SAPROLEGNIA: THERE'S A FUNGUS AMONG US

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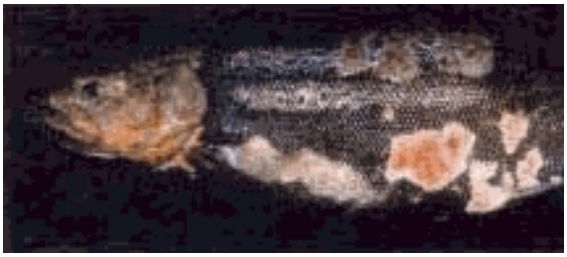
This report provides information mainly about the *S. declina* Humphrey-*S. parasitica* Coker complex (Willoughby, 1985), the main fungal pathogen of salmonids (Beakes *et al*., 1994; Pickering and Willoughby, 1982).

For readability, citations have been limited in this report. Many of the authors under "References" present similar descriptions and essential details about *Saprolegnia*. Therefore, to acknowledge contributions, the notation "and others" is used to inform the reader about a specific detail that was also provided by additional authors.

What is *Saprolegnia*?

Saprolegnia (pronounced: "Sap-ro-leg-ni-ah") is ubiquitous in freshwater ecosystems and is the main genus of water molds responsible for significant fungal infections of freshwater fish and eggs. Almost every freshwater fish is exposed to at least one species of fungus during its lifetime (Neish, 1991; Noga, 1996; and others), especially from the egg stage through smoltification (Bruno and Wood, 1999; Pickering, 1994). The infection of fish with *Saprolegnia* is termed "saprolegniasis" (Beakes *et al*., 1994; Roberts, 1989; and others).

On fish, *Saprolegnia* invades epidermal tissues, generally beginning on the head or fins (Neish, 1977; Willoughby, 1994; and others) and can spread over the entire surface of the body. Visible as white or gray patches of filamentous mycelium (Bruno and Wood, 1999; Beakes *et al.*, 1994; and others), *Saprolegnia* is characterized by an external, cotton-like appearance that radiates out in a circular, crescent-shaped or whorled pattern. Pickering and Willoughby (1982) suggest that there are differences in the patterns of infection between hatchery fish and wild fish. The pictures below (Bruno and Poppe, 1996) show fish infected with *Saprolegnia*:



Saprolegnia also infects moribund eggs by adhesion to and penetration of the egg membrane (Willoughby, 1994), and can spread from dead eggs to live eggs via positive chemotaxis (Bruno and Wood, 1999). The picture below (Bruno and Poppe, 1996) shows eggs infected with *Saprolegnia*:

Life cycle of *Saprolegnia*

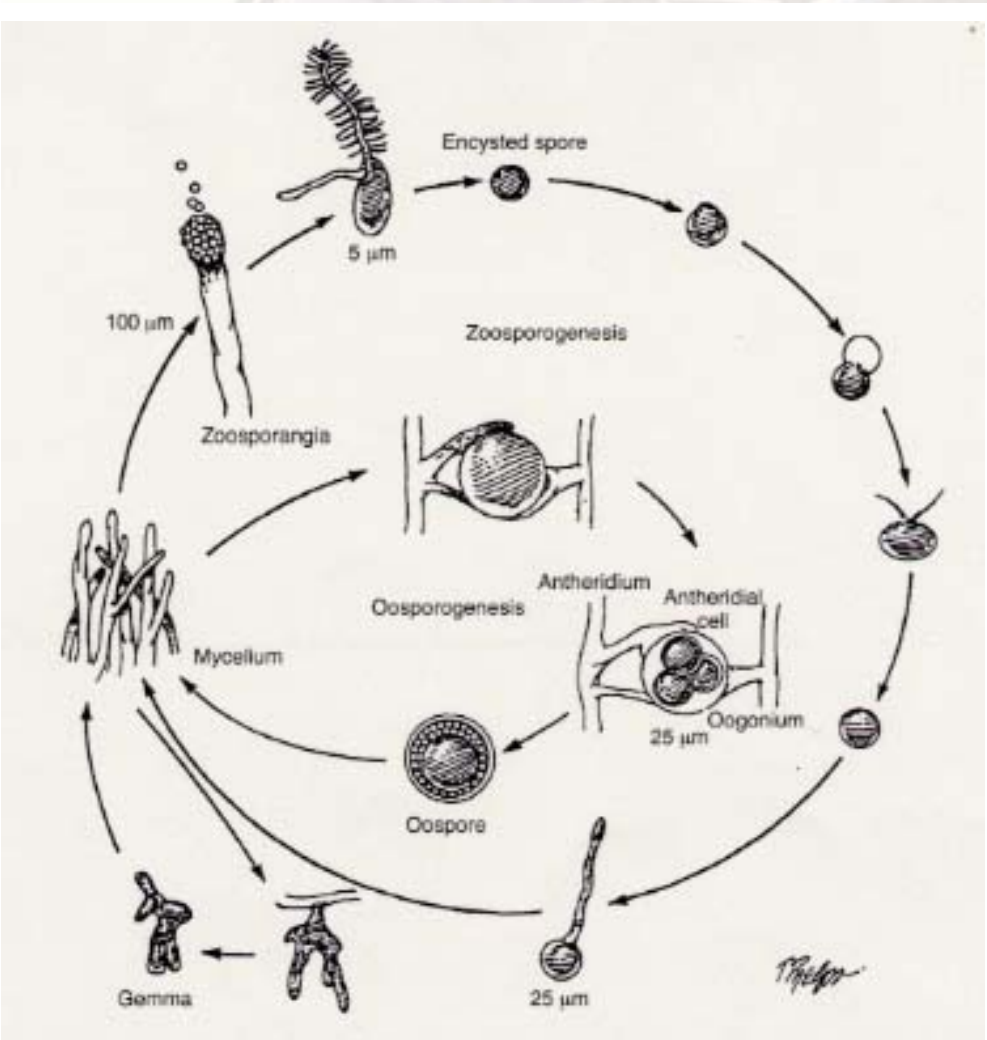
Bruno and Wood (1999) provide the following taxonomic classification for *Saprolegnia*:

Kingdom:	*Protoctista*
Division:	*Oomycota*
Phylum:	*Heterokonta*
Class:	*Oomycotea*
Order:	*Saprolegniales*
Family:	*Saprolegniaceae*
Genus:	*Saprolegnia*
Species:	*declina* Humphrey-*parasitica* Coker complex

Saprolegnia has a complex life cycle, which includes both sexual and asexual reproduction. Sexual reproduction involves the production of antheridium and oogonium gametangia, which unite for fertilization (Pickering and Willoughby, 1982; Seymour, 1970; and others).

The asexual spore of *Saprolegnia* release motile, primary zoospores (Bruno and Wood; Willoughby, 1994). Primary zoospores are active only for a few minutes before they encyst, germinate, and release a secondary zoospore (Seymour, 1970; Willoughby, 1994; and others). Secondary zoospores are more motile for a longer period of time than primary zoospores (Willoughby, 1994) and are considered the main dispersion phase of Saprolegnia (Beakes *et al.*, 1994; Pickering and Willoughby, 1982; and others). The repeated cycles of encystment and release, called "polyplanetism" (Beakes, 1982), allows secondary zoospores to make several attempts to locate a suitable substrate (Beakes, 1982; Bruno and Wood 1999). Secondary zoospores are considered the infectious spore of *Saprolegnia* (Bruno and Wood, 1999; Hatai and Hoshiai, 1994).

The *Saprolegnia* life cycle is presented in the diagram (Neish and Hughes, 1980) below:



Following encystment, secondary zoospores release hairs for attachment (Beakes, 1982; Willoughby, 1994). It has been suggested that these hairs are also used for buoyancy (Hatai and Hoshiai, 1994;

Pickering and Willoughby, 1982; and others), to decrease the sedimentation rate (Beakes *et al*., 1994), and for fungal-host recognition response (Beakes, 1982). The most pathogenic species of *Saprolegnia*, *S. parasitica* (Beakes *et al*., 1994; Hatai and Hoshiai, 1994; and others), have long, hooked hairs (Beakes, 1982; Pickering and Willoughby, 1982; and others).

Different species of *Saprolegnia* are able to germinate under different environmental conditions and nutrient levels (Bruno and Wood, 1999; Willoughby, 1985). Some *Saprolegnia* isolates are able to grow in water alone (Willoughby, 1986) and on waste products from hatcheries (Willoughby and Roberts, 1992). *S. parasitica* can sprout and grow in dilute nutrient mediums such as fish mucous (Murphy, 1981; Pickering and Willoughby, 1982). Willoughby (1985) developed a ‘rapid preliminary screening’ system which uses a low nutrient agar to confirm the existence of *S. parasitica* by the presence of cysts with long hairs, sometimes called "boathooks" (Beakes, 1982).

Identification of different species of *Saprolegnia* is difficult (Hughes, 1994) and can only be done by taxonomic analysis of the sexual structure (Willoughby, 1985; and others) combined with limited morphological characteristics (Pickering, 1994; Seymour, 1970; and others) of the organism. Fish-lesion isolates commonly do not produce any sexual structures and cannot be identified to species (Hughes, 1994), and are therefore grouped in the generic classification "*Saprolegnia* spp." (Beakes *et al*., 1994; Pickering and Willoughby, 1982). DNA fingerprinting is becoming an important technique for identifying of *Saprolegnia* isolates (Whisler, 1996).

How *Saprolegnia* Affects Fish

As a member of the class *Oomycete*, the genus *Saprolegnia* is considered an opportunist facultative parasite (Neish, 1977), which is saprotrophic and necrotrophic (Bruno and Wood, 1999). Fungal spores may be transmitted by hatchery fish, wild fish, eggs, water supplies, and equipment (Bruno and Wood, 1999). Fungal patches may consist of one or more species of *Saprolegnia* (Pickering and Willoughby, 1982; Whisler, 1996; and others) and become grayish due to the presence of bacteria and debris (Bruno and Wood, 1999; and others). It has been suggested that certain bacteria may repel (Beakes *et al*., 1994) or are antagonistic to *Saprolegnia* (Peterson *et al*., 1994).

Saprolegnia has a large impact on salmonids, especially those in aquaculture (Beakes *et al*., 1994; Hatai and Hoshiai, 1994; and others). However, it can also infect a number of other teleosts as well (Bruno and Wood, 1999). Channel catfish (Howe *et al*., 1999), pike (Willoughby, 1985), bass (Noga, 1996), elver and suckers (Roberts, 1989), roach, orfe, carp, tench, lamprey, sturgeon, barramundi, tilapia, and mullet (Bruno and Wood, 1999) have been infected with *Saprolegnia*. It has also been associated with tropical fish, including the kissing gourami, guppy, swordfish and platyfish (Roberts, 1989; Willoughby, 1994)

Willoughby (1989) determined that fish have 3 types of defenses against *Saprolegnia*. First, the physical removal of attached spores by the renewal of mucous. Second, a morphogen in the mucous inhibited the growth of mycelium but not killing it. And third, a cellular response in the mucous is directed at growing mycelium. Therefore, the mucous acts as a primary physical barrier (Bruno and Wood, 1999; Pickering, 1994), by continuous replenishment of the mucous layer (Pickering and Willoughby, 1982), although not

for complete, i.e., 100%, removal of fungal spores (Murphy, 1981; Willoughby and Pickering, 1977). However, a fish having an intact epidermis is probably the best defense against saprolegniasis (Hatai and Hoshiai, 1994; Pickering, 1994).

Generally considered a secondary pathogen, *Saprolegnia* can also act as a primary pathogen (Neish, 1977; Whisler, 1996; Willoughby and Pickering, 1977; and others). *Saprolegnia* causes tissue destruction and loss of epithelial integrity (Bruno and Poppe, 1996; Neish, 1991), due to cellular necrosis or dermal and epidermal damage (Pickering and Willoughby, 1982; and others), including hyphae penetration of the basement membrane (Bruno and Wood, 1999; Neish, 1991). However, *Saprolegnia* does not appear to be tissue specific (Neish, 1991). Pickering (1994) suggests that *Saprolegnia* lesions are not randomly located.

If untreated, *Saprolegnia* leads to death by heamodilution, i.e., osmoregulatory failure (Hatai and Hoshiai, 1994; Pickering and Willoughby, 1982; and others). Time to death by saprolegniasis is dependent on the initial site of the infection, type of tissue destroyed, growth rate of the fungus, and the ability of the individual fish to withstand the stress of a fungus invasion (Neish, 1991; Pickering and Willoughby, 1982).

While there is no evidence that *Saprolegnia* causes systemic infections or produces toxins (Neish, 1977), there can be a slight inflammatory response on the fish to fungal infections (Pickering and Willoughby, 1982). Fish with severe *Saprolegnia* infections appear lethargic, lose equilibrium and generally do not recover (Bruno and Poppe, 1996; Pickering and Willoughby, 1982; and others).

Causes of Saprolegniasis

In salmonids, the physiological state of the fish generally determines if a fungal infection will be successfully established (Neish, 1977; Snieszko, 1974; and others). *Saprolegnia* generally invades fish that have been stressed or otherwise have a weakened immune systems (Bruno and Wood, 1999; Pickering, 1994). Since fungus is almost always present in freshwater, it is assumed that some change in the fish occurs which allows a *Saprolegnia* infection to take hold (Bruno and Wood, 1999). Neish (1991) suggests that immunosupression provides a mechanism that causes the transformation of normally non-pathogenic organisms, including *Saprolegnia*, to become pathogenic.

Conditions that render fish susceptible to saprolegniasis include, but are not limited to, the following:

Conditions for *Saprolegniasis*	**References**
Broodstock	Meyer, 1991
Crowded hatchery conditions	Beakes *et al*., 1994; Whisler, 1996; and others
Epidermal integrity	Hatai and Hoshiai, 1994; Pickering, 1994; and others
Handling	Bruno and Wood, 1999; Hatai and Hoshiai, 1994

High corticosteroid level/androgen metabolism	Murphy, 1981; Neish, 1977; and others
Human error	Meyer, 1991
Mature males	Bruno and Woods, 1999; Pickering, 1994; and others
Pathogens and parasites	Bruno and Wood, 1999; Meyer, 1991
Physical activity on spawning beds	Bruno and Woods, 1999; Richards and Pickering, 1978
Pollution	Snieszko, 1974
Sexual maturity	Neish, 1977; Pickering and Willoughby, 1982; and others
Social hierarchies	Pottinger and Pickering, 1992**;** Whisler, 1996; and others
Water quality	Bruno and Wood, 1999; Pickering, 1994; and others
Water temperature changes	Bruno and Wood, 1999; Howe *et al*., 1999; and others

Saprolegnia has a fairly wide range of temperature tolerance, from 3 ºC to 33ºC, which appears to reflect the thermal preferences of the host (Pickering and Willoughby, 1982). However, sudden changes in temperature can make fish vulnerable to saprolegniasis (Bruno and Wood, 1999; Willoughby, 1994), due to the increased physiological stress. Channel catfish may suffer "winter kill" (Willoughby, 1994), a condition which occurs during winter months. Winter kill follows colder than normal weather when zoospore production of the ubiquitous *Saprolegnia* spp. is high. The colder weather suppresses the catfish immune system rendering them susceptible to saprolegniasis.

Treatment of *Saprolegnia*: What Works

Fungal infections are difficult to prevent and treat. Therefore, proper use of chemicals may be necessary when a *Saprolegnia* is diagnosed. However, there are few chemicals approved for use in aquaculture in the United States (Fitzpatrick *et al*., 1995; Meyer, 1991; and others).

Malachite green is considered the most effective chemical for controlling *Saprolegnia* (Bruno and Wood, 1999; Willoughby and Roberts, 1992; and others). However, because of concerns about potential carcinogenicity, i.e., its teratogenic (Fitzpatrick *et al*., 1995) and/or mutagenic properties (Bruno and Wood, 1999), malachite green is banned in the United States and some other countries (Marking *et al.*, 1994; and others).

Formalin, a solution of 37% formaldehyde (Van Waters and Rogers, 1988), is effective in treating *Saprolegnia* (Fitzpatrick *et al*., 1995; Mitchell and Collins, 1997; and others), and is the only fungicide registered for use in aquaculture in the United States (Bruno and Wood, 1999; Marking *et al*., 1994; and

others). However, there are concerns about its affect on both the environment and personnel who handle it (Fitzpatrick *et al*., 1995; and others).

Hydrogen peroxide is a promising chemical for the treatment of *Saprolegnia* (Fitzpatrick *et al*., 1995; Marking *et al.*, 1994; and others) with minimal impact to the environmental (Bruno and Wood, 1999; Mitchell and Collins, 1997). However, it is important to consider the species, life stage and water temperature when treating *Saprolegnia* with hydrogen peroxide (Rach *et al*., 1997).

Sodium chloride at high concentrations, i.e., sea water at 29 gm/liter and salt water at 15 gm/liter, is lethal to *Saprolegnia* (Marking *et al.*, 1994; Pickering, 1994), and effective for controlling *S. parasitica* (Willoughby, 1994).

Currently, the most effective strategy for controlling and preventing *Saprolegnia* infections is a combination of good fish management and husbandry techniques, combined with chemical treatment (Bruno and Wood, 1999), especially during the 2 to 4 day period after handling (Hatai and Hoshiai, 1994). Meyer (1991) states "well-nourished fish reared in highly favorable environmental conditions will be resistant to most pathogens." The reduction of stress appears to be the single greatest factor to help fish resist saprolegniasis.

Economic Information on *Saprolegnia*

Disease is the single largest cause of economic losses in aquaculture (Meyer, 1991), and fungal infections are second only to bacterial diseases in economic importance. Fungal infections are generally restricted to chronic, steady losses (Bruno and Wood, 1999; Pickering and Willoughby, 1982). Hatai and Hoshiai (1994) indicate that in Miyagi Prefecture, Japan, there is an annual mortality rate of 50% in coho salmon (*Oncorhynchus kisutch* Walbaum) due to *Saprolegnia parasitica* Coker. Fifty percent per year losses have also been reported in elver (*Anguilla anguilla*) culture in Japan (Bruno and Wood, 1999). And in the southeastern United States, major financial loses occur in channel catfish farming due to a condition called "winter kill." Some catfish farmers have reported losses of up to 50%, an economic loss of $40 million (Bruno and Wood, 1999).

Links to Other Web Sites about *Saprolegnia*

http://web1.manhattan.edu/fcardill/plants/protoc/sapro1.html

http://carroll1.cc.edu/~jclausz/

http://130.158.208.53/www/PDB/Images/Eumycota/Saprolegnia/index.html

http://www3.mistral.co.uk/xalan/i_fungal.htm

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This web page last updated 6/1/00.