

CHAPTER 29. Parasites and Pathogens: Saprolegniosis

Among the Saprolegniaceae there is an assemblage of individuals that can exist at the expense of fish and other freshwater vertebrates. Since these fungi respect neither exotic aquarium inhabitants nor the catch of the fisherman -- and are thereby a troublesome and costly lot -- a lengthy literature has accumulated about them. Mycologists and others have given much study to these fungi, but the results are not always in agreement. Moreover, from the very beginning, attention was focused on describing the diseased animals, with investigators forthrightly stating (or guessing at) the causes of those diseases, and suggesting ways to combat these obnoxious creatures. To a very real degree the fungi were ignored taxonomically as is reflected in the names by which the supposed causal organisms were referred to: filaments, fungus, threads, bacteria, algae, *Saprolegnia ferax*, *S. parasitica*, or merely *Saprolegnia* or *Achyla* or Saprolegniales.

At the outset, it must be confessed that there are no incontrovertible answers to three fundamental questions regarding fungus and fish associations -- are these water molds virulent pathogens? are they able alone to cause primary infections? to what degree must the subject be compromised to enable a fungus to cause disease? Because the great majority of fish appear to escape saprolegniosis even though obviously exposed to the spores (Willoughby, 1970; Willoughby and Pickering, 1977), the fungi involved must be opportunistic facultative parasites (Neish, 1977). We will not be so bold as to answer the foregoing questions, but will present a summary of what is known of saprolegniosis, beginning with a historical survey, and ending by considering -- in a lump sum, as it were -- the various suggestions for control. Between these two ends are accounts of factors thought to lead to or encourage saprolegniosis, the relation of this disease to others (primarily to ulcerative dermal necrosis, UDN), recognition signs and histopathology of infected tissue, and, of course, the host range of the various disease-associated water molds.

Since there is not yet proof of primary pathogenicity by these water molds, to speak of a "disease of fish" may be an unjustified oversimplification. Without some name to apply, however, one is helpless in trying to convey what has been discovered about the relationships between the water molds and fish. The name "saprolegniosis" is chosen because it relates particular disorders of fish much more succinctly to the water molds than do such provincial names as "fish mold" and "fungused fish".

For purposes of this chapter, reference to saprolegniosis does not carry with it implications of primary cause, it merely says that some saprolegnians are associated with freshwater animals. In other circumstances, in the absence of suspects these fungi are successful saprotrophs living on various parts of dead animals in the water (Minden, 1902) as well as vegetable debris.

The scientific and public awareness of saprolegniosis naturally has encouraged several general publications and reviews, promoted popularized accounts, and stimulated some unusual conclusions and suppositions. Some examples follow.

In 1973, Hester reported the results of a national survey to determine which of the problems associated with fish rearing and production were most important. His account leaves no doubt that agencies concerned with these activities considered damage by "aquatic Phycomycetes" to be the least worrisome. This certainly confirmed H. S. Davis' (1944) contention that it was erroneous to believe that saprolegniosis was a persisting "trouble" to be borne resignedly by fish hatcheries. He thought that bacterial diseases were more lethal than fungal ones, but the latter being more easily seen seemed more prominent.

Saprolegniosis has been related, usually in a tentative way, to particular biological phenomena. Swan (1889) thought that epidemics of fungal diseases might in time prove beneficial by insuring more vigorous races of fish as survivors reproduced and subsequent generations developed. To Rushton (1933), fungal infection possibly served a useful purpose in preventing overpopulation. Sex reversion by *Lebistes reticulatus* (in the laboratory), DeWit and Verster (1953) suggested, might be a reaction to toxins secreted by infecting saprolegnians. Stokoe (1966) believed that *Saprolegnia* (species?) was air-borne as well as being distributed by water.

One of the earliest general accounts of diseases of fish appears in a series of papers by Maurizio (1895b, c; 1897a); his articles are of particular historical value because they reflect accurately the mycological thinking of the time. Other publications that treat saprolegniosis in a general way are those by Allison (1950); Alderman (1976), marine biflagellates, largely; Bauer *et al.* (1973), and Shereshevskaya (1932), Russian summary accounts; Chiappelli (1933); Coutière (1900); Gopalakrishnan (1966), Asiatic and Far East fisheries; Hatai and Egusa (1976), Japanese account; G. L. Hoffman (1963), references to control practices; Ivankov (1971); Lacaz *et al.* (1970); F. P. Meyer (1966); Neish (1976), excellent modern account; Noll (1882); Plehn (1924), practical aspects of fish diseases; R. H. Richards (1978); R. C. Srivastava (1980a-c); Wolke (1975); and Wood (1974). Textbooks, handbooks, and reference sources to fish diseases -- all treating saprolegnial infections to a greater or lesser degree -- are abundant. The prominent ones in this category are those by Ainsworth (1949), Amlacher (1961, 1970, 1976), Duijn (1956, 1967, 1973), Hofer (1906), G. L. Hoffman (1967), G. L. Hoffman and Meyer (1974; many references to control methods); Neish and Hughes (1980); Reichenbach-Klinke (1966, 1973), Reichenbach-Klinke and Elkan (1965), Roberts and Shepherd (1974), and Schäperclaus (1933). Popular accounts abound as well. Three examples suffice: S. Lockwood (1890), Gordon (1936; refers to *Saprolegnia* as a renegade alga), and Wold (1950). The first two of these popular publications illustrate all too strikingly the dangers -- to accuracy -- of popularizing serious scientific information.

Reports of research on various aspects of the physiology of water molds associated with fish are treated in prior chapters: Chong (1973), Dop (1905a, b), Duff (1929), J. L. Harrison and Jones (1971), and Kanouse (1932), among others.

AN HISTORICAL VIEW -- TO 1900

In Chapter 1 we referred to Arderon's (1748) description of "mortification" in a species of *Rutilus*. This account seems to be the first written record of a fish disease that can be clearly identified with a watermold. Among those who later wrote of fungi associated with fish and other animals, and also described (to some extent) the diseased conditions, were Hannover (1839, 1842), Goodsir (1842), Areschoug (1844), and Bennett (1841-42, 1842a, b). In these papers, the descriptions of the fungi -- thought of by some to be confervoid algae -- are vague and inaccurate, yet sufficiently detailed to confirm that the authors saw saprolegnians.

Confusion over the nature of the organisms involved in fish diseases persisted almost throughout the mid- to late 1800's. The account of the morphology of some Saprolegniaceae associated with fish hardly qualifies as a dependable treatise. An unfortunate coincidence may have led H. Hoffman (1867) to conclude that a *Saprolegnia* on a fish generated a *Mucor*, which then succeeded the watermold. Gerard (1879) also suffered from imaginative intuition, concluding that *Empusa muscae* was merely *S. ferax*, the fish parasite, in an aquatic environment. Because he propagated on moist bread a *Saprolegnia* sp. obtained from salmon and could return the fungus to an aquatic habitat where it grew in the fashion of a watermold, Edington (1889) thought that he had discovered a terrestrial form of *Saprolegnia*. In spite of this erroneous generalization, he provided a sound, early description of the disease. Edington also stated that the fungus was the primary cause of the disease of salmon; later workers were to deny this.

Predictably, bacteria came into their share of the blame for microbial diseases of fish. The white mass that was evident on dead fish, W. G. Smith (1878a, b) maintained, was a mass of bacteria. Rutherford (1880) was a strong proponent of the view that bacteria were the primary organismic agents of salmon disease. He found such creatures in the musculature of obviously sick fish, concluded that the malady began in the bloodstream, and then argued that the blood became "diseased" through food taken in by the animals or by some deleterious matter in the water. In either case, Rutherford claimed, bacteria developed in endogenous decaying matter, exuded through the skin of the fish, and then became the centers for the germination of fungal spores. Contradicting this viewpoint, M. C. Cooke (1880) wrote that Rutherford had offered no proof of his claims. According to Bataillon (1893), and Bataillon and Dubard (1893), trout and trout eggs -- even when artificially inoculated -- were invaded and destroyed by bacteria. Cavara (1898) thought these investigators probably were correct, but at the same time advocated further experimental work. As will be evident in a later section, the possibility that bacteria are in fact the primary etiologic agents of "fungal diseases" of fishes persists.

Descriptions of the disease, under various names, appeared with some frequency during the latter half of the 19th Century. To Ogle (1873), reporting on work by others, the white patches on infected fish were simply moss-like material, the real cause being bacteria in the animal's circulatory system. Almost all descriptions (for example, Buckland *et al.*, 1880; Clinton, 1893; Cavara, 1898; Fatio, 1887) referred to the white

patches on head, fins, and tail, and most observers concluded that infection began at the tail or head, then spread to other parts of the body (Buckland *et al.*, 1880; W. G. Smith, 1878a, b). Stirling (1880a) believed that two conditions accounted for the fact that symptoms of infection first appeared on the head of the animal. Fish generally swam or rested against a water current, he reasoned, and thus would be hit headfirst by spores. In addition, the natural mucus of the fish body was thickest in the head region, and thus spores would adhere very readily (but *see* Willoughby, 1971a, 1972). Only the epidermis and dermis of fish were infected by the patches of surface growth Huxley (1882a, b) maintained. Robin (1853) regarded the characteristic localized “spots” as altered places on the body surface where nitrogenous compounds were undergoing putrefaction, and he considered that the “humors” secreted by the animals were suitable materials on which the fungus could grow. Most of the early observers of saprolegnians on fish were of the opinion that the fungi did not grow on the gills directly, but would develop on gill covers, or in the gill openings (Stirling, 1880b; Walpole and Huxley, 1882), as is well illustrated by Robin’s (1853) belief that the invading fungi affected parts associated with “respiration.” Stirling (1880b) reported that one effect of saprolegniosis was to cause suffocation; Blanc (1887) agreed, but Walpole and Huxley (1882) concluded that infected fish died of exhaustion, irritation, and from a “drain on their resources”. Rolleston, reporting in the extensive survey conducted by Buckland and his collaborators (1880), did not believe that predisposition of fish to attack was an important contributing factor, and concluded that the damage done to the fish was mechanical rather than chemical. Generally, too, there was agreement that internal organs and the circulatory system were not invaded by the fungi (Blanc, 1887; Buckland *et al.*, 1880; Walpole and Huxley, 1882).

Ryder (1881, 1882) first gave an extensive account of the development of saprolegnians on fish eggs, concluding that fungi always invaded egg masses when there was poor water circulation over them or detritus accumulated on them. If hatchery eggs were kept in constant motion, the “egg-fungus” could not develop (*see* Harsbarger and Porter, 1979).

Stirling (1878, 1880b) was satisfied, as were Walpole and Huxley (1882), that the disease condition in salmon followed the appearance of the fungus, and did not therefore precede this agent. Thus, in effect, they considered the saprolegnians to be primary invaders. In defense of his argument that salmon disease did not depend on some prediseased condition, Stirling reviewed the current theories regarding the cause of the disease. These were pollution, overcrowding, absence of frost, diseased kelts (weakened after spawning), or “addled” ova. From observations and experimental work (purposely overcrowding hatchery ponds, for instance), he concluded that none of these theories was correct.

In an addendum to the report by Buckland, Walpole, and Young (1880), F. Buckland commented, as had Stirling almost simultaneously, on several supposed causes of salmon disease. Most of Buckland’s conclusions were in agreement with those reached by Stirling, but the former believed that overcrowding contributed to the intensity and development of the disease. In an appendix to the report by Buckland *et*

al. (1880), M. C. Cooke wrote that if fish became weakened or exhausted they were predisposed to attack. Low water, he argued, induced a general "debility" of fish because of the accompanying reduction in available food.

Three different infections of fish were possible, according to Walpole and Huxley (1882): that due to *Saprolegnia*, disease resulting from *Achlya* invasion, and a third associated with thickening of the skin but without any visible fungus. Like Stirling and others, Huxley (1882a), and Swan (1889) did not believe that pollution was the cause of salmon disease.

Some investigators carried on inoculation experiments -- crude by modern standards -- in attempts to determine how the water molds invaded and spread within and among fish, and whether or not the fungi actually were causing the diseased condition. As Stirling (1880a), among others, discovered, fish did not become infected simply by feeding on the hyphae of water molds. In like manner, Jeunet (1891) decided that the disease was not contagious and could be cured provided the fish's gill tissue was not invaded. One of the earliest attempts at inoculating healthy animals with fungi was that by Huxley (1882a); he succeeded only in transferring saprolegnians from fish to flies. Walpole and Huxley (1882) achieved no more success in their experiments. Stirling (1880a), similarly, could transfer the "fish fungus" from fish to flies and spiders, but not the reverse. In 1885, however, Murray reported that he had succeeded in inducing the disease in "healthy" salmon and dace by introducing into them mycelium (from flies) bearing oogonia and sporangia. When he abraded the head of a fish with sand and then rubbed a fungus-infected fly on the wound, infection developed.

The troublesome question to these early investigators seemed to be that of the source of the saprolegnians that were implicated in the disease. It generally was agreed that the fungi were freshwater inhabitants, and that salmon migrating from the sea did not bring the organisms with them. Edington (1889) disagreed, believing that salmon regularly harbored the fungi, but simply were healthy at the time they migrated into freshwater rivers. Swan (1889), too, was satisfied that fish and fungi coexisted -- when the disease was not prevalent -- and de Bary (1884) observed that fish could live healthy lives in water infested with motile spores of the saprolegnians.

It was commonly accepted before the turn of the 20th Century that the bare wooden troughs used in hatching eggs harbored the fish fungus, but C. G. Atkins (1897) could not attribute the white spot disease of salmon eggs to such a source. Clinton (1893) traced infection of fish in aquarium tanks to the water pumped into them and circulated through the system. Finding oospores of a *Saprolegnia* " ... in the earthy contents ... " of earthworms, and seeking the nidus of an outbreak of fish fungus, Murray (1885: 306) concluded that the disease agent got into fishponds through earthworms that had picked up the fungus from dead fish cast out onto the ground. Indeed, he reported recovering a fungus from an earthworm, and successfully infecting a fish with a culture of it.

Inevitably, in the early years of investigation of saprolegniosis differences of opinion arose as to the seriousness of these fungi as pathogens of fish (Armistead, 1891). Valette St. George (1878) considered eggs and adults alike to be seriously threatened by

the disease, yet Seagle (1897) thought that "fungus" was not a serious trouble of adult fish provided the animals were not spawning or were handled carefully to avoid injury. If F. W. Clark's (1874) experiment was reported correctly, a bit of harsh treatment of infected fish encouraged their recovery. He observed that a fish which had lost its tail fin not only sloughed off the fungus filaments, but also regenerated new fin tissue after a few drops of nitric acid had been put on the infected part! Once an infected fish died, W. G. Smith (1878a, b) reported, the fungus disappeared, but other investigators recognized that water molds also grew saprobically on dead animals.

Among the pre-1900 accounts of water molds and fish diseases the most exhaustive treatments are to be found in the papers by Clinton (1893; includes a lengthy bibliography), Huxley (1882a, b), W. G. Smith (1878a, b), Stirling (1878, 1880a, b), Swan (1889), and Walpole and Huxley (1882). While the mycological aspects of the disease conditions reported in these papers are not entirely accurate (especially those by W. G. Smith) these publications illustrate well the early -- and diverse -- viewpoints.

MACROSCOPIC AND HISTOLOGICAL SIGNS OF SAPROLEGNIOSIS

Shortly after 1905, and extending to about 1970, reports of saprolegniosis reflected increasing respectability, and while the cause of the disease was still in some dispute, investigators looked more carefully than had their predecessors at infected material at hand. From several accounts there emerge rather accurate descriptions of saprolegniosis, and the extent of infection possible after the fungus gains a rhizoidal-hold, as it were, in the epidermis of a fish.

THE INFECTION

It seems generally agreed (Bootsma, 1973; Huntsman, 1918; Johnston, 1917; Mawdesley-Thomas and Jolly, 1967; Nolard-Tintigner, 1967; Stokoe, 1966, among others) that saprolegniosis begins at the epidermis, and probably most often (if not exclusively) where the epithelial cells are ruptured (Rucker, 1944). It was Tiffney's (1936) contention that *Saprolegnia parasitica* (= *diclina*) could gain entrance into susceptible animals by penetrating directly into the intact epidermis, or entering into natural body openings or wounds. The invading organism also may enter at points where the epidermis is not scaly (Willoughby, 1971a) or is thin (Florinskaya, 1969).

Precisely what region of the animal is first invaded -- as judged largely by where the signs (the extramatrical filaments) first appear -- is disputable. Kanouse (1932) believed that infection began in the vicinity of the mouth of a fish, as did Petersen (1909a). Others found signs of infection only in the caudal region (Griffon and Maublanc, 1911), in the tail region (Jolly, 1967), or at several points (Huntsman, 1918) on the body: mouth, jaws, top of head, dorsal midline, and at fin margins. There is disagreement in the various accounts of saprolegniosis as to whether the fungus attacked the gills; Bootsma (1973) thought yes. Duijn (1956, 1967) argued that gill infection (gill rot) resulted from invasion by species of *Branchiomyces*. Neish (1977)

stated that gill infection was rare Aleem *et al.* (1953) reported that *Isoachlya parasitica* (= *S. diiclina*) invaded the gill tissue of *Atherina riqueti* Roule causing asphyxiation.

According to D. A. White (1975), the position of the epidermal lesions in diseased *Salmo trutta* L. was related to spawning activities. The female fish, he found, had statistically significantly more lesions on the caudal peduncle area than on any other part of the body. Conversely, most of the lesions on the males were along the anterior surfaces of the body. Willoughby (1971a) concluded from his observations on infected trout and perch that there was no uniformity as to where the disease became established on the body surface.

Lesions associated with saprolegniaceous hyphae develop at the site of infection; subsequently, necrosis, ulceration (Bootsma, 1973; Harz, 1906; Jong, 1914; Stokoe, 1966), and inflammation (Vishniac and Nigrelli, 1951; Wolke, 1975) develop. The epidermis and then the dermis of the infected animal are breached and, according to Huntsman (1918:171), the disease becomes restricted to "... subadjacent parts." The hyphae of some water molds in fish doubtless penetrate into the dermal areas (connective tissue) and also can adhere closely to the stratified epithelial cells of the epidermis. Necrosis may be accompanied by cell proliferation according to Bootsma (1973).

Detailed studies of the nature and distribution of skin lesions on infected fish have been undertaken in addition to D. A. White's (1975) observations on lesion development in relation to spawning. Four grades of skin disruptions were detected by Pickering and Willoughby (1977) on diseased *Perca fluviatilis* L. The first two grades -- characterized by relatively small injuries, epidermal degeneration, and infiltration of erythrocytes and leucocytes into the superficial musculature, among other features -- were not associated with species of Saprolegniaceae. Grade III areas of tissue disruption appeared as open sores accompanied by connective tissue necrosis, invasion of blood cells into superficial musculature and subcuticular area, and signs of fungus filaments. Profuse growth of hyphae through the dermis, loss of scales, muscle fiber bundle necrosis, and disintegration of superficial melanophores constituted the characteristics of grade IV lesions. Hyphae of representatives of *Achlya*, *Aphanomyces*, and *Saprolegnia* were to be found in these latter two types of lesions. Pickering and Willoughby (1977) postulated that the fish louse, *Argulus foliaceus* L. might be responsible for the initial skin disruptions because the macroscopic appearance of the early damage was similar to that from the bites of this parasite. In any case, they concluded that the exact cause of the early lesions was unknown.

Several noteworthy observations emerged from the study by R. H. Richards and Pickering (1978) of lesion distribution on *Salmo trutta* and *Salvelinus alpinus* L. There were significantly larger infected areas on the body and fins of hatchery-reared fish than on wild fish of the same species, and the former yielded more fungus colonies on isolation. Hatchery-raised, sexually mature brown trout were more severely infected than immature individuals of either sex. The males of *S. trutta* were heavily infected along the dorsal surface, and ventrally toward the lateral line (flanks). The tail and peduncle region of the females of this species showed most frequently the signs of infection. Prior to spawning, hatchery-raised male char had noticeably higher

incidences of infection than did the mature females. Considering both the wild and reared fish of both species, Richards and Pickering found that large areas of the flanks of the males were infected more frequently than those of the females. However, the tail and ventral fin of the females of both wild and reared fish were more heavily infected than comparable parts of the males. Significantly, these investigators discovered that the areas around the lower jaws, the operculum, and the pectoral region (exclusive of the pectoral fin) were uninfected in all individuals examined.

In contrast to observations reported by Pickering and Willoughby (1977) for coarse fish, R. H. Richards and Pickering (1978) noted that infection appeared to be associated with a particular type of *Saprolegnia* having a low degree of homothallic sexuality (Neish, 1977). Richards and Pickering suggested that the heavy infections in the tail and ventral region of spawning female brown trout could have been associated with redd digging. It should be noted that these authors were unable to discount the possibility that saprolegniosis was secondary to viral or bacterial invasion.

In some cases of fish infection -- perhaps only exceptional ones, as Stokoe (1966) mentioned -- the invading saprolegniaceous hyphae breached the musculature (causing necrosis), seemingly along the capillary system (Nolard-Tintigner, 1967). Precisely how much penetration occurs beyond the musculature is in dispute. Bootsma (1973) stated that hyphae grew into the animal's body cavity, yet Griffon and Maublanc (1911) earlier had found no evidence of mycelium in the organs or blood of infected individuals. Some investigators reported that they had not seen invading filaments in the kidneys, liver, digestive tract, heart, or brain (Rucker, 1944; W. N. Tiffney, 1936; Nolard-Tintigner, 1971). Hyphae have been detected in the circulatory system (caudal aorta and veins, in particular) and spinal cord (Nolard-Tintigner, 1971), the endoskeletal system (Duijn, 1956; Stokoe, 1966), and on the cornea of eyes and in the lens (Plehn, 1924; Dukes, 1966). The extent of infection as determined by histopathological sections is considered in the next section.

Histopathology: -- As the infection associated with lesion development proceeds, the mycelium of the invading fungus is found (Nolard-Tintigner, 1967) only at the lesion margins. Nolard-Tintigner (1973) explored the histological aspects of four "zones" of a lesion: the center, the margin, an area where scales were damaged, and a region with no visible damage. There was noticeable destruction of tissue in the first three of these zones. The invading watermold caused a deep mycosis, penetrating into the vascular and nervous system, and grew very rapidly into the infected area. For example, guppies exposed to spores of *Saprolegnia ferax*, Nolard-Tintigner found, showed some paralysis at 16 hours after inoculation, and at this time hyphae were present in the spinal cord. Hemorrhaging began about 20 hours after the animal was exposed to the fungus. Nolard-Tintigner demonstrated that visible external symptoms in infected animals corresponded to the onset of necrosis in the spinal cord and thrombosis and hemorrhage in the caudal vein and aorta. Hyphae also were found (Nolard-Tintigner, 1974) in the cranial cartilage.

Neish (1976) observed the course and progression of saprolegnian infection in some Pacific salmon. Hyphae at first destroyed the multicellular, stratified squamous epithelium, then penetrated into the basement membrane. Subsequently, as infection proceeded, the mycelium grew into the *stratum spongiosum* of the dermis and then into the collagen bundles of the *stratum compactum*. From the dermis, hyphae could penetrate into the hypodermis, and thence into the subtending musculature. The histopathology of the invasion of salmonid fry by *Saprolegnia diclina* was investigated by Miyazaki and collaborators (1977). They found hyphal embolisms in the blood of infected fish, and mycelium throughout the abdominal cavity. Filaments of *S. diclina* penetrated into the stomach lumen at the pyloric region, and grew from sites in the stomach wall into the pancreas, pyloric caecae, spleen, abdominal musculature, and visceral blood vessels. This pattern and sequence of penetration described by Miyazaki and his colleagues recalls very strongly the course of a fish disease believed by H. S. Davis and Lazar (1940) to be caused by *S. invaderis*.

The Mucus Layer: -- What function (if any) does the mucus layer on living fish perform in relation to saprolegniosis? Rucker (1944) thought that this material served to free the fish of adhering spores as the substance was sloughed off and replaced. Some experimental work by Hattingh and van Warmelo (1975), Willoughby (1969, 1971a, 1972), and Willoughby and Pickering (1977) indicate that the role of the mucus layer is not a simplistic one.

The mucus layer, or cuticle, consists of mucopolysaccharides, albumin, lipids, free amino acids, and various ions (Hattingh and van Warmelo, 1975; Willoughby, 1971a). As Hattingh and van Warmelo discovered, the mucus actually supported the growth of *Saprolegnia* (species?), and had no mycostatic or mycocidal effect. They proposed that the mucus was a mechanical barrier. When the fish were handled the layer was thrown off by the mucus-producing goblet cells, thus exposing the skin surface to infectious agents.

Willoughby (1969) put motile spores of *Saprolegnia* Type I (see systematic account) into freshly collected mucus from trout, perch, and eels. Within seconds, the spores encysted, and, in time, germinated and produced mycelium in the mucus. When Willoughby (1971a) examined the mucus from fish exposed in tanks to suspensions of motile spores, he found that the spores could not physically attach to any portion of the fish body that was heavily covered by this layer. The spores would adhere only where the mucus was thin, or on roughened areas such as the edges of the caudal and dorsal fins.

It was Willoughby's (1971a) hypothesis that the animal's resistance to saprolegnian invasion was affected (or lost) at the mucus layer. Observations by Willoughby and Pickering (1977), however, suggested that there were two critical aspects of mucus as a potential physical barrier. In the first place, when fish were put into spore-free water after exposure to spore suspensions of *Saprolegnia* Type I, the majority of spores that had contacted the animals were removed or inactivated within the first 24 hours. The mucus layer, however, was not replaced in 24 hours. Secondly,

because secondary planonts germinated and grew vigorously on globules of mucus, any biochemical defense property of the mucus was obviously lost rapidly. The precise role of the mucus in saprolegniosis is yet to be discovered, it would appear.

Toxins: -- Experiments were designed by W. N. Tiffney (1936) to determine if *Saprolegnia parasitica* alone or in conjunction with bacteria produced an exo- or endotoxin. Culture filtrates and comminuted mycelium were injected into fish. The filtrate from fish broth and a proteose peptone broth killed fish within 32 hours to 5 days after being injected into animals. Filtrate from macerated mycelial mats also was toxic as was that from mixed cultures of bacteria and the fungus. Tiffney concluded that both endo- and exotoxins were elaborated and suggested that the death of infected fish might be the result of toxins rather than mere mechanical injury from the mycelium itself. The considerable time lapse between injection of filtrates and the death of animals does not strongly support the toxin hypothesis.

DeWit and Verster (1953) thought that a toxin secreted by a saprolegnian could have been responsible for sex reversal in *Lebistes reticulatus* Peters but offered no experimental support. Neish (1977) found no evidence of a toxin factor, and much earlier, Rucker (1944) had reached the same conclusion. Proof of a toxic effect accompanying saprolegniosis is wanting (but see discussion by Peduzzi *et al.*, 1976, of a chymotrypsin-like proteolytic enzyme system).

Serology and Enzymology: -- Employing the Ouchterlony double diffusion technique, Peduzzi (1973, 1975), Grimaldi *et al.* (1973), and Peduzzi and Bizzozero (1977), demonstrated antigenic properties in various species of *Saprolegnia* and other fungi. The serological reactions were suggested as useful tools to distinguish among the various water molds known to attack fish when ordinary morphological criteria failed or were inadequate. The test animals were shown (Peduzzi *et al.*, 1976) to develop antibodies when they were invaded by such species as *S. diclina* and *S. ferax*. The various antigenic reactions are summarized in Chapter 22; a general account of serological/immunological reactions in fish appears in J. G. M. Wilson's (1976) review.

A chymotrypsin-like enzyme system was detected by Peduzzi *et al.* (1976), and Peduzzi and Bizzozero (1977) in culture filtrate and mycelial extracts from some water molds associated with saprolegniosis of fish. They noted that an extracellular, proteolytic enzyme complex produced by a fungus would favor the deep penetration by invading hyphae into the host tissue. Moreover, Peduzzi and his collaborators hypothesized that the ability of a fungus to synthesize such enzymes had adaptive significance by favoring the evolution from a saprotrophic to a biotrophic (necrotrophic?) existence. The point made by Peduzzi and Bizzozero (1977) that an experimental approach to the etiology of saprolegniosis through enzymology could lead to a better understanding of the disease is well taken.

By means of serum analyses, R. H. Richards and Pickering (1979) investigated changes in serum osmotic pressures, ionic content, and serum protein patterns in relation to the degree of infection in *Salmo trutta* (tested during the spawning season) by

Saprolegnia diclina. Infection of brown trout by *S. diclina* resulted in significant declines in the concentration of sodium, potassium, calcium, magnesium, and protein in the blood serum. There was a direct correlation between serum sodium concentration and the percentage of body surface showing infection; the degree of infection also was correlated with the osmotic pressure of the serum. Severe hypoproteinaemia of the serum and a reduction in the ratio of albumin to globulin accompanied infection. Richards and Pickering suggested that lethal haemodilution due to breakdown of serum osmoregulation might have been responsible for the death of infected animals.

THE INFECTION IN EELS

Scanty but informative reports of the invasion of eels (*Anguilla japonica* T. and E.) by water molds -- the disease known as "sure" -- have come from the work of Japanese mycologists. In an initial study of *Saprolegnia parasitica* on *A. japonica*) Hoshina and his collaborators (1960) gathered data on the growth of the fungus (Chapters 17, 20), and determined experimentally that it did not produce a toxin. The peak period for occurrence of *S. parasitica* was in January, with a minimum frequency in April and March. The disease appeared in the eels, Hoshina (1963) reported, in early spring (March to May).

Fungal filaments first become evident on the snout or tail region of diseased eels, followed by necrosis and eventually ulceration (Hoshina and Ookubo, 1956; Egusa, 1963). Although mycelium of the water mold invades the musculature of eels (Hoshina and Ookubo, 1956), the liver and intestine are not penetrated yet can become inflamed. Egusa (1963) found that in artificially wounded and inoculated specimens of *Anguilla japonica* the mycelium of *Saprolegnia parasitica* did not penetrate into "healthy" tissue beyond the margin of lesions. In cases of severe wounding in eels, the necrotic tissue would slough off, taking with it the fungus. In the case of small primary lesions following very slight abrasion of the skin, on the contrary, the water mold spores germinated and the resulting mycelium would then penetrate healthy tissue. According to Egusa (1965), then, the causal agent in the disease of eels could take two courses, either deep penetration or superficial growth without penetration of healthy tissue.

Even though eels were exposed to *Saprolegnia parasitica*, Egusa (1965) found, they did not invariably become infected. Moreover, visible lesions on the eel were not necessarily caused by the water mold but could be the result of invasion by *Aeromonas liquefaciens* (see Bergey's Manual of Determinative Bacteriology, 8th Ed., p. 346, for a discussion of this binomial), with the fungus infection appearing only secondarily (Egusa and Nishikawa, 1965). Hoshina and Ookubo (1956) believed the disease of eels to be caused by a fungus, yet admitted that some individuals showing signs of the fungus also were contaminated with bacteria. Unidentified fungi also have been found associated with infections in eels (Hatai, Egusa, Takahashi, and Ooe, 1977).

Hoshina (1963) reviewed the eel disease situation in Japan, and Egusa (1966) commented on the three maladies to which elvers (young eels) were susceptible: a

disease caused by *Aeromonas* sp., a second one of a bacterial nature, and a third of unknown etiology. Saprolegnians were secondary invaders in the bacterial diseases. In a later review, Egusa (1973) again cited the disease caused by *Aeromonas* sp. as the one principally damaging to eels, and therefore of most concern to the fishing industry.

The extensive host range study by W. N. Tiffney (1936, 1939a) bears on the eel disease problem. Among the test species, *Anguilla chrysypa* Raf. (whether injured or not) was not infected by *Saprolegnia parasitica*.

THE FUNGUS

The identity of the various water molds associated with saprolegniosis has long been a problem. Until Coker's monograph appeared (1923), it was common practice to identify any water mold associated with fish disease as *Saprolegnia ferax*; subsequently the blame shifted to *S. parasitica*. As Willoughby (1970) and Willoughby and Pickering (1977) so clearly demonstrated, fish are subjected to all manner of water-borne spores, but only certain fungi seem to be involved consistently in the disease. Willoughby (1970), for example, found that although the spores of *S. ferax* predominated in water, the fungus did not appear on the animals themselves.

According to Willoughby (1971a), *Saprolegnia* Type I, a form with restricted growth capabilities and distinctive sporangia (Willoughby, 1969, 1972), consistently was parasitic on salmonid fish. Neish (1976) analyzed the characteristics of many water molds recovered from Pacific salmon, and was able to recognize four categories of isolates separable largely by reliance on the consistency with which they produced oogonia. Individuals having only sporadic production of oogonia -- category C (the same as group D in Neish, 1977) -- he suggested, would be assigned to *Saprolegnia parasitica sensu* Kanouse, and were strikingly like those described by Willoughby as *Saprolegnia* Type I. Neish could not separate the "classical" (morphological) species associated with saprolegniosis by their DNA base composition. Pickering *et al.* (1979) have attempted to shed some light on the identity of water molds associated with saprolegniosis by giving attention to the ultrastructure of the hair-like protuberances on the secondary spore cysts (Manton *et al.*, 1951; H. Meier and Webster, 1954). They found the hooked (bifurcate) hairs on the cysts of various species to vary in length, inter-hook distance, and number of hairs per bundle. A major point to emerge from the study by Pickering and his associates touched upon the fact that bundles of long, hooked hairs characteristic of isolates identified as *S. diclina* Type I also were found on some secondary spore cysts of sexually sterile isolates of *Saprolegnia* from fish.

Willoughby (1978) attempted to define and circumscribe the fungus complex associated with disease of salmonid fish, stressing the taxonomic importance of length to breadth ratios in the oogonia. He concluded that *Saprolegnia diclina* and *S. parasitica* could not be retained as separate species, even though water molds isolated from salmonids could be distinguished from those recovered from infected perch. Accordingly, he proposed that only a single species, *S. diclina* be retained, but recognized as a pathogen having three types or forms.

A rather significant discovery yet to be fully explored and exploited is found in Willoughby's (1977) report of "starved germings" of the *Saprolegnia diclina* complex. Such a deprived germling consisted of a slender hypha producing one terminal sporangium in which one or rarely two functional spores were delimited. When released, the spores were laterally biflagellate. Willoughby regarded the germling as a distinct morphological entity capable of producing a single planont if no nutrients were available. These "starved" germings, he proposed, could have considerable adhesive and entangling properties when in contact with the animal's cuticle.

Hatai, Egusa, and Awakura (1977) have described a new species, *Saprolegnia shikotsuensis*, capable of infecting wounded and unwounded specimens of *Oncorhynchus nerka* var. *adonis*. A descriptive analysis of this species, and further remarks on the taxonomy of the "fish saprolegnians" particularly those isolated by Neish and Willoughby are treated in the systematics account.

THE PRIMARY ETIOLOGIC AGENT

Whether water molds are the primary agents in saprolegniosis, or are only opportunistic invaders is a troublesome point. Huxley (1882a, b) and Walpole and Huxley (1882) were satisfied that the "fish Saprolegnia" was the cause of salmon disease, even though admitting that the fungus was not dependent on fish for its existence. Maurizio (1897b) regarded these invaders of fish as virulent parasites; Hardy (1907) thought it possible that they were primary invaders, but in 1910, wrote that fungi prepared the way for invasion of freshwater fish by bacteria. Similarly, Griffon and Maublanc (1911) believed that fungus in carp was indeed the primary cause of the disease. On the other hand, Rushton (1925) did not think saprolegniaceous fungi were parasitic and was satisfied that these organisms grew only on dead organic material.

Some investigators hedged on the question of the cause of saprolegniosis. While H. S. Davis (1944) believed that bacteria were the more lethal agents, he admitted that fungi did cause diseases of fish also. However, in a 1946 paper he stated that it was doubtful that *S. parasitica* was the primary agent. According to F. P. Meyer (1966), only two species were primary invaders of fish, *S. parasitica* and *S. invaderis* (= *ferax*). It was suggested by M. E. Brown (1968) that *Saprolegnia* sp. might well be necessary for the full development of the disease of salmon. She (M. E. Brown, 1966) argued that lesions caused by bacteria became invaded by *Saprolegnia* when the fish migrated from the sea into fresh water. She regarded the fungus as a superficial agent; bacteria were responsible for symptoms of deep ulceration. Wolke (1975) decided that only some species of "fish fungus" were in fact primary pathogens.

The accumulated literature leaves the impression that most investigators seem to have regarded saprolegniaceous fungi in fish diseases only as secondary agents. The proponents of this view cite water mold development in fish following invasion by *Branchiomyces* species (Wunder, 1938), or after the trauma of various stresses (such as temperature shock) or injuries from other parasites (G. L. Hoffman, 1976). In the case of fish eggs as the substrate, it has been argued, water molds only infest the dead eggs

initially (Martin, 1956) or simply attack ones associated with foreign organic material (Davis, 1953). Among those who have regarded saprolegnians as opportunistic invaders are Ajmal and Hobbs (1967); Baudouy and Tuffery (1973); M. E. Brown and Collins (1966); Christensen (1972); Drew (1909); Duijn (1967); Estes (1957); F. C. Harrison (1918); Johnston (1917); Johnston and Bancroft (1921); Oberstein (1913); J. H. Patterson (1903); Reichenbach-Klinke (1966, 1973); Reichenbach-Klinke and Elkan (1965), Rucker (1944); Rushton (1925, 1926); Rutherford (1880); H. H. Smith (1912); Stokoe (1966); Tomasec (1966); Wood (1974); and Wunder (1954).

Of the foregoing, the paper by J. H. Patterson (1903) was the first lengthy account of experimental inoculations cited in proof of the theory that salmon disease was caused by bacteria. He reported, for instance, that wounded fish inoculated with *Saprolegnia ferax* did not become infected. Moreover, he isolated a bacterium (*Bacillus salmonis pestis*) from all cases of the disease. When fish were inoculated concomitantly with the bacterium and the fungus, the animals subsequently died. Unwounded fish were not infected either by the fungus or the bacterium.

A number of cases of fish disease have been described in which there was no invasion by saprolegniaceous fungi whatsoever, or only doubtfully so. Included among such reports are those by Forel (1867), Bataillon and Dubard (1893), M. L. Stevens and Keil (1931), Rabanal *et al.* (1951), and Tashiro *et al.* (1977). In a 1950 publication, L. F. Whitney concluded that the white, cottony masses of material in the mouth region of diseased fish were not "mouth fungus" but rather bacterial infestation.

Other than J. H. Patterson's early (1903) study, there has been very little experimental work to determine whether or not saprolegniaceous fungi are primary, virulent pathogens. It was W. N. Tiffney's (1936) belief that watermold hyphae penetrated the epidermis of fish directly, so these fungi were therefore not just secondary invaders. A few years later, Rucker (1944) stated that the environmental conditions under which W. N. Tiffney (1936, 1939a) did his experimental inoculations were detrimental to the fish themselves. This being true, there is no way to be certain that Tiffney's data were proof of pathogenicity to healthy animals.

Monsma (1936) performed a series of inoculations on eggs and fry of *Micropterus dolomieu* and *Lepomis pallidus*. Living eggs of the former were not penetrated by any of the species of Saprolegniaceae tested, but seven species (*Achlya* and *Saprolegnia*) infected -- by hyphae, not by germ tubes from spores -- some eggs of the latter fish. None of the fry of either fish species was infected when artificially inoculated with *Achlya oblongata* var. *globosa* (=var. *oblongata*), *A. americana*, *A. klebsiana*, *A. racemosa*, *Saprolegnia anisospora*, *S. diclina*, and *S. ferax*. Of the successful cases of infection, Monsma wrote that all test fish had been handled, and were therefore injured prior to inoculation (an injury, of course, can provide the site for a primary infection).

Extensive experimental work by Nolard-Tintigner (1973) involved testing *Achlya ambisexualis*, *Saprolegnia* sp., *S. diclina* and *S. ferax* for possible pathogenicity to *Lebistes reticulatus* and *Xiphophorus helleri* Heckel. She inoculated test animals -- by scarification of the epidermis or by direct intramuscular injection -- with oogonia, gemmae, hyphae, sporangia and spores (planonts? encysted?) of the fungi. Only when sporangia

containing spores were used as inoculum was any infection detected. Bacteria-free cultures produced as much as 75% infection in the test fish. As has been noted, Nolard-Tintigner traced the course of infection, finding that there was a constant progression of the fungus into the inoculated animal. Moreover, she detected a definite, orderly relationship between the time necessary to kill the fish and the time "needed" by the invading fungus to reach and destroy the vital areas of the spinal cord and the cardiovascular system. Nolard-Tintigner (1973) was satisfied that this correlation proved pathogenicity, and that death of the animal ultimately was traceable to asphyxiation resulting from the destruction of the blood. Death was preceded by a loss of equilibrium in infected fish as the invading fungus caused necrosis of the spinal cord. To be sure, Nolard-Tintigner's observations are strongly supportive of a primary pathogenic role for saprolegnians. Final proof -- the ability of one or more of these fungi to serve as the virulent, primary invader in nature -- is wanting.

Experimental work by Neish (1976) on the etiology of saprolegniosis in Pacific salmon also involved inoculating test animals. Fish were inoculated and subsequently became infected when hempseed colonies of the fungi were put into aquaria with individuals lacking any visible signs of injury (that is, ones not purposely wounded).

In some instances, infection occurred when water mold colonies were actually stitched onto the fish, but this method did not insure that infection would develop. Neish also noted that wounded and macroscopically uninjured fish when put into direct contact with fungal spores did not necessarily become infected. Moreover, in one case where the fungus was sewn onto the animal, lesions developed in unwounded areas on the fish as well as at the site of the attached colony.

To support his belief that it was possible for species of Saprolegniaceae to act as primary pathogens, Neish (1976, 1977) proposed a theory based on physiological reactions in the salmon. Since wounding did not insure that the fungus would gain entry, some other factors impairing the natural defense mechanisms obviously were functioning. Under the stress of overcrowding or temperature shock, for example, the corticosteroid level in an individual increases, and the animal is less resistant to infection at that point. In addition, since maturing salmon do not feed, Neish pointed out, their ascorbic acid reserves were depleted and repair to damaged tissue accordingly was retarded or suppressed. This combination of physiological changes, Neish hypothesized, made the fish susceptible to saprolegnian invasion. Vulnerability of the individuals was coincidental with the onset of sexual maturity. In 1980, Neish and Hughes further emphasized the importance of stress in modifying the host such that invasion was facilitated.

OTHER FUNGAL AGENTS

From time to time, diseases of various freshwater fish have been described that seem not to result from invasion by the usual saprolegniaceous species. Among the most familiar of these diseases is one caused by species of *Branchiomyces* (Plehn, 1912, 1914), a genus which Grimaldi *et al.* (1973) assigned to the Saprolegniales. A disease of

ayu (*Plecoglossus altivelis*) is characterized by granulomata (Miyazaki and Egusa, 1973), but the fungus has not been identified. Hatai, Egusa, Takahashi, and Ooe (1977) also reported on the same disease condition. The fungus induced granulomata in the musculature, but the white cottony masses that are the obvious signs of saprolegniosis were absent in even the late stages of the disease. A panzootic in *Coregonus lavaretus* (L.) in Scotland was reported by R. J. Roberts *et al.* (1970). In one year, dead or moribund animals showed heavy infestation by a fungus in the head and fin regions, but in a successive year, the evidence of fungus was all but gone yet the head lesions ("bald spot disease") persisted. They were unable to isolate an agent that could be confirmed as the pathogen involved in this disease.

Members of the nonzoosporic groups of fungi also cause disease in various fish. For example, *Ochroconis* (= *Scolecobasidium*) *humicola* Barron and Busch has been associated with a phaeohyphomycosis of rainbow trout (Ajello *et al.*, 1977) and coho salmon (Ross and Yasutake, 1973). McGinnis and Ajello (1974) described a species of *Exophiala* isolated from channel catfish, and R. H. Richards and associates (1978) reported another species, *E. salmonis*, from *Salmo salar*. *Phoma herbarum* Westendorp has been implicated (Ross *et al.*, 1975) in a disease condition of *Oncorhynchus tshawytscha*, *O. kisutch*, and *Salmo gairdneri*, a most unusual role for this species ordinarily thought of as a saprotroph.

INTERNAL MYCOSES

In 1933, Agersborg described an intestinal mycotic infection of *Salvelinus fontinalis*. The causal agent, identified as *Saprolegnia ferax*, was alleged to invade the intestinal tract but yet not induce initially any outward symptoms (lesions) or signs (hyphae). A few years later, H. S. Davis and Lazar (1940) described *Saprolegnia invaderis* (= *ferax*), recovered from rainbow trout, and believed to cause a disease similar to that reported by Agersborg. Infection, Davis and Lazar stated, began within the stomach and intestine of the animal with the hyphae then growing through the body wall upon the death of the individual and then producing the reproductive elements exogenously. Blood congestion, tissue necrosis, and a bloating of the visceral blood vessels were the chief internal symptoms. Development of saprolegniosis endogenously before any external signs appear is most unusual.

Saprolegnia diclina has been implicated by Miyazaki *et al.* (1977) in a visceral mycosis of *Oncorhynchus rhodorus* f. *macrostomus*. The organism evidently gains entrance into the animal through the pyloric region. Thus, this disease begins internally -- unlike the usual progression in saprolegniosis -- and clearly recalls that described by H. S. Davis and Lazar (1940) and Agersborg (1933). Hatai and Egusa (1977) also have studied this mycotic infection; they isolated a fungus with septate hyphae, in addition to *S. diclina*. A mycotic granuloma from dermal ulcerated individuals of *Mugil cephalus* was described by McKenzie and Hall, in 1976. Nonseptate hyphae thought to be those of one of the Saprolegniaceae appeared in the degenerating musculature. The tissues

surrounding the lesions were oedematous, congested, and infiltrated by mononuclear inflammatory cells.

FACTORS INFLUENCING SAPROLEGNIOSIS

In papers published in 1973 and 1974, Snieszko commented upon the interrelationships of three factors leading to the establishment of infectious diseases in fish: (1) a susceptible animal, (2) a virulent pathogen, and (3) a combination of environmental stresses. Whether, in saprolegniosis, a virulent pathogen -- the watermold -- is always present is open to question; perhaps the fungus involved could be equated with a fourth factor, proposed by Wedemeyer (1974), a chronic pathogen. In any event, there appear to be a multitude of biotic and abiotic factors that are involved in the disease.

As will be evident in the subsequent account, the bulk of attention has been given to the effect of environmental parameters on growth, infectivity, or virulence of the potentially pathogenic fungus. It should be recognized, however, that these fungi may, in fact, possess wider ranges of tolerance to the various factors in their surroundings than do the potential suspects. This being so, the major effect of environmental change on the incidence of saprolegniosis may well be on the host's resistance rather than on the fungus directly. Moreover, differences in the degree of tolerance or resistance are likely to be expressed not only in relation to the suspect species, but to the stage of development of the individual host animal as well.

TEMPERATURE

Whether in terms of an influence on the invading fungus, the host, or the disease, *per se*, temperature has been singled out by most investigators as an important factor in saprolegniosis. There are exceptions. For example, D. A. Webster (1945) reported that temperature changes were not responsible for the destruction of egg masses of *Micropterus dolomieu*, and in some situations (D. A. White, 1975) temperature fluctuations are probably insufficient to have much effect on the disease.

Both W. G. Smith (1878b) and Gerard (1879) believed that low water temperatures "collapsed" the hyphae of saprolegnians on fish, and conversely (by inference), warm waters favored the development of the malady. A view quite the contrary emerges from an account published by Jeunet in 1891. He maintained that fish first contracted the disease at the onset of low winter water temperatures. In his study of salmon disease in Irish waters, Stirling (1880a) pointed out that infected salmon had been taken from rivers frozen over, and thus the view that the "absence of frost" was the cause of salmon kill was not correct. Bauer *et al.* (1973), Dudka (1964a), G. L. Hoffman (1963), Hoshina (1963), Petersen (1909a, b), R. J. Roberts and Shepherd (1974), Ryder (1881), and Wolke (1975) all reported that low temperatures favored the development of the disease in fish or eggs. According to Graham (1956), *Pomobolus pseudoharengus* (Wilson) was "fragile" when acclimated to cold water, and therefore

displayed increased susceptibility to invasion by saprolegnians. Dudka (1964b) reported saprolegniosis of fish from waters within the range of 0.8-23.2 °C; most cases of infection, however, appeared on animals held at 9-17 °C. Cool waters, Pickering and Willoughby (1977) reported, favored the growth of *Achlya* species on invaded animals.

Views quite the contrary to the foregoing -- that low water temperature retarded saprolegniosis -- were held by Hardy (1910), D. L. Koch and Contreras (1973), Swan (1889), Valette St. George (1878), and Nolard-Tintigner (1970). Plehn (1924) wrote that the disease was most common in warm water, and Chidambaram (1942) was of the opinion that warm temperatures augmented its spread. Rucker (1944), too, blamed high water temperature in part for leading to the establishment of fungal disease. Invasion of *Achlya bisexualis* into wounded *Platygoecilius maculatus*, O'Bier (1960) reported, was favored by warm water. According to W. N. Tiffney (1936), temperatures of 10 °C or less or 25 °C or more retarded saprolegniosis. Dioni and Reartes (1975) observed that *Saprolegnia* (species?) was a factor common to many dead fish exposed to low water temperatures. Cold alone, they concluded, was not the sole cause of death in experimental animals. The watermold disease in eels, Hoshina (1963) noted, vanished when water temperatures exceeded 20 °C.

Little has been done experimentally to determine what effect temperature actually has on the inception and course of the disease. There are two notable exceptions. Hoshina and Ookubo (1956) inoculated eels with mycelium or spores of *Saprolegnia parasitica* and held the animals at various water temperatures. Although animals kept at 18-19 °C after inoculation with mycelium escaped infection, they did not do so when mycelium and spores were used as the inoculum, with incubation at 13-15 °C. A study by D. L. McKay (1967) on *S. diclina* and *Oncorhynchus kisutch* included inoculation experiments at 8, 13, and 18 °C, and involved temperature shock resulting from removing animals from 8 °C water to that at 3.5-4.5 °C, 9-10 °C, 13°, and 18 °C. *Saprolegnia diclina* did not infect fish at 8 or 9 °C, but inoculated animals showed symptoms of the disease after more than a week of incubation at 13 °C. Within seven days after inoculation, the fungus infected the test salmonids held at 18 °C. Cold temperature shock did not affect the natural resistance of the fish to saprolegniosis.

OXYGEN

As in the case of temperature as a modifying factor in saprolegniosis, there simply is no agreement as to the influence of dissolved oxygen on the disease, *per se*, or the fungus. The more "air" available in the water, according to Benecke (1886), Reichenbach-Klinke and Elkan (1965), and Swan (1889), the less favorable are the conditions for the fungus, and therefore the less frequent -- or harsh -- is the disease. Indeed, Johnston (1917) predicted that a rainy season, bringing fresh, well-aerated water into ponds would be followed by the disappearance of epidemics of saprolegniosis. The invading watermolds thrived in poorly aerated water, Stokoe (1966) contended, thus agreeing with the earlier views held by Plehn (1924) and

Rushton (1936), but contradicting Jeunet's view (1891) that the watermold disease was not associated with fish living in stagnant water.

Perhaps the comment by Brook (1879) that *Saprolegnia ferax* developed sooner on fish in still rather than in running water could indicate that a low oxygen level promoted the development of saprolegniosis. The same might be said for O'Bier's (1960) comment that *Achlya bisexualis* as a fish pathogen was favored in turbid waters. Rucker (1944) maintained that he had demonstrated experimentally an inability of *S. parasitica* to grow in water deprived of oxygen (by boiling). Dudka's (1964b) observations greatly magnified the results of Rucker's experiments. She found infected fish (saprolegniosis) in waters ranging from 0.82-11.47 mg L⁻¹ oxygen.

An evaluation of the influence of oxygen on saprolegniosis should at least allow for the possibility that saprolegnians may function actively at oxygen tensions less than those tolerated by most fish. While the fish, then, might be stressed by lack of O₂ the potential invader still would be adequately supplied.

OTHER HYDROGRAPHIC FACTORS

Schuster (1952) reported that there was no infection whatever of *Chanos chanos* in Javanese ponds because the fish were kept in saline waters. Sea water killed the *Saprolegnia* that invaded fish, Clinton (1893) said, yet he also reported that the fungus he collected on fish grew in a mixture of one part seawater to five parts fresh water. Even Swan (1889) considered that the cause of the salmon disease he encountered did not originate in salt water. It was J. P. Stevenson's (1970) view that salmon could be infected by saprolegnians prior to migrating to the sea, and the invading fungus then remained dormant until the fish returned to fresh water. This view is in agreement with reports by Hearth and Padgett (1990) and Shafer *et al.* (1990) both of which discussed fish infections being initiated in estuaries. These papers described significant salinity tolerance of non-sexual isolates of *Aphanomyces* and *Saprolegnia* recovered from Atlantic menhaden, *Brevoortia tyrannus* Latrobe. It further is apparent from the publications of Padgett (1978a, b; 1980, 1984) that salt tolerant ecotypes of saprolegnians inhabit estuaries and exhibit significant potential for dispersal by fish. We note that Padgett's findings are not inconsistent with the view expressed by Swan (1889) that the fungi etiologic in salmon disease do not originate in salt water. When infected fish moved into the sea during their natural migratory pattern, R. J. Roberts and Shepherd (1974) contended, the disease incidence was correspondingly reduced. The pH of the water is not by itself a factor contributing to the incidence of saprolegniosis, W. H. Tiffney (1936) suggested.

In his review of the prevailing theories to explain salmon kill in English waters, Stirling (1880a) pointed out that there were infected fish in unpolluted as well as polluted waters, and thus this factor was not the cause of the damage to fish. Walentowicz (1885) was of the opinion that pollution favored fish diseases, but Swan (1889) took a contrary view regarding the salmon disease in Irish waters. Much later, Chidambaram (1942) was to regard pollution as a major factor contributing to the

fungus disease of *Osphromenus goramy* in India. Willoughby and Collins (1966) argued that fish mortality was greatest in polluted habitats, and there occurred in such waters also (in southern England) a species complex -- *Saprolegnia ferax/mixta* -- that often was associated with fish mortality. The common denominator in reports on pollution and incidence or severity of saprolegniosis is that for the most part specific agents are not identified. This softens the impact of any conclusions that are reached.

NONHYDROGRAPHIC FACTORS

Whether put in positive terms -- that is, encouraging saprolegniosis -- or in negative ones (avoiding particular conditions to thus lessen the chances of the disease), the fact remains that many contributing factors have been singled out. Among those conditions surfacing often as ones favoring the development and severity of saprolegniosis are these: overcrowding, low nutrition (for the fish), animals weakened from some debilitating activity such as spawning or sexual maturity, individuals kept in unsanitary conditions, changes in environment to which the fish could not readily adjust (as Wolke, 1975, put it, subjecting the animals to some "environmental insult"), or simply a weakened, sickly condition perhaps resulting from some other disease (E. H. Brown, 1968; Chidambaram, 1942; Clinton, 1893; M. C. Cooke, 1880; Fiebiger, 1903-04; Florinskaya, 1969; G. L. Hoffman, 1963; Johnston, 1917; Plehn, 1924; R. H. Richards and Pickering, 1978; R. J. Roberts and Shepherd, 1974; Rosenberg, 1908; Rucker, 1944; Rucker *et al.*, 1953; Rushton, 1935; Schäperclaus, 1933; Stokoe, 1966; Vincent, 1908). Keeping the animals healthy, it was very early observed, certainly tended to suppress or reduce but not eradicate the frequency and severity of saprolegniosis (Brook, 1879; Swan, 1889). With respect to fish eggs, unfavorable hatchery conditions, as well as such factors as clumping of the ova or their inherent nonviability, have been singled out as factors influencing the inception and spread of saprolegnians (Ryder, 1882; E. H. Brown, 1960). In working with eggs of *Salmo trutta*, Harbarger and Porter (1979) concluded tentatively that ova confined in transplant boxes were more susceptible to fungal attack (saprolegnians?) than they were in intragravel plants.

Precisely what triggers susceptibility to saprolegnian invasion has not been determined satisfactorily. A study by Roth (1972) on corticosteroids suggests one possible explanation. He gave intravenous doses of steroids, and infusions of thyroid-stimulating hormone (TSH) to individuals of *Catostomus commersonii commersonii* Lacépède. Infection by the water mold (unidentified) was facilitated in fish with a high plasma level of estradiol, progesterone, TSH, and the 17-hydroxy-corticosteroids, but not in individuals injected with corticosterone or testosterone, or infused with ACTH. Roth suggested that the action of steroids in favoring or "permitting" infection related to their ability to impair antibody formation and suppress reactive tissue inflammation.

INJURY

Accounts attributing saprolegnian invasion into fish through abiotic, mechanical injuries or wounds of a biotic source are many, for example: Bauer *et al.* (1973), Benecke (1886), Chidambaram (1942), Clinton (1893), Duijn (1967), Dukes (1966), Egusa (1963), Florinskaya (1969), Gopalakrishnan (1963), Gopalakrishnan and Jhingran (1972), Hatai, Egusa, and Awakura (1977), G. L. Hoffman (1976), Johnston (1917), Marnell and Hunsaker (1970), Nigrelli (1943), Plehn (1924), R. H. Richards and Pickering (1978), R. J. Roberts *et al.* (1973), R. J. Roberts and Shepherd (1974), Rucker *et al.* (1953), Rushton (1926, 1935, 1936), Schäperclaus (1933), Seagle (1897), W. G. Smith (1878a, b), G. C. Srivastava and Srivastava (1977a), Swan (1889), Tempère (1904), and D. A. White (1975). Conversely, there are only a few investigators who maintained that it was not necessary for some injury to provide an entryway for saprolegniaceous hyphae: among others Stirling (1880b) and Edington (1889) took this view. Kanouse (1932) was not certain that injury was a predisposing factor, and McKay (1967) stated that wounding alone was insufficient to allow development of the disease. Experimental inoculations by Monsma (1936), O'Bier (1960), and Neish (1976, 1977) support the notion that injury or wounding is not invariably necessary for infection to become established. It is, of course, difficult to prove that injury is not a prerequisite to infection because of the possibility of microscopic breaches of the fish's epidermis.

Several investigators have utilized some artificial means of wounding fish prior to inoculating them with spores or mycelium of the water molds being tested (Bhargava *et al.*, 1971; Monsma, 1936; Neish, 1976; G. C. Srivastava and Srivastava (1977c, d), R. C. Srivastava (1978a, b), W. N. Tiffney (1936, 1939a), and Vishniac and Nigrelli (1957), among others. Methods for wounding the animals have consisted of such procedures as removing scales, or incising or abrading the skin below the mucus layer of test animals. Perhaps the most unusual treatment for abrading fish prior to inoculation was that employed by Estes (1957). Animals were removed from the water, covered with sand, and then rotated in a wire basket for a few minutes before being returned to water. Surely this treatment meets almost all the "requirements" for favorable disease development -- suffocation, physical stress, debilitation -- plus the painful additive of severe abrasion: truly an environmental insult of exceptional quality!

ASSOCIATIVE EFFECTS

In a foregoing section, we summarized what is known (or suspected) of the supposed relationships between saprolegnosis and bacteria. Additionally, mention has been made from time to time of injuries resulting from parasite infection, and the role of such invertebrates in disease inception. Thus, there are certain associative effects resulting from contacts with biotic elements in the environment that may (or may not) contribute to the inception and development of saprolegnosis.

PARASITES

Bites of the fish louse, Bower-Shore (1940) reported, provided puncture wounds in which *Saprolegnia ferax* quickly became established. Ivasik (1953) and Reichenbach-Klinke and Elkan (1965) likewise associated injuries inflicted by parasites with avenues of entry for fungi as secondary invaders. Bauer (1961a) reported that wounds inflicted by the copepod *Lernacea cyprinacea* became centers of concentration for saprolegnians and *Costia necatrix*. Fish suffering from invasion by the latter also were attacked by water molds (Bauer, 1961b), thus aggravating the diseased condition.

ULCERATIVE DERMAL NECROSIS (UDN)

The association that has by far received the most attention of pathologists and mycologists alike is that of saprolegniaceous fungi and UDN, reported in 1964 as a disease of salmon, sea trout, and brown trout in Irish waters. Ulcerative dermal necrosis appeared as the animals migrated from the sea into fresh water. Salmonids from British, French, and Swedish waters also are known to have UDN (R. J. Roberts and Bullock, 1976).

In 1934, Fish published an account of an ulcerative disease of trout in which saprolegnians evidently were not present. This diseased condition recalls some of the symptomatology of UDN. However, if, R. J. Roberts (1972) and Munro (1970) are correct, UDN actually was known -- but not by this name -- much earlier. Munro has pointed out that the descriptive characters of saprolegnian diseases of fish recorded by W. G. Smith (1878a, b), Stirling (1878), and Brook (1879) were very similar to those of UDN. According to R. J. Roberts (1972), UDN was first described by W. G. Smith as the "salmon disease," and believed to be caused by *Saprolegnia ferax* (a decision with which Huxley, 1882a, b concurred).

Ulcerative dermal necrosis (it is the epidermis, actually, that is necrotized) is characterized (Carbery, 1968a, b) by the development of small, necrotic areas on unscaled regions of the head of salmon. Subsequently, these areas enlarge and ulcerate. Severe acantholysis of the desmosomes and the malpighian cells occurs, and the basement membrane is breached. With this event, the so-called terminal stage (R. J. Roberts and Bullock, 1976; T. Murphy, 1973) of UDN is reached. The necrotic areas in time then may become invaded by *Saprolegnia parasitica* or other water molds. The observations by Pyefinch and Elson (1967) on bleached areas of fish skin leave little doubt that such a visible symptom is not necessarily accompanied by fungal colonization.

It is the rapid invasion by fungi of UDN-infected fish that has complicated the search for a cause for the ulcerative necrosis. Indeed, for some investigators, the presence of fungus filaments was the only sign by which the disease could be recognized (Willoughby, 1968b), and these hyphae clearly were a feature of the disease in its advanced stages (Willoughby, 1969). It has been pointed out by Willoughby (1972) that the blanching symptom (changes in the melanophore cells) in UDN is like that associated with lesions where *Saprolegnia* Type I occurs. Earlier, however, Carbery (1968b) stated that it was possible to distinguish UDN complicated by secondary

mycotic infection from primary mycotic infections *sans* UDN. In saprolegniosis, the development of the fungus begins, Carbery pointed out, at various sites on the fish body and then spreads. UDN with secondary mycosis, however, is characterized by the inception of fungal growth only at the UDN lesion sites. According to Carbery (1968a), UDN infection begins on unscaled portions of the animal's body and the dermis ulcerates, while fungus infection can commence at any point on the individual's body.

Carbery (1968a, b), Carbery and Strickland (1968a, b), and O'Brien (1976) thought it possible that the etiologic agent of UDN was a virus. Elson (1968a, b), W. Meier, Klingler and Müller (1977), W. Meier, Klingler *et al.* (1977), and Munro (1970), clearly accepted the premise that water molds were secondary agents as did Reichenbach-Klinke (1974). On the other hand, having consistently isolated *Saprolegnia parasitica* (provisional identification) from lesions on fish suffering from ulcerative dermal necrosis, Stuart and Fuller (1968a) were unable to accept the view that water molds were only secondary invaders. They in fact isolated water molds from sites that others had considered to be premycotic UDN lesions. Reichenbach-Klinke (1974) has reported being able to provoke UDN symptoms by using mucus-dissolving agents, by inducing certain hormonal changes in fish, and possibly also by subjecting animals to water-borne poisons. Certainly this would be convincing evidence, if confirmed, that the cause was not some invading saprolegnian.

There is some biochemical evidence to suggest that UDN is not caused by saprolegnians. Hodkinson and Hunter (1970a) using antigens prepared from lesioned tissue and from the mycelium of an unidentified *Saprolegnia* induced antibody reactions in salmon. The production of anti-saprolegnian antibodies, they reported, was not necessarily related either to the colonization of the salmon by the fungus or to the UDN condition. Experimenting with polyacrylamide gel patterns of serum protein levels in salmon (with and without UDN or fungal infections), Mulcahy (1967, 1969) found that individuals showing the early stages of UDN infection had a serum protein pattern unlike those occurring in individuals showing late stages of dermal necrosis. However, serum proteins from fish infected with *Saprolegnia ferax* were similar to those found in fish showing these late stages of UDN infection. O'Brien (1974) injected filtrates from UDN lesions directly into healthy salmonids, and these individuals subsequently developed UDN. Neither bacteria nor fungi, alone or in combination, induced the disease.

Ulcerative dermal necrosis obviously is a complicated disease in which the role of several factors such as low temperature (R. J. Roberts, 1966, 1972; R. J. Roberts *et al.*, 1971), fungi (notably *Saprolegnia parasitica*) and some other agents (perhaps viruses) are involved. As R. J. Roberts (1972:60) has pointed out, the persistence with which *S. parasitica* hyphae are associated with UDN suggests that the fungus is "...much more than a mere opportunist." Indeed, to emphasize the nature of the association of fungus to UDN, Roberts proposed in 1972 that the disease should be designated as the ulcerative epidermal necrosis-saprolegniosis complex.

It would appear that the cause of UDN is yet to be uncovered, and its relationship to saprolegniosis still to be unraveled. The review by T. Murphy (1973)

and the several papers by Roberts and his colleagues are reference sources adequate for a full account of UDN: R. J. Roberts (1972), R. J. Roberts *et al.* (1971), R. J. Roberts and Bullock (1976), R. J. Roberts *et al.* (1969), R. J. Roberts, Shearer, Elson, and Munro (1970), and R. J. Roberts, Shearer, Munro, and Elson (1970).

CONTROL

Because fish disease is a topic of intense commercial interest, it is not strange that one is confronted with an extensive literature on methods to battle, avoid, or coexist with saprolegniosis. In accord with self-imposed restrictions, we are merely listing in this section some of the combative techniques, grouped into general categories. A review by W. Steffens *et al.*, appearing in 1962, provides a basic bibliography of control of saprolegniosis and other fish diseases. Earlier, Mellen (1928) published the results of a survey designed to determine what control methods were in general use, and R. C. Srivastava and Srivastava (1978b) have surveyed the use of several chemicals to determine effective concentrations.

Little has been done to explain how control methods actually perform their intended function. One exception worthy of being singled out for emphasis is Draggan's (1977) work on chromates. He noted that the mortality of carp eggs and the growth of saprolegnians attacking them increased coincidentally with the use of low concentrations of chromates to control the "parasite". He hypothesized that microorganisms (bacteria and microbial grazers) associated with the invading fungus were supported by metabolites diffusing from that fungus during its growth. In turn, the microorganisms functioned as a stress factor to limit the parasite's development. Growth of the bacteria and grazers, sensitive to and inhibited by low concentrations of chromates, became limited upon the application of the chromium-containing chemicals, and substantial fungal growth thus was "allowed." High concentrations of chromates, on the other hand, acted directly to inhibit the parasitic fungus.

Salt: -- Inasmuch as saprolegniosis was early associated (W. G. Smith, 1878a, b; Stirling, 1880a, b, for example) with salmon migrating into freshwater from the sea, the use of ordinary salt (or sea water) was among the first of the methods proposed to combat the disease. Often, the application of salt either directly onto the diseased part of individual fish, or as a solution in which to bathe the animals was combined with some other method such as removing dead animals or eggs, and possibly burning them (C. G. Atkins, 1897; H. S. Davis, 1953; H. S. Davis and Lazar, 1940; Schneberger, 1941; Tempère, 1904), or using an additional chemical agent. The following publications are an adequate sampling of control methods by application of sodium chloride, sea water, or brine: Armistead (1891; said always to be effective unless the invaded animal's gills were infected), Astakhova and Martino (1968; an unsuitable method), C. G. Atkins (1897), Benecke (1886), Clinton (1893), H. S. Davis (1946), Franke (1908: 922), Gerard (1879), Gopalakrishnan (1963), Gopalakrishnan and Jhingran (1972), Hoshina *et al.* (1960; chlorine), Maurizio (1897b), Mawdesley-Thomas and Jolly (1967), Nakamura

(1962), Rice (1884), Seagle (1897), Spencer (1908), Stirling (1880b), S. G. Taylor and Bailey (1979). Perhaps salting as a control measure was best summed up by E. M. Robinson (1894) in quoting from an unidentified source to the effect that "the price of all fish is eternal vigilance," to which he added "and a free use of salt."

Malachite Green: -- The weakly basic, diamino triphenyl methane dye malachite green is perhaps the most popular and widespread chemical recommended and used for the control of fish diseases, including saprolegniosis. Malachite green was first reported as an effective agent by Foster and Woodbury (1936), and subsequently was utilized in several concentrations and applied by a variety of methods. The dye has also been recommended for use concomitantly with another antifungal agent, formalin (Oláh and Farkas, 1978). Nelson (1974) published a review of the chief literature on malachite green as a control agent.

The chemical evidently is not without its own harmful elements. For example, Lieder (1961), among others, regarded malachite green as both a carcinogen and mutagen. It was W. Steffens and his collaborators (1962) who pointed to chromosomal aberrations in fish resulting from the use of the dye. Some strains of water molds implicated in saprolegniosis supposedly are more resistant to malachite green than are others, according to Arasaki *et al.* (1958a, b).

A sampling of accounts that report experimental work with malachite green and treat the practical aspects of its application are: Allison (1953), Astakhova and Martino (1968), Bootsma (1973), Burrows (1949), Cummins (1954), Gottwald (1961), Hatai and Egusa (1977), Herman (1970, 1972), Hodgkinson and Hunter (1970b), Hoshina (1963), Hoshina *et al.* (1958, 1960), H. E. Johnson *et al.* (1955), Knittel (1966), D. L. Koch and Contreras (1973), Lennon (1955), R. L. Martin (1968a, b), Merriner (1969), Nakamura (1962), Noguchi and Tsunokai (1958), O'Donnell (1941, 1944), Poupard (1978), Rankin (1953; fin rot), R. J. Roberts and Shepherd (1974), Sakowicz and Gottwald (1958, 1960), Scott and Warren (1964), Sharp *et al.* (1952), G. C. Srivastava and Srivastava (1978a), R. C. Srivastava and Srivastava (1978b), Suchebyanu (1966), Wood (1974), Wright (1976).

Bauer and his collaborators (1973) recommended against the use of malachite green. Another common dye, methylene blue, has been suggested as a suitable control agent in certain concentrations (Gottwald, 1967; Hatai and Egusa, 1977).

Formalin: -- Fish (1944) considered that formalin had not been proven to be a safe chemical to use in the treatment of fish diseases. Sills and Allen (1979), on the contrary, could not detect any free formaldehyde in the musculature, liver, or blood plasma of four species of fish exposed to a solution of 300 $\mu\text{g L}^{-1}$. Nevertheless, the compound has been employed in some treatments even though its effectiveness is suspect, and it may have detrimental effects on the patients it is intended to cure. Selected references are: Astakhova and Martino (1968), Bauer *et al.* (1973), Bootsma (1973), Clemens and Sneed (1958), Cline and Post (1972), Herman (1970, 1972), Ryder (1882), W. Steffens (1962), R. C. Srivastava and Srivastava (1978b), Tanaka (1935), Watanabe (1940), Wright (1976). See also review by Schick (1973).

Other Chemicals: -- The kinds of chemical agents that have been experimented with as possible control materials are legion: aryloxyalkanols, various antibiotics (amphotericin, acriflavin, penicillin, nalidixic acid, and others), mineral salts (copper sulfate, notably), glutaraldehyde, Dexon and a variety of like fungicides, DDT, permanganate, merthiolate, hydrogen peroxide, chromates, iodine compounds, and 6-hydroxymehtyl-2-[2-(5-nitro-2-furyl) vinyl] pyridine, to name a few. Representative accounts of the use -- or avoidance -- of such chemicals are those by Ali (1968), Auld and Schubel (1974), Bauer *et al.* (1973), M. J. Berkeley (1864), Bootsma (1973), Cline and Post (1972), Conroy and Vasquez (1976), Coutière (1900), Detwiler and McKennon (1929), Draggan (1977), Duijn (1956, 1967; recommended against the use of any mercuric compounds), Estes (1957), Gopalakrishnan (1963), Gopalakrishnan and Jhingran (1972), Herman (1972), Hodgkinson and Hunter (1970b), Hoshina *et al.* (1960), R. L. Martin (1968a), Maurizio (1897b), Mawdesley-Thomas and Jolly (1967), Mayer (1976), Mekrani (1980), O'Donnell (1941, 1944), Oláh and Farkas (1978), Reichenbach-Klinke and Elkan (1965), Ross and Smith (1972), Rucker (1944), Schneberger (1941), Scott and Warren (1964), Shimizu and Takase (1967), Snow (1972), Spencer (1908), R. C. Srivastava (1976), Stokoe (1966), Vasconcellos and d'Oliveira (1947), Warthmüller *et al.* (1932), Wold (1950), Wright and Snow (1975).

Miscellaneous Methods: -- A sampling of the great variety of methods employed to control saprolegniosis would not be complete without reference to Valette St. George's (1878) observation that attempting to brush away the fungus on adult fish and eggs was wasteful of time. Several investigators essentially recommended control through avoidance, that is, by proper handling of the animals themselves, or by establishing and adhering to sanitary measures: Duijn (1956), Holder (1908), Woynárovich (1959), and Snow (1972). Ozone has also been tested as a therapeutic method (Benoit and Matlin, 1966; Dodge, 1895; Zirzow, 1908), as has ultraviolet (Bauer *et al.*, 1973; Astakhova and Martino, 1968; Kokhanskaya, 1970; Vlasenko, 1969). Shackley and King (1978) devised a circulating seawater system in which the water could be sterilized by ultraviolet light. Eggs could be cultured in jars in this system without danger of contamination by fungi (including water molds). Vlasenko (1969) concluded that irradiating water in egg-rearing portions of hatcheries was an efficient and commercially feasible method of ridding the water of parasites and pathogens, including the spores of *Saprolegnia thureti* (=ferax). While the planonts of this species were highly sensitive to UV [coefficient of resistance (K) = 5850 $\mu\text{w} \times \text{sec cm}^{-2}$], they could be photoreactivated after being subjected to near-lethal doses. One of the most novel approaches to control of saprolegniosis of fish eggs was reported by Oseid (1977). *Gammarus pseudolimnaeus* Bousfield, by its feeding habits, prevented the growth of *Saprolegnia* sp. from occurring, and individuals of *Asellus militaris* Hay fed on the fungus as well. Oseid concluded that both of these amphipods eliminated fungus-related egg mortality.

THE HOST RANGE

The literature on saprolegniosis makes it unavoidably clear that three elements have figured into the accumulated knowledge of host range of the various water molds implicated in the disease, namely, natural infections, artificial inoculations, and reports of water molds on dead fish and other aquatic animals. The latter of these three elements we dismiss arbitrarily as being meaningless for host range determinations. Mikheeva's (1969) account is a case in point. He reported species of water molds to occur on such substrates as dead roach, chironomid larvae, catfish eggs, and individuals of *Daphnia*, *Bosmina*, and *Cyclops*. He concluded that although these fungi (*Achlya* and *Saprolegnia* species, including *S. parasitica*) were found on animal substrates they contributed only to decomposition (and disposal) of the organic remains. Whether ranges based on inoculations in the laboratory -- often with the added assurance of positive results through prior wounding (Mekrani, 1980; G. C. Srivastava and Mekrani, 1980b; G. C. Srivastava and Srivastava, 1976a, 1977a-f, for example) -- should be considered truly reflective of pathogenesis is a debatable point.

Several listings of hosts for saprolegnians have been published (as have lists of fungal agents) notably those by W. N. Tiffney (1936, 1939a), Scott (1961b, 1964), Scott and O'Bier (1962), Scott and Warren (1964), J. G. M. Wilson (1976), and Wolke (1975). The ranges have been determined both from the collection of naturally infected fish and from animals purposely inoculated with water molds. Some investigators such as Monsma (1936) experimented at length using inoculations of a variety of hosts with an assortment of fungi. Water molds that generally are recognized as saprotrophs also have been used in pathogenicity studies (Nolard-Tintigner, 1974), and various methods for determining pathogenicity have been devised (R. C. Srivastava, 1976, among others). There seems little doubt that differences of opinion on potential pathogenicity of some water molds -- G. L. Hoffman's report (1949) that *Achlya racemosa* was not pathogenic as one example -- have arisen because methods of study are not standardized. Host range determinations, then, may at best be approximations.

In spite of obvious limitations, and recognizing an inherent degree of artificiality, we are including in Table 47 a partial host range pared of some elements, but including a sufficient coverage of the literature to illustrate breadth of the participants, hosts and fungi. The names of hosts are those given by the respective authors that are cited.

APHANOMYCOSIS

An incompletely known *Aphanomyces*, *A. pisci* (see systematic account), has been implicated by R. C. Srivastava (1979a) in an infection of *Cirrhinus mrigala* Hamilton. The fungus causes blackening of the scales, a symptom that is followed by exfoliation. Srivastava believed that the disease caused by *A. pisci* is not the same as saprolegniosis, but it is not entirely clear on what basis this decision was made. Aphanomycosis is alleged to be difficult to diagnose in the early stages of its development, but exfoliation seems to be a distinctive feature of the disease in its later stages. Artificially wounded

adults of *Puntius sophore* and *Colisa lalia* died within 6-8 days after being inoculated with *A. pisci*, but exfoliation was not a symptom in these species.