Winter School on

'RECENT ADVANCES IN DIAGNOSIS AND MANAGEMENT OF DISEASES IN MARICULTURE'

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Course Manual

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MYCOTIC DISEASES OF FISH AND SHELLFISH

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Several species of fungi are responsible for a range of serious economically important diseases of fish and shellfish. However, far less is known about fungal diseases of fish compared to bacterial or viral disease. Major problem is in identifying the fungus responsible and in determining whether it is truly pathogenic or simply a saprophyte taking advantage of an existing lesion.

Most of Ichthyoparasitic fungi are members of Eumycota (true fungi) under the subdivisions

- Mastigomycotina
- Zygomycotina
- Ascomycotina
- Deuteromycotina (Fungi imperfecti)

Some of the important fungal fish pathogens (eg. *Branchiomyces* and *Ichthyophonus*) are still of uncertain taxonomic affinity.

I. Mastigomycotina

Characterised by spores with a typically motile sexual stage and either a motile or non-motile asexual stage.

I.1. Class : Oomycetes:

This is the most important group of fish fungal pathogens. These are widely distributed in the aquatic habitat and only very few are parasitic. These produce motile, biflagellate spores. Asexual reproduction is by means of zoospores produced in zoosporangium and sexual reproduction is by producing a thick-walled oospore. Hyphae do not generally possess cross walls. There are four orders within oomycetes having representatives as fish pathogens.

I.1.1.Order: Saprolegniales
I.1.1.1. Fly : Saprolegniaceae

Majority of the significant fish pathogens are with in this family. Members are known as water molds, possessing profusely branching non-septate mycelium and appearing like cotton-wool tufts in water. Reproductive structures are separated from the somatic hyphae by means of a septate zoosporangium containing biflagellate zoospores. Some species are found in brackish water, but salinities higher than 2.8% limit their distribution. Eg. *Saprolegnia, Achlya, Aphanomyces*

Saprolegniasis

Infection with fungi of the *Saprolegnia parasitica* complex is known as saprolegniasis. These fungi normally lead saprophytic lifecycle and generally result in surface infections, but pathogenesis is complex. These are secondary opportunistic

invaders, commonly seen after handling and after traumatic damage to skin in overcrowded conditions and in conjunction with pollution or bacterial or viral infections. High temperature and organic loading are the other predisposing factors. Spores of saprolegnia are always present in water, but in healthy fish, spores get rare chances to settle and establish because of mucus production and sloughing. In fish with damaged skin, spores settle, germinate and spread hyphae in all directions damaging epithelium.

Saprolegnia lesions are focal gray-white patches on the skin of the fish, which when examined under water have cotton wool like appearance. The early lesions are often almost circular and grow by radial extension around the periphery until lesions merge. At this later stage the fungal patches are often dark gray or brown in colour as the mycelium traps mud or silt.

The fungus usually establishes itself focally, invading the stratum spongiosum of the dermis and then extending laterally over the epidermis, eroding as it spreads. Relatively superficial invasion of the dermis rapidly leads to fluid imbalance and peripheral circulation failure due to inability to maintain circulating blood volume. In histological sections of skin infected with *Saprolegnia* , numerous hyphae can be seen on the skin surface, enmeshing cellular debris and material trapped from the water by the hyphal strands. Beneath this surface mat of mycelium, areas of degenerating tissue can be seen ranging from superficial dermal necrosis and oedema to deep myofibrillar necrosis and extensive hemorrhage. There is often only a slight inflammatory response but, when concomitant bacterial infection occurs, especially at haigh temperatures, a marked inflammatory infiltrate is usually apparent. The fungal hyphae are PAS positive and easily demonstrated by silver impregnation methods, such as Grocott's technique. They are branching, non-septate and approximately 20 μm in diameter.

Skin and gill lesions are by far the most frequently observed but there are reports of infection of internal organs. *Saprolegnia* is also a common invader of incubating fish eggs, usually establishing itself first on dead eggs and extending to neighboring healthy ones.

Prevention of the disease may be aided by maintaining the fish under good husbandry conditions. Correct feeding, avoidance of overcrowded conditions and good water quality are essential for preventing incidence of saprolegniasis.

Achlya spp.

The genus, *Achlya* consists of a number of species parasitic on fish. In contrast to *Saprolegnia*, the zoospores do not swim away from zoosporangium, but encysts as a hollow ball at its mouth. Thus there is no freely motile primary zoospore. The infecting secondary zoospores emerge from the cyst coming directly from the mouth of zoosporangium. There are many reports of different *Achlya* spp. infecting fish, however, there is no consistent and regularly observed clinical conditions as with *Saprolegnia*.

Aphanomyces spp.

Aphanomyces invadens :

Associated with Mycotic granulomatosis (MG) of freshwater and esturine fishes in Japan, Red spot disease (RSD) of grey mullets in Australia and Epizootic Ulcerative syndrome of freshwater and esturine fish in Asia.

Aphanomyces astaci :

Known as the crayfish plague fungus responsible for catastrophic mortalities of feral crayfish (*Astacus* sp.) in European waters. Causes mortality due to fungal proliferation in the internal tissue of susceptible species.

I.1.1.2. Fly: Leptolegniaceae

Leptolegnia marina: infects eggs and gills of crabs and lobsters

I.1.1.3. Fly: Haliphthoraceae

Haliphthoros milfordensis :

Obligatory marine, filamentous, biflagellate fungus. Infects ova, larval stages and juveniles of crabs, lobsters and shrimps.

I.1.2. Order: Lagenidiales

The order Lagenidiales contains a primarily parasitic group of endobiotic fungi occurring both in fresh and marine waters.

I.1.2.1. Fly : Lagenidiaceae:

These fungi produce secondary zoospores either entirely within the sporangium or exogenously, with or without surrounding vesicles.

Lagenidium spp :

Causes larval mycosis (systemic non-inflammatory mycoses) responsible for major epizootics of penaeid larvae. Eggs and larvae are highly vulnerable.

Lagenidium callinectes :

Internal parasite of the eggs of blue crab, *Callinectes sapidus.* Zoosporangia are formed on short hyphae which penetrate through the host wall and act as discharge tubes.

Lagenidium scyllae :

Infects ova and larvae of *Scylla serrata*

I.1.2.2. Fly: Sirolpidiaceae

Sirolpidium spp. :Fungi have branched, septate hyphae, causes mycosis (systemic non-inflammatory mycoses as with *Lagenidium*) in shrimps, clams and oysters.

I.1.3. Order: Leptomitales

Fly: Leptomitaceae :

Leptomitus spp.:

Causes mycosis in salmonids and perch.

I.1.4. Order: Peronosporales

Fly. Pythiaceae :

Pythium thalassium :

Infects eggs of crabs and shrimps.

Pythium undualtum :

Infects eggs of salmonids.

1.2. Class: Chytridiomycetes:

Order : Chytridiales

Dermocystidium spp :

Infects skin and gills of carps, salmon and trout. Parasite encysts in the gills and excite a typical chronic inflammatory response comprising a fine fibrous capsule surrounded by epithelioid cells and often with marked epithelial hyperplasia. Lesions may also be seen in the spleen and occasionally in muscle, kidney or other organs. Mortalities confined to fry and young ones.

Dermocystidium marinum :

Parasites of oysters.

II. Zygomycotina:

Fungi under this subdivision produce non-motile sporangiospores (aplanospores)

Order : Entomophthorales:

Basidiobolus ranarum and *Basidiobolus meristophorus* :

Reported to cause infection in fish.

III. Ascomycotina:

Fungi posses septate mycelium and does not have flagellated structures. Produce ascospores in specialized sac like structures known as ascus.

Trichomaris invadens :

Causes blackmat syndrome in tanner crab (*Chinocetes bairdi*)

IV. Deuteromycotina (Fungi imperfecti) :

The fungi imperfecti are fungi brought together by the common feature that they lack a sexual stage in their life cycle. Possess septate mycelium (with cross walls) and reproduce by asexual methods by producing conidia on conidiophores. Although they are generally considered to be opportunistic pathogens. When infections occur, they are generally chronic, progressive and fatal and all are associated with systemic granulomata. Invariably the fungi imperfecti occur internally in fish.

Phoma herbarum :

Infects salmonid fry and fingerlings

Fusarium solani:

Causes burn spot disease in crustaceans. Localised mycosis in juvenile and adults accompanied by inflammatory response.

Ochroconis spp.:

Infects salmons and trouts

Exophiala :

Causes cerebral mycetoma in channel catfish and extensive granulomatous inflammatory response with necrosis of cartilage in salmonids.

Phialophora :

Causes systemic infection in salmonids

Aspergillus :

An important fish pathogen especially in the tropics. Systemic infection with fungal hyphae causes Aspergillomycosis in cultured Tilapias.

Other aseptate fungi :

Branchiomyces spp.:

There are two species of *Branchiomyces*, both known only as parasites of fish gill tissues. Both have branched, coenocytic hyphae, which produce aplanospores (non-motile spores) by endogenous cleavage. Two species are distinguished based on the size of hyphal wall and spore and on specific habitat in the gill. The two species, *Branchiomyces sanguinis*, which occurs within the blood vessels of the gill and *Branchiomyces demigrans*, which can penetrate through the gill tissues to the surface and grow well outside blood vessels as a mass of invading hyphae, are generally associated with infection of cyprinid fishes, tench, sticklebacks and Japanese eels. The branched, non-septate hyphae of *B.sanguinis* measure 8-30 μm in diameter, with spores 5-9 μm in diameter. Those of *B.demigrans* are larger.

Branchiomycosis, otherwise known as gill rot, is the disease condition caused by *Branchiomyces*. Characterized by areas of infractive necrosis in the gill due to intravascular growth of the fungus. Both species are involved in the disease. Both species prefer gill tissue, because they prefer high oxygen tension. Obstruct circulation in gill tissue resulting in white necrotic patches in gills, clumping of gills and excessive mucus production. Histopathological features include, hyperplasia, fusion of gill lamellae, and areas of massive necrosis resulting from thrombosis of vessels by fungal hyphae, telangiectasis and vascular necrosis. Affected fish may die as rapidly as within two days of infection.

Infection is probably by spores liberated from necrotic gill tissue but it is not known whether infection occurs directly through the gill or after ingestion of spores. Waters rich in organic fertilizers, algal blooms and temperatures exceeding 20°C encourage the disease. Disease may be prevented by strict hygiene, the removal of dead fish and the avoidance of overfeeding, especially at high water temperatures.

Critical taxonomic analysis of *Branchiomyces* has not been undertaken, except for the systematic studies by Peduzzi (1973). Antigenic studies by Peduzzi suggest that *Branchiomyces* belongs within the Saprolegniaceae.

Ichthyophonus hoferi :

Ichthyophonus hoferi is an internal fungus, causing systemic granulomatosis in both fresh water and marine fish. Regarding the taxonomic classification of the organism there is still considerable amount of confusion. Clinical signs of ichthyophoniasis include

roughened skin surface due to the proliferating dermal granuloma (ring nodules) and raised white nodules in internal organs. These nodules are produced by the severe granulomatous response with large number of epithelioid cells, macrophages and occasional giant cells, along tip of fungal hyphae or newly developed spores.

Although the parasite appears to be confined to the marine environment, the disease can be readily transferred to freshwater fish by feeding them on infected trash fish. Source of infection is ingestion of infected material (oral route). The spores germinate to form amoeboblasts in the digestive tract, penetrate the gut mucosa, reach blood vascular system and spread via, the portal system and usually localizes in liver, spleen, kidney and particularly in the muscle including the cardiac muscle. Here, the amoeblasts germinate into often extensive invasive branched hyphae which excite a significant host reaction with epithelioid tissue, lymphoid infiltrates and fibrous cyst formation. Some spores may remain in the gut mucosa and germinate there to produce a patent infection. Spores are released when hyphae rupture the surface of the gut or penetrate the skin to allow infection to spread via, the water.

There appear to be a number of stages in the life cycle and the microscopic appearance varies depending both on the stage in the life cycle and the nature of the host. *Ichthyophonus* is frequently observed in the resting or spore stage, and this is an oval structure, 10-250 µm in diameter. This stage has a double wall, which stains PAS positive, and is also positive to silver stains such as Grocott's methanamine silver. There are multiple nuclei. These spores may be present singly or in large numbers in a variety of organs and are often found in association with other stages. The germinating spore is often seen, especially in *post mortem* material and consists of cytoplasmic elongation bounded by the inner spore wall which herniated through the thicker outer wall. Further differentiation then takes place to form non-septate macrohyphae up to 40 µm in diameter. New spores, termed hyphal bodies may be formed within these hyphae.

Ichthyophoniasis was first recorded in Atlantic herring along East Coast of US and Canada. Since herring forms major component of fishmeal, which are fed to trout and carps, carry spores and infect the latter. *Ichthyophonus* regularly causes severe natural epizootics in Atlantic herring, cod and whiting. Outbreaks in herring usually occur in winter and spring and infection is apparent as a roughened, 'sandpaper-like' skin texture occurring principally on the ventral surface. The sandpaper effect is due to the loss of epithelium over the proliferating fungal granulomata. Infections in rainbow trout are common when trash fish is fed. Heart, liver, spleen, kidney, muscle and even brain may be infected and may result in serious mortalities.