

CHAPTER 3. Ecology: Sampling Methods and Environmental Factors

The beginning of ecological studies on the Saprolegniaceae can be traced to the first chance collections of specimens on dead flies and fish. These encounters yielded geographical distribution data, and information on habitat types -- all valuable ecological measures. As mycologists became more proficient at finding water molds these aspects were expanded, and in time, interests on the part of some shifted from identification and descriptive taxonomy of isolates to the characteristics of the communities in which the fungi were discovered. The early development of collection and culture techniques permitting taxonomic work with the water molds were precisely the ones that allowed ecological investigations to proceed, and this has not changed radically in spite of advances in both the systematics and ecology of the family's representatives.

The field aspects of the ecology of water molds lagged behind the laboratory investigations of such parameters as the effects of temperature, nutrition, and pH on growth and reproduction. This sequence of emphasis in the development of knowledge of the ecology of water molds was to leave the discipline barren for some time. It can be argued in defense of such an approach that mycologists have had to become acquainted with the growth requirements of these fungi under controlled conditions before they could hope to devise sound explanations for their distribution and occurrence in nature.

Ecology as a discipline developed with little attention being given to fungi, let alone to the Saprolegniaceae. Accordingly, the information now at hand on distribution and abundance of water molds does not always fit easily into the terminology and concepts of that discipline. It is not possible to relate the accumulated knowledge of water molds to the sophisticated principles of modern ecology because of the inconsistencies in the ways in which investigators have sought, interpreted, and presented data. Meaningful statistical analyses are all but absent from even the most extensive ecological studies of water molds, hence the significance and applicability of most data can be judged only subjectively.

From the several existing accounts of the general principles of fungal ecology and reviews of ecological work on the water molds specifically, certain ideas surface repeatedly (W. B. Cooke, 1970b, 1976b; Dick, 1976; Hawker, 1957; T.W. Johnson and Sparrow, 1961; Liu and Volz, 1976; Meshcheryakova, 1977; Park, 1972b; Pirozynski, 1968; Sparrow, 1968; Weston, 1941). Like many other fungi, water molds are opportunistic, and display a remarkable plasticity. At the same time, they surely must be subject to competition in their natural habitat yet be able to contribute to that habitat. Some environmental factors may act differently on the various species of Saprolegniaceae, and even on the vegetative and reproductive phases of the same individuals. Finally, these reviews demonstrate that it is still impossible to state with confidence in just what physical condition (hypha, gemma, spore, or oospore) the individuals exist in nature. At best, methods devised to provide quantitative data on the distribution and occurrence of water molds serve to demonstrate the inadequacy of known techniques.

One can call to mind all manner of reasons why ecological studies of the Saprolegniaceae have not attained prominence or appear to lack the preciseness of science, but to dwell on such a negative approach is to do injustice to a substantial array of efforts. In this and the following two chapters we treat principally the field ecology of members of the family. What in another context might be termed the ecology of water molds under domestication is accounted for in the chapters on the physiology of growth and reproduction. Ecological aspects of the pathogenic water molds are dealt with primarily (though not exclusively) in the respective chapters on the diseases themselves. This is not to say that we are unaware of parasitism as an ecological factor in natural or disturbed communities. However, the ecology of diseases caused by water molds is so intimately associated with epidemiology and control (management) measurements that it is best treated in the context of pathology. We have divested the accounts to follow of incidental facts of an ecological nature coming from strictly taxonomic papers, or from those treating primarily geographic distribution (Harder and Persiel, 1962; Kobayasi *et al.*, 1968; and Paterson and Rooney, 1972, among others). The details of routine methods for baiting and collecting are left to the previous chapter.

ECOLOGICAL SAMPLING

Various techniques have been devised to measure species density¹ or abundance¹ and to provide quantitative information on which to construct conclusions. Unfortunately, some methods have not been adequate, and the conclusions drawn from the resulting data are unreliable. Each of the quantitative techniques applied to ecological studies of the water molds have shortcomings as will be apparent from the following account.

One of the earliest attempts to quantify the results of collection and isolation of water molds from soil was that by K. B. Raper (1928). He sought to determine the abundance of these fungi in a given surface area and volume of soil. By diluting and plating standardized quantities of soil he determined the species composition in each sample from the study area. The resulting data simply showed the number of times particular species were isolated from the various sites.

Quantitative data assembled by V. G. Collins and Willoughby (1962) were based on the recovery of water molds from lake water and sediment samples plated on a

¹ These terms have precise meanings in ecology. Abundance refers to the number of individuals per unit area or unit volume; when put in quantitative terms, this becomes the taxon's density. The ecological distribution of a taxon is based upon its occurrence through space and time: what environments within the geographical distribution of that taxon does it occupy? Frequency refers to the uniformity of the geographical or ecological distribution of a taxon in a given unit area of volume. Not all authors cited in these chapters on ecology of the water molds apply these terms in this fashion.

medium containing potassium tellurite (*see* Chapter 2). Numerous subcultures were then propagated from the resulting colonies. The frequency at which representatives of *Saprolegnia* and *Achlya* appeared on the plates was determined from the number of positive subcultures in the total. In a set of 908 subcultures eleven members of *Saprolegnia* and one of *Achlya* appeared. By calculation (V. G. Collins and Willoughby, 1962:303) this meant that there were "... 833 *Saprolegniales* spores per litre ..." of sample (center and marginal surface water, and center or marginal bottom mud). Relatively few specimens of water molds were recovered by this method: a yield of 6% of 176 isolations from samples at one site to less than 0.4% of 266 isolations from another.

As has been mentioned (Chapter 2), Park (1972a) held that small bits of bait were likely to yield a greater diversity of individuals than large pieces of substratum such as hempseed. He sought to accumulate some data on occurrence of zoosporic fungi by three methods: baiting, dilution plating, and particle plating. Neither dilution (1:100,000 to 1:700,000) nor particle plating [seeding bits of organic matter directly on W.B. Cooke's agar (1954) with rose bengal and streptomycin] yielded any members of the Saprolegniaceae, and the number of water molds was low even in baited cultures (Park, 1972a). Of course, possible toxic effects of culture adjuvants have not been determined and evaluated.

Some relatively easily managed methods for obtaining quantitative data on the frequency and abundance of water molds were used by Ho (1975c), Maestres (1977), and Ulken and Sparrow (1968). Ho (1975c) investigated the occurrence of saprolegniaceous fungi at sites on a river system. He prepared discs of hempseed extract agar, and put these into dishes containing water samples. The number of such discs that (with incubation) supported growth of water mold hyphae was taken as the "isolation percentage." In her study of the frequency of water molds in a Newfoundland river, Maestres (1977) centrifuged 20 L of water at each sampling time then resuspended the concentrate in sterile water to obtain the desired dilution of propagules. She inoculated the surface of plated growth medium with aliquots of the various dilutions, analyzed the yield quantitatively, and related resulting figures to the presumed number of propagules in the original sample volume. Ulken and Sparrow (1968) applied the most probable number method primarily to determine the frequency of chytrids in water samples, but they also attempted to estimate the numbers of saprolegniaceous fungi in the same samples. The resulting data were scanty and led to no conclusions. Whether MPN determinations can yield meaningful quantitative analyses with respect to water mold populations is yet to be determined.

To analyze quantitatively the distribution and abundance of Saprolegniaceae in soils of southeastern England Dick and Newby (1961) and Dick (1962) developed a quadrat sampling system. The method provided data concerning the position of a particular species in one quadrat as well as information on the frequency of species in several quadrats over a period of time. Sampling from the established quadrats was done in two ways. In one experimental design, a single quadrat 90 cm² was divided into 16 subsquares, and samples of soil (to be baited for water molds) taken from each. From such quadrats nearly 4000 samples were collected for analysis. The second

experimental design utilized twelve 90 cm² quadrats partitioned into 16 equal subunit areas, from each of which 16 samples were taken at various intervals of time (results from a series of test replicates established 16 as an adequate sampling base from which to assess relative abundance, Dick alleged). The samplings showed that the saprolegniaceous flora was relatively constant at each site. More than 50% of the species (Dick, 1962) were in an "aggregate pattern," that is, they occurred repeatedly in the same part of the quadrat over a limited time (up to 2 1/2 years).

Three culturing methods have been designed to provide quantitative information on the frequency and local distribution of water molds: Willoughby (1962, 1965), Dick (1966), and Suzuki (a series of papers beginning in 1960).

The culture technique devised by Willoughby (1962, 1965) is as follows. Water samples of a standardized amount are collected, shaken thoroughly, and aliquots mixed into melted oatmeal agar and poured into sterile Petri dishes. When the nutrient medium solidifies, it is cut into eight equal sections, and each section placed in a dish of sterile, distilled water. After these dish cultures have incubated, the edge of each section is examined for hyphae of water molds. In developing the method Willoughby had determined that for each agar section showing a fringe of growth, the morphology of the mycelium generally was consistent over the entire surface of that section, suggesting that the fringe mycelium came from a single propagule. To correct for errors introduced by those sections in which the mycelium at the edge obviously consisted of two or more species of fungus, Willoughby applied a correction factor -- the number of sections that could be expected, in a Poisson distribution, to harbor more than one propagule. Using spores from a nonsexual *Saprolegnia*, he tested experimentally the correction factor and confirmed its validity in axenic culture. In practice, then, Willoughby could calculate with reasonable accuracy the number of propagules L⁻¹ of water. Further, he found that spores retained their viability even though embedded in the agar sections. There were, however, differences in longevity of spores on storage prior to seeding the plates. Spores of *Achlya* and *Aphanomyces* species survived in water samples stored for 24 hours, but substantial numbers of propagules from isolates of *Saprolegnia* lost viability after about two hours of storage.

Thakur Ji and Dayal (1966) combined Willoughby's quantitative technique with sample dilution. Their data were reported simply in terms of genera represented, thus contributing nothing additional to Willoughby's method.

The technique developed by Dick (1966) for quantitatively determining water mold populations in soil was based on the preparation of fractions from the samples. A soil sample was mixed with water, stirred mechanically, and three portions were selected for plating out and baiting: coarse plant material, supernatant fluid, and slurry (the fine particulate sediment remaining after the coarse material and liquid were removed). Dick then analyzed the results of baiting these fractions for water molds. The supernatant portion yielded (with few exceptions) a species composition unlike that recovered from the slurry. The spectrum of water mold species found in the coarse plant material hardly differed from that in the baited slurry. Dick concluded that the yield from slurry cultures of one soil core in a particular site was an adequate base on

which to assess quantitatively the population of Saprolegniaceae in that site. An analysis of results using various types of bait demonstrated that hempseed was eminently satisfactory, and did not introduce appreciable errors that might be linked to competition for the substratum. On the other hand, the magnitude of dilution of the slurry fraction was especially critical: if too great, the variety of species appearing on the baits was not representative of the saprolegniaceous flora in the particular soil tested. Modifications in treating samples such as mixing slurry with an agar medium, cutting standard-sized agar blocks from the solidified substratum and plating these blocks in water (with hempseed) also gave reliable estimates of propagule numbers in the raw sample. With respect to the composition of representatives in the samples Dick (1966:281) wrote that the methods did not yield "...information on activity of these fungi, since they sample total propagules, the majority of which are resting propagules."

Beginning in 1960, Suzuki published extensive ecological studies on the Saprolegniaceae in Japan and reported much of his data on the frequency of species in terms of the numbers of motile (flagellate) spores per unit volume of raw sample of water or sediment. The method devised to provide such quantitative information was as follows. Samples were placed in Petri plates, and three halves of boiled hempseed floated on the surface of each dish. The cultures were incubated 2-4 days, and at that time the hyphae on each hempseed half were counted. The number of hyphae was equated directly with the number of spores in the original sample. Suzuki assumed, of course, that the incubation period was sufficiently brief to allow a spore to produce only one germ hypha that neither sporulated nor branched. Since hyphae could very well have branched within the substratum, the method has serious inherent defects. Accordingly, the quantitative data in Suzuki's papers are not necessarily reliable, but perhaps in a very broad sense may be somewhat useful for general comparisons.

Methods in studying the ecology of water molds are not limited to those that provide quantitative analyses. Two techniques are illustrative. Willoughby (1974) employed a cylindrical, rubberized tube (45 m in diameter) positioned vertically in a shallow lake to impound a large mass of water. The tube was anchored in such a fashion that no water entered at the bottom (mudline) and a circular, inflated section held the upper open end above the surface. This device was a barrier to the lake's surface water (and to runoff from the adjacent shoreline), but because of the volume the tube enclosed, the natural physicochemical properties of the lake water were maintained. The preliminary data from samplings of the Saprolegniaceae in the impounded and free sectors of the lake were scanty (Willoughby emphasized the chytridiaceous population) but sufficient to indicate that, with time, a prominent dissimilarity developed between the population density and constituency of water molds inside the tube and those outside.

To investigate the depth distribution of Saprolegniaceae in response to hydrographic factors in a forest lake, Johannes (1957) devised a novel bait submergence technique. Ant eggs (see Chapter 2) were fastened onto a paraffin layer over slips of paper, and these devices attached at intervals to poles. The poles were then submerged

upright in such a fashion as to position the eggs at various depth intervals. After an average submergence time of five days the "eggs" were removed, put in Petri plates of water and examined. Johannes (1957) observed that there was a relationship -- he termed it an "antagonism" -- between the extent of vegetative growth and degree of reproduction in the water molds he recovered, and he concluded that this opposition of one stage toward another had seasonal implications. These results recall Lilienshtern's (1924) theory of antagonism in *Saprolegnia monoica*[†] between vegetative growth and sporangium production.

OCCURRENCE IN RELATION TO THE ENVIRONMENT

The relative constancy of water molds in culture in the haven of the laboratory safe from competitors is doubtless not a characteristic of their existence in nature. Abundant nutrient, constant temperature, and adequate moisture (among other factors in culture) simply do not exist over extended periods of time in most habitats that harbor water molds. Individuals lacking the ability to adjust to the changing natural environment are not necessarily eliminated from the community, but certainly their performance is constrained. Conceivably, the water molds simply shift over to a resistant stage when confronted with the stresses of their surroundings, but how to measure and analyze their adjustments escapes current methodology.

It has been stated that an ecological approach to the study of organisms aims at finding out what those organisms do, the conditions under which they act or react, and the factors preventing them from doing other things (Andrewartha, 1970). These conditions and factors constitute the biotic and abiotic environment, the totality of all components that directly influence an organism's chance to survive and reproduce in a particular situation. In this section, we examine -- insofar as information is at hand -- the various external stimuli and resources that influence the water molds in nature.

TEMPERATURE

Some reports of the occurrence and distribution of water molds with respect to temperature are general indeed. Stoll (1936), for instance, simply stated that there were fewer species of these fungi in colder waters than in warmer ones. His conclusion reflects A. Lund's (1934) earlier view that species of *Achlya* thrived best in cool temperatures, and gave as a possible reason for their poor performance in warm waters severe competition created by algae, among other organisms. Temperatures extant at the time of sampling were recorded by A.W. Ziegler (1958b) and subsequently correlated with the recovered species arranged according to their oospore type. About 75% of 1191 isolates had eccentric oospores, and these representatives of the family were found in both cool and hot weather. Some 80% of the remaining isolates -- those with centric and subcentric oospores -- were collected only in cool weather. Later (1962), A.W. Ziegler calculated from an analysis of another 615 isolates that the

optimum temperature range for species with centric or subcentric eggs was 16-19 °C, while that for eccentric-egged forms was 19-30 °C.

With respect to temperature the species of water molds collected by G. C. Srivastava (1967b) fell into two categories. One group, the so-called high-temperature species, consisted of some representatives of *Achlya*, *Aphanomyces helicoides*[†] and *Dictyuchus sterile* (an excluded name; see systematic account). In the second or low-temperature group, only species of *Saprolegnia*, *Leptolegnia* sp., and *D. monosporus* appeared. Dayal and Tandon (1963) simply reported that a temperature range of 23-25 °C favored the growth of the Saprolegniaceae in water. In his search for a procedure to standardize determinations of the abundance of water mold propagules Ho (1975c) recognized that a correlation existed between water temperature and isolation frequency (see previous section). The percentage of propagules in his samples was lowest at temperatures below 5 °C and at 25-26 °C and above; maximum yields were recorded when the temperatures were between 7 ° and 20 °C. Studying the occurrence of two water mold species in a granite outcrop, Nesom (1969) found that the frequency of isolation of *A. klebsiana*[†] decreased as the soil temperature increased, whereas that of *Leptolegnia* sp. showed a reverse trend. In connection with low temperatures and yield of water molds, on culture, H. J. Fletcher (1979) has posed an intriguing hypothesis. From some samplings of water molds at three sites, he found that the number of specimens recovered declined to zero when the water temperature reached 20 °C. Later, when the temperature dropped to 5 °C, the yield of specimens rose in the sites to a level only slightly below that of the peak recovery time. Fletcher's question: are there psychrophilic saprolegnians?

Grouping species of Saprolegniaceae according to temperatures extant at the time samples were collected have been attempted. Sampling a Newfoundland river periodically, Maestres (1977) was able to place the species she recovered into three categories: (1) cold temperature ones -- *Aphanomyces stellatus*, *Pythiopsis cymosa*, *Saprolegnia eccentrica*, *S. monilifera*[†], *S. terrestris*; (2) warm temperature ones -- *Achlya oblongata*, *Protoachly paradoxa*, *S. unispora*, *S. parasitica*[†] [Florinskaya (1971), on the contrary, found this species in waters of a wide temperature range: 0.8-20 °C], and (3) those species characteristic of intermediate temperatures -- *Aphanomyces laevis*, *Leptolegnia caudata*, *S. anisospora*, *S. australis*, and *S. diclina*. Not one species producing eccentric oospores appeared in Maestres' collections, but it must not be concluded that such water molds are absent from cold waters. We have ample evidence from numerous collections that eccentric-egged forms occur in glacier-fed streams and other bodies of cold water in Iceland (Howard *et al.*, 1970; T.W. Johnson, unpublished) and northern Alaska (Seymour, unpublished).

Three additional studies have yielded temperature-limited species groupings. Khulbe and Bhargava (1977) recognized four clusters of saprolegniaceous forms in the mycoflora of certain Indian lakes. Constant species -- *Aphanomyces laevis* and *S. ferax* -- were those found in samples collected at 10-28.5 °C. There were two low temperature species (most frequent in waters at 10-18 °C), *Brevilegnia linearis* and *S. parasitica*, and three high temperature (15-20 °C) ones: *Aphanomyces* sp., *A. scaber*, and *S. diclina*. Most

species in the lakes sampled, however, made up the moderate temperature (15-23.5 °C) group: *A. apiculata*, *A. flagellata*[†], *Isoachlya anisospora* var. *indica*[†], *I. monilifera*, *Isoachlya* sp., *Protoachlya* sp., *S. hypogyna*, *S. lapponica*[†], *S. mixta* var. *asplundii*[†], *S. crustosa* var. III[†], and *Thraustotheca clavata*. It should be emphasized that Alabi (1971b) considered *Aphanomyces laevis*, *B. linearis*, and *T. clavata* to be “high temperature species”, but Khulbe and Bhargava list these same species as constant, low, and moderate temperature ones, respectively. Mer *et al.* (1980) found three species groups with respect to temperature in the Indian lakes they investigated. Their data are not in total agreement with those assembled by Khulbe and Bhargava (1977). For example, *A. laevis* and *T. clavata* are “moderate temperature” species according to Mer and his associates, in contradiction to the findings by Khulbe and Bhargava. Among other questions, one may ask whether such categories of species based on field temperature determinations at the time of sampling are truly a measure of the influence of this parameter in the natural habitat, or simply reflect temperatures of incubation needed to bring the fungi out in culture?

Data on planont density (the data base is suspect) in relation to temperature in various bodies of water in Japan were published by Suzuki (1960f, 1961a, 1963). He (1960f) combined spore counts of all species collected, and found two maxima, and at 9 °C, and a second at 20-23 °C. The lowest spore loads in the samples (see previous section for method of calculation) were at 3, 21, and 27 °C. Further analysis of the field data revealed that there were certain optimum temperatures for spore production for each species; these were: *Achlya flagellata*, 9-21 °C; *A. racemosa*, 3-12 °C, *Aphanomyces laevis*, 15-18 °C, and *Saprolegnia monoica*[†], 3-18 °C. From sediments where the bottom water temperature was 3-11.5 °C, Suzuki (1961a) found only *A. racemosa*; *A. americana*, on the contrary, was collected in the mud when the temperature of the bottom water was 11.5-28 °C. In 1963, Suzuki published additional figures on the frequency of various watermold species at particular temperature again using calculated numbers of planonts in the water to provide the raw data. *Achlya racemosa* (maximum spore production at 3-11 °C) and *S. monoica*[†] (maximum at 6-17 °C) were considered cold water species, while *Aphanomyces laevis* (peak sporulation at 15-17 °C) was a warm water one, and *A. flagellata*[†] was indifferent to temperature with respect to its tendency to reproduce asexually (9-20 °C).

OXYGEN

Petersen (1909a, 1910) was among the first to speculate that a low level of dissolved oxygen in water hindered the growth of saprolegniaceous fungi. The alleged necessity for an oxygen-rich environment may have been interpreted from his observation that anything (mud or blue-green algae, for example) which prevented the renewal of water around mycelium growing on submerged twigs was detrimental to the fungus. Petersen remarked that summer was not a propitious time for watermolds to infest twigs: these branches (not yet decayed) sank quickly and, he reasoned, the fungi on them would be deprived of air. Moreover, bodies of relatively quiet waters

with an open surface were in his estimation the most favorable habitats for the growth of water molds. Maurizio (1899) had earlier concluded that species of Saprolegniaceae thrived best where oxygen was abundant. Although A. Lund's (1934) work was largely with pH as a determining factor in the occurrence of water molds, he was aware that most species grew on substrates near the surface of lakes rather than on deeply submerged ones. Species of *Achlya* and *Saprolegnia* required high oxygen levels, Lund said, for proper growth and reproduction.

A few investigators have remarked specifically on the general conditions under which one could expect to find representatives of the Saprolegniaceae in water. Ramsbottom (1931), for instance, wrote that these fungi did not occur (that is, were not collected) in flowing water, and Forbes (1935b) thought they were scarce in stagnant aquatic areas. Later, Stpiczynska-Tober (1965) reported that water molds in two tributaries of the Vistula River were equally as frequent in clear water as in water with an abundance of plant debris. Data from other studies have led to additional conflicting conclusions. From the results of a year-long investigation of water molds in a Newfoundland river Maestres (1977) proposed that oxygen had no substantial influence in establishing the distributional patterns she uncovered. On the other hand, Khulbe (1977) reported that in general the species he collected thrived in conditions of low oxygen tension. Barbier (1969) concluded that since members of the Saprolegniaceae occurred in stagnant water rich in organic debris they could at least adapt successfully to low oxygen tensions. When all data on the water molds they collected in a Utah lake (species of *Achlya*, *Saprolegnia*, *Pythiopsis* and *Protoachlya*) were combined, Rooney and McKnight (1972) noted that the frequency of these fungi increased as the dissolved oxygen content of the water diminished. Clausz (1970) found few propagules of the Saprolegniaceae in oxygen-deficient waters of an impoundment (North Carolina), but at those times when the lake was richly oxygenated, the spores of water molds were essentially distributed throughout the water.

Suzuki (1960e, 1961d) studied spore distribution in relation to the overturn and "stagnation" (thermocline) periods in certain Japanese lakes. During the circulation period, with its accompanying high levels of dissolved oxygen, spores of some species (*Achlya* sp., for instance) were distributed vertically from surface to a depth of 12 m, while those of others such as *Saprolegnia diclina* were distributed only in the upper two meters of the lake. During the thermocline period, all the species Suzuki collected had a shallow distribution (0-5 m) vertically. Similar data from analyses of additional lake systems were reported by Suzuki (1961h) and Suzuki and Nimura (1961c).

Other papers dealing with species distribution in relation to oxygen have come from Suzuki's efforts in the field and laboratory. Only an unidentified *Aphanomyces* was recovered (Suzuki, 1961c; 1962b), from mud on lake bottoms where the oxygen level was low, and the number of spores of other species in these lakes dropped sharply as the dissolved oxygen content of the water decreased with depth. Accordingly, the spores of some water molds were limited to near-surface waters. Suzuki claimed (1962b) to be able to rank individual, closely associated lakes on the basis of their productivity of motile spores, as did Suzuki and Hatakeyama (1960, 1961) for additional lake

systems. The latter also found few spores of water molds in the lake sediments, yet were able to determine that *Aphanomyces* sp. predominated there. Vertical distribution of planonts in the water of those lakes studied by Suzuki and Hatakeyama (1960) was alleged to be related to oxygen content. However, in spite of almost identical oxygen and temperature levels in water of one lake in two samplings a year apart, the vertical distribution patterns of spores were very different. This suggests an error in calculation, an improper database, or the involvement of some additional hydrographic factor.

Water molds in shallow lakes appear not to follow necessarily the same model of distribution exhibited by those in deep lakes. Suzuki and Nimura (1961d) did not recover any spores from water of certain very shallow Japanese lakes, while the bottom muds harbored aquatic species in frequencies of 90% and above. This shallow water distribution of spores is evidently not a consistent phenomenon, since in one lake Suzuki and Hatakeyama (1960) found more planonts in the waters of a shallow arm than in the deeper main basin. Moreover, there were more viable spores of water molds in samples of the bottom sediments of the shallow water than in those under deeper overlying water. It should be recognized that numbers of spores reported to occur in the bottom muds of these Japanese lakes may be quite misleading, since there is no evidence in the paper by Suzuki and Hatakeyama that their sediment samples contained free spores. Indeed, it is more likely that the muds contained resistant oospores. Upon germination in culture these zygotes would yield considerable numbers of planonts.

Suzuki's studies of the distribution and abundance of water molds (and other fungi) were quite varied, as is shown, for example, in his investigation (1961c) of diurnal migration of planonts in a shallow lake. Unfortunately, he did not publish names of the species collected during the course of this study, but it may be assumed he found representatives of *Achlya*, *Saprolegnia*, and *Dictyuchus*. Water samples were removed from the shallow lake both on clear days and on cloudy and rainy ones in the spring and autumn. The number of spores found in samples taken in the morning and evening of a clear day was approximately the same at the surface, bottom and middle region of the lake. Suzuki believed that the spores occurring in these horizontal regions had migrated up from the bottom layers of water. At night, the heaviest concentration of water mold spores was in the surface waters. Samples taken on cloudy days pointed to the fact that the motile spores were concentrated at the lake surface throughout the day. During prolonged rain water mold propagules were evenly distributed in the lake from surface to bottom.

It was oxygen level, Suzuki speculated (1961c), that was most influential in the diurnal vertical migration of spores. During the early morning on clear days in the spring and autumn the spores accumulated at the lake surface. As the oxygen content of the bottom waters, in the shallow lake rose during the day the spores migrated toward the bottom and thus were reasonably evenly distributed vertically. After sunset, with a coincidental decrease in bottom water oxygen the spores then migrated back to the surface. Suzuki argued that since on a cloudy day the surface water oxygen

level was not accompanied by an increase in oxygen in the bottom waters, the planonts tended to remain at the surface. He further stated that during the summer and winter, there was no diurnal migration of watermold spores in the shallow lake, but the data in his paper indicate otherwise. So far as we are aware, no other accounts of diurnal migration have been published. Additional work on this aspect of watermold distribution is much needed, if only to test further Suzuki's observations. Data from his analyses suggest that migration takes place over substantial distances. If this is so, how are the spores supplied with sufficient energy for the necessary sustained movement? The study by Clausz (1970) of a somewhat deeper lake than that explored by Suzuki, pointed to a nonrandom distribution of watermolds. Assuming the nutrient resources in the water to be adequate, Clausz attributed the observed distribution to an influence by oxygen level.

SOIL MOISTURE

Information on the occurrence and abundance of watermolds in soil is scanty and by no means consistent in what it reveals. According to Höhnk (1952a) an increasing number of watermold species could be isolated from shoreline areas from which the water level was receding, and the results of an ecological study by Dayal and Thakur Ji (1966) are not substantially different. They found the greatest diversity of species in samples taken in temporary ponds. Dogma (1966) did not isolate any species of *Aphanomyces* from Philippine soils, and except for *Achlya klebsiana*[†] and *A. proliferoides*, all representatives of *Achlya* came from water samples. Nesom (1969) specifically studied soil moisture as a possible factor in modifying the relative abundance of *A. klebsiana* and *Leptolegnia* sp. In her study she compared the number of isolates recovered from soil samples at the time of collection and after a two-month drying period during storage. She found that a decrease in field soil moisture, accompanied by an increase in soil temperature, lowered the frequency at which *A. klebsiana*[†] was isolated, but *Leptolegnia* sp. was collected most frequently from dry soils at high ambient temperatures. Storage, of course, changes the nature of soil samples in other ways apart from moisture.

Studies by Höhnk (1935a) and Dick (1968a) dealt with soil moisture and occurrence of watermolds in a zoned and linear series of habitats, respectively, and we are therefore treating their papers in the next chapter. Data accumulated by Dick (1963) from a distribution analysis in soils in southeastern England pointed to a richer saprolegniaceous flora in general (and a higher percentage of species of *Achlya* specifically) in wet than in dry soil sites. Inasmuch as there were fewer species of *Saprolegnia* than of *Achlya* in soil, Dick suggested that most but not all members of the former genus were probably primarily aquatic (*see*, in Chapter 4, Höhnk's habitat and sporulation types). *Achlya* species appeared to predominate in sites subject to flooding or waterlogging. *Saprolegnia terrestris* was most frequent in poorly drained sites, or ones occasionally inundated, while *Aplanopsis terrestris*, *Isoachlya subterranea*[†], *Leptolegnia*

eccentrica, and *S. megasperma* were characteristic elements of the zoosporic mycoflora of well-drained soil sites (Dick, 1966).

Detailed observations on the occurrence of members of the Saprolegniaceae in relation to soil moisture were published by Apinis (1960, 1964). He concluded that the water molds were confined largely to marshes, swamps, and waterlogged soils, and regarded such species as *Achlya racemosa*, *Dictyuchus monosporus*, *Saprolegnia ferax*, and *S. glomerata* as hydrophilic fungi in which minimum growth was only attained if the relative humidity of the soil was 99%. Such water molds, he contended, would not grow below a diffusion pressure deficit (water potential) of four atmospheres. In an investigation of the water molds of cultivated and uncultivated soils in Denmark, A. Lund (1978) noted that wet soils were richer in these fungi than were dry habitats (*see also*, Chapter 4).

According to Prabhujii (1979) six groups of species of Saprolegniaceae could be recognized with respect to the percent soil moisture at the time of their collection. However, we believe that the magnitude of differences in moisture content among the various soils sampled does not bear out recognition of six categories. At most, Prabhujii's data show two species clusters. One group of species occurred in soils at or near the lowest moisture level (7.5%) and also at 50-90% (*Achlya diffusa*, *A. orion*, *Aphanomyces helicoides*[†] are examples). The second group comprised those species (for instance, *A. proliferoides* and *Isoachlya luxurians*[†]) not found in soil with a moisture level below 30%.

MINERAL AND ORGANIC CONTENT OF SOIL AND WATER

In his account of Danish fungi, Petersen (1909a, 1910) wrote that water favorable for growth of the saprolegniaceous fungi was not very rich in decaying organic matter, and had little iron. Moreover, he thought that the amount of calcium in the water was unimportant. Petersen's broad conclusion (nutritional studies tend to refute it) was not confirmed by A. Lund (1934), who noted that species of water molds reacted differently to amounts of submerged organic matter. Species of *Achlya* were plentiful only in water in wooded areas where there was a substantial supply of twigs, while members of the genus *Saprolegnia* occurred equally well in such habitats or in water with little in the way of woody organic substrates. Sampling at intervals in a river system in Japan, Okane (1967) found that the highest numbers of water mold spores appeared in samples of water with a high "chemical oxygen demand" (probably measured by the potassium permanganate method). According to Dayal and Tandon (1963), nitrates, nitrites, and ammonia in solution in water did not limit the occurrence of Saprolegniaceae. Maestres (1977) found that sulfides did not modify the frequency of water molds, but a winter increase in micronutrients in the Newfoundland river system she studied was reflected in a rise in populations in the spring. She proposed that trace elements might influence propagule germination, and this would account for the abundance of fungi she encountered. High levels of sodium and phosphorus were correlated at particular seasons with peaks in the richness of some species of the family; Khulbe (1977) detected

similar patterns with respect to the phosphorus content of water. Schmitt (1967) concluded from the results of some collections made in Lake Texoma that, as the lake's water level receded there was an increase in the concentration of minerals and a concomitant decrease in the diversity and abundance of the water molds in the exposed soil. The results of this study by Schmitt are reflected in data from Khulbe's (1977) work: there is a high frequency of saprolegnians in waters low in some minerals (nitrates, sulfates, calcium).

It was Suzuki who explored more fully than any other investigator the relationship between mineral content of aquatic habitats and occurrence of Saprolegniaceae. Suzuki and Nimura (1961a) reported that in a group of five lakes three species of water molds -- *Saprolegnia* sp., *S. diclina*, and *Aphanomyces* sp. -- were found only in the one lake having no detectable iron, manganese, calcium, chlorides, or sulfates. On the other hand, in another set of lakes characterized by water with a low mineral content (no chlorides, for example) all habitats yielded some species of water molds. Suzuki and Nimura (1962) analyzed the water mold populations and hydrochemical characteristics of yet another cluster of three lakes, these being rich in sulfates, chlorides, and calcium, but deficient in nitrites and phosphate. There were spores of these fungi in each lake, but the species composition differed among the three bodies of water. This is unusual in that the three lakes were connected by a common watercourse.

Information on the occurrence of Saprolegniaceae in relation to the organic content of the soil is sparse. Investigating *Aphanomyces euteiches*, the cause of root rot of peas, J. E. Mitchell *et al.* (1969) discovered that the number of test seedlings infected by this species was proportional to the amount of debris in the soil in which the seedlings were growing. Infection percentage was highest in plants in soils richest in organic matter. The epidemiological implications of this fact are treated in the account of the disease (Chapter 27). Nesom (1969) attempted to show a relationship between the organic matter content of soil and the presence (or absence) of *Achlya klebsiana*[†] and a nonsexual *Leptolegnia*. She could not isolate either species from any soil with a measurable organic content less than 1%. Soils above 5% organic matter yielded both species, but in higher levels *Leptolegnia* sp. increased in frequency while *A. klebsiana*[†] declined. Prabhuji (1979), on the other hand, found no correlation between organic matter level in the soil and the occurrence of any particular species of Saprolegniaceae. It may be recalled that Garrett (1951) applied Burges' (1939) term "sugar fungi" (unable to hydrolyze cellulose and lignin) to the Phycomycetes (inclusive of the Saprolegniaceae). Water molds undeniably occur in organic-rich soil, hence if Garrett's conclusion is correct, these fungi depend on other organisms in nature to provide carbon in a simple, readily utilizable form.

Members of the Saprolegniaceae are not limited in the natural environment to sources of simple carbohydrates alone. Dick (1970) demonstrated that some species are more likely to colonize insect exuviae (presumably in the absence of more readily available substrates) than are others: *Saprolegnia diclina* (*sensu lato*) and *Aphanomyces laevis* being the chief colonizers, followed, in order, by species of *Achlya*, *Leptolegnia*, and

Dictyuchus. *Saprolegnia ferax* was present on calcareous shell fragments (Zebrowski, 1936) collected by Höhnk (1969) in coastal habitats from the tidal zone out to depths of 62 m. He suggested that the zoosporic fungi he found were actively engaged in the breakdown of the calcareous substratum, and therefore contributed to organic matter turnover in the marine environment. This is a problem inviting attention.

Like other members of the soil mycoflora, saprolegnians respond to herbicides and pesticides. *Aphanomyces eutiches*, Ko and Lockwood (1968a) found, was insensitive to DDT or DDD [1,1-dichloro-2,2-bis(p-chlorophenyl) ethylene] in concentrations up to 100 ppm, and was able to accumulate DDT and the fungicide PCNB (pentachloronitrobenzene) in quantities above the amounts ambient in soil. This same watermold sequestered the pesticide Dieldrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1, 4-endo,-exo-5,8-dimethanonaphthalene) as well. Hodkinson's (1976) review paper summarized the work by Dalton and Smith in which they demonstrated that *Isoachlya* sp. could accumulate ¹⁴C-DDT. Within 15 minutes after isolates were exposed to DDT 62-83% of the compound (incorporated into the medium initially) was recoverable from the mycelium. It is suggested (Hodkinson, 1976) that fungi might contribute to both retention and concentration of DDT in the environment.

HYDROGEN ION CONCENTRATION

More investigations have been conducted to determine the relationship between pH and the occurrence and abundance of watermolds than of any other factor in the environment. The results of the several studies are disparate.

It was Aage Lund (1934), in Denmark, who was most instrumental in calling attention to the importance of pH as a factor influencing the occurrence of watermolds in aquatic situations. The five habitat types -- as to pH level -- recognized by Lund and the species he collected in each are recorded in Table 13A. He concluded that *Aplanest* spp., *Saprolegnia diclina*, and *S. litoralis* "preferred" acid habitats, while *A. radiosa* alone was limited to alkaline waters. Those species that seemed not to be influenced by pH either in their occurrence or frequency included *A. racemosa*, *A. colorata*, and *S. ferax* (*sensu lato*). At least with respect to the occurrence of *A. racemosa* and *S. ferax* Dudka's (1965) findings agree precisely with Lund's observations.

Perrott (1960) and G. C. Srivastava (1967b) also have assembled data on occurrence of watermolds in relation to pH (Tables 13B, C) as have R. E. Roberts (1963), working in England, Stamak (1971), Stpiczynska (1962) Stpiczynska-Tober (1965), Zaborowska (1965), and Żebrowska (1976a) in Poland, and Ergashev and Kirgizbaeva (1978) in Russia. Some of the data, selected to show contrasts and similarities with those assembled by A. Lund are given in Table 14 (Staniak, 1971, found no distributional patterns with respect to pH). These data suggest that *Saprolegnia ferax* and *Aphanomyces laevis* are distributed generally in low acid alkaline waters. Florinskaya's (1969) isolations of watermolds from some neutral or alkaline fisheries ponds in Russia are at least in agreement with this distribution pattern for *S. ferax*. On

the other hand, Milanez (1966) pointed out that these two species were not restricted to any particular pH range. The occurrence of some other representatives of the family in response to pH -- *Achlya americana* and *S. litoralis*, for instance -- is quite inconsistent (Table 14). Hasija and Batra (1978) collected *A. americana* in neutral waters (pH 7.1-7.9), but R. E. Roberts (1963) found the highest frequency of this species in acid environs (pH 3.6-4.8). *Saprolegnia hypogyna*, which Perrott (1960) found in both acid and alkaline waters, is a species characteristic only of alkaline waters according to Ergashev and Kirgizbaeva (1978). Such discordant distributional data may signify wide tolerances to pH levels on the part of these fungi, or may merely reflect differences in methods (and magnitude) of sampling and testing.

Further comparisons among the distributional patterns of representative saprolegnians in acid and alkaline habitats are revealing. According to Suzuki (1961a, e) and Suzuki and Nimura (1960), *Achlya americana* occurred in Japanese lake waters only at pH 7.4-8.0, while *A. racemosa* could be isolated from samples having a pH range of 6.2 to 7.6. To the contrary, R.E. Roberts (1963) and Zaborowska (1965) collected the former in acid waters (Table 14), but the distribution of the latter in Japanese lakes seems to be in accord (Table 13B, C) with the findings by Perrott (1960) and G. C. Srivastava (1967b). Some observations indicate that species of *Aphanomyces* are distributed generally in either alkaline or neutral waters (Table 14), but there are exceptions (Table 13A, B). Unidentified species in the genus *Aphanomyces* were collected by Suzuki (1961e) and Suzuki and Nimura (1960) in acid lakes (pH ranges of 4.4-6.8 at the time of sampling) and also (Suzuki and Nimura, 1962) in nearly neutral water (pH 7.1-7.4).

The existing distribution and occurrence data do not permit the general conclusions that a given species of Saprolegniaceae can be expected to inhabit waters of a particular pH level. *Achlya androgyna* is a notable exception, and *Saprolegnia turfosa*, common in Scandinavian waters, appears to be another. These species are "weedy" occupants of acid bogs (*Sphagnum* spp.) in Norway and Sweden (unpublished). Dick (1971c) considered *A. treleaseana*[†] to be a member of an "acidophile" community, and Milanez (1966) found it only in acid bogs in Michigan. Suzuki and Nimura (1960) recovered the species (identified as an *Aplanes*) and a form closely akin to it, *Aplanes braunii*[†] from Japanese lakes where the water was pH 2.75 and 4.6-5.2, respectively. *Saprolegnia monoica* var. *acidamica*[†] was described by Suzuki (1961f) as a new variety because of its restriction to highly acid waters (as low as pH 2.9; Suzuki, 1961e). This variety was also collected in very acid lakes by Suzuki (1962a), Suzuki and Nimura (1960, 1961b), and Suzuki and Tatsuno (1964).

Not all accounts of the distribution of Saprolegniaceae report that there are specific water molds in acid environments. Kobayasi and Ookubo (1952b) did not find a single species typical of acidic peat bogs and swamps (stagnant waters at pH 4.0-5.0). Essentially the same conclusion was reached by Beverwijk (1948); saprolegniaceous fungi were sporadic in acid waters, and she collected (once) only one species at a pH of 4.9 (*Achlya colorata*). In their papers reviewing the ecological work on Japanese water molds Suzuki and Tatsuno (1965a, b) remarked that there were no consistent

patterns of occurrence of Saprolegniaceae with respect to hydrographic factors. This does not agree, of course, with Suzuki's contention that *Saprolegnia monoica* var. *acidamica*[†] was a regular -- and at times exclusive -- inhabitant of highly acid lakes. Without a doubt the conclusions by Dayal and Thakur Ji (1966), Dayal and Tandon (1963), Muhsin (1977), and Talukdar and Baruah (1952) that pH was not an influential factor in watermold occurrence in the lakes and ponds they sampled was simply a reflection of an extremely narrow range of pH in those bodies of water. Khulbe (1977, 1980a), however, also dealing with a narrow pH range (7.3-8.8), was able to sort out the species he collected into three groups: "wide", "moderate", and "narrow" tolerance ranges. In the latter range (not above pH 8.0), Khulbe only found such species as *Achlya apiculata* and *Brevilegnia linearis*. *Aphanomyces laevis* and *S. ferax*, among others, had a "wide" tolerance range, that is, they were found in samples having a pH of 7.3-8.8.

Maestres (1977) believed that the influence of pH in the Newfoundland river waters she sampled was on reproduction and not growth (Maestres and Nolan, 1978) of the fungi. Even that influence was suppressed provided the nutrient level was sufficiently high to support vigorous growth. Stoll (1936) concluded that pH was not an important factor in occurrence because some zoosporic species could be found with equal frequency both in highly acid and in alkaline ponds.

Two reasonably extensive accounts of the distribution and frequency of watermolds in soil in relation to pH are those published in 1963 by Dick and in 1978 by A. Lund. The quadrat-delineated soils from which Dick isolated members of the family were grouped into four pH classes: 4.2, 4.3-4.7, 4.9-6.0 and >6.0. *Leptolegnia eccentrica*, *Saprolegnia asterophora*, *S. litoralis*, and *S. turfosa* predominated in soils below pH 4.2, and were not found at all when the level was above pH 6.0. *Achlya caroliniana*, *Achlya* sp., *Brevilegnia* sp., *Calyptrolegnia achlyoides*, *Geolegnia* sp., and *S. anisospora* were recovered almost exclusively in those quadrats where the soil was above pH 6.0, and Dick did not collect these same species if the samples were below pH 4.2. *Aplanopsis spinosa*[†] occurred throughout the pH range expressed in the area sampled. More than half of the species recovered developed in gross cultures of soils in which the pH was not below 4.2. In summary, the greatest frequency of occurrence of all species that Dick collected was realized by culturing soils within a pH range 4.3-4.8. In Denmark, A. Lund (1978) analyzed the saprolegniaceous flora of 185 samples of uncultivated soils, and 142 samples of cultivated ones in relation to pH of those soils. The greatest number of isolates of species known to occur both in soil and water (for example, *Achlyas* and *Saprolegnias*) were found in the Danish soils having a pH range of 4.4-6.5. The largest number of specimens of species known primarily or exclusively from soil (members of *Thraustotheca* and *Geolegnia*) occurred in those soils with a pH greater than 5.8. These observations by A. Lund seem to agree in general with Dick's (1963) data.

While the extant data on the occurrence and distribution of watermolds in relation to pH are scanty, two inferences can be derived. Most species are not commonly found in highly acid waters, (Tables 13A, C; 14), but exceptions have been noted. Acid soils, on the contrary, are more likely to yield a diverse flora of

saprolegniaceous fungi than are alkaline ones. These conclusions need to be validated by further exploration.

SALINITY

While it was Willy Höhnk (T.W. Johnson and Sparrow, 1961) who did the bulk of the pioneer work on the distribution and occurrence of nonparasitic freshwater fungi (including Saprolegniaceae) in relation to salinity, Stoll (1936) had earlier commented on this factor. He concluded that the brackish water ponds he sampled harbored an insignificant freshwater mycoflora [he collected *Achlya oblongata*, *A. polyandra* Hildebrand, *A. prolifera*, *A. racemosa*, *Aphanomyces laevis*, *S. dioica*[†], *S. thureti*[†] and *S. monilifera*] and because of the small numbers of isolates he simply could not be certain that salinity had any effect at all.

In an early account of the aquatic mycoflora of brackish water Höhnk (1939) concluded that *Saprolegnia* sp. (collected in loam soil eight meters from the water's edge) was retarded in its growth by increases in salinity. Later, he isolated *S. ferax* (among other species) from brackish water sediments, but maintained that the water molds were freshwater forms (Höhnk, 1952c, d). In a paper published in 1953(c), Höhnk reported that members of the Saprolegniaceae could not "conquer" an oligohaline environment, yet a year later (1954:413) stated that brackish water had a particular mycoflora of Oomycetes, with members of the Saprolegniales occurring in salinities averaging 5-7 ppt. Höhnk's paper of 1956(b), however, is his chief ecological contribution with respect to the water molds (the major components of this publication were repeated by him in 1957b, and appeared in English in 1961).

Höhnk (1956b) divided brackish water habitats into four regions beginning with a zone (I) in which the salinity of the water and bottom sediments was less than 7 ppt. The second zone (II) included those saline habitats at 7-15 ppt, and additional ones were characterized by salinities beyond 15 ppt. He isolated saprolegniaceous forms from water and sediment in brackish zones I and II, but not from any region where the salinity exceeded 15 ppt; *Saprolegnia variabilis*[†], *S. monilifera*, *Dictyuchus sterile*[†], and unidentified members of *Saprolegnia*, *Aphanomyces*, and *Calyptralegnia* appeared in his collections. Although Höhnk obviously found water molds in saline habitats, the ones to appear in cultures from high saline habitats, were nonsexual "species" (Table 15). Höhnk's extensive data on distribution of representatives of the major groups of fungi in relation to salinity demonstrate that in a linear series of samplings from seawater to adjacent terrestrial and fresh water habitats the numbers of zoosporic fungi (including water molds) which can be isolated increases. In general, Artemchuk's study (1974) of Oomycetes in the White Sea supports Höhnk's observations: most such fungi occurred in littoral regions of low salinity and abundant detritus.

Harder and Uebelmesser (1955) found that members of the Saprolegniaceae (not identified) could be collected from maritime coastal habitats including submerged sediments. Ulken (1966) likewise isolated "Saprolegniales" in coastal waters (Brazil).

Leptolegnia caudata, *Aphanomyces* sp., and *Achlya racemosa* were collected by Scott (1962) from water and bottom muds (of coastal Virginia) at 16.13 ppt. These fungi survived submersion in salt water, even if they did not grow in such an inhospitable environment.

Three field studies of the estuarine distribution of Saprolegniaceae along a salinity gradient are prominent. TeStrake (1958, 1959) collected several common species of the family in the freshwater reaches of a North Carolina estuary, but the highest salinity at which a representative was found was 2.8 ppt (*see* Chapter 16 for an account of her experimental data, *in vitro*). Reporting on work of a similar nature on a Welsh river, R. A. Couch (1951) wrote that *Aphanomyces laevis* was the only species to occur in the most saline part of the river (*see* also I. Wilson, 1960). Padgett (1978a) submerged bait (others had collected water samples to be baited in the laboratory) to trap species in that portion of a narrow estuary having a relatively constant salinity gradient of 0-15 ppt. The salinity ranges of water from which he collected particular species were as follows (numbers in ppt): *Achlya americana*, 0-3; *A. flagellata*, 0-6; *Achlya* sp., 0-12; *Dictyuchus* sp., 0-3; *Saprolegnia* sp., 0-8, and *S. australis*, 0-6. Padgett thus found saprolegnians in waters with much higher salinities than had been reported by TeStrake (1959).

How do individual species of water molds react to salinity fluctuation that is one of the hallmarks of the estuarine habitat? The pioneer (if not the only) experimental study to approach this question is that by Padgett (1978b). By means of an ingenious variable medium culture chamber (Padgett and Lundeen, 1977; *see* Chapter 2) he subjected *Saprolegnia australis* to an artificially controlled diurnal tidal cycle with low tide waters having a magnitude of 0-4 ppt and that of high tide waters in the range of 8.5-14.3 ppt. Sporulation was sought in the various cultures. In water that did not fluctuate in salinity, the mortality of spores of *S. australis* was approximately doubled with each 4 ppt increase in salinity. On the other hand, the total percentage of spore germination increased with lengthened exposure time to particular levels of salinity. It seems very likely -- if Padgett's data from simulated tidal cycles can be extrapolated -- that this one species of *Saprolegnia*, at least, can produce functional sporangia in a diurnal cycle of salinities in which high tide values reach 12 ppt provided the low tide salinity drops to 3 ppt or less.

While the spores of *Saprolegnia australis* are not demonstrably tolerant of salinity stress (Padgett, 1978b), disarticulating gemmae are produced by the mycelium of this species in a saline environment. Padgett (1980) has explored in the laboratory the ability of certain water molds to produce functional vegetative propagules in fluctuating salinity regimes obtained by use of the estuarine tidal cycle simulator. Vegetative propagation can occur in a simulated saline cycle that does not permit sporulation, but the potential for that propagation varies among species. In a simulated tidal cycle whose low and high tide values were 0 and 10 ppt respectively, *Achlya americana* was unable to colonize bait by vegetative means (sporulation was not a factor), whereas *Thraustotheca clavata* successfully did so in a regime where the salinity cycled between 5 and 18 ppt. Even some nonaxenic, vegetatively-propagating colonies were able to

compete with contaminating organisms for substrate. There is at least good evidence from laboratory-imposed conditions that the inhibitory effect of salinity on sporulation may not necessarily restrict water molds to a low saline environment if they are capable of producing gemmae.

To explain the occurrence of water molds in low saline waters TeStrake (1958) proposed that the level of nutrition was the controlling factor. In summarizing some of the observations from the thesis by J. L. Harrison, Bremer (1976) remarked that mere vegetative growth of a fungus in a saline environment was not a reliable measure for assessing its salinity response. By implication, then, reproductive ability in a saline habitat must be considered. Dick (1968b), commenting on a paper by Stuart and Fuller (1968b) on the occurrence of *Saprolegnia parasitica*[†] in estuarine waters, also criticized work designed to explain saline tolerance by freshwater fungi. He emphasized that investigations such as TeStrake's only showed that viable propagules were present in a saline habitat but not that they were generated at that site. This is a pertinent observation that should figure prominently in any future experimental and interpretive work on salinity tolerance in natural habitats. Unpublished observations (Padgett, communication) suggest that ecotypes of some members of *Saprolegnia*, *Achlya*, and *Thraustotheca* may become established in estuarine habitats by means of vegetative propagules released from populations in freshwater (or very low saline situations). Once established, these ecotypes frequently do not sporulate yet grow quite well in simulated estuarine conditions. Thus, the failure of a water mold to sporulate in a particular saline region is not necessarily evidence that it is absent from that region or is ecologically "inactive" there.

While an explanation for the appearance of water molds in saline waters is still to be found, Padgett's (1978b) experimental work demonstrating that sporulation can occur during tidal cycles cannot be ignored. His results are diametrically opposed to the viewpoint expressed earlier by R.A. Couch (1951: 56): "... fungi may find a fluctuation in salinity more difficult to withstand than they would a fixed high salinity with no variation." The discovery by R. Mitchell and C. Wirsén (1968) that some water mold hyphae are lysed by raw seawater and by a marine bacterium is also a factor to consider in the general ecology of Saprolegniaceae in maritime habitats.

MISCELLANEOUS FACTORS AFFECTING DISTRIBUTION

Soil Depth: -- Utilizing E. J. Butler's (1907) method of collecting water molds in soil, J.V. Harvey (1925a, b) recovered abundant specimens from soil down to depths of six inches (15 cm), and sparsely from samples taken as deep as 12 inches (30 cm). During an initial analysis of the microfungi of alluvial grassland soil, Apinis (1958) collected but one species of Saprolegniaceae, *Brevilegnia unisperma*. Later work published by him in 1964 (a shorter version appeared in 1960) provided considerable information on depth distribution of these fungi. Soil samples from depths of 1-135 cm were cultured for zoosporic fungi. The data showed that water molds were most often isolated from soils at depths of 1-5 cm, and also revealed that species could be placed in

one of four groups (Apinis, 1964). *Thraustotheca clavata* alone occupied Group A, being distributed only at one depth level, 1-5 cm. *Achlya* sp., *A. apiculata*, *Aphanomyces scaber*, *Dictyuchus pseudodictyon*, *Isoachlya unispora*[†], *Saprolegnia* sp., and *S. ferax* -- Group B, according to Apinis -- were found at two depths, 1-5 cm and 20-25 cm. In Group C were those water molds distributed vertically at three depths: *A. racemosa*, *A. spinosa*, and *B. unisperma*, but having a maximum level at 55-60 cm. The fourth group (D) was represented only by *S. glomerata*; this species was distributed at four levels in soil, with a maximum at 100-105 cm. In a study of the water mold flora of Danish soils A. Lund (1978) reported that there were no differences in the composition of that flora with respect to soil depth.

Environmental data do not accompany these various reports on vertical distribution of Saprolegniaceae. In all likelihood, the species found in very deep soil samples (those reported by Apinis, 1964, for example) were there as dormant propagules -- oospores possibly -- rather than as active components of the mycoflora.

Elevation: -- Altitude, *per se*, is not an environmental factor (nor is latitude or soil depth), although characteristic climatic edaphic, and biotic factors accompany this physical feature. For example, some of the most productive sites for water molds on oceanic islands occur in the high elevation bogs and swamps of cloud forests. We have found an abundant and luxuriant saprolegniaceous mycoflora in the Alakai Swamp (Mt. Waialeale) on Kauai (1500 m), on El Yunque (900 m) in Puerto Rico, and in water-filled depressions on Mt. Victoria in Fiji.

Reports of water molds in relation to elevation are scanty indeed, but very likely only because mycologists have been somewhat provincial in their explorations. The collections made by E.M. Brown (1938) from ponds and pools at three levels [sea level to about 1200 feet (366 m)] in Wales yielded nothing to indicate patterns of altitudinal distribution. From samplings made in the western United States, J.V. Harvey (1942) concluded that a higher elevations, and in the northerly latitudes at low elevations, water molds were sometimes scarce or absent. Conversely, at lower levels in southerly latitudes (in the western U.S.) they were usually plentiful, but on occasion were absent from soils at sea level. Harvey collected some specimens in soils from an elevation of 6700-8500 feet (2044-2593 m); Kuehn (1960) found seven species of Saprolegniaceae in water from rivers, reservoirs, ponds, and a fish hatchery in northern New Mexico at these same altitudes. On the island of Madeira Höhnk (1962) isolated three water molds from soil samples taken at elevations of 675-1007 m.

Light: -- Illumination is not ordinarily thought of as a factor influencing the occurrence and distribution of water molds in nature, and the literature certainly reflects this view. In some general comments on the occurrence of aquatic fungi Petersen (1909a, 1910) stated that shady locations were not favorable for collecting such fungi, although he remarked that the absence of light was not detrimental. Prowse (1954a) reported that the planonts of *Aphanomyces daphniae* were usually released at night, but made no comments beyond this point. The diurnal migration which Suzuki (1961c)

detected in saprolegniaceous spores in shallow lakes was presumed to be related to oxygen level that, in turn, changed with alternating periods of light and dark.

Distribution by Animals: -- Citing as evidence the notably extensive distribution in English soils of *Saprolegnia asterophora*, *Achlya radiosa*, and *Aplanopsis spinosa*[†], for example, Dick (1962) suggested that the indigenous mesofauna might "assist" in dispersal. That the oogonia of these fungi are ornamented, he speculated, would seem to favor their transport by this means. Thornton (1970) recovered *Achlya dubia* and *Aplanopsis spinosa*[†] from earthworm casts, but not from washings of the whole animals. Bird droppings also, Thornton (1971) found, harbored viable propagules of *Geolegnia* sp. and *Thraustotheca clavata*. While these isolated cases of transport do not prove that water molds are distributed in this fashion, propagules at least survive animal passage.

Other fungi: -- The concept of the influence of other fungi on the occurrence and distribution of water molds has been almost entirely neglected. There are instances of parasitism of water molds by other fungi (see Appendix), but these have not been accorded ecological significance. Dylewski and Miller (1983) conducted cross-inoculation studies of *Woronina pythii* Goldie-Smith and 26 species of *Achlya*, four of *Saprolegnia*, three of *Brevilegnia*, and single isolates each of *Aplanes*, *Aphanomyces*, and *Dictyuchus*. None was susceptible to the *Woronina*. Such a finding and experimental approach suggests that an exploration of resistance/susceptibility might prove informative to the knowledge of the ecology of water molds.