ON THE ECOLOGY OF THE SAPROLEGNIACEAE

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The aquatic environment has been examined throughout the world by numerous investigators for the identification of isolated fungal species. Journal literature holds many reports in taxonomy, morphology, anatomy, genetics, nutritional requirements, and related studies on the fresh water fungi, however, much less attention has been directed to the environment of the fungi in terms of microbial ecology. An attempt is made here to view the environment of the filamentous species with emphasis on definitions of microbial ecology.

General Description of the Saprolegniaceae: The Saprolegniaceae is the largest family in the order Saprolegniales. The terms 'water mold' and 'fish mold' are ordinarily referred to the members of this family. Although the designation 'water mold' suggests that the Saprolegniaceae occur exclusively in water, investigations have shown other habitats. Numerous species have been isolated from the soil. They are widely distributed and are among the most ubiquitous of aquatic fungi.

The mycelium of Saprolegniaceae is a variably and profusely branched system of tubular, nonconstrictional coenocytic hyphae, easily visible as it forms a colony around some bit of decaying organic matter, either animal or plant, in water. Structurally, the hyphal walls are composed mainly of cellulose. No septa are formed except in the mycelium just below the reproductive organs and in the delimitation of gemmae (chlamydospores).

Asexual reproduction is effected by zoospores in zoosporangia which are typically long, cylindrical, and terminal. Zoospores generally mature prior to the formation of the sexual reproductive organs. Sporangial proliferation is an interesting phenomenon characteristic with the representative genera. It may occur in various ways. In the genus Saprolegnia it takes place as follows. When a sporangium has emptied its contents of spores, another or secondary sporangium is often initiated at the basal septum, and grows through the first sporangium, maturing within the other, each maturing and shedding its spores before the next one is formed. As a general rule, the sporangia of the Saprolegniaceae remain attached to the somatic hyphae throughout their lives. The genus Dictyuchus is an exception, the sporangia commonly fall off the hyphae at maturity.

Zoospores in the Saprolegniaceae are biflagellate. Two zoospore types occur in this family. One is the primary zoospore which is pear-shaped with the flagella at the apex. The other is the secondary kidney-shaped zoospore with two oppositely directed flagella at the concave side.

Diplanetism is the successive formation by a single fungus of 2 different types of zoospores, whereas monoplanetism has only one swarming period and only the primary type of zoospore. In addition, the form of sporangial proliferation and the behavior of zoospores are of great diagnostic importance in distinguishing genera.

Sexual reproduction is accomplished by the formation of oogonia and antheridia. One or several uninucleate eggs (oospheres) are formed in each oogonium which is usually a terminal structure on a hypha. The antheridia are elongated and multinucleate. Four types of antheridia characterized by their point of origin may be distinguished in this family (Ivankow, 1971 and Seymour, 1970).

Fertilization takes place through gametangial contact, the passage of the male gametes into the female gametangium through a fertilization tube. Fertilized oospheres develop into thick-walled oospores. In the mature oospores the fatty reserve is stored in the form of oil droplets characteristically arranged in different species. These arrangements are of considerable taxonomic value. After a period of rest the oospore germinates to produce a tube which gives rise to a zoosporangium typical of the species.

Both homothallous and heterothallous species can be found in the family. Raper (1936, 1949) demonstrated conclusively that at least four distinct hormones are involved in the initiation of sexual organs when potentially male and female thalli grow in close proximity.

Composition of the Community: Constituents of a community are the organisms inhabiting a given site. Members of the Saprolegniaceae are found in most bodies of fresh water. The genera belonging to the Saprolegniaceae have been identified in taxonomic reviews by Coker (1923) and Ainsworth, et al. (1973). A minimum of 10 genera belong to the family, however, more representatives may be found in the literature depending on the reference. Some species are in question due to similarities in established organisms.

In regards to the dominant species of the community, Achlya and Saprolegnia are the two genera which exhibit large populations in water, and they are also predominant on the special host habitat of fish. Tiffney and Wolf (1937) reported in observations from a fish parasite study that Saprolegnia parasitica is the primary parasitic form. At times A. flagellata becomes parasitic and causes severe losses of fish.

Interaction Between Organism and Environment: Environmental factors such as temperature, light intensity, O_2 tension, water level and quality, and substrate are all important to the development of the community composition. In the various papers of Suzuki

(1960a-d, 1961a-f, 1960 w/T. Hatakeyama), it is evident that under field conditions, temperature has a profound effect upon zoospore formation; instances of lowered zoospore production due to unfavorable temperatures are cited. It has been noted that no water molds have been isolated from thermal springs (Cooney and Emerson, 1964). Zoospores are the most efficient unit in establishing and distributing the organisms of the family Saprolegniaceae. Absence of the zoospores of species in an ecosystem usually correlate to the populations in the community. Seasonal occurrence of species is regarded as an environmental selection due primarily to changes in temperature. In spring most species grow well, only a few species can be collected in other seasons (Coker, 1923).

Oxygen is a very important requirement for growth and development for the majority of members belonging to this family. The variation in the correlation of fungi with the oxygen dissolved in the water layers has been reported (Suzuki, 1961a). Achlya flagellata, A. racemosa, Dictyuchus sp., Aphanomyces sp., and Saprolegnia sp. were obtained when the bottom layer of water contained oxygen. Laboratory studies by Suzuki confirmed these results. He reported that the bottom of a lake of the Nikko volcanic group was characterized by jet black reduced mud "resulting in the removal of oxygen from the contacting water" (Suzuki, 1961e). Here aquatic Phycomycetes were very scarce, and Pythium sp. was the only isolate from such a habitat. In anaerobic layers of a lake bottom, Aphanomyces was the only fungus of the Saprolegniaceae found in this extreme condition. The diurnal migration of the zoospores distribution is also considered to be influenced by O_2 (Suzuki, 1961b). An increase in O_2 level will increase the zoospore population.

Water or moisture and nutritional factors also influence species selection. Extremely dry conditions will kill all of the zoospores as well as the hyphal filaments. Low pH and high mineral content deplete saprolegniaceous fungi in various habitats.

The inhabitants in turn modify the composition of their surroundings. Metabolites of the existing members of the community will influence the chemical composition of their surroundings. Some groups are favored by environmental changes and assume greater prominence, while others are not able to cope with the new circumstances. Population density and composition of the community are thus affected. In laboratory conditions, some saprolegniaceous fungi produce metabolites which are toxic to the same fungal species thus inhibiting growth.

Species Diversity: Species diversity clearly varies from ecosystem to ecosystem. It is reported that small populations and great species diversity seem to be associated with nutrient deficiencies. When there is an inflow of nutrients, species diversity frequently declines, for a nutrient-rich area is poor in microorganisms (Harvey, 1942). Zoospores of the Saprolegniaceae may easily be injured or killed if the total osmotic concentration of the medium is high.

Other conditions such as seasonal effects are responsible for fluctuation in the intensity of an ecological variable. Suzuki (1961a) reported that Saprolegnia monoica, Achlya racemosa, and A. flagellata were found flourishing in winter whereas S. diclina was found throughout various seasons. It was found that Saprolegnia and Achlya occur below the sewage effluent in a stream, whereas species of Aphanomyces were restricted to the cleaner portions of the stream above the entrance to the effluent (Reischer, 1951). Environmental factors previously described would certainly influence the presence of species in an ecosystem as well as the density of existing species.

Habitat: Because the Saprolegniaceae is ubiquitous in distribution, representative species have been found in nearly every aquatic habitat previously investigated such as ponds, streams, stagnant pools, aquaria, sinkholes and the like. Roberts (1963) in a study of Saprolegniales from 21 natural waters in the United Kingdom found that typically more species were collected from the bottom than from surface waters. However, Suzuki (1961f) noted that during the stagnation period in lakes, aquatic fungi were scarce on the bottom. Some vascular plants, fish, and fungi provide habitats for the parasitic Saprolegniaceae. The composition of these habitats is relatively uniform. However, habitats change with food or organic material intake by the animal. The species composition of the residing organisms are thus affected. Some pink salmon in rivers have recovered from disease induced by Saprolegnia when the salmon were transported to marine water (Ivankow, 1971).

Dispersal: Dispersal is essential for the continued existence of many species. This is particularly true for parasites that are capable of independent existence in vitro, yet they are restricted in nature to life in association with a suitable host. In such instances individuals of the species must escape from the locally detrimental environment and find a new habitat conducive to the continued existence of the species. The lack of means of dissemination could mean the elimination of the species.

Dissemination may occur by virtue of active movement or growth, or it may result solely from the passive transport of the microorganism by means not under its own control. On the other hand, if the microorganisms are not able to escape in space from unfavorable locales, they have a mechanism for an escape in time: a structure allowing the species to endure adversity such as oospores or gemmae.

Unit of Dispersal: Zoospores are typically produced as an asexual means of reproduction in species of Saprolegniaceae. Two types of zoospores occur, i.e., primary zoospores which are pear-shaped, and secondary zoospores of a kidney shape. The behavior of the zoospores varies in different genera and thus serves as an important taxonomic character. The typical manner and activity of zoospore liberation from zoosporangia, variation in the discharge and behavior of the spores are well described by Coker (1923). He concluded that the variations are the results of environmental conditions.

Under normal environmental conditions, the zoospores of Achlya encyst immediately after emergence at the mouth of the sporangium in the form of a hollow sphere. The zoospores undergo a second motility period which is followed by encystment and germination. This zoospore development and germination sequence is fairly constant, although under varying culture influences minor changes have been reported (Collins, 1920 and Lechmere, 1911). If the concentration of specific nutrient present is above a certain threshold, the encysted spores germinate by a small tube which eventually forms mycelium but if the quality of nutrient material is below this value, then the encysted zoospore emits an active laterally biflagellated spore for a third motility period. Three, four or five motility periods can occur with each zoospore with environmental manipulation.

"Selective power" of fungal zoospores for suitable substrates was studied. In Achlya (Salvin, 1940), if a zoospore on emerging from the encysted condition is unable to find a suitable substrate, it can pass again into a resting state, and then once more emerge as an active entity until it finally finds a favorable substratum to locate and start a new colony. This kind of phenomenon was also observed in Dictyuchus (Weston, 1919). This rejuvenscence of motile cells is dependent upon stored energy, and also on reserve food material. Weston also found that Thraustotheca clavata was unable to form the repeated emergence of zoospores under similar conditions. It appears that some inherent factor may control this phenomenon.

Chemotactism was long ago shown to be characteristic of aquatic Phycomycete zoospores. Fischer and Werner (1958a,b, 1955) found that chemotactic sensitivity of saprolegnian zoospores was largely dependent upon their age and solutes in the medium. Alkaline metal chlorides and alkaline earth metal chloride such as $CaCl_2$ and $MgCl_2$ are positively chemotactic substances. Protein freed of the dialyzable fractions had no tactic effect. Very small amounts of different amino acids function to increase the chemotactic activity of the aforementioned salts. Solutions containing primarily NaCl and KCl and traces of amino acid mixtures induce both the chemotactic attraction of zoospores to natural substrate and their subsequent encystment.

Other environmental factors, such as temperature and oxygen tension also play an important role in determining the success of zoospore liberation. Turbidity of the water was found to affect the spore dispersal (Petersen, 1910). When silt is in suspension in water, zoospores are prevented from reaching a proper substratum.

Gemma or chlamydospore is another kind of propagule found under environmental stress, such as dry conditions and high temperatures.

Certain parts of the hyphae give rise to gemmae. Upon the advent of favorable conditions, the gemmae germinate to produce either hyphae or short stalked zoosporangia. At 27°C, Saprolegnia ferax produced only gemmae, whereas sexual organs were not formed. Efficiency of dispersal is operated by three factors in the fresh water fungi. Repeated formation of motile cells occurs until a hospitable environment is reached. Formation of resistant propagules or gemmae assist in species survival. The release of a vast number of spores increases the chances of locating a suitable substratum for the organism.

Colonization: Changes in species composition of ecosystems invaded or inhabited by microorganisms are seen in numerous places. The innoculation of a medium initiates a dramatic sequence of changes in the types of organisms present. This kind of change in response to the modification of environment results from a successful colonization.

Colonization and the Pioneer: The development of a colony of mycelium of Saprolegniaceae in a natural habitat is usually initiated by zoospores. Germination of zoospores begins when a suitable substratum is invaded. Growth and development of hyphae are followed by the formation of a colony or colonies.

If the substratum is initially devoid of saprolegniaceous fungi, a succession is initiated by the first invaded species, the pioneer species. In general, the pioneers have a transitory existence and are rapidly eliminated. As they grow, they influence their surroundings by secreting or producing some substances which are toxic or unsuitable for themselves. Later species arrive as the surroundings are modified. Some species of Saprolegniaceae in laboratory storage need an early or frequent transfer of cultures due to the availability of nutrients and water.

Factors Favorable to Colonization: The substratum character and availability are influencing factors for colonization of heterophilic organisms. Submerged twigs, floating fruits, dead insects, dead vertebrates and invertebrates, and other organic debris have been found covered with saprolegnian mycelium in ordinary aquatic habitats. These nutritional preferences appear in nature but may not be so evident under laboratory conditions. The growth requirements of nitrogen, sulfur, and carbon are available from many amino acids and carbohydrates. Both O_2 and moisture are critical factors for zoospore dispersal. An efficient means of zoospore dispersal undoubtedly favors colonization if the substratum is not in question. Other physical factors such as temperature, pH value, etc. also influence colonization.

In parasites, suitable hosts and mechanical characters of the host are important for the initiation of an infection. Wounds on the bodies of hosts such as fish are required factors for the parasites (primarily Achlya and Saprolegnia) to have a successful infection. Saprolegnia sp., Achlya bisexualis, and A. flagellata have been placed in the category of wound parasites (Tiffney and Wolf, 1937 and Vishniac and Nigrelli, 1957). Artificial factors such as a direct innoculum to a proper substratum would result in growth of the fungal species. For example, hyphal colonies rapidly develop on corn meal agar.

Under natural conditions, hyphal colonies of Saprolegnia and Achlya are frequently found in pure culture. The hyphae grow in a radiate form spreading from a central point. Mycelium of one colony is usually found with similar characteristics for they usually are the same species. Such findings may explain the fact that already well established colonies produce chemical substances that inhibit the growth of other microorganisms. The removal of nutrients necessary to other forms and the simple fact of taking up space by one species limits the introduction of other species into a community. Achlya flagellata found as a parasite was the only species isolated from the water inhabited by diseased trout (Sorenson, 1964). The established community is among the most effective barriers against invasion by new arrivals.

The absence of carbonaceous nutrients in habitats, anaerobic conditions, extreme drought, low pH, extreme high temperature, etc. are physical environmental conditions that are beyond the tolerance range inhibiting the growth and development of a species in a community. Chemical barriers in hosts may be present. The mechanism and reason of some hosts immunity to the parasites are not known. The immunity of fingerlings of brown trout and rainbow trout to Achlya flagellata (Tiffney and Wolf, 1937), however, might be due to a chemical barrier of an unknown substance or substances present in fingerlings. Adult fish, however, are susceptable to the fungus.

Mechanical barriers establishing environmental resistance can be found in fish hosts. It is reported that wounded fish are invaded by Achlya and Saprolegnia (Sorenson, 1964, Sparrow, 1960). The skin of fish serves as an excellent mechanical barrier for the colonization of parasites. The gill region also serves as a suitable invasion area for representative species of the genera.

Environmental Feedback: An environmental feedback is a modification of the habitat resulting from the presence of one or more microbial populations. The change can affect the size, activity, or survival of the invading population or of one or more segments of the community. It is a dynamic component of the environmental resistance of the suscept. The antagonistic action among the saprolegniaceous species and Fungi Imperfecti has been reported. Of the antagonists, 46.9% had inhibitory responding action on Saprolegniaceae. Penicillium rugulosum depressed the growth of all tested organisms (Raper, 1936). The amount of concentration of the antibiotics produced, and the properties of the invader have a profound influence on the ecological success of an invader. Environmental feedback may also be attributable to the isolation and immune mechanisms of the host.

Succession: Succession is characterized by the shifts both in species in the community and in the relative abundance of the resident species of a community. A successful colonization would initiate a succession. The areas where heterotrophs were previously unable to colonize is usually inhabited by autotrophs such as green algae. As they grow, they influence the surroundings by providing some organic substances which are necessary for the growth of heterotrophs. Then pioneer organisms invade the habitat creating colonization. Once colonized, wave after wave of populations appear and recede. The pioneer community continually modifies its surroundings which are now becoming more suitable for growth of new species. At this situation, succession occurs. The reciprocal interaction between the microbial and non-microbial components of the ecosystem ultimately leads to a form of sterilization. This final microbial assemblage in the area is known as the climax community.

Evidence of succession can be found in laboratory gross cultures. Pond water samples are baited with halved hemp seed. Within a period as short as 48 hours, there may be an explosion of a single species of Achlya or Saprolegnia. In time this population is succeeded by another, or several others until activity seems to be at a standstill.

Suitable substratum, abundant oxygen, and temperature are the most influential environmental factors for a successful succession as well as colonization. Petersen (1910) and Lund (1934) emphasized that Phycomycetes are found in calm water since there is little violent wave action, little mechanical damage, and near the shore where oxygen is available in abundance. Constant rolling of substrata by wave action close to shore will no doubt eliminate a substratum of Phycomycetes. The composition of the community is thus indirectly modified. Temperatures and oxygen tension are more or less variable in different depths of water. In warm (22-27°C) surface layers down to 2 meters with an oxygen content of 100-110%, a strong colonization of various bait by Saprolegnia, Achlya, Dictyuchus and Aphanomyces occurred. Below this depth at 4 meters lies a zone of 15°C water with diminished oxygen content. Here only a moderate degree of colonization of bait occurred and no fungi with Saprolegnia-like discharge were recovered. Only Achlya-type of discharge occurred at 4 meters.

Seasonal variation of the composition in a community has been studied. The appearance of characteristic species in different seasons is attributable primarily to the fluctuation of temperature. It was found that Saprolegnia spp. were dominant in spring and Achlya spp. in autumn (Hughes, 1962). Barrier and environmental feedback previously described determine the course of a progression. Other factors relating to colonization would, of course, influence the succession.

The Climax Community: At the climax community the species composition is maintained reasonably constant with the passage of time. Nevertheless the climax may be modified from time to time as the ecosystem is exposed to drastic exogenous forces. Water molds in pools and lakes may be subjected to drought. The climax community in the lower water layers is less influenced by exogenous conditions compared with the surface. Environmental conditions in the bottom area are as a rule more steady which is important for a climax community.

Modification of the climax may be recurrent. The seasonal distribution of Saprolegniaceae is a recurrent process. On the other hand, when the environment becomes irreversibly altered, the climax is deflected and shifts to a steady state. To maintain the climax, energy must be supplied either frequently or continuously. In conclusion, the evidence for the occurrence of ecological succession in Saprolegniaceae has not yet been well recorded and assembled in the literature.

Nutrition: The members of a community obtain their nutritional elements or compounds primarily from their surroundings. From these elements and compounds they synthesize their cellular constituents and obtain energy necessary for their life processes. The nutritional requirements for growth of some species of this family have been investigated and a general picture is beginning to appear (Papavizas and Davey, 1960; Reischer, 1951; Whiffen, 1945). The requirements may be few in number. In general, micronutrients were essential for growth whereas vitamins were not. Glucose seems to be the best source of carbon for most species. Maltose, starch, and glycogen are available to several while fructose, mannose, sucrose and ethanol are utilized by some. Most of the Saprolegniaceae cannot utilize nitrate nitrogen under any condition, but in some investigations it appears that some species are able to utilize ammonium nitrogen as NH_4Cl or NH_4NO_3 under optimum pH range (5.4-6.5) (Sorenson, 1964). In general the Saprolegniaceae grow well on media containing organic nitrogen in the form of peptone or any one of the amino acids. Inorganic requirements include Mg, Ca, Zn, Mn, Fe, and S. Sulfate cannot be utilized, but sulfur can be supplied in organic form as cysteine, cystine, glutathione, methionine, or inorganic sulfide.

Because most fungi are saprobes, they depend on dead organic matter for nutrition. In nature, the greater abundance of individuals in open sunny marshes and ditches is probably due to the large amount of organic substances in such places. In collecting the species, the "baiting" technique has usually been employed. It is widely known that hemp seeds would be the most suitable bait. Hemp seed not only contained sufficient stored food material for growth of saprolegniaceous fungi but the seed also has further advantage in reducing culture contamination, especially bacteria (Harvey, 1925). Hemp seeds are commonly used for the collection, propagation, and maintenance of aquatic isolates.

Various isolation techniques were developed by workers (Johnson, 1956 and Raper, 1937). Natural media and synthetic media have been used. Natural media included: corn meal agar, potato dextrose agar, malt agar, yeast extract agar. A medium of glucose glutamate agar (Seymour, 1970) was adopted as a synthetic medium for isolation of Saprolegnia.

Patterns of Nutrition: Most of the members of Saprolegniaceae are saprobes, however, some deviate from these nutritional requirements. A few species are important parasites. Some species of Saprolegnia, i.e., Saprolegnia parasitica, cause disease of fish, fish eggs, and frog eggs. Some Achlya species had been reported as fish parasites. Olpidiopsis is a genus causing diseases to some members of this family. Aphanomyces contains several destructive parasites to the roots of vascular plants.

Undoubtedly, all of the parasitic species obtain their organic nutrients from their hosts. As a general rule, they can be isolated and grown on natural media or on synthetic media containing beef extract, yeast extract, or peptone (Sparrow, 1960). With most fungi, exhaustion of the food supply favored sporulation (Lilly and Barnett, 1951). Klebs (1899) kept a culture of Saprolegnia mizta in the vegetative condition for 2½ years by constant renewal of the nutrient solution. Yet this fungus produced spores within a few days when the food supply became exhausted. Factors affecting the nutritional uptake include enzymes, hydrogen-ion concentration, and other biotic factors in the ecosystem. The limiting factors remain uncertain.

Tolerance Range: Each species grows, reproduces, and survives within a definite range of external conditions which represent its tolerance range or ecological amplitude for critical factors. The environmental factors (abiotic factors) controlling the distribution and growth of the community of Saprolegniaceae include temperature, moisture, light, oxygen, hydrogen-ion concentration (pH), salinity, and other inhabital factors.

Temperature: Of all the various factors operating at a site, temperature obviously is of great importance. It acts directly upon such processes as germination of overwintering structures for vegetative growth, and for induction of asexual reproduction by means of zoospores. In the various papers of Suzuki, it is evident that under field conditions, temperature has a profound effect upon zoospore formation, and instances of lowered zoospore production due to unfavorable temperatures are cited. Temperature also affects zoospore activity (Salvin, 1941). It is indicated that at 10°C primary zoospores swarm abnormally, some of them (in Saprolegnia) encysted almost immediately after emerging from the zoosporangia, others whirled about in narrow spirals, or rotated their anterior ends slowly while the posterior remained stationary. At another extreme of 30°C, primary zoospore activity was also abnormal. The zoospores, instead of swimming in a relatively straight line, circled, spiraled, twisted, and carried on vigorous vibratory movements. The phenomenon of fusion of two zoospores was found under this high temperature extremity. It was also reported that the encysted spores, whether within or without the sporangium, mostly ceased their motile activity.

In a comparative study of the primary and secondary zoospore rate of Saprolegniaceae, Salvin (1941) reported that the secondary spore typically carried on normal activity over wider extremes of temperature than the primary spore. The optimum temperature of primary and secondary zoospores is 15-25°C and 10-30°C respectively. It was also shown that the secondary zoospore progressed at a more rapid rate than the primary under a given temperature (25°C). The author explained the faster rate may be due to a higher rate of O_2 intake compared with the primary spore.

Numerous investigations of the temperature effect on formation of oogonia and antheridia in this family have been made. Barksdale (1960) reported that the induction of sexual organs, both on single thalli and on paired thalli, is temperature-dependent. Sexual organs were initiated at 15-20°C in many strains of *Achlya* that were usually sterile at 25°C. Another report from Szaniszlo (1965) indicated that in continuous darkness 21°C was the highest temperature for the formation of oogonia, while temperature range 18-20°C was optimum. Some other temperature ranges (20-30°C) for the oogonial formation were also found (Milanez and DoVal, 1969 and Krause, 1960). The optimum temperature for producing sexual organs of one of the strains of *A. ambisexualis* lay between 28-30°C.

The effect of temperature upon interthallic sexual reactions was investigated (Barksdale, 1960). At 20°C in most of the paired cultures, oospores were produced, but not at 25°C. One exception was that in *A. bisexualis* male strain and female strain, temperature as high as 30°C exerted little effect on the nature of the sexual reaction. In conclusion, lower temperature seems to favor the formation of sexual organs in *Achlya* (Peterson, 1910).

It has been suggested that *Achlya* grows best in cold water and completes its life cycle, whereas at high temperatures in seasons of bright sunlight, it has difficulty competing with algae and in forming sex organs (Sparrow, 1973). In laboratory conditions, cultures of Saprolegniaceae are always incubated at 18-22°C which would undoubtedly be the optimum growing temperature of these fungi. Powell *et al.*(1972) reported in their study of *Saprolegnia parasitica* that excellent growth occurred between 15-30°C. Stock cultures can be preserved at the temperature as low as 5°C in a certain stock medium. The minimum and maximum temperature for growth and asexual spore formation of the species *S. mixta* has been recorded as: growth, 0-1 to 36-37°C; asexual spores, 1-2 to 32-33°C.

Petersen (1910) thinks that freezing for a short time need not have a deadly effect on the mycelium, but that freezing for a long time is absolutely destructive. Coker (1923) found that in all but one of the species (Pythiopsis cymosa), a culture allowed to freeze solidly overnight is killed except for the eggs.

Moisture or Water Level Availability: The members of the family Saprolegniaceae are ordinarily referred to by the term 'water mold'. It suggests that water is a very important environmental factor affecting the growth of these fungi, though numerous species have been isolated from soil. When the growth habitat dries, gemmae instead of zoosporangia are produced. No mycelium or gemmae in any species can survive in a rather dry habitat. Experiments revealed that mature eggs may resist drying. Petersen (1910) made efforts to secure cultures from dry materials but failed.

Light: Although light for most fungi growing in water does not seem to be an important factor, laboratory studies show otherwise in some instances. Szanisalo (1965) indicates that light totally or partially inhibited the production of oogonia in Saprolegnia diclina, but did influence the mycelial growth.

In continuous darkness, 21°C was the highest temperature for oogonial formation. Lower light intensity that suppressed oogonia in culture maintained at 20°C was between 17 and 22 ft.c. A repeated 250 ft.c. photoperiod of 10 hours duration each day also caused inhibition, but daily photoperiods of 2 hours did not do so. Blue and green spectral range completely suppressed the formation of oogonia rudiments in S. ferax has been reported (Krause, 1960 and Szanisalo, 1965).

Oxygen: Quite predictably oxygen proved to be a major factor in determining which fungi will grow in water. For a variety of reasons natural habitats vary greatly in the amount of oxygen available. Dissolved oxygen in a lake, for example comes principally from the atmosphere through the exposed surface of water, and from photosynthesis of green plants. Members of the Saprolegniaceae typically require an abundance of oxygen. Most species of water molds are incapable of growing in a culture bottle tightly sealed which contained the ordinary growth media. Growth is rapid when cotton plugs are used instead of the plastic screw caps. Oxygen seems to be one of the limiting factors of these organisms.

The diurnal migration of zoospores (Suzuki and Hatakeyama, 1960) of aquatic fungi in a shallow (50 cm) lake was closely correlated with the distribution of the dissolved oxygen. On a clear day in the morning the zoospores gather at the surface, where oxygen is abundant. As the oxygen concentration increases in the bottom lake area with time, the zoospores move toward the bottom, and they became distributed uniformly from surface to bottom. By 4:00 p.m. most zoospores are at the bottom. During summer and winter the oxygen concentration is most abundant at the surface and middle region of the lake. Zoospores are found in the surface layer throughout the daytime, apparently in an inactive state.

Hydrogen-ion Concentration (pH): A study concerning pH and the distribution of the Saprolegniales was made (Roberts, 1963). It was found that species fell into three groups; acid group in waters with pH below 5.2, alkaline group in waters with pH above 7.8, and a neutral group of species found over a pH range of 5.2-7.8. Lund (1934) pointed out that some species will thrive vegetatively on both acid and alkaline substances, whereas the formation of reproductive organs seems to be conditioned by a specific range of pH. Krause (1960) found oogonia of Saprolegnia ferax formed only in a range of pH 5.2-7.2, while pH 5.8-6.9 was optimal.

Saprolegnia diclina and Aplanes braunii were recovered only from acid lake water (Suzuki and Hatakeyama, 1960). In the lakes of lowest pH (1.9-2.75), Suzuki (1961c) indicated no fungi were found. In the others, which varied from pH 2.9-5.8, one or more species were found. The greatest number of organisms in any one lake was in the lake with a pH of 5.8. However, some investigators felt that pH was not the decisive factor, primarily because many of the fungi which are obtained from acid habitats did well in water at pH 7.2-7.7 (Lund, 1934).

Osmotic or Hydrostatic Pressure: Some of the water molds, as might be expected, seem unable to tolerate high osmotic concentrations, and media containing relatively small amounts of salts and nutrients are certainly advisable for initial isolations. Dilute media also induce more rapid spreading of the filamentous forms, often allowing normal zoospore emergence.

Salinity: The fungi present in such extreme saline aquatic conditions as that provided by the small pool called 'Bad Water' in Death Valley, CA, face rigorous conditions for life. Nonetheless in this pool Traustochytrium pachyderm was discovered (Sparrow, 1973). Hohnk (1973) found that the fresh water Saprolegnia ferax grew and reproduced best in fresh water. A salinity of 3% inhibited sex organ formation, 7% prevented sporangial formation, at 13% there were no gemmae, and at 25% only a few feeble hyphae were developed.

Other Factors: Several other habitat factors might be briefly mentioned. Harvey (1942) has suggested that altitude affects the distribution of water molds belonging to the Saprolegniaceae. In California at 1100 feet he found water samples from aquatic sites to be rich in water molds whereas at increased altitudes such fungi became fewer and fewer. In a lake at 4600 feet, an asexual strain of Aphanomyces was found in abundance (presumably nothing else); in another at 6750 feet only one specimen, an asexual saprolegnian was found, while at several nearby lakes at this same altitude, no members of the group were found. Neither water nor soil samples taken at 6000 feet produced members of the Saprolegniales. Other factors in addition to altitude must have been at work at the above sites. A species of Achlya has been reported from a small lake at the summit of Mauna Kea on the island of Hawaii (Gregory and Wentworth, 1937), at an altitude of 13,000 feet.

Toxic substances arising from the inanimate constituents produced by algae, fungi, and other members of the aquatic community will no doubt be found to be habitat factors of influence in the ecology of water molds.

Geography: It is apparent that members of the Saprolegniaceae are world-wide in distribution. Nevertheless Petersen's extensive investigation of Danish water molds showed that in Denmark Apodachlya is common while Leptomitus was not recorded (Petersen, 1910). In North Carolina, Coker (1923) stated that Leptomitus was very common, while Apodachlya has been found only once. Similarly other representative genera may be absent from one collecting site while one genus is present in abundance. Such occurrences may be local or on a larger geographic scale within the limitations of similar climatic regions.

The range of a species is limited to regions containing the particular substrates it can use and on which it has a selective advantage over other potential colonizers. In Saprolegniaceae, the greater abundance of individuals or species in open sunny marshes and ditches is probably due to the large amount of organic life in such places.

Temperature is a very important determinant in distribution of diverse species, excessive heat or cold serves an effective means of regulating the geographic range of some fungi. Seasonal distributions are considered to be the result of temperature influence. Apparently spring seems to be the most favorable season for growth (Coker, 1923). Suzuki (1960a) followed seasonal changes in aquatic fungi of Senshun-ike pond through a period of one year. Achlya flagellata was found throughout the year, A. racemosa and Apodachlya brachynema were found only in winter. In Denmark, because of the cold climate, the growth of the Saprolegniaceae begins in the spring, and ends in November. But in Coker's studies (1923), no closed season was found in his findings. He also noted that water molds are present when the water is open any day in winter.

Other factors such as salinity and soil type will influence microorganism distribution patterns. One of these ecological factors occasionally appears to be of prime significance in itself, but frequently a combination acting together probably regulates the extent of spread of a particular organism.

The Microenvironment: The microenvironment is a special microcosm, a distinct ecosystem posing a characteristic community made up of populations coexisting and interacting with one another. The mean physical, chemical, and biological composition of the macrohabitat is often far removed from that of the many microenvironments it contains. Sites at points remarkably close to one another may be vastly different in their nutrient condition, moisture status, oxygen content, light intensity, temperature, pH, osmotic pressure, or the kinds and amounts of toxins.

Changing the macroenvironmental factors described previously would directly or indirectly influence the microenvironments. The diurnal migration of the zoospores of water molds was closely correlated to the distribution of the dissolved oxygen in different water levels of the substrate. Members of the Saprolegniaceae require oxygen for their metabolic processes in life. The vertical distribution of water molds have been studied by Suzuki (1961d) and Willoughby (1961). Some of the saprolegnian fungi are collected from the bottom mud, but their occurrence is rare. This localization is explained by the nutritional availability. Fish, frog eggs, and the water mold itself when parasitized by other aquatic fungi may be viewed as a microenvironment. But biodegradation of these organic substances also provides nutrients for the aquatic fungi that in turn add to the decomposition process. The physical or chemical changes in the organs or tissues where these microorganisms locate will certainly influence the growth and distribution of these parasitic fungi.

Natural Selection: From the existing literature, one may find that the Saprolegniaceae rank high among the ubiquitous filamentous water molds. They are found not only in pools, ponds, lakes, rivers, streams, bogs, but also in marginal as well as terrestrial habitats. Soil samples from below the humus layers in deciduous forests, from stream banks, and associated with lichens and various green cryptogams are particularly satisfactory sources of achlyoid fungi (Klebs, 1899). Sand and soil from wooded areas of predominantly coniferous vegetation yield some isolates but not in abundance. Wet soils from the profundal regions of fresh water lakes may bear species of Achlya.

The presence of the species in the above mentioned habitats indicates that the biotic and abiotic factors in the surroundings are suitable for the growth and development of these fungi. The traits underlying fitness of the species to their special surroundings are not well understood. The physical, chemical, and biological stresses are considered to be influential factors.

Interspecific Selection: Factors of interspecific natural selection include nutrition, temperature, light intensity, oxygen tension, osmotic pressure, availability of free water, pH value, toxic substances and biotic factors. Growth rate is often stated to be the ultimate determinant of selection, populations with higher rates of development alledgedly overgrow and displace slow growers. This is evident in the isolation when the culture is contaminated by bacteria. Because the filamentous fungal cultures always grow much faster than bacteria, a pure fungal culture can be obtained by repeated innoculation of the marginal hyphae to a fresh medium. Under strong light intensity, green algae naturally grow and develop vigorously, and thus the components of the heterotrophs in the same community such as the members of the Saprolegniaceae are strongly affected.

Concerning the substrata, many of the bait types used in collecting the saprophytic members of the Saprolegniaceae provide favorable nutritional sources for the development of bacteria and protozoans. These contaminating organisms bring about such alterations of the environment as to change drastically the morphology of the water fungi with which they become associated. The unsuitability of hemp seed to the growth and development of bacteria might be due to the inability of the bacteria to utilize this substance because of the absence of certain enzymes. Chemical ingredients of hemp seed could also be toxic to the bacteria.

Other abiotic factors such as temperature, osmotic pressure, and pH also strongly affect the interspecific selection. Although most of the members grow well in a mild situation, the extreme environment may favor the development of few species. No fungi have been isolated from thermal spring water according to Cooney and Emerson (1964). In sphagnum bogs, Achlya treleaseana was found to be highly characteristic of such bogs. Saprolegnia ferax grew and reproduced best in fresh water. Saprolegnia monoica var. acidamica was a predominant species and was widely distributed in acidotrophic lakes of a pH 1.9-2.9 (Suzuki, 1960b, 1961c). Water is the universal requirement of this family. The extreme drought condition of the environment would certainly not permit any water mold to survive, and would prevent zoospores from reaching a proper substratum.

Host as a Selective Factor: Parasites have novel physiological qualities which, when the appropriate host is present and the environment permits the expression of these characteristics, an enormous selective advantage occurs. Some species of Saprolegnia such as S. parasitica cause diseases of fish and fish eggs. The genus Aphanomyces, primarily A. euteiches, attacks a number of economically important hosts, while A. parasiticus invades the mycelium sporangia and oogonia of certain other fungi. The biochemical bases for the selective advantage possessed by the parasites are poorly understood. Nevertheless, a profound host-parasite relationship exists.

Adaptation: Microorganisms respond in different ways to an alteration in the environment. In the process of adaptation, the individual or population of individuals adjusts to new circumstances, and has the advantageous quality which permits the organisms to live in the new surrounding. Adaptation at times results from the synthesis of a new enzyme or set of enzymes, or it may on occasion be linked to the formation of additional morphological features. Each organism has a certain adaptive capacity to the altered environments. In Saprolegniaceae, the dimorphism of the zoospore is a phenomenon which not only is physiologically and genetically controlled but also is an environmental adaptation. Motility may be omitted by adapting to the altering environments, such as in the case of bacterial contamination or the presence of certain chemical components in the habitat. The formation of chlamydospores (or gemmae) from vegetative hyphae is an adaptation phenomenon to the dry condition. The chlamydospores or gemmae are the vegetative reproductive units that form to enable the cultures to adapt and survive unfavorable environments.

There are two possible adaptation mechanisms which are taken by the population to a new set of conditions. One is phenotypic adaptation, which is a temporary modification and can be readily reversed by a return to the original circumstances. The other is genotypic adaptation which requires genetic modification, the change is inherited by daughter cells. The genotypic adaptations result from mutation. This kind of genetic study in Saprolegniaceae heretofore received little attention. No experimental works are available. The variation in spore discharge is a phenoadaptation.

Interspecific Competition: Numerous organisms frequently arrive during the colonization of previously unpopulated sites, yet owing to the interactions initiated soon after growth commences at the site, few of the arrivals survive. This may be due to the process of competition. When the inhabiting biotic components are heterogenous, and the nutrients in the site are limited, the competition among the species is especially strong. Natural habitats of the aquatic fungi such as sewage treatment plants, fresh nutrients are constantly added. As a result, the species closely similar in nutrient requirement in a given site can grow without discernible competition if space is not limited.

In a laboratory study of two species with similar growth rates in axenic and in two-membered cultures, a critical point is reached where the available supply of some factor is exactly equivalent to that necessary to meet the demands of the two populations, thereafter the concentration or level of the factor is insufficient to allow for maximum growth rates. At this stage each population will get less of the new developing limiting factor than it would obtain if it was growing alone. Nevertheless one of the competitors will obtain or assimilate more of the limiting resource than the other; the latter, therefore, is more severely affected as a consequence.

The technique of freeing saprolegniaceous fungi from bacteria is an application of the result of growth competition, since the majority of isolates of these fungi (at least of Achlya) grow more rapidly on corn meal agar than the ubiquitous bacteria.

Intraspecific Competition: The struggle for the limited store of nutrients among individuals of the same species identifies survival. In axenic cultures, the density of cells usually rises until the supply of a component in the medium is exhausted. The evidence of competition in natural ecosystems is difficult to obtain. In the climax community, the operation of competition leads to a selection for better competitors and elimination of the less fit, so that much of the evidence may have been destroyed.

Parasitism: A parasite is an organism living on or in, and getting its food from its host, another living organism which is commonly injured in the process of parasitism. Two kinds of parasites can be designated, facultative parasites living independently as well as by parasitism and obligate parasites living on their living host for part or all of their life cycles. The so-called endoparasites live inside an organism. Ectoparasites, on the other hand, are localized on the external surface of their host. Parasitism in the Saprolegniaceae has been well studied. Literature reveals the list of the Saprolegniaceae parasites is so diverse as to suggest that any saprolegniaceous fungus might be capable of parasitism under proper conditions (Vishniac and Nigrelli, 1957). From the results obtained, at least 27 species in 10 genera of the family have been found capable of attacking animal hosts. Potential parasitism is a familial characteristic. But from an ecological point of view, the 12 of the 27 species not known to be involved in natural infections are not parasites. Many of the parasitic species of this family are pathogens on fish and fish eggs. Frog eggs have also been reported as the host of water molds as previously mentioned. The genus Aphanomyces was reported to contain several destructive parasites of the roots of vascular plants, causing serious diseases of sugar beets, peas, and other crops (Alexopoulos, 1966). This genus is particularly known in parasitism. Other saprolegniaceous fungi and pythiaceous fungi have been found to be the hosts (Madelin, 1973). In addition certain species of the water molds can be attacked by fungi other than the Saprolegniaceae. Species of Olpidiopsis, Petersenia, Pringsheimiella, Rhizidomyces, and the Legnidiales have been reported as the parasites (Milanez and DoVal, 1969, Papavizas and Davey, 1960).

Host-Parasite Relationship: Microorganisms differ markedly in their susceptibility to parasites and in the influence the invaders exert on them. Such differences are governed by inherent properties of the cells under attack, the virulence of the particular parasite, and prevailing environmental conditions.

If the host is viewed as an environment for the invader and the host-parasite relationship is essentially an interaction between a small organism and its habitat, then virulence is essentially a measure of the parasite's colonizing ability, and the resistance of the host may reflect the suitability of the environment for the invader. Tiffney (1939) found that there was a marked difference in the resistance of various species of hosts to the pathogen, Saprolegnia parasitica. In his experiment, some species of fish were found to have a very low resistance to the disease, some showed a somewhat greater resistance while others had a high resistance to the disease. Only Anguilla chrysypa was found to be immune.

Aphanomyces parasiticus invades mycelium, young sporangia and young oogonia of certain other saprolegniaceous fungi. It was reported that once a saprolegniaceous thallus begins to reproduce, it becomes immune to infection by Olpidiopsis incrassata (Slifkin, 1961). If made to resume vegetative growth, the thallus again becomes susceptible. This phenomenon suggests that the host at that particular stage would produce a chemical or possess a physical structure which prevents the pathogen from invading.

Infection of pink salmon with Saprolegnia was reported (Ivankow, 1971). Some diseased fish recovered which indicates the host may produce a toxin to the pathogen. The effects of the pathogen on the host are primarily the diverse symptoms produced by the hosts. In fatal infections of fish, hyphae often emerge from the gills and mouth in tufts over the body. In parasitism of other fungi, the host produced more or less morphological adaptation to the invader. The ultimate result is the death of the host, and the association between host and parasite disappears.

There is no specificity between the parasite and host of most members of the Saprolegniaceae. Saprolegnia parasitica is universally reported as a common parasite to numerous fish species. Nearly 35 species of fish have been reported susceptible to this pathogen. On the other hand, Vishniac and Nigrelli (1957) reported that 16 species of saprolegniaceous fungi infected platyfish. A few species of this family are obligate parasites although most of them are facultative parasites. Aphanomyces parasiticus, a saprolegniaceous organism was unable to grow when apart from its hosts (Coker, 1923).

Conclusion: In the association of parasitism, the parasites appear to gain two main advantages. They avoid competition for food from less specialized saprophytic fungi. To a certain extent they are protected from those changes in environmental conditions which are adverse to their mycelial growth. Success in parasitism may be judged by the extent to which these advantages are enjoyed. The characteristics of the parasites of Saprolegniaceae could be identified by three distinct features. The organism causes lesions and finally death of the host. Most of the species are capable of saprobic growth under natural conditions. Each species has a wide host range. Saprolegnia parasitica is universally reported as a common parasite, others are reported in frequency down to only a single established case. The reason for the rarity or absence of the parasitic habitat in some species probably may be found in their specific ecology. Tiffney (1939) in his study of hostrange of Saprolegnia parasitica suggested three possible causes of death to the host: the destruction of tissue, the formation of toxic materials, and the dilution or dehydration of body fluids resulting from the destruction of areas of protective epidermis. Species of Saprolegnia (predominantly S. parasitica) and Achlya are generally the predominant fungi associated with fish diseases in nature. The evidence for initiating primary infection on wholly uninjured fish, however, is not convincing. Vishniac and Nigrelli (1957) found that uninjured fish were not attacked.

Species belonging to the Saprolegniaceae have diverse yet specific habitats. Frequently these habitats influence the growth and development of the species. It is also equally evident that the establishment of saprolegniaceous fungi also influences and changes the habitat in which they are found. Numerous studies could be initiated to identify new habitats of the water molds and possibly locate new representative forms of the family. It is quite evident that increased dependence on aquatic habitats will be needed for the needs of human food resources. The aquatic fungi will certainly have influencial effects upon the aquatic environment as an area of crop production in the future.

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