CHAPTER FOUR

Fungal diseases

Eduardo M. Leaño

Many diseases of aquatic animals are caused by organisms that are part of the normal biota of their surrounding environment. Fungi and fungal-like organisms (straminipilous organisms) occur in most waters. They are either saprobes that colonize decaying organic matter, or parasites, which attack a great variety of aquatic organisms leading to disease outbreaks. Among the parasitic species, many are known to be important causative agents of aquatic animal diseases. They are generally opportunistic invaders, but once established, are often fatal and difficult to treat. Thus, fungi and straminipilous organisms may be problematic pathogens under stressful conditions in an aquaculture system.

This chapter lists the important fungal diseases of freshwater and marine animals that are commonly encountered in the Philippines and other Asian countries.

WHAT ARE FUNGI?

Fungi constitute a group of heterotrophic organisms, which contain no chlorophyll and are historically compared to plants. They are usually filamentous and multicellular, although some are non-filamentous and unicellular. The filaments known as hyphae (sing. hypha) constitute the body of a fungus. These filaments elongate by apical growth (growth is active at hyphal tips), in contrast to intercalary growth of other filamentous organisms. The hyphae are either septate (divided by cross walls) or non-septate (coenocytic, without cross walls) (Fig. 4-1). They branch successively behind the tips, resulting in a network of hyphae called mycelium (pl. mycelia).

Figure 4-1. Non-septate (A) and septate (B) hyphae
Fungal cell walls are primarily made up of chitin. This characteristic is one of the basis for separating lower fungi (also known as zoosporic fungi) into another kingdom – Kingdom Stramenopila; as the cell wall of this group of organisms is made up primarily of cellulose. Lower fungi are now referred to as stramenopiles or straminipilous organisms. This group of zoosporic organisms causes most fungal diseases of aquatic animals.

As heterotrophs, fungi exhibit absorptive nutrition. They have a remarkable ability to utilize almost any carbon source as food. Many fungi are saprobes (saprotrophs), which obtain their food from dead or decaying organic matter. On the other hand, a considerable number of species live as parasites of plants, animals, and in some cases, even other fungi. Majority of fungi are also capable of living on dead organic materials, as shown by their ability to grow on synthetic media. These organisms are known as facultative parasites or facultative saprobes. There are some parasitic species, which cannot be cultured in synthetic media, referred to as obligate parasites or biotrophs. Still others can form mutualistic relationships with either animal or plant host.

Fungi and straminipilous organisms (oomycetes) may be isolated from nearly any organic detritus or biological surfaces. Most are ubiquitous in freshwater, estuarine or coastal marine habitats. For straminipilous organisms, there are two isolation methods commonly used:

a) placing of appropriate substrate on a more or less selective medium supplemented with antibiotics to inhibit bacterial growth; and,

b) baiting water with selective substrate such as pollen, insect exuviae, and cellulose.

Freshwater oomycetes (e.g. Saprolegnia and Achlya) are readily isolated by baiting pond waters, and from fish with fungal infection. Holocarpic oomycetes such as Lagenidium callinctes and Haliphthoros milfordensis, which are facultative parasites of eggs and larvae of marine crustaceans, may also be isolated.
with a bit more effort and at the appropriate season, when female crabs and shrimps are carrying eggs.

Isolation of pathogenic oomycetes is usually done by inoculating infected tissues into a suitable medium. Using this method, the inoculated medium needs to be observed for fungal growth within a week (sometimes after 24 to 48 h) to facilitate subculture and identification. Isolation using baits may stand for several weeks without being overgrown by contaminants.

Identification of fungal pathogens are usually based on the fruiting bodies (zoosporangia or conidia) produced by the organism, either on the infected tissues or from axenic cultures. Hyphae of straminipilous organisms are usually wide, hyaline, and non-septate, while those of the higher fungi are thin and septate.

### MAJOR FUNGAL DISEASES OF FISH

Mycotic infections among freshwater fish species are commonly caused by straminipilous organisms. The pathogens can infect eggs, fry, fingerlings, and adult fish. Stress factors such as mechanical injury after handling, exposure to extreme pH levels, prolonged exposure to low water temperatures, lack of food, and presence of other microbial infections (e.g. bacterial, viral) increase the susceptibility of fish to fungal infections. Infection is normally restricted to superficial tissues and, unless the fish can be treated, the condition is usually lethal. Listed below are fungal diseases of freshwater fish caused by zoosporic stramenopiles.

**Saprolegniosis (Saprolegniasis)**

**Causative agents:**
Saprolegnia spp., Achlya spp., and Aphanomyces spp.

**Species affected:**
Many freshwater fish (e.g. carps, goldfish)

**Gross signs:**
Formations of white cottony growth on fish eggs and on affected tissues of fish. Virtually, any area on the surface of a fish may become infected but it is usually the integuments that are involved. Gills, eyes and olfactory pits may also become infected (Fig. 4-3). The color of the mycelium may also vary from white to brownish, depending on the color of the particles, which get trapped on the mycelium.

**Effects on hosts:**
Once infection is initiated, it is generally progressive and terminal. Affected fish become increasingly lethargic, tires more easily and becomes less responsive to external stimuli. Loss of equilibrium often occurs shortly before death. Histopathologically, evidence of rapid destruction of epidermis (tissue necrosis) with slight inflammatory response can be observed.
**Diagnosis:**

Microscopic examination of the cottony growth from the affected tissues will reveal the characteristic hyaline and coenocytic mycelia. Usually, numerous sporangia will be present. If sporangia are present, one can often make a tentative generic identification. However, it must be remembered that other infections (viral, bacterial, protozoan) that are less easy to diagnose might also be present, and that the occurrence of a saprolegnian infection can mask the characteristic signs of these diseases.

**Prevention and Control:**

Prevention and treatment of saprolegnian infections of fishes and fish eggs have attracted much attention for a long time now. A vast array of chemicals has been tested for effectiveness against these fungi in vitro. Common chemicals used as chemotherapeutants include bath treatments of either of the following:

- Zinc-free malachite green (0.1% on wound and rinse; 67 mg/L for 1 min; 0.2 mg/L for 1 h; 0.1 mg/L indefinite). Cautions: persistent tissue levels; mutagenic; teratogenic; treatment can result in gill damage; more toxic at warm temperatures. Malachite green is considered as the most popular antmycotic agent, being inexpensive and highly effective fungicide. This compound also allows a wide margin of error between therapeutic and toxic dosages.
- Sodium chloride (22 g/L for 30 min; 30 g/L for 10 min; 1-3 g/L indefinite). This compound is safe to use and inexpensive.
- Formalin (0.4-0.5 ml/L 30% formaldehyde for 1 h). Inexpensive and popular chemoprophylactic and chemotherapeutic agent.

**Causative Agents:**

* Aphanomyces invadans is associated with the disease outbreak together with rhabdovirus and the bacteria *Aeromonas hydrophila* (Lio-Po 1988). Refer to chapters 2 and 3 for details. Other straminipilous organisms that may superinfect lesions include saprobic Aphanomyces strains, Saprolegnia spp., and *Pythium* spp.

**Species Affected:**

More than 30 freshwater fish species (e.g. snakeheads, catfish, guoram, goby, etc.)

**Gross Signs:**

Early signs observed among affected fish are: darker discoloration and loss of appetite; fish floats just below the water surface, or in some species, with the head just breaking the water surface; occasionally, fish may be hyperactive with a very jerky movement. Ulcerative lesions can be observed throughout the body, which may vary from small areas of rosacea occasionally on the side of the jaw or head, to larger, deep, ulcerative lesions found anywhere on the body.

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**Epizootic Ulcerative Syndrome (EUS)**

**Causative agents:**

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PREVENTION AND CONTROL:

Since EUS mostly occurs among wild fish stocks, it can be very difficult to control outbreaks within a local area. Therefore, where EUS is not endemic, the most effective means of control would be to prevent entry of any infected fish into the area.

For areas where EUS is presently considered endemic, prevention program should include:

• Eradication of the causative agent (e.g., fungi) by removal of all fish from ponds, reservoirs, and water channels prior to restocking; drying-out and liming of ponds; and disinfection of contaminated equipment.
• Once the causative agent has been eradicated from an affected site, reintroduction should be prevented.
• Proper management by reducing stocking densities when EUS prevalence is high in adjacent wild fish populations.
• Farming of EUS-resistant fish species (e.g., tilapia) would also be effective in preventing the occurrence of the disease.

Potentially useful treatments for the causative fungus include:

• 5 ppm Coptrol (a chelated copper compound);
• 0.1 mg/L malachite green.

Fungal diseases

EFFECTS ON HOSTS:

Fish affected by the disease become lethargic (inactive or comatose in later stages). Advanced stage of the disease often results in exposed head and bone tissues, visceral organs, and vertebral column. Total erosion of the tail is also common. There is severe hydration problem. Histopathologically, massive infiltration of the muscle tissues by the fungus (Fig. 4-4) accompanied by severe tissue necrosis and minimal inflammatory response can be observed. Fungal hyphae may reach the cranium, kidney, and spinal cord.

DIAGNOSIS:

Outbreaks occur at certain times of the year, normally after flooding followed by cool weather (usually from December until February). Presence of mycotic granulomas which can spread throughout the lesions and also affect some internal organs. Isolation of A. invadans from internal tissues.

CAUSATIVE AGENTS:

Branchiomycetes spp.

SPEICES AFFECTED:

Carps, goldfish, eels

GROSS SIGNS:

Gills become pale with brownish areas due to hemorrhage and thrombosis, or
grayish as a result of ischemia. Necrotic areas might slough-off at a later stage becoming a focus for saprolegnian infections.

**Effects on hosts:**

Fungal hyphae in the gills obstruct the circulation of the blood. Necrosis and proliferation of lamellar epithelial cells and lamellar fusions may be observed. The disease can appear suddenly and often has a rapid course with losses as high as 30-50% occurring in 2-4 days. Death is due to anoxia.

**Diagnosis:**

Microscopic examination will reveal the branched and coenocytic mycelia of the pathogen within the affected gill tissues.

**Prevention and control:**

Various chemicals have been used to treat branchiomyocosis, which include:

- malachite green (0.3 mg/L for 24 h)
- benzalkonium chloride (1-4 ppm active ingredient for 1 h)
- copper sulfate (100 ppm for 10-30 min)
- sodium chloride (3-5%)

If an outbreak occurs, feeding of the fish should be stopped and dead fish should be removed from the ponds and buried in a lime pit. To help prevent further outbreaks, the pond should be drained, dried-out and disinfected with quicklime.

**Causative agent:**

*Ichthyophonus* sp. (= *Ichthyosporidium* sp.)

**Species affected:**

Groupers, trouts, flounders, herrings and cods

**Gross signs:**

External manifestation of the disease varies from species to species, while some affected fish don’t show any external sign. Erratic swimming behavior and swelling of the abdomen are sometimes observed among affected fish. Internal organs (spleen, liver and kidney) become swollen with numerous whitish nodules up to 2 mm diameter. Nodules can also be observed in the muscle tissues of some affected fish.

**Effects on host:**

Infected fish lose their appetite and become lean and anemic.

**Diagnosis:**

The nodules formed in internal organs or muscle will show different stages of the pathogen (early cyst stage, developed cysts, fungal hyphae) when observed under the microscope. These are surrounded by the hosts’ connective tissues forming lumpy granulomas which are typical of a fungus-infected tissue.
PREVENTION AND CONTROL:

There is no known treatment of ichthyophoniasis. Cultured fish become infected when they are fed raw trash fish contaminated with the pathogen. Therefore, contaminated trash fish should be carefully avoided to prevent outbreaks.

MAJOR FUNGAL DISEASES OF CRUSTACEANS

Most marine fungal diseases are encountered among crustaceans, and rarely among fish species. Similar to freshwater condition, most of these diseases are caused by straminipilous organisms, and occasionally by other groups of fungi (e.g. mitosporic and ascomycetes). The eggs and different larval stages are most commonly affected. Listed below are some fungal diseases of cultured shrimps and crabs.

Larval Mycosis

CAUSATIVE AGENTS:
Lagenidium spp., Sirolpidium spp., Haliphthoros spp.

SPECIES AFFECTED:
All Penaeus species, crabs (e.g. Scylla serrata)

GROSS SIGNS:
Sudden onset of mortalities in larval stages of shrimps and crabs. Crab eggs are also susceptible for mycotic infection. The commonly affected larval stages among shrimp species are the protozoeal and mysis stages.

EFFECTS OF HOSTS:
Progressive systemic mycosis that is accompanied by little or no host inflammatory response can be observed. Infection is apparently lethal, accumulating mortality of 20-100% within 48-72 h after onset of infection.

Figure 4-5. Lagenidium infection in crustacean larvae. A - Larva of Penaeus monodon heavily infested with the fungus (note the mycelia [arrows] completely replacing the body tissues of the larva; and the vesicles [arrowheads] ready to release the zoospores). B - Infected Scylla serrata larva in brain-heart-infusion (BHI) broth after 2 days (fresh mount, 40x)
Diagnosis:

Microscopic examination of affected larvae will reveal extensive, non-septate, highly branched fungal mycelia throughout the body and appendages (Fig. 4-5). Specialized hyphae or discharge tubes, with or without terminal vesicles, may be present, and could be the basis for identification of the causative agent. Motile zoospores may be observed being released from the discharge tubes in the case of some species. Classification of the type of organism causing particular epizootic of larval mycosis is dependent upon the microscopic examination of sporogenesis as follows:

Lagenidium - zoospores are released from terminal vesicle (Fig. 4-6).

Sirolpidium and Haliphthoros - absence of terminal vesicles; zoospores are released through discharge tubes formed by the zoosporangia (Fig. 4-7).

Prevention and Control:

Disinfection of contaminated larval rearing tanks and chlorination and/or filtration of the incoming water can prevent outbreaks. Different antymycotic compounds have been tested in vitro. Recommended chemicals for therapeutic and prophylactic treatments include the following:

- 0.2 ppm Treflan
- 1-10 ppm formalin
- egg disinfection with 20 ppm detergent followed by thorough rinsing before hatching

Black Gill Disease (Fusarium Disease)

Causative Agent:
Fusarium solani

Species Affected:
All Penaeus species
**Fungal diseases**

**Gross Signs:**
Appearance of "black spots" that preceded mortalities in juvenile shrimps grown in ponds.

**Effects on Hosts:**
Infection usually starts on damaged tissues such as wounds, gills damaged from chemical treatments or pollutants, and lesions resulting from other disease processes. Once infection is established, it is usually progressive with 30% remission rate. Lesions may also serve as a route of entry for other opportunistic pathogens.

**Diagnosis:**
Microscopic examination of wet mounts of infected tissues will reveal the presence of canoe-shaped macroconidia (Fig. 4-8). Fusarium spp. are ubiquitous soil fungi. Infection may begin at different loci and spread slowly. Fusarium solani is an opportunistic pathogen of penaeids and are capable of establishing infection in shrimps compromised by other stresses or overcrowding.

**Prevention and Control:**
Preventive measures include the elimination of sources of Fusarium conidio- phores and destruction of infected individuals. Several fungicides show promise in vitro but none proved to be effective in actual field trials.

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**Aflatoxicosis (Red Disease)**

**Causative Agent:**
Aflatoxin produced by Aspergillus flavus and other Aspergillus spp. (Fig. 4-9) which are common contaminants of not-properly stored or expired feeds.

**Species Affected:**
Penaeus monodon, other Penaeus spp.

**Gross Signs:**
Yellowish, and eventually reddish discoloration of the shrimp body and appendages can be observed among pond-cultured shrimp juveniles. Affected animals become lethargic with weak swimming activity near pond dikes. Soft shelling can also be observed.

**Diagnosis:**
Affected shrimps will not survive for more than 30 seconds when collected from the feeding trays. There will also be loss of appetite. Confirmation is by chemical analysis for the presence of aflatoxin in the suspected feed/ingredient.

Additional information on aflatoxicosis in shrimp can be found in chapter 7.

**Effects on Hosts:**
Histopathologically, necrosis in the tubule epithelium that proceeds from proximal portion of the tubules to peripheral tubule tips in the hepatopancreas can be observed. Growth will be retarded.
PREVENTION AND CONTROL:

Do not use moldy feeds. Feeds should be properly stored (for not more than 6 months) in dry and well-ventilated areas to prevent, or at least minimize growth of fungal contaminants.

SUMMARY

Over the past 20 years, aquatic animal mycopathogens have become the focus of considerable research. The many known occurrences of fungal diseases in wild populations and the documented devastating disease outbreaks indicate that fungal and fungal-like pathogens are important in nature. Fungal diseases can act as major limitations on natural and cultured populations of aquatic animals. However, knowledge on fungal diseases is rudimentary consisting primarily of the identification and pathology of etiological agents. Detection of fungal infections relies only on the observation of gross pathology, histological examinations, and standard mycological isolation and identification procedures. As a result, there are some cases where the implicated fungal pathogen cannot be demonstrated as the primary cause of a particular disease. In such cases, the fungal pathogen is usually regarded as secondary invader.

Continued research in basic mycology is still an essential resource for fish pathologists in diagnosing diseases caused by fungi. Although fungi reportedly affect very few species, fungal diseases, if not properly controlled or prevented, can still pose a threat to the aquaculture industry.

REFERENCES/SUGGESTED READINGS


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