Fish Fungal Diseases

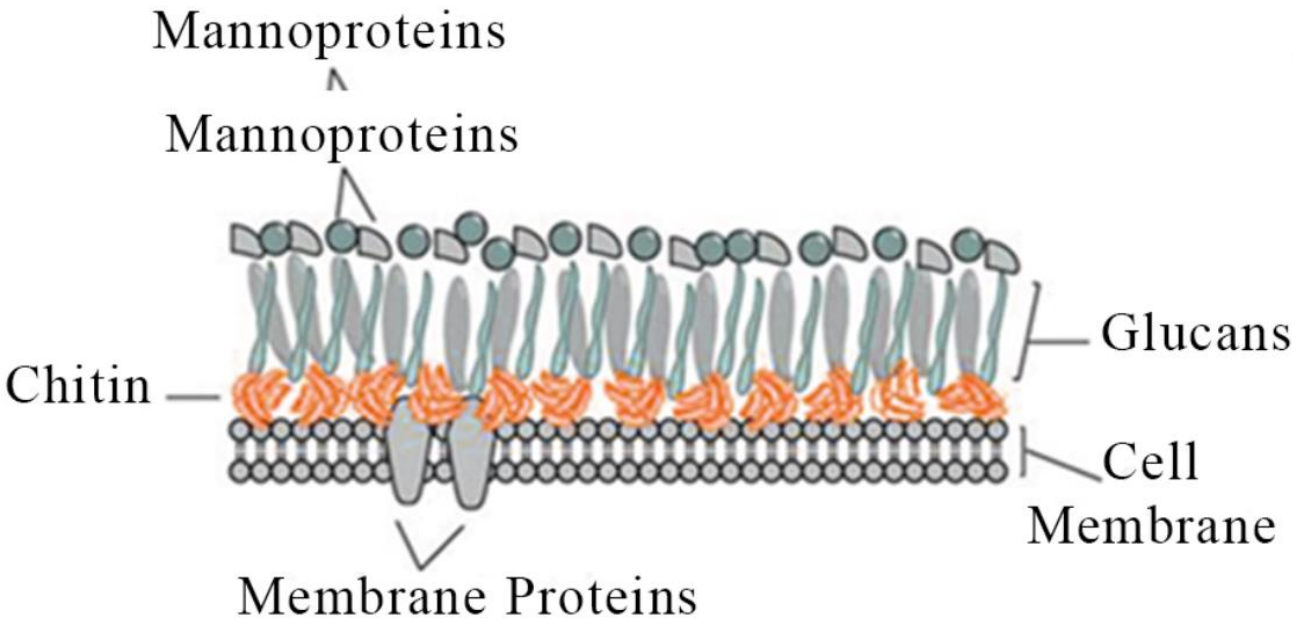
- As other vertebrates, aquatic animals are susceptible to a variety of pathogenic organisms, including fungi.
- **Fungal Infections** (are called **mycoses**) are among the most common diseases seen in temperate fish. Because fungal spores are found in all fish ponds and create problems in stressed fish.
- **In fact,** the first clinical report of a fungus infecting a vertebrate was of a **fish**.
- **The ability of aquatic fungi to cause diseases** is encountered in eggs, fry, fingerling and adult fishes.
- **fungi can become a problem** if fish are stressed by disease, by poor environmental conditions, receive poor nutrition, or are injured. If these factors weaken the fish or damage its tissue, fungus can infect the fish.
- They are primarily regarded as a **secondary invader** to some other factor or pathogen, a consequence of water quality problems, poor condition, trauma (rough handling or aggression), bacterial disease, or parasite and are known to attack the host when it gets injured either mechanically or as a result of infection other than fungi.

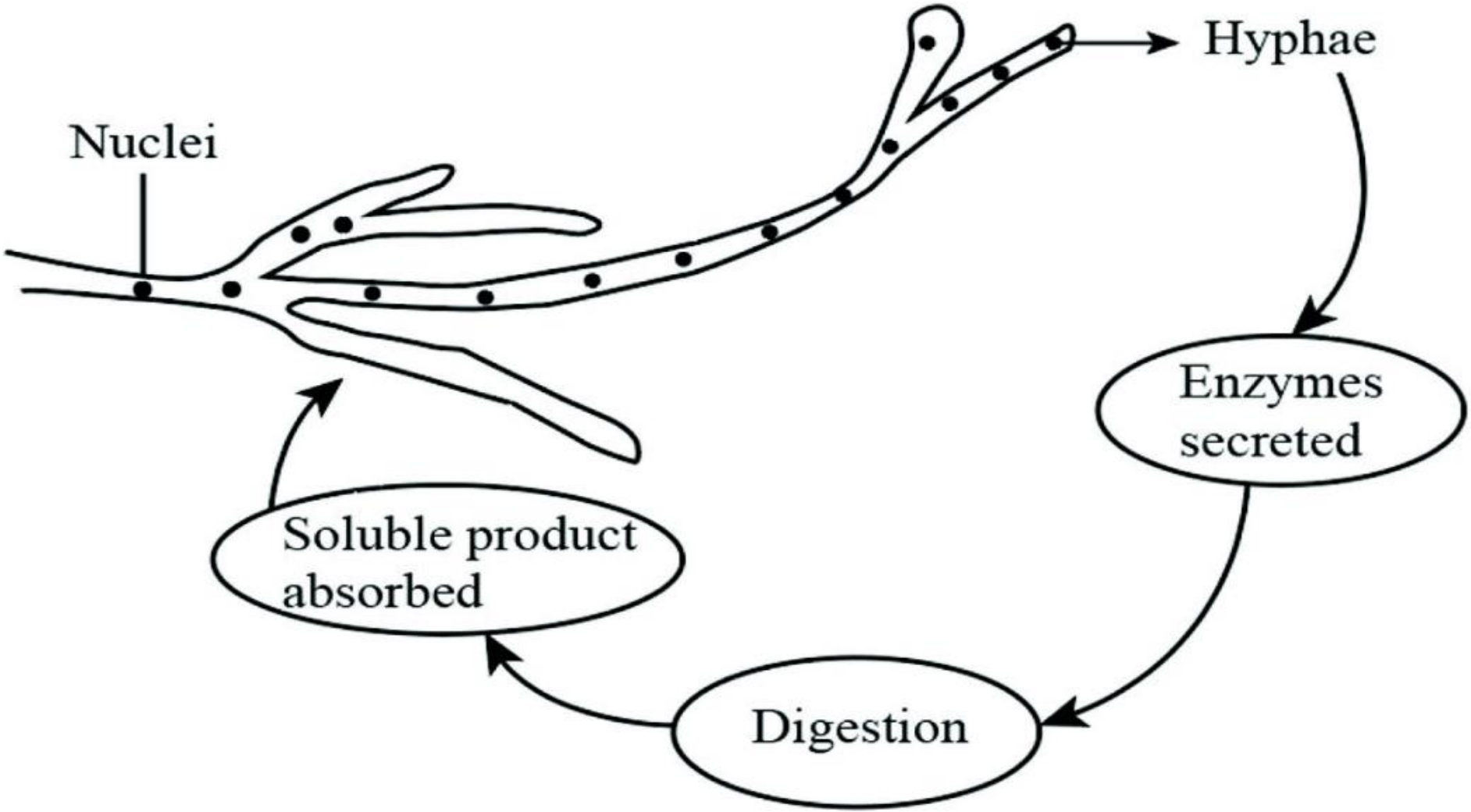
- **Fungi** are a group of organisms called **heterotrophs** that require living or dead matter for growth and reproduction. Unlike plants, they are incapable of manufacturing their own nutrients by photosynthesis.
- Fungi are member of the kingdom **Thallophyta**.
- Fungi are organisms containing no chlorophyll (**colorless**), and have **eukaryotic cell** (that can be unicellular or multicellular), but most fungi are **eukaryotic** and **multicellular**.
- Fungal colonies have a **filamentous** shape.
- **Nuclei** within the fungal **hyphae** are **diploid**. Their cell wall is comprised of **cellulose** and **glycans**, and **not chitin** and reserve food (**Glycogen**).
- **Majority** of the aquatic fungi are **saprophytic** and derive their nourishment by decomposing organic matter and could be at a **parasitic** forms have evolved and can cause significant disease in fish.

- Fungi assimilate nutrients by means of **extracellular digestion** (by excreting digestive enzymes into their environment) and **absorption of resultant simple biomolecules** through the cell wall and membrane.

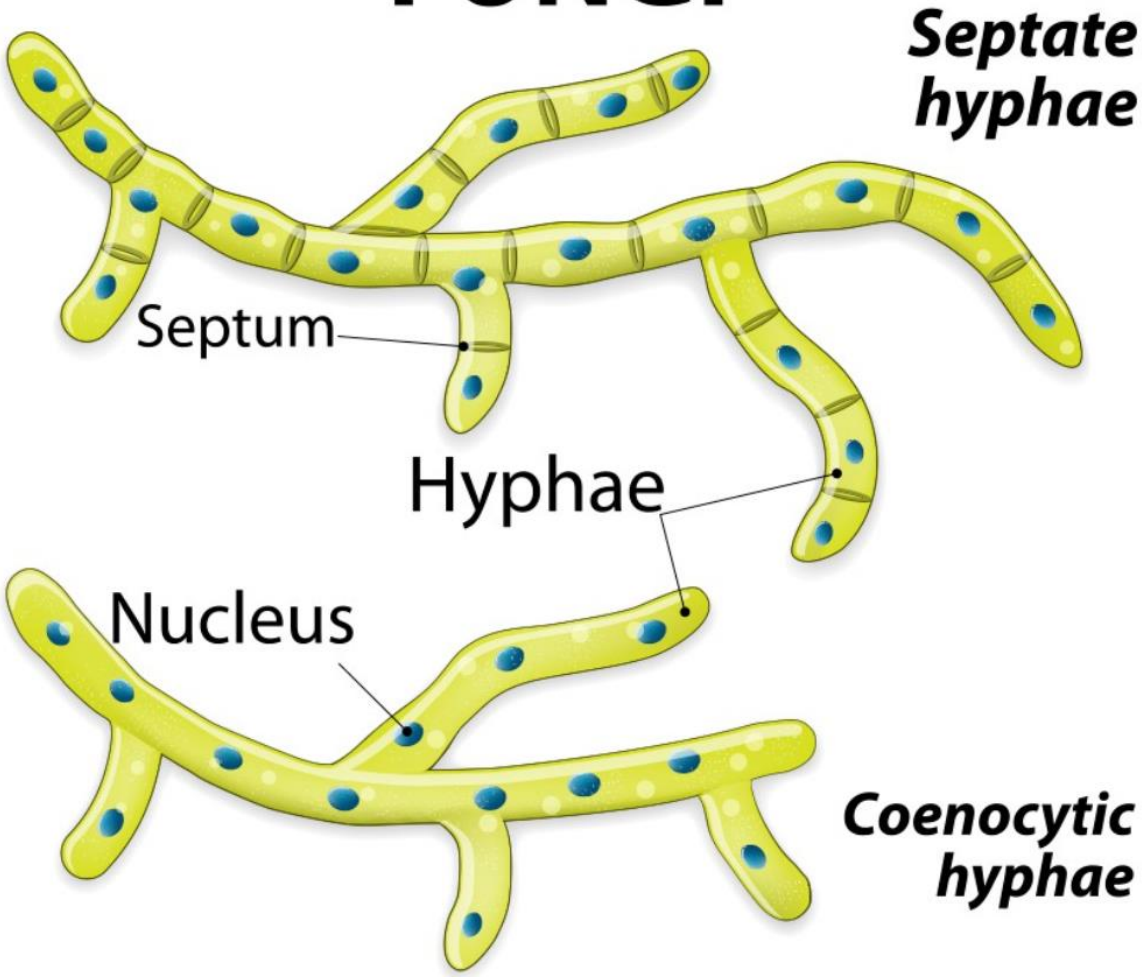
- All fungi produce **spores** which readily spread disease.
- Most fungi reproduce **sexually** and **asexually** by spores.
- **The fungal spore** is like a seed which is resistant to heat, drying, disinfectants and the natural defense systems of fish.
- Fungi can cause problems during **reproduction**, for example, by infecting fertilized **eggs** in spawns.
- Certain species of fungi can grow in **poorly stored feeds** and produce **mycotoxins**.

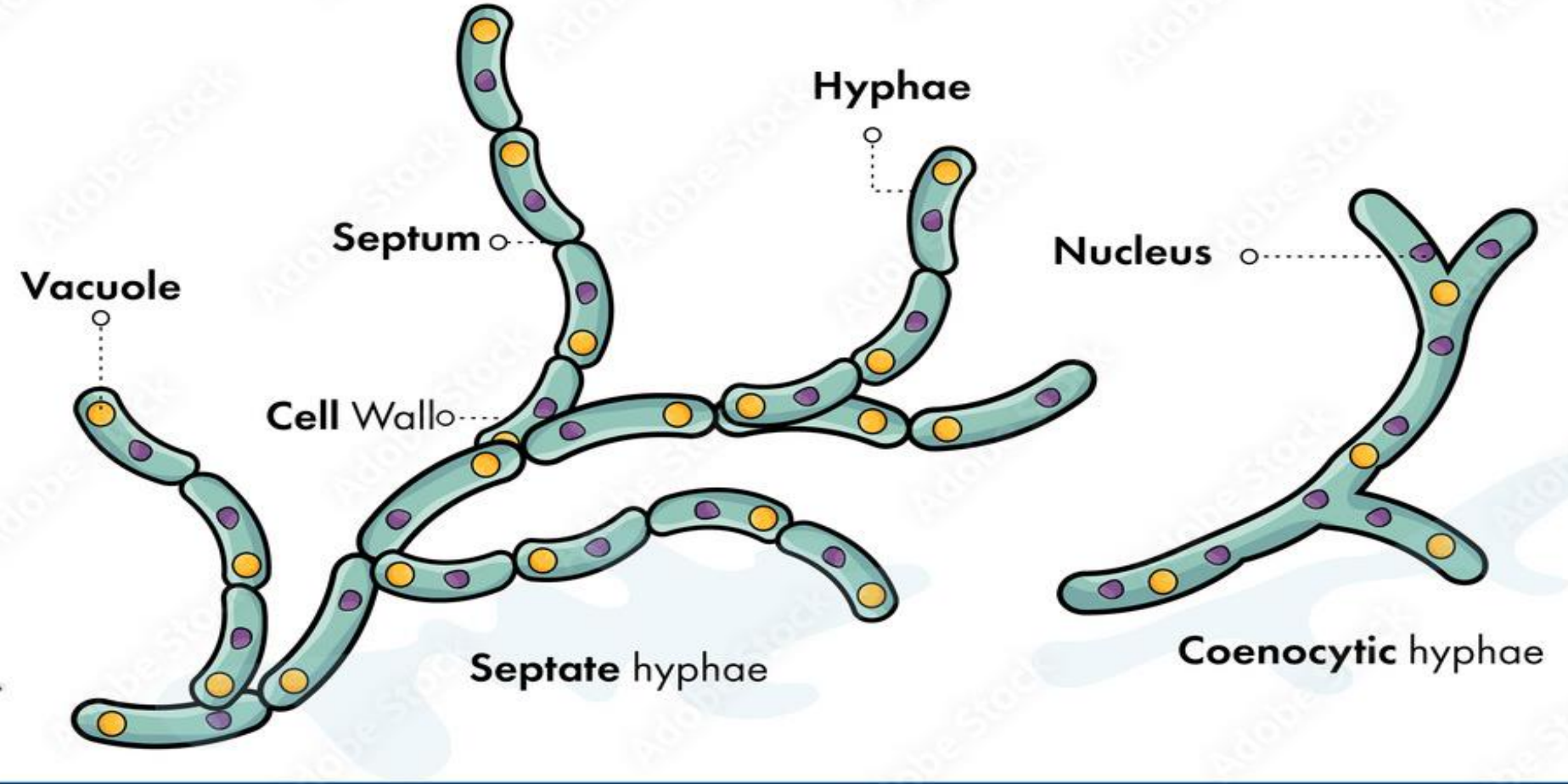
Fungal Cell Wall




Hyphae
Nuclei
Enzymes secreted
Soluble product absorbed
Digestion

FUNGI




Hyphae
Septum
Vacuole
Cell Wall
Septate hyphae
Nucleus
Coenocytic hyphae

Fungi

Surface Fungi Ecto

Localized **Saprolegnia**

Systemic Fungi Endo

Branchiomycosis
Ichthyophonous hofri
Aspergillus flavus
Fusarium
Scolecobasedium humicola
Phoma herbarum

Fungal Taxonomy

- **The “groups” of fungi can be divided over several different kingdoms.**

- **Fungi** that have been reported to cause clinical disease in fish are primarily from the kingdoms **Chromista**, **Protoctista**, and **Eumycota (true fungi)**.

- **The Oomycetes**, previously placed in the kingdom **Protoctista,** now are considered members of a new kingdom, **Chromista (Stramenopiles),** which includes **diatoms, kelps,** and **brown algae.**

- **Another new taxonomic group,** the class **Mesomycetozoea,** in the kingdom **Protoctista (Protista),** comprises organisms at the animal/fungal evolutionary boundary.

- **The third major kingdom, the true fungi (Eumycota), i**ncludes a large number of species pathogenic to fish, some increasing in reported incidence.
- 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.

- **Four orders** are recognized in this class and the most important are the **Saprolegniales**. Although eight genera have been reported in naturally or artificially induced infections, Saprolegnia, Achlya, Aphanomyces, Calyptratheca, Thraustotheca, Leptolegnia, Pythiopsis and Leptomitus, only **Saprolegnia**, **Achlya** and **Aphanomyces** are significant in **aquaculture**.

Non-Taxonomic groupings

- **The Mitosporic Fungi** (formerly Fungi **Imperfecti** / **Deuteromycota**) are a large group of organisms that traditionally has been considered fungi, but is not strictly a taxonomic grouping.
- **Members of this group have either lost the ability to reproduce sexually**, or have not been observed with sexual forms (sexual reproduction is considered perfect).
- **Some fungi that do not have known sexual forms**, known as **anamorphs**, are placed into specific groups primarily based on **anatomic morphology**. To add even more confusion, however, for some previously described anamorphic species, **sexual forms** known as teleomorphs have been identified.
- These sexual forms frequently are given a different genus.
- **For those species with both known sexual and asexual forms, the following relationship holds:**
- **Holomorph** (whole fungus) = **Anamorph** (asexual form) + **Teleomorph** (sexual form).

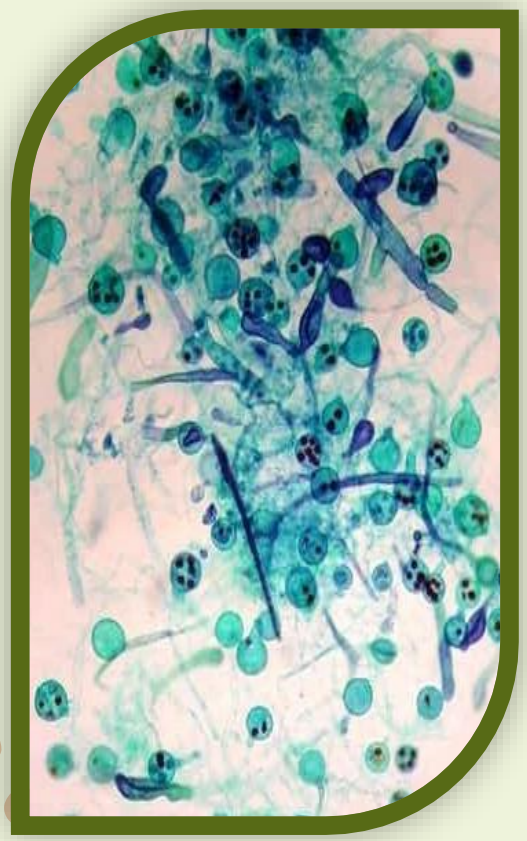
The Possibility of Fungal Infections

- **Increased Temperature** which is important for **growth** and **multiplication** and **development** of **fungi** (Except: **Saprolegnia** which grow at **low temperature**).
- Prolonged exposure to **low water temperatures**
- **Using of fertilizers** by high amounts in fish farming where this materials increase the organic matter which is a good media for growth of most fungi.
- **Absence of filtration system in fish culture** that leads to passing of high numbers of different kinds of fungal spores.
- **Poor quality of water** and **poor hygiene system** in the farm.
- Presence of large amount of **dead fish** and **organic materials** in the pond.
- Fish that are injured have other **diseases** or **microbial infections**.
- **Mechanical Injury** after handling.
- Exposure to **extreme pH levels**.
- Excessive use of **chemotherapeutants**.

Classification of Fungi

according to reproduction and morphology

Un-certain Class	Class fungi : Imperfecti	Class Oomycetes or Phycomycetes
Un-Known Reproduction	**Asexual** Reproduction only present. Abssence of sexual type	Both **sexual** and **asexual** reproduction present
Un-differentiated hyphae	**Septataed** hyphae	**Non-Septataed** hyphae
Included: **Branchiomycosis** Mycophlus	Included: Phoma herbarum Scolecobasidium humicola **Aspergillus flavus** **Candida albicans** Fusarium	Included: **Saprolegnia** Achlya Aphanomyces Dectychus F. Dermocystidium Ichthyophonous hoferi



Saprolegniasis

- Cotton-wool like disease
- Winter-kill disease
- Water mold
- Dermatomysis
- Dermatophytes of fish

Saprolegniasis

- One of the mycotic diseases in **fresh** and **brackish** water specially young stages (fingerlings or juveniles), cultured and aquarium fishes, **eggs** (problems in hatcheries) but not in the marine fishes (inhibiting its growth and killing its spores).
- Cured by adding **Nacl** to increase salinity and killing the spores so don't found in **marine water**.
- Characterized by **fluffy white** (in water) to **greyish** (out water)
- **cotton-like growth** on the skin, gills and fins.
- **High morbidity** and **mortality** among young fish.
- **Local ulceration** and **subdermal necrosis** in advanced cases.
- Members of the family: **Saprolegniaceae** (Saprolegnia sp.).

Saprolegniasis

- They are **opportunistic** saprobes (if the condition are not suitable for it to attack the fish, it bound itself by the flagella forming its own cyst until occurring changing (lowering) in the temperature or the environmental condition are not good for the fish it get out its flagella and attack the fish), spread in **freshwater** & **brackish** water and salinities over **2.8 %** limited its distribution.

- They are characterized by long branched **non-septated hyphae** with many zoosporangia

- **Zoosporangia** are long, slender, double walled sacs slightly larger in diameter than the hyphae to which they are attached contain the spores that cause infection to the host.

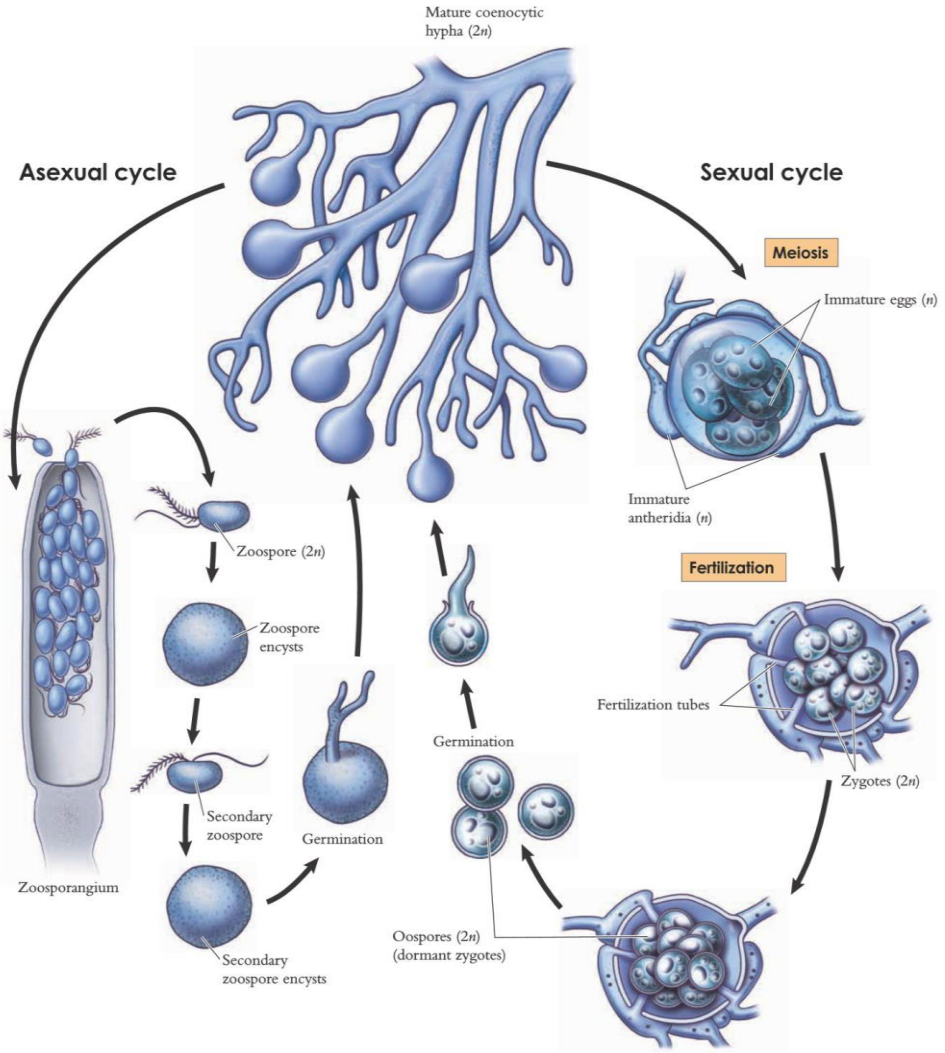
Saprolegniasis

1- Asexual reproduction:

- The zoosporangium get out the **primary zoospores** which are (motil pear shaped with 2 flagella at the apex which encyst its self with their flagella if the condition is not suitable for its growth) then grow into motile **secondary zoospores** (kidney or bean shaped with 2 flagella) and all their end there is **hyphae**.
- if the condition is **suitable** for its growth then grow into hyphaewhich develop into mycelium.
- If the condition is **not suitable**, its flagella round around itself and keep its body within the cyst that formed until the condition is suitable and so get out and continue its life cycle.

2- Sexual reproduction:

- **Antheridium** (male) and **oogonium** (female) under certain condition give oospore within the fertilization tube which germinate into **oospores** and into hyphae which develop into new **mycelium**.


Mature coenocytic hypha (2n)
Asexual cycle
Sexual cycle
Meiosis
Immature eggs (n)
Immature antheridia (n)
Fertilization
Fertilization tubes
Zygotes (2n)
Zoospore (2n)
Zoospore encysts
Secondary zoospore
Germination
Zoosporangium
Germination
Oospores (2n) (dormant zygotes)
Secondary zoospore encysts

Susceptibility and transmission

- Saprolegniasis is **world-wide** in distribution, It is **seasonal** disease, where it spreads in **winter** but also may affect fish and eggs specially in spring and autumn.
- **The optimum temperature** for disease outbreaks is between **15-18 C°**.
- Both **wild** & **cultured** & **dead fish** & **eggs** fish are susceptible.
- **Freshwater** and **brackish** water fishes are susceptible while **marine** are **not** (carps, goldfish).
- **Dead fish & eggs** act as fertile media for fungus growth & production of **zoospores** which increase the possibility of invasion of other living fish or eggs.
- Disease is **secondary** and transmitted by **direct contact between fish or eggs**.

Pathogenesis

- **Saprolegnia** is **not tissue specific**, invading any damaged tissue.
- Damage done by **necrotizing action** of their **proteolytic enzymes** which is secreted for damaging any attacked tissue, but they do not produce any toxin so its effect is **localized**.
- **Saprolegnia** establish themselves firstly on **dead tissue or eggs**, then spread rapidly to **viable ones** causing **suffocation** to them and finally **died**.
- **Zoospores** are attracted toward **viable eggs** due to presence of **chemotactic factors** (chorionic membrane of the live eggs).

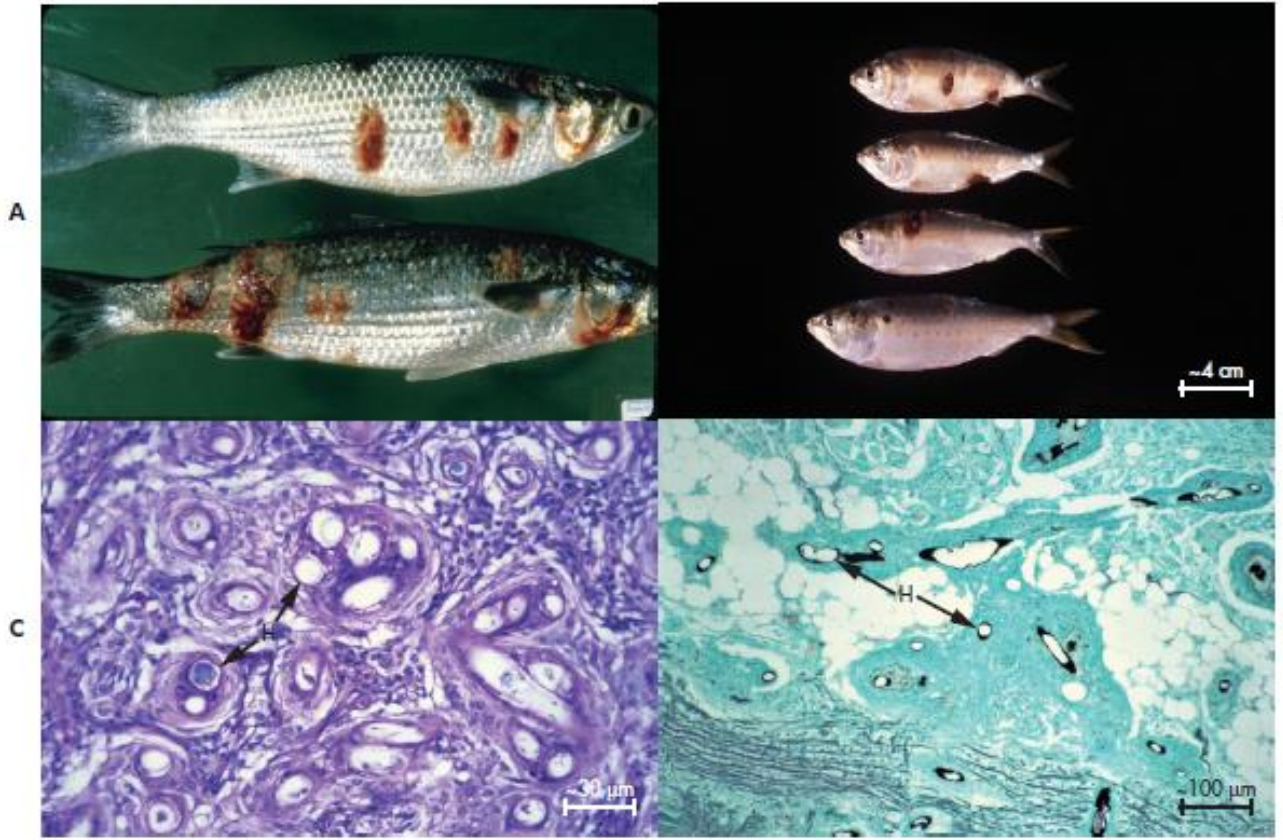


Fig. II-35. A. Relatively early, atypical water mold infection on grey mullet from the Clarence River, Australia. B. Advanced atypical water mold infection on Atlantic menhaden from Pamlico River, United States. C. Histological section of an atypical water mold infection showing chronic inflammatory response to broad, aseptate hyphae (*H*). Hematoxylin and eosin. D. Silver stain of atypical water mold lesion. *H* = hyphae. Gomori methenamine silver. (*A* photograph courtesy of R. Callinan.)

Predisposing factors

Rapid decrease in water temperature is an essential factor for saprolgenia infection as it causes.

A-Lack of an inflammatory response i.e lack of leucocytic infiltration.

B-Increase in cortisol level causing immunosuppression.

C-Increase in cytotoxic factor secreted by the fungus.

Integument injury facilitates saprolegnian infection

A- Destruction of the epithelium which is physical barrier

B-Loss of goblet cells leading to decrease in mucification which is chemical barrier as mucus has fungistatic action.

Other stress by poor water quality, bad hygiene, infection by bacteria or parasites or virus increase fish susceptibility to the disease.

Clinical signs

- **White to brown cotton wool like growths** on the external surface of the fish body or eggs which is prominent in water but after getting out, fungal growth adhere to fish body and cannot be detected (detected as **ulcerations**).
- **Superficial skin erosions** or **deep ulceration** with **extending fungal infection** to the underlying musculature or organs may occur.
- The infected fish are **lethargic** with **loss of equilibrium**.
- **Respiratory disorders** seen when fungal infection extends to gills.
- **Osmotic imbalance** with subsequent haemodilution & death.
- The **inflammatory signs** around saprolegnian lesions.

Figure 4-3. Mycelial filaments of *Saprolegnia* sp. on the gills of red drum with saprolegniasis (fresh mount, 100x)

Fig. 1. (*A*) African cichlid with Saprolegniasis. Note dark areas (hemorrhages/ulcers) at edges of fungal growth. (*B*) Life cycle of water molds (*From* Noga EJ. Fungal and algal diseases of temperate freshwater and estuarine fishes. In: Stoskopf MK, editor. Fish medicine. Philadelphia: WB Saunders; 1993. p. 278–83; with permission).

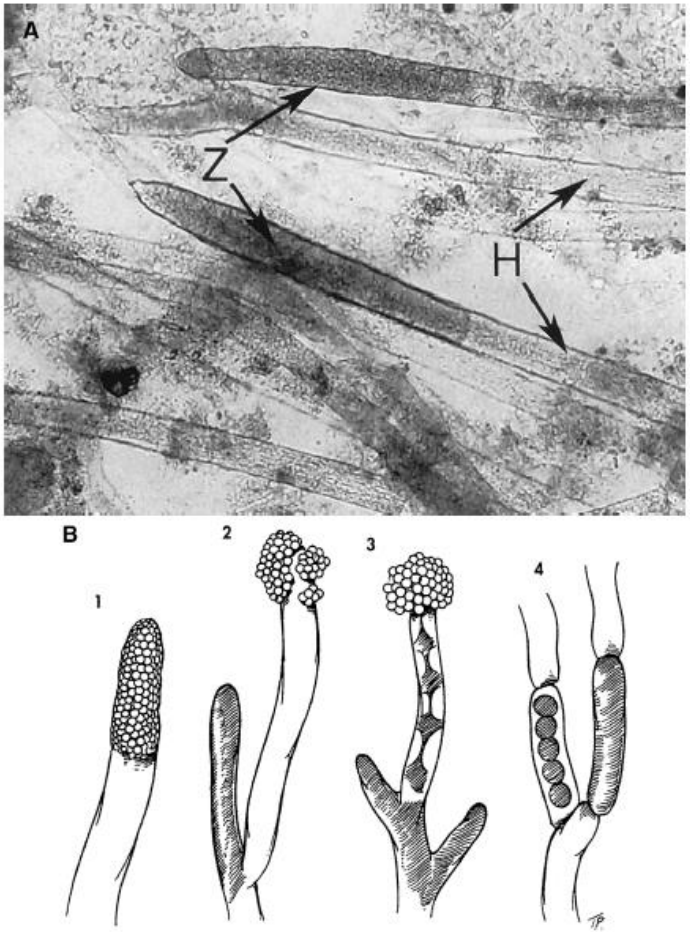


Fig. 2. (*A*) *Saprolegnia* species, wet mount. (100×) H, hyphae; Z, zoosporangia. (*B*) Zoosporangia of some fish pathogenic Oomycetes. 1, Saprolegnia; 2, Achlya; 3, Aphanomyces; 4, Leptolegnia (*From* Noga EJ. Fungal and algal diseases of temperate freshwater and estuarine fishes. In: Stoskopf MK, editor. Fish medicine. Philadelphia: WB Saunders; 1993. p. 278–83; with permission.)

Diagnosis

- **Confiramatory diagniosis:**
- **A-Microscopical examination** of a fresh squash preparations or lacto phenol cotton blue or methylene blue stained squashes from infected tissues show as Long branched non septated hyphae with many zoosporangia are detected on using dark field or phase contrast microscopy.
- **B-Isolation:** Saprolegnia species can be isolated by:
- Culturing on SDA (sabouraudś dextrose agar media) at 15-18 C° for 3-5 days and take swab and stained by lactophenole cotton blue (low nutrient, antibiotic- supplemented media).
- Peptone glucose yeast extract agar to reduce growth of saprophytic species.
- **C- Identification:**
- IT is not practical as no need for identification as control of all fungus species is same.

Prevention and Control

- The most successful strategy for the prevention of saprolegniosis in farmed fish is **“husbandry practices”** to avoid physical injury during decrease in temperature (Good Managment).

- During spawning season, **sexually mature fish** need special care.

- For eggs, use of an **elevated water flow** to **roll eggs** is an **effective** method for saprolegiosis prevention.

Several chemicals as a prophylactic treatment can be used

- **A. In infected fish:**
- a-Malachite green:-
- b-Sodium chloride: (if available, sea water flush into aquaculture facility)
- c-Formalin solution:
- d-Hydrogen peroxide & buffered iodophores:
- e-Potassium permanganate:
- f-Acetic acid:
- Best treatment: (mixture of **Malachite** green and **formalin**)
- **B. In infected fish eggs:**
- removing dead eggs at regular periods of time during incubation (in order not to occurring suffocation to the healthy eggs from the growing parts of the hyphae from the dead tissue so it dies and the disease spreads rapidly so removing dead eggs preventing this from being happened).
- Then flowing **malachite green** as a **fungicide** as a **flushing treatment**.

Common chemicals used as chemotherapeutants include bath treatments

• **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: treatment can result in gill damage; more toxic at warm temperatures.

Malachite green is considered as the most popular antimycotic 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.

Ichthyophonosis

- Swinging disease
- Zygomycotina
- Ichthyosporidiosis
- Ichthyophonus infection

Ichthyophonosis

- It is a **chronic** systemic (**internal**) fungal disease affecting all fish species with **no external signs** but in extreme infection, affected **scale less fish** show **rough** or **granulomatous skin** (**sand-paper effect**) with **lordiosis** & **scoliosis**.
- Usually seen as **thick**, **fungus-like resting spores**.
- **Swinging** and **chronic mortality** in some endemic **cold water areas**.
- **Ichthyophonus hoferi** fungus an **obligate pathogen**.
- The most commonly observed stage of the fungus is **the resting spores** (latent cyst- Quiescent cysts).
- Mostly, they present in **muscle**, **heart**, **liver**, **kidney** & **spleen** and **discharge with its excreta**.
- **Transmission:** orally
- Produce large numbers of **endospores** and **resting spores** in most internal organs.
- **infective stage:**
- amoeboblasts

Life Cycle

- The lifecycle of Ichthyophonus hojeri is complicated, involving **production** of **multinucleated spores**, which produce **endospores**. Hyphae may not be produced before endospore formation.

- Endospores are disseminated to new hosts or to other parts of same host. **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.

- **Spores** are released when **hyphae** rupture the surface of the gut or penetrate the skin to allow infection to spread via the water.

Ichthyophonosis

- **Susceptibility**:
- The disease is **worldwide** in distribution.
- It affects **all fish species** (Groupers, trouts, flounders, herrings and cods).
- It affects mainly **cold**, **wild marine fishes**, but also **cultured fishes** can be infected on feeding **raw marine fishes**.

- **Predisposing factors:**
- It mostly spread in **cold season (3-20◦c)**
- **Overcrowding** increase disease incidence.
- Presence of large amount of **organic matter** and **water blooms**.
- **Nutritional deficiencies** and **malnutrition**.
- **Injuries of the gills** either by **trauma** or **ectoparasites**.

Clinical signs

1- Slight to moderate infection

- No clinical signs.

2- Extreme infection:

- The clinical signs differ according to the affected organ:
- In case of **skin affection** in scale less fish e.g. **herring**:

  Sandpaper texture (rough or granulomatous skin) in the advanced stages.
- In case of **liver affection:**

  Ascites Exophthalmia Erected scales
- In case of **muscle affection:**

  scoliosis (lateral bending of the body) lordiosis (dorsoventrally bending of the body),

  this deformation can be severe and interfere with swimming ability.
- In case of brain or **spinal cord affection:**

  abnormal swimming or spiral swimming (swinging disease)

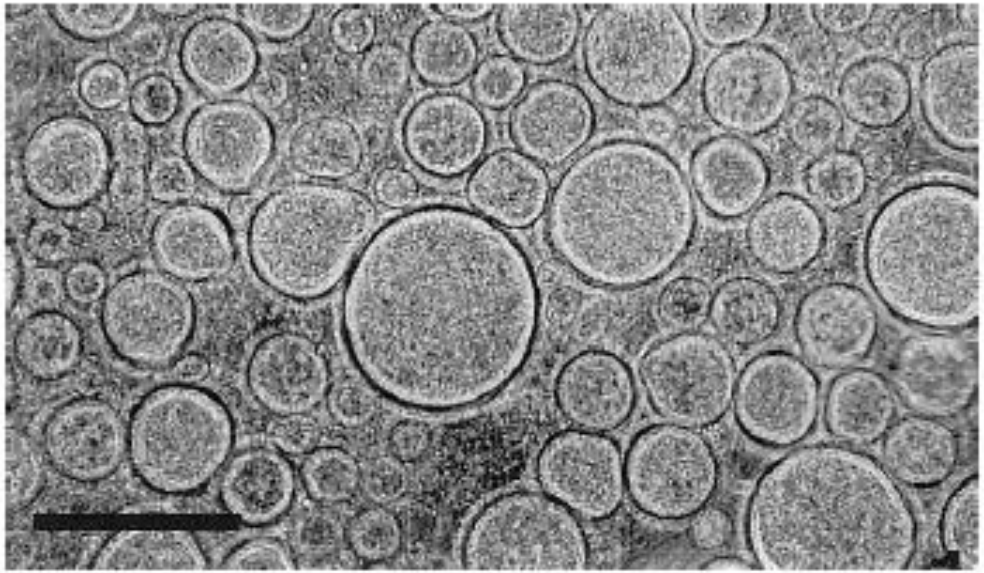


Fig. 7. Squash preparation of plaice liver infected with *Ichthyophonus*. Scale bar = 100 μm. (*From* Woo PTK, Bruno DW, editors. Fish diseases and disorders. Volume 3. Viral, bacterial and fungal infections. New York: CABI Publishing; 1999; with permission.)

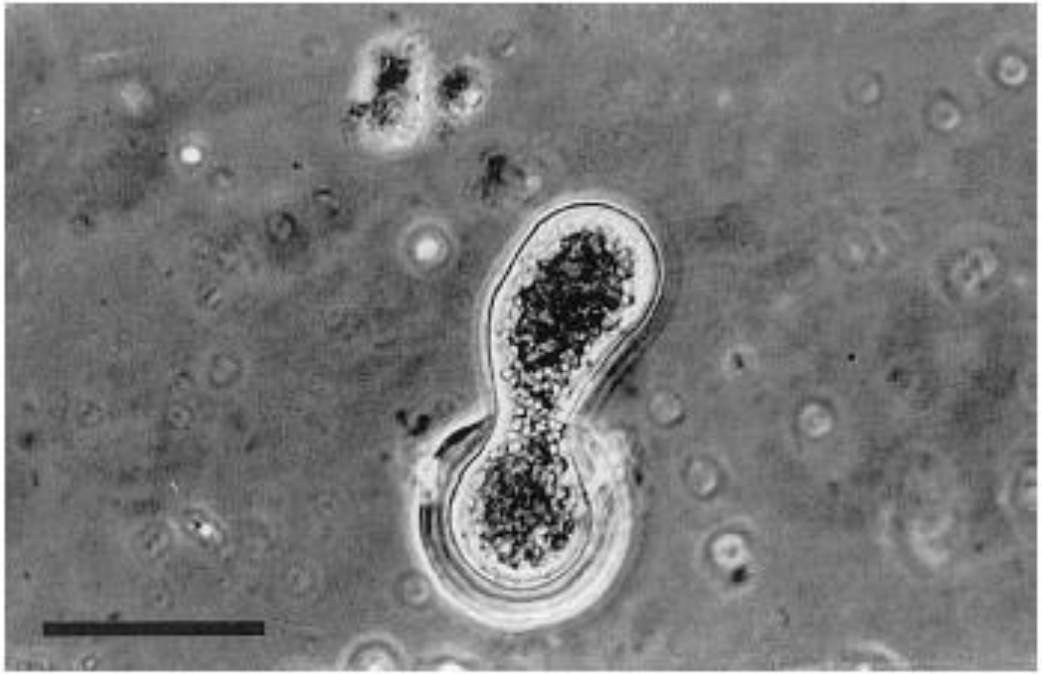


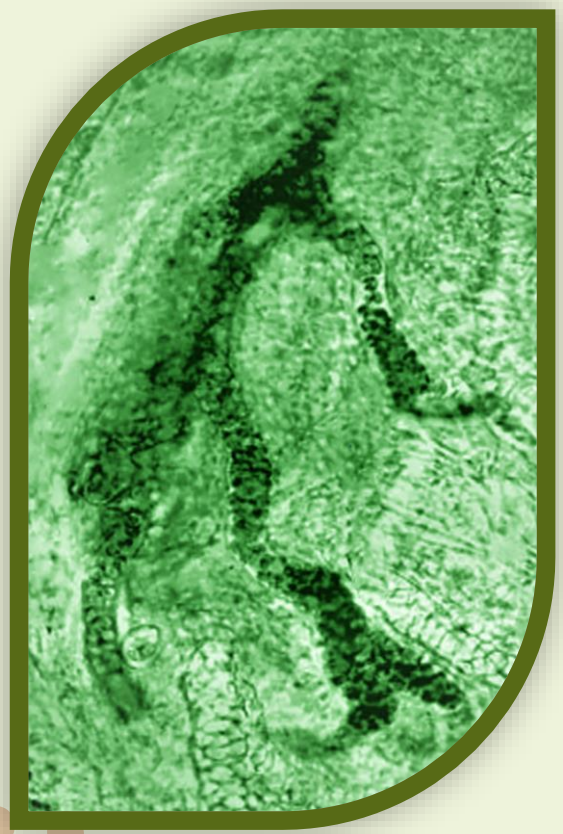
Fig. 8. Germination of *Ichthyophonus* spherical bodies in the intestine of an experimentally challenged rainbow trout 4 to 6 hours after feeding. Scale bar = 100 µm. (*From* Woo PTK, Bruno DW, editors. Fish diseases and disorders. Volume 3. Viral, bacterial and fungal infections. New York: CABI Publishing; 1999; with permission.)

Diagnosis

- Ichthyophonus can often be identified from **fresh lesion material**.
- 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 **icytoplasmic 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 diameter**.
- **Ichthyophonus** has a double wall, which stains **PAS positive**, and is also **positive** to **silver stains** such as **Grocott's methanamine silver**.
- Culture from lesions can be established using **Sabouraud dextrose agar** with **1% serum**.

Control and Treatment

Disease transmitted **orally**; obviously, don't feed **infected fish** to fish (as some people feed raw marine fish offal to hatchery fish).



Branchiomycosis

- Gill rot
- Bad management disease
- Environmental gill disease

Branchiomycosis

- **Definition**:
- It is **acute**, **sub-acute** or **chronic** fungal disease of **freshwater** fish which cultured under unhygienic conditions **(Bad Management)** especially in **hot season (25-30 °c).**

- **SPECIES AFFECTED:**
- Carps, goldfish, eels.

- Characterized by **marbeling appearance** of the **gills**, **gill necrosis**, **respiratory distress**.

- High **morbidities (100%),** high **mortality (30-50%).**

- It is an environmental problem because disease induction & severity is closely related with bad environmental conditions.

Branchiomycosis

- **Etiology:**
- Two species from genus Branchiomyces associated with the disease:
- **Branchiomyces sanguinis**
- **Branchiomyces demigrans**
- They need oxygen from the gills to continue their life cycle.

- The causative fungui are **opportunistic facultative microbs** which need oxygen tension for their growth.
- They have **branched non-septated hyphae** with **spores**.
- The caustive species can be differentiated by differences in **morphology** and **growth habits**.

	Branchiomyces sanguinis	Branchiomyces demigrans
Predilection seat	Capillaries of gill filament & lamellae	Parenchyml tissues of gills (epith cells of gill lamellae)
Hyphal wall	Thinner (0.2 micrometer)	Thicker (0.5-0.7 micrometer)
Spores diameter	Smaller (5-9 micrometer)	Larger (12-17 micrometer)
Spores released into	Capillaries of gill filament & lamellae	Enviromental water
Growth temperature	15-35 °c , optimum 25-30°c	
oxygen tension required	High	low

Branchiomycosis

- **Sources of infection:**
- Infected fish.
- Water & pond detritus bottom carrying spores liberated from necrotic gill tissue.
- Carrier (chronic cases).
- Raw fish products.

Predisposing factors

1- Accumulation of organic matter

as in overfeeding, eutrophication, overcrowding, over fertilization enhance the growth of the fungus and the spore population in pond.

2- Acidic pH (5.8 - 6.5)

3- Do as with algal blooms, reducing in water flow (low oxygen)

4- Increasing water temp (25-30)

- under suitable temp 2-4 days.
- under unsuitable temp 14 days.

Mode of infection

- **Directly:**

• through penetration of the fungal elements (spors, hyphae or both) the gill epithelium and locate into the branchial blood vessels or tissue according to the fungus species.

- **Indirectly:**

• through ingestion of the spores go to the blood stream and to gill as their final target and germinate at the gill blood vessels or gills tissue according to the fungus species.

Pathogenesis

spores liberated from **necrotic gill tissue**

↓

germinate and produce **hyphae**

↓

penetrate **gill epithelium** and locate in the gill epithelium **(B. demigrans)**,

or

within **gill capillaries (B. sanguinis),** according to its oxygen demand

↓

Occur **fungal growth** to the **hyphae** so occur **blockage of the brancial capillaries.**

(partial then complete)

B. sanguinis

Inside capillaries

B. demigrans

Outside capillaries

Pathogenesis

homeostasis and **thrombosis** (blockage of capillarie)

↓

necrosis of gill tissue

↓

marbling appearance

↓

necrotic tissues are **sloughed** & **spores** released into the water, then:

↓

infect gill **directly** or remain **suspended** in water or fall to the **bottom**

↓

fish die with characteristic condition of **asphyxia**

Clinical signs

- **Acute case:**
- observed in warm months with **rapid** onset and **fatal termination**.
- affected fish appear **weak**, **lose their appetite**, **lethargic**, **can't tolerate handling** with **respiratory distress** (gasping at the water surface & collected at the water inlet).

- **In sub-acute cases**:
- the same clinical signs appear, but with less severity.

- **Chronic cases:**
- No disease signs appear.

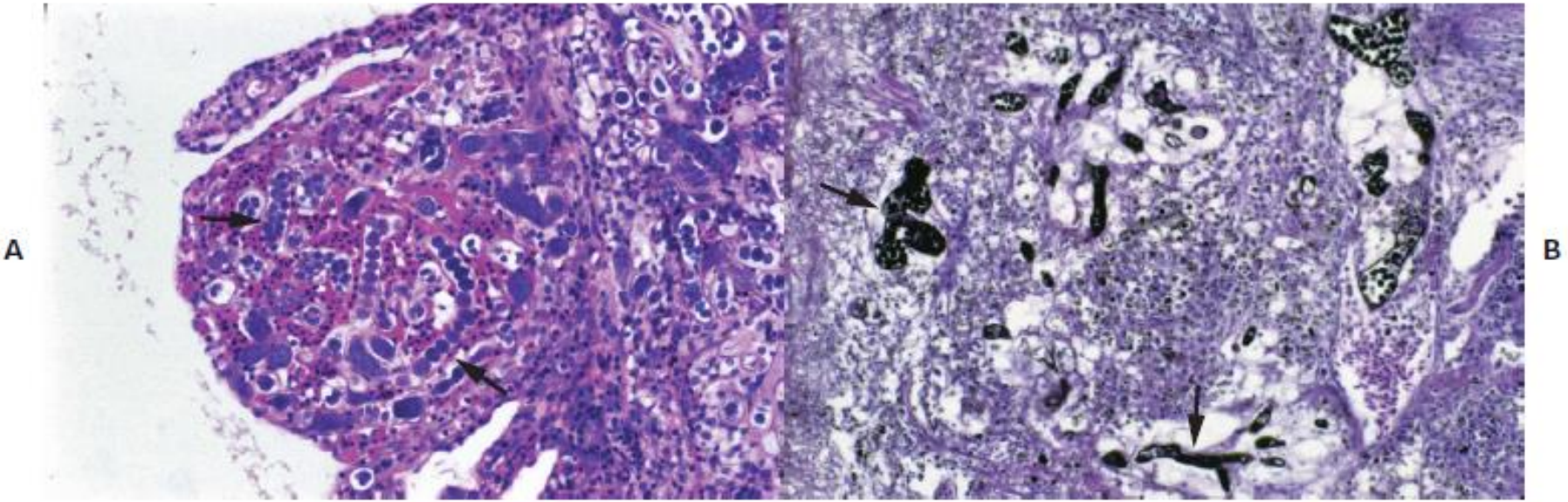


Fig. II-36. A. Histological section through *Branchiomyces*-infected gill. The key diagnostic feature is sporulating hyphae (*arrows*). Hematoxylin and eosin. B. Histological section through *Branchiomyces*-infected gill. The hyphae are black with silver-staining (*arrows*). Gomori methenamine silver.

Post mortem findings

- **Acute:**
- Gills may appear bright **red** from impaired circulation.

- **subacute cases:**
- **Marbling appearance** (**necrotic** patches with false membrane formation, & **ragged** corroded shape) of the gills together with **sloughing** of some **gill lamellae**.

- **Chronic cases:**
- **Pale** areas of the **gills** may or may not present.
- Slight to moderate **necrosis** of the **gill lamellae**.

Lab diagnosis (definitive diagnosis)

1- Microscopical examination of squash Preparation from gills detecting of branched non-septated hyphae with spores.

2- Isolation & identification

- Pieces of **gill tissue** → **Homogenization** → **formalin** addition → **Centrifugation** → Wash with **sterile saline** (**3**) times → Cultivation in **blood broth** and /or **blood agar** (within **pH 5.8-6.5** at **25-27 ° C** for **3-5 days**)
- Isolation on **sabauroud's dextrose agar** or **peptone dextrose agar** at **25°c** for **5-14 Days** (about 1 week).

- Colonies are thin **brown** & **pellicle like** .

3- Histopathology

Prevention and Control

- Depends upon avoidance of fish stressing with **good management** i.e. keep fish under adequate husbandry conditions.
- Depends upon **sanitation**, **disinfection** and **treatment**.
- **Dead fish** must be **collected, burned** or **buried**.
- **Increase water flow** to **decrease organic matter**, **decrease water temp.**, **increase DO** & **release of debris** & **necrotic tissue** .
- **Stop** feeding.
- the pond should be drained, dried-out and disinfected with quicklime.
- **Decrease** stock density.
- Treatment with **potassium permanganate** (give out nacent oxygen), **Nacl** , **formaline** or **malachite green**.
- **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%).

Epizootic Ulcerative Syndrome (EUS)

- Red spot disease (RSD).
- mycotic granulomatosis (MG).
- ulcerative mycosis (UM).

Epizootic Ulcerative Syndrome (EUS)

- **CAUSATIVE AGENTS**
- **Aphanomyces invadans** is associated with the disease outbreak together with **rhabdovirus** and the bacteria **Aeromonas hydrophila**. 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, guorami, goby, etc.).
- This agent is localized in **freshwater** aquaculture and also is seen in **river region** fishing.
- EUS occurs mostly at water **temperatures** of **18-22 ° C**.
- **The hypae** show **thick (> 7 µm)** , **multinucleated**, **non-segmented** and **branching**.
- **Susceptibility:**
- **Tilapia** (Orechromis niloticus) are considered relatively immune, whereas **snakehead** (Channa species) and some **barb** species are considered very susceptible, **goldfish** were much more susceptible, **common carp** showed no clinical signs.

Epizootic Ulcerative Syndrome (EUS)

- **A. Invadans** has an **aseptate fungal-like mycelia structure**. This oomycete has **two** typical **zoospore** forms.
- **The primary zoospore** consists of **round cells** that develop inside the sporangium. It is released to the tip of the sporangium where it forms a **spore cluster** transforming 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**.
- **Transmission:** horizontally.

Clinical Signs

- **darker** discoloration
- **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** throughout the body, (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).
- 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 accompanied by severe **tissue necrosis** and minimal
- **inflammatory response** can be observed.
- **Fungal hyphae** may reach the **cranium**, **kidney**, and **spinal cord**.

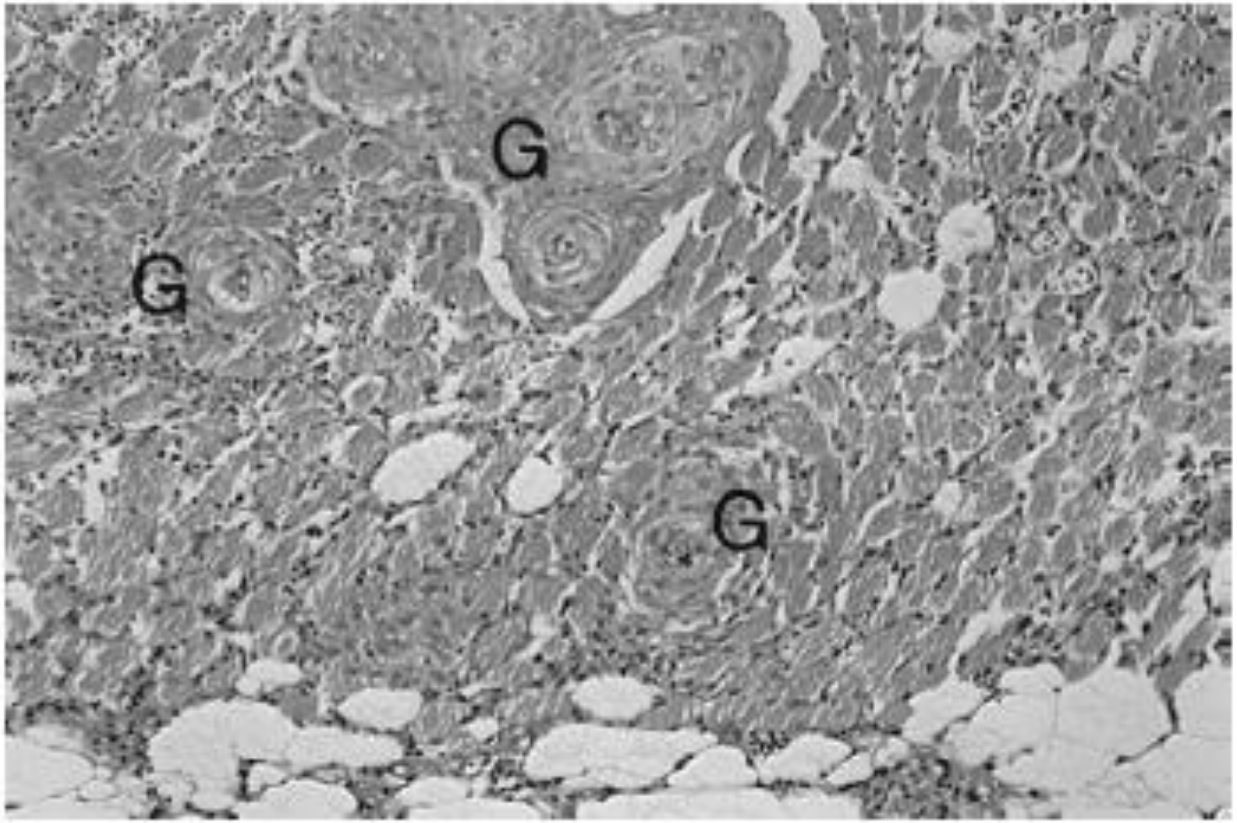


Fig. 4. H & E stained section. Granulomatous inflammation with necrosis of muscle. G, Granuloma. (Courtesy of Christine Densmore, National Fish Health Research Laboratory, US Geological Survey, Kearneysville, WV.)

Fig. 3. Atlantic menhaden (*Brevoortia tyrannus*) with deep ulceration caused by *Aphanyomyces invadans*. (*Courtesy of* Christine Densmore, National Fish Health Research Laboratory, US Geological Survey, Kearneysville, WV.)

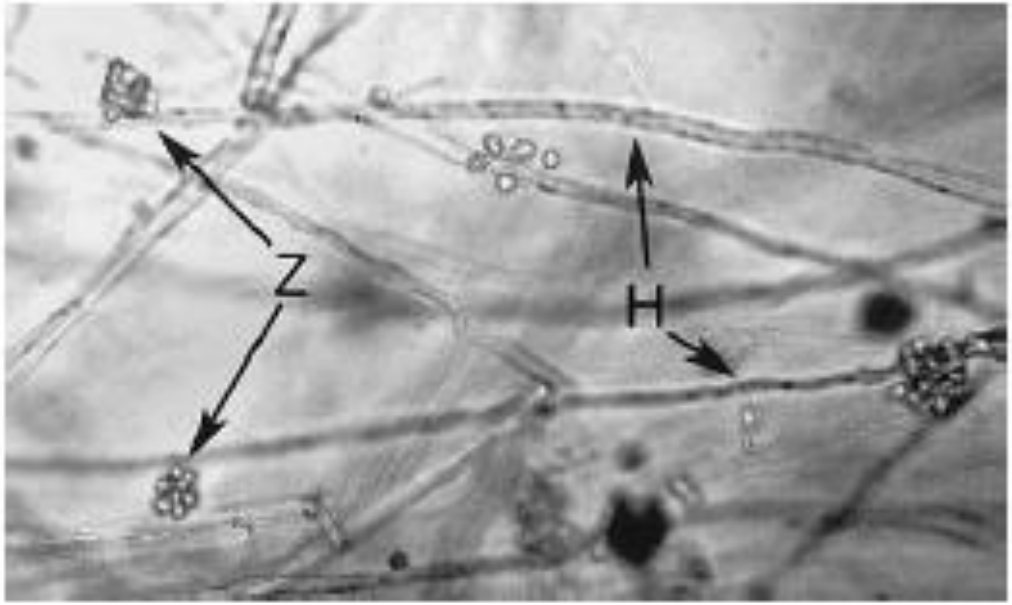


Fig. 5. *Aphanomyces invadans*, wet mount. H, hyphae; Z, Zoosporangia with primary zoospores. (Courtesy of Yasu Kiryu, Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA)

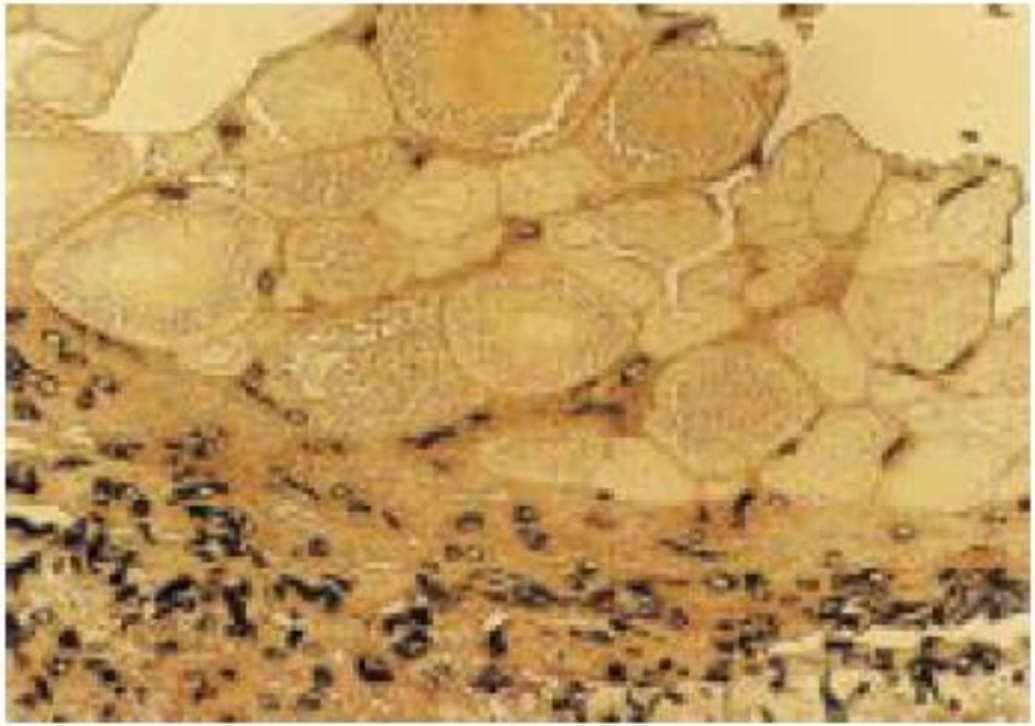


Figure 4-4. Fungal hyphae (black stain) in the connective tissue of the ovary of EUS-infected snakehead, *Ophicephalus striatus*. (Gomori methenamine silver stain, 100x)

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**.

Prevention and Control

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,
- hydrogen peroxide (100 ppm to 500 ppm),
- sodium chloride (10 ppm to 20 ppm).

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. Ichthyophonus disease is a systemic fungal disease and once it enters the fish, there is no cure. The best control for all fungal infections is good management : good water quality, good nutrition and proper handling.