a notable constraint related to organic enrichment and nitrogenous wastes, including ammonium and ammonia (NH_3). It is essential to have a sound understanding of the role of bacterial communities in live feed, investigate the potential benefits of probiotics and prebiotics in improving live feed cultures, and explore their feasibility as mediators to fish larvae cultures. This chapter delivers into the microbial aspects of live feed, explores the potential use of probiotics and prebiotics to enhance live

feed cultures, and examines the possibility of utilizing live feed as a tool to deliver prebiotics and probiotics to fish larval cultures.

Potential probiotic bacteria used in live feed culture

Application of potential probiotic bacteria with enrichment of live feed with probiotics will enhance the survival and density of fish larvae. Several probiotics are reported as beneficial for the enhancement of population density of rotifers and copepods. Supplementation of probiotics in Artemia increased HUFA and total lipids levels of live feed. The transfer of probiotic species at different levels of microflora of live feed, fish larvae and rearing environment still remains unclear. Potential probiotic bacterial species can be used as co-culture with microalgae and as enrichment feed for rotifer, copepod, and Artemia. Most commonly used probiotic bacterial species used in aquaculture are *Lactococcus* sp., *Lactobacillus* sp. *Pediococcus* sp., *Carnobacterium* sp., *Bacillus* sp., *Enterococcus* sp., *Pseudomonas* sp., *Pseudoalteromonas* sp., *Alteromonas* sp., *Microbacterium* sp., *Ruegeria* sp., *Streptococcus* sp., *Thermophilus* sp., and *Shewanella* sp.

Probiotic selection

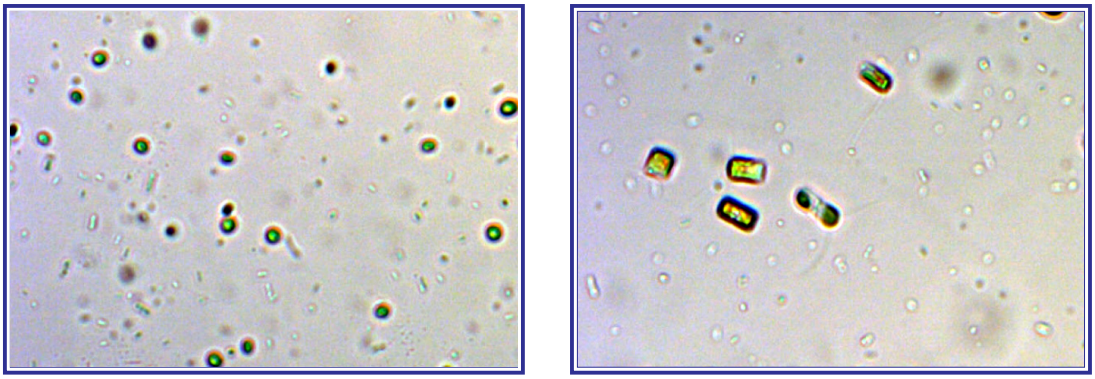
Selection of potential probiotics can be done by isolation of native species from live feed or obtaining the probiotics from commercial feed supplement suppliers. Prior to incorporating these probiotics into live feed for enrichment purposes, it is essential to evaluate their antagonistic effects against pathogens and assess their effectiveness in terms of other probiotic characteristics. Selection of the most effective and promising probiotics offers an efficient approach to enhance the growth of marine microalgae. These probiotics serve as a valuable addition to the culture medium, providing essential nutrients, and act as supplementary feed for rotifer, copepod, and artemia populations.

Probiotic enrichment methods in different live feed cultures

Co-culturing of probiotics with marine microalgae

Co-cultivation can take advantage of mutualistic interactions between bacteria and microalgae. The inhibitory effects of beneficial microbes on pathogenic bacterial species, especially *Vibrio*s, can be strengthened by the metabolites released by microalgae, like *Chlorella vulgaris* or *Nannochloropsis oculata*. The feasibility of coculturing microalgae with beneficial bacteria is well demonstrated.

The efficacy of this approach in delivering probiotics to live prey, such as Artemia, and controlling pathogens is proven by several researchers. Interactions between microalgae and bacteria are intricate, and specific bacterial strains may have distinct interactions



Co-culture of marine microalgae and probiotic consortium

with different microalgae species. The selection of the most efficient combination can be assessed by the potential specificity of different combinations of various bacteria with different microalgae. Additionally, this approach allows for the examination of potential changes in the bacterial communities that are associated with the microalgae. Microalgae are cultivated in marine fish hatcheries through batch or continuous cultures, under non-axenic conditions. Ensuring the preservation and transfer of probiotic bacteria during the expansion process in non-sterile environments can be achieved by initially introducing vibrio-antagonistic bacteria to colonize the microalgae at an early stage of colonization. This approach guarantees the continued presence and effective delivery of the desired probiotic bacteria on a larger scale.

Procedure:

1. Prepare pure strains of Marine Microalgal cultures in F/2 medium and scale up to batch and mass-scale production.
2. Pure probiotic bacterial strains (Laboratory developed or commercial strains) should be reactivated in marine broth and cultured for 24 - 48 hr (till the required concentration reaches).
3. Add an adequate volume of culture to sterile-filtered supernatants of microalgae cultures at an initial concentration of 10^5 colony-forming units (CFU) mL^{-1}.
4. Co-culture of marine microalgae and probiotic bacteria is to be done in 10 L culture flasks containing sterile culture medium by inoculating 100 ml of marine microalgae containing probiotic consortia which are developed in stock culture. Cultures should be made under constant temperature and light illumination for one week.
5. Then transfer the 5 litres of co-culture (with a concentration of $1x10^5$ cells/ml of microalgae and $1x10^7$ cfu/g consortium) to 1tonne microalgal tanks.

6. Marine microalgae which are co-cultured with different combinations of probiotic bacterial consortia for 3-4 days can be used as feed for mass production of rotifer, copepod, and artemia.

Probiotic enrichment in Rotifer culture

1. The rotifers should be washed with fresh seawater and stocked in a plastic bucket containing 50% volume of sterile seawater.
2. Probiotics (5 mg L^{-1}) are mixed with marine microalgae (single or in a combination of two species with an approx. con of $1x10^8$cfu/ml) and are blended with 100 mL of deionized water.
3. This mixture is then poured into the bucket containing rotifers. Based on the stocking densities of rotifer, the feeding rate of this mixture can be adjusted.
4. Rotifers can be fed to fish larvae after 6 hours of enrichment.

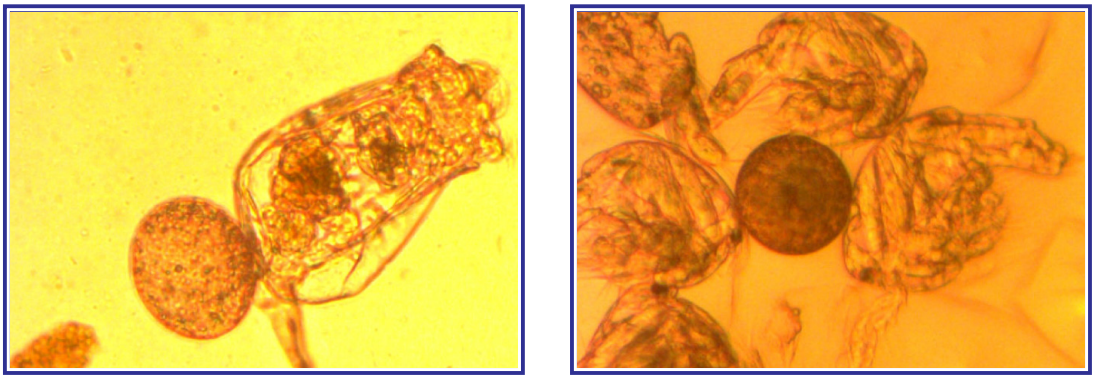
Enrichment of probiotics in Copepod and Artemia culture

1. Newly hatched copepod and Artemia are soaked in the probiotic suspension (10 mg L^{-1}) with strong aeration.
2. The nauplii, approximately 100 nauplii per mL, should be shifted to 5-liter buckets filled with 4 liters of seawater and 1 liter of the probiotic consortium containing 10^5 cfu/mL. and culture for 24 hrs at a temperature of 27 °C with gentle aeration.
3. This enrichment process should be carried out for 1 h in order to avoid nutrient losses in copepod and Artemia.

The enrichment protocol is as follows

1. Just hatched artemia is not completely developed and therefore cannot consume food from water. However, they are capable of absorbing water that contains probiotics.
2. Consequently, the *Bacillus* has the ability to inhabit both the gastrointestinal tract and the external surface of Copepod/Artemia.
3. In Long-term probiotic enrichment, the retention of probiotics in Copepod/ Artemia *nauplii* is compared over a long-term period of 24 hours which coincides with the commercial EFA enrichment period.
4. After the 22-hour hatch phase, copepod and Artemia nauplii are enriched with S. presso (INVE Aquaculture®, Belgium) and probiotics (1×10^{10} cfu/ ml).
5. Analyze Artemia and copepod samples at 10 min, 15 min and 24 hours after probiotic enrichment, following the methods described above.

6. Disinfection of samples with sodium hypochlorite (5 ppm) for 10 and 15 minutes intervals, gives best results during enrichment process.
7. In short-term probiotic enrichment, the Copepod and Artemia are rinsed and concentrated.
8. The stock is inoculated with probiotics at a concentration of 1mg/L.
9. The density of the Copepod and Artemia can be determined by diluting the culture 1:20 in aerated seawater and counting eight 100 µL samples of Artemia under a microscope.
10. Artemia and copepod nauplii can be disinfected with sodium hypochlorite (5 ppm) for 15 minutes prior to enrichment. Sodium thiosulphate (10 ppm) is used to neutralise the sodium hypochlorite.
11. Then the cultures are inoculated with the bacterial concentrate to attain 10^6 CFU/mL and remained at room temperature for 30 minutes after inoculation.
12. The enriched culture is stored in a refrigerator (4 p C) for 6 hours.
13. Samples (water, copepods, artemia) should be taken at 30 minutes and 6 hours for observation of enrichment and for determination of density, survival and also for elimination of pathogens in water.



Enrichment of rotifer, copepod nauplii with probiotic consortium

Application of enriched probiotic consortium in larval rearing of finfish

1. Probiotic consortium (1.6×10^{10} CFU g^{-1}), which is already tested for its potential antagonistic and other probiotic characteristics towards the cultured larvae, is supplemented with live feed diet to the fish larvae as a multi-strain probiotic.
2. The strains are to be kept in Modified Marine Broth, incubate at 35^0C for 24 h.

3. Then centrifuge the probiotic strains at 1800g (rpm) for 15 min and resuspend in a sterile saline solution.

4. Then, sprinkle the respective suspensions on the artificial feed.

The quantity of feed should be replenished every seven days on a weekly basis and stored at 4^0C.

Advantages of probiotic supplementation in live feed

Probiotics consist of live microbial supplements designed to optimize the host's microbial equilibrium for its potential benefit. In most of the high-intensity live feed culture systems, accumulation of organic carbon, nitrates, and phosphorous results in the release of nutrients at high levels, and this results in a decrease in the yield which can eventually cause the culture to break down. The removal of particulate matter and excess nutrients continues throughout the harvesting period in many culture systems. Hence, to improve the live feed culture condition, a probiotically enriched system will be the best solution to prevent the constant collapse of the live feed cultures. Therefore, the constant collapse of live feed cultures may be remedied by a bacterially mediated mechanism for enhancing the condition of the culture and maximizing the utilization of the nutrients. Adding carbon in this system can restore the correct C: N ratio, enabling bacteria to transform solid waste into biomass. Bacterial communities have the potential to shape the environmental conditions and population expansion of live feed cultures. When bacteria and microalgae are combined, the resulting population growth of rotifers is significantly higher compared to using bacteria alone as the primary diet. In the case of larger live feed sources, single bacteria cells may prove to be an inadequate diet, and the process of flocculation can be employed to boost the growth of these organisms. Enhanced utilization of microalgae, due to its increased digestibility of probiotic bacteria increases amictic reproduction with the availability of increased food. Enrichment of microalgae with probiotic bacteria enhances number of eggs, population density, locomotion and swimming behaviour of rotifers. Enrichment of probionts helps in dominating their population in culture systems and decreases or inhibits growth of other microbes. Probiotics play a significant role in balancing the microbial community of rotifer/copeod culture systems and also on the population density. Probiotic application enhances rotifer/copeod density significantly and the concentration of probiotics and strains vary with initial density of zooplankton and also mode of culture.

The density of copepod nauplii increase significantly (2.4 times) in tanks supplemented with probiotics. Water quality of live feed culture systems also improves with probiotic inoculation in culture tanks. In the mass culture tanks of copepod and rotifers,

supplemented with probiotic enriched diets, a decrease in *Vibrio* count and increase in the live deed density with an increase of probiotic loads is observed. The total bacterial loads and ammonia levels of water in copepod tanks decrease in probiotic-treated tanks and eliminate *Vibrio*s from tanks fed with marine microalgae supplemented with probiotics.

In conclusion, the usage of probiotics as co-culture with marine microalgae and as a supplementary diet along with the combination of live feeds (microalgae and rotifers/ microalgae and copepods/artemia) is suggested to improve the quality of water and density of copepod/rotifer in mass scale production which in turn helps to improve the survival rates of finfish larvae in early stages of development.

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STRATEGIES FOR BETTER MANAGEMENT OF LARVAL PRODUCTION OF MARINE FINFISH

Ritesh Ranjan

Chinni Babu, B, Ravi Avadhanula, Padmaja Rani

Introduction

A complete finfish larval production generally involves three phases of techniques i.e. i. broodstock management, ii. larval rearing, and iii. food preparation. Broodstock management involves techniques to collect and culture high quality broodstock, to induce maturation and spawning, to manipulate sex and to collect and incubate eggs. Larval rearing involves various techniques related to larviculture system, nutrient requirements, cannibalism prevention, and tank management. Nutritional content and size of food for larvae and fry determine the success of commercial-scale larviculture. Feed preparation for larviculture involves the use of highly unsaturated fatty acids HUFAs-supplemented feeds, the application of suitable live food for different larval stages, and the adoption of live food enrichment protocols.

Generally, tropical marine finfish larvae are altricial type, where the larvae possess very less yolk reserves at hatching. These yolk reserves exhaust early, when the larvae are still in undeveloped stage. 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 development of digestive system is also very primitive in these types of larvae. These all factors poses challenges in their larviculture, hence, the larval production protocol needs to be standardized for each species according to their requirements.

Broodstock management

Matured adult fish of appropriate size are generally collected either from the wild or from the pond or cage (farmed), and are further raised for broodstock development; for example Indian pompano, *Trachinotus mookalee* of more than 3 kg and Orange spotted grouper, *Epinephelus coioides*) of more than 2 kg are collected. Few criteria are applied during selection of adult fishes for broodstock development. They are as follows

- Normal body shape and colour
- Absence of skeletal deformities
- Overall health status, i.e. absence of large wounds, haemorrhages, infections and parasites

- Normal behaviour, such as a good response to feed, controlled buoyancy to maintain position in the water column
- Best growth and feed conversion rate within its age group, when we are selecting from cultured one.

Generally adult fish should be collected from nearby area as far as possible. The collected fish should be transported in covered tanks containing aerated or oxygenated water to reduce stress. Mild sedation, using approved sedatives for fish, such as 2 phenoxyethanol @ 50 ppm can be used to reduce stress and make handling the fish easier and safer. If the fish is collected from the culture system, they should not be fed for the previous 24 h to avoid deterioration of water quality during transportation. Generally, fish regurgitate the feed if they are handled after feeding, thus it will spoil the water quality as well as induce stress to the collected fish.

Once the fish are transported to the hatchery, the fish need to be shifted in quarantine area. It is advisable to quarantine them to reduce the parasitic or bacterial infections. Generally the quarantine period varies from 3 to 4 weeks and can be carried out in small tanks of 1 m^3 to facilitate easy handling. The quarantine protocol varies as per the species concern. For example, Indian pompano should be given a bath treatment of formalin in freshwater at the rate of 30 mg L^{-1} for 15 min, once in four days for a period of 3 to 4 weeks. Orange spotted grouper should be given a bath treatment of formalin in seawater at the rate of 200 mg L^{-1} for 30 min, followed by 5 minutes dip in freshwater, once in three days for a period of 1 to 2 weeks.

The sex of individual fish can be confirmed only by physical examination *i.e* live ovarian biopsy (LOB). Fishes need to be anaesthetized by using 2 phenoxy ethanol at the rate of 200 ppm for 2-3 minutes. The anaesthetized fish is then cannulated using the fish cannula or baby feeding tube CH 6 having an inner diameter of 1 mm and an outer diameter of 2 mm. The cannula is inserted into the urinogenital orifice of males and the oviduct of females. Fish to be cannulated are anaesthetised and a wet cloth or towel is placed over the eyes to assist in calming the fish. The cannula is guided into the fish for a distance of 6–7 cm and suction is applied to the other end of the cannula as it is withdrawn. After withdrawal, the sample within the cannula is expelled onto a microscope slide for immediate examination or into a vial containing 1% neutral buffered formalin for later measurement of egg diameter. To confirm matured male, the abdomen of an anaesthetised fish is gently massaged in a head-to-tail direction. A sexually ripe male spawner will extrude copious milt from its urinogenital pore. If no milt is expressed, the fish is immature, a male not in spawning condition. Once the sexes of the fishes are confirmed, they are tagged with

passive integrated transponder (PIT) and the tag number need to be maintained for future use.

Generally broodstock tanks are used for culture and maturation of broodstock as well as for spawning. Due to the size of the broodstock and the natural spawning behavior of the fish, larger tank of 50 -100 m^3 are preferred for broodstock tanks. Generally tanks should be round, or square or rectangular with rounded corners, however round tank is preferable. Medium-range blue, green or grey colour is preferable for the broodstock tank. Generally the broodstock tanks are used as flow-through system, however re-circulating aquaculture system are better for broodstock development and spawning. It is advisable that broodstock area should be roofed in order to reduce the growth of algae on the tank wall and bottom, which makes egg collection difficult and increases the risk of failure of re-circulating system. Moreover, frequent washing of uncleaned tanks may stress the broodstock and cause spawning failure or lower the quality of eggs. Broodstock tanks should have re-circulating facilities with 300% water re-circulation. Sea water used for broodstock development should be filtered and clear with stable salinity of 30-35 ppt and water temperature of 27-32^{o}C.

Broodstocks are fed to satiation at least once daily in the morning with good quality broodstock diet. For example, fresh or frozen squid for orange spotted grouper and squid along with clam meat for Indian pompano. Various vitamins namely, vitamin A, vitamin B-complex, vitamin C , vitamin E and vitamin–mineral mix are supplemented twice a week along with the feed to avoid any possible nutritional deficiencies.

Depending upon the species requirement, matured fish are either induced for spawning or they perform volitional spawning. For example, orange spotted grouper brooders don't need induction for spawning however Indian pompano are induced with inducing hormone hCG @350 IU per kg body weight. The eggs are collected at the eyed stage.

The fertilized eggs of fish needs to be collected in advanced stages of embryogenesis, otherwise the handling stress will lead to death of the eggs. The collected eggs should be disinfected with 20 ppm active iodine for 10 minutes in strong aeration. The treated eggs are then washed with deionized sea water and stocked in glass aquarium with water salinity of 30-32 ppt. Fertilized eggs will float on the water surface whereas unfertilized or dead eggs settle in the bottom. Only floating eggs are used for incubation and hatching because these are more likely to be good quality than sinking eggs, which are unfertilized or dead.

Larval rearing management

Generally, eggs are stocked at the eyed stage because they are more robust than the newly hatched larvae. Newly hatched larvae are very sensitive to physical shock or changes in water quality and moving them to the larval-rearing tanks may result in high levels of mortality. Because the hatching rate is not known before the eggs are stocked in the larval-rearing tanks, the number of eggs to be stocked needs to be estimated using historical hatching rates for that hatchery. Accurate estimates of the number of larvae stocked can be back-calculated using data from the actual batch stocked.

Generally round and rectangular tanks are used for larval rearing. The aeration should be mild during the early stages (at least upto 6 days) of larval rearing to avoid physical damage of the larvae. However, it is increased gradually with progression of the larval-rearing cycle, as the larvae become more robust. The tank should be cleaned either with liquid bleach or acid wash and dried atleast for two days before stocking.

Sea water should be filtered with sand bed filter and treated with ozone at the rate of 0.1 ppm for 5 minutes. The ozoned water should be passed through carbon filter before filling in the larval rearing tank. Larval-rearing tanks should be placed under roof to avoid direct sunlight, rain and it is preferable to have larval rearing tanks inside concrete building. Light is necessary for larval rearing. It should be either diffused light or artificially provided with the help of tube lights. These tanks should be maintained at a separate quarantined area within the hatchery, with restricted entry only to authorized few persons. Their hands and feet should be washed on every entry and exit, and disinfection of all equipments should be performed before and after use.

The recommended initial stocking density for larval rearing should be used, for example 10 larvae/L in case of Indian pompano. Live feeds used for larval rearing comprises microalgae (*Nannochloropsis* sp. and *Isochrysis* sp.), copepod nauplii and adult, small rotifers (*Brachionus rotundiformis*), large rotifers (*Brachionous plicatilis*) and brine shrimp (*Artemia*) nauplii.

The yolk sac (endogenous source) continues as the sole source of nutrients for the developing embryos immediately after hatching. The endogenous source provides nutrients till the mouth gape formed after which an external feed of appropriate size needs to be provided. The size of the feed should be 1/3rd of the mouth gape. Generally, initial feed used in the larval rearing is live zooplankton of appropriate size. The selecting of the initial live feed is the paramount requirement for the larval rearing of marine finfish otherwise larvae mortality is sure. The each species requirement is different and generally varies due to the mouth gape; hence the feeding protocol for each species needs to be

standardized. For example, Indian pompano and orange spotted grouper larval rearing protocol are given below.

Indian pompano

The yolk sac (endogenous source) continues as the sole source of nutrients for the developing embryos immediately after hatching. The endogenous source provides nutrients for 2 days in Indian pompano larvae. Then, the exogenous feeding starts when the mouth opens after 2nd day. Their initial mouth gape is around 230µm and hence appropriate size of feed *i.e.* copepod nauplii and rotifers needs to be provided. *Nannochloropsis* sp. and *Isochrysis* sp. in ratio of 1:3 is introduced into the larval-rearing tanks on 2nd DPH at algal cell density of 1× 10^{5} cells/ml. Rotifers and copepod nauplii filtered with 100 µm mesh are introduced into the larval rearing tanks on 2nd DPH, after the larval mouth opening has been formed. The rotifer and copepod nauplii density in the larval-rearing tanks is maintained at 10-15 and 2-3 individuals/ml during 2nd -5th DPH. After 5th DPH, rotifers are introduced at densities of 20 individuals/ml, which is gradually increased to 30 individuals/ml from 8th to 10th DPH. Rotifer density gradually decreases with increase in the rate of rotifer consumption by the larvae and eventually by 13th DPH, the rotifers disappear. Freshly hatched out *Artemia* nauplii are fed at density of 0.5 individual/ml from 8th DPH and their size increasing with advancement in rearing period. Weaning of pompano larvae with artificial diets started from 11th DPH. Artificial diet with a particle size of 200-300 µm is used initially. The formulated feed is sprinkled onto the surface of the water in small amounts frequently throughout the day. Formulated feed is added in small amounts so that the feed is consumed within 5 or 10 minutes, as excess feed should not be allowed to accumulate on the bottom of the tank where it get decomposed and degrade water quality. The size of particulate feed is increased to 400–800 ìm from 22nd DPH. High-quality micro diets, specifically formulated for marine finfish, should be used and these should be stored in a refrigerator or freezer to maintain their quality.

Larval-rearing tanks are bottom siphoned on the day of hatching to remove unhatched eggs as well as hatched out eggs shells and are further needed to maintain static until 3rd DPH, and then from 4th DPH, 5-10% of water exchange per day is required to maintain the rearing water quality. Bottom siphoning of the tank should be started on 4th DPH and are carried out once every 3 days. From 12th DPH, faeces, dead larvae and uneaten food accumulated on the tank bottom should be siphoned out at least once daily for maintaining water quality. Water exchange should be increased to 20%/day, when both rotifers and *Artemia* are being fed together (8th DPH). Water exchange gradually increased to 50%/day from 11th DPH, and is 100%/day from 16th DPH.

Orange spotted grouper

The yolk sac (endogenous source) continues as the sole source of nutrients for the developing embryos immediately after hatching. The endogenous source provide nutrient for 2-3 days in grouper larvae. Then, the exogenous feeding starts when the mouth opens after 2nd day. Their initial mouth gape is very less, so they have to be provided with appropriate size of feed i.e. copepod nauplii and screened rotifers. *Nannochloropsis* sp. is introduced into the larval-rearing tanks on 2nd DPH at algal cell density of 1×10^5 cells/ml. Rotifers and copepod nauplii filtered with 100 ìm mesh are introduced into the larval rearing tanks on 2nd DPH, after the larval mouth opening has been formed. The rotifer and copepod nauplii density in the larval rearing tanks is maintained at 5-7 and 2-3 individuals/ml respectively during 2nd -5th DPH. After 5th DPH, small rotifers (filtered with 150 ìm mesh) are introduced at densities of 10-15 individuals/ml, which is gradually increased to 20 individuals/ml from 11th to 18th DPH. Rotifer density gradually decreases with increase in the rate of rotifer consumption by the larvae, and eventually by 30th DPH, the rotifers disappear. Freshly hatched out Artemia nauplii are fed at density of 0.5 individual/ml from 17th DPH, and their size increasing with advancing in rearing period. Adult copepods are fed during 16th-20th DPH in larval rearing. Weaning of grouper larvae with artificial diets starts from 20th DPH. Artificial diet with a particle size of 200-300 ìm is used initially.

The formulated feed is sprinkled onto the surface of the water in small amounts frequently throughout the day. Formulated feed is added in small amounts so that the feed is consumed within 5 or 10 minutes, excess feed should not be allowed to accumulate on the bottom of the tank where it get decomposed and degrade water quality. The size of particulate feed is increased to 400–800 µm from 30th - 45th DPH. High quality micro diets, specifically formulated for marine finfish, should be used and these should be stored in a refrigerator or freezer to maintain their quality. In addition, minced fresh fish meat is fed from 30th DPH onwards. Bottom siphoning of the larval rearing tank Larval-rearing tanks are maintained static until 7th DPH, and then from 10th DPH, 5-10% of water exchange per day is required to maintain the rearing water quality. Bottom siphoning of the tank should be started on 7th DPH. From 12th DPH, faeces, dead larvae and uneaten food accumulate on the tank bottom, and should be siphoned out at least once daily for maintaining water quality. Water exchange should be increased to 20%/day, when both rotifers and Artemia are being fed together (15th-20th DPH). Water exchange gradually increases to 50%/day from 25th DPH, and is 100%/day from 35th DPH.

It is of utmost importance to measure water quality regularly in the larval-rearing tanks (Table 1). If water quality degrades, it should be necessary to exchange the water at

rates higher than the rates recommended above. However, replaced water should be of similar temperature and salinity to the water in the rearing tanks to avoid stress to the larvae.

The optimum physico-chemical parameters for larval rearing of marine finfish during early larval rearing

Sl. No.	Parameters	Value
1.	Temperature	28-30°C
2.	Salinity	16-24 ppt
3.	Light	500 to 700 lx
4.	Photoperiod	Natural
5.	aeration	0.62-1.25ml/min/L
6.	Dissolved oxygen	Near saturation
7.	Ammonia	<0.1 ppm
8.	Nitrite	<1.0 ppm

Nutritional enhancement of live foods

Larvae of marine finfish require high levels of the unsaturated fatty acids eicosapentaenoic acid (EPA, or 20:5n-3), arachidonic acid (ARA, or 20:4n-6) and docosahexaenoic acid (DHA, or 22:6n-3) for proper development, and provision of these fatty acids in the diet, via incorporation in the live foods used for larval rearing, improves survival, growth and pigmentation of the larvae and fingerlings. However the rotifers are poor in terms of high level of unsaturated fatty acid such as DHA and EPA. Even the *Artemia* nauplii are rich in EPA however they lack DHA. Due to this, these live feed need to be enriched either with natural phytoplankton or artificial enrichment product to increase the levels of these essential fatty acids.

Various commercial preparations have been developed for nutritional enhancement of rotifers and brine shrimp (Alava et al. 2004) such as Ori 1 from Skretting, Easy DHA from Inve Aquaculture and Algamac from Bio-marine. These enrichment products are packaged as liquid or spray-dried products. Generally, preparation involves measuring the required quantity, blending to hydrate (for spray-dried products) or emulsify (for liquid products) the material, then add to the live-food culture tanks. The manufacturers provide technical information on the application of their products. Of particular importance is the need to

maintain high dissolved oxygen levels in the culture tanks during the application period (usually <12 hours). This may require the use of pure oxygen, or oxygen-supplemented air, particularly if the live-food organisms are at high density.

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HUSBANDRY PRACTICES IN GROW-OUT SYSTEMS OF MARINE FISH CULTURE

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Introduction

A technological intervention has been the major impetus for the rapid development of farming of marine fishes across the world in different culture systems like marine cages, coastal ponds, and other systems. In all these production systems, fish growth is influenced by several factors, in which husbandry practiced helps for better fish growth by maintaining congenial growing conditions. This chapter mostly deals with marine cage farming of Indian pompano, which has been gaining importance in the farming sector among different stakeholders. Cage culture of Indian pompano is becoming popular technology, in which it is necessary to optimise the many factors periodically to avoid the adverse impact of environmental and ecological factors for long maintenance of cages and also to maintain the healthy animals in the cage. In this context, selecting optimum culture method following prescribed protocol and continuous monitoring both culture system and growing animal, plays vital role in any type of mariculture activity. Therefore, a well-conceived and designed monitoring programme is needed to promote good growth of fishes and to obtain optimal production in a sustainable manner from cages. Cage monitoring is an integral part of the cage culture and it should be continued starting from the installation of the cage to till harvesting the fishes. The following are the major aspects where the cage monitoring is essential and it includes maintenance of cage and its accessories, stocking of the fish, feeding, fish husbandry, health management, water quality and harvesting.

Maintenance of cage and its accessories

The materials are used in constructions of cages have a definite life span and will eventually wear out. Therefore, cage with net and mooring system should be checked periodically during the culture period. Generally algae grow on the cage frame, which makes the frame slippery. It should be scrapped once in a month to keep cage frame clean, so the worker can easily work by standing on the cage. The chain and floats attached to the mooring system should be regularly (once in a month) inspected for any damage such as loosening of shackle and damage of chain. If any damage is noticed, it should be repaired immediately. The mooring system should be compulsorily checked after any extreme

weather conditions such as cyclones, storms, depressions, etc. The net attached to the cage frame is always in the water, and it is susceptible to settlement of fouling organisms such as barnacle, algae, etc. Therefore, the nets should be frequently checked for assessing the extent of fouling and if it is observed that the more than 50 % of the net meshes are clogged and then the net must be changed. The inner net of the cage should be changed once in every 1-2 months or depends on the rate of fouling. Also, the mesh size used in the inner net should be selected according to the growth of stocked fish. The net must be checked frequently for any damage.

Stocking of the fish

The candidate species for which the seed is readily available is the ideal for cage culture. Seed may be obtained from wild source or can be procured from commercial hatcheries. However, they should be of uniform size for stocking. The seed collected from wild or hatchery should be acclimatized to the water condition in the cage so that mortality will be reduced during stocking. It is better to stock the cage during early morning or evening hours to avoid wide temperature fluctuations. The ideal size of the fish to be stocked in cage is around 10-15 cm (20-30g). If the size of fish is smaller than recommended size, then it has to be reared in nursery system either in tank or pond or cage itself. When smaller sized fishes are reared in the cage and pond, it is better to stock in hapa of appropriate mesh size till reaching optimum size. In this case, fishes have to be graded every week to avoid cannibalism depending on the species, if the fishes are in cannibalistic nature, e.g Asian Seabass and orange spotted grouper. Feeding of fish on transfer to the cage should commence 3-4 hours after transfer. The stocking density in the cage will vary

Cage management: Cleaning of cage frames

according to size of the fish. Generally, the recommended stocking density for Asian sea bass of 10-15 cm is 24-30 no/m^3, Indian pompano and Orange spotted grouper is 25 nos/m^3. Stocking density for pond culture for marine finfish varied between 1-1.5 nos/m^2

Feeding

Feed and feeding regimes need proper management for maintaining better health and growth of the cultured fish. Feeding should be done throughout the culture period at varying levels depending on the growth rate and natural feed availability in the system. Generally, fishes should be fed @ 10% of body weight during the starting period and slowly it should be brought down to 3-5%. Hand feeding is done in most cases and it is recommended for small-scale farmers. However, mechanical feeders such as demand feeders and automatic feeders are used in large scale farms. Feed rings can be used if floating pellets are used. Feed trays set inside the cages at different positions can also be used for distribution feed evenly. During hand feeding, the feeding of fish can be monitored and can be fed till satiation. While doing so, the stock health status can also be monitored. Type of feeding is depending on the feeding behaviour of the fish; Formulate floating feed of 40% crude protein and 10% crude fat is the optimum feed for Indian pompano in cages and coastal ponds; Mixed feed including low cost trash fish (Sardine, Decapterus & Tilapia) and formulated feed (floating pellet feed) is the suitable feed for orange spotted grouper, and Asian seabass. Asian seabass and orange spotted grouper can be culture with either formulated or low value fish or mixed feed. But selecting optimum feed depends on the feed availability, culture conditions, nature of the seed. The mixed feeding scheduled is good for proper growth of fish: orange spotted grouper and Indian pompano. When frozen trash fish is given as feed, should be thawed first, chopped and then broadcasted over the surface. The trash fish should be washed properly with fresh water to avoid any external parasite entering into the cage with feed. When floating pellet feed are given, a feed mesh should be used to avoid feed wastage by drifting along wind. Overfeeding of the stocked fish should be avoided otherwise it could lead to deterioration of water quality. The fish should be fed at least twice per day once in the morning and then evening. However, at the earlier stage, feeding frequency of more than two times, and maximum of 4-5 times is suggested for better growth.

Fish husbandry

Regular observation of fish stock is essential for any culture system. Therefore, farmers should observe the fish stock without unduly disturbing them which helps to understand the general behaviour of the fish under normal cycle of environmental condition prevalent at the site, i.e., dawn/mid day/dusk/high tide/low tide, feeding/non feeding, etc. If

something wrong is observed then fish should be sampled and examined further for changes in physical appearance in different body parts: spine - deformed spine; skin - abnormal colour, presence of lesion, rashes, spots or lumps, excessive mucus; eyes - bulging eyes, cloudy lens; fin and tail - erosion. These clinical signs indicate that the fish stocks are in abnormal condition, which might be due to effects of adverse environmental factors or infected with disease. These problems need to be properly addressed to adopt the further precautionary measures or to give proper treatments to the fishes.

In cage and coastal pond, fish sampling should be done at regular interval of at least once in a month to understand the growth rate of the fish. Periodic information on the growth rate of the fish is required for the calculating the feed requirement of the fish stock. This information will give a fair idea about the stock performance and the feed requirement for further coming days of the culture and then also help to avoid over feeding. Record keeping of the farming practices such as daily mortality, feed consumption, and growth rate should be maintained. It is crucial in understanding the epidemiology of diseases and this allows farmer to identify the critical management point in the production cycle. Observation, collection and storing the data during a culture practices help to take early preventive action in case of disease outbreaks/abnormal situation in the subsequent culture practices.

Monitoring of Indian pompano grow-out culture in floating marine HDPE cages

Monitoring of grow-out orange spotted grouper in sea cage

Health management

Implementation of the good sanitation practices is essential in any fish culture system. However, it is difficult to implement the practices in cage farming system since there are no barriers between the cage environment and its surroundings (where the pathogen can be found). However, it is easy to maintain barriers in coastal pond culture systems. Even

though, it is necessary to reduce the risk of contamination by adopting simple management practices to reduce the pathogen pressure in the environment. The important practices to be followed to reduce the pathogenic loads include, avoid the overfeeding to the stock; wash the trash feed with fresh water (if fed with low value fish); remove the moribund or dead animal immediately, maintain the optimal stocking density in cage, exchange of net at appropriate time (cage), provide proper aeration in pond through aerators. The uneaten food is a potential source of pathogens, so stock must be not overfed. The dead animal is another source of pathogens in the cage, if a moribund or dead fish is noticed it should be removed immediately. Washing trash fish with fresh water will kill almost all the external pathogens from sea origin; hence washing the feed with fresh water is must. The fish stocking density should not be more than the recommended stocking density otherwise it will lead to many complications and finally stock will collapse due to health problem. The net of the cage should be removed at appropriate time, otherwise the water flow in the cage will reduce which may leads to water quality related problems and finally stock will collapse.

Water quality

Maintenance of good water quality parameters in cage farming system is difficult, since the cage culture practiced in open water bodies and no boundary existing between the cage environment and its surroundings. However, the important water quality parameters are to be monitored frequently to avoid losses caused by lethal changes in water quality. It is essential to have long term data on the changes in the water quality parameters at the site; so that changes in the water quality parameters from the site could be observed and predicted and accordingly preventive decision could be taken in advance. Frequent recording of important water quality parameters liken ammonia, nitrite and nitrate, pH, turbidity and temperature will give a clear idea about cage environment and also will help to understand the health status of the animals in the system. In pond culture system, maintaining water quality is easier by applying optimum quantity of allowed farm remedies including prebiotics, probiotics, and chemical.

Harvesting

Harvesting of fish is done continually or in batches depending on how the production cycle is managed. Before harvesting, the fish may be starved for a day to have empty gut which will help to get long shelf life of the produce. Fish could be harvested in situ or the cages are towed to convenient places where the netting operation may be carried out more smoothly. The process of harvesting is simple where the net is lifted up and fishes are concentrated to a small volume and scooped out. In case of coastal pond culture

system, the fishes are harvested with help of drag net at the time of complete harvest, or cast net to be used for partial /small quantity fish harvest. Either in cage or pond, harvesting is preferred during morning hours.

Harvesting of Indian pompano and Orange spotted grouper

Some of best management practices that should be followed in open sea cages and coastal pond is given below.

Best Management Practices (BMP) for cage culture of Indian pompano

The following important BMP must be implemented while practicing grow-out culture in floating marine cages for better production with high economic returns.

1. A cage should be installed where the water movement is adequate for getting optimum concentration of dissolved oxygen and for washing away the accumulated waste generated from cultured fish
2. Fish fingerlings of > 30g should be stocked to obtain maximum survival
3. Feed mesh of 1 mm mesh size should be attached with inner cage net for avoiding feed wastage
4. Inner cage net should be additionally supported with a middle ballast pipe for maintaining the round shape and for avoiding net folding.
5. Feed should be broadcasted slowly in cages to ensure that all the fishes get the required feed and for avoiding feed wastage.
6. Periodical monitoring of fish, cage net and other cage system is essential.
7. Continuous observation for vibriosis and parasitic infestation to ensure the fishes are free from the disease, and immediate treatment of infected fishes.
8. Demand based fish harvest is recommended for better profit.

Best Management Practices (BMP) for grow out culture of orange spotted grouper

The following important BMP must be implemented while practicing grow-out culture in floating marine cages for better production with high economic returns

1. The fish is cannibalistic in nature during nursery phase, and thus periodical fortnight grading is essential to reduce cannibalism.
2. Cage should be installed where the water movement is adequate for optimum concentration of dissolved oxygen
3. Fish fingerlings of > 20g should be stocked to obtain maximum survival
4. Cage net depth of 2.0 m should be maintained till the fish reached 250g
5. Inner cage net should be additionally supported with a middle ballast pipe for maintaining the round shape and for avoiding net folding.
6. Low value fish feed should be given along with artificial pelleted feed for better growth and to avoid size variations
7. Periodical monitoring of fish, cage net and other cage system is essential
8. Stress should be avoided while harvesting and the harvested fish should be maintained in ice till packing.

Best Management practices to be adopted for grow out culture of Indian pompano

1. Fish fingerlings of > 30 g should be stocked to obtain maximum survival, and stocking bigger seed size will reduce the grow-out period to six months.
2. Suggested optimum stocking density should be of 5000 nos/acre for good economic return.
3. Pond should be fertilized at every fortnight to maintain water quality and water productivity (colour). Maintaining more zooplanktons and small crustaceans in the pond will help to reduce the use of formulated feed, increase the feed conversion efficiency, and also helps in the overall improvement of the fish pigmentation offering a better price at harvest.
4. The fish is active and fast moving fish, oxygen consumption by the fish is very high and thus, dissolved oxygen content should be always > 4 ppm for better survival. Thus, one acre water spread area of the pond should have minimum of 4 aerators and with minimum of 8-10 hours per day, especially late evening and early morning.
5. Creation of feeding zone in the pond will help to conditioning the fish for feeding to a particular area, and also will reduce feed wastage by dispersal over the entire pond area due to wind action.

6. Except for the first few months (3-4 months) of operation, the subsequently water exchange of 25% should be done in every month to maintain water quality. This practice may help in reducing the use of probiotics and water conditioners.

Best Management Practices (BPM) for pond culture of orange spotted grouper

The following steps are recommended for the coastal pond farming of orange spotted grouper for better economic returns

- Transportation of fingerlings of 10-15g size in polythene bags should be avoided
- Seed stocking during winter season should be avoided
- Grading in nursery is essential for reducing cannibalism
- Hide –out should be used in grow-out culture pond
- Mixed feeding (artificial and low value fish) helps for better growth

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WATER QUALITY INDICATORS FOR HEALTH MANAGEMENT IN MARICULTURE

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Introduction

Fishes live in water and depend on it for all their basic needs, so we have to provide a better water quality for its well being. The cultivation of fish under controlled conditions can be significantly affected by various diseases. The host (fish)-pathogen-environment relationship is a fundamental concept in understanding the dynamics of infectious diseases. It refers to the complex interactions between a host organism, a pathogen (disease-causing agent), and the environment in which they interact. This relationship plays a crucial role in determining the occurrence, transmission, and outcome of infectious diseases. Water quality is a critical environmental factor in mariculture, as it directly affects the health and growth of aquatic organisms. Understanding the intricate interplay between hosts, pathogens, and the environment is crucial for developing effective strategies to prevent, control, and manage infectious diseases. So a regular and comprehensive monitoring and control of these water quality indicators is essential for maintaining a healthy mariculture system and preventing potential health issues for the cultured species. Continuous data collection and prompt corrective actions based on monitoring results are crucial components of effective mariculture management.

Water parameters

The aquatic environment is a complex eco-system consisting of multiple water quality variables, so monitoring and maintenance of its quality is very much necessary for achieving higher production. The water obtained for fish culture may be filtered and treated but while culture is practiced, due to metabolic activities, accumulation of waste may pollute the water. Of the various water quality parameters few are highly important and play a decisive role, they are temperature, pH, salinity, dissolved oxygen, ammonia, nitrite, CO_2, hardness and total alkalinity. Each individual parameter is important, but it is the aggregate and interrelationship of all these parameters will have direct influence on the well being of the fish cultured and inturn slighter variation from its optimum will affect the growth of fishes. In the view of above a set of protocols has to be followed for maintaining good water quality for sustainable fish culture.

Temperature

Water temperature is one of the most important factors influencing the culture of tropical fishes. Fishes are generally characterized as warm water species (25-30°C), cool water species (15-25°C). Although majority of the fishes tolerate water temperatures between 21°C and 30°C, 24°C to 28°C, have been found to be optimum and most suitable for the tropical fishes. In order to maintain optimal temperatures, suitable devices have to be used. An increased temperature also decreases solubility of gases in water that leads to lower dissolved oxygen. Fishes are very sensitive to sudden variation of temperature. The metabolic rate of fish is closely correlated to the water temperature. The efficiency of many physiological processes in fish will change by 6-10% per 1! change in body temperature. A sudden change of 1°C even within the temperature tolerance range of that fish results in a serious stress to fish. Due to disruption in metabolism the food will remain undigested or half-digested in the digestive tract. This will lead to considerably increase the level of ammonia nitrogen in the blood serum can lead to ammonia autointoxication and death. Therefore, fish should be gradually acclimatized whenever shifted from one tank to another. Water temperature also has a great influence on the initiation and course of a number of fish diseases. The immune system of the majority of fish species has an optimum performance at water temperatures suitable to it. Water temperature also plays significant role in nitrification process as 10°C drop in temperature will lead to a 23% reduction in nitrification rate.

pH

pH is an indicator of acidity or alkalinity of water which ranges from 1-14. A value of 7 is considered neutral whereas values below 7 are acidic and above 7 are basic. A buffering system to avoid wide swings in pH is essential in aquaculture. Fish have an average blood pH of 7.4; hence a pH range of 6.5 to 8.5 is more optimum and conducive to fish life. The pH of water usable for fish farming may vary from acidic to alkaline depending upon its source, chemical and biological factors. The optimal pH for the growth of majority of the fishes should be neutral or slightly alkaline i.e. 7-8. Largely, pH in culture system is reduced by production of CO_2 by cultured fish as well as that produced by the microbes present in the system. The loss of alkalinity together with the increase in dissolved CO_2 will also cause a reduction in pH of the system.

During sudden change or fluctuations in pH as a defence against the effect of a low or high water pH, fish can produce an increased amount of mucus on the skin and on the inner side of the gill covers. Extremely high or low pH values cause damage to fish tissues, especially the gills, and haemorrhages may occur in the gills and on the lower part of the

body. Excess amounts of mucus, often containing blood, can be seen in post mortem examination of the skin and gills. The mucus is dull-coloured and watery.

pH of pond / tank water can be corrected by following simple methods

- Low pH (<7.0) can be corrected by application of limestone-calcium carbonate ($CaCO_3$) @ 30-50 mg/L.
- High pH (>9.5) is corrected by repeated application of Alum - aluminium sulphate [$Al_2(SO_4)_3$] @ 5-10 mg/L. However, alum should not be used in waters with total alkalinity of less than 20 mg/L as $CaCO_3$ because even small amount will drastically lower the pH to a dangerous level.

Dissolved Oxygen

Dissolved oxygen content of the water plays a crucial role in fish culture. Different fish species have different requirements for the concentration of oxygen dissolved in water. Generally oxygen requirements of fish decreases with increasing body weight. Fish must be cultured at optimum levels of dissolved oxygen (DO) to achieve high survival and good growth. It should remain well above 5 mg/L. A value of less than 5 mg/L can result in undue stress to the fish resulting in mortality below 2 mg/L. Fishes under culture would be under stress and be liable for parasitic attack if optimal oxygen levels are not maintained. Oxygen deficiency causes asphyxiation and leads to fish death. Fish exposed to oxygen deficient water do not take food and ultimately die. However supersaturation of water with dissolved gas can also cause gas bubble disease. Apart from fishes nitrifying bacteria also consumes large amount of oxygen. It is consumed at the rate of 4.57 g for oxidizing every gram of ammonia-nitrogen to nitrate-nitrogen. The solubility of oxygen in water depends on its temperature and also on the rate at which it is kept in contact with water. Factors influencing depletion of oxygen in water are

- Higher water temperature
- Accumulation of excessive organic mater
- Formation of phytoplankton/algal blooms

Oxygen is dissolved in water by direct diffusion at the air-water interface. In emergency situations oxygen level of ponds can be enhanced by constant aeration, circulation of water, sprinkling of water, surface agitation, etc.

Carbon dioxide

Fishes try to avoid free carbon dioxide. Free carbon dioxide at a concentration of more than 15 ppm is detrimental to fishes. Fishes can tolerate high level carbon dioxide provided dissolved oxygen content of the water is sufficiently high. But the presence of high free

CO_2 may hinder solubility of oxygen. Generally CO_2 content in water may increase during night and decrease during day time. 1.375 grams of CO_2 is released back into the system for every gram of dissolved oxygen is consumed both by fish and bacteria. Exposure to higher concentration of CO_2 reduces respiratory efficiency of fishes, higher concentration of CO_2 in blood and lowering of blood plasma pH. High level of free CO_2 can be removed by application of following for every 1 ppm of free CO_2.

- add 0.84 mg/L of $Ca(OH)_2$ or
- add 0.64 mg/L of CaO

Water Hardness

The total hardness of the water is due to the sulphite and chlorides ofsoluble calcium and magnesium salts present in the water expressed as its calcium carbonate equivalent. The total hardness is mainly used to classify waters into 'hard water' or 'soft water'. Water with a total hardness of 0-75 mg/L is considered soft, 75-150 mg/L as moderately hard, 150-300 mg/L as hard and above 300 mg/L as very hard. Water with hardness of 50-200 ppm have been found to be optimal for the normal growth of majority of fish. Water with less than 12 ppm requires liming for higher production of fish. Hard water is also known to influence feed intake and growth of fishes.

The total hardness can be improved by application of agriculture lime ($CaCO_3$) as per requirement. In case, total alkalinity is in desirable range, total harness alone can be enhanced to optimum levels by using gypsum ($CaSO_4$) without affecting the alkalinity. A proper management of hardness and alkalinity will usually eliminate the need to worry about pH.

Water hardness can be reduced by following methods

- apply EDTA at 1 kg/acre
- softening of water by application of 'Zeolite' (Sodium alumina silicate)
- addition of sodium carbonate

Total Alkalinity

Alkalinity is the capacity of water to neutralize acids without an increase in pH. This parameter is a measure of the bases i.e. bicarbonates & carbonates (CO32- and HCO3-) and in rare instances, hydroxide (OH-). Total alkalinity is the sum of the carbonate and bicarbonate alkalinities. Some waters may contain only bicarbonate alkalinity and no carbonate alkalinity. Fish grow within a narrow range of pH values and either of the above extremes will be lethal to ûsh. A culture system is considered well buffered when pH varies between 7.5 - 8.5 and total alkalinity ranges from 80-150 mg/L. There will be a

reduction in alkalinity due to conversion of total ammonia-nitrogen (TAN) to nitrate-nitrogen(NO3-N) by nitrifying bacteria. It is consumed at the rate of 7.14 g for reducing every gram of ammonia-nitrogen to nitrate-nitrogen. The total alkalinity of water can be increased by addition of lime in day time, which should be done in phases by monitoring the value of pH. Sodium bicarbonate at 20 kg/ha will improve alkalinity. A pH value of above 8.5 is not desirable for most of the species. There is no practical method to reduce total alkalinity of ponds. However, it can be reduced significantly by regular replenishing of water and suspending feeding and manuring for some time.

Total ammonia

Ammonia content of the water is due to the release of metabolic wastes and decomposition of organic matter including feed remains and faecal matter. Every 1 mg/L of oxygen consumed by the fish will produce 0.14 mg of ammonia. Ammonia occurs in aquaculture systems in two forms i.e. ionized or ammonia ion (dissociated) and un- ionized or molecular (non dissociated) form. Of which molecular form (NH_3) can readily diffuse across the tissue barriers and passes to fish blood, and is therefore the potentially toxic form to fish, while the ionized form (NH_4+) is not. Both forms are grouped together as "total ammonia". The unionized or free ammonia is safe below 0.02 mg/L and toxic above 0.02mg/L in freshwater and 0.01mg/L in seawater. Above this level, free ammonia causes the fish to stress and at higher levels it may cause damage to gills and many internal organs, eventually resulting in fish deaths.

As the temperature and pH of the culture system increases the level of free ammonia increases and of ammonium ions decreases. Hence, the balance between free ammonia and ammonium ions is determined by the pH and temperature of the water. The skin of ammonia poisoned fish is light in colour, and covered with a thick or excessive layer of mucus. The gills will be heavily congested and dark red in colour. The digestive tract of those fish with severe poisoning symptoms will be filled with undigested food. The toxic level of ammonia can be best reduced in a culture system by immediate water exchange, control feeding rate and increase beneficial bacteria and carbon source.

Nitrite and Nitrate

Nitrites are usually found together with nitrates and ammonia nitrogen but their concentrations are usually less because of their instability. The nitrites are produced in culture waters by bacteria (including *Nitrosomonas* spp.) through nitrification process when ammonia is broken down. At any situation, nitrite should not exceed 0.125 mg/L in seawater. The toxic action of nitrite on fish is incompletely known. Nitrite poisons fish by binding the haemoglobin forming methaemoglobin in the blood preventing it from carrying

oxygen, in effect suffocating the fish. The increase in the amount of methaemoglobin can be seen from the brown colouration of the blood and gills. If the amount of methaemoglobin in the blood does not exceed 50% of the total haemoglobin content, the fish usually survives. The pH value has also been considered as important factor influencing nitrite toxicity along with temperature. The toxicity of nitrite increases by decreasing pH. Another factor that influences nitrite toxicity is the dissolved oxygen concentration. Fully oxygenated water increases oxygen in the blood and reduces the formation of methaemoglobin.

Nitrates are produced in culture waters by bacteria (including *Nitrobacter* spp.) through nitrification process when nitrite is broken down. Nitrate is generally of low toxicity though some species, especially marines, are sensitive to its presence. But mortalities have only been recorded when concentrations have exceeded 1000 mg/L. However, as with ammonia, water quality standards need to be set for nitrate to prevent excessive growth of algae and plants and its resulting effect on fish. The high levels of nitrite and nitrate can be best reduced by immediate water exchange, control feeding rate and increase beneficial bacteria and carbon source.

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MICROBIAL BIOREMEDIATION IN MARINE CULTURE SYSTEMS

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Introduction

Health Management in Mariculture, especially farming of finfish and shellfish in marine and coastal waters, is a highly economically viable culture system in the world, due to its high production and export market value. The aquaculture industry worldwide is undergoing a transition from an extensive to an intensive system. This shift is accompanied by a growing inclination towards augmenting inputs such as over stocking, overfeeding, and this high-density production in a confined volume will always cause disease outbreaks, and this results in increase the use of fertilizers and various chemicals including antibiotics, herbicides, and pesticides. However, these inputs have the potential to alter the aquatic environment, causing detrimental effects on the organisms residing within it and ultimately resulting in fish mortality. The manifestation of diseases in mariculture systems, particularly in closed systems, exhibits variation across species and environments, predominantly attributable to the absence of proper management practices. Due to the ubiquitous nature of *Vibrios*, and its intricate interplay with the host and surrounding environment, the transmission of *Vibrio* spp. in marine fish is still not clear. Wild and prey fishes also act as reservoirs and carriers of the pathogen. The dynamic nature of the marine environment allows the survival of pathogenic *Vibrio* species, which may enter as viable but in non-culturable state under unfavourable conditions, but still infective for longer periods. In hatcheries and closed marine culture systems, intake water and feed are considered as the reservoirs for *Vibrio* and serve as natural transmission path for *Vibrio*s towards susceptible fish. In addition, infected eggs, juveniles and broodstocks also contribute to the proliferation of *Vibrio* spp. in Marine hatchery systems, apart from water and feed. Vibriosis can be controlled by chemotherapy and the application of antibiotics. Oxytetracycline is the most common antibiotic used in hatchery and culture systems. However, continuous usage of antibiotics is not suggestible in culture systems which may cause resistance against those drugs and also to avoid quality control issues. Hence, the water quality management of the culture system through a beneficial microbial consortium gives the best preventive measure at hatcheries to prevent the attack of any disease.

To improve the water quality in culture systems, Gram-positive bacteria is used as it converts organic matter back to CO2 better than Gram negative bacteria. High levels of Gram-positive bacteria can minimize the buildup of dissolved and particulate organic carbon and the use of *Bacillus* sp. improves water quality, survival, and growth rates, and the health status of cultured shrimp/fish in aquaculture systems. Beneficial bacterial Consortium as a Biological control strategy, mainly use of marine beneficial bacteria in mariculture systems is the best way of approach to eliminate or prevent infectious diseases. Probiotics can be applied to the feed, or they can be added directly to the water and the other administration strategy is encapsulation. Encapsulation helps by improving nutritional value and proper delivery of the microbe to the host without waste of live organisms. Many microorganisms are evaluated as probiotics in aquaculture. *Bacillus subtilis, Lactobacillus acidophilus, L. sakei, and Shewanella putrefaciens* are the most commonly used probiotics in indoor mariculture systems.

In closed/indoor mariculture systems, the levels of ammonia and nitrite are a major concern, as the toxicity of these nitrogen species varies depending on the pH level and the specific aquatic organism being cultivated. Ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) play a crucial role as dominant microorganisms in the conversion of ammonium to nitrite, which is a vital process in the complete breakdown of organic matter. This ultimately results in the buildup of nitrate in oxygen-rich environments. Usage of the potential marine nitrifying bacterial consortium with a combination of different AOB and NOB species like *Nitrosomonas sp.*, *Nitrobacter sp., Nitrotoga sp. Nitrospira sp., N. oligotropha and N. winogradskyi* is the best way of biological control measures for ammonia reduction and *Vibrio* elimination in Marine Hatchery systems.

Major Ammonia Oxidizing and Nitrite Oxidizing Bacteria used in Closed Marine Aquaculture systems

Nitrosomonas oligotropha,

Nitrosomonas europaea,

Nitrosospira briensis

N. winogradskyi

Nitrosococcus sp

Arthrobacter globiformis

Thiosphaera panthotropha

Nitrosospira and *Nitrosomonas* have the ability for ammonia oxidation rate within 6 hours, *Nitrosospira* cells maintain a low but steady ammonia oxidation rate.

Water Probiotics in water quality management in Indoor systems

Name of the probiotics	Beneficial effects
Bacillus spp.	Reduces the load of ammonia and nitrite
Enterococcus faecium ZJ4	Improves water quality and enhances immunity
Lactobacillus acidophilus	Improves water quality
Bacillus NL110, Vibrio NE1	Reduces ammonia and nitrite concentration
Nitrosomonas sp.,Nitrobacters sp.	Reduces the concentration of ammonia, phosphates and nitrite in culture pond
Rhodopseudomonas palustris, Lactobacillus plantarum, Lactobacillus casei, Saccharomyces cerevisiae	Reduces nitrate load, maintains water pH andenhances dissolve oxygen concentration
Paenibacillus polymyxa	Enhances immunity and reduces pathogenic stress
Lactobacillus rhamnosus	Reduces pathogen load in culture tank
Pseudomonas sp.	Enhances transcription rate of anti-microbial peptide
Bacillus spp	Promotes the growth of beneficial algae andreduces the growth of harmful algae
Nitrosomonas sp., Nitrobacters sp.	Reduces pathogen load in culture pond andincreases dissolved oxygen content

Protocols for the development of Potential ammonia oxidizing bacteria from sediment/water samples

1. Collect sediment /water samples from marine coast or mangroves in axenic condition
2. Mix the sample with a standard AOB medium to form slurries.

3. Incubate these slurries upright in an orbital shaking incubator (175 rpm) at 25°C for 6 h.
4. Collect the samples of the sediment slurry after 2 and 6 h of incubation, and determine ammonia and nitrite levels.
5. The potential ammonia oxidation rates can be calculated based on the change in nitrite concentration.
6. Measure temperature, pH, oxidation-reduction potential, Ammonia, Nitrate and Nitrite at every 6 hours.
7. The nitrifiers that are developed from sediment and water samples should be enriched in sand based biological filtration systems and add the ammonia substrate for the multiplication of AOB and NOB in the filtration systems.
8. The enrichment cultivation aims to enhance the proportionate abundance of a specific AOB in order to promote their development, viability, or spatial isolation from other individuals within the community.
9. Biofilters can be the best support media in batch enrichment studies.
10. Periodic sterilization of biofilters and the entire system helps in the removal and elimination of organic load and pathogenic bacterial loads.
11. The ammonia and nitrate reduction in waters and complete elimination of *Vibrio*s and zero ammonia will be varied with the AOB and NOB bacteria developed in the biofiltration units and also on the density of aquatic organisms in the culture systems and development of Ammonia.
12. An increase of pathogenic *Vibrio* loads due to high levels of ammonia in the Marine culture systems cause fish mortalities.
13. Management of biofilteration system is the most crucial element in Marine culture systems.
14. Development of the best nitrifying bacteria is essential, as level of ammonia and nitrite oxidation by microorganisms in a biofilter depends on the percentage of nitrifying bacterial inoculum and type of media in biofilters and also the volume of the bifiltration unit.
15. In order to decrease the ammonia levels and pathogenic bacterial abundance in Marine culture systems, nitrification stability need to be improved. This can be successfully achieved by precoating of biofilter material with nitrifying bacteria,

which occupies the inner most layers of biofilm and can be transferred easily into the microbial populations.

16. Periodic sterilization of the biofilter is essential in Recirculating systems that encounter with frequent disease outbreaks.
17. A dual biofiltration system helps in reducing the pathogenic load in the water. In addition to this, usage of commercially available water and feed probiotics in Marine culture systems are the best preventive measures for the occurrence of any bacterial infections.
18. Further investigations on microbiome dynamics of water, biofilm and biofilter is essential to develop health management protocols for Marine Aquaculture systems.
19. A prolonged incubation period is necessary for the AOB to fully oxidize the ammonia-nitrogen.
20. The concentration of 100 mg/L ammonia-nitrogen in 100 mL of bacteria suspension is sufficient to complete ammonia oxidation.
21. In order to enhance the growth of ammonia oxidizers in the serial batch cultures with and without bio-filters, lower concentrations of ammonia-nitrogen (Â100 mg/L NH3-N) can be suggested.
22. The utilization of bio-filters proves to be beneficial when compared to systems lacking bio-filters for the retention of slow-growing bacteria.

Development of AOB and NOB consortia from Marine sediment in biological filters

General Protocols for Environment and Fish Health Management in Indoor Mariculture Systems

Health management of closed culture systems of marine finfish is always a challenging aspect due to its dynamic nature in open waters and also dealing with broodstock management practices. Hence, the following protocols should always be followed as a part of better health management practices in hatcheries and closed mariculture systems

- Biosecurity measures with effective quarantine methods should be implemented at all the marine hatcheries, so as to eliminate pathogens in the larval development process.
- All the broodstock must be screened before initiating the breeding programmes for larval development and also eggs, fry and fingerlings must be checked before going for further rearing in nurseries.
- Nursery reared fish fingerlings must be screened for the occurrence of parasites and pathogens in order to avoid the vertical transmission of pathogens.
- It is always suggestible to undertake regular monitoring of the fish health and environmental health to understand the health condition in relation with water quality.
- Maintenance of optimum stocking density is always suggestible in order to avoid stress due to over stocking in indoor nursery and grow-out systems which may lead to the development of opportunistic secondary infections due to stress factors
- It is suggested to take precautions while using feed (live/artificial) which may be one of the reason for the transmission of parasites and pathogens
- Avoid the usage of chemicals and antibiotics in the hatchery systems which creates a problem of the development of residues and drug-resistant strains
- Application of tested and approved probiotics, immunostimulants and vaccines always gives a better management practice to produce sustainable, pathogen-free, and disease-resistant fish.
- Strict quarantine measures such as egg disinfection, water treatments, clean feed and disposal of mortalities, should be maintained for the rearing of fish in Marine Hatchery systems.

- Application of tested and approved probiotics, immunostimulants, and vaccines always gives a better management practice to produce sustainable, pathogen free and disease-resistant fish in Mariculture systems.

Biosecurity measures in mariculture systems can keep the safety of indoor culture facility from certain disease-causing agents that are absent in particular system. Biological control strategies, such as, using probiotics, prebiotics, and medicinal plants will play a major role in providing safety culture systems. Both AOB and AOA play a significant role as microbial bioremediation in the development of zero ammonia and Zero *Vibrio* levels in closed culture systems or sustainable fish and shellfish production in indoor conditions.

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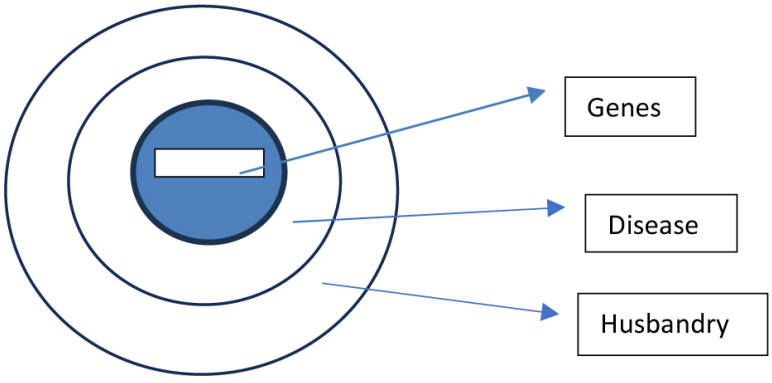
HEALTH MANAGEMENT IN AQUACULTURE: BIOSECURITY PROTOCOLS AND CONCEPTS

Joe K. Kizhakudan

The growth of any industry is tied to investment and the prospects for investment are determined by a combination of risk and rate of return. Greatest risk in aquaculture is the sporadic and unpredictable availability of high-quality animals for culture, basically related to the quality of seed and broodstock's and overdependence on the wild capture and long duration transports and crowding and environmental variations. The shrimp industry has moved into almost an exclusively hatchery produced seed and seed raised broodstock through selection programmes.

The demand from the farmer community is high and for them to be certain that the purchased animals for rearing or broodstock meet the expectations is very uncertain on many occasions, farmers buy seed from variety of firms and hatcheries rely on others for broodstock and as several hands change their history becomes highly obscure.

The 3 critical factors affecting the overall quality of production of animals are the



Genes, disease and husbandry practices involved, and any one alone cannot decide the production. Animals carrying a severe pathogen or grown in poor conditions cannot assure a good crop even though the animals are from a pure genetic background. As all three factors are vital in quality they are to be treated logistically separately and with utmost care for a successful venture.

Quality assurance is the set of policies of an establishment or company or industry through which the confidence in the nature of a product is developed and documented. This is achieved only through a series of operating principles followed closely.The Team and management has to be certain and should be committed for the reliable results and sustained success of the firm.

The following principles are essentially to be looked into

Data quality:

Accurate, regular large volume data keeping and documentation, the source of the collection, personnel involved, how, when and where.

High health and genetically improved stocks, services and technologies designed to ensure adequate and reliable supplies of high quality broodstock and seed. This helps in reconstructing the group of animals, whole populations for eg. analysis of pathogen detection, genetic marker verification, performance evaluation etc. Any sample from a group of animals must be traceable to their origin and their history retrieved from signed and dated records. Establish a comprehensive system of documentation and record keeping, indicating daily numbers, larval health, treatments/chemicals used, water quality and other relevant information for each tank stocked. This will help determine the cause of any problems and any remedial action required.

Log books:

Data regarding actions performed on animals e.g., sampling for diagnostics, feed distribution, sorting /grading, treatments, stunting and packaging getting recorded in log books or soft files by operating individuals, with date, name, doses, intervals, numbers etc. Helps in reconstructing the data base for further events and understanding.

Chain of custody record:

Records to be maintained on changes in possession or responsibility for a group of animals or samples or product, normally accompanying them with an identification number or code, signatures of personnel involved in the chain of the entire production buying and selling.

Labels:

Water proof IDs or labels on the samples or animals or tags or containers to avoid misidentification and ease of documentation at each entry levels and ease for digitisation.

Branded /Custody seals or logos:

These are generally used for interfacility transfers, import or export, for shipping of samples to avoid tampering and unauthorised adulteration or contamination.

Origin-destination sheet:

The shipper particulars of the samples and specimen or animals with details on number, sizes, strain, age, purpose of shipment, origin identification number, country name, hatchery or farm name and Reg no, certificates etc and similarly the recipient customer with destination, purpose, number and dates.

Education and Training:

All the personnel should be trained in their specific areas and in concurrence with the requirements and standards. The employees and technicians should be familiar with the objectives and principles of the program and the importance of the adhering to the policies and procedures of the quality assurance and health management in the production system.

General hatchery requirements and principles

A well-designed hatchery will consist of physically separate facilities for quarantine, maturation, spawning, hatching, larval rearing, indoor and outdoor algal culture (where applicable), and for Artemia/rotifer/live feeds/micro alga preparation and hatching

Larger hatcheries may have separate units within each of these categories, which should be run like mini-hatcheries for reasons of biosecurity. This should include attempts to stock the entire hatchery (or at least the individual units) as quickly as possible in order to reduce problems with internal contamination. Supporting infrastructure is also required for the handling of water (facilities for abstraction, filtration, storage, disinfection, aeration, temperature adjustment and distribution), larval laboratories, feed laboratories (for analysis and preparation) and storage facilities, maintenance areas, packing areas for nauplii and PL, offices, storerooms and staff living quarters and facilities. In existing hatcheries with no physical separation, effective isolation may also be achieved through the construction of barriers and implementation of process and product flow controls. If possible, the hatchery facility should have a wall or fence around the periphery of the property, with enough height to stop the entrance of animals and unauthorized persons. This will help to reduce the risk of pathogen introduction and increase security.

Water source and treatment:

More specific water treatment procedures to be used for each phase of maturation and larval rearing are detailed in the appropriate sections. Each functional unit of the hatchery system should have the appropriate water treatment systems and, where necessary, should be isolated from the water supply for other areas. Separate recirculation systems may be used for part or the entire hatchery to reduce water usage and further enhance biosecurity, especially in high-risk areas . Ensure that all water discharged from the facility is free from pathogens, particularly that known (or suspected) to be

contaminated, for example, water originating from the quarantine areas. Discharge water should be held temporarily and treated with hypochlorite solution (>20 ppm active chlorine for not less than 60 min) or another effective disinfectant prior to discharge. This is particularly crucial where the water is to be discharged to the same location as the abstraction point

Treatment schedules and protocols:

Effective tank, pipework and general hatchery preparation protocols should be used prior to each cycle/use to ensure that diseases affecting one cycle are not transmitted to the next. Vibrio Sp. bacteria, viruses, fungi, microsporidians and protozoans are able to survive and multiply from small founder populations, which may escape disinfection within the bare concrete of hatchery or reservoir tanks or within un-disinfected facilities, pipes and other equipment. Diseases which can affect one tank of larvae can be easily spread to other tanks through contamination of hands or equipment, if they are used for more than one tank. Therefore, all equipment's should be maintained separate, with one set for each tank. Procedures for disinfection of hands and feet should be strictly followed.

Broodstock care and health management:

Before broodstock are transported to their destination hatchery, they must be prepared so that they will have the best chance of arriving alive and in good condition, with minimum stress. Correct transportation of broodstock is critical to the provision of high quality broodstock that will survive the stress of transportation and be able to produce healthy seed in the hatchery maturation system. Once the broodstock holding facilities have been prepared correctly and the broodstock arrive at the hatchery, they should be slowly acclimated to the water quality conditions in order to avoid stress and maximize survival. Once in the hatchery, the broodstock to be used should be disease checked and those selected should be kept in a stress-free environment with oceanic

water quality and fed adequate quantities of high quality fresh and dry feeds so that they are able to produce high quality seed. Broodstock must be maintained, spawned and hatched individually so that any infected broodstock cannot infect the others in the facility. Spawning and hatching techniques must be used which promote production of high-quality, disease-free eggs and larvae.

Larval rearing:

To optimize water quality and reduce disease and stress levels for the growing larvae, it is important to stock the correct number of larvae and exchange sufficient water to maintain optimum water quality conditions throughout the larval rearing process. (In tiger shrimp hatchery -Gently collect eggs in a 50-60 micron mesh net (*a preliminary 300 micron net is used to catch and discard any faeces from the spawning tank) gently rinse in running seawater for 5 minutes 30-second dip in 100ppm formalin solution 1 minute dip in 50 ppm povidone iodine solution ,gently rinse in running seawater for 5 minutes Stock eggs in hatching tanks, gently collect nauplii in a 100 micron mesh net, gently rinse in running seawater for 5 minutes 30-second dip in 200 ppm formalin solution 1 minute dip in 50 ppm povidone iodine and gently rinse in running seawater for 5 minutes and stock the nauplii in larval rearing tanks*).

Diagnostic level in hatchery systems- Disease control encompassing screening, diagnosis prevention and treatment technologies as well as services and product to protect health.

Level 1 Observation of animal and environment. - Examination based on gross features. i.e. Health examination of broodstock, sex determination, moult and ovarian development staging, removal of sick/moribund individuals, selection of larvae by phototactic response, stage feeding activity by observation of faecal strands, larval activity, postlarval health, activity and behavior, stress testing.

Swimming activity/ Phototaxis / Faecal string/ Luminescence /jerking response/ flashing/ gaping /swirling/ erratic swimming

Level 2 More detailed examination using light microscopy and squash mounts, with and without staining, and basic bacteriology i.e. -Checking bacterial flora of shrimp, feed and water, microscopic examination of egg/ larvae quality, routine microscopic examination of larval and postlarval quality.

Hatcheries should have a microscope that is used to make more detailed examinations of larval condition, on microscopic examination and squash mounts, if necessary, of a randomly taken sample of at least 20 larvae per tank (more for larger tanks). Special

attention is paid to the state of the hepatopancreas and intestinal contents, necrosis and deformity of limbs, fouling organisms and the presence of baculovirus in the faeces or hepatopancreas of older larvae. Hatcheries should routinely (daily) employ basic bacteriology to identify possible pathogens when the larvae become weak or sick and determine sources of infection (i.e. algae, Artemia or incoming water). If they do not have these facilities, samples should be taken and sent to a competent laboratory for analysis. This information may then be used to make a decision on how the tank should be treated or whether it should be discarded.

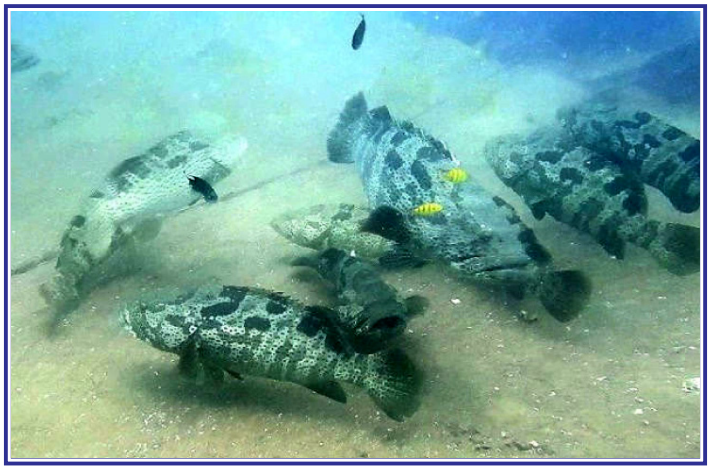
Level 3 Use of more complex methods such as molecular techniques and immunodiagnostics (e.g. PCR) i.e. Screening of broodstock, larvae and postlarvae by PCR-techniques are becoming more commonly employed in shrimp hatcheries and laboratories servicing such hatcheries. Polymerase chain reaction (PCR) methods may be used for the screening of postlarvae and broodstock for viral diseases, as can dot blot and other immunodiagnostic tests.

Advanced culture technologies, services and products to ensure high quality inputs and operating culture conditions yielding yields at acceptable costs.

Larval growth and survival, and the water quality of the larval rearing tanks depend to a large extent on the quality and quantity of food offered to the larvae. Optimization of feeding regimes based on live feeds helps maintain good water quality, whilst promoting fast growth and high survival of the larvae and hence optimal production from the hatchery. Live (or preserved, if live are unavailable) diatom microalgae are the perfect food or media for early larval stage not only do they offer the perfect nutrition, they also are self-suspending in the water column, enhance water quality (by absorbing ammonia, nitrite and carbon dioxide etc., they maintain shade in the water, they produce natural and helpful antibiotics, and they act to enrich the nutritional value of Artemia/rotifers/copepods. The supplementation of artificial foods with live microalgae should therefore be a prerequisite.

A certification is therefore essential such programs ,where a set guidelines are to be followed and can vary facility to facility .A potential pathogen should be on a list of certifiable agent if the pathogenicity, diagnosability and excludability are certain and established and the pathogen causes serious economic losses too and are a threat to the industry .Similarly a procedure must be available that will provide sensitive, reliable and relatively simple and efficient diagnosis of the pathogen and should be cheaper. Routine procedure cannot be very expensive methods .And most importantly the list of pathogens or species should actually be possible to be kept out of the facilities if at least on a short term.

A thorough certification of a facility asks for a complete cleanup or a partial one or a stepwise procedure, but both the last two options are not acceptable for longer durations. Guidelines are prepared for some as explained above and certain new pathogens may emerge in the classifications, and undescribed pathogens might be found to be problems and further studies on biology and identity of the pathogen would be intensively taken up subsequently. And this will need the industry -R&D and the farmer community standing together in leading the sector to the next century.



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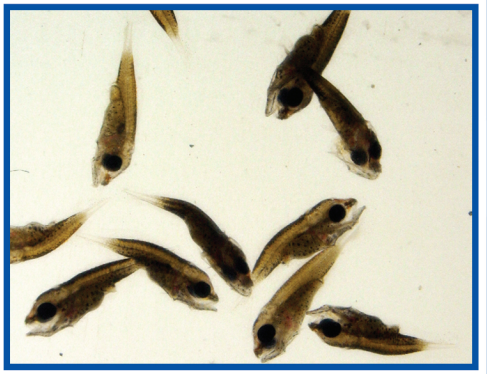

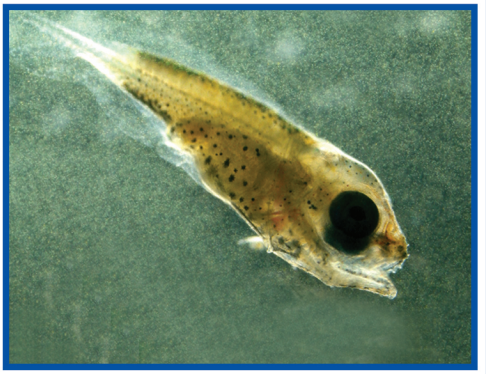

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