

CHAPTER 30. Parasites and Pathogens: the Crayfish Plague and Other Associations

Saprolegniosis (Chapter 29) is a widespread, familiar disease (or a complex of diseases, perhaps) seemingly accorded only a lowly place in the economics of fisheries. Save for occasional outbreaks, it is a malady of endemic proportions with which the freshwater fisheries industries coexist. Not so the crayfish plague (Krebspest, Kräftpesten), caused by *Aphanomyces astaci* (Schikora, 1906). This is a disease of epidemic extent, and although it affects only a small industry -- the sale and export of edible crayfish, particularly in the Scandinavian Peninsula -- it seriously reduces populations of an animal regarded by many as an irreplaceable delicacy.

In this chapter we consider what is known of the crayfish plague, and, as well, chronicle a miscellaneous assortment of case histories of other associations between animals and water molds.

THE CRAYFISH PLAGUE

It generally is assumed (Unestam, 1969a) that crayfish plague was first discovered in Italy in 1860 (Seligo, 1895), but perhaps the malady that Ninni referred to in 1865, traced from the Italian province of Lombard, was this disease. In any case, the cause of crayfish disease epidemics was unknown at first.

Seligo (1895) published the first extensive account of crayfish plague, noting that the cause had been variously attributed to ectoparasites, bacteria, and even to a vaguely defined "mycosis astacinus." Although he was not certain that a real connection existed between the annelid worms inhabiting *Elodea canadensis* Mich. and certain Branchiobdellans (the latter allegedly caused the disease, and were transferred by the annelids), Seligo did not rule out such a possibility. As late as 1934, the supposed relationship between crayfish plague and aquatic vascular plants was being explored. Schiemenz (1934) concluded that these weeds did not really make the aquatic conditions "unhealthy" for crayfish; bacteria were responsible for the disease, he maintained.

The first authentic record of crayfish plague in Germany appeared in 1864, where the pestilence evidently had spread from Italy through France. The disease dispersed quickly through Central Europe, reaching Russian waters about 1891 or 1892 (Arnold, 1900), then migrated into Finland in 1893 where it extended its range rapidly throughout the chief crayfish regions (Westman *et al.*, 1973). Subsequently, the plague reached Sweden sometime between 1907 and 1910, and there also became distributed rapidly and widely (Alm, 1929; Vallin, 1932, 1933).

In 1900, Mühlen reported a disease outbreak in crayfish in rivers of what is now the southern Estonian and northern Latvian region (formerly USSR). Mannsfield (1942) made an extended study of the plague in this area. The report by Happich (1900) of a "blight" of crayfish may or may not have been of the plague (as it was later to be known). In any case, he believed the cause of the disease he studied was *Oidium astaci*

Happich. Kozłowski (1968) commented on crayfish infection in Polish waters. As of 1969, Vik noted that crayfish plague had not reached Norwegian waters; but in 1972, Håstein and Gladhaug reported finding diseased animals in that country in a river at the Swedish-Norwegian border. Although there seems little doubt that crayfish plague moved through France on its north and eastward spread from Italy, Vey and Vago, in published reports in 1972 and 1973, did not list *Aphanomyces astaci* as a cause of crayfish disease in France. Vey (1976a, b) did not detect this organism on diseased crayfish in France, and has been unable to authenticate any cases of the plague there (Vey, 1979).

THE CAUSAL AGENT

Although Hofer had in 1898 ascribed the cause of crayfish plague to *Bacillus pestis astaci*, it was in 1906 that his chief summary account of the malady appeared. In 1899, A. Weber wrote that Hofer was correct, but Mühlen (1900-01) believed that there were several causes of the disease, among which was a bacterium. By 1903, Schikora had proven to his satisfaction that the plague was caused by a species of *Aphanomyces* (not then named), and cited experimental supporting evidence from culture work and artificial inoculations. Hofer (1906), in defense of his view that the cause was a bacterium, criticized Schikora for using cultures, because, Hofer maintained, these simply eliminated the organism that was the actual culprit. Prior to Hofer's account, Hilgendorf (1884) and Leuckart (1884) reviewed the nature of the crayfish disease, and discussed the various hypotheses put forth to explain the cause.

It was Schikora, then, who first proposed that crayfish plague was an "aphanomycosis." He was to repeat this view -- and answer criticisms of it -- in a series of papers culminating in 1926 with an analysis of the previous five decades of work (in part his own) on the disease (Schikora, 1904, 1905, 1906, 1913, 1914, 1922, 1926). Schäperclaus (1927, 1928, 1935) confirmed Schikora's observations that a species of *Aphanomyces* was the cause of the disease, chiefly by proving that bacteria were not responsible for the necrosis of crayfish chitin. The very intimate position of the hyphae in and below the chitinous layers of diseased animals was further evidence, he thought, supporting Schikora's view. Contrary to Schikora's conclusion that the damaging effect of the fungus was on the invaded animal's musculature and vital organs, Schäperclaus believed that the *Aphanomyces* attacked the exoskeleton and nervous system.

To Nybelin (1931, 1934, 1936, 1954), working in Sweden, goes the credit for proving through pure culture means that *Aphanomyces astaci* was the cause of the plague. He isolated the fungus from the ventral nerve cord in the tail of a crayfish after having inoculated the posterior musculature with a small piece of infested cuticle (Nybelin, 1934, 1936). Such inoculations repeatedly resulted in typical symptoms in the test animals within 28-37 days. Nybelin became convinced that *A. astaci* was in fact specialized for existence in crayfish and unable to survive in nature as a saprophyte (we have collected abundantly in Swedish waters where crayfish plague is known to occur, but have not once recovered the fungus, on baits, in the absence of the host).

Rennerfelt (1936) studied extensively the growth of *Aphanomyces astaci* in culture, giving particular emphasis to the effect of various factors on sporulation and development (Chapters 17, 19). Although he described oogonia and oospores for *A. astaci*, these have not since been seen, and the illustrations he provided are not convincing.

It has been demonstrated on one occasion that genetic strains of *Aphanomyces astaci* may exist. Unestam and Svensson (1971) experimented with various isolates of the species finding that loss of virulence was reversible (by passage of a virulent strain through a test animal). When a strain no longer was capable of sporulating, however, that loss was permanent.

There are several general technical and popular accounts of crayfish plague designed to alert the crayfish-hunting public to the disease and the fungus: Gulbrandsen (1976), Håstein and Unestam (1972), Spitzzy (1972), Unestam (1961, 1962, 1964, 1965b, 1968b, 1969a,b, 1970, 1973a, b, 1974b), Wennberg (1963), and Westman (1973). Pauley (1975) regarded the crayfish plague as one of the four most serious infectious diseases of economically important crustaceans. An excellent historical summary of the introduction, use, and economics of the host animal was written (1969a) by Sture Abrahamsson. The series of review accounts and progress reports edited by Fürst (1978) provides especially broad coverage of what is known of the crayfish plague.

CHARACTERISTICS OF THE DISEASE

Several accounts treat the general symptoms and signs associated with infection in crayfish: Amlacher (1954, 1961, 1970), Benish (1940), Kozowski (1968), Schäperclaus (1935), Tsukeris (1964), and Unestam (1968b), among others. Hyphae of the causal fungus penetrate directly into the chitinous layers, particularly in articulations where the integument is thin. The mycelium grows into the chitin, eventually reaching the musculature and the main elements of the nervous system. In severe cases of infection, filaments may in time grow outward through the chitinous exoskeleton as well.

Internally, certain reactions seemingly of a defensive nature appear after the animal is invaded: blood clotting, encapsulation by blood cells, and melanization (Unestam, 1968b, 1974a). There is some evidence (Unestam, 1968b) that exotoxin production damages the nervous system, but according to Benisch (1940), the infected individuals die from bacterial invasion following fungal penetration, or as the result of being unable to molt because of the intramatrix mycelium. These two hypotheses have not gained general acceptance.

INOCULATION

In view of the persistent spread of the disease geographically, and the rapidity by which *Aphanomyces astaci* transfers from individual to individual, the fungus must possess a rather efficient mechanism for inoculation. Since *A. astaci* has no strong

chemotactic response -- its planonts are only weakly attracted to crayfish blood (Unestam, 1969d) -- inoculation of susceptible animals in nature would seem chancy at best. The epidemiology of the disease, however, contradicts such a suggestion. The work of Svensson and Unestam (Svensson, 1978) has provided some information on the matter of inoculation.

By means of osmotic induction experiments Svensson and Unestam (Svensson, 1978) demonstrated that the secondary planonts of *Aphanomyces astaci* could be induced to encyst in a weak solution of NaCl (among other compounds) but retained their reniform configuration. Moreover, that shape persisted even when the spore contents disintegrated, indicating that a thin but structurally functional wall had been formed around the cell. Indeed, fifteen seconds after a planont was induced to encyst a thin wall was evident, and a complete cell wall was produced in about two minutes. Spores (including the reniform ones) could be stimulated osmotically to germinate about one hour after they had been induced to encyst.

Svensson and Unestam (Svensson, 1978) also investigated the effect on germination of the osmolarity (and constituency) of the solution bathing encysted spores, and compared responses by spores of *Aphanomyces astaci*, *A. euteiches*, and *A. stellatus*. In *A. astaci*, osmolarity effected by low concentrations of salts, for example, was far more stimulatory to germination than were nutrient solutions. Although the spores of *A. stellatus* germinated following osmotic triggering, nutrient level was a more efficient stimulator. Germling hyphae (emerging from any point in encysted reniform spores) of *A. astaci* were not thigmotropic.

Attachment of *Aphanomyces astaci* spores to crayfish cuticle (and to other hydrophobic but not hydrophilic materials) appears to result from a lipophilic substance secreted by encysted spores (Svensson and Unestam, *in* Svensson, 1978). Planonts, on the contrary, are devoid of adhesive material.

Experimental data provided by Svensson and Unestam (Svensson, 1978) suggest that salts (or perhaps proteins) diffusing outward through the crayfish cuticle might have two functions. First, such molecules could trigger rapid encystment without accompanying structural changes of motile spores and thus in effect act as a surface trapping mechanism. Secondly, concentration gradients of salts at the cuticle/water interface conceivably act as the stimulus necessary to induce the germ tube from a spore to grow toward the cuticle, thus circumventing any disadvantage conferred by the absence of a thigmotropic reaction.

PENETRATION, REACTION, AND DEFENSE

The bulk of evidence points to *Aphanomyces astaci* as an organism well adapted to penetrating the host chitinous exoskeleton, as Unestam (1964) believed. By what countering mechanisms does the host resist or contain (at least temporarily) the foreign adversary? Experimental work and methodology (Unestam and Ajaxon, 1974) bearing on this question come exclusively from the laboratories of Unestam and his collaborators in Sweden (Unestam *et al.*, 1977). The physiological properties and

biochemical activities of the pathogen also have been characterized by Unestam and associates (Chapters 17, 23).

Epicuticular Penetration. -- As Unestam (1968b, 1969a) remarked, the first line of defense offered by the host is the nonchitinous, proteolipid epicuticular layer, but *Aphanomyces astaci* can penetrate this barrier readily and enter the subtending chitinous layer. Four characteristics of the fungus (Unestam, 1969a) reflect its adaptation to a parasitic existence. These are: (1) restriction to glucose and amino acids for maximum growth; (2) constitutive chitinase in the mycelium; (3) growth in crayfish blood, and (4) maximum sporulation (Unestam, 1965a) in water with a low mineral content (as would be expected in those freshwaters inhabited by *Astacus astacus*). According to Svensson and Unestam (Svensson, 1978), the mechanism for spore adhesion, salt-stimulated encystment, and rapid encystment and germination (*see* foregoing section on inoculation) further testify to this adaptation.

Once the epicuticle is ruptured, Unestam and Weiss (1970) report, *Aphanomyces astaci* rapidly invades the chitin. When spores are injected into the abdominal hemocoel of susceptible animals, they aggregate around nerve cords. Blood cells encircle these clumps, an event that is followed by melanization in the region surrounding the spores and the tips of penetrating hyphae. Thus, the accumulation of melanin it has been argued (Unestam and Weiss, 1970), constitutes a defense mechanism. There is, of course, supportive evidence in the report by Kuo and Alexander (1967) that melanins inhibit protease and chitinase activity.

Penetration of the host cuticle by the fungus evidently is a combination of mechanical pressure and enzyme activity (Nyhlén and Unestam, 1975; Söderhäll and Unestam, 1975). A spore on the outer lipoid layer of the cuticle appears to push the layer aside as the cell enlarges. A penetration peg then thrusts into the epicuticle, and upon reaching the chitinous and proteinaceous endocuticle produces a hypha. At this time, but not earlier, chitinase activity can be detected *in vitro* in germinating spores (Söderhäll *et al.*, 1978).

Ultrastructural features of penetration have been characterized to some extent by Nyhlén and Unestam (1975). They could not detect any mucoid coatings or surface structures on the spore wall that would promote adhesion, but noted that cuticular folds could serve to trap the spores. The spore nucleus (0.3-0.6 μm in diameter) divides prior to germination and to formation of a penetration peg. There is no appressorial apparatus, hence the peg alone seems to be involved in both digestion and mechanical disruption of the cuticular material. Hyphae developing from the infection peg initially grow in the endocuticle approximately parallel to the epicuticle.

Subsequently, growth becomes more random as the cuticle is further digested enzymatically. Of course hyphae also grow from the inside outward through the epicuticle, but in such cases, the hyphal tips are swollen. Hyphae growing along the surface of the cuticle do not penetrate it (Unestam and Nyhlén, 1974), and appear not to cause any surface "corrosion."

Melanization. -- The host blood and cuticular reactions to the penetrating hyphae were studied chemically and ultrastructurally by Unestam and Nyhlund (1972). In the invaded animal blood cells clump quickly around hyphal tips, and prophenol oxidase is activated. Within a few hours after blood cell clumping (Unestam and Nyhlén, 1974), a refractive zone appears around the hyphae, and subsequently the area turns brown as melanin is deposited. This reaction occurs in the presence of *l*-dihydroxyphenylalanine, but this compound evidently is not the natural substrate for pigment formation, with its source in the clumped host cells (Unestam and Nyhlén, 1974; Unestam, 1975). Encapsulation followed by melanization of the hyphal wall occurs also in musculature penetrated by hyphae of the *Aphanomyces* (Unestam, 1974a; Unestam and Nyhlén, 1974; Unestam and Weiss, 1970).

The initial clumping reaction by the hemocytes Unestam and Nyhlén (1974) found, was nonspecific, and took place even in crayfish blood circulating in an artificial system into which hyphae or spores were introduced. Encapsulation occurred around hyphae of Ascomycetes and Basidiomycetes, an alga, and even in response to nylon and cotton fibers, but melanization occurred only with fungi having β -1, 3-glucans in the hyphal wall (Unestam and Söderhäll, 1977, Unestam and Beskow, 1976).

By spectrophotometric methods and the use of a microcuvette constructed of a glass slide (Unestam, 1976), Unestam and Beskow (1976) demonstrated that crayfish blood cells in contact with *Aphanomyces astaci* and some other plant materials (but not cellulose, laminarin, or purified chitin) formed phenol oxidase, followed by melanization (phenols are oxidized to melanins). Moreover, prophenol oxidase of crayfish hemocytes is activated by water-soluble material [containing β -1, 3- glucans active in concentrations to 10^{-10} M (Unestam and Söderhäll, 1977)] from hyphal walls of various fungi and *A. astaci*. Activation of this enzyme is specific for β -1, 3-glucans and for a linear pentasaccharide composed of (1 \rightarrow 3-linked β -D-glucopyranosyl residues (Söderhäll and Unestam, 1979). Heat-inactivated, purified glycoproteins of *A. astaci*, containing β -1, 3-glucans, are also active against prophenol oxidase.

Unestam and Ajaxon (1976) reasoned that if phenol oxidase activity was a defense mechanism, the enzyme would be very effective if adsorbed on the surface of the hyphae of the invading fungus; such a response has been demonstrated (Unestam and Nyhlén, 1974) to occur both in the blood and in the crayfish cuticle. Söderhäll and his associates (1979) demonstrated that prophenol oxidase from crayfish hemocytes could attach to many foreign surfaces; the attached enzyme was specifically activated on fungal hyphal walls containing β -1, 3-glucans. Cuticle phenol oxidase was also attached to hyphal walls when applied onto a naked endocuticle. Apparently the "agglutinin behavior" of the phenol oxidase renders the enzyme more effective when interfering with an invading parasite. Unestam and Ajaxon (1976) proposed that the cuticle was an active transmitter of a signal from the point where it was breached by the invading fungus to the subtending epidermis where the "ingredients" of melanization originated.

Figure 58 depicts diagrammatically the possible defense points at which melanization is effected. Melanin production occurs also at those sites where spores or

hyphae contact the cuticular surface. It has been suggested (Unestam, 1974a) that phenol compounds accumulate at the nidus of penetration -- as, for example, at a hyphal tip -- under the action of phenol oxidase in the crayfish cuticle. The phenols then could interfere with the synthesis of proteases and chitinase required by the fungus for continued growth and subsequent penetration. However, except for melanization in the musculature, there are essentially no reactions to invasion in the internal organs of the crayfish. It thus seems well established that the response to attack is limited almost exclusively to the integumentary parts and the hemolymph (Unestam and Nyhlén, 1974).

Although a function for phenol oxidase in crayfish resistance cannot be denied, this is perhaps not the only enzyme system involved in the host/pathogen relationship. Lang and his colleagues (1977) have studied mixed-function oxidases in *Astacus astacus*. There was no (or only very low) activity of such enzymes (for example, arylhydrocarbon hydroxylase) in microsomal fractions from gills, hepatopancreas, and intestine of the animals. Moreover, individuals invaded by *Aphanomyces astaci* showed no increase over uninfected ones in microsomal oxidase enzyme activity.

Resistance Reactions: -- The mechanisms of a mechanical barrier afforded by the cuticle, and of a phenol oxidase-glucanase-melanin response (foregoing section) appear to provide a defense against *Aphanomyces astaci* for the highly susceptible *Astacus astacus*. Do the more resistant crayfish species display a comparable reaction?

Although it is not immune to invasion by *Aphanomyces astaci*, the American crayfish *Pacifastacus leniusculus* is highly resistant (Unestam, 1969a) even to infection by growth from injected spores (Unestam, 1968b). Hyphae of the fungus developed in the cuticle of *P. leniusculus*, Nyhlén and Unestam (1975) observed, when the epicuticle was removed. Unestam (1969a) put forward the hypothesis that during natural selection processes, a number of parallel defense systems were developed by *P. leniusculus* against *Aphanomyces* penetration. Through selection the fungus only had to breach one system to invade the cuticle (where it was protected against competition with other microorganisms). Other defense mechanisms, however, hindered rapid growth of the fungus so that it did not penetrate throughout the cuticle and into the blood and other organs. Thus, such an animal exhibited high but not complete resistance, but in a highly susceptible host none of these defense mechanisms developed in the absence of a potent pathogen. Accordingly, when *A. astaci* is introduced into a susceptible population, the hosts are unprotected and die before the necessary resistance factors can appear during natural selection.

It has been demonstrated by Unestam and Nyhlén (1974) that when hyphae of *Aphanomyces astaci* penetrate *Pacifastacus leniusculus* during cases of natural infection, the filaments become more highly melanized than do those of the fungus in the cuticle of *Astacus astacus*. Melanization effected in the presence of whole blood also is much more intense in the resistant than in the susceptible crayfish (Unestam and Nylund, 1972). Moreover, Unestam and Weiss (1970) noted, melanin is always produced much more rapidly in individuals of *P. leniusculus* than in those of *A. astacus*.

HOST RANGE

Aphanomyces astaci as a parasite is limited largely to decapod crayfish in the Astacidae and Parastacidae (Crustacea). The fungus is found widely on the European continent, and also occurs in some of the more resistant crayfish of North America. The *Aphanomyces* disease appears not to occur in animals in Australian or Japanese waters (Unestam, 1972), but some crayfish species from these countries are nonetheless susceptible (Unestam, 1969c, 1975). Planktonic crustaceans, Unestam (1969c) has suggested, possibly function as carriers of *A. astaci* in the absence of susceptible crayfish. There are no authenticated instances of the fungus existing as a free-living organism.

Because much of the information on hosts for *Aphanomyces astaci* has come from experimental inoculations (Unestam, 1969c, 1972, 1975), the natural host range of the parasite is open to debate. Table 48 lists those species that show some degree of susceptibility (that is, are not completely resistant) to *A. astaci*. The literature is not entirely clear on the intended implications of "resistance" but *Gaxonella clypeata*, *Mysis relicta* (a planktonic crustacean), most species of *Oronectes* and *Cambarus*, and two species of *Procambarus* have not been artificially infected under laboratory conditions (Unestam, 1969c). Amlacher's (1970) statement that Shanor and Saslow (1944) found *A. astaci* on fish is incorrect.

DISSEMINATION

In 1929, Alm wrote that crayfish plague in Swedish waters was spread by steamer restaurants discarding overboard infected animals taken on at various ports. Gabran (1939) thought that birds were responsible for much of the spread of the disease by feeding on infected crayfish, and dropping remnants into other bodies of water as they migrated locally. Pleasure craft and fishing boats were able to spread the plague readily and widely, according to Vik (1969) and H. M.-K. Lund (1975). Unestam (1973b) suggested that man was the chief vector of the causal agent through such activities as handling infected animals in traps and other fishing equipment. Where crayfish harvesting was particularly heavy, Unestam wrote, the plague always spread most rapidly.

CONTROL

The early observations linking water traffic to the spread of crayfish plague led to attempts to control both the distribution of harvested animals and the use of fishing gear. In 1932, Sweden established laws to regulate the harvesting of crayfish (Wennberg, 1963): forbidding the transport of crayfish from one body of water to another, and limiting the fishing season, among other restrictions. Norway similarly enacted very restrictive laws (Håstein and Gladhaug, 1972; H. M.-K. Lund, 1975).

Among these were regulations prohibiting the use in Norwegian waters of any fishing gear that previously had been employed outside the country, requiring disinfection of any harvesting equipment before it could be used in different lakes, and specifying that animals had to be processed at the site of harvest, and not transported as live catches. As early as 1929, Alm recommended that transport of infected animals be halted and Amlacher (1961) suggested that quarantine and sanitation measures might be necessary to control the disease.

Chemicals have been screened as possible agents to prevent the spread of the crayfish *Aphanomyces*. Rennerfelt (1936) was the first to determine the effects of various compounds on the fungus, *in vitro*, but the data from his experimental work could not be applied to practical control methods. Reporting on the spread of crayfish plague in Sweden, Vallin (1932) suggested that dead animals could be treated with formalin to diminish the chances of inadvertently spreading the causal organism. Tsukeris (1964) found *A. astaci* to be sensitive to the sulfate ion and suggested that such a compound might control the disease. The chief chemical employed in attempts to delay spread of diseases crayfish, and hence of the fungus, has been slaked lime, Ca(OH)_2 .

Vallin (1933) used lime to kill indigenous populations of infected animals so that uninfected individuals moving into those waters could not be inoculated. This method was partially effective, and in 1936, Vallin wrote that freshly prepared calcium hydroxide so drastically increased the pH of the water that the susceptible animals were killed, and, in essence, a crayfish-free zone was created. It was assumed that the causal agent could not exist where there were no hosts, and therefore spread of the disease was halted. Svensson, Söderhäll, Unestam, and Andersson (1976) found that lime had little effect in suppressing the disease in laboratory and field tests unless it was used in a saturated concentration so that the resulting pH of the water exceeded 11.5 for one day.

Vallin's (1933) concept of creating crayfish-free zones to block the spread of the fungus is at the basis of management of the plague by an electrical barrier (Håstein and Unestam, 1972; H. M.-K. Lund, 1975; Söderhäll *et al.*, 1977; Svensson *et al.*, 1976a; Unestam *et al.*, 1972). Submerged electrified fences, Unestam and his colleagues found (1972), created a buffer zone that prevented infected animals from moving upstream into disease-free regions. Since the plague would spread rapidly downstream from the barriers and kill the animals in those waters, the fungus subsequently would die out. In ponds (Svensson *et al.*, 1976a) liming could be used to prevent animals from entering into the infected area, and to insure that the only entrance was that served by the electrical barrier. When the residual animals in the pond succumbed to the *Aphanomyces* they could be flushed out, and subsequently disease-free animals from upstream allowed into the pond by opening the electrical fence. Such a barrier simply permitted the disease to run its course in isolation, resulting in the death of the fungus. The method of control by encouraging such natural eradication of hosts is of course temporary.

As an introduced species (Fürst, 1977, Svärdson, 1965, 1968, 1970) *Pacifastacus leniusculus*, which carries *Aphanomyces astaci* naturally (and spreads the disease to

Astacus astacus (Fürst, 1977), but is little harmed by it, could serve to circumvent crayfish plague to some extent, but there are dangers in the use of such introductions (Spitzzy, 1972). Unestam (1973b) warned that simply replacing the susceptible *Astacus astacus* with a resistant species could introduce new problems such as ones accompanying different parasites, pathogens, or commensal forms. In a popular account of the plague, Karlsson (1977) noted that twelve countries had begun to introduce *P. leniusculus* ("Signalkräftan"). In Sweden, for example, this species is propagated under strictly controlled conditions in hatcheries. After the second molt the animals are released for stocking (see Abrahamsson, 1969b, and Brinck, 1977 for comments on introductions). Fürst and Boström (1978) have provided an informative analysis of the impact of the introduction of *P. leniusculus* into Swedish waters (in 1960). Symptoms of the crayfish plague appeared in nearly all established populations of this species, and the frequency of infection by *A. astaci* seemed to rise as the size (length) of the individual and its age increased. The report by Fürst and Boström confirms that *P. leniusculus* can harbor the plague fungus. However, the degree of infection (number of animals infested and number of infection sites) in populations of this crayfish in Sweden is less than in comparable populations in Canadian and American waters.

Orconectes limosus (Raf.) was introduced into Germany in 1890 from the United States, and thence into France and the Netherlands. This species was expected (Geelen, 1975) to replace the plague-prone *Astacus astacus*, but a stocking experiment with *O. limosus* reported by Svärdson (1976) failed.

In sum, suitable controls for crayfish plague must prevent or decelerate spread. A combination of barriers, liming, and enforced sanitary measures appears to be the most satisfactory procedure. Perhaps, Fürst and Boström remark (1978), there is no better way known to restore Sweden's crayfish waters than to continue introducing the resistant (though not immune) species.

ECONOMIC IMPACT

Alm (1929) reported that for Sweden alone, there essentially was no export of crayfish during the period 1920-25 after the severe epidemic of plague prior to 1920. During the same five years, imports of crayfish more than tripled in Sweden. In an analysis of the impact of the plague, Unestam (1969a) reported that in one Swedish lake of 484 km² five million animals were harvested annually before the epidemic. Svensson *et al.* (1976b) suggested that crayfish plague could be eradicated in about five years from Swedish waters by strict and inclusive application of control practices. The cost for such a program would be about one million Swedish kroner; the value of crayfish to the harvesting and processing industry in Sweden is estimated at 20 million kroner annually. To provide crayfish to meet the demand of this luxury item (harvested only in a 2-4 week period in August, in Sweden; Svärdson, 1965) import of edible crayfish into Sweden in 1971 was 442 metric tons; in 1976, import totaled 2783 tons (Fürst, 1977).

A survey conducted by Järvi in 1910 confirmed the rapid spread of the disease in Finland and the drastic reduction in export of crayfish. By 1900, Finland was exporting

some 20 million crayfish annually. In 1907, the plague reached epidemic proportions, and in less than ten years, exports declined to under one million animals per year. According to Westman (1973), the annual loss due to crayfish plague in Finland was about \$2.3 million.

By 1972, the crayfish plague had appeared in two Norwegian rivers, and threatened the industry in that country. In 1975, according to H. M.-K. Lund, the catch of crayfish in Norwegian waters was about 50 tons, at an estimated value of 2.5 million kronor. For the two rivers invaded by infested animals prior to 1972 estimates are that the loss to the Norwegian economy is about 30,000-40,000 kronor annually (Håstein and Unestam, 1972).

Revenue loss is only one aspect of the economics of crayfish plague. Of singular importance biologically is the impact of the disease on the natural balance among the elements of the aquatic biota following removal of the crayfish segment.

In 1939, Gabran called attention to a decline of mussels (species of *Anodonta* and *Unio*) in Estonian and Latvian waters in which the crayfish plague was epidemic. He suggested that this coincidence should be explored experimentally to determine if the mussels also might be infected by *Aphanomyces astaci*. From a study of crayfish populations in isolated ponds in Sweden, Abrahamsson (1966) concluded that the plague had a marked effect on the submerged vegetation in those bodies of water. Ordinarily, populations of submerged plants were kept in check by the grazing *Astacus astacus*, but when the crayfish were killed in substantial numbers by *A. astaci*, the vegetation (species of *Ranunculus*, *Potamogeton*, *Myriophyllum*, and *Chara*) quickly increased. Populations of some sedentary invertebrates also increased during the same period. Stands of *Chara* and *Elodea canadensis*, Unestam (1974b) noted, were suppressed by the crayfish, but rapidly expanded and became abundant when the animal was eliminated.

OTHER PARASITES AND PATHOGENS OF CRAYFISH

Achlya sp. was collected on crayfish eggs in the United States by Clinton (1893), but nothing further is known of this "association". In 1940 (p. 212), R. I. Smith reported that strains of *Aphanomyces laevis* were found as "... wound parasites..." of *Cambarus clarkii* Girard. These fungi simply prevented healing of natural wounds, Smith argued, and thus provided an entry portal for other organisms. He concluded that the fungi he isolated were not *A. astaci*, basing that decision on Rennerfelt's (1936) characterization of this species.

Certain other water molds grow on eggs of various crayfish. *Dictyuchus* sp. attacks the eggs of *Pacifastacus leniusculus* (Vey 1976a, b, 1977), and *Pontastacus leptodactylus* (Vey, 1979). The *Dictyuchus* which Vey studied penetrated the egg chorion, but did not attack adult animals. A second unidentified saprolegnian (Vey, 1977) invades the gills of *P. leniusculus*, resulting in the detachment of the epithelium from the basal membrane. Possibly this second pathogen is a *Pythiopsis* (Vey, 1977: fig. 50), but this is only speculative. An unidentified saprolegniaceous fungus also has been found,

in France, on the eggs of *Austropotamobius pallipes* (Vey and Vago, 1973). *Saprolegnia parasitica* (= *diclina*) has been implicated (Avault, 1972) as a parasite of crayfish adults and eggs in the United States, but is not considered a serious problem.

A "smut disease" on three species of crayfish was reported by Mann and Pieplow, in 1938. They believed the cause to be previously undescribed species of *Didymaria*, *Ramularia*, and *Septocylindrium*, and maintained that "smut" of *Astacus astacus* was not the same as crayfish plague. A disease of *Atlantoastacus pallipes* Lereboullet (= *Austropotamobius*), in France has been traced to bacterial infection (Boemare and Vey, 1977).

WATERMOLDS AND OTHER AQUATIC ANIMALS

A number of saprolegniaceous fungi have been implicated as pathogens or parasites of various aquatic and amphibian animals, but with few exceptions the reports are little more than records. There is no evidence among the earliest reports of watermolds on animals -- Hannover (1839), a "*Conferva*" on *Triton punctatus* (see Buchwald, 1971); Stilling zu Cassel (1841), a "*Conferva*" on a frog, for example -- that the fungi involved were anything more than saprotrophic opportunists. Table 49 lists some representative species and their hosts. These so-called diseases generally were reported without any supporting experimental or observational data, hence the pathology of the cases is essentially unknown.

ASSOCIATIONS WITH MOSQUITO LARVAE

Although it is not at all unusual to find dead mosquito larvae infested by various saprolegnians (*Saprolegnia ferax*, *Aphanomyces laevis*, and *A. stellatus* are common), cases where watermolds are actively parasitic in these insects are indeed rare, and the fungus is not identified (Marshall, 1938). Chorine and Baranoff (1929) found two unidentified watermolds (probably members of *Saprolegnia*) infesting, respectively, the larvae of *Anopheles maculipennis* Mg., and *Odagmia ornata* Mg. The watermold in the latter also would infect the larvae of the former. Neither fungus would sporulate until the infected larvae died. Another unidentified *Saprolegnia*, ubiquitous in rice fields of the People's Republic of China was found by Jettmar (1947) to attack and invade inactive or injured larvae of *Anopheles hyrcanus*. According to Jettmar, the invading fungus was not normally a pathogen. *Saprolegnia monoica* (= *ferax*) has been found to kill larvae of *Culex fatigans* (Hamlyn-Harris, 1932). An *Aphanomyces* (not identified) from dead larvae of *Anopheles gambiae* (Nigeria) has been implicated experimentally in infection of *Anopheles albimanus* in larva maintenance units (Seymour and Briggs, 1985). By certain management practices, for example by discontinuing use of plant infusions in rearing units, and heating or filtering the water in such units, the *Aphanomyces* sp. could be eliminated. Artificial infection experiments with *S. ferax* (reported as *S. thureti*) from dead mosquito larvae, pupae and adults caused mortality in *Culex pipiens pipiens* L. (Kal'vish and Kukharchuk, 1974).

The most extensive published observations on a watermold --*Saprolegnia diclina* -- parasitic in mosquito larvae appeared in 1956 (Rioux and Achard 1956). Rioux and Achard demonstrated by means of inoculation experiments that this species was a primary pathogen in *Aedes berlandi* Séguy, *A. detritus*, *A. geniculatus*, and *Orthopodomyia pulchripalpis*. *Saprolegnia diclina*, they maintained could play an ecological role in reducing larval populations of susceptible mosquitoes.

In Ohio, Seymour (1976) isolated a parasitic *Leptolegnia* (identified at the time as *Leptolegnia* sp.) from larvae of *Aedes triseriatus* (Say). Preliminary tests demonstrated that the fungus was a virulent pathogen of various culicine and anophiline mosquitoes. In larvae of *Ae. triseriatus* infection is usually initiated in the vicinity of the bilateral tracheae with subsequent involvement of the hemocoel (communication, R. L. Berry).

Subsequent to Seymour's initial account of the larvicidal *Leptolegnia* (*L. chapmanii*: Seymour, 1984) accounts of more detailed studies on etiology, parasitism, and pathogenicity appeared. Inoculation can occur by two routes, encysted spores on the larval cuticle (mechanical pressure alone does not seem the sole force for cuticular penetration; Zattau and McInnis, 1987), or endogenously by cysts that germinate in the alimentary canal (McInnis and Zattau, 1982). Entrance gained subsequent to the former mode may be only minor (Nnakumusana, 1986b). Within the midgut, penetration of germling hyphae into the hemocoel may occur in a little as six hours after inoculation. In the hemocoel, the blood cells are invaded, fatty tissue is penetrated, and then mycelial growth into musculature and mesenterion occurs (Nnakumusana, 1986b). According to Nnakumusana, there are no hyphal appressoria, but Zattau and McInnis reported such structures in their study of the infection process.

Within the cuticle, hyphae grow laterally (McInnis and Zattau, 1982). The epicuticle and exocuticle may show evidence of lysis (Nnakumusana, 1986b).

At the sites of entry, either at the cuticle or at the coelomic gut lining, blackening may occur in association with penetration or adjacent to lateral hyphae growing within the cuticle (McInnis and Zattau, 1982). This host response (noted by Zattau and McInnis, 1987, as well) recalls the melanization reaction seen in crayfish invaded by *Aphanomyces astaci*. However, only rarely in mosquito larvae does retarded development of the invading fungus accompany the discoloration. Observations on infection and subsequent growth of the *Leptolegnia* suggest that the chief "target" of the invasion is the hemocoel (Zattau and McInnis, 1987). The work on McInnis and Zattau (1982) and Nnakumusana and Seymour (1987) established the first and second instar larvae as being the most susceptible to *L. chapmanii* (100% infection) while third and fourth instar larvae show some resistance (<40- <15% infection).

Larval age is thus a factor in the degree of infection. Water temperature also is influential (McInnis and Zattau, 1982). In inoculation/infection experiments, 100% infection was achieved at incubation temperatures of 15-25 °C; there was no infection if the host and fungus were incubated at 35 °C. A sterol-rich medium, or hempseed extract in the growth medium may enhance sporulation and degree of infectivity (Nnakumusana, 1986c). Various other environmental factors may have some effect on fungal growth and resulting infection (Nnakumusana, 1986c). Organic pollutants

reduce planont longevity, and limit the degree of infection. Calcium in the medium enhances growth slightly, and magnesium and sulfur (inorganic, most favorably) are essential, even though sporulation may be reduced. Phosphate, borate, and acetate buffers in the propagation medium also contribute to reduced sporulation. Optimum pH for the fungus seems to be between 6 and 8.3. Oospore germination occurs at 28 °C but not at 35 °C (Nnakumusana and Seymour, 1987). *Leptolegnia chapmanii* loses its pathogenicity (Nnakumusana, 1986b) in culture, but this can be somewhat restored by growing the organism in a sterol-rich medium.

The host range of *Leptolegnia chapmanii* is rather broad (natural infection, and infection of test species under laboratory conditions). Nnakumusana (1986c), for example, reported 15 species of mosquitoes in Uganda to be susceptible. *Anopheles gambia*, the vector for malaria, *Culex fastigans* (filaria vector), *Aedes aegypti*, *Ae. africanus*, and the *Aedes* carrying the yellow fever agent, all showed very high mortality (98.8-100% infection) in 24-72 hours after exposure to the *Leptolegnia*. Other species known to be hosts, in the larval stages, for the fungus are (Nnakumusana and Seymour, 1987) *Ae. triseriatus*, *Haemagogus equinus*, *Culex pipiens*, *C. restuans*, *Anopheles quadrimaculatus*, and *Toxorhynchites nutilus* (its larvae are predaceous on other mosquito larvae). Inoculation studies by Mc Innis *et al.* (1985) extend this list to include *Culex salinarius*, *Culiseta inornata*, and *Ae. taeniorhynchus*. *Culex pipiens quinquefasciatus* and *Anopheles albimanus* (McInnis and Zattau, 1982; McInnis *et al.*, 1985) also are susceptible to *L. chapmanii*. Although very low levels of mortality resulted from infection experiments (Nnakumusana, 1986a), larvae of *Simulium neavei* and *S. damnosum* (blackflies) can serve as hosts for the fungus. Representatives of Dixidae, Crustacea, and Tabanidae are not susceptible (Nnakumusana, 1986c), nor are test species (larval, adult, and naiad stages) in such orders as Diptera, Noteridae, Odonata, Plecoptera, and Cladocera (McInnis *et al.*, 1985).

Two principal factors would seem to limit the use of *Leptolegnia chapmanii* as a biological control (larvacidal) agent (McInnis and Zattau, 1982). The fungus is sensitive to high water temperatures (commonly encountered in tropical areas where vectors of important disease agents exist), and the hyphae and spores appear to have only a short life span. Thus, inoculum viability is a limiting factor.

A curious phenomenon involving the invasion of mosquito larvae by an asexual strain of *Achlya* has been observed in Nigeria (RLS). Sensitivity tests were conducted on larvae of *Culex latigans* Weidemann. During their ascent to the water surface in dishes containing a colony of the *Achlya* on hempseed, the larvae occasionally would dislodge with their siphon a spore cluster which then adhered to the setae of the breathing apparatus. The encysted spores, triggered perhaps by a thigmotropic or chemotropic response, produced germ hyphae which subsequently penetrated the animal's siphon and hemocoel. Although the *Achlya* was capable of invading healthy larvae in this manner, inoculation tests using motile secondary spores gave no subsequent evidence of infection.

ASSOCIATION WITH CLADOCERANS

According to Petersen (1909a, 1910), P. E. Müller (1868) was the first to report *Leptolegnia caudata* as a parasite of *Leptodora kindtii*. The fungus reached epidemic proportions during the host's maximum frequency, and subsequently reduced the population substantially. Petersen thought that *L. caudata* entered the animal at the oral opening; he also believed that the production of oogonia within the diseased individual caused it to sink. It should be recognized that if Müller indeed saw clavate reproductive cells protruding from infected cladocerans, it is unlikely that the fungus was a *Leptolegnia* as Petersen believed.

Specimens of *Daphnia hyalina* var. *lacustris* collected by Prowse (1954a) were invaded by an *Aphanomyces* (*A. daphniae*). By means of germ tubes from encysted spores, the fungus penetrated integumentary chitin directly; ingested spores, however, were incapable of establishing infection. Prowse maintained that infection took place within three seconds after a spore had settled on the chitinous surface, and invaded individuals died about eight hours later. *Aphanomyces daphniae* would not infect living specimens or invade dead individuals of species of *Eurycerus*, *Bosmina*, *Diatomus*, or *Cyclops*. The fact that under some conditions the spores of the fungus swam away from the exit orifice of the sporangium immediately upon discharge recalls *Leptolegnia*, not *Aphanomyces*.

Aphanomyces daphniae has been implicated (Seymour *et al.*, 1984) in the infestation of *Daphnia magna* populations in aquatic toxicology aquaria and tanks. The fungus evidently gained access to the fish containers from water pumped in during spring and fall homothermal periods (Lake Huron). Under certain stress conditions the daphnids were especially sensitive to fungal attack. Poor ambient nutrition, reduced dissolved oxygen, and high ambient temperatures predisposed the daphnids to invasion. Seymour *et al.* (1984) postulated that the *Aphanomyces* was maintained in the tanks and aquaria in conjunction with the fish kept there.

Fritsch (1895:82, fig. 5) described and illustrated a fungus (in *Daphnia galeata* and *D. kalbergensis*) that he with reservation, evidently, identified as *Ancylistes cladocerarum* Fr. The description suggests that the invading organism was a watermold, and certainly the single figure Fritsch provided is not of a species of *Ancylistes*.

ASSOCIATION WITH SHRIMP LARVAE

Noting that laboratory-reared larvae of *Palaemonetes kadiakensis* were invaded by *Saprolegnia parasitica* and *Achlya flagellata* Hubschman and Schmitt (1969) explored the pathogenicity of the former. Depending upon when the larvae were inoculated by *S. parasitica* -- that is, before or after the first molt -- 100% mortality could be realized in 2-5 days. The fungus entered the shrimp larvae through the base of the eyestalk or an antenna, or in the articulation between the telson and last abdominal segment. Within the animal, *S. parasitica* appeared to develop preferentially in the central nervous system and musculature. Infected animals, Hubschman and Schmitt found, were immobilized and killed before visceral organs were invaded.

ASSOCIATION WITH COPEPODS

In 1951, Vallin reported finding infected, planktonic *Eurytemora hirundoides* Nordquist in brackish water (4-6 ppt salinity) of the Baltic Sea. He suggested that the fungus -- later described by Höhnk and Vallin (1953) as *Leptolegnia baltica* -- invaded the host by means of spores ingested during feeding. The fungus spread internally into the soft parts of the animal, and eventually burst (mechanically?) through the exoskeleton because of the mass of growth. Vallin (1951) believed that summer temperatures and the influx of freshwater into the Baltic favored the development and spread of the fungus.

The eggs of three calanoid copepod species (in New Zealand waters), *Boeckella dilatata* Sars, *B. friarticulata* (Thomson), and *B. hamata* Brehm, may be invaded by an *Aphanomyces*. First reported by Burns in 1979, the fungus tentatively was identified a year later (Burns, 1980) as *A. ovidestruens* Gicklhorn. As this species is not a valid one (see systematic account), the invading fungus subsequently has been (Burns, 1985a, b) referred to as *Aphanomyces* sp. (its affinities seem to be with de Bary's *Aphanomyces scaber*).

Burns (1985a) characterized seven stages (at 15 °C incubation) in the development of *Aphanomyces* sp. in the copepod eggs. The fungus attaches to the females before their ovulation, and then develops within the clutch of eggs deposited and carried by those animals. Eventually all eggs in the clutch are invaded, the fungus sporulates, and mature oogonia are formed. Susceptibility of the copepod eggs to infection is not related to the size either of the female or the clutch of eggs (Burns, 1985a). Some individuals escape infection within a population, and some level of recovery exists. In any case, it is clear from Burns' time course studies that the complete development of the invading *Aphanomyces* (through oospore formation) can be accomplished within the time frame of the copepod's egg production. While infection by the *Aphanomyces* does not hinder females' egg production in a population, the birth rate (and thus population renewal) is depressed (Burns, 1985b).

There is low fungal incidence in the copepods in winter months (May-September), but a high incidence in the summer (January and February), with the peak in the autumn period (Burns, 1989). No correlation could be demonstrated between the percentage of parasitized females and the number of mature females, or the total population size. Burns (1989) hypothesized that infected ovigerous copepods were more likely to be subject to predation than were uninfected ones. In times of low predation pressure, the lowered population renewal (birth from eggs) resulting from parasitism by the *Aphanomyces* was more evident than at times of high predation pressure.

ASSOCIATION WITH AN ISOPOD

The study by Marcus and Willoughby (1978) of the feeding behavior of the isopod *Asellus aquaticus* L. in relation to *Saprolegnia* sp. is not properly pathology, but may nonetheless be treated here as an association. These investigators grew mycelial pellets of *Saprolegnia* sp. in shake culture, and fed them to test animals. The isopods so fed attained essentially the same growth level as those given softened (but not skeletonized) leaves of *Quercus* sp. The walls of hyphae later found in fecal pellets of the animals had not been digested, but there was evidence of this process having occurred within the filaments themselves. The data did not substantiate the view that it is fungal material not leaf tissue which really serves as food for aquatic invertebrates.

ASSOCIATIONS WITH NEMATODES AND MIDGES

Xiphinema rivesi Dalmasso and *X. americanum* Cobb are two species of nematodes known to transmit tomato ringspot virus. Jaffee (1986) reported that these two pests were parasitized by unidentified *Aphanomyces* and *Leptolegnia* species. When freshly extracted nematodes were put in spore suspensions in soil extract, positive infections were obtained. Higher incidences of infection occurred if the nematodes were aged (four days in soil extract) before being exposed to planonts of the water molds. The majority of infections began (Jaffee, 1986) near the stylet and esophagus, and the cuticle was almost always penetrated by hyphae from the developing fungi. Observations on the frequency of infection indicate that neither water mold is an aggressive parasite and pathogen.

The egg masses of three species of Chironomidae -- *Chironomus attenuatus* (Walk.), *Tendipes decorus* (Joh.) and *Pentaneura carnea* (Fabr.) -- can be invaded by an unusual water mold, *Couchia circumplexa* (W. W. Martin, 1981a). *Couchia* representatives and species in other fungal genera have been shown to contribute significantly to infection and mortality levels in midge populations (W. W. Martin, communication). W. W. Martin (1981b) has suggested that fungi capable of attacking midge eggs are important regulatory agents in natural populations of chironomids in Virginia waters.

Couchia circumplexa exhibits an unusual growth pattern in the inoculation and subsequent infection of midge eggs (W. W. Martin, 1981a). From an encysted planont on the surface of the egg mass a filiform tube develops and grows through the egg mass matrix. Upon contact with an egg the tube gives rise to a small, pyriform appressorium on the egg surface, and penetrates the chorion. An endogenous, branching haustorial system forms, and as the exogenous appressorium enlarges, a septum develops to delimit it from the penetration system. Subsequently, hyphae originate from the appressorium, and eventually ramify within the matrix of the egg mass. Main hyphae (or branches from them) in contact with an egg form specialized appressorial complexes (Fig. 118) which are involved, by means of penetration pegs, in entering through the chorion. Endogenously, these pegs form haustoria that, with continued growth, ramify throughout the infected egg. New appressorial complexes may be formed as the mycelium proliferates through the egg mass. According to W. W. Martin (1981a), circular, dark, callus material is deposited at the sites of penetration peg entry. Such

deposits recall the melanin accumulation in cuticular entry of crayfish by *Aphanomyces astaci* (Unestam and Nyhlén. 1974, for example).

Two additional species of *Couchia*, both occurring in eggs and egg masses of chironomids, have been described by W. W. Martin (2000). *Couchia amphora* parasitizes eggs of *Polypedilum simulans*, and *C. limnophila* invades eggs of *Glyptotendipes lobiferus*. The progression of fungal development in these two parasitic associations follows that in *C. circumplexa* (W. W. Martin, 1981a).

MISCELLANEOUS NONSAPROLEGNIAN ASSOCIATIONS

Four species of fungi reported as parasites or pathogens of aquatic (marine) animals, and originally assigned to Saprolegniaceae, are no longer accepted as members of the family. *Leptolegnia marina*, first described by D. Atkins in 1929 -- and named by her in 1954(a) -- as a parasite of the pea crab, *Pinnotheres pisum* (but also invading *Barnea candida* and *Cardium echinatum*), has been placed, and correctly so, in the Leptolegniellaceae by Dick (1971a). Atkins' species was retained in *Leptolegnia* by T. W. Johnson and Sparrow (1961:330) a decision followed by T. W. Johnson and Pinschmidt (1963:413 *et seq.*). The latter investigators found the fungus in ova of *Callinectes sapidus* Rathbun, but recognized that it was not characteristically saprolegniaceous (*see the review by Alderman, 1976:242*). Artemchuk's (1968, 1981) *Leptolegnia* occurring in the ova of *Balanus improvisus* and *B. eberneus*, is likewise not a species in this genus. The fungus produces endoconidia, and the oospores, alleged to be eccentric, are not at all like those of *Leptolegnia*. Artemchuk's species also is best treated as a member of some other family if, indeed, it is valid at all.

The fungus occurring in the ova, zoeae, and prezoae of *Pinnotheres pisum*, and described by D. Atkins (1954b) as *Plectospira dubia* has been renamed *Atkinsiella dubia* by Vishniac (1958), and settled in the family Haliphthoraceae. *See remarks in the systematic account of Plectospira.*

Hydatinophagus americanus described by Bartsch and Wolf (1938) in a rotifer belonging to the genus *Monostyla*, was renamed and placed in *Aphanomyces* (as *A. americanus*) by Scott (1961a). Since neither Bartsch and Wolf nor Scott saw sporangia, the assignment is in doubt, and we are excluding the species from the genus (*see taxonomic account of Aphanomyces*). The fungus reported by Bartsch and Wolf very likely was pythiaceous.