

# Chapter 4. Parasitic Diseases

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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:

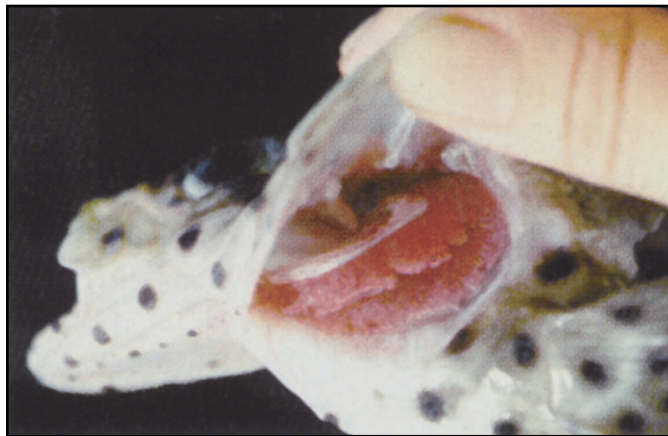
The causative agent of white spot disease is the dinoflagellate *Amyloodinium ocellatum*. The parasite attaches to the host's tissue through a short stalk or peduncle that ends in a flattened attachment disk bearing numerous projections or rhizoids and a mobile tentacle-like stomopode. Mature trophonts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

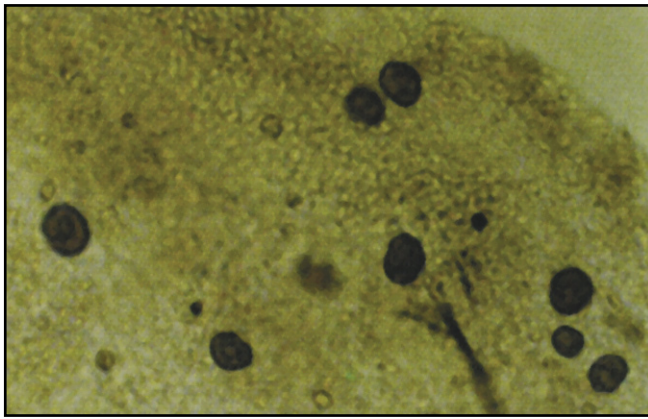
Heavy infections can cause high or mass mortality. Disintegration of the affected tissues has been observed. Histopathological changes include severe gill lamellar epithelial hyperplasia accompanied or followed by reduced or absence of mucus cells.

**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,  $150\text{-}350 \times 15\text{-}70 \mu\text{m}$  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 ( $\text{CuSO}_4$ ) 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:**

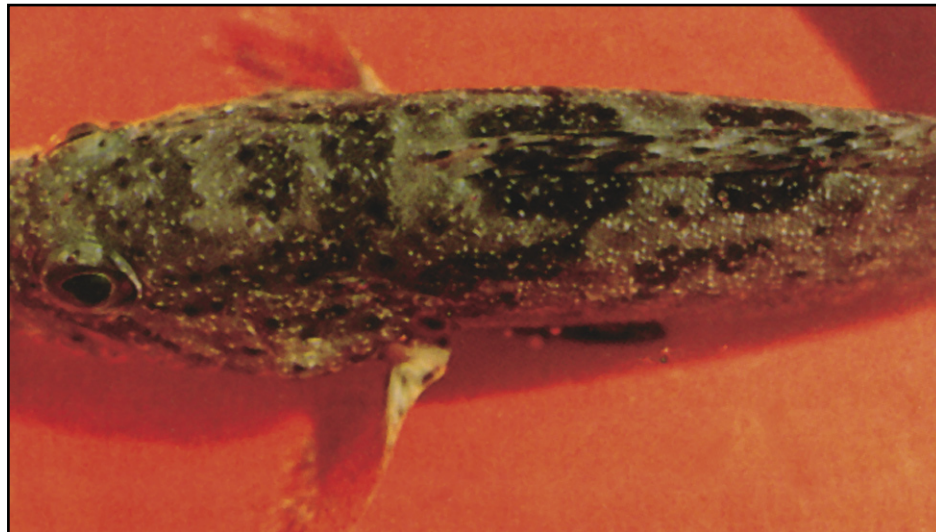
Cryptocaryonosis is caused by *Cryptocaryon irritans*. The parasites are round to spherical in shape, 0.3-0.5 mm in size, with cilia on the surface.

### **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:

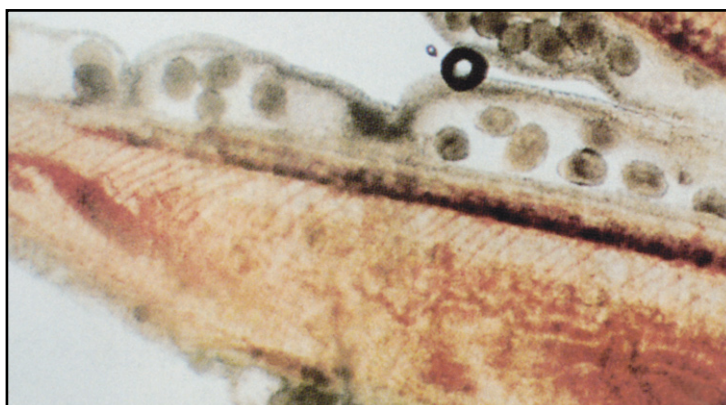
Transmission is horizontal through infected fish and water. The mature trophont leaves the fish as a free-swimming tomont, secretes a cyst to undergo multiple cell division and produces 200 or more tomites. The tomites differentiate into free-swimming infective theronts that attaches onto the host. Pre-disposing 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 ( $\text{CuSO}_4$ ) 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:

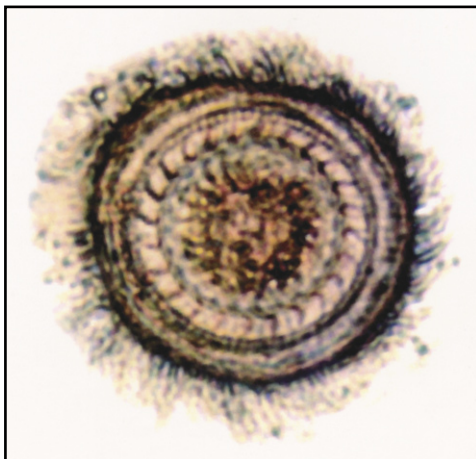
Trichodinids have a saucer-shaped body with cilia around the perimeter of the body (Fig. 4-5). Trichodiniosis can be caused by *Trichodina* (45-78  $\mu\text{m}$  diameter), *Trichodinella* (24-37  $\mu\text{m}$  diameter) and *Tripartiella* (up to 40  $\mu\text{m}$  diameter). The three genera can be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

#### Stages affected:

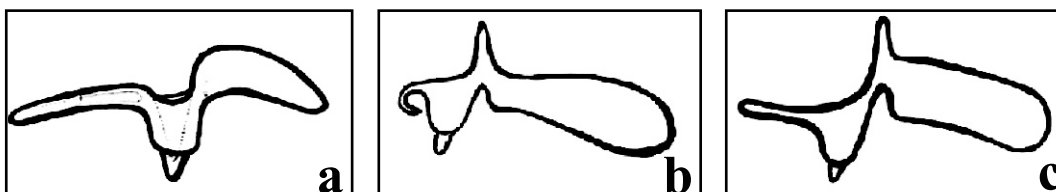
Trichodinids can affect all phases of culture, hatchery, nursery and grow-out.

#### 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*. Silver nitrate 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:**

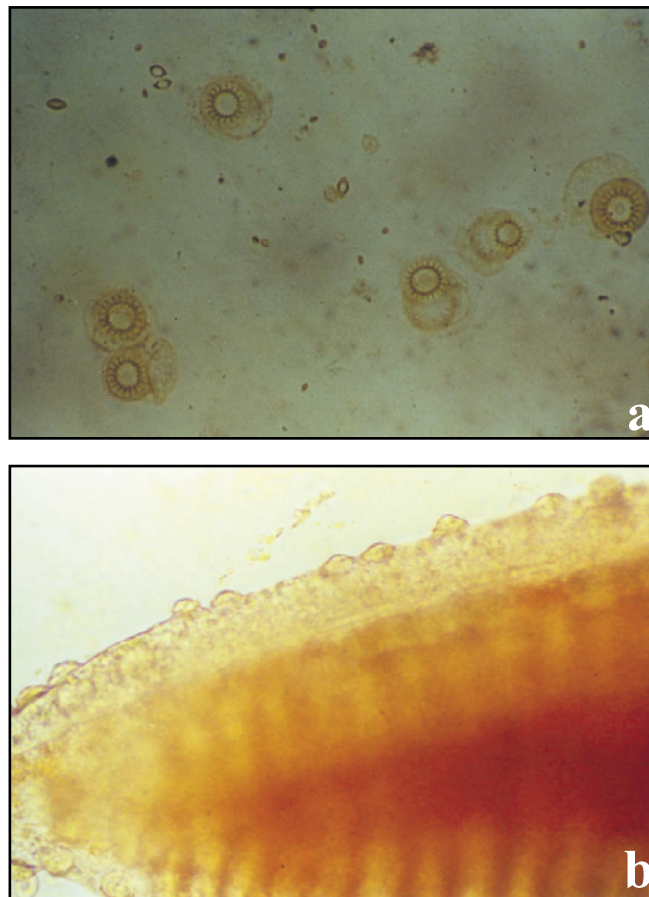
Transmission is horizontal through infected fish, water, contaminated farm equipment and live feed. Pre-disposing factors are high levels of organic matter in the water, poor water exchange and handling stress.

**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 ( $\text{AgNO}_3$ ).

**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  $\mu\text{m}$  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  $36\text{-}86 \times 32\text{-}50 \mu\text{m}$  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  $8.7 \times 8.2 \mu\text{m}$ , 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.

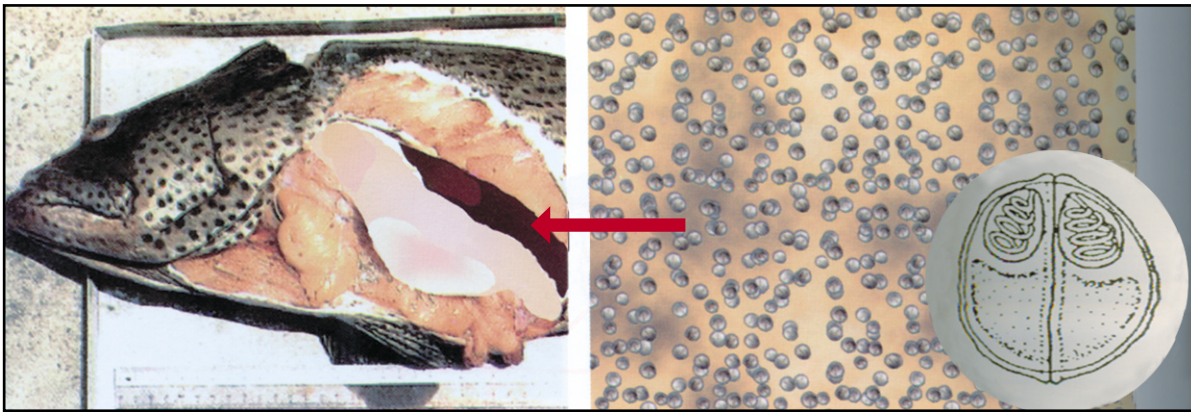
#### **Gross clinical signs:**

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 pseudoplasmodia 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  $\mu\text{m}$  in length, 12.3-14.5  $\mu\text{m}$  in thickness and 7.0-9.5  $\mu\text{m}$  in width, and with two spherical polar capsules, equal in size with a diameter of 2.9-4.4  $\mu\text{m}$ . 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  $\mu\text{m}$ ) containing sporoplasm and coiled polar filament. The disease has been reported in *Epinephelus tauvina* and *Epinephelus* spp. in China and India.

**Causative agent:**

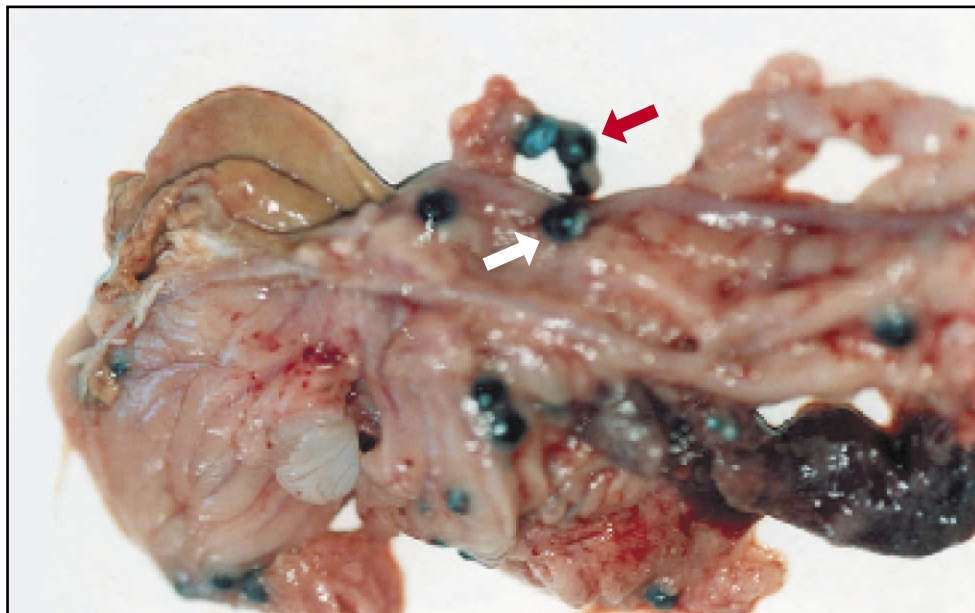
Microsporidiosis form small nodules on the affected tissue and these are filled with pear-shaped spores. *Glugea* sp. and *Pleistophora* sp. have been reported in grouper culture. The spores are 6  $\mu\text{m}$  in size.

**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:**

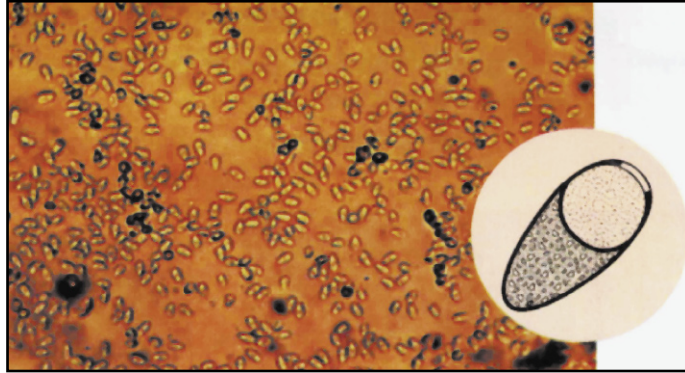
Pre-disposing factors are poor water quality and poor nutrition. Transmission may be horizontal through oral ingestion of spores. The life cycle is unknown.

**Diagnosis:**

Microscopic examination of fresh-squashes of Giemsa-stained smears from infected tissues will reveal oval-shaped spores, 5-6.5  $\times$  2-2.5  $\mu\text{m}$  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:

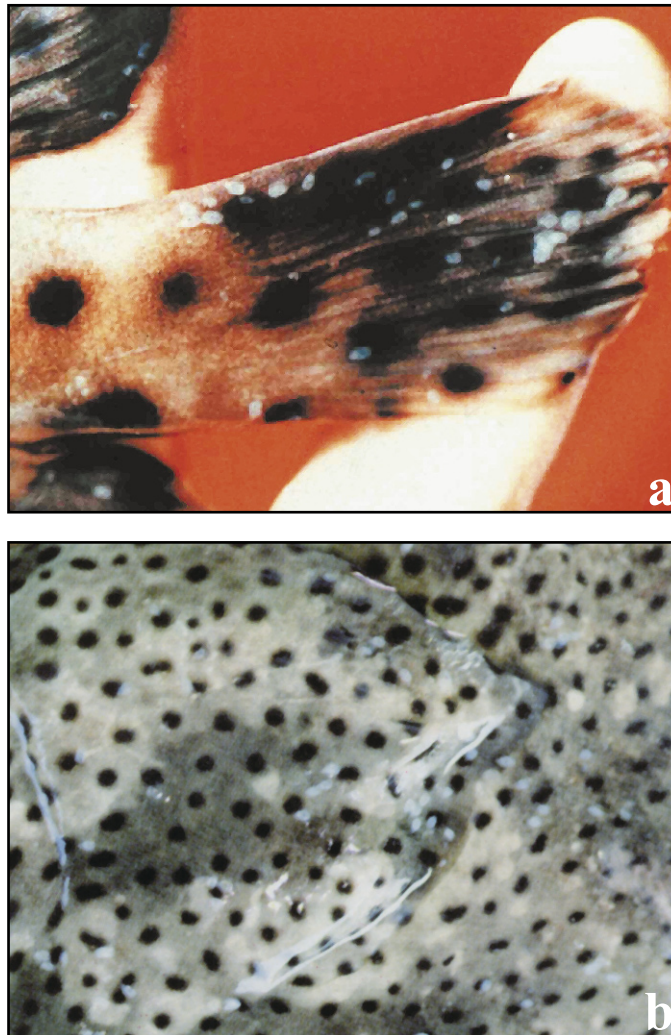
Skin monogeneans are 2-6 mm long. Infections reported are caused by the capsalid monogeneans *Benedenia epinepheli*, *Benedenia* spp., *Neobenedeniagirellae* and *Neobenedenia* spp.

#### 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.

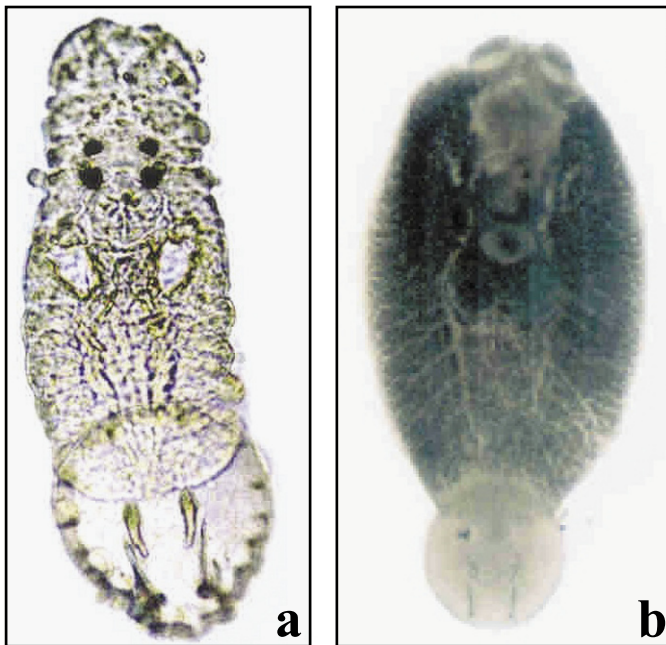
#### Diagnosis:

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<sub>2</sub>O<sub>2</sub>) 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.

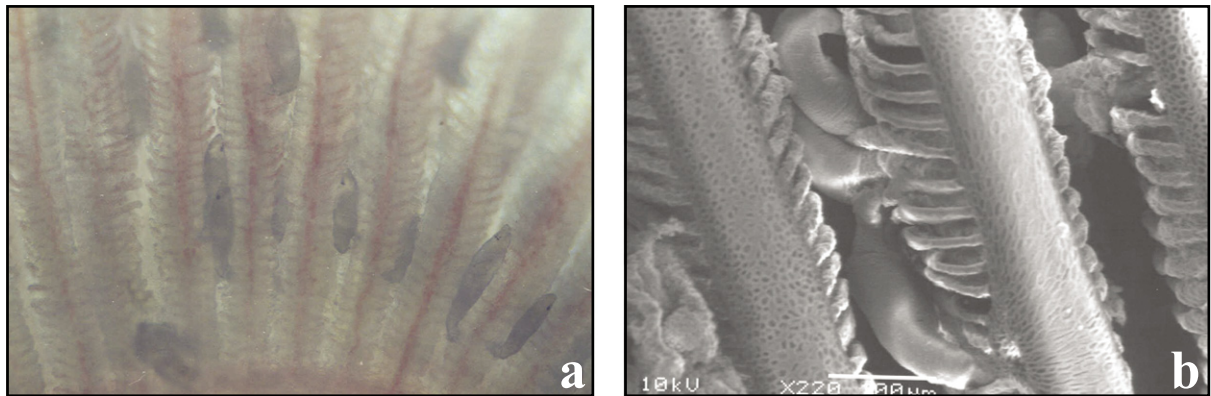
#### **Causative agents:**

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:

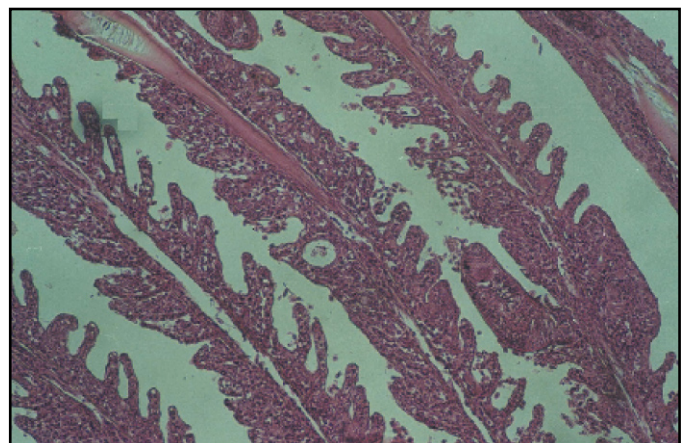
Affected fish display abnormal swimming behavior near the water surface and loss of appetite. An increased mucus production is observed on darkened body surface with frayed fins and pale gills. Hemorrhagic lesions on the body surface are common in heavy infection.

### 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:

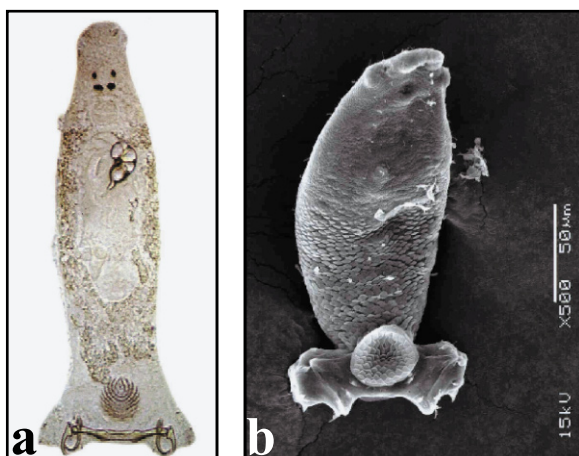
Transmission is horizontal and pre-disposing factor is overlapping generation of fish. High stocking density provides greater opportunity for faster infection. The egg of a gill monogenean is elongate with a long, spiral filament. Hatching of eggs occurs in 5 days to produce the free-swimming larval 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<sub>2</sub>O<sub>2</sub>) 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:**

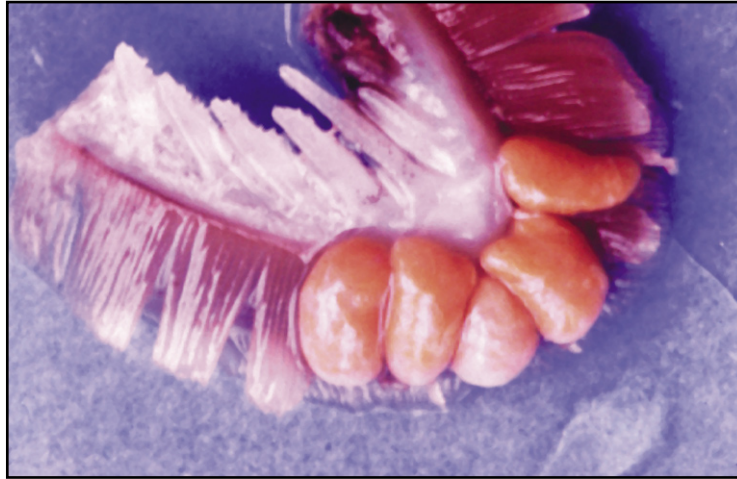
The species *Gonapodasmius epinepheli* has been reported from *E. coioides* in the Philippines and from *E. malabaricus* in Thailand.

**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:**

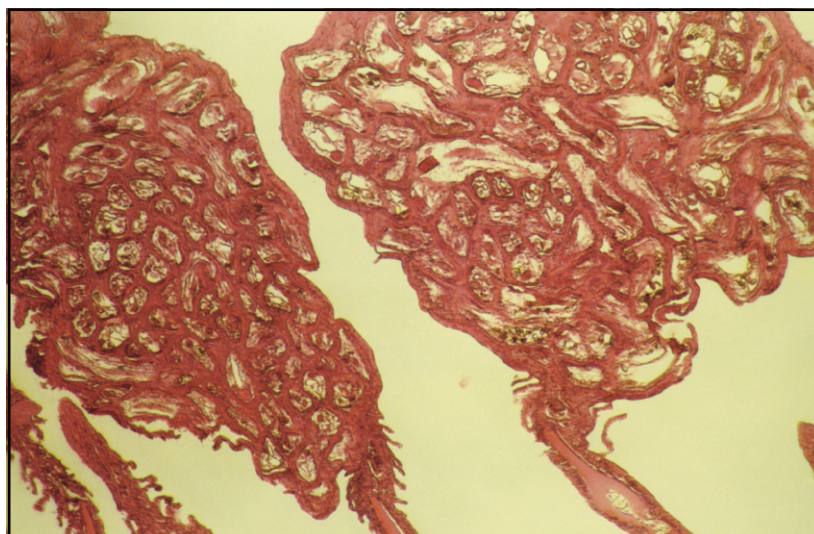
The complete life cycle is unknown but it is likely that the first larval stage, the free-swimming miracidium, is taken by a gastropod mollusc that acts as the first intermediate host. The second larval stage, the cercaria, is released in the water and encysts in the second intermediate host. Small crustaceans 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 *Raphidascaaris* 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.

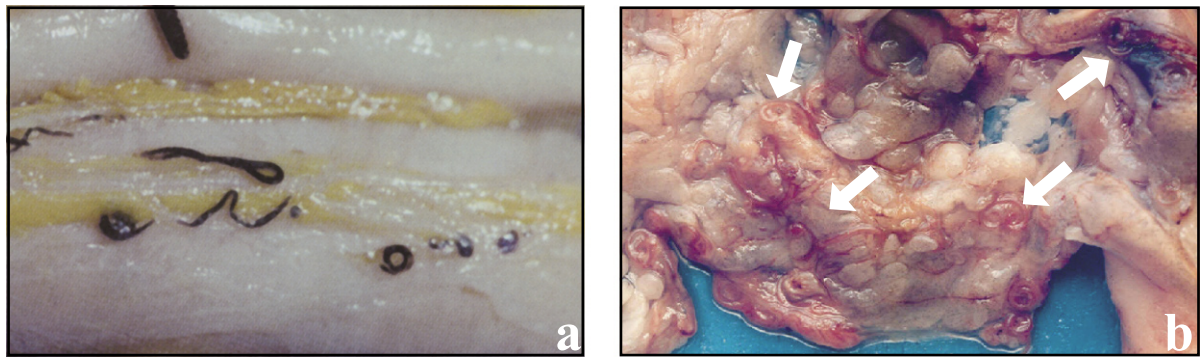
### Effects on host:

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.

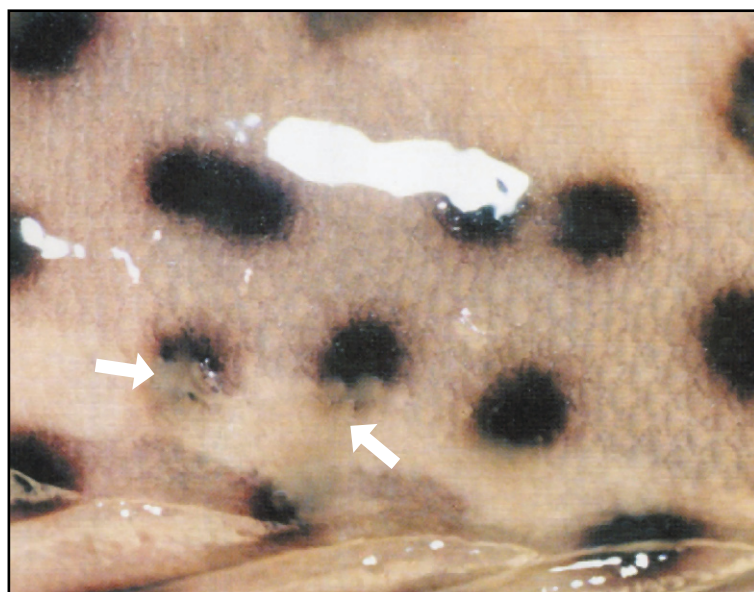
#### Stages affected:

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:**

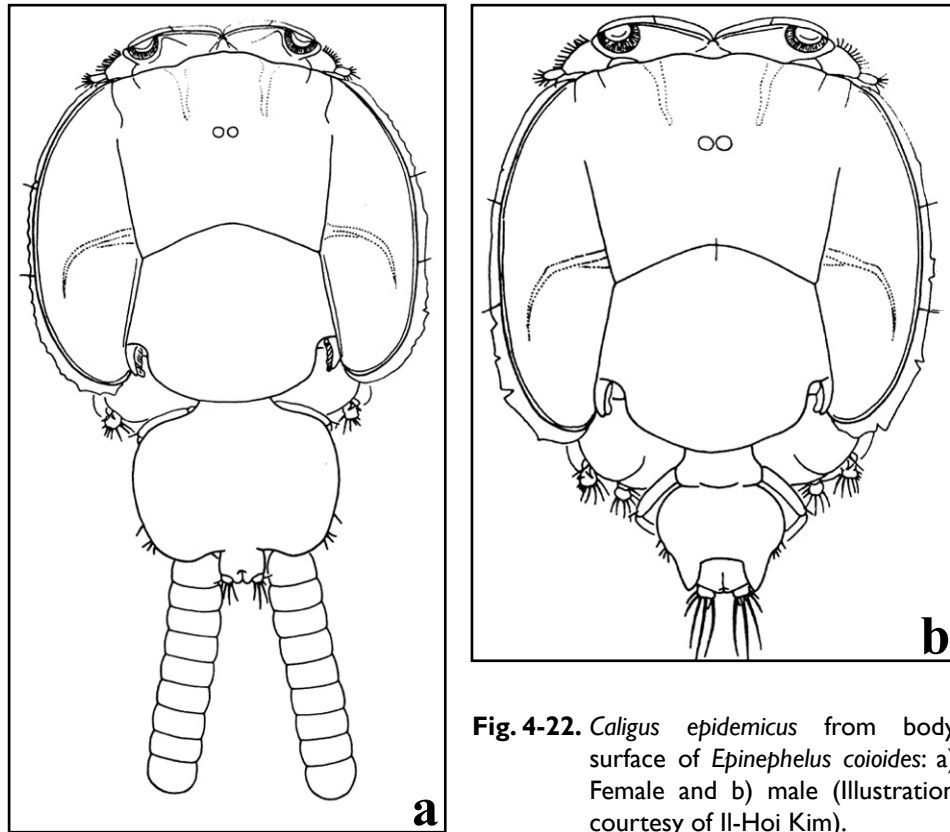
Pre-disposing factor is poor water exchange. Transmission is horizontal. The life cycle of *C. epidemicus* has 11 stages: two nauplii, one copepodid, six chalimus, one pre-adult and one adult. The whole life cycle is completed in 15 days.

**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<sub>2</sub>O<sub>2</sub>) 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:

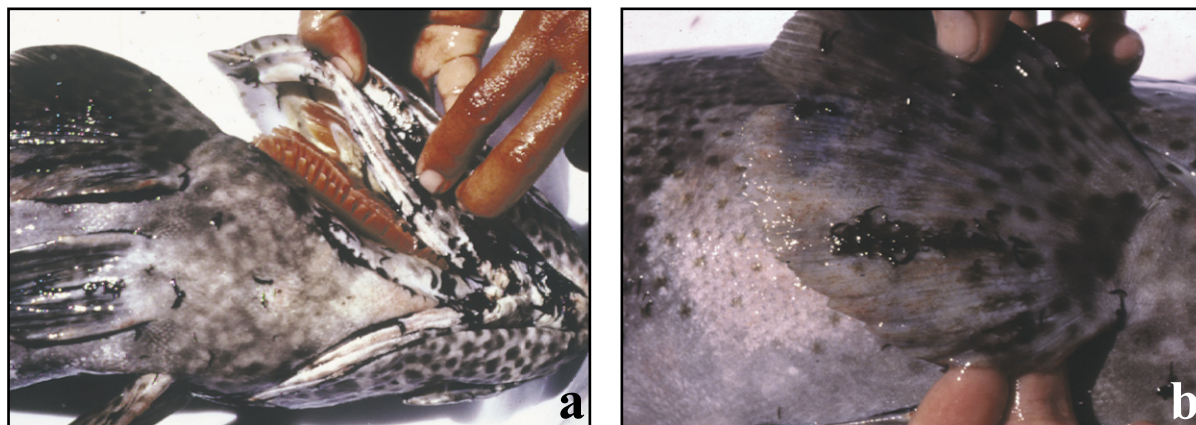
The brownish-black parasites are attached in patches in affected areas such as the body surface, fins, eyes, brachial and mouth cavities (Fig. 4-25). The fins of affected fish are frayed and the attachment and feeding sites are hemorrhagic and swollen. Affected fish lose their appetite, show sluggish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

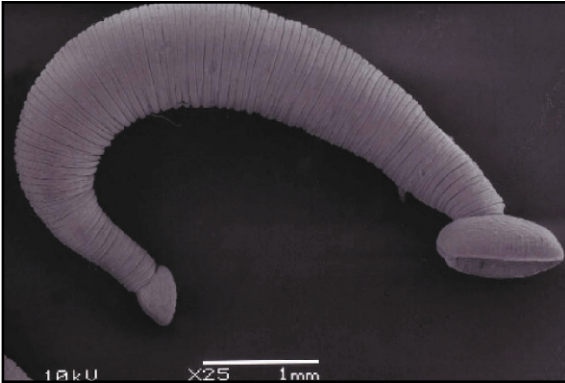


**Fig. 4-25.** *Zeylanicobdella arugamensis* on opercular cavity (a) and pectoral fin (b) of *Epinephelus coioides* broodstock. The attachment and feeding sites of the parasite are hemorrhagic.



### 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.



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