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## Economically important freshwater fishes infected with fungi causes EUS

Podeti. Koteswar Rao \*

Department of Zoology, Kakatiya University- 506 009, India.

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### Abstract

The aim of this study was to investigate fungal infections in *Channa punctatus* and *Channa striatus* twenty specimens of each species were studied for the presence of fungal infections. Infected fish's shows clinical signs such as fungal growth on skin, fins, eyes, eroded fins and scales, hemorrhages on body surface and abdominal distension. The ability of aquatic fungi to cause diseases in fish is well known potentially all freshwater fishes their incubating eggs are susceptible to fungal infection. The most common fungal diseases of fish are saprolegniasis, disease caused by Achlya, branchiomycosis, epizootic ulcerative syndrome (EUS). Saprolegniasis is one of the most common infections of freshwater fishes in warm temperature and tropical waters; distributed throughout worldwide. Although there are many reports of different Achlya species infecting fish, there is no consistent and regularly observed clinical condition such as occurs with Saprolegnia. EUS is a seasonal epizootic condition of great importance in wild and farmed freshwater.

**Keywords:** *Channa punctatus*; Saprolegniasis; Branchiomycosis; EUS

### 1. Introduction

The Freshwater fishes are an important protein source for people of many countries [1-2]. However, globally fish from freshwater and marine sources are in severe decline, driven in large part by economic and human population growth [3]. Fish farming in various parts of the world has increased many folds in the last decade. As a result, fish culture has now become commercially an important industry worldwide. The aquatic animals are susceptible to a variety of pathogenic organisms, including fungi. In fact the first clinical report of a fungal infecting a fish [4]. The ability of aquatic fungi to cause diseases is encountered in eggs, fry, fingerling and adult fishes. Majority of the aquatic fungi are saprophytic and derive their nourishment by decomposed organic matter. They are primarily regarded as a secondary invader and are known to attack the host when it gets injured either mechanically or as a result of infection other than fungi [5]. Fungal infections of fish by Oomycetes, commonly known as water moulds, are widespread in fresh water and represent the most important fungal group affecting wild and cultured fish. The most important are the Saprolegniales. Although eight genera have been reported in naturally or artificially induced infections, Saprolegnia, Achlya, Aphanomyces, Calyptrotheca, Thraustotheca, Leptolegnia, Pythiopsis and Leptomitosis, only Saprolegnia, Achlya and Aphanomyces are significant in aquaculture [6-8]. On the other hand, the most common fungal diseases of fish, saprolegniasis, disease caused by Achlya, branchiomycosis, epizootic ulcerative syndrome (EUS). The behavioral changes appear as the infection progresses; fish becomes lethargic, irregular in swimming and equilibrium is lost. [9].

\* Corresponding author: Podeti. Koteswar Rao

## 2. Results and discussion

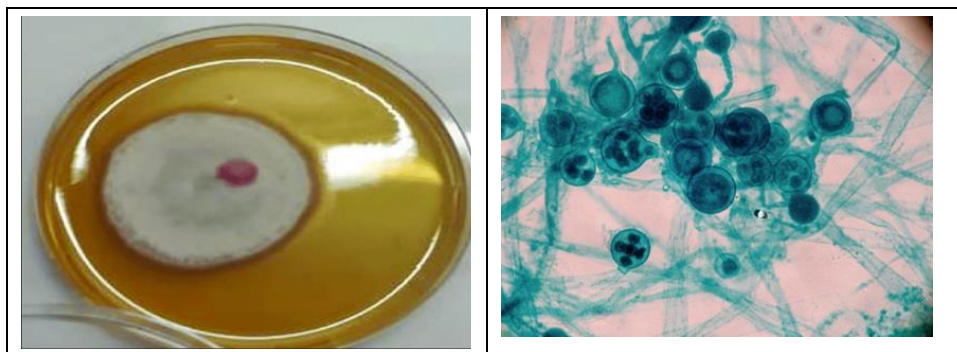
### 2.1. Saprolegniasis

Saprolegniasis is caused by fungus *Saprolegnia* sp. (that is why the disease is called saprolegniasis), one of the most important fish pathogen. *Saprolegnia* comes under family Saprolegniaceae, order Saprolegniales one of the four orders within the Oomycetes. Species mainly causing Saprolegniasis was defined as *Saprolegnia parasitica* by Coker [10] to accommodate all asexual isolates of *Saprolegnia* that were parasitising fish. *S. parasitica* and *Saprolegnia diclina* are probably the two species most commonly isolated from Saprolegniasis infected fish; they are closely related to each other and are often referred to as the *S. parasitica*–*S. diclina* complex [11-15]. *Saprolegnia* are filamentous producing a profusely branching septate vegetative mycelium. The life cycle of *Saprolegnia* was first described by Kanouse [15]. *Saprolegnia* development begins with a slight enlargement of the terminal portion of a hypha and cytoplasm and nuclei accumulate in the enlarged tip, called sporangium which produces masses of zoospores. The tip of sporangia rupture to release the primary zoospore has two flagella at the anterior end. They move for some time and encyst. The cyst after a period germination into hyphae or burst to release secondary zoospores which are reniform with two flagella laterally placed on the concave side. After swimming for some time, they encyst and finally germinate into hyphae. Sexual reproduction in *Saprolegnia* takes place by the sex organs developing at tip of the hyphae. The female organ oogonium is a globose body and the male organ antheridium is slender and sinuous. Sexual reproduction occurs by gametangia contact, resulting in the fusion of the haploid oosphere with nuclei to produce a zygote [17]. The zygote then transform into a sporangium where the protoplast divides into a number of the zoospores. From fertilization to germination into hyphae it takes nearly 3 months. Saprolegniasis is one of the most common infection of freshwater fish and also some estuarine fish in warm temperature and tropical waters, distributed worldwide. *Saprolegnia* are classical opportunists that normally feed saprophytically on dead organic matter. These infections in fish are often associated with immunosuppression. Temperature has a significant effect on development of *Saprolegnia* infection and outbreak often occur when temperature are near the physiological low end for particular fish species. This may be due not only to lower immunity, but also to the fact that many oomycetes are more active in cooler months of the year [18]. When saprolegniasis affects fish during cold temperature, it is often called winter kill.

The disease occurs when pond temperature drop below 15 °C. However stress of high temperature and poor oxygen levels may also induce *Saprolegnia* invasion [19]. Lesions are focal gray-white patches on the skin, have a cotton wool-like appearance where hyphal filaments extend out into the water. The early lesions are often almost circular and grow by radial extension around the periphery until lesions merge. At later stage, the oomycete patches are often dark grey or brown in color as the mycelium traps mud or silt. Skin and gill lesions are by far the most frequently observed, but there have been reports of infection of internal organs [5]. *Saprolegnia* are important pathogens of fish eggs. Infections most often begin in unfertilized or otherwise nonviable eggs. Once established, they can rapidly spread to healthy eggs, eventually resulting in complete loss of the brood [20- 21]. Among the four species of *Achlya*, *A. Americana* dominated even though all the *Achlya* species were perennial. Within the two *Pythium* species, *P. aphanidesmatum* occurred only during the rainy season while among the two species of *Saprolegnia* both were absent during the summer season. [22].

#### 2.1.1. Diagnosis

*Saprolegnia* are more closely related to algae than to true fungi however hyphae of true fungi are septate while those of oomycetes are aseptate [21]. Observation of a cottony proliferative growth on the skin or gills is possible diagnosis of *Saprolegnia* infection. (Fig. 1)



**Figure 1** *Saprolegnia* cultured images

### 2.1.2. Treatment

Saprolegnia are among the most difficult disease to treat. Prevention of the disease may be aided by maintaining fish under good husbandry condition. Correct feeding, the avoidance of overcrowded conditions and good water quality are essential. For treatment a variety of external disinfectant may be used, but except salt, most agents legally approved for food fish [24] are of limited effectiveness. Malachite green is highly effective for treating Saprolegnia infection, but it is not approved for food fish use in most countries because of its teratogenic and mutagenic properties. Saprolegnia infection are inhibited by even low prolonged immersion salt concentrations (>3ppt), which is probably why they do not affect marine fish in high salinities [25].

## 2.2. Epizootic ulcerative syndrome (EUS)

It is a seasonal epizootic condition of great importance in wild and farmed freshwater and estuarine fish. It has a complex infectious aetiology and is clinically characterized by the presence of invasive *Aphanomyces invadans* infection and necrotising ulcerative lesions, typically leading to a granulomatous response. EUS is also known as red spot disease (RSD), mycotic granulomatosis (MG) and ulcerative mycosis (UM). In 2005, scientists proposed that EUS should be named as epizootic granulomatous aphanomycosis or EGA [26]. However the term EUS has been used by most scientists. The oomycete that causes EUS is known as *A. invadans*. EUS has spread widely since the first outbreak in 1971 in Japan and to date only one genotype has been recorded. Parasites and rhabdoviruses have also been associated with particular outbreaks, and secondary Gram-negative bacteria invariably infect EUS lesions. *A. invadans* (Saprolegniales, Oomycetes) has an aseptate fungallike mycelia structure. This oomycete has two typical zoospore forms. The primary zoospore consists of round cells that develop inside the sporangium. The primary zoospore is released to the tip of the sporangium where it forms a spore cluster. It quickly transforms into the secondary zoospore, which is reniform with laterally biflagellate cells and can swim freely in the water. The secondary zoospore remains motile for a period that depends on the environmental conditions and presence of the fish host or substratum. Typically, the zoospore encysts and germinates to produce new hyphae, although further tertiary generations of zoospores may be released from cysts (polyplanetism) [27]. The EUS occurs mostly at water temperatures of 18–22°C and after periods of heavy rainfall [28].

EUS is transmitted horizontally if the secondary zoospores cannot find the susceptible species or encounter unfavorable conditions, they can encyst in the pond environment. The cysts may wait for conditions that favor their transformation into tertiary generations of zoospores that are also in the free-swimming stage. The encysting property of the *Aphanomyces* pathogen may play an importance role in the cycle of outbreaks in endemic areas. EUS causes disease and mortality in farmed and wild fish, worldwide. Around 94 species of fish have been confirmed by histological diagnosis to be naturally affected by EUS. Suspect cases of natural infection with *A. invadans* in species other than those listed should be referred immediately to the appropriate OIE Reference Laboratory, whether or not clinical signs are associated with the findings. Some fish, such as common carp (*Cyprinus capio*), Nile tilapia (*Oreochromis niloticus*) and milk fish (*Chanos chanos*), have been considered to be naturally resistant to EUS. (Fig. 2 & 3).



**Figure 2** EUS infected *C. striatus*



**Figure 3** EUS infected *C. punctatus*

### 2.2.1. Diagnosis

EUS can be readily detected in diseased fish specimens collected from EUS-infected areas using histological techniques. However, *Aphanomyces* can be isolated only from fish with mild or moderate clinical signs of EUS, exhibiting red spots or small ulcers. Fish with severe clinical signs or large ulcers are not suitable for isolation. Fish skeletal muscle is the target organ and exhibits major EUS clinical signs with mycotic granulomas.

### 2.2.2. Treatment

There is no effective treatment for EUS-infected fish in the wild and in aquaculture ponds. To minimise fish losses in infected fish ponds water exchange should be stopped and lime or hydrated lime and / or salt should be applied. Attempts at using green water, ash, lime, and neem seeds or branches (*Azadirachta indica*) for prophylactic treatments of the EUS-infected fish in fish ponds gave variable results (Inland Aquatic Animal Health Research Institute, Thailand, internal report, 2001). In outbreaks occurring in small, closed water bodies or fish ponds, liming water with agricultural limes and improving water quality, together with removal of infected fish, is often effective in reducing mortalities and controlling the disease. Ensuring no leakage of water from EUS-infected areas into fish ponds is a normal practice that easily prevents the spread of EUS into farms. Sodium chloride or salt and agricultural lime are safe and effective chemicals for treating or preventing the spread of EUS.

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## 3. Conclusion

Fungal diseases are often indicative of a more serious problem. Saprolegniasis is a common fungal disease which affects the external surfaces of fish. It can be eliminated easily after the primary cause of illness has been identified and corrected. On the other hand, Branchiomycosis has caused high mortalities in cultured fish, and is difficult to control. EUS causes disease and mortality in farmed and wild fish, worldwide, especially in the topical areas. The best control for all fungal infections is good management good water quality, good nutrition and proper handling.

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## Compliance with ethical standards

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