Diseases of Cultured Groupers

Kazuya Nagasawa and Erlinda R. Cruz-Lacierda
Editors

Southeast Asian Fisheries Development Center
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Preface

Aquaculture production has been growing for over two decades in Southeast Asia. Fish disease is always a major constraint and threat to aquaculture production in this region. Numerous infectious diseases have been reported from fish and shrimp cultured in the region. Currently, several emerging diseases are also recognized. These diseases cause large-scale mass mortalities of cultured species, inducing devastating losses to regional aquaculture production.

Groupers are recognized as economically-important marine fish and abundantly cultured in Southeast Asia for domestic consumption and overseas export. Various diseases occur in grouper aquaculture and frequently create serious problems.

The Government of Japan is funding the Aquaculture Department of the Southeast Asian Fisheries Development Center (SEAFDEC/AQD) based in Tigbauan, Iloilo, Philippines, through the Regional Fish Disease Project to address various fish disease problems in Southeast Asia. Under the title of "Development of Fish Disease Inspection Methodologies for Artificially-Bred Seeds," the first phase of the project was implemented for five years from 2000 to 2004. During this period, many activities including research, hands-on trainings, international meetings and dissemination of information were conducted.

The present publication entitled "***Diseases of Cultured Groupers***" is one of the outputs from the Regional Fish Disease Project. This book compiles information on various diseases of groupers and was written by the staff of Fish Health Section of SEAFDEC/AQD (Dr. Gilda D. Lio-Po, Dr. Erlinda R. Cruz-Lacierda, Dr. Celia R. Lavilla-Pitogo, Dr. Leobert D. de la Peña, Ms. Eleonor A. Tendencia, Dr. Edgar C. Amar, Dr. Elena S. Catap and Ms. Gregoria E. Erazo-Pagador).

I hope that the book will provide practical information to prevent and manage disease outbreaks, reduce mortality, and improve fish production, and thus utilize more efficiently the existing grouper seed resources.

I sincerely thank three scientists, Dr. Leong Tak Seng (LTS Consultancy, Penang, Malaysia), Dr. Supranee Chinabut (Department of Fisheries, Bangkok, Thailand) and Dr. Toshihiro Nakai (Faculty of Applied Biological Science, Hiroshima University, Higashihiroshima, Japan) for carefully reviewing the draft of this book. I also gratefully acknowledge the following persons for giving permission to reproduce their photographs or illustrations for this book: Mr. Yukio Maeno (Seikai National Research Institute of Fisheries, Nagasaki, Japan), Dr. Kei Yuasa (National Research Institute of Aquaculture, Tamaki, Japan), Dr. Somkiat Kanchanakhan (Aquatic Animal Health Research Institute, Bangkok, Thailand), Mr. Zafran (Gondol Research Institute for Mariculture, Bali, Indonesia), Ms. Isti Koesharyani (Fish Health Research Laboratory, Jakarta, Indonesia), Mr. Lin Li (Guandong Daya Wan Fisheries Development Center, Guandong, China), Dr. Il-Hoi Kim (Department of Biology, Kangreung National University, Kangreung, Korea) and Dr. Leong Tak Seng.

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Introduction

The groupers belong to the subfamily Epinephelinae and the family Serranidae. They are widely distributed in the tropical and subtropical regions. The groupers are of great economic value and are a major component of the coastal fisheries in Asia. At least 21 species of groupers are cultured in Asia with the following as the most popular (English and scientific names follow suggestions of FishBase, http://www.fishbase.org/): orange-spotted grouper (*Epinephelus coioides*, junior synonym: *E. suillus*); malabar grouper (*E. malabaricus*, junior synonym: *E. salmoides*); greasy grouper (*E. tauvina*); brown-marbled grouper (*E. fuscoguttatus*); duskytail grouper (*E. bleekeri*); giant grouper (*E. lanceolatus*); palemargin grouper (*E. bontoides*); camouflage grouper (*E. polyphekadion*); convict grouper (*E. septemfasciatus*); Hong Kong grouper (*E. akaara*); longtooth grouper (*E. bruneus*, junior synonym: *E. moara*); yellow grouper (*E. awoara*); leopard coralgrouper (*Plectropomus leopardus*); spotted coralgrouper (*P. maculatus*); and humpback grouper (*Cromileptes altivelis*).

In 1997, the Asia-Pacific region contributed about 90% to the total world aquaculture production. The regional production of cultured groupers was estimated at 15,000 metric tons (MT), with China as the biggest producer contributing 8,000 MT followed closely by Indonesia. Other countries in the region commonly produce 1,000-2,000 MT annually in 1990-1997.

Groupers are generally cultured in floating net cages or earthen ponds, but cage culture is more common in Southeast Asia. Although grouper culture is popular in the region, its sustainability is hampered by the limited availability of seed stocks. Majority of the grouper fry and fingerlings are wild-caught, with some produced from hatcheries.

A major production constraint in grouper culture is heavy mortality due to diseases. Aside from the health status of the fish during culture, they are also subjected to considerable stress during collection and transportation. Diseases of cultured groupers may be caused by infectious disease agents such as viruses, bacteria, fungi and parasites. Non-infectious disease agents such as nutritional imbalances and environmental factors may also lead to disease.

The objective of this book is to provide information on diseases observed among the major species of groupers cultured in the region. It includes the common name of the disease, causative agent, stages affected, gross clinical signs, effects on the host, transmission, diagnosis, and methods of prevention and control. A list of references at the end of each chapter is included for detailed information about the diseases. Appendices and Glossary are found at the end of the book. In cases where treatment is indicated, it is best to do this under the guidance of a fish health specialist to avoid unnecessary loss of stock.

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Chapter 1. Viral Diseases

Gilda D. Lio-Po and Leobert D. de la Peña

Some viral infections are serious diseases of groupers causing heavy mortalities. In most cases, larval stages are the most susceptible stage. With the carnivorous nature of groupers, they can readily ingest viral pathogens from live fish food or trash fish that carry the viral pathogens. Moreover, viruses are able to effect vertical transmission from broodstocks that are likely carriers of the virus. Survivors of viral epizootics can be carriers of viral pathogens.

This chapter focuses on current information on the major viral infections of groupers, i.e., viral nervous necrosis (VNN) and viral infections attributed to the family Iridoviridae.

VIRAL NERVOUS NECROSIS (VNN)

This disease is also known as viral encephalopathy and retinopathy (VER), viral vacuolating encephalopathy and retinopathy, paralytic syndrome, spinning grouper disease, fish encephalitis, piscine neuropathy and whirling disease. Grouper species susceptible to VNN include *Epinephelus akaara, E. coioides, E. tauvina, E. fuscoguttatus, E. septemfasciatus, E. malabaricus, E. bruneus* and *Cromileptes altivelis*. The VNN has also been reported in other fish species worldwide, i.e., *Anguilla anguilla* (Anguillidae), *Gadus morhua* (Gadidae), *Lates calcarifer*, *Lateolabrax japonicus* (Centropomidae), *Dicentrarchus labrax* (Percichthydae), *Latris lineata* (Latridae), *Pseudocaranx dentex, Seriola dumerili*, *Trchinotus blochii* (Carangidae), *Sparus auratus* (Sparidae), *Sciabips ocellatus, Umbriana cirrosa, Atactoscion nobilis* (Sciaenidae), *Oplegnathus fasciatus, O. punctatus* (Oplegnathidae), *Oxyeleotris lineolatus* (Eleotridae), *Racycentron canadum* (Rachycentridae), *Verasper moseri, Hippoglossus hippoglossus* (Pleuronectidae), *Paralichthys olivaceus, Scopthalmus maximus* (Bothidae), *Solea solea* (Soleidae) and *Takifugu rubripes* (Tetraodontidae). The geographic distribution of VNN is worldwide having been reported to occur in Australia, Asia (Japan, Taiwan, People's Republic of China, Korea, Thailand, Indonesia, Brunei Darussalam, Singapore, Philippines), Tahiti, North America (United States of America) and Europe (France, Greece, Italy, Malta, Norway, United Kingdom).

Causative agent:

Piscine nodavirus of the genus *Betanodavirus* (25-30 nm) is the causative agent of VNN (Fig. 1-1). Piscine nodavirus consists of 4 genotypes: red-spotted grouper nervous necrosis virus (RGNNV), striped jack nervous necrosis virus (SJNNV), barfin flounder nervous necrosis virus (BFNNV) and tiger puffer nervous necrosis virus (TPNNV).

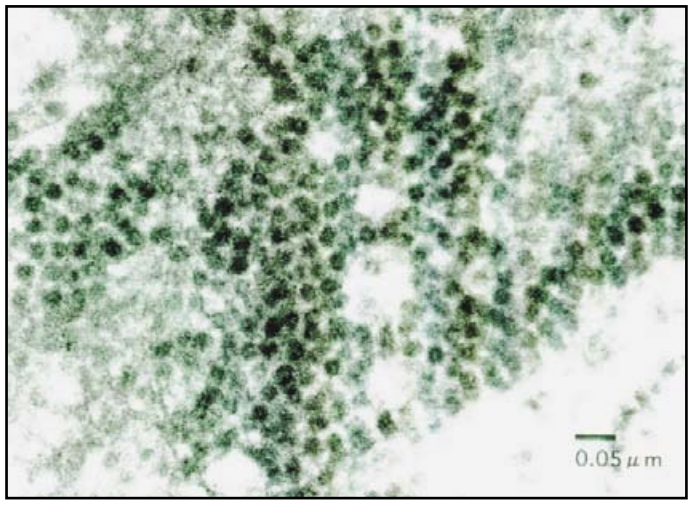


Fig. 1-1. Electron photomicrograph of VNN virus isolated from *Epinephelus coioides* 7 days post-inoculation of infected tissue to SSN-1 cells (Photo courtesy of Y. Maeno).

Stages affected:

All growth stages are affected but heavy mortalities were reported among larvae less than 20 days old.

Gross clinical signs:

Diseased fish swim in darting, corkscrew manner. Some fish sink to the bottom then float to the surface again. Affected juvenile and broodstock fish develop bloated belly (Fig. 1-2). In addition, affected fish also show lethargy, pale coloration and loss of appetite.

Fig. 1-2. *Epinephelus coioides* broodstock with bloated belly associated with VNN infection.

Effects on host:

The VNN disease is the most devastating viral infection among marine finfish. Outbreaks of VNN caused up to 70% mortality in fry (Fig. 1-3), up to 100% mortality in 2.5-7.5 cm fish and <20% mortality in >15 cm size fish. Among hatchery-reared larvae of *E. coioides* in the Philippines, 5-10% mortality was observed daily that reached 100% within 10 days at water temperatures of 24.5-28°C. Disease signs of reduced feeding, darkened pigmentation, lethargy and abnormal swimming started 34 days after hatching. In Japan, more than 50% mortalities among 170 g-1.85k g *E. septemfasciatus* were frequently observed during summer and early autumn (July to October) at water temperatures of 25-28°C. In the People's Republic of China, mass mortalities attributed to VNN occurred among 7-45 day-old *E coioides* and *E. akaara*. Taiwan also reported mass mortalities associated with VNN in hatchery-reared groupers, *E. fuscoguttatus* and *E. akaara*. In Thailand, outbreaks in *E. coiodes* larvae and juveniles were observed at 26-30°C. Affected fish showed whirling swimming, darkened color and hyperinflated swimbladders.

Internal disease signs include pale livers, empty digestive tracts and intestines filled with greenish to brownish fluid. The virus propagates in the eye, brain and distal spinal cord of affected fish causing marked vacuolations leading to vacuolating encephalopathy and retinopathy. It also multiplies in the gonad, livers, kidney, stomach and intestine.

Temperature plays an important role in the replication and pathogenicity of piscine nedavirus. For example, an RGNNV isolated from grouper produces cytopathic effects

Fig. 1-3. *Epinephelus coioides* larvae with showing bloated belly.

(CPE) in GF-1 cells at 24-32°C but not at 20°C or 37°C. In larvae challenged with RGNNV at 28°C, mortality reached 100% 50-80 hours post-inoculation. In infection experiments on *E. akaara* juveniles, RGNNV caused 100% mortalities of the test fish at 24-28°C. At 16 and 20°C, mortalities were reduced to 57-61% and onset of abnormal swimming and deaths were delayed. In addition, the virus antigen was detected among survivors 50 days post-infection at 16°C.

Transmission:

The virus can be waterborne-transmitted from diseased to healthy fish within 4 days of contact. Nodaviruses were, likewise, detected in fish without clinical disease signs. As such, grouper broodstocks can be virus reservoirs and can therefore be a source of virus for its larvae.

Diagnosis:

Typical external signs of VNN-affected fish are rotating, spinning and horizontal looping swimming behavior and inflation of the swimbladder. Diagnostic, also, is the presence of necrosis and marked vacuolations in the eye retina, brain and spinal cord of infected fish when examined by light microscopy (Fig. 1-4). By electron microscopy, abundant numbers of the virus were detected in the cytoplasm of affected nerve cells in infected fish.

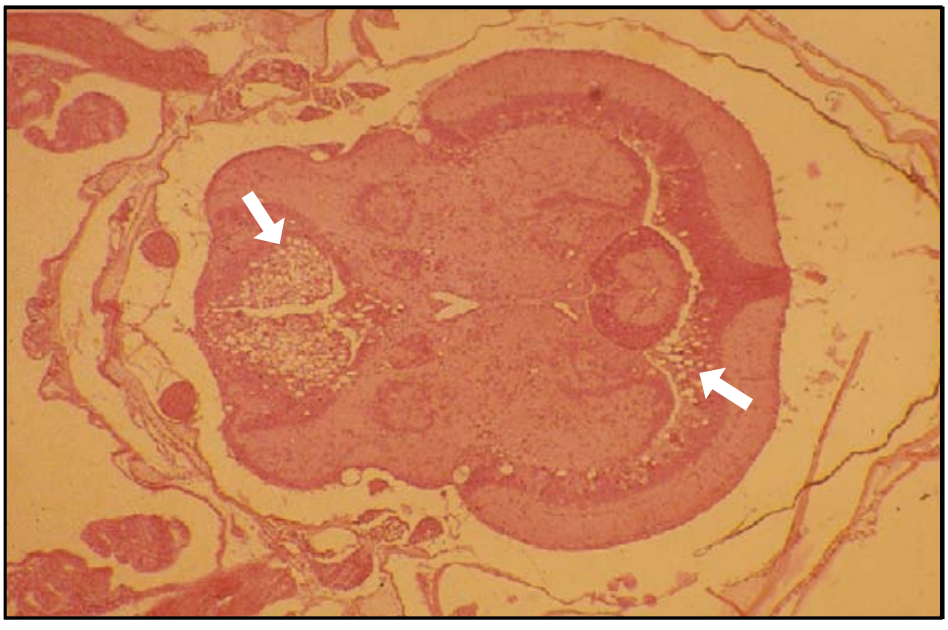


Fig. 1-4. Vacuolations (arrows) in brain of VNN-infected fry of *Epinephelus coioides*. H & E stain.

The betanodavirus can be isolated *in-vitro* in several fish cell lines, i.e., SSN-1 (Snakehead fry), E-11 (cloned from SSN-1), GF-1 (Grouper fin), SB (Asian Seabass fry), GB (Grouper brain), GF (Grouper fin) and GL (Grouper liver) at 28°C. Cytopathic effects (CPE) in GF and GL cells are characterized by typical vacuolations from 3-5 days post-inoculation. In SSN-1

cells, CPE is detected approximately 5-10 days post-inoculation. In addition, virus-neutralizing antibodies in fish serum can be assayed using susceptible cell lines.

The virus can, also, be detected by immunological methods like indirect fluorescent antibody technique (IFAT) and enzyme-linked immunosorbent assay (ELISA) using rabbit anti-VNN serum.

Reverse transcriptase-polymerase chain reaction (RT-PCR) is, by far, the most powerful tool to detect the virus. A primer pair designed to amplify the T4 region of SJNNV coat protein gene is possible to detect almost all genotypic variants of betanodaviruses. The sensitivity of the assay is further improved by the nested step (Fig. 1-5). Moreover, when tissue filtrates are inoculated onto E-11 cells at 20 or 25°C for 48 hours, the virus can be more rapidly detected by RT-PCR. This is particularly useful in detecting the virus from subclinically infected fish with very low viral load.

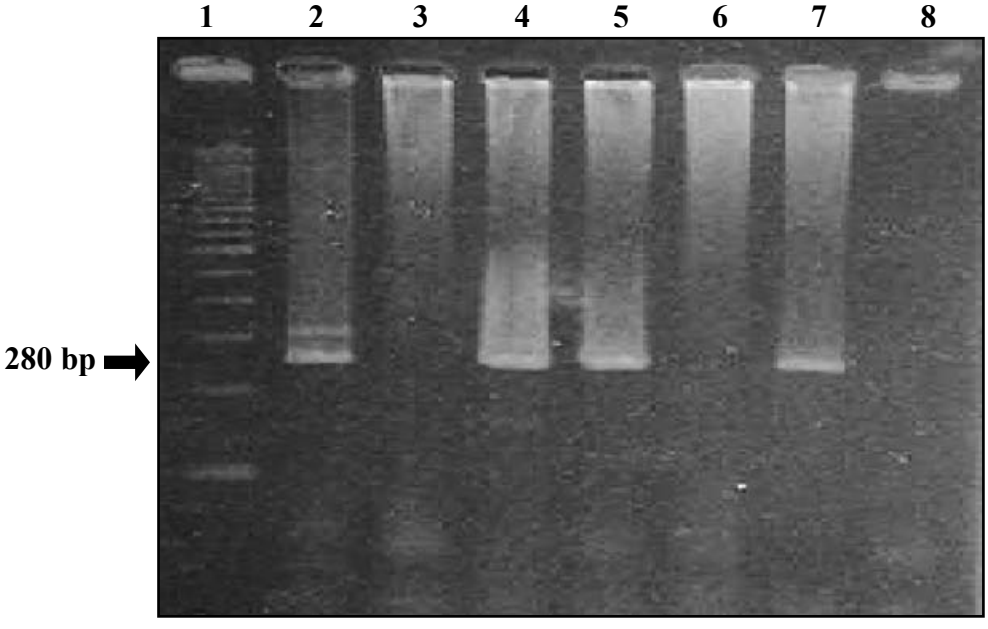


Fig. 1-5. Agarose gel electrophoresis of nested PCR amplification products of VNN. Lanes: (1) 100 bp DNA marker; (2 and 7) positive control; (3-6) *Epinephelus coioides* broodstocks; (8) negative control.

Prevention and control:

VNN-carrier broodstocks were found to be a source of inoculum of the virus to their larvae. Pre- and post-spawning screening of broodstocks for VNN using PCR is very important. Only VNN-negative broodstocks shall be allowed to spawn, followed by disinfection of the fertilized eggs using ozone or iodine. These procedures are very effective to prevent the vertical transmission of the virus.

Strict husbandry management in the hatchery phase is very important in the management of VNN infection. Betanodaviruses are quite resistant to some environmental parameters, thus it is highly possible that the virus could be easily translocated via contaminated rearing water

and paraphernalia. In Australian barramundi hatchery, the use of non-recycled, chemically treated rearing water and decontamination of tanks after every hatching cycle were effective in preventing VNN infection. Other measures in the control of VNN in larval striped jack are: disinfection of eggs with iodine or ozone and hatchery paraphernalia with chlorine; rearing of each batch of larvae and juveniles in separate tanks supplied with UV or ozone-sterilized seawater; and separation of larvae and juveniles from broodfish.

Vaccination is a promising method of preventing VNN in groupers. Immunization of groupers with recombinant coat proteins prepared from RGNNV genotype strains induced virus-neutralizing antibodies that resulted in high protection against experimental infection of the virus. However, a multivalent vaccine may be needed for total protection from infection by different genotypic variants of piscine nodavirus.

In addition, reduction of identified stress factors in the culture system is very important. Piscine nadavirus-free broodfish should be induced to spawn up to 7-10 times only since the stress of multiple spawnings may activate the residual virus. Reduction of larval stocking density in the tank may help reduce the possibility of viral transmission. Rearing water temperature influenced disease development wherein higher mortality and earlier appearance of the disease signs were observed at higher rearing water temperatures. This suggests that manipulation of water temperature will help reduce disease outbreaks.

IRIDOVIRUS INFECTIONS

Iridoviruses are a group of emerging viral pathogens causing infections in many marine finfish, e.g., *Pagrus major*, *Evynnis japonica*, *Oplegnathus punctatus*, *O. fasciatus*, *Latelabrax* sp., *Seriola quinqueradiata*, *S. dumerili*, *S. aureovittata*, *Caranx delicatissimus*, *Takifugu rubripes*, *Epinephelus akaara*, *E. malabaricus* and *E. tauvina*. The International Committee on Taxonomy of Viruses classifies the family Iridoviridae into four genera: *Iridovirus*, *Chloriridovirus*, *Ranavirus* and *Lymphocystivirus*. Grouper infections attributed to iridoviruses have been invariably identified as Fish Lymphocystis Disease (FLD), Blister Disease, Red Seabream Iridovirus Disease (RSIVD), Sleepy Grouper Disease (SGD), Grouper Iridovirus Disease of Taiwan (TGIVD), Grouper Iridovirus Disease (GIVD), Grouper Spawner Iridovirus Disease (GSIVD) and Singapore Grouper Iridovirus Disease (SGIVD). Except for FLD, the other iridovirus pathogens cause systemic infections in fish. However, the exact relationship among these systemic piscine iridoviruses is not clear. Hence, these diseases are presented separately.

1. Fish Lymphocystis Disease (FLD)

Lymphocystis is a chronic viral infection that occurs among finfish worldwide over a wide range of water temperatures including tropical climates. Among groupers, this disease was reported in *E. bruneus*, *E. malabaricus* and *E. chlorostigma* cultured in marine net cages in Guangdong, China, and also among *E. fuscoguttatus* in Malaysia.

Causative agent:

Fish Lymphocystis Disease virus (FLDV) is an iridovirus measuring 130-330 nm.

Stages affected:

Fingerlings, juveniles and adult fish are affected.

Gross clinical signs:

Infected fish develop small (0.5-2 mm in diameter) pearl-like nodules occurring singly or in clusters on the body surface, fins and occasionally on the gills. The apparent nodules are a consequence of the enlargement of tissue cells infected by the virus. These cells are hypertrophied fibroblasts or the so-called Lymphocystis giant cells.

Effects on host:

Fish exhibiting the clinical signs of FLD have decreased market value. It is a chronic infection that can cause low mortality and is rarely fatal in older fish.

Transmission:

The viral pathogen is released after rupture of the nodules. The virus is thus transmitted from diseased to healthy fish through exposure to contaminated water via abraded skin or by cohabitation.

Diagnosis:

The disease can be readily diagnosed on the basis of the presence of the characteristic external nodules. The appearance of the Lymphocystis giant cells upon histological examination of sections of nodules is confirmatory for FLD.

2. Blister Disease

Causative agent:

The causative agent of blister disease is an iridovirus measuring 140-160 nm.

Stages affected:

The disease has been observed in 5-100 g *Epinephelus malabaricus* in Thailand.

Gross clinical signs:

Affected fish manifests signs of loss of appetite initially and whitish blister development on the body and fins.

Effects on host:

Fish develop localized, severe inflammation of the epidermal and dermal layer (Fig. 1-6). Necrotic dermis shows the presence of exudates and hemorrhagic infiltration. The virus is seen in the liver, spleen, kidney and lesions of infected fish. Natural infection results in 30-80% mortality within one month. Pathogenicity experiment showed that clinical signs and mortality occur within 5 days after exposure to the virus, and can reach 100% within 10 days. High stocking density can predispose susceptible fish to this disease.

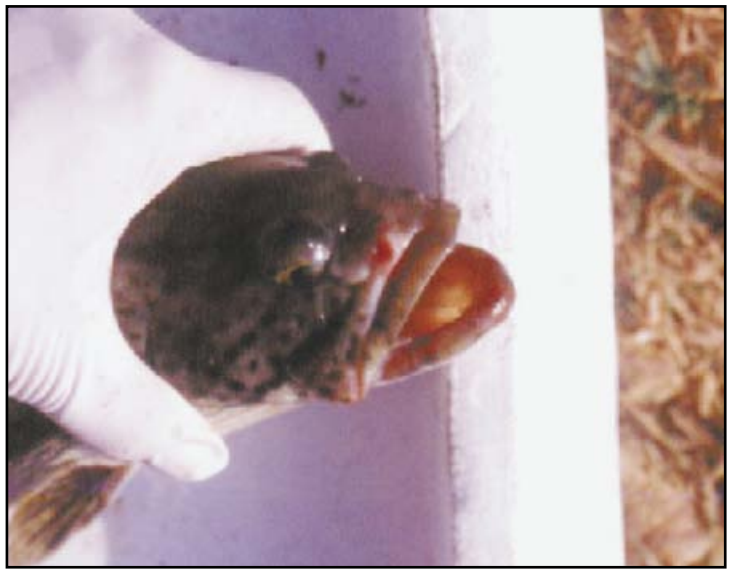


Fig. 1-6. Blister disease associated with iridovirus infection in *Epinephelus malabaricus* (Photo courtesy of S. Kanchanakhan).

Transmission:

The virus can be transmitted to healthy fish via rearing water.

Diagnosis:

The virus can be isolated in GF (grouper fin) and *Epithelioma papulosum cyprini* (EPC) cells.

3. Red Seabream Iridovirus Disease (RSIVD)

This disease is the most extensively studied iridovirus-causing infection. It is most frequently reported among red seabream in Japan. Subsequently, the virus was reported to cause infections in many grouper species, i.e., *Epinephelus akaara*, *E. septemfasciatus*, *E. malabaricus*, *E. bruneus*, *E. coioides*, *E. awoara* and *E. fuscoguttatus* in Japan, Taiwan, Thailand, Malaysia and Indonesia.

Causative agent:

Red seabream iridovirus (RSIV) is 130-196 nm in size.

Stages affected:

The less than 1 year-old fish are more frequently infected but 1 and 2 year-old groupers can also be infected.

Gross clinical signs:

There is reduction in food consumption and is associated with excretion of mucoid and opaque feces. Moribund fish shows darkened body color. Mortalities occur 8-10 days after exposure to the virus.

Effects on host:

Experimental studies have shown that the RSIV multiply initially in the cytoplasm of cells of the spleen and head kidney of *E. malabaricus* as early as 2 days post-infection. This may result in diffused necrosis of the spleen that may be associated with hemorrhage. Subsequently, the virus was detected in the kidney and liver by the 6th day post-inoculation. The number of cells infected with the virus in the small sized grouper, 7.7 g, was higher than in the large test fish weighing 84.3 g. Eosinophilic, degenerated cells and basophilic, enlarged cells were seen in the spleen and head kidney of dead infected fish. In contrast, surviving fish had granulated eosinophilic cell debris but no enlarged cells were observed. The virus eventually infiltrates the blood vessels of the spleen and head kidney, causing degeneration of these organs. The virus also migrates to the liver, heart, gills, esophagus, stomach and intestine without inducing degenerative changes.

Intraperitoneal injection of *E. malabaricus*, 7.7 g and 84.3 g in size with the RSIV, exhibited mortalities in 8-11 days and 8-10 days, respectively, post-exposure. Cumulative mortalities in both sizes of challenged fish were 90%.

Transmission:

The viruses are contained in inclusion bodies, within the infected cells and are rapidly released upon lyses of the cell. Since the gills and the intestine have direct contact with the environment of the fish, transmission of the virus among fishes in the same culture system can readily occur. However, there is no indication that RSIV is transmitted vertically. The RSIVD reported in Malaysia was introduced through fish imported from Taiwan.

Diagnosis:

Giemsa staining of fish spleen imprints is commonly used for the rapid, presumptive diagnosis of RSIV-infected fish. The disease is characterized by the appearance of large numbers of blast-like inflammatory cells in the blood, spleen, kidney, heart, liver, digestive tract, pancreas, gills, swim bladder, eyes, meninges, bone and musculature (Fig. 1-7). The inclusion body bearing enlarged cells are intensely basophilic with expanded nucleus and prominent nucleolus. Confirmatory diagnosis of the disease is done by direct fluorescent antibody technique (IFAT) using monoclonal antibody against RSIV. PCR amplification using primer sets based on the ATPase gene, DNA polymerase gene or Pst I-restriction fragment of RSIV genomic DNA is useful for diagnosis (Fig. 1-8). Quantitative estimation of RSIV is also possible using the real-time PCR assay. The virus can also be propagated in grunt fin (GF) cells incubated at 25°C.

4. Sleepy Grouper Disease (SGD)

Causative agent:

The causative agent of sleepy grouper disease is an iridovirus measuring 130-160 nm.

Species affected:

The disease was reported in 100-200 g and 2-4 kg *Epinephelus tauvina* in Singapore and Malaysia.

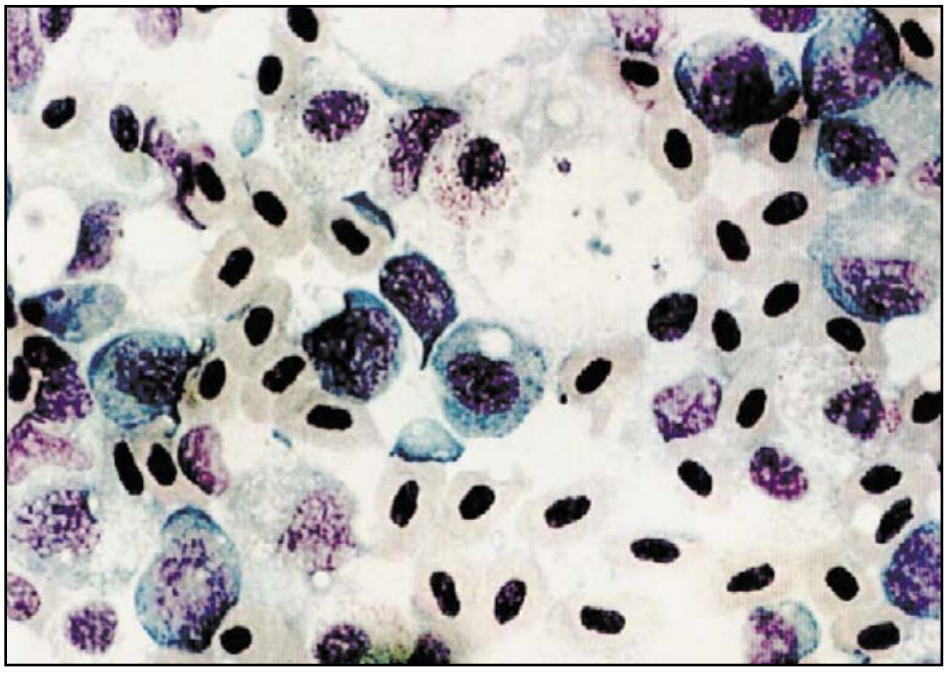


Fig. 1-7. Tissue imprint of spleen stained with Giemsa showing large numbers of blast-like inflammatory cells infected with RSIV (Photo courtesy of Y. Maeno).

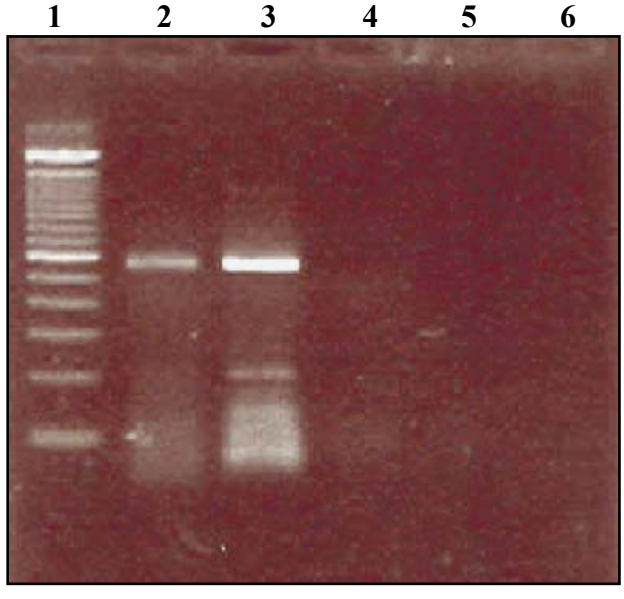


Fig. 1-8. Agarose gel electrophoresis of PCR amplification products of RSIV. Lanes: (1) 100 bp DNA marker; (2) positive control; (3-5) grouper broodstocks; (6) negative control.

Gross clinical signs:

Infected fish exhibit extreme lethargy, low appetite and swim either alone or hang at the water surface or remain at the bottom. At the terminal phase of infection, fish display gill pallor, rapid opercular movements and frantic dashing to the water surface to gulp air. Fish usually die at night or at dawn.

Effects on host:

The disease affected farmed groupers, 100-200 g and 2-4 kg in size, in 10 of 33 farms in Singapore and Malaysia. Acute disease causes up to 50% mortality mostly occurring during the night or at the early hours of the morning. Gradual mortalities follow after fish become sluggish over 3-5 days, after which the fish lie at the net or tank bottom exhibiting weak fin movements. Acute mass mortalities may occur 12-24 hours after handling or excessive feeding.

Internal pathology consists of enlargement of the spleen or anterior kidney and heart inflammation. The virus can be detected in the spleen, heart and kidney of affected fish.

Experimentally injected fish develop signs of SGD and die in 3-4 days.

Transmission:

The virus is introduced into farms with some imported fish and subsequently spread to neighboring farms.

Diagnosis:

In severe cases, histological sections show generalized splenic necrosis and focal or generalized myocarditis/endocarditis of the spleen and heart. The virus can also be seen by electron microscopy showing viral particles in the spleen, heart, anterior and posterior kidney samples.

5. Grouper Iridovirus Disease of Taiwan (TGIVD)

Causative Agent:

The causative agent is an iridovirus with a size of 200-240 nm.

Stages affected:

TGIVD affects *Epinephelus* sp. from larvae to broodstock.

Gross clinical signs:

Fish infected with TGIV swim in circles and are anemic, lose appetite, and are underweight and lethargic.

Effects on host:

Natural infections associated with TGIVD can cause up to 60% mortalities among farmed groupers, 5-8 cm in length at 25-28°C. Transport stress of previously exposed fish predisposes TGIVD outbreaks. The spleen of affected fish has abnormal hypertrophied cells containing numerous icosahedral virions. Experimentally infected fish reach a cumulative mortality of 100% in 11 days without other clinical signs.

Transmission:

This disease has been reported in Taiwan since 1995, through importation of latently-infected grouper fry from Malaysia.

Diagnosis:

The TGIV virus can be propagated in grouper cell line (KRE). Histological and tissue imprints show basophilic enlarged cells in the spleen, heart, kidney, liver and gill. The virus can also be seen by electron microscopy. This virus has antigenic similarities with the red seabream iridovirus (RSIV) isolated in Japan, the epizootic haematopoietic necrosis virus, and the iridovirus isolated from sheatfish and the grouper iridovirus isolated in Thailand. A nested PCR amplification using primers from RAPD-derived amplicons is very useful for confirmatory diagnosis.

6. Grouper Spawner Iridovirus Disease (GSIVD)

Causative agent:

The causative agent is an iridovirus measuring 120-135 nm.

Stages affected:

Fingerling to spawners of *Epinephelus malabaricus* can be infected.

Gross clinical signs

Infected fish do not exhibit any lesion but its body becomes pale before sudden death.

Effects on host:

Up to 90% mortalities of 20 g-5 kg sized grouper reared in net cages have been associated with outbreaks of this viral infection in Thailand. Cells of the spleen, kidney, heart and digestive tract become enlarged. The virus is found within the enlarged cells of the spleen and head kidney causing necrosis of these organs.

Transmission:

Horizontal contact and waterborne transmission appear to be the principal mechanism for virus spread.

Diagnosis:

Histopathologic analyses of the spleen and head kidney show basophilic, larged cells.

7. Grouper Iridovirus Disease (GIVD)

Causative agent:

The causative agent is an iridovirus, genus *Ranavirus*, 200-240 nm in size that is distinct from RSIV.

Stages affected:

Epinephelus aurowa and *E. malabaricus*, 1.0-1.5 cm and 10-12 cm in size, were infected in Taiwan and Thailand, respectively.

Gross clinical signs:

Infected fish exhibited abnormal swimming with spasm, reduced feeding, lethargy and darkening of the tail and fins. Moribund fish floats at the water surface then finally sink to the bottom of the tank and die.

Effects on host:

The icosahedral virions were detected in the cells of fins, spleen and kidney of affected fish. Fish mortality runs to 20-30%.

Transmission:

The GIV can be transmitted to grouper fry and fingerlings but not to adults. Up to 60 % mortality was observed in intraperitoneally challenged fish, 1.5-2.0 cm in size, 6 days post-exposure. Cohabitation with infected fish caused 30% mortality after exposure for 14 days.

Diagnosis:

The GIV induces cytopathic effects (CPE) in grouper kidney (GK), grouper liver (GL), grunt fin (GF), EPC and grouper fin (GF) cells 3 days after inoculation at 28°C. The affected cells become rounded, granular and refractile. Complete disintegration of the monolayer occurs 7 days later. Cells form small dense grayish patches, then rounding and lysis. GIV produces cytoplasmic inclusions in infected cells. In addition, round plaques are formed in agarose overlays. PCR amplification using primer pair from MCP gene of GIV is useful for confirmatory diagnosis.

8. Singapore Grouper Iridovirus Disease (SGIVD)

Causative agent:

The causative agent is an iridovirus closely related to the genus *Ranavirus*, with a mean diameter of 200 nm.

Stages affected:

Fry and adult *Epinephelus malabaricus* and *E. tauvina* in Singapore were affected. The grouper fry were imported from other Southeast Asian countries.

Gross clinical signs:

Infections caused by SGIV are characterized by hemorrhage and enlargement of the spleen of infected fish.

Effects on host:

The SGIV causes serious systemic infection in cage-farmed *E. malabaricus* and *E. tauvina.* This virus can induce more than 90% mortality over several weeks. After experimental challenge of 10 g *E. tauvina*, a cumulative mortality of 96% was observed in 3-10 days post-infection.

Transmission:

The SGIV was experimentally transmitted via intraperitoneal injection of cell-cultured virus. No other information is available on transmission of the virus in culture systems.

Diagnosis:

Tissue filtrates from pooled samples of spleen, kidney, liver and heart, when inoculated into Grouper (GP) embryo cells, induce CPE at 25°C in 24 hours. PCR amplification using primers from the MCP gene of frog virus 3 (FV3) is useful for confirmatory diagnosis.

Prevention and control of iridovirus infections:

Fish Lymphocystis Disease virus (FLDV) may be prevented by avoiding skin damage and by quarantine of new fish. Early detection of viral pathogens in the hatchery can prevent continued culture in grow-out systems. Monitoring of viral pathogens in broodstocks may prevent vertical transmission. Reduction of known stress factors like transport stress and high stocking density will lessen the possibility of infection. A formalin-killed vaccine developed in Japan from the cell culture supernatant of RSIV-infected cells showed higher survival rates among the vaccinated group after experimental and natural infections of the virus.

REFERENCES

Boonyaratpalin, S., Supamattaya, K., Kasornchandra, J. and Hoffmann, R.W. 1996. Picorna-like virus associated with mortality and spongious encephalopathy in grouper *Epinephelus malabaricus*. Dis. Aquat. Org. 26: 75-80.

Caipang, C.M., Hirono, I. and Aoki, T. 2003. Development of a real-time PCR assay for the detection and quantification of Red Seabream Iridovirus (RSIV). Fish Pathol. 38: 1-7.

Chao, C.B., Chen, C.Y., Lai, Y.Y., Lin, C.S. and Huang, H.T. 2004. Histological, ultrastructural, and *in situ* hybridization study on enlarged cells in grouper *Epinephelus* hybrids infected by grouper iridovirus in Taiwan (TGIV). Dis. Aquat. Org. 58: 127-142.

Chao, C.B., Yang, S.C., Tsai, H.Y., Chen, C.Y., Lin, C.S. and Huang, H.T. 2002. A nested PCR for the detection of grouper iridovirus in Taiwan (TGIV) in cultured hybrid grouper, giant seaperch and largemouth bass. J. Aquat. Anim. Health 14: 104-113.

Chi, S.C., Lin, S.C., Su, H.M. and Hu, W.W. 1999. Temperature effect on nervous necrosis virus infection in grouper cell line and in grouper larvae. Virus Res. 63: 107-114.

Chi, S.C., Lo, C.F., Kou, G.H., Chang, P.S., Peng, S.E. and Chen, S.N. 1997. Mass mortalities associated with viral nervous necrosis (VNN) disease in two species of hatchery-reared grouper, *Epinephelus fuscoguttatus* and *Epinephelus akaara* (Temminck & Schlegel). J. Fish Dis. 20: 85-193.

Chou, H.Y., Hsu, C.C. and Peng, T.Y. 1998. Isolation and characterization of pathogenic iridovirus from cultured grouper (*Epinephelus* sp.) in Taiwan. Fish Pathol. 33: 201-206.

Chua, F.H.C., Loo, J.J. and Wee, J.Y. 1995. Mass mortality in juvenile greasy grouper, *Epinephelus tauvina*, associated with vacuolating encephalopathy and retinopathy. *In*: Diseases in Asian Aqauaculture II, M. Shariff, J.R. Arthur, R.P. Subasinghe (eds.), p. 235-241, Fish Health Section, Asian Fisheries Society, Manila, Philippines.

Danayadol, Y., Direkbusarakom, S. and Supamattaya, K. 1995. Viral nervous necrosis in brownspotted grouper, *Epinephelus malabaricus*, cultured in Thailand. *In*: Diseases in Asian Aquaculture II, M. Shariff, J.R. Arthur and R.P. Subasinghe (eds.), p. 227-233, Fish Health Section, Asian Fisheries Society, Manila, Philippines.

Danayadol, Y., Direkbusarakom, S., Boonyaratpalin, S., Miyazaki, T. and Miyata, M. 1997. Iridovirus infection in brown-spotted grouper (*Epinephelus malabaricus*) cultured in Thailand. *In*: Diseases in Asian Aquaculture III, T.W. Flegel and I.H. MacRae (eds.), p. 67-72. Fish Health Section, Asian Fisheries Society, Manila, Philippines.

Fukuda, Y., Nguyen, H.D., Furuhashi, M. and Nakai, T. 1996. Mass mortality of cultured sevenband grouper, *Epinephelus septemfasciatus*, associated with viral nervous necrosis. Fish Pathol. 31: 165-170.

Iwamoto, T., Nakai, T., Mori, K., Arimoto, M. and Furusawa, I. 2000. Cloning of the fish cell line SSN-1 for piscine nodavirus. Dis. Aquat. Org. 43: 81-89.

Iwamoto, T., Mori, K., Arimoto, M. and Nakai, T. 2001. A combined cell-culture and RT-PCR method for rapid detection of piscine nodavirus. J. Fish Dis. 24: 231-236.

Jung, S., Miyazaki, T., Miyata, M., Dayanadol, Y. and Tanaka, S. 1997. Pathogenicity of iridovirus from Japan and Thailand for the red seabream *Pagrus major* in Japan and histopathology of experimentally infected fish. Fish. Sci. 63: 735-740.

Kasornchandra, J. and Khongpradit, R. 1997. Isolation and preliminary characterization of a pathogenic iridovirus in nursing grouper, *Epinephelus malabaricus*. *In*: Diseases in Asian Aquaculture III, T.W. Flegel and I.H. MacRae (eds.), p. 61-66. Fish Health Section, Asian Fisheries Society, Manila, Philippines.

Kawakami, H. and Nakajima, K. 2002. Cultured fish species affected by red seabream iridoviral disease from 1996 to 2000. Fish Pathol. 37: 45-47.

Kongpradit, R., Kasornchandra, J., Krachaiwong, V. and Boonyaratpalin, S. 1997. Blister disease in malabar grouper, *Epinephelus malabaricus*: Isolation and some characterization of causative agent. Technical Paper No. 9/1997. National Institute of Coastal Aquaculture. Department of Fisheries, Songkhla, Thailand. 14 p. (in Thai with English abstract).

Lai, Y.S., Chiu, H.C., Murali, S., Guo, I.C., Chen, S.C., Fang, K. and Chang, C.Y. 2001. *In vitro* neutralization by monoclonal antibodies against yellow grouper nervous necrosis virus (YGNNV) and immunolocalization of virus infection in yellow grouper, *Epinephelus awoara* (Temminck & Schlegel). J. Fish Dis. 24: 237-244.

Lai, Y.S., John, J.A.C., Lin, C.H., Guo, I.C., Chen, S.C., Fang, K., Lin, C.H. and Chang, C.Y. 2003. Establishment of cell lines from a tropical grouper, *Epinephelus awoara* (Temminck & Schlegel), and their susceptibility to grouper irido- and nodaviruses. J. Fish Dis. 26: 31-42.

Lin, L., He, J., Mori, K., Nishioka, T., Wu, J.L., Weng, S., Musiake, K., Arimoto, M. and Nakai, T. 2001. Mass mortalities associated with viral nervous necrosis in hatchery-reared groupers in the People's Republic of China. Fish Pathol. 36: 186-188.

Lio-Po, G.D. 2001. Viral diseases of fish and penaeid shrimps. *In*: Aquatic Animal Health Management, G.D. Lio-Po, C.R. Lavilla and E.R. Cruz-Lacierda (eds.), p. 9-23. SEAFDEC Aquaculture Department, Iloilo, Philippines.

Lio-Po, G.D., Cruz-Lacierda, E.R., de la Peña, L.D., Maeno, Y. and Inui, Y. 2002. Progress and current status of diagnostic techniques for marine fish viral diseases at the SEAFDEC Aquaculture Department. *In*: Disease Control in Fish and Shrimp Aquaculture in Southeast Asia–Diagnosis and Husbandry Techniques, Y. Inui and E.R. Cruz-Lacierda (eds.), p. 172-180. SEAFDEC Aquaculture Department, Iloilo, Philippines.

Maeno, Y., de la Peña, L.D. and Cruz-Lacierda, E.R. 2002. Nodavirus infection in hatchery reared orange-spotted grouper *Epinephelus coioides*: First record of viral nervous necrosis (VNN) in the Philippines. Fish Pathol. 37: 87-89.

Munday, B.L., Kwang, J. and Moody, N. 2002. Betanodavirus infections of teleost fish: a review. J. Fish Dis. 25: 127-142.

Murali, S., Wu, M.F., Guo, I.C., Chen, S.C., Yang, H.W. and Chang, C.Y. 2002. Molecular characterization and pathogenicity of a grouper iridovirus (GIV) isolated from yellow grouper, *Epinephelus awoara* (Temminck & Schlegel). J. Fish Dis. 25: 91-100.

Nakajima, K., Ito, T., Kurita, J., Kawakami, H., Itano, T., Fukuda, Y., Aoi, Y., Tooriyama, T. and Manabe, S. 2003. Effectiveness of a vaccine against red seabream iridoviral disease in various cultured marine fish under laboratory conditions. Fish Pathol. 37: 90-91.

Nishizawa, T., Furuhashi, M., Nagai, T., Nakai, T. and Muroga, K. 1997. Genomic classification of fish nodaviruses by molecular phylogenetic analysis of the coat protein gene. Appl. Environ. Microbiol. 63: 1633-1636.

Nishizawa, T., Mori, K., Furuhashi, M., Nakai, T., Furusawa, I. and Muroga, K. 1995. Comparison of the coat protein genes of five fish nodaviruses, the causative agents of viral nervous necrosis in marine fish. J. Gen. Virol. 76: 1563-1569.

Office International des Epizooties (OIE). 2003. Viral encephalopathy and retinopathy. *In*: Manual of Diagnostic Tests for Aquatic Animals, p. 135-141. OIE, Paris, France.

Qin, Q.W., Chang, S.F., Ngoh-Lim, G.H., Gibson-Kueh, S., Shi, C. and Lam, T.J. 2003. Characterization of a novel ranavirus isolated from grouper *Epinephelus tauvina.* Dis. Aquat. Org. 53: 1-9.

Sano, M., Minagawa, M., Sugiyama, A. and Nakajima, K. 2000. Susceptibility of fish cultured in subtropical area of Japan to red seabream iridovirus. Fish Pathol. 36: 38-39.

Sano, M., Minagawa, M. and Nakajima, K. 2002. Multiplication of red seabream iridovirus (RSIV) in the experimentally infected grouper *Epinephelus malabaricus*. Fish Pathol. 37: 163-168.

Tanaka, S., Aoki, H. and Nakai, T. 1998. Pathogenicity of the nodavirus detected from diseased sevenband grouper *Epinephelus septemfasciatus*. Fish Pathol. 33: 31-36.

Yongjia, Z., Zeyang, W. and Kangrong, C. 1996. Ultrastructural study of lymphocystis in kelp bass (*Epinephelus moara*; Serranidae). *In*: Biology, Fisheries and Culture of Tropical Groupers and Snappers, F. Arreguin Sanchez, J.L. Munro, M.C. Balgos and D. Pauly (eds.), p. 385-398. ICLARM, Makati City, Philippines.

Zafran, Koesharyani, I., Johnny, F., Yuasa, K., Harada T. and Hatai K. 2000. Viral nervous necrosis in humpback grouper, *Cromileptes altivelis*, larvae and juvenile in Indonesia. Fish Pathol. 36: 95-96.

Chapter 2. Bacterial Diseases

Eleonor A. Tendencia and Celia R. Lavilla-Pitogo

Bacteria are very common in the aquatic environment. Most bacterial disease agents are part of the normal flora of the water. They cause disease only when the fish are stressed due to poor environmental conditions, inadequate diet and poor husbandry techniques.

This chapter focuses on the most common bacterial diseases of groupers.

VIBRIOSIS

Vibriosis is also known as *Vibrio* hemorrhagic septicemia and is often associated with another disease, the red boil disease, which is caused by *Streptococcus* sp. The disease has been reported in *Epinephelus malabaricus*, *E. tauvina*, *E. coioides* and *E. bleekeri*. Vibriosis has been recorded in cultured groupers in Brunei Darussalam, Malaysia, Taiwan, Indonesia, Kuwait, Thailand, Singapore and the Philippines.

Causative agents:

The causative agents of vibriosis are *Vibrio parahaemolyticus, V. alginolyticus, V. vulnificus* and *V. carchariae*.

Stages affected:

The bacteria may affect grouper fry, fingerlings, juveniles, adults and broodstocks.

Gross clinical signs:

The first sign of the disease is anorexia or loss of appetite with darkening of the fish coloration. The fish may be lethargic, swimming near the water surface. Affected fish may lose equilibrium and exhibit abnormal swimming behavior. One of the signs of the disease is body ulcer that may be hemorrhagic (Fig. 2-1a). Fin rot, which usually starts with erosion of the tip of the fin and gradually becomes necrotic, may also be observed (Fig. 2-1b). Exophthalmia and corneal opacity are also common signs of the disease. Internally, bloody discharges may be observed in the abdominal cavity due to internal organ hemorrhage (Fig. 2-1c). In the case of *V. carchariae*, gastroenteritis manifested by a swollen intestine containing yellow fluid may be observed.

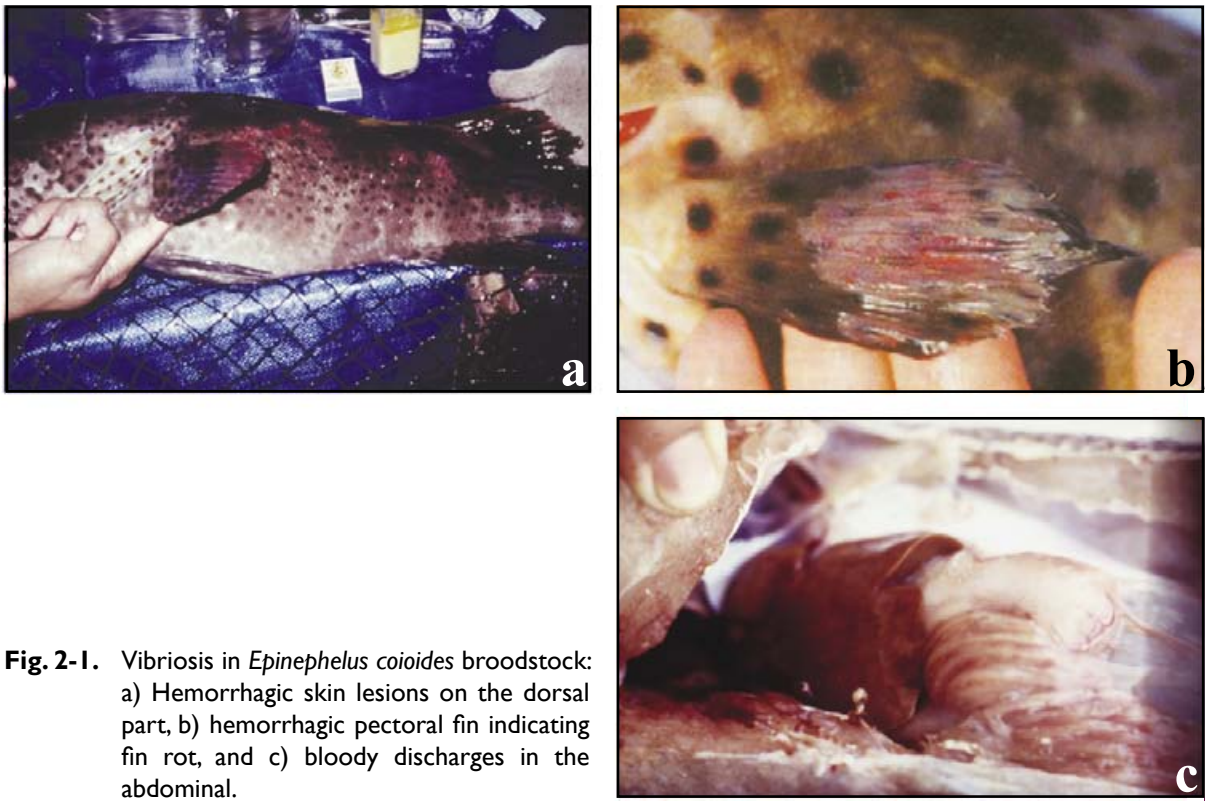


Fig. 2-1. Vibriosis in *Epinephelus coioides* broodstock: a) Hemorrhagic skin lesions on the dorsal part, b) hemorrhagic pectoral fin indicating fin rot, and c) bloody discharges in the abdominal.

Effects on host:

Ten to 50% mortality may be observed in affected populations depending on the farm management. It has also been observed that infected grouper harbor monogeneans and protozoans on the gills and skin. *Vibrio* sp. infection is also associated with red boil disease. In relatively colder countries like Taiwan, outbreaks may occur in summer when ambient temperatures rise.

Transmission:

The spread of the disease has been correlated with high salinity (30-35 ppt). The bacteria enter the fish through damaged areas. The disease may be transmitted through the water and trash fish given to the grouper. Infection is enhanced by parasitic infestation and mechanical injuries during transport, and grading.

Diagnosis:

Squash preparation of affected areas examined under the microscope may reveal the bacteria. Vibrios are Gram-negative straight or curved rods, 0.5-0.8 µm in width and 1.4-2.6 µm in length. The bacteria may be isolated from the infected organ using tryptic soy agar (TSA), nutrient agar (NA), or brain heart infusion agar (BHIA) supplemented with NaCl. Thiosulfate citrate bilesalt sucrose agar (TCBS), a *Vibrio* selective medium, may be used to isolate and primarily identify associated main groups of vibrios. Opening of the abdominal cavity will reveal hemorrhagic internal organs. The kidney may be swollen and filled with yellow fluid.

Preventive methods:

Rough handling of the fish during stocking, sampling, changing of nets, grading and overcrowding should be avoided. Good water quality must be maintained.

Control methods:

The disease may be controlled through freshwater bath for 10-15 minutes. Affected fish may be treated with oxalinic acid mixed with feed at 20 mg/kg of fish. Terramycin added to feed at 7.5 g/kg for 5 days, reduced to 3.75 g/kg for the succeeding 5 days also proved effective. Prefuran bath treatment for 1 hour at 2 ppm may also be implemented (see Appendix 1 for points to consider before using antibiotics and a list of anti-infectives recommended for use in marine food fish together with the withdrawal period).

PSEUDOMONAS INFECTION

The disease is also known as pseudomonad hemorrhagic septicemia. The only reported case of the disease is in cage-cultured *Epinephelus tauvina* in Malaysia.

Causative agent:

The causative agent is *Pseudomonas* sp.

Stages affected:

The bacteria may affect grouper at all stages.

Gross clinical signs:

Infected fish have extensive hemorrhagic erosions of the body. Ulcerations on the skin, fins and tails may also be observed. Other common signs of the disease are exophthalmia and corneal opacity.

Effects on host:

Twenty to 60% mortality may be observed in affected populations. A secondary epibiont fouling may also be observed. Internally, there is renal fragility, and dark red multifocal hepatic discoloration. Histologically, pathological changes consistent with subacute bacterial septicaemia are observed in the internal organs. Diffuse pericarditis and marked multifocal endocardial thrombosis and embolism are observed in the heart (Fig. 2-2a). Thrombosis and embolism are also observed in the hepatic vein (Fig. 2-2b). Diffuse pancreatic acinar cell atrophy and mononuclear cell infiltration are observed in the pancreas (Fig. 2-2c).

Transmission:

Pseudomonas spp. are ubiquitous in the aquatic environment. *Pseudomonas* infects fish when it is subjected to environmental stressors such as extreme water temperature changes, overcrowding, poor water quality and sub-optimal nutrition.

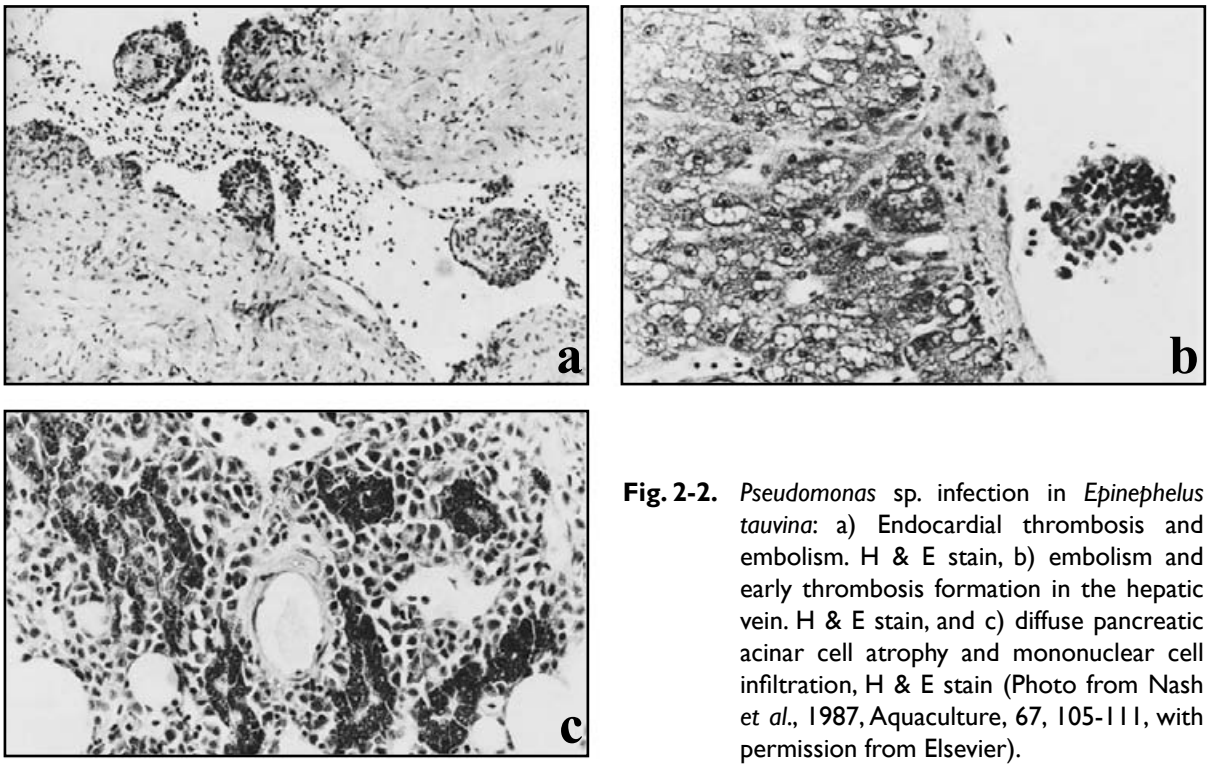


Fig. 2-2. *Pseudomonas* sp. infection in *Epinephelus tauvina*: a) Endocardial thrombosis and embolism. H & E stain, b) embolism and early thrombosis formation in the hepatic vein. H & E stain, and c) diffuse pancreatic acinar cell atrophy and mononuclear cell infiltration, H & E stain (Photo from Nash *et al*., 1987, Aquaculture, 67, 105-111, with permission from Elsevier).

Diagnosis:

Squash preparation of the kidney and other internal organs as well as the affected areas may reveal large colonies of *Pseudomonas* sp. Pseudomonads are Gram-negative, straight or slightly curved rods but not helical in shape, 0.5-1.0 µm in width and 1.5-5.0 µm in length. The bacteria may be isolated from the infected organ and affected areas using glutamate starch phenol red agar (GSP) or *Pseudomonas-Aeromonas* selective agar, a medium that selectively promotes growth of *Aeromonas* and *Pseudomonas* but inhibits growth of other bacteria. Internal examination of affected fish will show renal fragility and dark red multifocal discoloration of the liver.

Preventive methods:

Avoid the predisposing factors such as extreme water temperature changes, overcrowding, poor water quality, and sub-optimal nutrition to prevent pseudomonad infection.

Control methods:

Transferring affected fish into another tank with clean water may control the infection.

STREPTOCOCCAL INFECTION

The disease is also known as red boil disease. The disease is often associated with vibriosis. The disease has been reported in *Epinephelus malabaricus* and *E. bleekeri* in Brunei Darussalam, Malaysia, Singapore and Thailand.

Causative agent:

The causative agent is *Streptococcus* sp.

Stages affected:

Streptococcus sp. could infect grouper at all stages but is common in fry and fingerling stages.

Gross clinical signs:

Affected fish are weak and display disoriented whirling motion. Exophthalmia and hemorrhages on the cornea, operculum, around the mouth and the anus could also be observed. Infected fish have red boils in the skin.

Effects on host:

The red boils on the skin enlarge and eventually burst, exposing the necrotic musculature underneath and form small ulcers, which act as portals of entry for other bacteria. The disease may also cause systemic infections with few external signs. The infection could cause 10% mortality in affected fry but is not fatal to older fish.

Transmission:

The bacterium is ubiquitous in the environment, in the water and in carrier fish. Spread of the disease is associated with the presence of parasites, handling stress and sub-optimal water quality.

Diagnosis:

The bacteria grow well on BHIA, Todd-Hewitt agar, horse agar and TSA supplemented with 0.5% glucose. The colonies on agar plates appear small (0.5-1.0 mm diameter), yellowish, translucent, rounded and slightly raised. *Streptococcus* spp. are Gram-positive bacteria with ovoid or spherical cells, less than 2 µm in diameter, occurring in pairs or chains.

Preventive methods:

Avoid the predisposing factors such as the presence of parasites, handling stress and sub-optimal water quality to prevent disease occurrence.

Control methods:

Affected grouper could be treated with oxolinic acid mixed with feed at 20 mg/kg of fish, and perfuran bath for 1 hour at 2 ppm (see Appendix 1 for points to consider before using antibiotics and a list of anti-infectives recommended for use in marine food fish together with the withdrawal period).

FLEXIBACTER INFECTION

Flexibacter spp. are long rod-shaped, Gram-negative bacteria with parallel sides and rounded ends, typically 0.5 µm wide and 1-3 µm long. The bacteria do not possess flagella and move by gliding, thus are also known as the gliding bacteria. Some *Flexibacter* spp.

are widespread opportunistic bacterial pathogens. Some species of these yellow-pigmented bacteria have been associated with diseased fish, including *Flexibacter columnare* and *F. maritimus*, as well as other unidentified gliding bacteria referred to as *Cytophaga*-like bacteria. *Flexibacter* spp. are reported to cause bacterial gill disease and fin rot in groupers.

1. Bacterial Gill Disease

Bacterial gill disease has been reported in *Epinephelus malabaricus* and *E. bleekeri* in Brunei Darussalam and in *Plectopromus leopardus* in Indonesia. *Cytophaga* sp., *Flexibacter* sp. and *Flavobacterium* sp. cause bacterial gill disease in groupers in Brunei Darussalam. However, no bacteria were isolated from *Plectropomus leopardus* with bacterial gill disease in Indonesia, although histopathological examination showed rod shaped bacteria in the gills, which could possibly belong to the genus *Cytophaga*, *Flexibacter* or *Flavobacterium*.

Causative agents:

The disease is caused by *Cytophaga* sp., *Flexibacter* sp. or *Flavobacterium* sp.

Stages affected:

The bacteria usually attack fingerlings.

Gross clinical signs:

Affected fish become anorexic, lethargic and dark in color. Fish tend to remain near the surface and may be flaring their operculum. The gills produce excessive amounts of mucus and the gill filaments may stick together. The gills of affected fish become yellowish in color indicating gill rot.

Effects on host:

A high mortality rate of >80% may be observed within a week in affected populations. The bacteria attach to the gill surface, grow in spreading patches and eventually cover individual gill filaments that result in cell death. Gill lesion may cause respiratory difficulty and the fish eventually dies. Histologically, fusion of the secondary lamellae, epithelial hyperplasia and presence of rod-shaped bacteria could be observed in the gills.

Transmission:

The disease starts when the water quality deteriorates after a heavy rain. Silt and suspended organic particles from run-offs could irritate the gills and increase susceptibility to the disease. Low dissolved oxygen and high ammonia levels are often observed during disease outbreaks. Stress during grading makes fish susceptible to bacterial infection.

Diagnosis:

The disease is diagnosed by the presence of brown to yellow brown growth of bacteria in the gills (Fig. 2-3). Microscopic examination of wet mounts of the gill will reveal bacteria that are in a slow gliding movement. *Flexibacter* sp. is a thin, long Gram-negative rod with parallel sides and rounded ends, typically 0.5 µm wide and 1-3 µm long, that grows in layers, one on top of the other, giving it the appearance of "columns" or "haystacks" under the microscope.

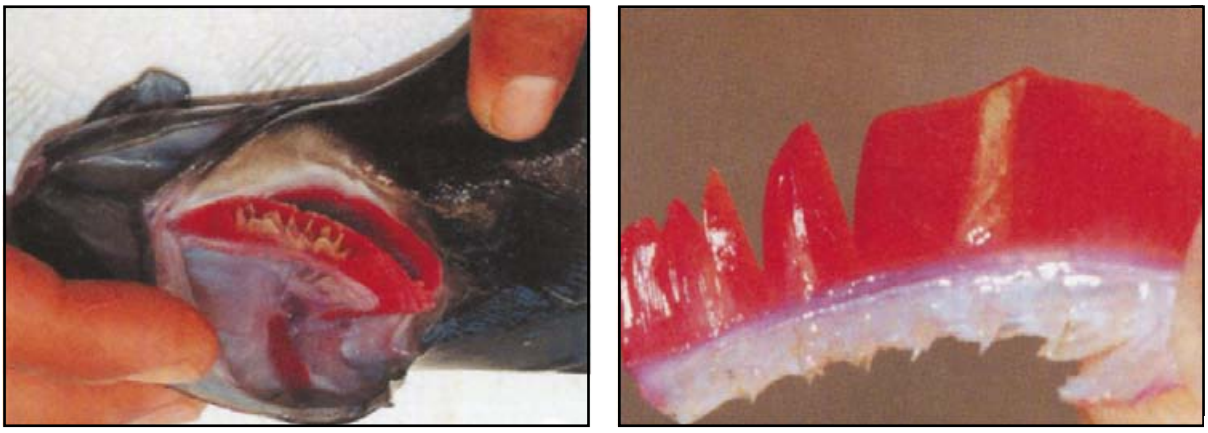


Fig. 2-3. Bacterial gill disease in *Plectropomus leopardus*. Note the presence of brown to yellow brown growth of the bacteria in the gill filaments indicating gill rot (Photos from Koesharyani *et al*., 2001).

The bacteria may be isolated using a selective media such as Selective *Cytophaga* agar and Hsu-Shotts medium. Colonies produced are pigmented yellow with rhizoids or root-like appearance.

Preventive methods:

Maintain good water quality and minimize stress by avoiding overcrowding, low dissolved oxygen and high ammonia levels. The occurrence of the disease could also be prevented by vaccination.

Control methods:

Transferring affected fish into another tank with clean water may control the infection. Affected fish may be treated with oxolinic acid mixed with feed at 20 mg/kg of fish and oxytetracycline at 75 mg/kg of fish/day for 10 days. Acriflavin dip at 100 ppm for 1 minute, and potassium permanganate at 2-4 ppm added to the water and allowed to dissipate over time could also be used to treat diseased fish (see Appendix 1 for points to consider before using antibiotics and a list of anti-infectives recommended for use in marine food fish together with the withdrawal period).

2. Fin Rot

Fin rot with hemorrhages usually affects *Cromileptes altivelis* from the wild in Indonesia. *Flexibacter maritimus* was isolated from the lesions. Secondary *Vibrio* infection may worsen the fish condition if not treated. Fin rot is also observed in fish infected with *Vibrio*.

Causative agent:

The disease is caused by *Flexibacter maritimus* (synonyms: *Cytophaga marina*, *Tenacibaculum maritimum*).

Stages affected:

The bacteria usually attack fingerlings.

Gross clinical signs:

Affected fish become anorexic, lethargic and dark in color. Initially, the tip of the fin becomes grayish, and then it becomes eroded and hemorrhagic (Figure 2-4a). The lesions progress into fin rot or extensive fin loss (Figure 2-4b). Eventually, even the muscle fibers will be affected.

Effects on host:

Mortality rate of 80% may be observed within a few days if the infected fish are not treated. The bacteria could destroy the tail region within 2 days.

Transmission:

The occurrence of the disease is correlated with water salinity. The disease is observed when the fish are exposed to high salinity of 30-35 ppt. The bacterium infects the fish through damaged area on the fin region.

Diagnosis:

Squash preparation of affected areas examined under the microscope will reveal long rod-shaped bacteria (0.5 × 2.5 µm) gliding slowly without flagella. The bacteria may be isolated from the infected tissue using *Cytophaga* agar prepared with seawater forming yellowish colonies.

Preventive measures:

Avoid rough handling of fish to minimize lesions, which could be portals of entry for the bacteria, due to mechanical damage.

Control methods:

Treatments should be implemented before secondary *Vibrio* infection sets in. Freshwater bath for 10-15 minutes and prefuran bath treatment at 1-2 ppm for 24 hours are effective in controlling the disease (see Appendix 1 for points to consider before using antibiotics and a list of anti-infectives recommended for use in marine food fish together with the withdrawal period).

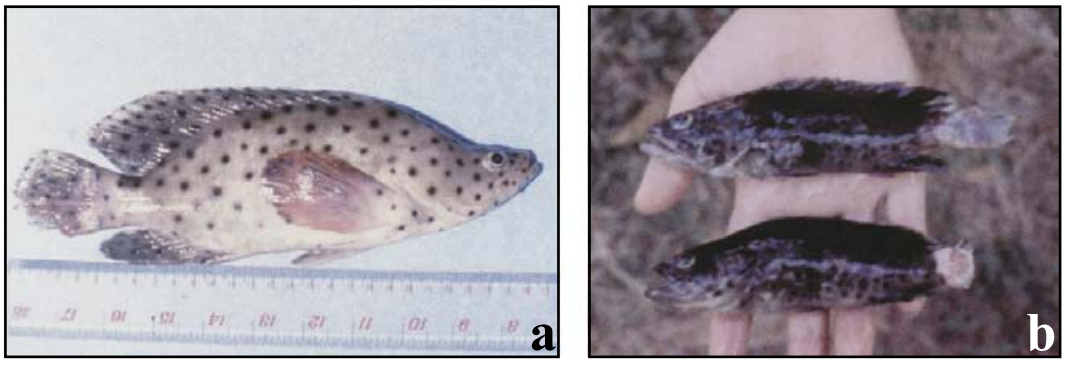


Fig. 2-4. *Cromileptes altivelis* infected with *Flexibacter maritimus*: a) hemorrhagic and eroded caudal and pectoral fins and b) extensive loss of fin (Photos from Koesharyani *et al.*, 2001).

REFERENCES

Cruz-Lacierda, E.R., de la Peña, L.D. and Lumanlan-Mayo, S. 2000. The use of chemicals in aquaculture in the Philippines. *In*: Use of Chemicals in Aquaculture in Asia, J.R. Arthur, C.R. Lavilla-Pitogo and R.P Subasinghe (eds.), p. 155-184. SEAFDEC Aquaculture Department, Iloilo, Philippines.

Hamid, H.I.H. 2001. Cage culture of grouper in Brunei Darassalam. *In*: Report and Proceeding of APEC FWG Project 02/2000 "Development of a Regional Research Programme on Grouper Virus Transmission and Vaccine Development", M.G. Bondad-Reantaso, J. Humphrey, S. Kanchanakhan and S. Chinabut (eds.), p. 51-54. Asia Pacific Economic Cooperation (APEC), Aquatic Animal Health Research Institute (AHHRI), Fish Health Section of the Asian Fisheries Society (FHS/AFS) and the Network of Aquaculture Centers in the Pacific (NACA), Bangkok, Thailand.

Holt, J.G. (ed.) 1984. Bergey's Manual of Systematic Bacteriology, Vol. 1. Williams and Wilkins, Baltimore, U.S.A. 964 p.

Holt, J.G. (ed.) 1994. Bergey's Manual of Determinative Bacteriology, 9th Edition. Williams and Wilkins, Baltimore, U.S.A. 816 p.

Kanchanakhan, S. 1996. Diseases of cultured grouper. AAHRI Newsletter 5(2): 3-4.

Kasornchandra, J. 2002. Major viral and bacterial diseases of cultured seabass and groupers in Southeast Asia. *In*: Diseases in Asian Aquaculture IV, C.R. Lavilla-Pitogo and E.R. Cruz-Lacierda (eds.), p. 205-212. Fish Health Section, Asian Fisheries Society, Manila, Philippines.

Koesharyani, I., Roza, D., Mahardika, K., Johnny, F., Zafran and Yuasa, K. 2001. Manual for Fish Disease Diagnosis–II. Marine Fish and Crustacean Diseases in Indonesia. Gondol Research Institute for Fisheries of Indonesia and Japan International Cooperation Agency, Indonesia. 49 p.

Lavilla-Pitogo, C.R., Castillo, A.R. and de la Cruz, M.C. 1992. Occurrence of *Vibrio* sp. infection in grouper*, Epinephelus suillus*. J. Appl. Ichthyol. 8: 175-179.

Lee, K.K., Liu, P.C. and Chuang, W.H. 2002. Pathogenesis of gastroenteritis caused by *Vibrio carchariae* in cultured marine fish. Mar. Biotechnol. 4: 267-277.

Lee, K.K., Yang, T.I., Liu, P.C., Wu, J.L. and Hsu, Y.L. 1999. Dual challenges of infectious pancreatic necrosis virus and *Vibrio carchariae* in the grouper, *Epinephelus* sp. Virus Res. 63: 131-134.

Leong, T.S. 1992. Diseases of brackishwater and marine fish cultured in some Asian countries. *In*: Diseases in Asian Aquaculture I, M. Shariff, R.P. Subasinghe and J.R. Arthur (eds.), p. 223-236. Fish Health Section, Asian Fisheries Society, Manila, Philippines.

Leong, T.S. 1998. Grouper culture. *In*: Tropical Mariculture, S.S. de Silva (ed.), p. 423-448. Academic Press, San Diego, U.S.A.

Leong, T.S. and Wong, S.Y. 1990. Parasites of healthy and diseased juvenile grouper (*Epinephelus malabricus* Bloch et Scheider) in Malaysia. Aquaculture 68: 203-207.

Nash, G., Anderson, I.G., Shariff, M. and Shamsudin, M.N. 1987. Bacteriosis associated with epizootic in the giant sea perch, *Lates calcarifer*, and the estuarine grouper, *Epinephelus tauvina*, cage cultured in Malaysia. Aquaculture 67: 105-111.

Ong, B. 1988. Characteristics of bacteria isolated from diseased groupers, *Epinephelus salmoides*. Aquaculture 73: 7-17.

Saeed, M.O. 1995. Association of *Vibrio harveyi* with mortalities in cultured marine fishes in Kuwait. Aquaculture 136: 21-29.

Yii, K.C. Yang, T.I. and Lee, K.K. 1997. Isolation and characterization of *Vibrio carchariae*, a causative agent of gasteroenteritis in the grouper, *Epinephelus coioides*. Curr. Microbial. 35: 109-115.

Zafran. 1998. *Flexibacter maritimus* infection in humpback grouper, *Cromileptes altivelis*. Lolitkanta Newsletter No. 9: 1-2.

Chapter 3. Fungal Disease

Elena S. Catap and Gilda D. Lio-Po

ICHTHYOPHONIOSIS

The incidence of ichthyophoniosis in groupers has been reported in *Plectropomus* sp. in Singapore and *Cromileptes altivelis* in Indonesia. It has also been known to infect at least 80 other species of teleost fish from marine, estuarine and freshwater habitats in both temperate and tropical regions (e.g., rainbow trout, yellowtail, mackerel, herring, flounder and cod). The etiologic agent of this disease is reportedly of uncertain taxonomic affinity but is often described as a fungus.

Causative agent:

The disease is caused by *Ichthyophonus* sp.

Stages affected:

Due to the chronic progression of ichthyophoniosis, the disease has been diagnosed only in market-sized grouper fish.

Gross clinical signs:

External manifestations include nonspecific signs such as loss of appetite, emaciation, lethargy and color changes. Infected fish exhibits rough skin texture or "sandpaper effect" and occasional skin ulcerations (Fig. 3-1).

Fig. 3-1. Skin ulcer of *Plectropomus* sp. due to *Ichthyphonus* sp. infection (Photo from Chong and Chao, 1986).

Effects on host:

Internal organs such as the spleen, liver and kidney become swollen and develop numerous white or cream-colored nodular lesions, up to 2 mm in diameter. Infected fish could also develop these nodules in the muscle tissues (Fig. 3-2). These nodular lesions in affected tissues are granulomas consisting of inflammatory cells surrounding spores or invading fungal hyphae (Fig. 3-3). When viewed under the microscope, these nodules could include the various life stages of the organism (early cyst, developed cyst and hyphae) in the affected tissues. The prevalence of ichthyophoniosis in most fish species affected increases with age.

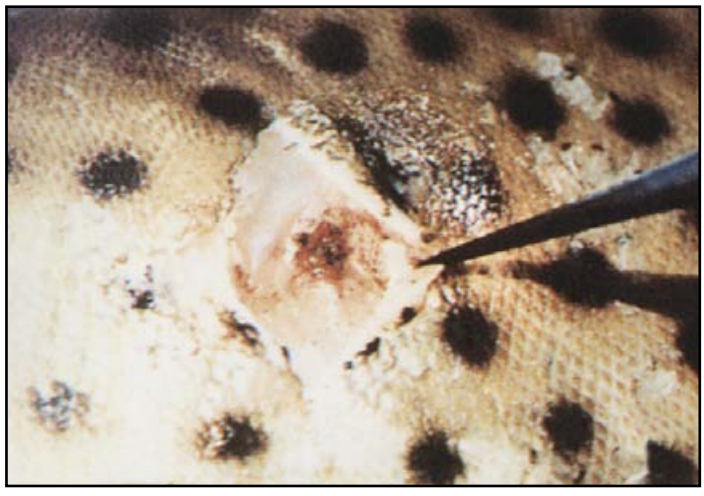


Fig. 3-2. Whitish nodular lesions or granulomas observed in the muscle tissues of *Cromileptes altivelis* (Photo from Zafran *et al.*, 1998).

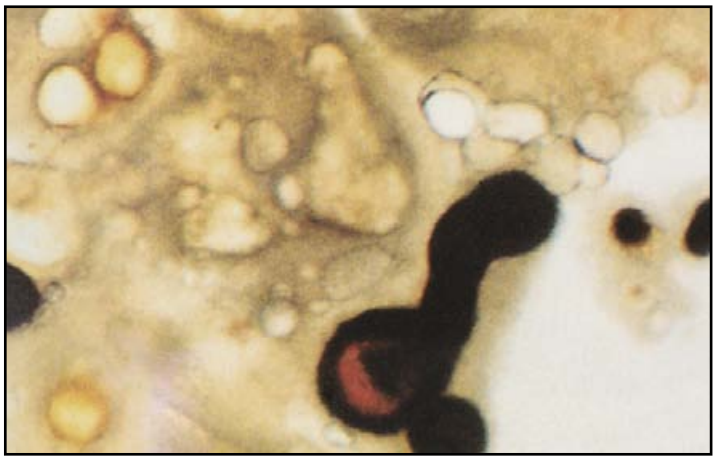


Fig. 3-3. Growing fungal hyphae surrounded by the host's cells which form the nodular lesions in affected tissues of *Cromileptes altivelis* (Photo from Zafran *et al.*, 1998).

Transmission:

The disease was experimentally reproduced in some fish species through exposure to pure cultures of the pathogen by injection or by feeding healthy fish with tissues infected with the organism. Zooplankton may possibly facilitate transmission of the causative organism.

Diagnosis:

Ichthyophoniosis may be diagnosed based on gross clinical signs, and confirmed by microscopic examination. Fresh squash preparations and histological analyses of infected fish tissues will show the presence of 50-100 µm cyst-like structures. Hyphae may be seen branching from the cysts. Alternatively, *in-vitro* culture of the heart, liver and spleen tissue excised from fish with ichthyophoniosis was reported effective in detecting subclinical infections. Tissues are cultured in Leibovitz medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 µg/ml Gentamicin, 100 units Penicillin and 25 µg/ml Streptomycin and buffered to pH 7.2 with 10 mM HEPES buffer. Tissue cultures are incubated at 12, 15 or 20°C for 10-14 days. In tissue culture, the pathogen is confirmed by the presence of fungal spores and hyphae.

Preventive and control methods:

There is no reported treatment for this disease. Infections in farms or culture areas have been commonly associated with the use of contaminated marine fish as feed; therefore caution should be exercised when using raw trash fish feed as these could be infected with the pathogen.

REFERENCES

Arthur, J.R. and Ogawa, K. 1996. A brief overview of disease problems in the culture of marine finfishes in East and Southeast Asia. *In*: Aquaculture Health Management Strategies for Marine Fishes, K.L. Main and C. Rosenfeld (eds.), p. 9-31. The Oceanic Institute, Hawaii, U.S.A.

Bruno, D.W., Alderman J. and Schlotfeld, H.J. 1995. What Should I do? A Practical Guide for the Marine Fish Farmer. European Association of Fish Pathologists. 60 p.

Chong, Y.C. and Chao, T.M. 1986. Common Diseases of Marine Foodfish. Fisheries Handbook No. 2, Primary Production Department, Singapore. 34 p.

Kocan, R.M., Hershberger, P., Mehl, T., Elder, N., Bradley, M., Wildermuth, D. and Stick, K. 1999. Pathogenicity of *Ichthyophonus hoferi* for laboratory-reared Pacific herring *Clupea pallasi* and its early appearance in wild Puget Sound herring. Dis. Aquat. Org. 35: 23-29.

Lio-Po, G.D., Lavilla, C.R. and Cruz-Lacierda, E.R. (eds.) 2001. Health Management in Aquaculture. SEAFDEC Aquaculture Department, Iloilo, Philippines. 185 p.

Okamoto, N., Nakase, K., Suzuki, H., Nakai, Y., Fujii, K. and Sano, T. 1985. Life history and morphology of *Ichthyophonus hoferi in-vitro*. Fish Pathol. 20: 273-285.

Woo, P.T.K. and Bruno, D.W. (eds.). 1999. Fish Diseases and Disorders. Vol. 3. Viral, Bacterial and Fungal Infections. CABI Publishing, Oxon, U.K. 874 p.

Zafran, Roza, D., Koesharyani, I., Johnny, F. and Yuasa, K. 1998. Manual for Fish Diseases Diagnosis. Gondol Research Station for Coastal Fisheries, Central Research Institute for Fisheries, Indonesia. 44 p.

Chapter 4. Parasitic Diseases

Erlinda R. Cruz-Lacierda and Gregoria E. Erazo-Pagador

A wide variety of parasitic organisms have been reported as causing significant problems in grouper aquaculture. In the hatchery and nursery stages, parasitic diseases of groupers are caused predominantly by protozoans, particularly the ciliates. When grouper fry are transferred to grow-out facilities, they are subjected to handling and transport stress. These fish often carry a large variety and high intensity of ciliated protozoans, skin and gill monogeneans and caligid copepods.

This chapter deals with the major parasites of cultured groupers including infections caused by protozoans, monogeneans, didymozoid digeneans, nematodes, caligid copepods, isopods and leeches.

INFECTIONS CAUSED BY PROTOZOANS

Protozoans are one-celled microscopic organisms with specialized structures for movement, food gathering and attachment. They can be external or internal parasites. They can multiply on or within their hosts. The major protozoan parasites of grouper are the dinoflagellates, ciliates, myxosporeans and microsporidians.

1. Amyloodioniosis

Amyloodioniosis is also known as velvet disease because the body surface sometimes shows the characteristic gray patches on the skin and gills. The disease is caused by a dinoflagellate. Dinoflagellates are external microscopic protozoan parasites with long, hair-like structures called flagella used as a locomotory organelle. They occur on the body surface and gills of fish. The disease has been reported in Malaysia and Indonesia affecting *Epinephelus* spp. and *Cromileptes altivelis*.

Causative agent:

The causative agent of white spot disease is the dinoflagellate *Amyloodinium ocellatum*. The parasite attaches to the host's tissue through a short stalk or peduncle that ends in a flattened attachment disk bearing numerous projections or rhizoids and a mobile tentacle-like stomopode. Mature trophonts of *A. ocellatum* measures up to 120 μm in diameter.

Stages affected:

The parasite is common in hatchery phase of culture. It can also affect fingerling and broodstock stages.

Gross clinical signs:

This dinoflagellate causes white patches or a dusty appearance on the body surface and gills, which show excessive mucus secretion. The affected fish rubs its body against objects and exhibit abnormal surface swimming characterized by spasmodic gasping and uncoordinated movements. Fish crowd together at the water surface or near the source of aeration. There is also darkening of the body and gills are pale (Fig. 4-1). Localized hemorrhage and increased respiratory rates are also reported.

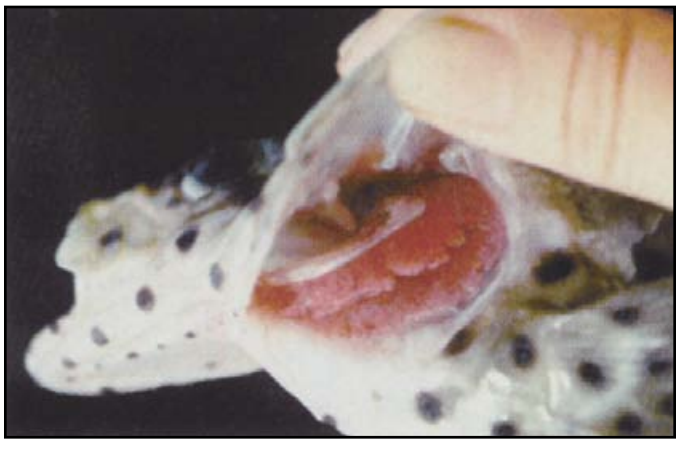


Fig. 4-1. *Cromileptes altivelis* with pale gills caused by *Amyloodinium ocellatum* (Photo from Zafran *et al*., 2000).

Effects on host:

Heavy infections can cause high or mass mortality. Disintegration of the affected tissues has been observed. Histopathological changes include severe gill lamellar epithelial hyperplasia accompanied or followed by reduced or absence of mucus cells.

Transmission:

Transmission is from fish to fish following the reproductive and cell division phase of the life cycle outside the host. Pre-disposing factors include high stocking density, high levels of organic matter in water and handling stress. After feeding, the trophont leaves the host, retracts its rhizoids and becomes a trophont. Asexual division occurs several times, motile dinospores are then released to infect a new host.

Diagnosis:

Microscopic examination of skin scrapings or gill filaments shows pear or ovoid-shaped trophonts, 150-350 × 15-70 µm in size, with elongate red stigma near the attachment site (Fig. 4-2). These trophonts appear white under reflected light.

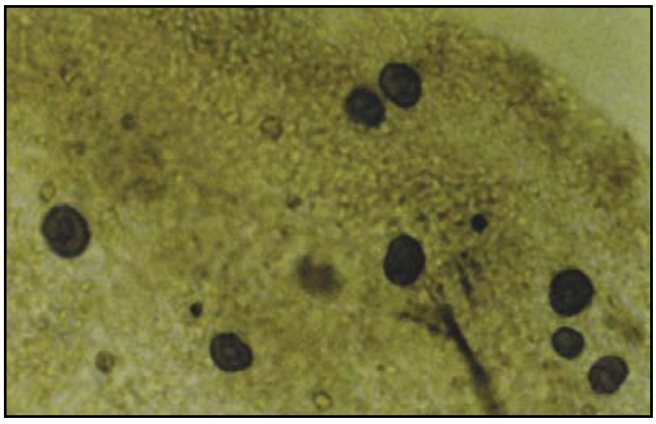


Fig. 4-2. Trophonts of *Amyloodinium ocellatum* on gills of *Cromileptes altivelis*. Fresh mount (Photo from Zafran *et al.*, 2000).

Preventive and control methods:

The parasite can be prevented through filtration of rearing water or disinfection with ultraviolet irradiation. New stocks must be quarantined. Freshwater bath will cause the parasite to drop-off from the skin and gills. Chemical bath treatments reported are 0.5 ppm copper sulfate ($CuSO_4$) for 3-5 days, or 200 ppm formalin for 30-60 minutes, provided with strong aeration. Treated fish must be transferred to clean, parasite-free tank twice at 3-day interval.

2. Cryptocaryonosis

Cryptocaryonosis is also known as white spot disease because of the presence of a few to numerous whitish or grayish spots on the body surface and gills of affected fish, which are actually nests of these parasites. It is caused by a motile ciliate. Ciliates have short, fine cytoplasmic outgrowths called cilia as the locomotory organelle. The disease has been reported in Indonesia, Malaysia, Singapore and Thailand infecting *Epinephelus bontoides, E. coioides, E. malabaricus, E. tauvina* and *Cromileptes altivelis*.

Causative agent:

Cryptocaryonosis is caused by *Cryptocaryon irritans*. The parasites are round to spherical in shape, 0.3-0.5 mm in size, with cilia on the surface.

Stages affected:

The disease causes severe epizootic especially in intensive culture systems. It may affect the hatchery and nursery phases of culture.

Gross clinical signs:

The parasites manifest in the form of whitish or grayish spots on the body surface and gills (Fig. 4-3). Diseased fish lose their appetite, are lethargic with abnormal swimming behavior, darkened body, hemorrhages on the body surface and opaque or hemorrhagic, exophthalmic eyes. Heavily infected fish show respiratory distress and produces a lot of mucus and rub their bodies against objects. Erosion of the skin may result in ulcers that are susceptible to secondary infections.

Fig. 4-3. White spots on body surface of *Epinephelus tauvina* infected with *Cryptocaryon irritans* (Photo from Chong and Chao, 1986).

Transmission:

Transmission is horizontal through infected fish and water. The mature trophont leaves the fish as a free-swimming tomont, secretes a cyst to undergo multiple cell division and produces 200 or more tomites. The tomites differentiate into free-swimming infective theronts that attaches onto the host. Pre-disposing factors are high stocking density, decreased water temperature, high organic load, and handling stress.

Diagnosis:

Microscopic examination of mucus scrapings from the body surface and gills reveals round or oval parasites revolving slowly in the host, propelled by cilia (Fig. 4-4).

Preventive and control methods:

Affected fish can be maintained in freshwater for 1 hour over 2-3 days or treated with 0.5 ppm copper sulfate ($CuSO_4$) for 5-7 days with strong aeration. Treated water must be replaced daily. Infected stocks should be transferred to parasite-free tanks 2-3 times at 3-day interval.

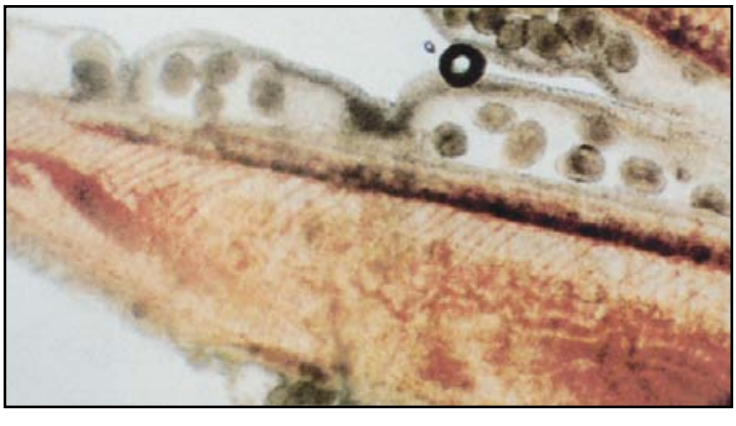


Fig. 4-4. *Cryptocaryon irritans* on gills of *Cromileptes altivelis*. Fresh mount (Photo from Zafran *et al.*, 1998).

3. Trichodiniosis

Trichodiniosis or infection caused by the ectoparasitic motile ciliate protozoan trichodinid is a common parasitic disease in intensive culture system. It has been reported in Brunei Darussalam, Indonesia, the Philippines, Malaysia, Singapore and Thailand infecting *Epinephelus bleekeri, E. bontoides, E. coioides, E. malabaricus, E. suillus, E. tauvina* and *Cromileptes altivelis*.

Causative agents:

Trichodinids have a saucer-shaped body with cilia around the perimeter of the body (Fig. 4-5). Trichodiniosis can be caused by *Trichodina* (45-78 µm diameter), *Trichodinella* (24-37 µm diameter) and *Tripartiella* (up to 40 µm diameter). The three genera can be differentiated by the shape of their denticle (Fig. 4-6).

Stages affected:

Trichodinids can affect all phases of culture, hatchery, nursery and grow-out.

Gross clinical signs:

Affected fish show excessive mucus production on the body surface and gills with frayed fins and pale gills. Heavily infected fish rub their body against objects. Fish are weak during heavy infection.

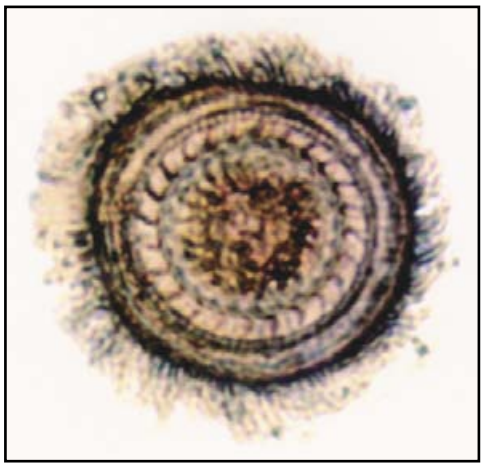


Fig. 4-5. *Trichodina* sp. from body surface of infected *Epinephelus coioides*. Silvernitrate trichrome stain.

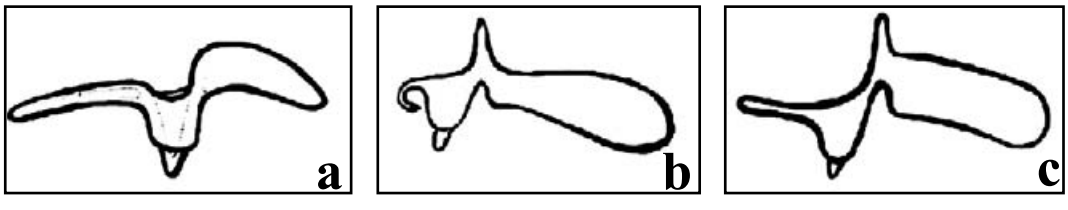


Fig. 4-6. Shape of a single denticle of a) *Trichodina*, b) *Trichodinella* and c) *Tripartiella* (Figures modified from Kabata, 1985).

Effects on host:

When present in excessive numbers on the skin and gills of fish, the parasites may interfere with respiration, leading to high mortality among young fish. The spinning motion and adhesive disc of the parasite can cause direct damage to the branchial epithelium, resulting in gill lesions.

Transmission:

Transmission is horizontal through infected fish, water, contaminated farm equipment and live feed. Pre-disposing factors are high levels of organic matter in the water, poor water exchange and handling stress.

Diagnosis:

The parasites can be demonstrated by microscopic examination of wet mounts of scrapings from the skin and gills (Fig. 4-7). The adhesive disc, which is the taxonomic characteristic, can be differentiated by staining with silvernitrate trichrome ($AgNO_3$).

Prevention and control methods:

Control methods include bath treatment with freshwater for 1 hour for 3 days, 200 ppm formalin for 30-60 minutes with strong aeration or 25-30 ppm formalin for 1-2 days.

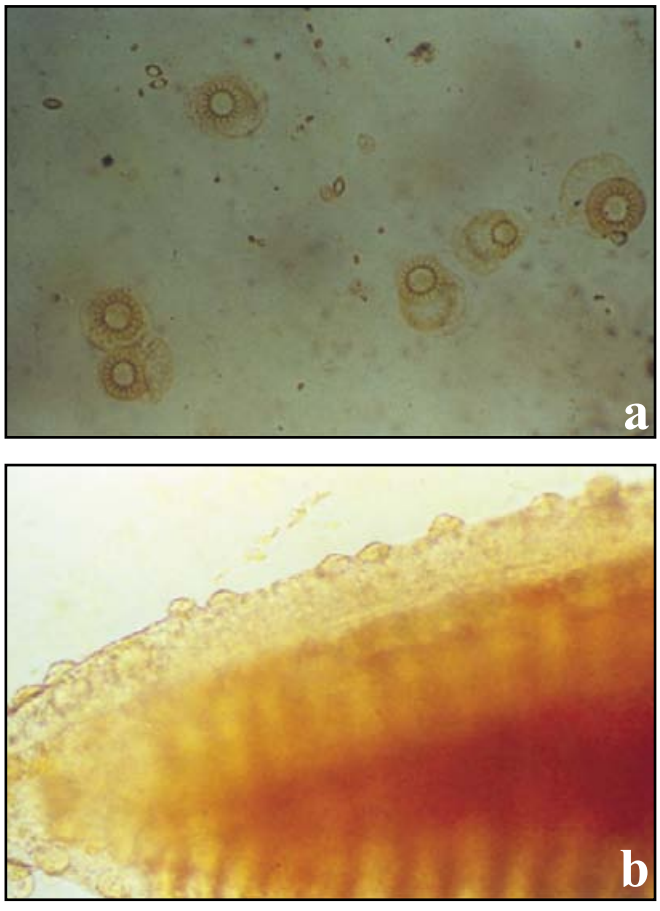


Fig. 4-7. *Trichodina* sp. from *Epinephelus coioides*: a) On body surface and b) on gill filaments. Fresh mount.

4. Brooklynelliosis

Brooklynelliosis is an infection caused by an ectoparasitic motile protozoan ciliate. The disease has been reported infecting cultured *Epinephelus tauvina* in Singapore.

Causative agent:

Brooklynelliosis is caused by *Brooklynella* spp., kidney-shaped ciliates, up to 60 µm in size, with long parallel lines of cilia.

Species and stages affected:

The parasite may affect fry and fingerling stages.

Gross clinical signs:

Affected fish rub their body against objects. This parasite causes extensive skin damage and subcutaneous hemorrhage after it has attached to the skin and gills (Fig. 4-8).

Effects on host:

The parasite causes subcutaneous and respiratory problems. The hosts may also develop secondary bacterial infection. It may also cause mass mortality.

Transmission:

Pre-disposing factors are high stocking density, poor water quality and handling stress. Transmission is horizontal through infected fish and water.

Diagnosis:

The parasite can be demonstrated by microscopic examination of wet mounts of mucus from the skin and gills of affected fish. The bean-shaped 36-86 × 32-50 µm protozoans with long parallel lines of cilia beat in wave-like motion (Fig. 4-9).

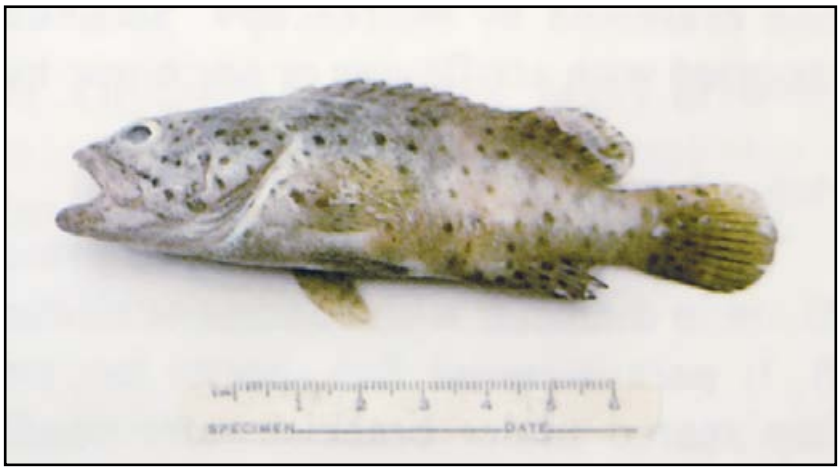


Fig. 4-8. Brooklynelliosis in *Epinephelus tauvina* showing extensive damage on body surface and subcutaneous bleeding (Photo from Chong and Chao, 1986).

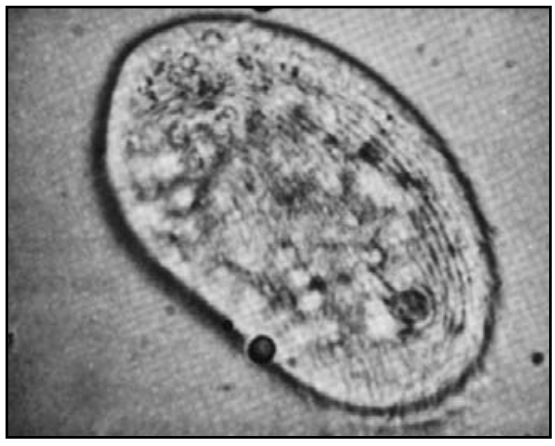


Fig. 4-9. *Brooklynella* sp. from body surface of *Epinephelus tauvina*. Cilia that form parallel lines along the body surface of the parasite are seen. Fresh mount (Photo from Chong and Chao, 1986).

Preventive and control methods:

Exposure to freshwater bath for 1 hour for 3 days, or 100-200 ppm formalin for 30-60 minutes for 2-3 days provided with strong aeration.

5. Renal Sphaerosporosis

Renal sphaerosporosis is an infection caused by an endoparasitic myxosporean. Myxosporeans are microscopic protozoans composed of several spore shell valves and are obligate parasites in organ cavities and tissues of fish, thus, they cannot survive outside the host. The myxosporean *Sphaerospora epinepheli* has been reported to cause renal sphaerosporosis in *Epinephelus malabaricus* in Thailand.

Causative agent:

Sphaerospora epinepheli, with spores 8.7 × 8.2 µm, containing two spherical polar capsules is the causative agent of renal sphaerosporosis (Fig. 4-10).

Stages affected:

The parasite can affect nursery, grow-out and broodstock stages.

Gross clinical signs:

Infected fish exhibit loss of equilibrium, floating or turning upside down, some with hemorrhages on the mouth and body surface. A few affected fish show hemorrhages in the swim bladder and swollen abdomen.

Effects on host:

The spores and psuedoplasmodia of the parasite invade and destroy the kidney, liver, gall bladder, intestine, spleen and blood cells. There is necrosis in the tubular epithelium and renal corpuscles of the kidney. Affected fish may be more susceptible to other pathogens.

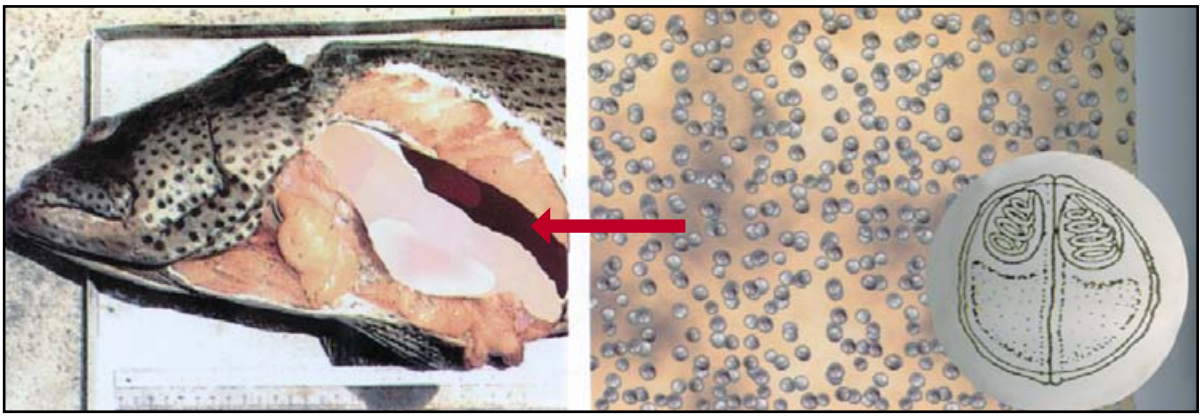


Fig. 4-10. Myxosporeans in kidney of *Epinephelus malabaricus* (Photo from APEC/SEAFDEC, 2001).

Transmission:

Pre-disposing factors include poor water quality such as fluctuating salinity and low dissolved oxygen, high stocking density and lack of quarantine. Transmission is uncertain and the developmental cycle is unclear. An alternate host may be included in the life cycle. Generally, when the host fish ingests a spore, the coiled polar filament in the polar capsule is released and attaches to the intestinal mucosa. The valves then separate and the infective sporoplasm is released. Two sporoplasms fuse to form a zygote that migrates to the target tissue. The zygote then proliferates into multinucleated plasmodium. The plasmodium grows, multiplies and produces spores that are released either in the water or after death of the host.

Diagnosis:

Demonstration of the parasite is done by microscopic observation of spores and developmental stages in affected tissue such as imprints from kidney. Mature spores are sub-spherical to spherical in shape, measures 7.8-10 µm in length, 12.3-14.5 µm in thickness and 7.0-9.5 µm in width, and with two spherical polar capsules, equal in size with a diameter of 2.9-4.4 µm. Mature spores are found in the lumen of renal tubules while pseudoplasmodia are mostly located in the peripheral brush border of the epithelium of the renal tubules.

Prevention and control methods:

Preventive methods include efficient water exchange and quarantine of new stock. Infected water should not be used for rearing fish. Ultraviolet treatment of inflow water can control the infective stage. Affected stock must be discarded.

6. Microsporidiosis

Microsporidiosis is an infection caused by a microsporidian. Microsporidians are protozoans intracellular parasites with unicellular spores (3-10 µm) containing sporoplasm and coiled polar filament. The disease has been reported in *Epinephelus tauvina* and *Epinephelus* spp. in China and India.

Causative agent:

Microsporidiosis form small nodules on the affected tissue and these are filled with pear-shaped spores. *Glugea* sp. and *Pleistophora* sp. have been reported in grouper culture. The spores are 6 µm in size.

Stages affected:

The parasite can affect nursery and grow-out stages.

Gross clinical signs:

Infected fish have swollen abdomen. Brown to black nodules of various sizes has been observed in fat tissue and internal organs (Fig. 4-11).

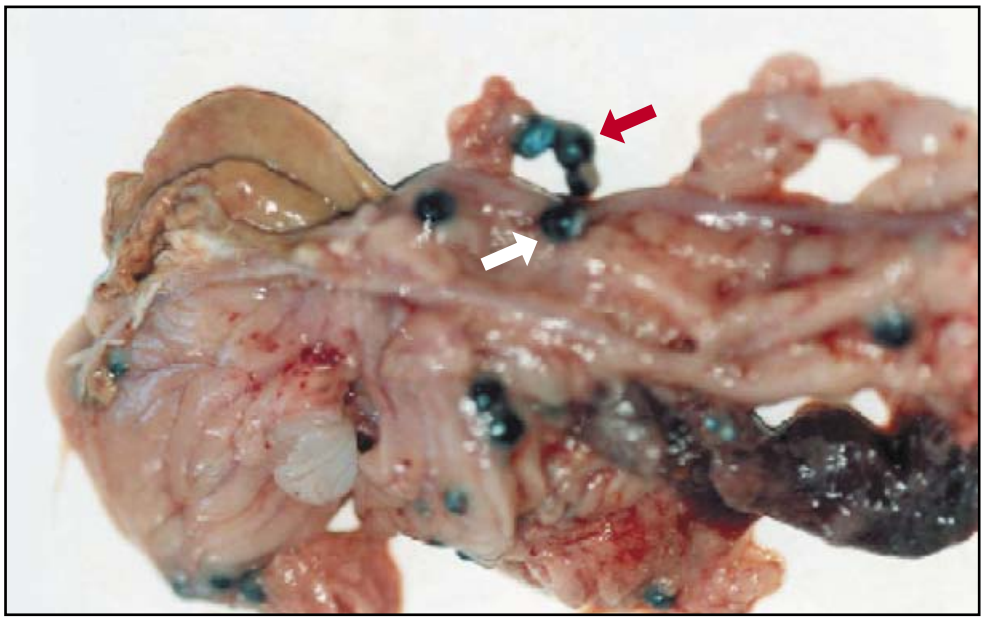


Fig. 4-11. Brownish-black cysts (arrows) on parenchyma of digestive organs of *Epinephelus tauvina* (Photo courtesy of Lin Li).

Effects on host:

Mortality is variable.

Transmission:

Pre-disposing factors are poor water quality and poor nutrition. Transmission may be horizontal through oral ingestion of spores. The life cycle is unknown.

Diagnosis:

Microscopic examination of fresh-squashes of Giemsa-stained smears from infected tissues will reveal oval-shaped spores, 5-6.5 × 2-2.5 µm in size (Fig. 4-12).

Prevention and control methods:

Good water exchange, isolation and destruction of infected fish, disinfection of culture systems with chlorine or iodine solutions, avoidance of feeding with contaminated trash fish are some preventive methods.

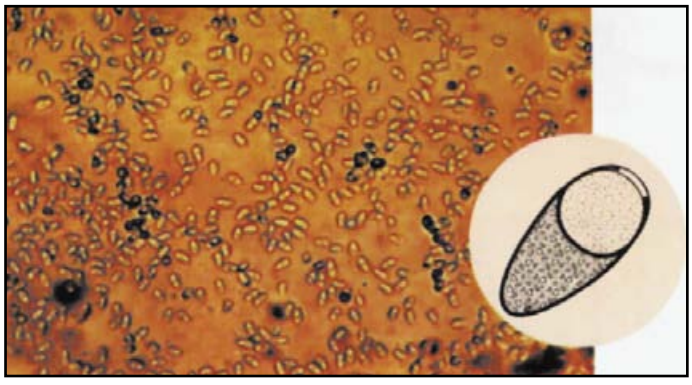


Fig. 4-12. Microsporidians from parenchyma of digestive organ of grouper. Fresh mount (Photo from APEC/ SEAFDEC, 2001).

INFECTIONS CAUSED BY MONOGENEANS

Monogeneans are ectoparasites with posterior organ of attachment called haptor armed with hooks and/or suckers. Some of the monogeneans are large enough to be seen by an unaided eye, while most are microscopic. The major monogeneans of groupers are the skin and gill parasites.

1. Skin Monogeneans

Skin monogeneans have been reported in several grouper species including *Epinephelus bleekeri, E. coioides, E. fuscoguttatus, E. lanceolatus, E. malabaricus, E. tauvina* and *Cromileptes altivelis*. The geographic distribution of the disease includes Brunei Darussalam, China, India, Indonesia, Kuwait, Malaysia, Myanmar, the Philippines, Singapore and Thailand.

Causative agents:

Skin monogeneans are 2-6 mm long. Infections reported are caused by the capsalid monogeneans *Benedenia epinepheli, Benedenia* spp., *Neobenedenia girellae* and *Neobenedenia* spp.

Stages affected:

The skin monogeneans may affect the nursery, grow-out and broodstock stages.

Gross clinical signs:

The parasite attaches on the eyes, body surface and gills of fish (Fig. 4-13). Affected fish rub their body against objects and aggregate near the source of aeration with flashing swimming

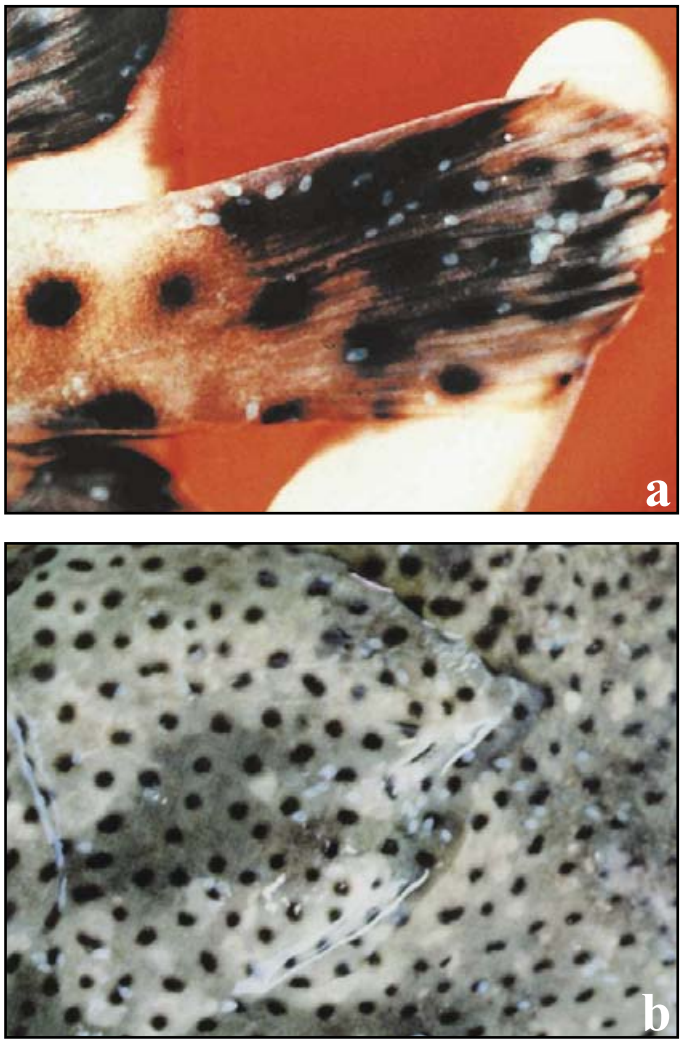


Fig. 4-13. Skin monogeneans appear opaque white on a) caudal fin and b) body surface of *Cromileptes altivelis* (Photos from Zafran *et al*., 1998 and Koesharyani *et al*., 2001).

behavior. Fish lose their appetite and are lethargic. The body surface of heavily infected fish is hemorrhagic and eyes are opaque.

Diagnosis:

Diagnosis is done by gross examination of the body surface of fish and confirmation by low power microscopic examination of the parasite. The transparent parasite turns white and detaches from the host when infected fish are placed in freshwater. These capsalid monogeneans are flat, oval in shape, with a pair of anterior sucker on the anterior margin and a large opisthaptor on the posterior region, and two pairs of eye spots behind the anterior sucker (Fig. 4-14). The length and width of mature *Benedenia* sp. ranges from 1.8-9.5 mm and 0.82-2.5 mm, respectively, while *N. girellae* is 3.3-6.1 × 1.8-3.7 mm.

Effects on host:

The parasite may cause blindness if eyes are affected. The lesions can serve as portals of entry for secondary bacterial infection. High or mass mortality have been reported.

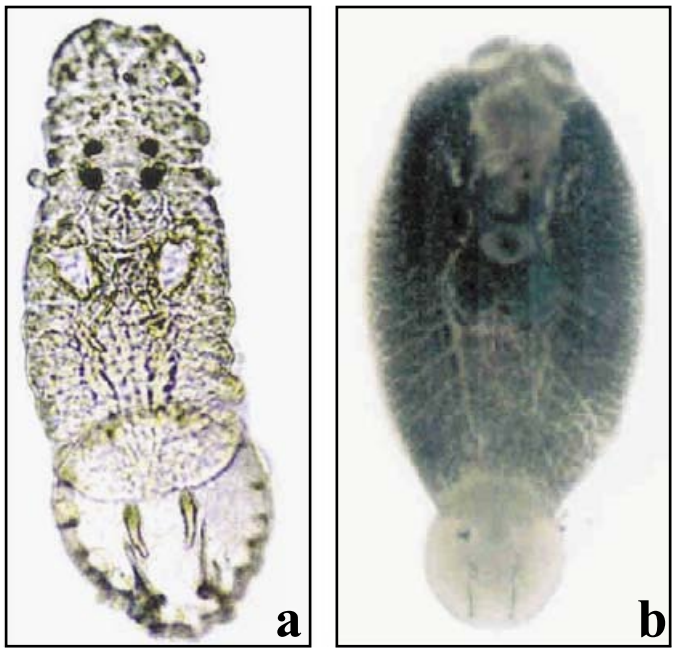


Fig. 4-14. *Benedenia* sp. from body surface of grouper: a) Larval stage and b) adult stage.

Transmission:

Transmission is horizontal and pre-disposing factor is overlapping generation of cultured fish. High stocking density provides greater opportunity for infecting the fish host by the monogenean. The eggs of skin monogeneans are tetrahedral in shape with long spiral filament and are usually attached on tank walls and nets. The egg hatches into a free-swimming larval stage, the oncomiracidium, in 4 days. The larvae attach onto a suitable host and mature in 7 days after hatching.

Preventive and control methods:

Freshwater bath for 5-30 minutes depending on tolerance of host or 150 ppm hydrogen peroxide (H_2O_2) for 10-30 minutes is effective in dislodging the parasites from skin and gills. Strong aeration must be provided during treatment.

2. Gill Monogeneans

The gill monogeneans have been reported in *Epinephelus bleekeri, E. bontoides, E. coioides, E. malabaricus, E. tauvina* and *Cromileptes altivelis*. The geographic distribution of the disease includes Brunei Darussalam, Indonesia, Malaysia, Myanmar, the Philippines, Thailand and Singapore.

Causative agents:

The gill monogeneans *Pseudorhabdosynochus* spp., *Megalocotyloides* spp. and *Diplectanum epinepheli* are commonly reported. They are <1-5 mm long. *Pseudorhabdosynochus lantauensis* is the most prevalent gill monogenean in *E. coioides* (Fig. 4-15).

Stages affected:

The gill monogeneans are common in nursery, grow-out and broodstock stages.

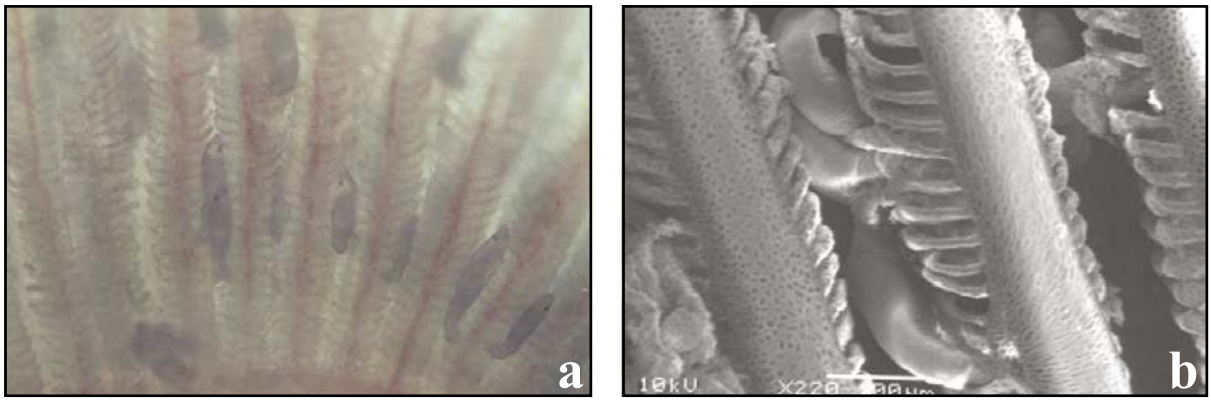


Fig. 4-15. *Pseudorhabdosynochus lantauensis* on gill filaments of *Epinephelus coioides*: a) Fresh mount and b) scanning electron microscopy.

Gross clinical signs:

Affected fish display abnormal swimming behavior near the water surface and loss of appetite. An increased mucus production is observed on darkened body surface with frayed fins and pale gills. Hemorrhagic lesions on the body surface are common in heavy infection.

Effects on host:

The most common effect on affected fish is hyperplasia of the epithelial cells on the gill lamellae (Fig. 4-16). When there is extensive damage to the gill epithelium, respiration is affected. Heavy infection is aggravated by low level oxygen and may result in mortality. Vibriosis is commonly associated with this parasite.

Transmission:

Transmission is horizontal and pre-disposing factor is overlapping generation of fish. High stocking density provides greater oppurtonity for faster infection. The egg of a gill monogenean is elongate with a long, spiral filament. Hatching of eggs occurs in 5 days to produce the free-swimming larval stage, the oncomiracidium. The larvae then attach to the body surface and migrate to the gills of the fish. The life cycle is completed in 14-21 days.

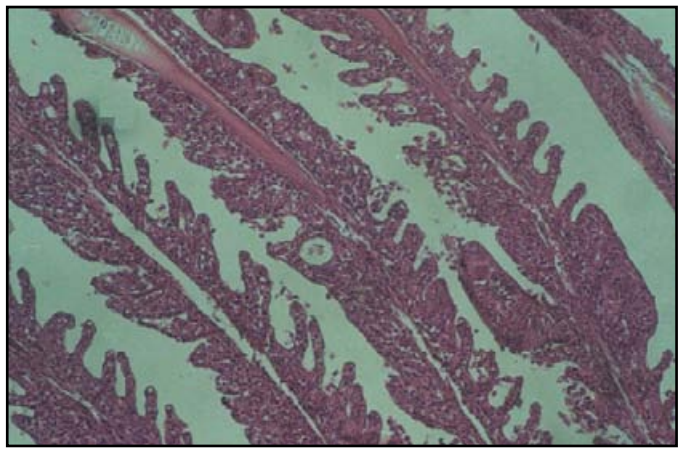


Fig. 4-16. Epithelial hyperplasia of gill lamellae of *Epinephelus coioides* infected with *Pseudorhabdosynochus lantauensis*. H & E stain.

Diagnosis:

Diagnosis is done by gross macroscopic examination of the body surface and gills of affected fish. Confirmation is by microscopic examination of mucus from the gills. The parasites attach themselves to the gill filaments (Fig. 4-17).

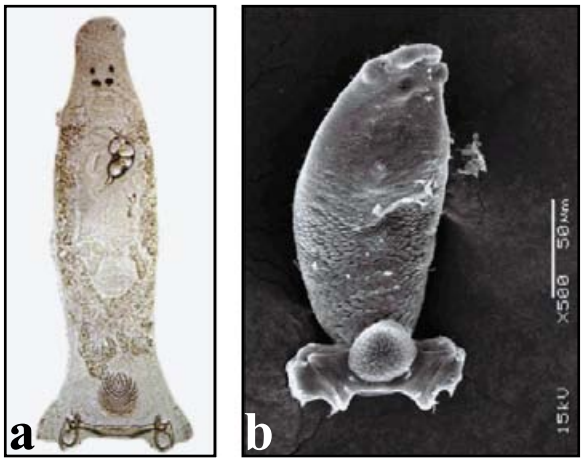


Fig. 4-17. *Pseudorhabdosynochus lantauensis* from gill filaments of *Epinephelus coioides*: a) Stained specimen and b) scanning electron microscopy.

Preventive and control methods:

Control methods include short bath treatment of 200 ppm hydrogen peroxide (H_2O_2) for 1 hour or 100-200 ppm formalin for 30-60 minutes. Strong aeration should be provided during treatment.

INFECTIONS CAUSED BY DIDYMOZOID DIGENEANS

Didymozoid digeneans are long, up to 80 cm, parasitic flatworms that form capsules or cysts on the gills of the host fish. It has been recorded in *Epinephelus coioides, E. malabaricus, E. tauvina* and *Epinephelus* sp. in Indonesia, Kuwait, Malaysia, Myanmar, the Philippines and Thailand.

Causative agent:

The species *Gonapodasmius epinepheli* has been reported from *E. coioides* in the Philippines and from *E. malabaricus* in Thailand.

Stages affected:

Didymozoids have been reported in nursery and grow-out stages.

Gross clinical signs:

Small, opaque-white to yellow cysts are found on the first gill arch of affected fish (Fig. 4-18).

Fig. 4-18. Yellow capsules containing didymozoid digenean on gill arch of *Epinephelus coioides* (Photo courtesy of K. Yuasa).

Effects on host:

Infected gill filaments are distorted in shape. The parasite causes focal hyperplasia of the epithelial cells of the gill lamellae and an increase in the number of mucus cells (Fig. 4-19).

Transmission:

The complete life cycle is unknown but it is likely that the first larval stage, the free-swimming miracidium, is taken by a gastropod mollusc that acts as the first intermediate host. The second larval stage, the cercaria, is released in the water and encysts in the second intermediate host. Small crustaceans have been implicated as the second intermediate hosts while small fish have been reported to act as probably the paratenic host. The encysted metacercaria is eaten by the final host fish.

Diagnosis:

Gross and microscopic examinations of the gills reveal opaque-white or yellow capsules attached lengthwise along the posterior surface of the gill filaments. The capsules contain tubular, long (several cm), thread-like worms tightly and neatly-arranged and packed inside. The worms are encapsulated between the basement membrane of the epithelium and the efferent artery of the primary gill filament. Digeneans generally have two sucker-like attachment organs located at the anterior and ventral portions.

Preventive and control methods:

The intermediate hosts (gastropod molluscs) which may be carriers of the larval stage of the parasite should be eliminated from the culture facility.

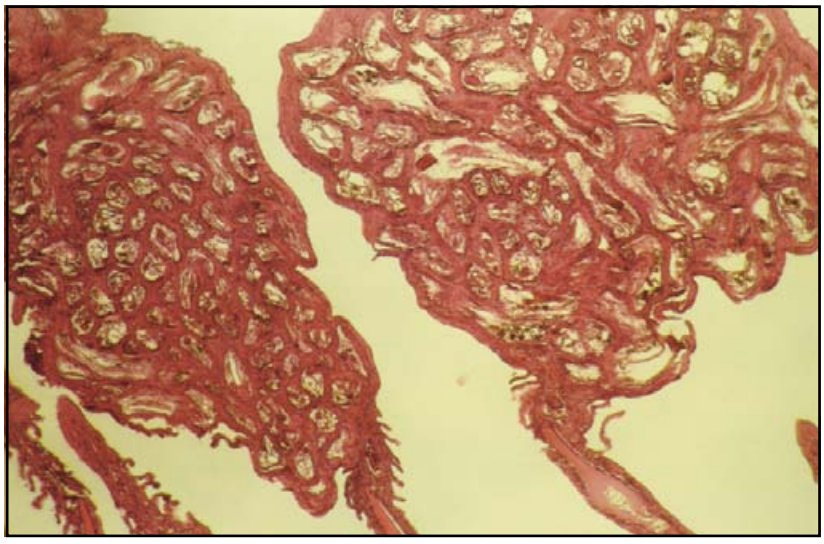


Fig. 4-19. Gill filaments of *Eiphenelus coioides* infected with the didymozoid digenean *Gonapodasmius epinepheli*. H & E stain.

INFECTIONS CAUSED BY NEMATODES

Nematodes or roundworms are internal parasites with un-segmented bodies, usually 1-2 cm long. The adult stage of nematodes is big enough to be seen by the naked eye. Nematodes have been reported to infect *Epinephelus coioides, E. malabaricus, Cromileptes altivelis* and *Plectropomus leopardus* in Indonesia, Malaysia and Thailand.

Causative agents:

The most common nematodes of groupers are *Philometra* sp., *Anisakis* sp. and *Raphidascaris* sp.

Stages affected:

Nematodes may affect nursery, grow-out and broodstock stages.

Gross clinical signs:

Reddish or black, non-segmented roundworms are attached on affected organs of the fish such as the fins, branchial cavity, muscles, parenchyma of digestive organs, and gonads (Fig. 4-20). The body surface of heavily affected fish may be discolored and emaciated.

Effects on host:

The parasite probably impairs feeding, resulting in reduced growth rate and emaciation. Such appearance of fish will result in reduced market value. When gonads are affected, there is atrophy and may lead to sterility of the host.

Transmission:

Transmission is horizontal through feeding of infected intermediate host or trash fish. The adult nematode releases egg that hatches into a free-swimming larva. This is eaten by

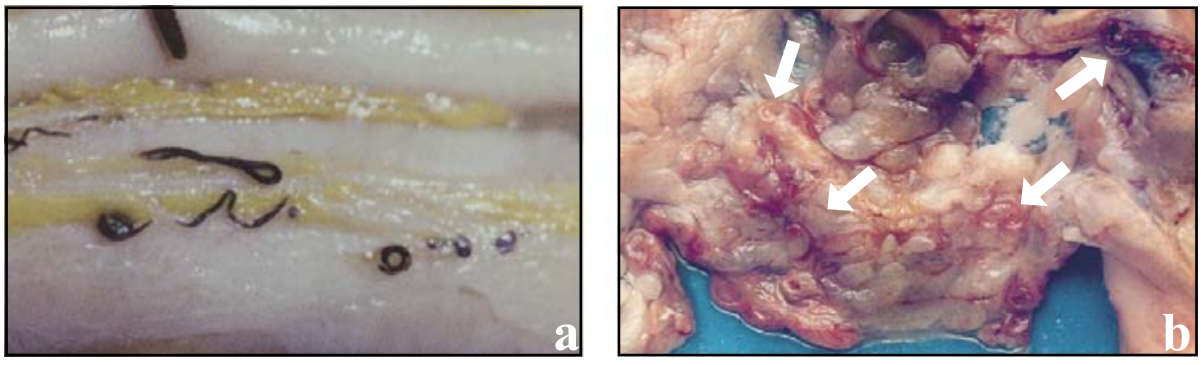


Fig. 4-20. Nematodes on parenchyma of digestive organ: a) *Plectropomus leopardus* and b) *Epinephelus coioides* (Photos from Koesharyani *et al*., 2001 and T.S. Leong).

an invertebrate intermediate host. Larval development takes place in the intermediate host, which is preyed on by the final fish host. Small fish can serve as the paratenic host of anisakid nematodes, and *Anisakis* sp. in the infected cultured grouper remains in the larval stage until eaten by the aquatic mammalian host.

Diagnosis:

The parasites are observed by gross and microscopic examinations. The affected tissues are dissected to reveal the parasite. A mature *Philometra* sp. can reach more than 20 cm in length.

Preventive and control methods:

Avoid feeding with infected trash fish; eliminate intermediate hosts (copepods) and dry the pond bottom; disinfect the culture facilities with quicklime to destroy the eggs of the nematode; filter the water used for rearing.

INFECTIONS CAUSED BY CALIGID COPEPODS

The caligid copepods are external crustacean parasites with segmented bodies covered by shell with jointed appendages. They are oval in shape, up to 3 mm in length and 1.6 mm in width, with four pairs of legs. Caligid copepods have been reported to infect several grouper species including *Epinephelus coioides, E. fuscoguttatus, E. malabaricus, Cromileptes altivelis* and *Plectropomus leopardus*. It has been recorded in Indonesia, Malaysia, the Philippines, Thailand and Vietnam.

Causative agents:

The most common caligid copepods in grouper culture are *Caligus epidemicus*, *Caligus* sp. and *Lepeophtheirus* sp.

Stages affected:

Caligids are common in nursery, grow-out and broodstock stages.

Gross clinical signs:

These parasites are transparent and are not permanently attached to the body surface and fins of fish. They appear as white patches (Fig. 4-21). The areas infected are devoid of scales and are hemorrhagic or ulcerated. Affected fish have lumpy body surface, swim sluggish near the water surface or show flashing behavior, with loss of appetite, and excessive mucus production. Fish are weak during heavy infection.

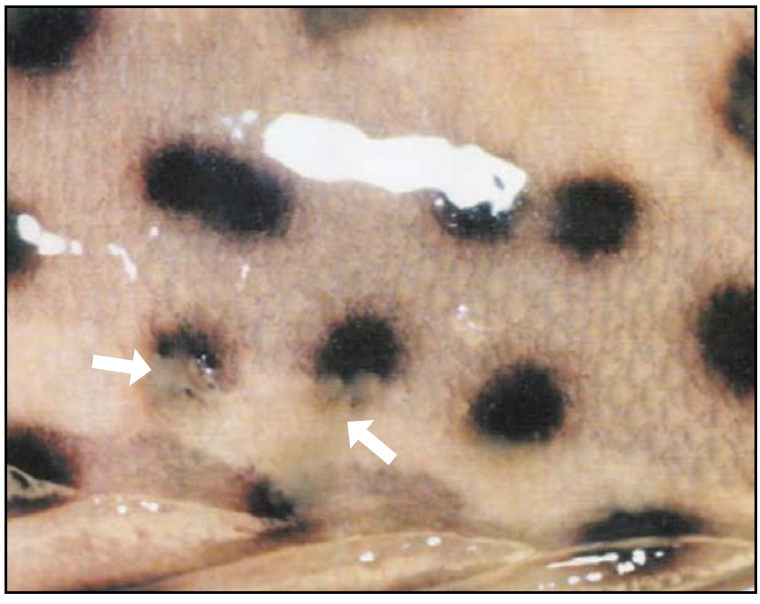


Fig. 4-21. Caligid copepods appear as white patches (arrows) on body surface of *Cromileptes altivelis* (Photo from Koesharyani *et al.*, 2001).

Effects on host:

Skin and muscle erosion have been observed. After heavy infection, secondary bacterial infection may occur, resulting to high or mass mortality.

Transmission:

Pre-disposing factor is poor water exchange. Transmission is horizontal. The life cycle of *C. epidemicus* has 11 stages: two nauplii, one copepodid, six chalimus, one pre-adult and one adult. The whole life cycle is completed in 15 days.

Diagnosis:

The parasites are observed by gross and microscopic examinations of scrapings from areas which are infected. Caligids are transparent, with segmented bodies covered by shell with jointed appendages (Fig. 4-22).

Preventive and control methods:

Sufficient water exchange can prevent infection. The parasites can be controlled by freshwater bath for 10-15 minutes, or chemical bath treatment using 150 ppm hydrogen peroxide (H_2O_2) for 30 minutes or 200-250 ppm formalin for 1 hour. Strong aeration must be provided during treatment. Treated fish should be transferred to clean, parasite-free facility.

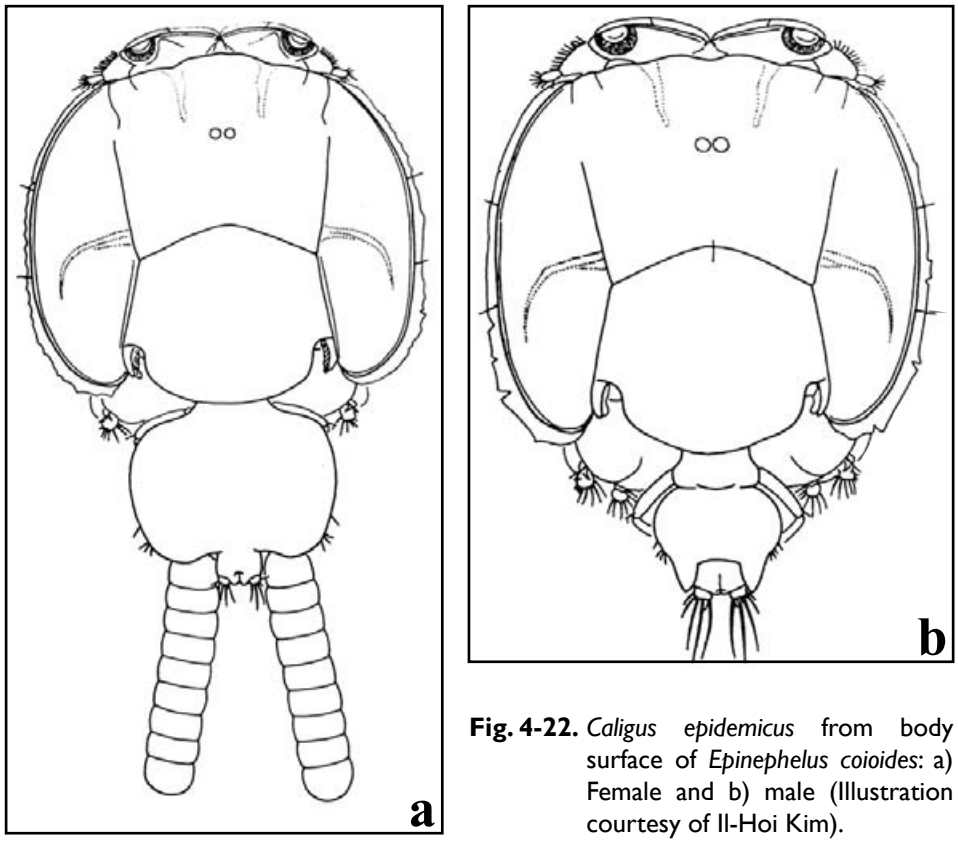


Fig. 4-22. *Caligus epidemicus* from body surface of *Epinephelus coioides*: a) Female and b) male (Illustration courtesy of Il-Hoi Kim).

INFECTIONS CAUSED BY ISOPODS

Isopods are 10-50 mm in size, and the body is divided into narrow segments, with a pair of eyes. The parasite has been recorded in *Epinephelus coioides* and *E. malabaricus* in Indonesia and Thailand.

Causative agent:

The isopod *Rhexanella* sp. has been observed in *E. coioides*.

Stages affected:

The isopod can affect nursery, grow-out and broodstock stages.

Gross clinical signs:

The parasite attaches on the body surface, mouth, nasal cavity and opercular cavity (Fig. 4-23). There is loss of appetite and the fish exhibit reduced opercular movement and slow growth rate. The fish rub its body against objects. Fish become weak when the isopod resides in the buccal cavity.

Effects on host:

The host tissue is destroyed brought about by the pressure of the parasite's body. There is necrosis of the dermis and the gill filaments. Swimming and feeding behavior are affected. Rapid death occurs in 1-2 days particularly in young fish during heavy infection.

Fig. 4-23 Isopod (arrow) attached to body surface of *Epinephelus coioides* (Photo from Koesharyani *et al.*, 2001).

Transmission:

Pre-disposing factor is high stocking density. Transmission is horizontal.

Diagnosis:

The parasites are observed by gross and microscopic examinations. The size of *Rhexanella* ranges from 10-50 mm in length, with 7 pairs of legs and a pair of eyes (Fig. 4-24).

Preventive and control methods:

The parasites may be removed manually and destroyed by crushing or other physical means; bath treatment using 200 ppm formalin for 30-60 minutes, provided with strong aeration; spraying of 1% formalin on nets; transfer treated fish to clean, parasite-free facility; disinfect infected facility by drying of the pond bottom for several weeks, followed by liming.

Fig. 4-24. *Rhexanella* sp. from body surface of *Epinephelus coioides* (Photo from Koesharyani *et al.*, 2001).

INFECTIONS CAUSED BY LEECHES

Leeches are external parasites with striated bodies, muscular body wall and two suckers used for feeding and movement. The parasite has been recorded in *Epinephelus bleekeri, E. coioides, E. fuscoguttatus, E. lanceolatus, E. malabaricus* and *Cromileptes altivelis* in Malaysia, the Philippines and Thailand.

Causative agent:

Zeylanicobdella arugamensis has been reported in *E. coioides* in the Philippines.

Stages affected:

Leeches are reported to affect nursery, grow-out and broodstock stages.

Gross clinical signs:

The brownish-black parasites are attached in patches in affected areas such as the body surface, fins, eyes, brachial and mouth cavities (Fig. 4-25). The fins of affected fish are frayed and the attachment and feeding sites are hemorrhagic and swollen. Affected fish lose their appetite, show sluggish movement, swimming at water surface.

Effects on host:

The affected fish have anemia and may show secondary bacterial infections. These leeches are known to act as vectors of viruses, bacteria and protozoan blood parasites. Mortality may occur in heavily infected fish.

Transmission:

Pre-disposing factors are poor maintenance of facilities and poor water quality. Transmission is horizontal. Mature leech leaves the host fish to deposit cocoons on hard substrate such as rocks, shells or vegetation. A cocoon contains a single egg which hatches onto a young piscicolid leech. The young leech then attaches to a host where it matures. Leeches usually die after deposition of cocoon.

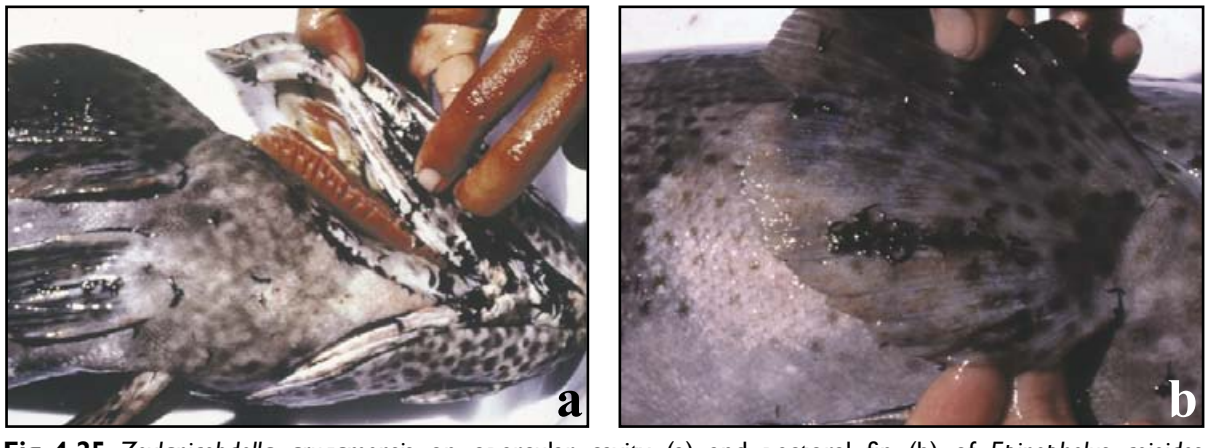


Fig. 4-25. *Zeylanicobdella arugamensis* on opercular cavity (a) and pectoral fin (b) of *Epinephelus coioides* broodstock. The attachment and feeding sites of the parasite are hemorrhagic.

Diagnosis:

The leech has an elongated and cylindrical body narrowing at both ends containing the suckers. The characteristic oral (anterior) and caudal (posterior) suckers of the leech can be seen by gross and microscopic examinations (Fig. 4-26). These suckers are alternately, strongly attached on the host. Mature leech can reach 15mm in length.

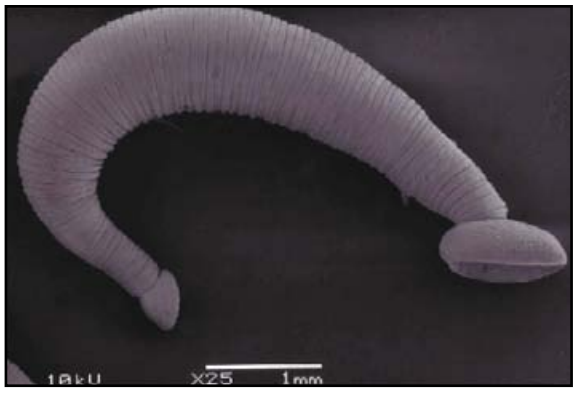


Fig. 4-26. Scanning electron micrograph of *Zeylanicobdella arugamensis* showing striations on body surface and oral and caudal suckers.

Preventive and control methods:

Leeches may be removed from culture water by filtration. Manual removal using wet cloth has been effective in removing large patches of the parasite (Fig. 4-27). A bath treatment of 200-250 ppm formalin for 1 hour, provided with strong aeration will detach most of the parasite. After treatment, treated fish should be transferred to clean, parasite-free facility. Culture facilities must be cleaned with detergent, disinfected with chlorine and exposed to intense sunlight for several weeks prior to use to eliminate cocoons of the parasite.

Fig. 4-27. Manual removal of leeches attached to *Epinephelus coioides* using a wet cloth.

REFERENCES

Al-Marzouq, A. and Al-Rifae, K. 1994. *Benedenia* sp., a monogenetic parasite of cultured brown-spotted grouper, *Epinephelus tauvina*, in Kuwait. J. Aqua. Trop. 9: 255-258.

APEC/SEAFDEC. 2001. Husbandry and Health Management of Grouper. APEC, Singapore and SEAFDEC Aquaculture Department, Iloilo, Philippines. 94 p.

Bondad-Reantaso, M.G., Kanchanakhan, S. and Chinabut, S. 2001. Review of grouper diseases and health management strategies for groupers and other marine finfishes. *In*: Report and Proceeding of APEC FWG 02/2000 "Development of a Regional Research Programme on Grouper Virus Transmission and Vaccine Development", M.G. Bondad-Reantaso, J. Humphrey, S. Kanchanakhan and S. Chinabut (eds.), p. 121-146. APEC/AHHRI/FHS-AFS/NACA, Bangkok, Thailand.

Chao, C.B. and Chung, H.Y. 1994. Study on *Cryptocaryon irritans* infection on captive grouper (*Epinephelus* sp.): Life cycle and pathogenicity. COA Fish. Ser. (46): 31-40. (In Chinese with English abstract).

Chong, Y.C. and Chao, T. 1986. Common Diseases of Marine Foodfish. Fisheries Handbook No. 2. Primary Production Department, Singapore. 34 p.

Cruz-Lacierda, E.R. 2001. Parasitic diseases and pests. *In*: Health Management in Aquaculture, G.D. Lio-Po, C.R. Lavilla and E.R. Cruz-Lacierda (eds.), p. 55-73. SEAFDEC Aquaculture Department, Iloilo, Philippines.

Cruz-Lacierda, E.R., Lester, R.J.G., Eusebio, P.S., Marcial, H.S. and Pedrajas, S.A.G. 2001. Occurrence and histopathogenesis of a didymozoid trematode (*Gonapodasmius epinepheli*) in pond-reared orange-spotted grouper, *Epinephelus coioides*. Aquaculture 201: 211-217.

Cruz-Lacierda, E.R., de la Peña, L.D. and Lumanlan-Mayo, S. 2000. The use of chemicals in aquaculture in the Philippines. *In*: Use of Chemicals in Aquaculture in Asia, J.R. Arthur, C.R. Lavilla-Pitogo and R.P. Subasinghe (eds.), p. 155-184. SEAFDEC Aquaculture Department, Iloilo, Philippines.

Cruz-Lacierda, E.R., Toledo, J.D., Tan-Fermin, J.D. and Burreson, E.M. 2000. Marine leech (*Zeylanicobdella arugamensis*) infestation in cultured orange-spotted grouper, *Epinephelus coioides*. Aquaculture 185: 191-196.

Koesharyani, I., Roza, D., Mahardika, K., Johnny, F., Zafran and Yuasa, K. 2001. Manual for Fish Disease Diagnosis–II. Marine Fish and Crustacean Diseases in Indonesia. Gondol Research Station for Coastal Fisheries and Japan International Cooperation Agency, Indonesia. 49 p.

Koesharyani, I., Zafran, Yuasa, K. and Hatai, K. 1999. Two species of capsalid monogeneans infecting cultured humpback grouper *Cromileptes altivelis* in Indonesia. Fish Pathol. 34: 165-166.

Leong, T.S. 1992. Diseases of brackishwater and marine fish cultured in some Asian countries. *In*: Diseases in Asian Aquaculture I, M. Shariff, R.P. Subasinghe, J.R. Arthur (eds.), p. 223-236. Fish Health Section, Asian Fisheries Society, Manila, Philippines.

Leong, T.S. 1997. Control of parasites in cultured marine finfishes in Southeast Asia-an overview. Int. J. Parasitol. 27: 1177-1184.

Leong, T.S. 1998. Grouper culture. *In*: Tropical Mariculture, S.S. de Silva (ed.), p. 423-448. Academic Press, San Diego, U.S.A.

Leong, T.S. 2002. Practical approaches to health management for cage cultured marine fishes. Aquaculture Asia 7: 42-45.

Leong, T.S. and Wong, S.Y. 1988. A comparative study of the parasite fauna of wild and cultured grouper (*Epinephelus malabaricus* Bloch et Scheider) in Malaysia. Aquaculture 68: 203-207.

Leong, T.S. and Wong, S.Y. 1990. Parasites of healthy and diseased juvenile grouper (*Epinephelus malabaricus* (Bloch and Schneider)) and seabass (*Lates calcarifer* (Bloch) in floating cages in Penang, Malaysia. Asian Fish. Sci. 3: 319-327.

Rasheed, V.M. 1989. Diseases of cultured brown-spotted grouper *Epinephelus tauvina* and silvery black porgy *Acanthopagrus cuvieri* in Kuwait. J. Aquat. Anim. Health 1: 102-107.

Ruangpan, L. and Tabkaew, R. 1993. Parasites of the cage cultured grouper *Epinephelus malabaricus* in Thailand. *In*: Proceedings of Grouper Culture, p. 106-111. National Institue of Coastal Aquaculture and Japan International Cooperation Agency, Thailand.

Supamattaya, K., Fischer Scherl, T., Hoffman, R.W. and Boonyaratpalin, S. 1990. Renal sphaerosphorosis in cultured grouper, *Epinephelus malabaricus*. Dis. Aquat. Org. 8: 35-38.

Supamattaya, K., Boonyaratplain, S. and Hoffman R. 1993. Parasitic myxosporea in grouper (*Epinephelus malabaricus*). *In*: Proceedings of Grouper Culture, p. 89-100. National Institue of Coastal Aquaculture and Japan International Cooperation Agency, Thailand.

Yambot, A.V., Song, Y.L. and Sung, H.H. 2003. Characterization of *Cryptocaryon irritans*, a parasite isolated from marine fishes in Taiwan. Dis. Aquat. Org. 54: 147-156.

Zafran, Roza, D., Koesharyani, I., Johnny, F. and Yuasa, K. 1998. Manual for Fish Diseases Diagnosis: Marine Fish and Crustacean Diseases in Indonesia. Gondol Research Station for Coastal Fisheries and Japan International Cooperation Agency, Indonesia. 44 p.

Zafran, Roza, D., Johnny, F., Koesharyani, I. and Yuasa, K. 2000. Diagnosis and Treatments for Parasitic Diseases in Humpback Grouper (*Cromileptes altivelis*) Broodstock. Gondol Research Station for Coastal Fisheries and Japan International Cooperation Agency, Indonesia. 8 p.

Chapter 5. Nutritional Diseases

Edgar C. Amar and Celia R. Lavilla-Pitogo

Nutritional diseases of fish may develop as a result of deficiency (undernutrition), excess (overnutrition), or imbalance (malnutrition) of nutrients present in their food. The disease usually develops gradually because animals have body reserves that make up for nutritional deficiency up to a certain extent. Disease signs develop only when supply of any diet component falls below critical level. When there is too much food, the excess that is converted to fat and deposited in fish tissues and organs, may severely affect physiological functions of the fish.

LIPODOSIS

Lipodosis is a common nutritional disease among cultured food fish and various degrees of lipodosis have been observed in the liver of cage-cultured groupers including *Epinephelus coioides, E. malabaricus* and *Cromileptes altivelis* in Indonesia, Thailand and the Philippines.

Causative agent:

Feeding with rancid formulated feeds or with fatty or poorly stored trash fish can cause lipodosis.

Stages affected:

Fish in the grow-out stage are susceptible to lipodosis.

Gross clinical signs:

Affected fish grow poorly, lethargic, with opaque eyes, and shows slight distention of the abdomen. The liver is also abnormally pale (Fig. 5-1a). The normal color of liver is shown in Fig. 5-1b.

Effects on host:

Affected fish exhibit poor growth and low mortality, the liver has a pale appearance and histological sections show presence of large fat droplets (Fig. 5-2).

Transmission:

Lipodosis is non-infectious as the causative agent is not a pathogen. The presence of affected fish in a farm will not endanger the other healthy individuals.

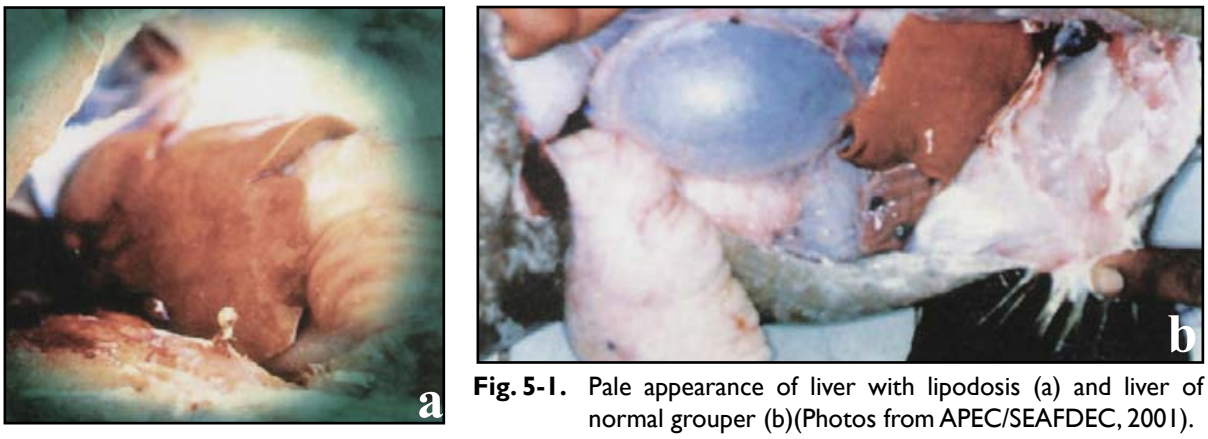


Fig. 5-1. Pale appearance of liver with lipodosis (a) and liver of normal grouper (b)(Photos from APEC/SEAFDEC, 2001).

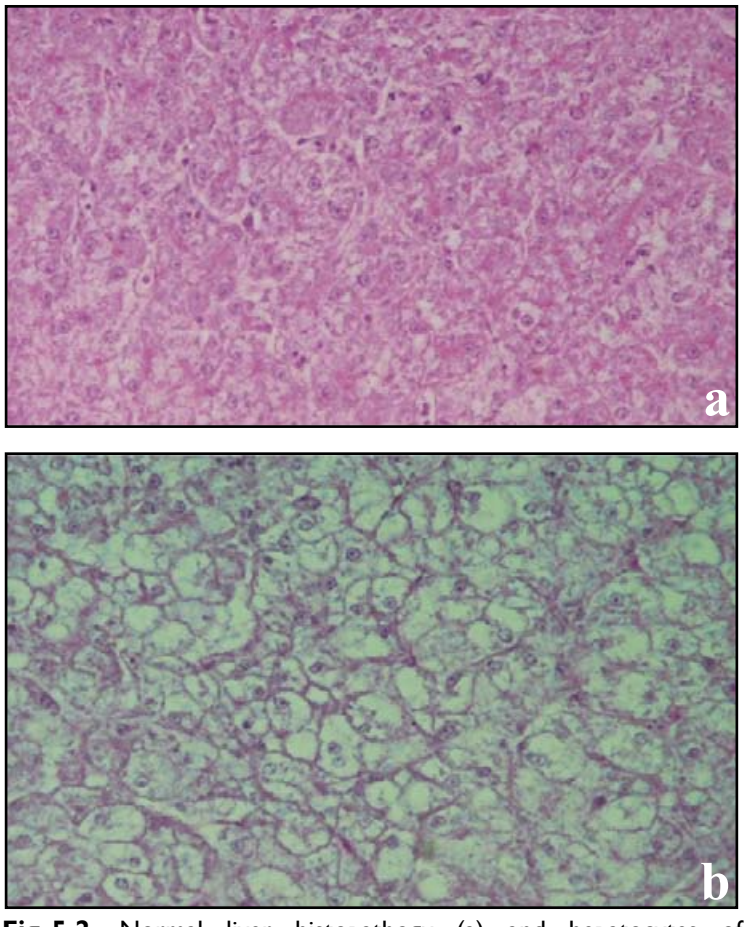


Fig. 5-2. Normal liver histopathogy (a) and hepatocytes of *Epinephelus coioides* filled with clear vacuoles containing fat droplets (b). H & E stain.

Diagnosis:

The disease may be diagnosed through histopathology of liver and analysis of proximate analysis of artificial feeds. Since histopathology is required, this may have contributed to lack of reports.

Preventive and control methods:

Handle feeds properly, observe good storage practices and avoid feeding fish with spoiled and poorly stored trash fish (see Appendix 3). When lipodosis is confirmed, immediately discontinue use of remaining feeds and replace with a totally new batch of feeds.

FISH SCURVY

Nutrient deficiency rarely occurs naturally when diets have been formulated and prepared based on the species' requirement. However, some commercially-available diets for another species may sometimes be used in the absence of a suitable formulation, resulting in deficiencies. Spinal deformity associated with ascorbic acid deficiency has been reported to occur naturally in *Cromileptes altivelis* postlarvae in Indonesia. Natural occurrence in grow-out farms has also been reported in *Epinephelus tauvina* and *E. malabaricus* in Thailand.

Causative agent:

Ascorbic acid deficiency is the primary cause of scurvy.

Stages affected:

Fish in the grow-out stages are usually affected but spinal deformity may occur at the postlarval stages when inappropriate larval feeds are used. Spinal abnormality can also be experimentally induced in *C. altivelis* fingerlings when they are given diets devoid of vitamin C.

Gross clinical signs:

Affected fish exhibit gross signs such as anorexia, short snout, erosion of opercula and fins, hemorrhaging of eyes and fins, exophthalmia, swollen abdomen, abnormal skull, falling pharyngobranchials, severe emaciation, and spinal column abnormality such as scoliosis (Fig. 5-3) and lordosis.

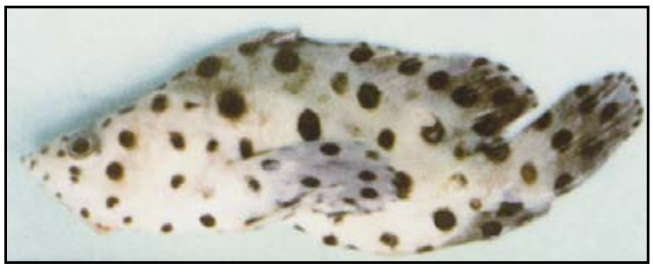


Fig. 5-3. Dorso-ventral curvature of the spinal column (scoliosis) in *Cromileptes altivelis* with ascorbic acid deficiency (Photo from Koesharyani *et al.*, 2001).

Effects on host:

Affected fish exhibit poor growth or severe emaciation in prolonged deficiency. Histologically, hyperplasia and detachment of gill epithelium can be observed and the hepatocytes may contain large fat droplets.

Transmission:

Scurvy is a deficiency condition and is therefore non-infectious.

Diagnosis:

The deficient condition of fish can be confirmed by the curvature of the body with the hemorrhagic lesion at the broken vertebral column, histopathology of gills and liver. The formulation can also be examined as to the form and level of inclusion of ascorbic acid and further by analysis of tissue and feed samples for ascorbic acid content.

Preventive and control methods:

Use adequate amounts and stable forms of ascorbic acid (e.g. L-ascorbyl monophosphate or L-ascorbyl polyphosphate) in diet formulation. Provide the minimum requirement for the species if information is available (e.g., 30mg L-ascorbyl-2-phosphate mg/kg diet for *E. malabaricus*) allowing for losses during manufacture.

EFA DEFICIENCY

Marine fish larvae require essential fatty acids (EFA) for normal growth and development. Fatty acids are essential components of biomembranes and precursors of some physiological modulators such as the eicosanoids. Marine fish in general, unlike their freshwater counterpart, cannot effectively elongate and desaturate saturated fats to unsaturated ones and so require the presence of unsaturated fatty acids in their diets. Essential fatty acids such as docosahexaenoic acid [DHA, 22:5(n-3)] and eicosapentaenoic acid [EPA, 20:5(n-3)] are commonly found in live food such as microalgae (e.g., *Nannochloropsis*), copepods, rotifers and *Artemia*. Deficiency in these fatty acids is associated with larval mortality known as "shock syndrome" in which the larvae display unusual sensitivity to stress. Any handling or disturbance (e.g., sorting, transfer, strong aeration) of grouper larvae with this condition invariably results in mortality. This disease has been reported in *Epinephelus malabaricus* in Thailand and in *E. tauvina* and *E. fuscoguttatus* in Singapore.

Causative agent:

This condition is associated with low levels of essential fatty acids in live food.

Stages affected:

Day 21 larvae for *E. malabaricus*, stage 1 (about day 12) and stage 2 (day 23) for *E. tauvina* and *E. fuscoguttatus* are affected.

Gross clinical signs:

General body weakness and mortality is observed starting day 21 for *E. malabaricus*. High mortality occurs in stages 2 and 3 for *E. tauvina* and *E. fuscoguttatus.*

Effects on host:

Total mortality is observed after day 30 for *E. malabaricus*.

Transmission:

The disease is not transmitted to other healthy individuals by affected fish.

Diagnosis:

Visual observation of larval behavior (weak movement) is confirmed by fatty acid analysis of live food.

Preventive and control methods:

Feed 15 day-old larvae with brine shrimps enriched with fish oil at 25-50 ml/m^3 of rearing water. While it is important to maintain a clean environment by sediment removal and water management, avoid unnecessary mechanical stress to the larvae by tender handling, using mild aeration and employing flow-through system of water management. The condition can be alleviated by enrichment of rotifers with n-3 HUFA or *Nannochloropsis* (syn. *Chlorella*). Bring the EFA levels of the live food to around 12% by enrichment. Supplementation with DHA is reported to slow down mortality in grouper while supplementation with EPA was less effective in other marine species.

NUTRITIONAL MYOPATHY ACCOMPANYING CEROIDOSIS

Lipid peroxidation can occur both in solution and in the cell because unsaturated fats are highly susceptible to oxidants. When peroxidation occurs in the cell membrane, cellular integrity is compromised that could lead to certain myopathies.

Causative agent:

This disease is associated with diets containing rancid fat or polyunsaturated fatty acids and low contents of vitamin E.

Stages affected:

Cromileptes altivelis fingerlings and broodstock are affected by this disease.

Gross clinical signs:

Affected fish show emaciation, darkening of body color (Fig. 5-4a), petechia at the base of the operculum (Fig. 5-4b) and occasional deformity of the spinal cord.

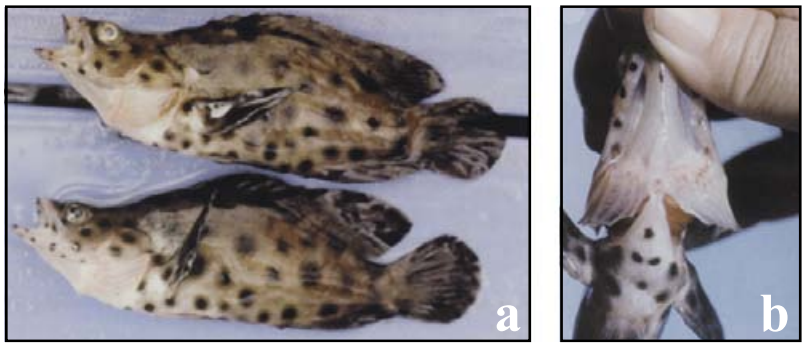


Fig. 5-4. Nutritional myopathy in *Cromileptes altivelis*: a) Darkening of body color and spinal deformity and b) petechia at the base of the operculum (Photos from Koesharyani *et al.*, 2001).

Effects on host:

The disease can cause low but continuous mortality in *C. altivelis* fingerlings. It can also cause mass mortality in *C. altivelis* broodstock.

Transmission:

The disease is not caused by an infectious agent; it is non-transmissible.

Diagnosis:

Histopathologically, myofibril degeneration including extensive myolysis and macrophage invasion in degenerated fibers are observed in the skeletal muscles (Fig. 5-5a). Ceroid deposits, a kind of lipo-pigment which stains pink with PAS reaction in the hepatocytes, is typical (Fig. 5-5b).

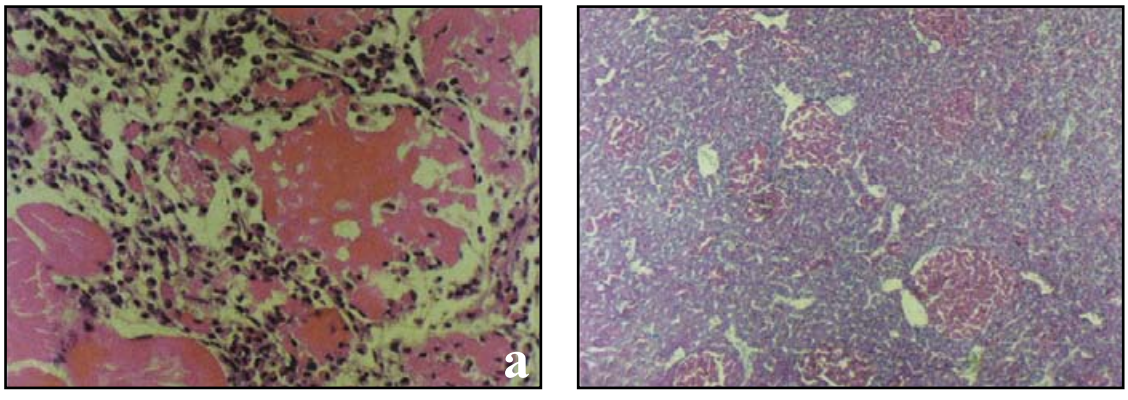


Fig. 5-5. Nutritional myopathy in *Cromileptes altivelis*: a) Macrophage invasion, H & E stain and b) ceroid deposits (pink in PAS reaction)(Photos from Koesharyani *et al*., 2001).

Preventive and control methods:

Proper food management can prevent this disease. To prevent rancidity of polyunsaturated fatty acids, food should be kept in freezers under –30°C and consumed as soon as possible. Antioxidants such as vitamin E are effective against this disease since they prevent peroxidative damage to cells. Enrichment of food with vitamin complex can stamp out mass mortality.

THIAMIN DEFICIENCY

Some species of fish belonging to the sardine and anchovy families contain enzymes that degrade thiamin contained in the trash fish itself. This is usually not a problem when trash fish are of mixed species or different species are fed alternately, but deficiency signs appear when single species are fed for extended periods.

Causative agent:

Deficiency of vitamin B_1 (thiamin) caused by thiaminase contained in sardine or anchovy as feed.

Stages affected:

This condition affects *Cromileptes altivelis* broodstock.

Gross clinical signs:

Affected fish exhibit whitish body color (Fig. 5-6a), erratic swimming behavior, mechanical injuries with hemorrhages on the body surface, especially around mouth, pectoral fins and abdomen (Fig. 5-6b).

Fig. 5-6. Thiamin deficiency in *Cromileptes altivelis*: a) Whitish body color and b) mechanical injuries around the mouth and pectoral fins associated with erratic swimming behavior (Photos from Koesharyani *et al*., 2001).

Effects on host:

Thiamin is a co-enzyme of many enzymes catalyzing carbohydrate metabolism and is essential for normal nerve functions, digestion and reproduction. Thiamin deficiency affects the functions of the nervous system.

Transmission:

The disease is a deficiency condition and is not transmitted.

Diagnosis:

Histopathological lesions are mainly found in the brain where hemorrhages and degeneration of the nuclei of nervous cells occur.

Preventive and control methods:

Avoid prolonged feeding with sardine or anchovy only as feed. A mixture of different species of trash fish should be fed and regular supplementation (e.g., once a week) of a vitamin complex in the feed should be adopted. Excess administration of vitamin B_1 supplement should be undertaken if fish become deficient in thiamin.

REFERENCES

APEC/SEAFDEC. 2001. Husbandry and Health Management of Grouper. APEC, Singapore and SEAFDEC Aquaculture Department, Iloilo, Philippines. 94 p.

Bautista, M.N., Subosa, P.F. and Lavilla-Pitogo, C.R. 1992. Effects of antioxidants on feed quality and growth of *Penaeus monodon* juveniles. J. Sci. Food. Agr. 60: 55-60.

Bautista, M.N., Lavilla-Pitogo, C.R., Subosa, P.F. and Begino, E.T. 1994. Aflatoxin B_1 contamination of shrimp feeds and its effect on growth and hepatopancreas of pre-adult *Penaeus monodon*. J. Sci. Food. Agr. 65: 5-11.

Boonyaratpalin, M., Wannagowat, J. and Borisut, C. 1993. L-ascorbyly 1-2 phosphate-Mg as a dietary vitamin C source for grouper. Paper presented at the Seminar on Fisheries, 16-17 September 1993, Department of Fisheries, Bangkok, Thailand.

Chong, Y.C. and Chao, T. 1986. Common Diseases of Marine Foodfish. Fisheries Handbook No. 2. Primary Production Department, Singapore. 34 p.

Dhert, P., Lim, C.C., Lavens, P., Chao, M. and Chou, R. 1991. Effect of dietary essential fatty acids on egg quality and larviculture success of the greasy grouper, *Epinephelus tauvina*, F): Preliminary results. *In*: Larviculture Symposium '91, P. Lavens, P. Sorgeloos, E. Jaspers and E. Ollevier, E. (eds.), p. 58-62. Spec. Publ. Euro. Aqua. Soc. No. 15.

Koesharyani, I., Roza, D., Mahardika, K., Johnny, F., Zafran and Yuasa, K. 2001. Manual for Fish Disease Diagnosis–II. Marine Fish and Crustacean Diseases in Indonesia. Gondol Research Station for Coastal Fisheries and Japan International Cooperation Agency, Indonesia. 49 p.

Lim, L.C. 1993. Larviculture of the greasy grouper *Epinephelus tauvina* F. and the brown-marbled grouper *E. fuscoguttatus* F. in Singapore. J. World Aquac. Soc. 24: 262-274.

Lim, C. and Webster, C.D. 2001. Nutrition and Fish Health. Haworth Press, Binghampton, New York, U.S.A. 437 p.

Phromkunthong, W., Storch, V., Suppamataya, K., and Boonyaratpalin, M. 1995. Effects of ascorbic acid deficiency on the gill and liver histopathology of grouper, *Epinephelus malabaricus*. *In*: Diseases in Asian Aquaculture II, M. Shariff, J.R. Arthur and R.P. Subasinghe (eds.), p. 503-512. Fish Health Section, Asian Fisheries Society, Manila, Philippines.

Ruangpanich, N. and Boonlipatanon, P. 1993. Some factors affecting the survival of grouper larvae (*Epinephelus malabaricus*). Technical Paper No. 20, National Institute of Coastal Aquaculture, Department of Fisheries, Thailand. 25 p.

Chapter 6. Environmental Diseases

Gregoria E. Erazo-Pagdor and Erlinda R. Cruz-Lacierda

This chapter focuses on swimbladder stress syndrome and gas bubble disease, the two most common disorders due to adverse environmental conditions.

SWIMBLADDER STRESS SYNDROME (SBSS)

Swimbladder stress syndrome (SBSS) is a malfunction of the swimbladder and is associated with a combination of abrupt changes in several environmental parameters. This syndrome has been a major limiting factor in fry production of *Epinephelus* sp. in Taiwan. It has also been reported in *E. bleekeri*, *E. coioides*, *E. lanceolatus*, *E. malabaricus, E. tauvina* and *Cromileptes altivelis* in Malaysia, Singapore and Thailand.

Causative agent:

The causative agent is unknown, but the syndrome is associated with abrupt changes in water quality such as high ambient temperature, high ambient illumination, dense algal bloom that may cause oxygen depletion at night and supersaturation during the day. In Malaysia, this syndrome usually coincides with the monsoon season when there is upwelling of bottom sediments under the net cages.

Stages affected:

In the hatchery this syndrome occurs during metamorphosis. It also affects marketable-sized fish and broodstock.

Gross clinical signs:

Affected fish exhibit hyperinflated swimbladder (Fig. 6-1), abdominal distention and swim in a head-down or sideward position near the water surface. There are bubbles in the gill lamellae.

Effects on host:

Affected fish have bloated swimbladder, which results in positive buoyancy. This condition is not lethal to the fish but they eventually die of starvation, overexposure to direct sunlight or secondary bacterial infection.

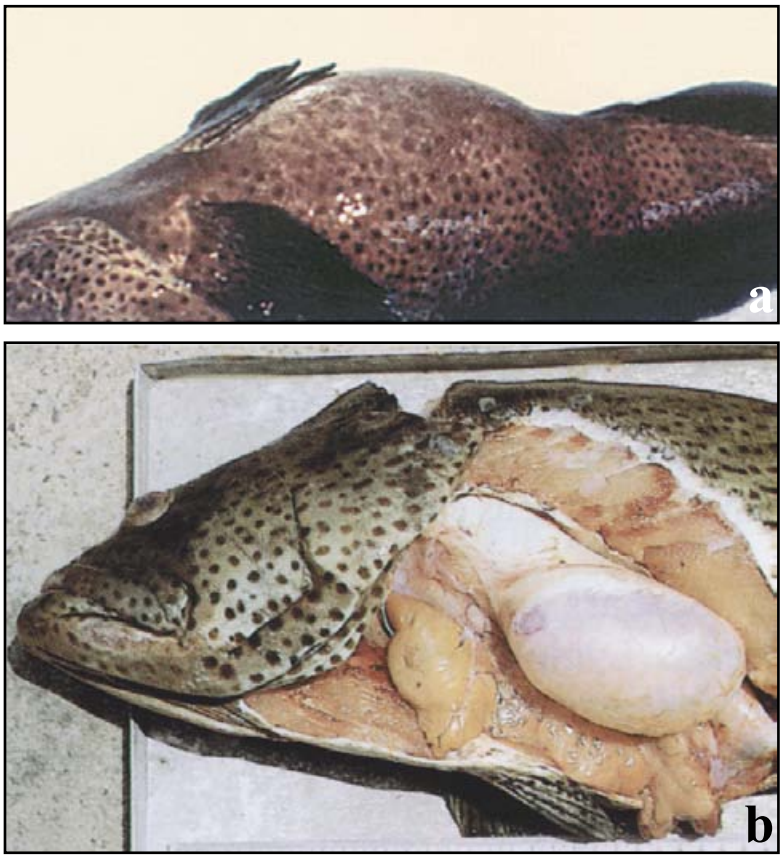


Fig. 6-1. *Epinephelus coioides* broodstock showing hyperinflated swimbladder: External (a) and opened (b) appearance (Photos from APEC/SEAFDEC, 2001).

Transmission:

This syndrome is non-infectious.

Diagnosis:

The disease is diagnosed through gross examination of the swimbladder.

Preventive methods:

If marketable-sized fish are affected, it is best to harvest the stock and sell. Smaller fish, which are affected, must be removed to avoid secondary bacterial infection and mortalities. For broodstock, gently pierce the abdomen with a sterile hypodermic needle, then press the fish into the water to allow gas to escape with water pressure (Fig. 6-2). Remove the needle and dab the pierced site with 0.1% acriflavine before returning the fish to the holding facility. Fish recovers in 3-6 days with a success rate of 50%.

GAS BUBBLE DISEASE (GBD)

Gas bubble disease (GBD) is due to supersaturation of dissolved gases, usually nitrogen and oxygen. All gases are more soluble in water at low temperatures. Solubility is diminished as temperature rises.

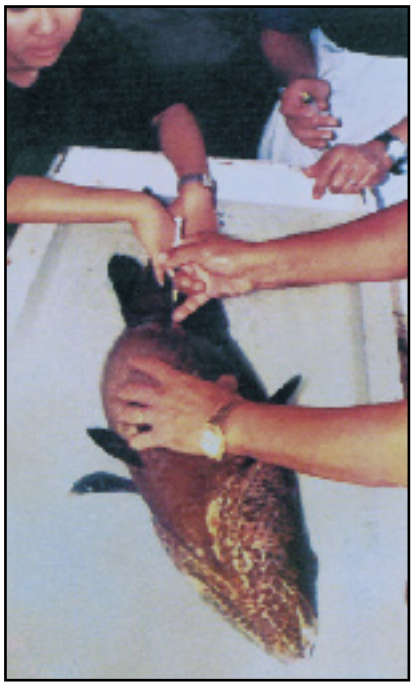


Fig. 6-2. Deflation of the swimbladder of *Epinephelus coioides* using a sterile needle.

Causative agent:

GBD is caused by a supersaturation of the water with the gas, nitrogen. Supersaturation occurs whenever the pressure of a gas in the water is higher than the pressure of the same gas in the surrounding atmosphere. It is also caused by the supersaturation of oxygen in water due to heavy algal blooms.

Stages affected:

This disease is common at the hatchery stage. It also affects broodstock.

Gross clinical signs:

Affected fish show bubbles in the eyes (causing exophthalmia or pop-eye)(Fig. 6-3a), body cavities, skin and gills (Fig. 6-3b). The bubbles can form in the gill lamellae and block blood flow resulting embolism of gill vessels. Affected fish also show erratic swimming patterns.

Transmission:

The disease is caused by an environmental factor, thus is non-infectious.

Diagnosis:

Wet mount examination of the gills and other organs under the microscope may show gas emboli within the bloodstream.

Effects on host:

Affected fish die due to embolism in blood, degeneration of the gill lamellae and bulging of the cornea. There is also an occurrence of abrupt mass mortalities.

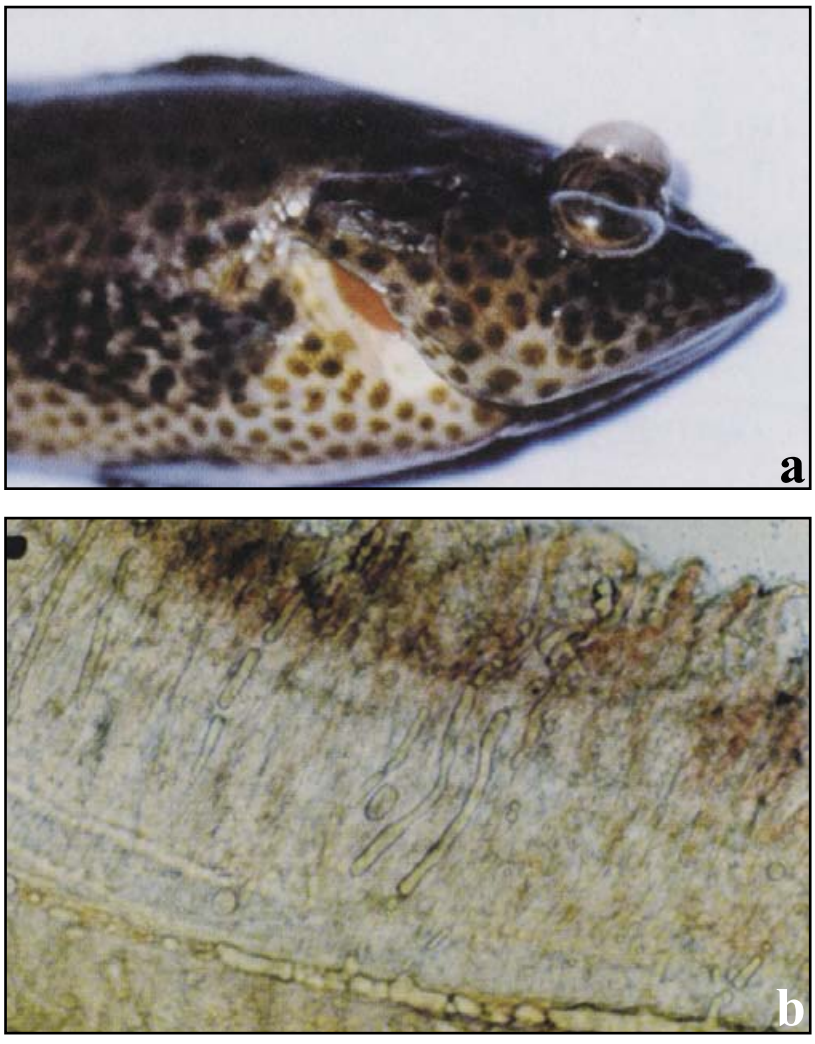


Fig. 6-3. Gas bubble disease in *Epinephelus coioides*: a) Exophthalmia and b) gas bubble on gill filaments (Photos from Koesharyani *et al*., 2001).

Preventive and control methods:

To treat fish suffering from GDB, first weaken water supply and supply strong aeration to remove saturated nitrogen. Repair cracked pipe, and position intake pipe above the water surface to remove the nitrogen.

REFERENCES

APEC/SEAFDEC. 2001. Husbandry and Health Management of Grouper. APEC, Singapore and SEAFDEC Aquaculture Department, Iloilo, Philippines. 94 p.

Bondad-Reantaso, M.G., Kanchanakhan, S. and Chinabut, S. 2001. Review of grouper diseases and health management strategies for groupers and other marine finfishes. *In*: Report and Proceeding of APEC FWG 02/2000 "Development of a Regional Research Programme on Grouper Virus Transmission and Vaccine Development", M.G. Bondad-Reantaso, J. Humphrey, S. Kanchanakhan and S. Chinabut (eds.), p. 121-146. APEC/AHHRI/FHS-AFS/NACA, Bangkok, Thailand.

Chong, Y.C. and Chao, T. 1986. Common Diseases of Marine Foodfish. Fisheries Handbook No. 2. Primary Production Department, Singapore. 34 p.

Chua, T., Loo, J.J., Wee, J.Y. and Ng, M. 1993. Findings from a fish disease survey: An overview of the marine fish disease situation in Singapore. Singapore J. Pri. Ind. 2: 26-37.

Danayadol, Y. and Kanchanapungka, S. 1989. Swimbladder syndrome disease in grouper (*Epinephelus malabaricus*). I. Occurrence and proposed primary cause. The Seminar of Fisheries 1989. Department of Fisheries, Thailand.

Koesharyani, I., Roza, D., Mahardika, K., Johnny, F., Zafran and Yuasa, K. 2001. Manual for Fish Disease Diagnosis–II. Marine Fish and Crustacean Diseases in Indonesia. Gondol Research Station for Coastal Fisheries and Japan International Cooperation Agency, Indonesia. 49 p.

Leong, T.S. 1998. Grouper culture. *In*: Tropical Mariculture, S.S. de Silva (ed.), p. 423-448. Academic Press, San Diego, U.S.A.

Liao, I.C., Mao-Sen, S. and Chang, S.L. 1995. A review of the nursery and grow-out technique of high-value marine finfishes in Taiwan. *In*: Culture of High-value Marine Fishes in Asia and the United States, K.L. Main and C. Rosenfeld (eds.), p. 121-138. The Oceanic Institute, Hawaii, U.S.A.

Appendix 1

POINTS TO CONSIDER BEFORE USING ANTIBIOTICS

1. Antibiotics should be used only as a last resort.
2. Definite disease diagnosis, including antibiotic sensitivity, should be done before administering antibiotics.
3. Chemotherapeutants that are less expensive and have negligible impact on the environment (or environment-friendly) are preferred.
4. The tolerance of the cultured species as well as the disease agent to the chemotherapeutant should be known.
5. The properties of the chemicals and its impact on non-target species, toxicities, effective doses, and spectrum of activity should be known.
6. The effect of the chemotherapeutant to human health, market and the environment should be considered.
7. Inappropriate use of antibiotics can lead to the development of resistant strains that may be difficult to treat.
8. Maximum residue limits and withdrawal periods for antibiotic used in food fishes should be considered before harvesting the fish.
9. In some cases such as the occurrence of a serious disease problem, eradication should be considered. Eradication includes removal of all susceptible species followed by drying out and liming of ponds and disinfection of contaminated paraphernalia.

Appendix 2

LIST OF ANTI-INFECTIVES RECOMMENDED FOR MARINE FOOD FISH USE AND THEIR WITHDRAWAL PERIOD (Cruz-Lacierda et al., 2000)

Chemical	Withdrawal period (days)
Oxolinic acid	30
Oxytetracycline	21
Ormetropin	NI
Sulfadimethoxine	NI
Sulfamerazine	25
Sulfisoxazole diolamine	NI
Trimethoprim/ sulfadiazine	20

NI: No information.

Appendix 3

PROPER STORAGE OF FEEDS (Bautista et al., 1994)

For dry ingredients and artificial feeds

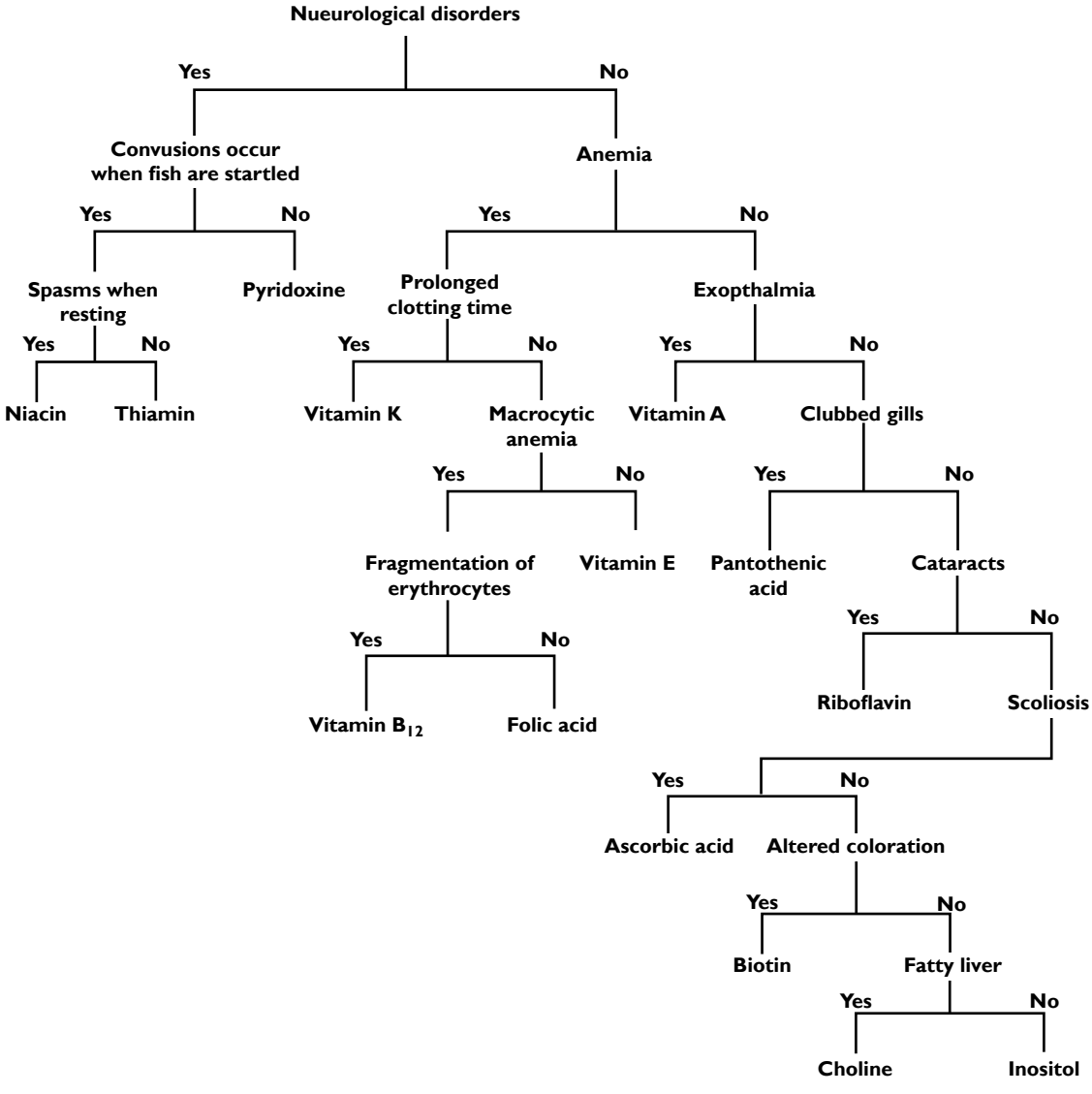
1. Provide a clean, dry, secure, and well-ventilated storage area. Avoid direct exposure to sunlight.
2. Label feeds and feed ingredients properly. Arrange feeds by kind and date.
3. Pile feed bags not more than 6 bags high on a platform 12-15 cm off the floor. To discourage insects, spread ash around and under the platform.
4. Store dry feeds not longer than 3 months. Use old feeds before the new deliveries. First in, first out.
5. Do not walk on the sacks of feed.

For moist or wet feed ingredients

1. Use fresh trash fish immediately or keep them frozen until use. Use moist compounded feeds fresh.
2. Keep oils and fats in sealed amber or dark-colored containers inside a cold store or refrigerator. Maintain the temperature of the cold storage below 10°C. Avoid overloading and unnecessary opening of the cold storage.
3. Keep vitamins in air tight, lightproof containers inside a refrigerator. Keep vitamins and minerals in separate containers.

Appendix 4

A GENERALIZED KEY TO VITAMIN DEFICIENCY FOR FINFISH (adapted from Lim and Webster, 2001)



Glossary

Acute – infection which develops rapidly, of short course, often fatal

Anchor – a posterior attachment organelle in monogeneans

Anemia – a condition characterized by a deficiency of hemoglobin, packed cell volume, or erythrocytes in the blood

Anorexia – loss of appetite

Anoxia – absence of oxygen in the tissues

Antibiotic – a chemical substance originally produced from molds or bacteria, but now from synthetic substances. Antibiotic can inhibit the growth of, or kill, other microorganisms

Ascites – the accumulation of serum-like fluid in the abdomen

Asphyxiation – deficiency of oxygen

Atrophy – a decrease in the amount of tissue or the size of an organ after normal growth has been achieved

Bacteria – one-celled microorganisms which lack well-defined nucleus

Blister – a thin vesicle, especially on the skin, containing watery matter

Carrier – one that transmits disease germs

Cercariae – free-swimming larval stage of digeneans

Chronic – of long duration or frequent recurrence

Cilia – short, hair-like structures used for movement

Copepods – small planktonic or bottom-dwelling crustaceans

Cyst – a non-motile, resistant, inactive stage; the term is also applied to changes in nucleic acid shape

Denaturation – a change in the shape of an enzyme that destroys its activity

DHA – docosahexaenoic acid

Diagnosis – the determination of the nature of a given disease

Dinoflagellates – a group of unicellular eukaryotic organisms which swims by means of a pair of whip-like flagella

DNA (deoxyribonucleic acid) – the nucleic acid that constitutes the genetic material of

all cellular organisms. It is a polynucleotide composed of deoxyribonucleotides connected by phosphodiester bonds

Dropsy – ascites; abnormal accumulation of liquid in internal organs or tissues

Ectoparasite – a parasite living in the external surfaces of the host

Edema – excessive accumulation of fluid in tissue spaces

EFA – eicosapentaenoic acid

ELISA (enzyme-linked immunosorbent assay) – an immunological test to detect minute quantities of an antigen or an antibody

Emaciation – become abnormally lean

Encapsulation – the covering of a parasite by the host with materials, mostly, if not entirely, of host origin

Encephalitis – inflammation of the brain

Encystment – the covering of a parasite with materials of parasite origin

Endemic – recurring in a locality

Endoparasite – a parasite living inside the body of the host

Endospore – the thick inner chitinous layer of the wall in a microsporean spore

Enteritis – the inflammation of the intestine

Enzyme – a protein catalyst with specificity for both the reaction catalyzed and its substrates

Epibiont fouling – presence of organisms on the surface of an animal

Epizootic – widespread outbreak of fish diseases

Exophthalmia – abnormal protrusion of the eyeball

Exospore – the proteinaceous outer layer of the wall in a microsporean spore

Fin rot – a progressive erosion and disintegration of fish fins

Flagella – long, hair-like structures used for locomotion

Fungus – a general term for a group of eukaryotic protista (e.g., mushrooms, yeasts, molds, etc.) marked by the absence of chlorophyll and the presence of a rigid cell wall

Granuloma – the aggregation and proliferation of defense cells, usually macrophages, which lead to formation of small nodules or granules

Haptor – the posterior and principal organ of attachment used by monogeneans

Hemoglobin – the respiratory pigment of red blood cells that takes up oxygen at the gills or lungs and releases it to tissues

Hemorrhage – internal bleeding and subsequent clotting caused by the rupture of blood vessels

Hepatocytes – liver cells

Histopathology – study of pathological lesions in a tissue

Holdfast – the attachment organ of some parasites

Host – a living organism harboring another orgnism

Hyperplasia – the increase in size of a tissue or an organ by the formation and growth of new cells

Hypertrophy – an increase in size of a tissue or an organ due to an increase in size of individual cells

Hypha(e) – a filament that develops from the germ tube of a fungus

IFAT (indirect fluorescent antibody technique) – a technique in which unlabelled antibody is incubated with the antigen then overlaid with a fluorescent conjugated anti-immunoglobulin to form a sandwich

Infection – a pathological condition due to the growth of microorganism in a host

Infectious disease – disease due to the microbial multiplication in the affected organism

Inflammation – a tissue reaction resulting from an irritation by a foreign material and causing a migration of leukocytes and increased flow of blood to the area, producing swelling, reddening, heat, pain and tenderness

Intermediate host – a host in which the larval stages of a parasite develop

Intracellular – situated or occurring inside a cell

Lesions – any morbid change in function or structure of an organ or tissue

Lethargy – weakness or sluggishness

Lipodosis – disease condition characterized by inflamed fatty liver

Lordosis – spinal curvature oriented laterally

Metacercariae – encysted cercariae of digeneans

Mycelium – mass of hyphae constituting the body (thallus) of a fungus

Mycosis – a fungus infection of an animal

Myopathy – degeneration or atrophy of the muscles

Necrosis – the alteration of tissue which results in cell death and formation of exudate

Nodule (nodular) – a small aggregation of cells

n-3 HUFA – highly unsaturated fatty acid with double bonds in every 3 carbon atoms

Obligate parasite – an organism that can, in nature, obtain food only from living protoplasms; organisms considered as obligate parasites usually cannot be grown in culture or non-living media

Oncomiracidium – a hatched larva of monogeneans

Parasite – an organism that lives at the expense of another, usually invading it and causing disease

PAS stain – periodic acid Schiffs stain

Pathogen – a disease-producing agent

Pathogenic – capable of producing disease

PCR (polymerase chain reaction) – an enzymic method for amplifying exponentially specific pre-selected fragment of DNA

Pharyngobranchials – part of the gills immediately next to the pharynx

Plasmodium – a multinucleate mass of protoplasm which is originally produced from a uninucleate stage

Polar capsule – a thick-walled vesicle in myxosporeans with an inverted polar filament

Parts per million (ppm) – or milligrams per liter or grams per ton

Primer – a short stretch of RNA or DNA used as a staring point for nucleic acid synthesis

Proboscis – a muscular, protrusible feeding organ in some parasitic organisms

Prophylaxis – preventive action

Quarantine – isolation of material or animal to prevent the spread of infectious disease it carries

Rancid – refers to the deterioration of fats

RNA (ribonucleic acid) – a polynucleotide composed of ribonucleotides joined by the phosphodiester bridges

RT-PCR (reverse transcription polymerase chain reaction) – a method to perform PCR for RNA amplification

Scoliosis – spinal curvature oriented dorsoventrally

Scurvy – a condition brought about by deficiency of ascorbic acid

Septicemia – a systemic disease caused by the invasion and multiplication of pathogenic microorganisms in the blood stream

Shell valve – one of the parts of the myxosporean spore wall

Spore – the infective stage of an organism that is usually protected from the environment by one or more protective membranes

Sporoplasm – the infectious component in spores

Stigma – a pigmented red spot in dinoflagellates; it may also be present in the dinospore and other stages

Thrombosis – the formation of presence of a blood clot within a blood vessel

Tomites – cells within the tomont which result from serial binary division

Tomont – a cyst-like structure formed by the trophont following detachment from the host

Trophont – the feeding and growing stage of a parasitic protozoan, which differentiates into the reproductive tomont following detachment

Ulceration – an open sore on skin or mucosal surfaces; it involves lesions with erosion of surface epithelium and inflammation of infiltration of leucocytes

Vacuolation – containing spaces or cavities in the cytoplasm of a cell

Vector – any agent that transmits an infectious organism

Virus – a minute infectious agent which can be resolved or viewed clearly only under a high-powered microscope. It lacks independent metabolism, and is able to replicate only within a cell

Vitamin – an organic compound occurring in minute amounts in foods and essential for numerous metabolic reactions in animals

The Southeast Asian Fisheries Development Center (SEAFDEC), a regional treaty organization based in Bangkok, Thailand, was established in December 1967 to promote fisheries development in the region. Its Member Countries are Japan, Malaysia, the Philippines, Singapore, Thailand, Brunei Darussalam, the Socialist Republic of Vietnam, Union of Myanmar, Indonesia, Cambodia and Lao Peoples Democratic Republic. The Council of Directors, who represent SEAFDEC Member Countries, is the policy-making body of the organization.

SEAFDEC conducts research on appropriate fisheries technologies, trains fisheries and aquaculture technicians, and disseminates fisheries and aquaculture technologies. Four departments were established to pursue these objectives:

- The Training Department (TD) in Samut Prakan, Thailand (1967) for marine capture fisheries training;
- The Marine Fisheries Research Department (MFRD) in Singapore (1967) for fishery post-harvest technology;
- The Aquaculture Department (AQD) in Tigbauan, Iloilo, Philippines (1973) for aquaculture research and development; and
- The Marine Fishery Resources Development and Management Department (MFRDMD) in Kuala Terengganu, Malaysia (1992) for the development and management of marine fishery resources in the exclusive economic zones (EEZs) of SEAFDEC Member Countries.

SEAFDEC/AQD is mandated to

- Promote and undertake aquaculture research that is relevant and appropriate for the region;
- Develop human resources for the region; and
- Disseminate and exchange information on aquaculture.

The Aquaculture Department (AQD) maintains two stations and two substations in the Philippines: the Tigbauan Main Station and the Dumangas Brackishwater Substation in Iloilo Province; the Igang Marine Substation in Guimaras Province; and the Binangonan Freshwater Station in Rizal Province.

Tigbauan Main Station

Dumangas Brackishwater Substation

Igang Marine Substation

Binangonan Freshwater Station

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