Fish health management and Biosecurity Measures in **Marine RAS**

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Mariculture, especially cage farming of finfish in marine and coastal waters is a highly economically viable culture system in the world, due to its high production and export market value. Recirculation technology is emerging in the marine aquaculture industry due to its unique form of fish farming and is gaining lot of attention all over the world. 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. RAS with proper management will have several advantages such as year round culture, harvest, flexibility in species selection and site of culture, conservation of water, improvement in water quality through filtration and recycling of clean water, reduction in stress and high survival and production of fish as compared to other mariculture systems. But high density production in a confined volume without proper management will always result in disease outbreaks, which leads to economic loss in a short span of time. Consideration of good health management is the most critical part of successful and efficient operation of recirculating systems. Occurrence of diseases in recirculating systems varies between the species and is mainly due to the lack of management practices. In recent years, prevalence and spread of diseases has been increasing enormously in mariculture systems 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 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 and pathogen profiling, surveillance and monitoring programmes and also development and implementation of preventive protocols as better management practices of RAS farming, is the need of the hour. Major common diseases encounter in cultivable marine fish and their management practices are mainly discussed in this chapter.

I. VIRAL DISEASES

A. VIRAL NERVOUS NECROSIS (VNN): Viral Nervous Necrosis (VNN) or viral encephalopathy and retinopathy (VER) is the most common disease that is encountered during almost all the stages of marine fish and is found to be a hazardous and devastating disease for many species of cultured marine fish worldwide. This disease is caused by Betanodaviruses, that damage the central nervous system in susceptible fish species and typically affect younger stages of fish (fry, fingerlings and juveniles). This disease is found to be very acute in early stages of larvae and cause mortalities ranging from 40%-100%. VNN infection is reported in more than 50 fish species (both wild and cultured). In culture systems, this disease encounters in marine finfish viz., Cobia, *Rachycentron canadum,* Asian Sea bass, *Lates calcarifer,* European sea bass (*Dicentrarchus labrax*), Grouper, *Epinephelus spp.,* Striped jack *(Pseudocaranx dentex*), Gilthead sea bream (*Sparus aurata*), Golden pompano (*Trachinotus blochii),* Florida pompano *(T.carolinus).*

i. Clinical Symptoms: The clinical signs of VNN are observed in the specific behavior of affected individuals, such as loss of appetite, spiral and whirling movements, belly up at rest, loss of equilibrium and alterations in pigmentations. Infected fishes exhibit external symptoms of anorexia, darkened body colouration and loss of reflexes and assemble as clusters on the surface. The infected individuals also adopt a stationary position (vertically), keeping caudal fin and head above the water surface.

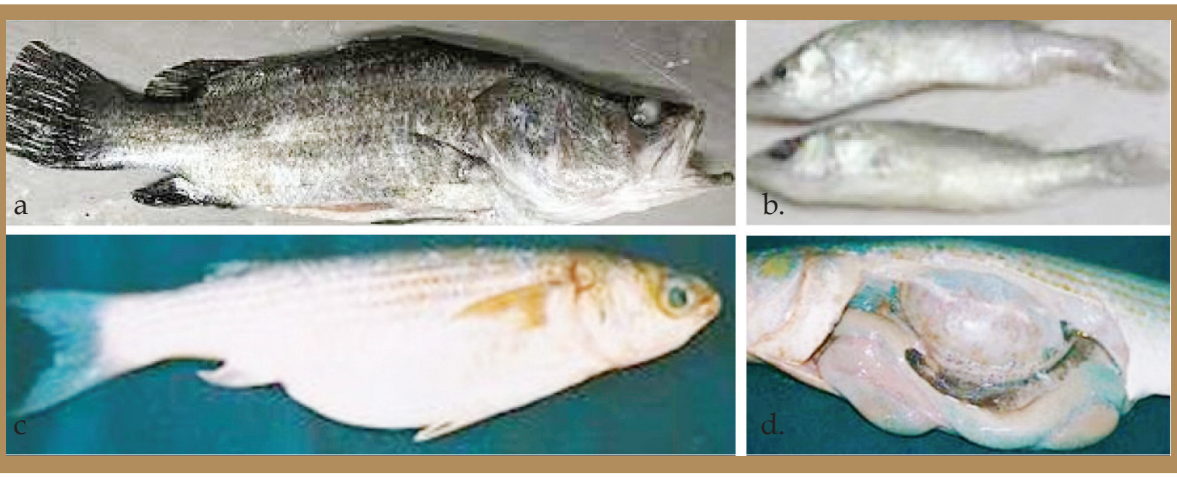


Fig.1: Viral Nervous Necrosis (VNN) infection in Asian seabass, *L. calcarifer* exhibiting darkened body colouration and exophthalmia (a & b); Swollen abdomen (c) and spleen (d).

ii. Geographical distribution: VNN virus which infects most of the cultured fish is found to be an emerging threat to global aquaculture industry. Betanodavirus infection is

one of the most disastrous diseases of mariculture due to its high virulence and broad range of hosts. Betanodavirus infections are reported in all continents including south and East Asia (Japan, Korea, Taiwan, China, Philippines, Thailand, Vietnam, Malaysia, Singapore, Indonesia, Brunei, India, China), Oceania (Australia, Tahiti), the Mediterranean (Israel, Croatia, Bosnia, Greece, Malta, Italy, France, Spain, Portugal, Tunisia), the UK, Scandinavia (Norway), and North America (USA, Canada). Recent outbreaks of VNN are reported in China, Indonesia, Singapore, Iran and India, affecting the mariculture and aquaculture industries.

iii. Aetiological agent: VNN belongs to Genus *Betanodavirus* and Family Nodaviridae and contains 2 single-stranded, positive-sense, nonpolyadenylated RNAs, RNAl and RNA2. The viral particle is non enveloped, spherical and 25 to 32 nm in diameter. Using molecular phylogenetic analyses based on partial sequences of the coat protein gene (RNA2), the betanodaviruses are classified into four genotypes: Striped Jack Nervous Necrosis Virus (SJNNV), Tiger Puffer Nervous Necrosis Virus (TPNNV), Bar Fin Flounder Nervous Necrosis Virus (BFNNV) and Red Spotted Grouper Nervous Necrosis Virus (RGNNV). Of these, the most commonly encountered betanodavirus in India is RGNNV.

Table 1: Betanodavirus genotypic and phenotypic variants :

Variants	Genotype/ Serotype	Target host fish	Optimum growth temperature
Striped jack nervous necrosis virus	SJNNV/A	Striped jack	20–25 °C
Tiger puffer nervous necrosis virus	TPNNV/B	Tiger puffer	20 °C
Barfin flounder nervous necrosis virus	BFNNV/C	Atlantic halibut, Atlantic cod, flounders	15–20 °C
Red-spotted grouper nervous necrosis virus	RGNNV/C	Asian sea bass, European sea bass, groupers, *Pompano, Seabreams*	25–30 °C

iv. Taxonomic tools: Histopathology and molecular (real time PCR) approaches are the best tools for confirmation of this outbreak in fish. Histopathological studies (H & E stain), with light microscopy reveals vacuolation and necrosis in the brain, spinal cord and eye.

The histological lesions of VNN in spinal cord and brain include severe degeneration, pyknosis, shrinkage and basophilic cells in affected areas and vacuolation throughout the central nervous system (CNS) of the fish. Vacuolated cells and vacuoles are present in the bipolar and ganglionic nuclear layer of the retina in the eyes.

The real-time PCR assay is more sensitive for detection of betanodavirus. This enhanced sensitivity can be harnessed to reveal sub-clinical VNN infections in carrier fish and to screen out infected spawners, to decrease or prevent the vertical transmission of the virus. The genome of NNV viruses consists of two single -stranded, positive-sense RNA molecules (RNA1 and RNA2) of about 3.0 and 1.4 kb in length respectively, without poly(A) extension at the 3′ end and sometimes possesses an additional segment designated RNA3. RNA1 encodes a non-structural protein, RNA-dependent RNA polymerase (RdRP) and RNA2 encodes a capsid protein (CP) of about 37-42 kDa.

v. Mode of Transmission: Viral nervous necrosis disease can be transmitted via horizontal and vertical transmission mode. In open seawater, betanodavirus can survive at low temperature for a long time; thus, its transmission outside the host from the infected area to the other area could be caused by tidal currents or boats coming from the infected area (Juniar et al. 2018; OIE 2018). This disease will be transmitted by either vertical i.e., through brood stock, eggs and larvae or horizontal through water. In some cases, the transmission will be through wild fish in cage farm. The disease is significantly influenced by water temperature and the disease was preliminary identified as Summer Disease. Betanodaviruses can infect tropical, sub-tropical, or cold temperate species. Optimal temperature ranges for the betanodaviruses vary depending upon the strain of the virus and the species of fish. Optimal temperatures for SJNNV are 20–25 °C (68–77 °F); for BFNNV, 15–20 °C (59–68 °F); and for TPNNV, 20 °C (68°F). For RGNNV, favourable temperature ranges are approximately 25–30 °C (77–86 °F) (Hata et al. 2007). Upper temperature limits for RGNNV appear to be ~32 °C (90 °F), based on laboratory studies (Hata et al. 2007).

vi. Disease Management: Control of viral infection in open waters is difficult once the disease outbreak occurs. VNN could be very resistant in aquatic bodies and water environments and it seems very difficult to eradicate when introduced to marine or aquaculture farms. Therefore, to recognize pathways of virus transmission is very critical for control strategies. For this reason, broodstock and larval fish could be considered as viral reserves and are responsible asymptomatic carriers for horizontal transmission. The best means for control of vertical transmission is the exclusion of vectors from the culture system. The best preventive measure to eliminate the entry of the virus into the hatchery is through the elimination or segregation of infected spawners. An effective control measure of the disease in hatcheries is through washing of eggs in ozone treated water, followed by

chlorination. To avoid the vertical transmission, it is always suggestible to disinfect the broodstock tanks, use of biological filters, avoid the usage of wet feed and screening of each brood stock fish for the presence of VNN and discard the positive specimens. Horizontal transmission of VNN infection may be: via contaminated influent and rearing water, utensils, vehicles and human activity. Some effective disinfectants can inactivate the virus and prevent spread of disease such as: ozone, acid peroxygen, sodium hypochlorite and benzalkonium chloride. A vaccination method is essential to prevent the disease especially during the primary stages. Vaccinating broodfish could reduce vertical transmission of VNN and will be more acceptable by the farmers. Also, feeding of immunostimulant components could be a beneficial way to increase immunity levels in larval fish against VNN infection. Strict hygiene can help to control viral nervous necrosis within hatcheries. Furthermore, applying biosecurity measures and general hygiene practices, such the UV treatment, sanitary barriers, regular monitoring and disinfection of tanks and biological filters, disinfection of utensils and decreasing stress factors and density of larvae and juveniles are strongly recommended (OIE, 2018). Usage of virus free water in hatcheries and also screening of the virus in the fish stock before stocking into the system are the preventive measures for this disease.

B. IRIDOVIRUS: Iridovirus is one of the most serious disease problems in mariculture systems and is recorded in tropical marine fish viz. seabream, grouper, pompano and Asian Seabass. This disease mainly occurs in the initial stages of growout phase (10-50 g size fish) and the mortality is of 80-90%. Several cultured marine finfish such as cobia, snapper, red seabream, grouper, seabass are found to be susceptible for Iridovirus across the world.

i. Clinical symptoms: Gross clinical signs are dark skin (change in skin colour is a significant gross sign), pale gills, red colouration of eyes, small patches on the gills with haemorrhages and enlarged spleen. The infected fish exhibits lethargy, severe anemia and enlargement of the spleen with pronounced opercular movement (indicating an increase in respiratory effort). Infected fish also exhibit enlargement of cells in kidney, heart and intestine.

ii. Geographical distribution: The first outbreak of red sea bream iridoviral disease caused by Red sea bream iridovirus (RSIV) was recorded in cultured red sea bream, *Pagrus major* in Shikoku Island, Japan in 1990. Since 1991, the disease has caused mass mortalities not only in cultured red sea bream but also in many other cultivable species. Currently, the World Organisation for Animal Health (OIE) lists approximately 40 fish species as susceptible to RSIVD. Pathogenic iridoviruses of fish (Iridoviridae, Megalocytivirus) are genetically classified into three major groups: Red sea bream iridovirus (RSIV) group; infectious spleen and kidney necrosis virus (ISKNV) group; and turbot reddish body iridovirus (TRBIV) group. Disease caused by RSIV-group viruses primarily occur in marine fish. Disease caused by ISKNV-group isolates occur in marine and freshwater fish. Disease

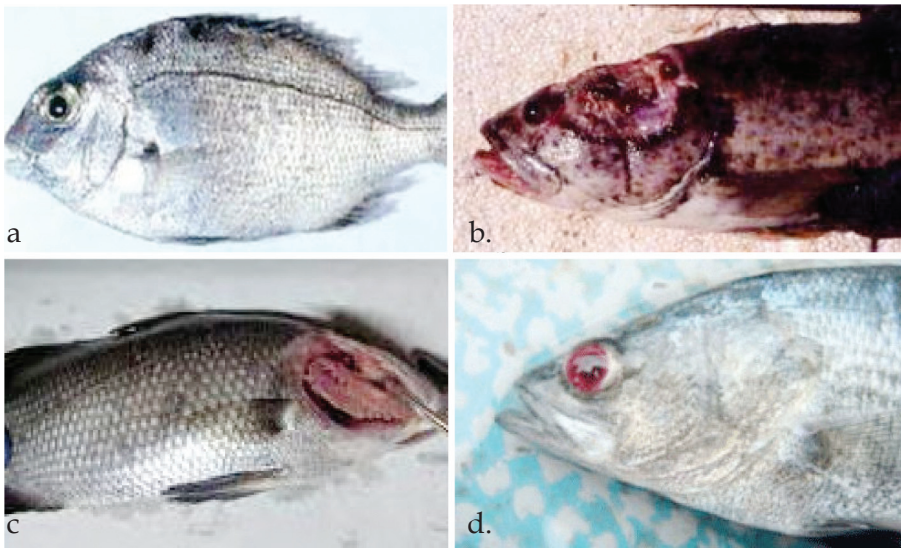


Fig. 2: Clinical symptoms of Iridovirus infection in Marine finfishes (a. Red sea bream, *Pagrus major;* b. Grouper, *Epinephelus tauvina;* c & d. Asian sea bass, *Lates calcarifer*)

associated with TRBIV-group viruses appear limited to Asian flounder species. The literature reports that RSIV-group viruses are widely distributed in East and Southeast Asia, including in China, Chinese Taipei, Hong Kong, India, Indonesia, Japan, North and South Korea, Malaysia, Micronesia, Papua New Guinea, the Philippines, Singapore, Taiwan, Thailand, and Timor-Leste (USDA 2022). Between 2010 and 2016, RSIVD occurred in maricultured Florida pompano (*Trachinotus carolinus*) in the Dominican Republic. The identified RSIV referred to as pompano iridovirus or PIV resembles (greater than 99 % nucleotide sequence) RSIV genomic sequence in Japan. As on date, there is no record of Irodovirus in Recirculating systems in India.

iii. Aetiological agent: The causative agent of RSIVD is a large, icosahedral, cytoplasmic DNA virus, RSIV classified as a member of the family Iridoviridae belonging to the genus Megalocytivirus. The pathogen is a DNA virus with a diameter of 200–240 nm. The size of viral particles ranged between 175-196 nm.

iv. Taxonomic tools: The disease can be confirmed by histopathological and molecular tools. Histopathological sections indicate that abundant blast-like inflammatory cells are clearly visible throughout the circulatory system. The enlarged cells usually have intensively basophilic and Feulgen positive cytoplasm, and an expanded nucleus with a prominent nucleolus. Enlarged cells, deeply giemsa positive, in the spleen, heart, kidney, liver and gills of infected fish, which are characteristic of this disease. Inclusion bodies containing compactly packed iridoviral virions can be seen through Electron microscopy. Necrotic

cells in the spleen and haematopoietic tissue exhibit diffuse multiplication of iridoviral virions in the cytoplasm. A simple and rapid PCR test can be done to detect irodovirus in diseased fishes using primers based on the sequence data of RSIV.

v. Mode of Transmission: Transmission is horizontal, via the water column from other infected fish. Outbreaks of disease occur at water temperatures greater than 20 °C, with viral multiplication increasing with water temperatures up to at least 28 °C. Mollusks and other invertebrates are reported to be the carriers of the virus as vectors. Vertical transmission is through brood stock fish, hence care should be taken to avoid the introduction of this virus into the system.

vi. Disease Management: Control methods currently rely on implementation of farm biosecurity and good management practices. There is no control measures of this disease once it attacks the fish and hence, it is always better to prevent the occurrence of this virus, through water exchange and screening of the fish and water regularly for the presence of the virus in order to eliminate the positive samples. Control/preventive measures such as avoidance of exposure to the pathogen, environmental manipulation, development of disease resistant-strains, health maintenance, and chemotherapy can be practised for prevention of outbreak of viral infections. Activation of non-specific defense and specific immunization based on general health maintenance seem to be the most promising prophylactic methods for the control of Iridovirus. The effectiveness of vaccination against RSIVD is evaluated using two kinds of vaccines in red sea bream under experimental conditions (Nakajima et al., 1997). A killed vaccine is commercially available in Japan for use in red sea bream, striped jack (*Pseudocaranx dentex*), Malabar grouper (*Epinephelus malabaricus*), orange spotted grouper (*E. coiodes*). It is also recommended to apply the commercially available vaccine (formalin killed) as a preventive measure. Mitigation practices include stocking with pathogen-free fish, improving biosecurity and management practices, disease surveillance testing, improving water quality, and avoiding practices that induce stress, caused due to overstocking and overfeeding. RAS reared marine fish are prone to this disease, hence attention is to be given as preventive measure. Screening of this virus in intake water and brood stock fish, that will be used for seed production and rearing in controlled conditions, is essential to prevent this disease.

II. VIBRIOSIS

Several bacterial diseases of cultivable fish in mariculture systems are reported worldwide, most of which are found to be opportunistic in nature. Vibriosis is a common disease outbreak in all stages of fish reared in Recirculating Aquaculture System. 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 harveyi*, *V. alginolyticus*, *V.*

anguillarum, V. vulnificus, V. damsela, Photobacterium damselae are the major pathogens recorded in marine fish. Among the *Vibrios, V.harveyi, V.alginolyticus, V.vulnificus, V.parahaemolyticus, V. fluvialis, V. furnisii, V. methcnikovii, V. vulnificus, V. ordalii, V. cincinnatiensis, V. carchariae, V. azureus, V. mimicus and V. damsela* and *Photobacterium damselae* are the most pathogenic bacteria of cultured fish, especially Cobia, Pompano, Snapper, Grouper, Seabream and Asian seabass which cause haemorrhagic septicaemia. Out of these, *V.harveyi, V.alginolyticus* and *Photobacterium damselae* are reported to cause mass mortalities in cultured marine fish in India. Reports on the bacterial populations in RAS and their impacts on health of marine fish are very rare. Vibrionales and Flavobaterionales are recorded as the most predominant bacterial communities in RAS. Few reports on colonization of fish pathogens on bio-film and water column of RAS are available.

i. Clinical signs: External lesions of vibriosis observed include ulcers on the skin, fins and mouth, corneal opacity, pop-eye and loss of one eye. In advanced stage, affected fish showed darkening of the skin, septicaemia, anorexia, exophthalmia, swollen abdomen, pale gill colour, erosion and hemorrhage in the fins and lesions on the skin. Infected fish exhibits lethargy, inappetance, sluggish swimming and frequent surfacing. Gills, liver and kidney are pale with profuse mucous secretions, ascitic fluid in the body cavity, yellowish-bloody fluid in the intestine, enlarged spleen and empty stomach are observed in the infected fish.

Fig. 3: Vibriosis in marine finfish (A. Cobia; B. Grouper; C. Asian seabass) : showing haemorrhages on skin, fins and tail region

ii. Geographical distribution: Bacteria of the genus *Vibrio*, which is included in the family Vibrionaceae are commonly found in coastal and estuarine waters. Many *Vibrio* species can cause disease in marine fish, crustacean and bivalve species. Moreover, many of them can infect human after a contact with these animals or consumption of these animals, which causes zoonoses. *Vibrio harveyi*, which was first isolated as a causative agent of a disease problem in a shark species in 1982, was responsible to cause diseases and mass mortalities especially in marine invertebrates and in a variety of fish species including cultured species such as salmonids, Cobia, snapper, sea breams, grouper, pompano, sea bass. Vibriosis is the most common disease outbreak of cultivable marine fishes affecting all stages of fish viz., hatchery, nursery and growout stages.

iii. Aetiological agent (s): Vibrio species are Gram-negative bacteria responsible for vibriosis disease in marine fishes and are becoming a major threat to the aquaculture industry. *Vibrio harveyi*, *V. anguillarum, V. alginolyticus, V. vulnificus, V. damsela, Photobacterium damselae* are the major pathogens recorded in marine fishes.

iv. Taxonomic tools: The disease can be identified by classical, microbiological taxonomy, histopathology and molecular diagnosis. Classical methods of identification can be done by conducting several morphological and biochemical tests using Bergeys Manual of Systematic Bacteriology (Imhoff, J.F. (2005) *Vibrio* spp. are Gram negative, motile, rod shaped facultative anerobes. Histopathological studies indicate hepatic lesions included congestion, haemorrhages and swollen hepatocytes, vacuolation, severe depletion of haemopoietic cells, deposition of haemosiderin in the spleen and peritubular vacuolar degeneration or necrosis in the renal tubules and hemorrhages and vacuolar degeneration in the liver of infected fish with vibriosis. Molecular taxonomy through 16s rRNA sequence gives the species identification as rapid diagnostic tools. Various molecular methods are developed to identify the source of pathogens and their transmission over space and time in different potential hosts. Major *Vibrio* species recorded in marine fish, which cause mortalities, are *V.harveyi, V. alginolyticus, V. damselae, V.anguillarum* and *Photobacterium damselae sub.sp.damselae*.

v. Mode of Transmission: 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 Recirculatory systems apart from water and feed.

vi. Disease Management: Vibriosis can be controlled by chemotherapy and application of antibiotics. Chloramphenicol, ciprofloxacin and oxytetracycline are recommended for the control of disease by various researchers. Oxytetracycline is the most common antibiotic used in marine 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, it is always suggested that the bacterial infections can be controlled by usage of probiotics in RAS, in addition to the other water quality management. Chlorination is the best preventive measure at hatcheries to prevent the attack of any disease. In addition to this, usage of commercially available water and feed probiotics in recirculatory

systems are the best preventive measures for occurrence of any bacterial infections. Vaccination is a well established method to control infectious diseases more safely and is an another alternative to antibiotic usage. Several advanced studies are reported on the development of species-specific vaccines for different species of vibrios, that are environmentally and clinically important in aquatic environments, and the antigenic diversities of the strains and serotype. Oral vaccination of VH1 vaccine is reported as a potential and cross-protective vaccine candidate for vibriosis. Increase of pathogenic *Vibrio* loads due to high levels of ammonia in the RAS cause fish mortalities. Management of biofilteration system is the most crucial element in RAS. 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 RAS, nitrification stability needs 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 RAS 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 RAS (water, biofilm and biofilter) is essential to develop health management protocols for RAS.

III. PARASITIC INFECTIONS

Among the parasitic infections, ectoparasites which feed on mucous, tissues and other body fluids cause the most serious disease outbreaks in larval and juvenile stages of Asian seabass, cobia and pompano. Most predominant ecoparasitic infections in fish reared RAS are *Cryptocaryoniasis*, Amyloodiniosis, Trichodiniosis, Myxosporidiosis, Microsporidiosis and other infections with monogeneans, digeneans, copepods and isopods. They cause damage to the epithelial layer resulting in haemorrhagic lesions on the skin. Many of these parasites act as vectors of bacteria and viruses which may lead to the outbreak of multiple infections in fish. Most common parasitic diseases which can encounter the marine fish reared in RAS are described in this chapter.

I. Cryptocaryoniasis: *C. irritans* is a holotrich ciliate protozoan that causes marine white spot disease or 'marine itch'. It is considered to be the most devastating parasitic disease in mariculture. *C. irritans* exhibits very low host specificity and is able to infect multiple fish species, with gilthead seabream being the most affected amongst the cultured species. *C. irritans* is widely distributed throughout the world. Cryptocaryoniasis was first reported from ornamental fish in Japan and later in USA, Thailand and China. Outbreak of Cryptocaryoniasis was recorded in various fish species and in various geographical regions

including America, Australia, England, Indonesia, Malaysia, India, China, Japan, Thailand, Korea, Israel, Spain and Greece. Cryptocaryoniasis is the greatest challenging disease-problem of cultured marine finfish viz., Pompano, red seabream, Grouper, Asian seabass and snapper.

i. Clinical signs: Fish infected with Cryptocaryon exhibit clinical symptoms such as small white spots/blisters, nodules, or patches on their fins, skin, gills, skin discoloration, anorexia, respiratory distress due to excessive mucus secretion, and a disruption of the lamellar structure of the gills. During its life cycle, the infective stage, the theront, infects the fish epithelial layer and develops to a trophont stage. This trophont feeds on tissue debris and body fluids and leaves the host after maturation, and becomes tomont cells, the external stage. Temperature is the most important factor for the outbreak of Cryptocaryoniasis. This parasite commonly encounters when temperatures are above 19 °C, mainly between 20–30°C.

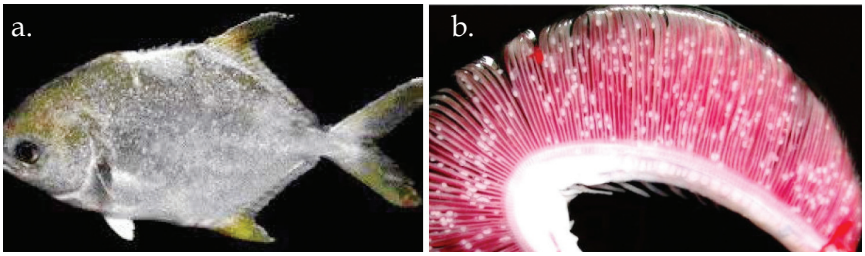


Fig. 4: *Cryptocaryon irritans infection in Golden Pompano, Trachinotus ovatus showing white nodules on the body (a) and gills (b)*

ii. Taxonomic tools: Observation of parasite trophonts under light microscopy is the basic, simple and rapid method for detection/identification of *Cryptocaryon irritans* infection. Trophonts can be detected by observing the skin, gills and fins under light microscopy. Trophonts are fusiform or pyroform, ciliated, opaque and are visible as revolving organisms under microscope. Molecular and immunological detection provides more accurate detection of this parasite. PCR assay (SYBR Green intercalating dye) can be used for rapid and quantitative detection of this parasite.

iii. Control / preventive measures: Control of Cryptocaryon infection is possible by using physical, chemical methods, plant components, biological control and vaccination methods. Several chemical agents including formalin, copper sulphate, dyes, quinine derivatives are more effective for control of Cryptocaryon infection. Administration of Sulphamethoxazole – Trimethoprim in optimum dosage will be the best therapy for inhibition of encystment of *C. irritans* infection. Several reports from China indicated removal of trophonts and tomonts by using herbal medicine extracts and antiparasitic phytochemical compounds. Biological control of this infection using live organisms viz., cleaner fish/cleaner

shrimp, which are not susceptible to this parasite, can eliminate the free swimming trophonts. Vaccination is another alternative method for prevention of this parasite. Research on development of vaccines using good candidate antigen, that will be safe, efficient and cost effective, will have practical application in control of Cryptocaryon infections.

II. Amyloodiniosis: Amyloodiniosis is one of the serious disease problem of marine fishes infesting both food fish and ornamental fish all over the world. *Amyloodinium ocellatum* is a dinoflagellate that infects gills and skin surface of both marine and brackish water fishes. The disease caused by these organisms is referred as Gold dust disease, due to its smooth shiny appearance in the heavily infected fish. Amyloodiniosis infections are reported in several marine cultivable food fishes with a wide geographical distribution viz., USA, Mexico, West Indies, Spain, Italy, France, Israel, Turkey, Kuwait, Australia, Taiwan, Thailand, Philippines and India.

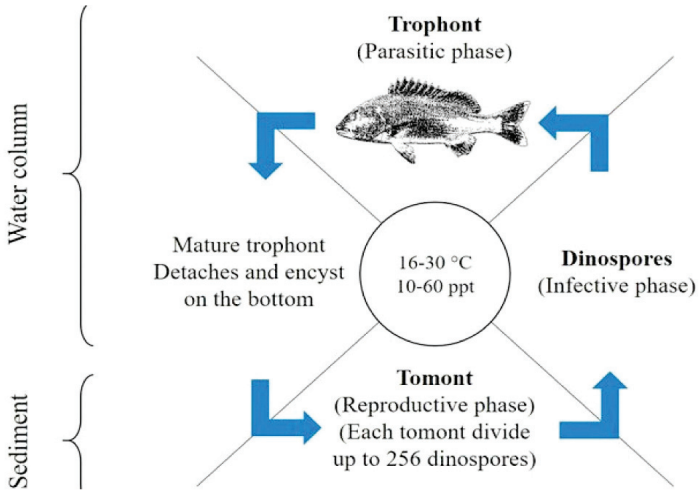


Fig. 5: Life cycle of *Amyloodinium ocellatum* (E. Brown & Hovasse, 1946)

i. Clinical signs: The infected fish exhibits clinical symptoms such as asphyxiation, respiratory distress, dark/brown discoloration, erratic swimming on surface, aggregation at the bottom and loss of equilibrium. External examination of skin surface, fins, and gills shows the presence of lesions on skin and are with velvety appearance. The infected fish exhibits haemorrhages, emaciation, deformities and abrasions on different body parts and fins.

ii. Taxonomic tools: Identification of the parasite can be done by microscopical examination of wet mount preparations and stained with iodine. Morphological features and measurement of dimensions of the parasitic infestations on skin and gills under microscope can be followed for identification of the *Amyloodinium* parasite. Skin parasites

are observed in a dark room using a shining flashlight on top of the fish and a dark background also helps easy identification of the parasite. Presumptive diagnosis of oodinid infestation can be done from the velvet structure. Scrapping of gills and skin under a dissecting microscope can help confirmation of presence of trophonts or tomonts. Specific Sequences of the SSU-rRNA genes from geographical isolates of *A. ocellatum* and development of *Amyloodinium*-specific oligonucleotide primers gives more than 99% identification of the parasite with a wide range of geographical distribution. PCR assay specific for *Amyloodinium* is highly sensitive and reliable for monitoring of pathogen load in susceptible populations.

a. *b.* c.

Fig. 6: Amyloodinium infection in silver pompano, *T. blochii* : (a. Erosion of caudal fin; b. Gills with trophonts; c. Tomonts under microscope 100X)

a. *b.* c.

Fig.7: Asian seabass, *L. calcarifer* infected with *Amyloodinium* showing a. erosion of fins and shedding of scales and b. primary gill lamellae; c. Wet mount of *Amyloodinium* Trophont

iii. Control/preventive measures: The parasite can be controlled using copper sulphate, Benzalkonium chloride, chloroquine phosphate, Odnonil and formalin. To control the outbreaks of infectious agents, including *A. ocellatum*, application of sufficient biosecurity measures in marine culture systems is essential. Disinfection of equipment, avoidance of introduction of parasite into the system, proper disinfection treatments in the entire system such as filtration of seawater using micro screen drum or bead filters and fresh water dipping gives better biosecurity measures to control amyloodiniosis. Vaccine development and application of immunostimulants to boost fish resistance against parasites is the best alternative measure for elimination or prevention of this disease in RAS.

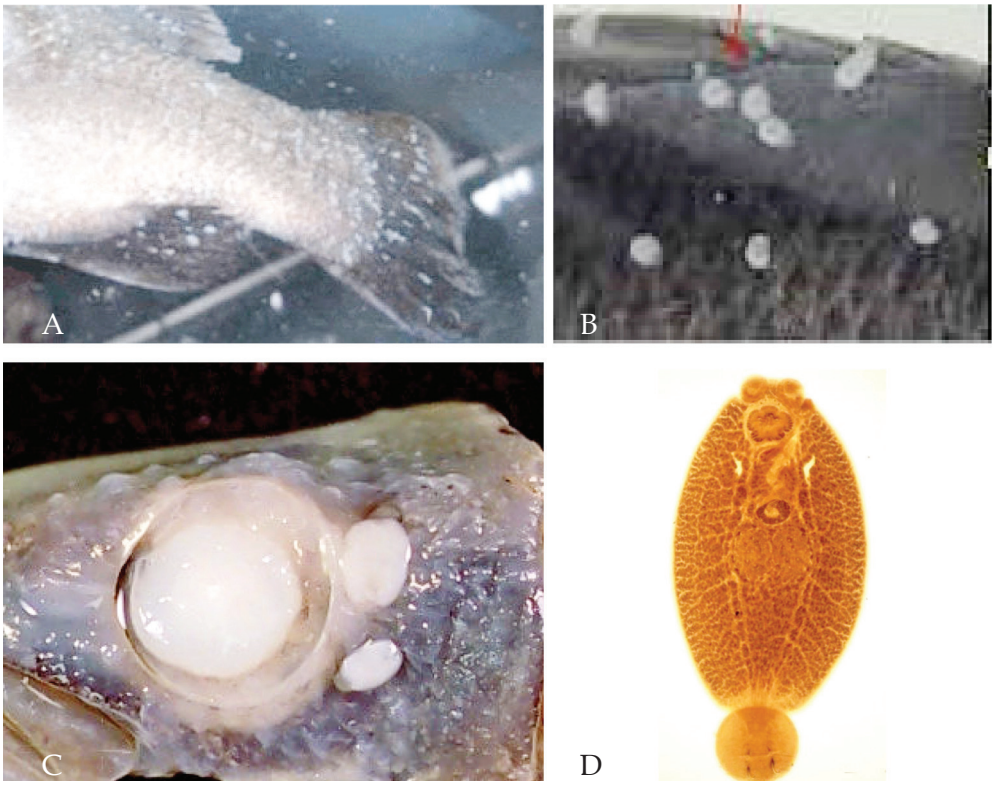
III. Trichodiniosis: Trichodiniosis or infection caused by the ectoparasitic motile ciliate protozoan trichodinid is a common parasitic disease of marine fish. It is reported in Indonesia, Philippines, Malaysia, Singapore, Thailand and Brunei Darussalam. This parasite infects most of the marine fish such as groupers, snappers and seabass. All phases of culture, hatchery, nursery and grow-out are found to be affected with this parasite. The infected fish exhibits clinical symptoms such as pale gills, eroded fins, excessive mucus production on the body surface, lesions on gills and the fish becomes weak and sluggish. Heavy infestation of this parasite interferes in respiration of fish which lead high mortalities. Transmission of this parasite is horizontal i.e., through water or feed. The parasite can be identified by microscopic examination of wet mounts of scrapings from the skin and gills and staining them with silver nitrate trichrome. Trichodiniosis can be caused by *Trichodina, Trichodinella and Tripartiella*. The three genera can be differentiated by the shape of their denticle. Freshwater bath treatment for 3 days and application of 200 ppm formalin with strong aeration for two days can be used as control measure for elimination of this parasite.

IV. Myxosporidiosis: Myxozoan infections, caused by *Hennegua* spp., can lead to major disease outbreaks and heavy losses in marine systems. *Henneguya* species target tissues including the skin, fins, urinary bladder, kidney, gall bladder, intestine and ovary. Myxozoans can be diagnosed based on the shape of the parasite on wet mount examination. Myxozoan-infected tissue appears either as a mass, nodule, granuloma, tissue discoloration or cyst. Microscopic examination of fresh tissues and histological sections using Giemsa stain shows presence of spores containing polar capsules in affected tissues. This parasite found to infect several cultivable marine finfish viz., *Rachycentron canadum, Trachinotus blochii, T.ovatus, Lutjanus argentimaculatus, Dicentrarchus labrax, Sparus aurata.* Currently, there are no effective drugs available for the treatment of myxozoan infections. Disinfection and quarantine measures are challenging as myxospores are often resistant to many common disinfectants. Application of chlorine (13 ppm) for 10 min, hydrogen peroxide (10%) for 10 min, and povidone-iodine (50%) solution (5000 ppm active iodine) for 60 min are effective for inactivation of actinospores of myxosporidians. Myxozoan infections can be avoided by using good biosecurity measures such as elimination of oligochaetes and polychaetes or through proper water management practices, as no effective treatment exists and disinfecting the environment is found challenging.

V. Microsporidiosis: Microsporidiosis is an infection caused by a protozoan parasite, microsporidian. The disease is reported in grouper, European seabass, gilthead seabream. *Glugea* sp. and *Pleistophora* sp. are reported from cultured grouper fish. The parasite can affect the fish in RAS. The infected fish shows clinical symptoms such as swollen abdomen and black/brown nodules in the infected tissues which are filled with pear-shaped spores

(6 µm size). Microscopic examination of fresh-squashes of Giemsa-stained smears from infected tissues reveals oval-shaped spores. Transmission is horizontal through oral ingestion of spores. Entry of this parasite can be prevented by disinfection of culture systems with iodine/chlorine solutions, good water exchange, avoidance of feeding with contaminated trash fish.

VI. Monogenean infections: Monogenean infections are commonly encountered in most of the marine finfishes with a wide geographical distribution. *Neobenedenia* sp., *Benedenia* sp., *Pseudorhabdosynochus* spp., *Megalocotyloides* spp. and *Diplectanum epinepheli* are the most common monogeneans that attack the gills and skin of marine fish. The parasite attaches on the eyes, body surface and gills of fish. The affected fish exhibits clinical symptoms such as darkened body surface, disolouration of gills, eroded fins. Affected fish exhibits loss of appetite and abnormal swimming behavior near the water surface. An increased mucus production and hemorrhagic lesions on the body surface, resulting in extensive damage of gill epithelium and repiratory problem and this leads into mass mortality of fish. Secondary infections like Vibriosis may also found to be associated with this parasite. Diagnosis is



Monogenean infections (*Neobenedinia* spp.) on dorsal fin of Asian seabass *(A & B)* and Cobia (C &D)

done by gross macroscopic examination of the body surface and gills of affected fish. Confirmation is by microscopic examination of mucus from the gills and attachment of parasites to the gill filaments can be seen under microscope. Transmission is horizontal and high stocking density provides greater opportunity for faster infection. Control methods include freshwater bath treatment and chemical treatment using hydrogen peroxide (200 ppm), formalin (100-200 ppm) and continuous aeration must be provided during the treatment. Oral administration of praziquantel can be used to control the parasite.

Most of the parasitic infections can be controlled by the application of formalin treatment with specific dosages as per the prevalence of parasitic occurrence. Health monitoring and early diagnosis is key for control of parasitic diseases. It is also suggested that the application of vaccines of respective parasites and pathogens always give the best management practice in Recirculating Aquaculture Systems.

VII. Digenean infections : Didymozoid digeneans are long, parasitic flatworms that form capsules or cysts on the gills of the host fish. It is reported in several cultivable species of groupers viz., *Epinephelus coioides, E. malabaricus, E. tauvina and Epinephelus* sp. from Indonesia, Kuwait, Malaysia, Myanmar, the Philippines and Thailand. The causative agent, *Gonapodasmius epinepheli* is the most common didymozoid which is reported from *E. coioides* in the Philippines and from *E. malabaricus* in Thailand. The affected fish exhibits clinical signs viz., presence of small, opaque-white to yellow cysts on first gill arch and distorted gill lamellae. Didymozoids are found to infect fish both in nursery and growout stages. Gross and microscopic examinations of the gills shows opaque-white or yellow capsules attached along the posterior surface of the gill filaments. The capsules contain tubular, long, thread-like worms tightly packed inside. Digeneans generally have two sucker-like attachment organs located at the anterior and ventral portions. The intermediate hosts (gastropod molluscs), which may be carriers of the larval stage of the parasite should be eliminated from the culture facility.

VIII. Copepod infections: The caligid copepods are external crustacean parasites with segmented bodies covered by shell with jointed appendages. Caligid copepods are reported to infect cultivable grouper species viz., *Epinephelus coioides and E. malabaricus*. It is recorded in Indonesia, Malaysia, the Philippines, Thailand and Vietnam. The most common caligid copepods in grouper culture are *Caligus epidemicus* and other *Caligus* sp. *and Lepeophtheirus* sp. and found to infect brood stock, nursery and grow out fish. These parasites are transparent and appear as white patches on the body surface and fins of fish. Affected fish exhibit symptoms such as erosions on skin, lumpy body surface with haemorrhages and ulcers, excessive mucus production, shedding of scales, loss of appetite and fishes become weak and swim sluggishly near the water surface or show flashing behaviour. Heavy

infections lead to secondary bacterial infections, resulting in mass mortality. Transmission is horizontal. The parasites are observed as transparent, with segmented bodies covered by shell with jointed appendages under microscopic examinations of infected areas. Control or prevention of the parasites can be done by freshwater bath for 10-15 minutes, or chemical bath treatment using hydrogen peroxide (150 ppm) for 30 minutes or with formalin (200-250 ppm) for 1 hour.

IX. Isopod infections: Isopod parasites are recorded *in Epinephelus coioides and E. malabaricus* in Indonesia and Thailand. The parasite attaches on the body surface, mouth, nasal cavity and opercular cavity. The affected fish exhibits necrosis of gill filaments and dermis, loss of appetite, reduced opercular movement and slow growth rate. Fish becomes weak when the isopod resides in the buccal cavity and heavy infections may lead to sudden mass mortalities in young fish. The isopod *Rhexanella* sp. are reported in *E. coioides* by gross and microscopic observations. Transmission is horizontal. Control or preventive measures include bath treatment using formalin (200 ppm) and the treated fish should be transferred to clean, parasite-free facility.

Biosecurity Protocols for Fish Health Management of RAsystems:

Disease and Health management of cultured marine finfish in RAS is always a challenging aspect due to its dynamic nature in marine waters and also when dealt with broodstock management practices. Hence, the following protocols should always be followed as a part of better health management practices for rearing of marine finfish in RAS.

- Development of rapid and sensitive disease diagnostic kits 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 nursery phase.
- 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, which may lead to development of 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 mariculture systems which create a problem of development of residues and drug resistant strains.
- Biological and chemical disease control strategies such as using probiotics, prebiotics, and medicinal plants are widely in use. 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 RAS.
- 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. Although several beneficial bacterial consortia are available in the market, development of potential marine nitrifying bacterial consortium is the best way of biological control measure for ammonia reduction and Vibrio elimination in RAS. Periodic sterilization of biofilters and the entire system helps in removal and elimination of organic load and pathogenic bacterial loads.
- 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 RAS.

Source :

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