#### **Fish Mycosis**

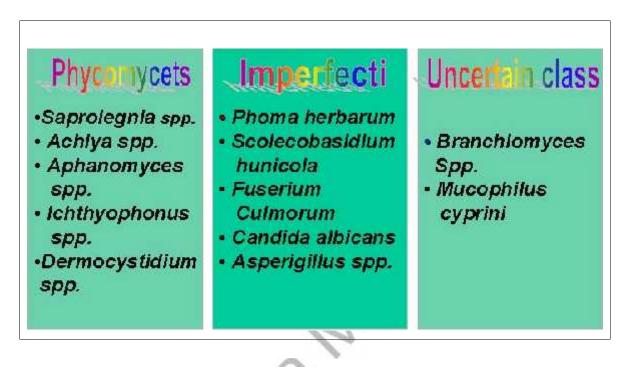
Relatively few genera and species of fungi are known to cause disease in fishes. Most epizootics of fish mycosis are usually facilitated by poor environmental conditions, malnutrition or primary disease.

This selection is going to discuss the most common mycotic diseases of fish as examples to demonstrate the complexity of fungal pathogens, how parasitic fungi cause diseases in the host and what may be done to reduce the effects on the host.

#### Facts about Fungi:

- > Fungi are belonging to the plant kingdom.
- Fungi are two major types, Sabrobes, those that live on dead organic mater, and Parasites, that live on living organisms.
- Many <u>sabrobes</u> are facultative parasites as well as many <u>parasites</u> are facultative sabrobes but all are <u>hetrophic</u> organisms, (ie. Need organic matter for their growth and reproduction).
- Some are made up one cell (yeast), while others are made up many cells joined together into long filament (hyphae) forming masses of hypha that may be septated or aseptated and called <u>mycillia</u>.
- Fungi reproduction is either by Asexual (Budding e.g. yeast, fragmentation of the hypha, spore formation e.g. sporngia or condiospore), or Sexual (usually occurred in unfavorable conditions) to produce resting or encysted spores.
- Resting spores are resistant to dryness, heat, disinfectants, and the defense mechanism of the host and when the conditions become favorable they become infective stages in the most cases of parasitic or facultative parasitic fungi.
- Most epizootics of mycotic origin in aquatic animals are facilitated by poor environmental conditions, trauma, malnutrition and / or primary disease (parasitic, bacterial ......etc).

## Classification of selected fungi in fish mycosis



Herein, three prevalent mycotic fish diseases will be discussed.

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#### Saprolegniosis

Ulcerative Dermal Necrosis (UDN), Salmon Disease, Winter Killer

Introduction Water molds belonging to Oomycetes, Saprolegniaceae, are the most important fungi affecting cultured freshwater fish and are considered by some specialists to occur secondary to bacterial diseases in terms of economic importance to aquaculture.

Among water molds, the genus *Saprolegnia* (family *Saprolegniacea*) those consider as a ubiquitous component of aquatic environment. However, the species of *Saprolegnia* implicated in fish pathology are probably best considered as those in which the fish are the major substratum.

On the other way, it would appear that many of *Sapolegnia* spp. causing out breaks of saprolegniasis act as primary pathogen rather than opportunistic one, and they are capable of infecting fish in the absence of existing bacterial or viral agent (s).

Historical viewBy the late years of eighteenth century, a fungal disease affectingofthe skin of salmons was first detected in the British Isles and nominatedsaprolegniosisas "Salmon disease". Then, the disease was recorded on specific riversof British, Wales and Scotland and the syndrome tock another namethat was ulcerative dermal necrosis.



In the early sixties, occasional fish with lesions characteristic for the disease were seen in the Waterville river system of south west of Ireland but the disease has since spread to the rest of Ireland. Since the disease first recognized it has spread throughout European water and in 1977, it was reported in Sweden for the first time.

Nowadays, such infection is usually categorized under the heading "saprolegniasis" and it is considered that *Saprolegnia* species behave as opportunistic necrotrophs.

In Japan the story of saprolegniasis has started dramatically by different way. The disease caused a great deal of damage and high mortalities in eel-culture ponds including different life stages of the fish and the causative agent was suspected to be *S. parasitica* Coker. A few years later, the disease spread among salmonid fishes and other fishes.

In USA, the disease caused great problems with catfish, which let fish specialists to nominate the disease as "Winter- killer". Since the disease recognized it has spread throughout American water and attacked salmonids as well as other fish.

"One of the prevalent chronic diseases of all freshwater and brackish water fishes specially young stages (fingerlings, Juveniles...etc), cultured and aquarium fishes, and even eggs <u>but</u> Definition <u>not affect marine fishes</u>, characterizing by fluffy white to grayish cotton-like fungal growth mass on the skin, gills, and fins, high morbidity & mortality among young fishes, local ulceration and subdermal necrosis in advanced cases".

Some members of the genus Saprolegnia:

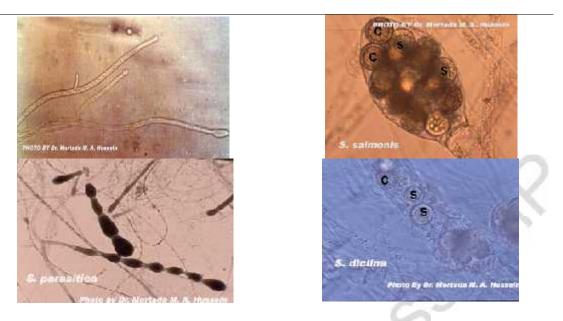
S. parasitica, S. salmonis, S. ferax, S. australis and S. diclina

Most of these *Saprolegnia* spp. are saprobes or facultative saprobes, however, it would appear that many of *Saprolegnia* spp. causing out breaks of saprolegniosis act as primary pathogen rather than opportunistic one (e. g. *S. parasitica, S. salmonis, S. declina* type I)

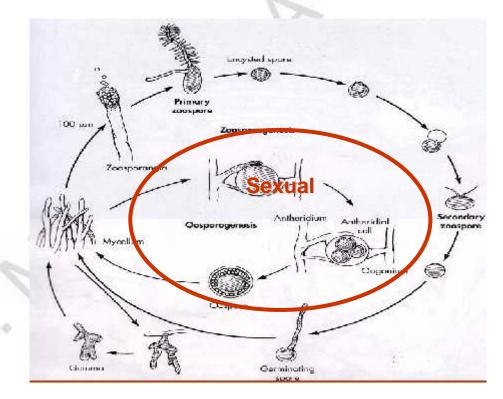


Etiology

These fungi have long branched aseptated hyphae, reproduce primarily asexually through formation of zoosporangia on fertile hyphae, zoosporangia long, cylindrical, double walled, and contain a large number of **zoospores** that discharged in the water, encysted and act as **infective stage** and germinate (under favorable) giving new hyphae.



Under unfavorable conditions, the fungus reproduces sexually through formation of Oogonia and Gemma elements. These structures could withstand the poor conditions and germinate again when the environment becomes suitable to give new hyphae, zoosporangia,..... and so on.



#### Susceptibility

 All freshwater fishes and their eggs are susceptible to catch the disease and infrequently brackish water fishes also could be infected (e. g. Mullets and drum fish).

Infections with the disease cause sever economic loss among fish

hatcheries. Living fish eggs are not susceptible to be attacked by any saprolegnia species, however, dead eggs considered as good fertile medium for the growth of the fungus leading to suffocation and invasion of the living ones by the growing hyphae.

- Overcrowding and physical trauma.
- Low dissolved oxygen.

Predisposing Causes (stressors)

- Presence of large amount of organic matter.
- Nutritional deficiencies and malnutrition especially among cultured fishes.
- Injuries of the skin or gill either by trauma or ectoparasites.
- Temperature variation (low temperature, 18~20° C) together with alternations with pH levels.
- Physiological alternation during spawning season.
- Rough handling especially during transportation.
- Sub-lethal level of toxic substances in the water (pollution).

Mode<br/>of infectionMainly through injuries of skin and /or gills. Zoospores from infected<br/>aquatic animals, infected dead carcasses as well as polluted water with<br/>them act as the source of infection.

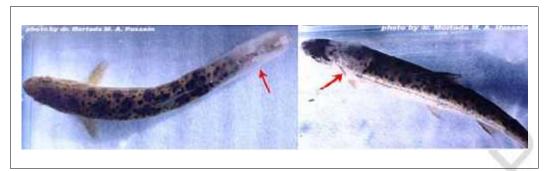
**Transmission** The transmission or the spread of the disease is usually horizontal (from infected material to the fish and /or aquatic animals).

Source of infection Contaminated water with the fungal elements consider as the main source infection particularly, inanimate sources (sporulated fungus on dead substrate). Infected fish and fish eggs are also important source of infection.

Pathogenesis

The lesion stated as superficial small focal infections that spread rapidly over the body surface and rarely penetrate beyond the superficial muscular layers.

Destruction of the integument (skin & gills) lead to loss of serum electrolytes and proteins, which is the primary cause for fatal termination.



#### Disease signs

Whitish to grayish cotton-like fungal growth on the skin, gills, and / or eyes of the infected salmonid fish, which can be seen only when the infected fish present in the water.



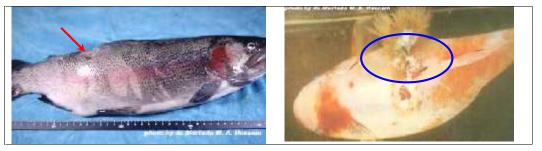


Glistening slimy matted mass on the body

When the gills infected respiratory distress occurred



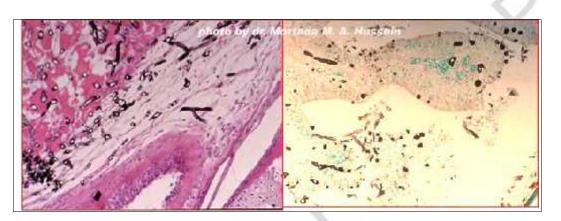
When the life eggs get infected suffocation and death occurred



In advanced cases, skin ulceration together with exposure of the muscular layer under the skin

#### Disease signs

- In mild cases gross pathology not clear, while in sever ones slimy grayish brown patches distributed on the affected areas together with haemorrhages, erosions and ulceration of the skin.
  - Destruction of the gills was also reported.
  - No internal lesions.



- Degenerative and necrotic changes in the skin & gills accompanied with fungal mycelia deeply into the underling muscle.
- Hyperplastic proliferation and \ or destruction of the epithelial linings accompanied with the invading fungal elements.
- I. Case history revealed that:
- Presence of Whitish to grayish cotton-like fungal growth on the skin, gills, and / or eyes.
- Sluggish swimming and loss of appetite.
- Presence of little mortality.

II. The disease signs (as mentioned above).

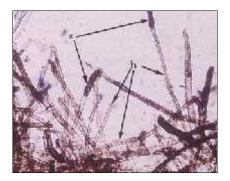
III. P. M. findings.



Diagnosis

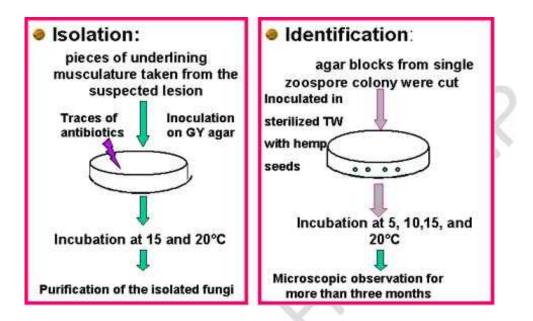
IV. Laboratory diagnosis:

 Wet mount preparation from skin and / or gills to detect the hyphal growth and /or the zoosporangia



#### Microscopic Pathology

Postmortem Findings Isolation and identification:



• Histopathological findings (as mentioned above).

#### Chemotherapy

# Therapy<br/>&Increase the water flow and aeration is the first step for treatment,<br/>then antiseptic bathes are recommended specially in early stages<br/>of infection.

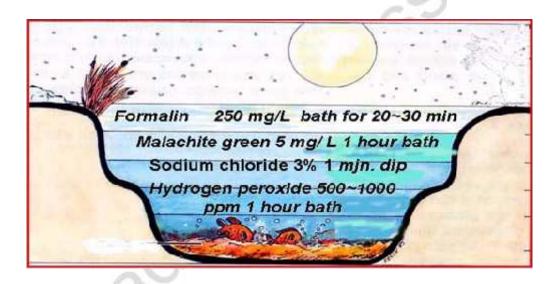
For nearly more than sixty years the compound of choice for the control of Oomycetes, particularly *Saprolegnia* infection, in aquaculture has been malachite green. In fact, **malachite green** is the most potent fish fungicide but it has an acute impact on aquatic ecosystems, has an immunosuppressive effect on repeatedly treated fish, teratogenic, and it result in hazardous residues in fish tissue.



For these reasons, the use of malachite green was terminated in United States of America and the need for a replacement fungicide has intensified.

Presently, **formalin** has been used as an effective fungicide, however, it is approved only for use on the eggs of salmonids and esocids. In addition, the use of formalin has increased causing more awareness about user safety and the chemical's impact on the environment. Recently, much current research has focused on **hydrogen peroxide** because it is safer to use than formalin and appears useful for hatchery water currents, as it is decomposed into water and oxygen via enzymatic catalysts found in most bacterial cells.

Yet, while attempts are being made to identify new antifungal agents against *Saprolegnia*, biological control of this organism has received little attention. As one of the methods for preventing infectious diseases, the **biocontrol** that is a microbial technique using the interaction of microorganisms to repress the growth of deleterious microbes or pathogens.



#### Control

Good hygiene and removal of all stressors is the proper way for disease control this can be achieved through:

- Avoid overcrowding.
- Proper disposal of dead and dying fishes either by burning or burying.
- Control of aquatic animals such as reptiles and amphibians.
- Proper disposal of infected fish if in small number.
- Proper drainage, drying, and disinfectant of the pond (quick lime 4 tone/acre.

#### Ichthyophonosis

Swinging disease, Ichthyophonus Disease

"One of chronic systemic granuolomatus disease affect freshwater, brackish water, marine water, and aquarium fishes characterizing by systemic granuoloma, emaciation, shallow skin ulcers, <u>sandpaper-like texture to the skin</u>, vertebral and spine curvature (Scoloisis & Lardiosis), swinging and chronic mortality in some endemic coldwater areas."

Ichthyophonus hofri, Obligatory fungal pathogen, resting stage (encysted stage) usually present in the host tissues, and discharged with its excreta, circular in shape, double walled each contains hundreds of endo-spores, 10~30μm (may be reached 250μm). The resting stage (Quiescent cysts) needs several months to ripe and become able to produce mature <u>amoeboblasts</u> as infective stage.

The germinating spores are flask shaped, with neck that consists of Aseptated hyphae that breaks through the outer wall.

The optimum temperature for outbreaks is 10° C, but it can be grow between 3~20° C.

Hos Dead fish tissues or fish feaces Fish issues Life Cyc Infected fish

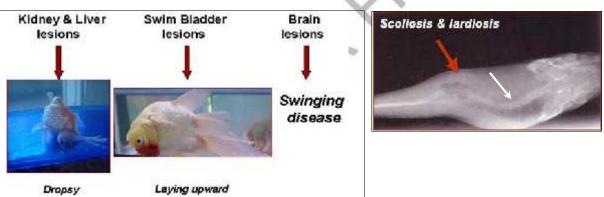


	Α	B, C & D	<b>E &amp; F</b>	
Life cycle		In dead fish	Alimontory tract	
	Inside infected fish tissue	tissues or feces of the	Alimentary tract of a new host	
	13500	infected fish		
	Reproduction occurs	Formation of the		
	by:	resting stage or Amoeboblast (E)		
		"Quiescent cysts"	Amoeboid embryos (F)	
	1.Filamentium.		$\sim$	
	2.Direct endospores.	■ Ripened cyst (C)	11	
	3.Plasmoium.		O	
	<b>J.FIdSIIIOIUIII.</b>		6	
		Germination (D)	6	
		Plasmodial G.		
		and/or Filamentous G.		
		K .		
Susceptibility	Almost all fishes are susceptible to catch the disease; in particular cold-			
	water fishes are more susceptible.			
	<ul> <li>Presence of large amount of organic matter and water blooms.</li> </ul>			
Predisposing Causes (stressors)	<ul> <li>Nutritional deficiencies and malnutrition.</li> </ul>			
	<ul> <li>Injuries of the gills either by trauma or ectoparasites.</li> </ul>			
	<ul> <li>Low temperature variation (3~20° C).</li> </ul>			
	<ul> <li>Sub-lethal level of toxic substances in the water (pollution).</li> </ul>			
Source	• Infected fishes (car	rriers) disseminating	the infection with their	
of	excreta.	le a construit de la contraction	and the state of the second	
infection	<ul> <li>Copepods and plankton organisms harboring the infective stage.</li> <li>Fish eating birds and mammals.</li> </ul>			
intection	<ul> <li>Dead infected fishes.</li> </ul>			
$\sim$				
$\sim$	Ingestion ° 🗐	н	eavy muscles	
~	orde.			
Mode of infection	CTA A	Н	cart Kidney	
of infection	here	Alimentary tract	jans with greatest	
&	1 2nd		blood supply	
Pathogenesis	May 2	Li	ver Spleen	
	And a			
	Injuries & abrasions	$\subset$	Brain.	
		JW	inging disease	

- Slight to moderate infected fish demonstrate no external signs.
- In advanced cases, the texture of skin become sandpaper-like, due to formation of granulomatus nodules in the dermal and sub-dermal layers.

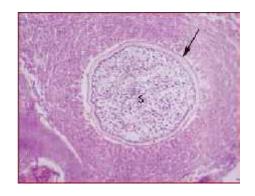
#### Disease signs

- Some skin granuolomatus lesions may rupture leaving open skin.
- Convulsion and swinging due to brain lesions.
- Exophthalmia, erected scales and dropsy due to liver and kidney affections.
- Loss of equilibrium together with laying upward due to affection of the swim bladder.
- Scoliosis & lardiosis.
- Emaciated carcasses.



#### Postmortem Findings

- According to the severity of infection the gross lesions, darken skin, sandpaper texture of the skin, swollen kidney, liver, spleen, heart, brain, and gills together with presence of whitish to grayish granular nodules.
- Muscles have purulent necrotic lesions filled with cellular debris, muscle fibers, and the fungal elements.





Microscopic Granular nodules within the infected tissues and inflammatory cells Pathology enclosed developing fungal stages in four layers that are,

## epitheliod cells, giant Macrophages, lymphocytes, and a thin layer of fibrous tissue.

Diagnosis I. Case histo

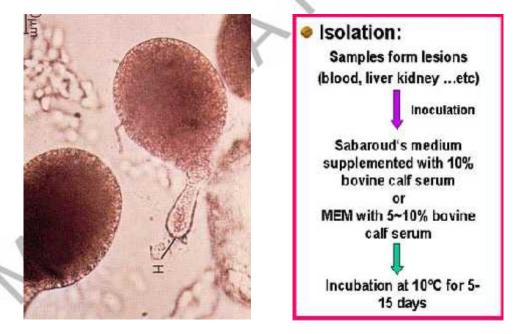
- I. Case history revealed that:
- Emaciation and abnormal swimming.
- sandpaper-like texture of the skin of the fish on handling.
- Season temperature variation.

II. The disease signs (as mentioned above).

III. P. M. findings.

IV. Laboratory diagnosis:

- Samples: different tissues including skin and internal organs from dead and dying fishes.
- Direct examination of gills tissue under microscope using <u>compressed slide</u> to detect the presence of fungal elements (spores and / or hyphae).





• Histopathological findings: As aforementioned.

#### Therapy & Control

#### Chemotherapy:

Up to date there is no therapy, for the reason, preventive hygienic measures are recommended to control the disease.

Good hygiene and removal of all stressors is the proper way for disease control this can be achieved through:

- •Quarantine and restriction of the fishes from infected areas.
- Complete drainage of the pond and draying till the soil cracks.
- Perfect disinfection using quick lime 1.5 ~ 4.0 tons/acre.

- Avoid feeding of infected fish offal.
- Improve all hygienic measures.
- Proper disposal of dead and dying fishes either by burning or burying.
- Control of aquatic animals such as reptiles and amphibians.
- Proper disposal of infected fish if in small number.
- Proper drainage, drying, and disinfectant of the pond (Perfect disinfections using quick lime 1.5 ~ 4.0 tons / acre)

or. Mortada MA Hussein

### Branchiomycosis

Gill Rot, European Gill Rot

Definition	"acute, subacute and chronic mycotic disease affecting freshwater and brackish water, specially cultured and aquarium fishes, and characterizing by gill degenerative changes (marbling appearance), gill necrosis (gill rot) and high morbidity (100%) & mortality (30-50%) among affected fishes ".		
Etiology	<ul> <li>Two members of Branchiomyces species are incriminated in disease occurrence they are:</li> <li>B. sanguinis and B. demigrans</li> <li>Both species need high oxygen tention for their growth.</li> <li>B. sanguinis: has thin hyphal wall (0.2μ) that contains small spores (5-9μ) in diameter. They usually locate within branchial blood vessels and discharge their spores into the blood stream.</li> <li>B. demigrans: has thick hyphal wall (0.5-0.7μ) that contains large spores (0.7-0.12μ) in diameter. They usually locate within branchial tissue and discharge their spores directly into the water stream</li> </ul>		
	These fungi have long branched aseptated hyphae, reproduce primarily asexually. They can grow between 14-35° C with optimal growth temperature of 25-32° C. They grow well on Sabauraud's		
dextrose agar (with or without blood supplement), blood broth and blood agar at 20-27° C.			
Susceptibility	All freshwater and aquarium fishes in particular cultured ones that depends on high amount of organic fertilizers are susceptible to catch the disease.		
Predisposing Causes (stressors)	<ul> <li>Overcrowding and physical trauma.</li> <li>Low dissolved oxygen.</li> <li>Presence of large amount of organic matter and water blooms.</li> <li>Nutritional deficiencies and malnutrition especially among cultured fishes.</li> <li>Injuries of the gills either by trauma or ectoparasites.</li> <li>High temperature variation (25~32° C) specially at late summer.</li> </ul>		

Sub-lethal level of toxic substances in the water (pollution).

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

Mode of infection &

**Indirectly:** through ingestion of the spores that undergo the blood Transmission stream through the intestinal lymphatic circulation and reach the gill as their final target (where as oxygen tension is high) and germinate at the aill blood vessels or aills tissue according to the fungus species.

Source Contaminated water with the fungal elements consider as the main of infection source of infection. Dead and dying fishes. Carriers particularly, chronic infected fishes.

Presences of the fungal elements (spores and / or hyphae) in the blood vessels of the gills reduce its blood supply leading to formation of thrombi and frequently infarctions occur. As a consequence many gill lamellae undergo different degrees of necrosis. Marbling appearance Pathogenesis of the gills is a characteristic for the disease. Fatal termination is the final result due to impaired respiration.

Although the fungal elements may reach the visceral organs via blood circulation, they doesn't chare in the epizootological finding because those organs have low oxygen tensions so the fungus fail to induce any lesions.

#### Disease Acute form:

signs

This form is characterized by rapid onset with rapid fatal termination:

- The fish lose their appetite and gathered in groups near the water inlet and water surface then going to die.
- Fish can't tolerate handling.
- Gills of died fish are severely congested and appear bright red or deep brownish red.







#### Subacute form:

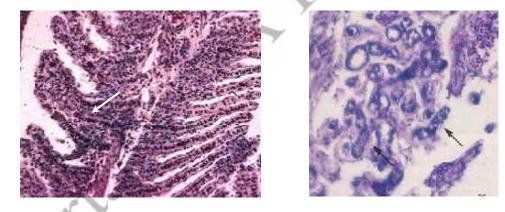
- Marbling appearance on different areas of the gills.
- Gills become ischemic, ragged and corroded.
- Sloughing of some gill lamellae.

#### Chronic form:

- Pale areas and / or muddy grayish strips on the gill.
- Swollen gill lamellae together with thrombi into gill epithelium.
- Slight to moderate necrosis of the gill lamellae



• In acute cases the gills appear bright red to brownish in color, while in sub-acute and chronic cases different alternations with the gill appearance are present ranged from ischemic areas, marbling areas, several degrees of necrosis.



#### Microscopic Pathology

- Hyperplastic proliferation and \ or destruction of the epithelial linings accompanied with fusion and edema of the gill Lamellae.
- Degenerative, necrotic changes, and thrombi formation within the gill tissues accompanied with fungal elements (spore and/or mycelia) deeply into the gill tissues.

Diagnosis

- I. Case history revealed that:
- Loss of appetite or may the fishes refuse food.
- Sluggish swimming and the fish together with respiratory distress.
- Presence of mortalities.

II. The disease signs (as mentioned above).

III. P. M. findings.

IV. Laboratory diagnosis:

- Samples: gills tissue from dead and dying fishes.
- Direct examination of gills tissue under microscope using

<u>compressed slide</u> to detect the presence of fungal elements (spores and / or hyphae).

#### Isolation and identification:

