Environment Management and Probiotics in Mariculture

Jayasree Loka

Visakhapatnam RC of ICAR-CMFRI
lokasree@gmail.com

Introduction

Marine cage aquaculture industry is gaining greater demand worldwide, due to its contribution as an alternate livelihood and also because of its protein and export value, for coastal communities. Mariculture is expanding significantly all over the world and is contributing 25.5 % to the global aquaculture production in the last two decades (FAO 2017). In India, Central Marine Fisheries Research Institute made significant contribution in standardization of breeding and cage culture technologies of marine finfishes viz., Cobia, *Rachycentron canadum,* Pompano, *Tachinotus blochii,* Grouper, *Epinephleus coioides,* Indian Pompano, *Trachinotus mookalee,* rabbit fish, *Siganus vermiculatus,* seabreams, *Acanthopagrus berda, Sparidentex jamalensis* and achieved a successful production of these species in different maritime states of India. High density intensive culture of fish in indoor tanks, with a controlled system will be highly beneficial compared to the traditional method of growing fish in open marine waters (cages/ raceways/ pens *etc*.), where control of environmental conditions is not possible. In order to sustain the growth and production of marine finfishes in India as well as in global level, it always suggestible to maintain a health protocol of fish and environment in any kind of culture systems. Hence, there is a need to understand the fish and environmental health status and their management for sustainable cage culture production.

Environmental Monitoring

Environmental quality is the most important determinant for maintaining sustainable marine cage farming. The most important physico-chemical and biological parameters to be considered in cage aquaculture include water temperature, turbidity, salinity, pH, dissolved oxygen, ammonia, nitrates, nitrites, phosphates and algal blooms. It is also understood that the effects of marine finfish cage aquaculture on water quality are of great concern to the development of an ecologically viable mariculture industry. To achieve a sustainable culture of marine fish, management of good water quality in the cage farm is of prime importance. This chapter summarizes the most predominant water quality parameters which are to be considered for management of marine cage farming.

Temperature

Water temperature has the maximum effect on fish and can be considered as a primary factor affecting the economic feasibility of a commercial aquaculture venture. Extreme temperatures can induce stress in the animal, and the metabolic activities of fish are affected, which ultimately affects the growth and health of fish. In cage culture, optimum water temperature depends on the type of cultivable species i.e., 26°C –32°C for most tropical species and 20°C–28 °C for most temperate species. Some of the fish species can survive even at varied temperatures but the growth of the fish may be affected due to temperature fluctuations. The sudden change in water temperature will affect fish metabolism, oxygen consumption, ammonia and carbon dioxide production, feeding rate, food conversion, as well as fish growth. The best solution is to select fast growing species and avoid the culture period during the months with unsuitable temperature.

Salinity

Salinity is the most important factor which can influence the ionic balance in the fish and extreme changes in salinity values further affect the growth of fish. In general, the optimum salinity required for cage culture of finfishes ranges between 10-30 ppt. However, the optimum salinity varies with the type of species cultured. Asian seabass can tolerate salinity ranging between 0-33 ppt, whereas, the salinity tolerance of cobia, pompano, snappers and groupers range between 15-35 ppt, 5-35 ppt, 15-33 ppt and 10-33 ppt respectively. Optimum salinity required for culture of Asian seabass, cobia, pompano, snappers and groupers, which are the potential candidate species for cage farming in India, are 15, 25, 15, 25 and 15 ppt respectively. It is suggested to have the culture of these species during the suitable season required for these fishes and also the area suitable and kind of water bodies. It is also suggested to culture Asian seabass in marine as well as brackishwater bodies, as the species can tolerate extreme salinity conditions. The culture of Asian seabass can be practised as in brackishwater areas and in controlled pond conditions as coastal farming. Cobia farming can be done preferably in marine water bodies as the growth rate of cobia is high under high saline conditions in marine water bodies. Pompano, *Trachinotus blochi*, Indian Pompano, *T.mookalee* can be cultured both in marine and brackishwater areas in cages and also in ponds as it tolerates all the salinities and the growth rate is more in brackishwater bodies.

Hydrogen ion index (pH)

The suitable pH for most marine species is from 7.0 to 8.5. The pH values vary directly or indirectly with other water parameters like salinity and temperature, which also influences the dissolved oxygen and ammonia levels. Extreme values of pH can directly damage gill surfaces, leading to death of fish.

Dissolved oxygen

Dissolved oxygen is one of the prime factor that influences the fish health and growth in marine farms. DO is found to be a very essential element for the maintenance of osmotic activity and also digestion and assimilation of food. DO levels are mainly influenced by other environmental factors, such as temperature and salinity, and the levels decrease with increase in temperature and salinity. Ideal dissolved oxygen levels required for cage culture of marine fish range between 6-9 ppm. However, the oxygen consumption of fish varies, with species, the pelagic fish like snapper and seabass requiring more than demersal species such as grouper. In general, dissolved oxygen should preferably be around 6 ppm or more and never less than 4 ppm for pelagic fish or 3 ppm for demersal species. In the case of cage culture, benthic organisms and sediment wastes may also reduce the oxygen level. Depletion of DO always occurs during night time at neap tide in summer. It is a known factor that the algal community forms a net oxygen consumer and the occurrence of algal blooms more in the areas where nutrient flux is more, and this can lead to the oxygen depletion in water columns. Hence, it is always suggestible to culture the fish in the open waters with sufficient currents that can remove the settled particulate matter and wastes at the bottom.

Turbidity

Turbidity indicates the degree of optical clearness of seawater affected by the existence of dissolved matters, suspended particles and also tides and water currents. The suspended particles should be < 2 mg/L for cage farming of fish in marine waters. Fish wastes and the feed particulates are two major sources of turbidity in cage culture. Increase in turbidity of water results in decrease in light penetration, which in turn affects the phytoplankton production and may further affect photosynthesis of benthic vegetation, and this leads to an increase in microbial loads and in ammonia levels at the cage culture site. During monsoon season, more freshwater runoff will influence the turbidity of the water. Freshwater runoff due to rains may lead to leaching of heavy metals from industrial effluents and suspension of organic and inorganic solids in the water column. Deposition of solid organic and inorganic materials to the bottom, due to heavy rains, may act as substrate for fouling organisms on the nets, which further prevents proper water circulation. Suspended sediments are also responsible for choking of fish gills, and may lead to mortality due to asphyxiation. Hence, in order to avoid the settlement of suspended particles in the cage, it is preferable to have the culture at sites where high flushing rate conditions are available. .

Nutrients

The ammonia-nitrogen levels in the water should be less than 0.1 mg l-1. Ammonia nitrogen levels in water increase by the decomposition of uneaten food and debris at the bottom, and can affect the fish. Normally in the coastal areas, sewage discharge and industrial pollution are the main sources of higher level of ammonia in seawater. The total inorganic nitrogen of water should be < 0.1 mg l-1 for a better fish culture operations. The excessive amount of nitrite in water leads to the oxidation of iron in fish haemoglobin, which causes hypoxia in fish. Total inorganic phosphorous plays an important role in growth of algae and other aquatic plants and it should always be < 0.015 mg l-1. Excess of phosphorous levels lead to algal blooms.

Algal blooms

A number of marine algae groups form blooms, including diatoms, Cyanobacteria and dinoflagellates, which interfere with fish gill function. Excessive algal blooms can happen whenever the suitable conditions, such as higher light intensity, higher nutrient level, warm water temperature, stagnant hydrological conditions, prevail. Algal blooms can affect fish by damaging fish gills by clogging and they also compete with fish for dissolved oxygen during night time. Red tides commonly occur in warm water, especially during summer months. Cage site should be selected in those areas where there is no occurrence of blooms and also where the waters are stagnant.

Maintaining good water quality of the marine cage culture operations is important to maintain the ecological balance and also for the health of the cage cultured fish. For maintenance of good water quality, it is essential to monitor all the parameters, which influence the growth and health of the fish, at regular intervals throughout the culture period. It is important to develop standard protocols for water quality management for the cultivation of different species. A standard policy should be clearly developed for the water quality criteria to be considered while selecting a site for cage culture operations.

Protocol for the environmental management of marine cage farms

i) Designing of the basic sampling data

Designing of sampling data collection on location, occupation, markers, features of the installation works, characteristics of production (species, quantities to be produced, etc.), characteristics of management (feeding, medication, waste treatment, production cycles, etc.) must be prepared prior to the sampling collection and monitoring of the data.

Sampling: Regarding the sampling design, in each case this should be decided on the basis of previous records existing on the zone. If no previous knowledge is available, the required minimum could be:

(a) A minimum of two samplings in extreme seasons: winter and summer must be taken.

(b) Five sampling points, whose design should be based on the main dispersal of the waste from the cages. Of these points, at least one should be below the point where the cages are to be installed and another should serve as a reference point for the future in an area unlikely to be affected.

(c) The sampling depths can be decided in accordance with the type of culture system.

(ii) Water column

Temperature, salinity, dissolved oxygen, optical properties (turbidity, suspended solids, Secchi disk transparency), nutrients (phosphorus, ammonium and nitrogen), chlorophyll. The data must be used to analyse and compare (once the culture is functioning) the real impacts correspond to the predicted ones.

(iii) Sediments

Distribution of the soft substrate in the area (linked to bathymetry) with data on granulometry, organic matter and redox potential must be studied before and after the culture period.

(iv) Bottom communities

Influence on the presence of communities with a high ecological value must be checked at regular intervals. Besides identification, data on richness of species, abundance, biomass and diversity should be available.

Environmental Health Management

Mariculture, especially cage farming of finfish and shellfish in marine and brackishwaters is highly economically viable culture system in the world due to its high production and export market value. But, high density production in a confined volume will always cause disease outbreaks which further leads to economic loss in a short span of time. Occurrence of diseases in cage culture varies between the species and environment and is mainly due to the lack of management practices. In recent years, prevalence and spread of diseases and 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, parasite and pathogens profiling, surveillance and monitoring programmes and also development and implementation of preventive protocols as better management practices of cage farming is the need of the hour. Many bacterial diseases of cage 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 *Vibrio*s, *V.harveyi* and *V.alginolyticus* and *Photobacterium damselae* are the most pathogenic bacteria of cage 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. Wild and prey fishes are also contemplated as reservoirs and carriers of the pathogen. The dynamic nature of 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. Intake water and feed are considered as the reservoirs for *Vibrio* and serve as natural transmission path for *Vibrios* 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 chaemotherapy and application of antibiotics. Oxytetracycline is the most common antibiotic used in hatchery and culture systems. But 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 its is always suggested that the bacterial infections can be controlled by usage of probiotics in hatchery and cage systems in addition to the water quality management of environment. Chlorination is the best preventive measure at hatcheries to prevent the attack of any disease.

Application of probiotics in Mariculture

Probiotic is a microbial supplement with living microorganisms, with beneficial effects on the host, by modifying its microbial community associated with the host or its cultivation environment, by ensuring improved utilization of the artificial feed or its nutritional value, enhancing the host response toward diseases and by improving its vigor in general (Csaba Hancz, 2022). The application of probiotics offers effective new and sustainable ways of maintaining good water quality and even increasing the biomass of natural food organisms in different pond cultures Usage of water and feed probiotics in hatchery tanks as well the feed probiotics along with feed in cage culture are the best preventive measure for occurrence of any bacterial infections. it is always suggested that the bacterial infections can be controlled by usage of probiotics in Mariculture systems (open and closed), in addition to the other water quality management.

Application of Probiotics for Enhancement of growth and survival

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

Methods of Probiotic Application

The main goals of using probiotics, similar to disease prevention and treatment, can be achieved by application through feed or water. Different administration modes can be applied depending on factors such as probiotics used, supplementation form, vector of administration, dosage level, and duration of application.

Oral Administration Via Diet

Mixing probiotics into the feed is the most widely used method. Probiotics can be applied in the feed or added to the tank or pond water to ensure protection against infection. Parabiotics are the inactivated microbial cells of probiotics containing cell components such as peptidoglycans, surface proteins, etc. which have advantages over probiotics such as availability in their pure form, ease in production and storage, and that they are even more likely to trigger only the targeted responses by specific ligand-receptor interactions. Probiotic administration can be continuous or at regular intervals. Most studies carried out continuous feeding of the host fish for a wide range of time, varying from 15 to 94 days.

Application of multistrain Probiotics

Using multiple-strain products has the advantage of being active against a wider range of conditions and species. Co-administrating probiotics with prebiotics and/or plant products are also widespread. Research on multistrain probiotic applications indicates multi-species probiotics containing Bacillus spp. ($1x10^9$ CFU/mL) and Lactobacillus spp. ($1x10^{11}$ CFU/mL) provided at concentrations of 0, 0.5, and 1.0 mL/L in water for 8 weeks enhanced the growth of fish by upgrading gut, liver, and muscle health. Several reports are available on impact of probiotics in aquaculture/mariculture including enhancement of fish growth performance, immune response, and resistance against some pathogenic bacteria.

Using Live Feed for Probiotics' Encapsulation

This method proved to be a viable and effective method since probiotics can even proliferate on the live feed. The enrichment of live feed such as rotifer, copepods, and Artemia with probiotics proved to be a success.

Improving Water Quality

Using probiotics for improving the quality of culture water is especially associated with Bacillus sp. because Gram-positive bacteria better convert organic matter back to CO2 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 to the water directly or 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. Usage of potential marine nitrifying bacterial consortium is the best way of biological control measure for ammonia reduction and Vibrio elimination in Marine Hatchery systems. Periodic sterilization of biofilters and the entire system helps in removal and elimination of organic load and pathogenic bacterial loads. In addition to this, usage of commercially available water and feed probiotics in Marine culture systems are the best preventive measures for occurrence of any bacterial infections. Increase of pathogenic *Vibrio* loads due to high levels of ammonia in the Marine culture systems cause fish mortalities. Management of biofilteration system is the most crucial element in Marine culture systems. 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. 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. Periodic sterilization of the biofilter is essential in Recirculating systems that encounter with frequent disease outbreaks. A dual biofiltration system helps in reducing the pathogenic load in the water. Further investigations on microbiome dynamics of water, biofilm and biofilter is essential to develop health management protocols for Marine Aquaculture systems.

General Protocols for Environment and Fish Health Management in Mariculture

Disease and Health management of cage cultured and hatchery reared 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 cage as well as hatchery systems

- Development of rapid and sensitive disease diagnostic kits for application in field will be more helpful for disease diagnosis in early stages which helps in taking further steps for control and management practices.
- 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 and wild collected fish seed 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 in the marine cage farm to understand the health condition in relation with water and sediment quality.
- Maintenance of optimum stocking density is always suggestible in order to avoid stress due to over stocking in cages which may lead to develop opportunistic secondary infections due to stress factors
- It is suggested to avoid use of trash fish which may be one of the reason for transmission of parasites and pathogens
- Avoid the usage of chemicals and antibiotics in the hatchery as well as in cage farm which creates a problem of development of residues and drug resistant strains in the open systems
- Application of tested and approved protiotics, immunostimulants and vaccines always gives a better management practice to produce sustainable, pathogen free and disease resistant fish in cage culture systems.

Recommendations for better environmental health management in cage culture

- Selection of a suitable site with sufficient depth (6-10 m) is recommended to have better water exchange and to avoid the deposition of suspended wastes at the bottom. It also helps to avoid the contact of cage bottom to the sea floor which eliminates the bacterial interactions and benthic foulers.
- Cages should be installed at a place where there is a continuous water current for good exchange of bottom fish wastes and suspended materials. The water current velocity should be between 0.05 m S-1 to 1 m S-1 with a tidal amplitude of < 1 m
- To avoid the fluctuations in salinity and dissolved oxygen levels, culture of marine finfish in cages should be carried out after monsoon period and also to avoid the current velocity, which further influences deposition of suspended solids at the bottom of the cage.
- Development of nutrient and water quality threshold values
- Development of feeding strategies to improve the FCR and reduce the nutrient influx into the waters
- Regular Monitoring of water quality parameters, at weekly intervals, is essential to understand the health status of the cage environment.
- Regular net exchange, at monthly intervals, also improves the water exchange in the cages and improves the environmental health. The nets which are with biofoulers are to be brought to the shore and should be thoroughly cleaned and can be reused.
- Measures should be taken while using the farm vessel, and properly operated with minimum spill and leaks, which may cause pollution in the farm site, that may further lead to fish mortalities.
- Rotation of cages should be implemented to decrease the waste deposition
- Fish wasters, dead organisms. debris and other suspended materials must be transported to the shore and properly disposed.
- Usage of antifouling agents must be avoided and mechanical cleaning of nets and frames is highly suggestible.
- Integrated Multi Trophic Aquaculture (IMTA) must be practised in combination with other species like mussels and seaweeds, which filter the waste particulates and absorb dissolved nutrients.
- It is suggested to avoid use of trash fish which may be one of the reason for transmission of parasites and pathogens
- Avoid the usage of chemicals and antibiotics in the Mariculture systems which creates a problem of development of residues and drug resistant strains.
- Biosecurity measures in mariculture systems can keep the safety of a facility from certain disease-causing agents that are absent in particular system. Strict quarantine measures such as egg disinfection, water treatments, clean feed and disposal of mortalities, should be maintained for rearing of fish in Marine Hatchery systems.

- Biological control strategies such as using probiotics, prebiotics, and medicinal plants are widely in use. Application of tested and approved protiotics, immunostimulants and vaccines always gives a better management practice to produce sustainable, pathogen free and disease resistant fish in Mariculture systems.

Further Reading

Csaba Hancz. 2022. Application of Probiotics for Environmentally Friendly and Sustainable Aquaculture: A Review. Sustainability 2022, 14, 15479. https://doi.org/10.3390/su142215479.

FAO. 2009. Environmental impact assessment and monitoring in aquaculture. FAO Fisheries and Aquaculture Technical Paper No. 527. Rome. 57 pp. Includes a CD-ROM containing the full document (648 pp.) (www.fao.org/ docrep/ 012/i0970e/i0970e00.htm).

FAO Aquaculture Newsletter. 2017: global Aquaculture updates. An Overview of Recently Published Global Aquaculture Statistics. No. 56 : 6-7.

OIE - Manual of Diagnostic Tests for Aquatic Animals, 20TH EDITION - IN WORLD ORGANISATION FOR ANIMAL HEALTH. 2017. ISBN 978-92-95108-45-5 WWW: http://www.oie.int. Pp.289.

Loka Jayasree, Shubhadeep Ghosh, Ritesh Ranjan, Biji Xavier , Sekhar Megarajan, and Narasimhulu Sadhu, Shiva, Ponnaganti and Gopalakrishnan, A. 2022. Potential of laboratory developed marine bacterial consortium as antibacterial and growth promoting agent in mariculture. Marine Fisheries Information Service, Technical and Extension Series (252). pp. 14-16. ISSN 0254-380X

Loka, Jayasree and Sonali, S M and Saha, Purbali and Devaraj, K and Philipose, K K. 2016. Use of commercial probiotics for the improvement of water quality and rotifer density in outdoor mass culture tanks**.** Indian Journal of Fisheries, 63 (4). pp. 145-149.

Loka Jayasree, Sonali, S M., Korabu Laxman Shankar, Philipose, K K. 2012.. Environmental monitoring in Sea cage culture. In: Handbook on Opensea Cage Culture. Central Marine Fisheries Research Institute, Karwar, pp. 118-124.

Loka, Jayasree. 2018. *Health Management in Mariculture.* In: Imelda, Joseph and Kuriakose, Somy and Rajesh, N (2018) Course Manual: International Workshop-cum-Training Programme on "Fisheries and Aquaculture". Manual. ICAR_Central Marine Fisheries Research Institute, Kochi.

Philipose, K K., Loka Jayasree, Sharma, S. R. Krupesha and Damodaran, Divu., eds. 2012. Handbook on Open sea Cage Culture. Central Marine Fisheries Research Institute, Karwar.

Prema, D. 2009. Importance of water quality in marine life cage culture. In: Course manual: National training on cage culture of seabass. Imelda, Joseph and Joseph, V Edwin and Susmitha, V (eds.) CMFRI & NFDB, Kochi, pp.81-86.

Price, C.S. and J. Beck-Stimpert (editors). 2014. Best Management Practices for Marine Cage Culture Operations in the U.S. Caribbean. GCFI Special Publication Series Number 4. 52 pp.

****