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REVIEW

HOST PARASITE INTERACTIONS BETWEEN FRESHWATER PHYTOPLANKTON AND CHYTRID FUNGI (*CHYTRIDIOMYCOTA*)[1]

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Some chytrids are host-specific parasitic fungi that may have a considerable impact on phytoplankton dynamics. The phylum *Chytridiomycota* contains one class, the *Chytridiomycetes*, and is composed of five different orders. Molecular studies now firmly place the *Chytridiomycota* within the fungal kingdom. Chytrids are characterized by having zoospores, a motile stage in their life cycle. Zoospores are attracted to the host cell by specific signals. No single physical–chemical factor has been found that fully explains the dynamics of chytrid epidemics in the field. Fungal periodicity was primarily related to host cell density. The absence of aggregated distributions of chytrids on their hosts suggested that their hosts did not vary in their susceptibility to infection. A parasite can only become epidemic when it grows faster than the host. Therefore, it has been suggested that epidemics in phytoplankton populations arise when growth conditions for the host are unfavorable. No support for such a generalization was found, however. Growth of the parasitic fungus *Rhizophydium planktonicum* Canter emend, parasitic on the diatom *Asterionella formosa* Hassal, was reduced under stringent nutrient limitation, because production and infectivity of zoospores were affected negatively. A moderate phosphorous or light limitation favored epidemic development, however. Chytrid infections have been shown to affect competition between their algal hosts and in this way altered phytoplankton succession. There is potential for coevolution between *Asterionella a*nd the chytrid *Zygorhizidium planktonicum* Canter based on clear reciprocal fitness costs, absence of overall infective parasite strains, and possibly a genetic basis for host susceptibility and parasite infectivity.

Key index words: **chytrids; coevolution; diatoms; epidemics; food webs; hosts; parasites; phytoplankton; succession; zoospores**

In 1963 Cook started his article with the following sentence: "The biology of algal parasites has received little attention despite the potential advantages these organisms offer for basic studies of host-parasite relationships." Forty years later, this statement still seems true. Although progress was made in the following decades (Van Donk 1989, Van Donk and Bruning 1992), algal parasites are still a neglected subject. Nonetheless, parasites in general are a widely studied group of organisms in biology. Parasites vary enormously in their diversity. It is probably fair to say that every species is affected by parasites in one way or another. Hence, parasites must be abundant in the phytoplankton too, although not much is known. Parasites are considered to be of prime importance for the evolution of their hosts (Buckling and Rainey 2002). One of the leading hypotheses for the evolution of sex, for instance, is based on the evolutionary struggle between host and parasite (Bell 1982, Ebert and Hamilton 1996). There is every reason to assume that parasites should be of importance for the evolution of phytoplankton species. Parasites, however, should not only be considered as relevant for the evolution of their algal hosts, but also for population dynamics and succession of phytoplankton. Parasites are able to decimate algal and cyanobacterial host populations during epidemics (Van Donk and Ringelberg 1983, Gons et al. 2002).

In this review we focus on a specific group of phytoplankton parasites, the chytrid fungi. Only freshwater phytoplankton is included. Chytrids are primitive fungi that produce zoospores and are often host specific, highly infective, and extremely virulent. Over 90% of all host cells in a population may be infested, and every infection commonly leads to the death of the host cell (Canter and Lund 1951). Selective parasitism on a species may abruptly terminate phytoplankton blooms and favor the development of other species, competing for the same resources. Parasitism can be a decisive factor in plankton dynamics, plankton succession, and food-web relations. Yet most phytoplankton and food-web models still ignore parasites.

Host-parasite interactions offer great potential for the study of coevolution because of the intimate nature of the interaction and the large reciprocal fitness costs

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involved (Thompson 2002). Coevolution is defined as the process of reciprocal adaptive genetic changes in two or more species (Woolhouse et al. 2002). The main result of coevolution is that species become specialized in their interaction with a limited number of species (Thompson 2002). Hence, coevolution shapes the way species interact in a community and is a dominant force in much of what we study in phytoplankton community ecology. Parasites impose selection on their hosts that in turn impose selection on their infective parasites. This reciprocal selection may result in rapid antagonistic coevolution. Studies of coevolution between host and parasites in freshwater ecosystems are among the best studied examples, providing strong evidence for local adaptation of the parasites to their host populations (Dybdahl and Lively 1998, Lively and Dybdahl 1998). Microparasites of the zooplankton, in particular of *Daphnia*, have been studied extensively (Little and Ebert 2000, 2001, Capaul and Ebert 2003). Although parasitic fungi of phytoplankton have been studied for decades, to the best of our knowledge there has been very little done on the potential for coevolution.

We emphasize that chytrids are indeed parasitic fungi of phytoplankton and not, for instance, host-specific predators. Definitions are given by Thompson (1992). Parasites live in intimate association in or on their hosts, are associated with their hosts for a major part of their life, derive all or almost all of their food from one host, and die if their host dies before the stage to which the parasite is adapted to leave its host. All these characteristics apply to chytrids and their algal hosts. Except for zoospores (as a free-swimming stage with a limited lifetime) and (in some species) resting spores, chytrids are attached to their hosts for their complete life cycle. They are nourished by nutrients that they extract from their host. A single parasite, during its lifetime, is only associated with a single host. If the host dies before the attached zoospore has developed into a fully mature sporangium (for instance, as a consequence of a so-called hypersensitivity response), the parasite dies together with the host. In contrast, predators kill their prey; eat several to many prey during their lifetime; and kill their prey quickly, all unlike most parasites (Thompson 1992). Of these characteristics, the latter two do not apply to a chytrid parasite, but chytrids like *Rhizophydium planktonicum* Canter emend clearly do kill their *Asterionella* hosts, just as predators do. Common wisdom has it that parasites should evolve to minimize damage to their hosts on which they depend for survival and reproduction. Ebert and Weisser (1997), however, distinguish a specific group of parasites, named obligately killing parasites, that convert host biomass into parasite transmission stages and obligately kill the host in the process, as is indeed the case with chytrids.

In this review we discuss advances in the study of algal chytrid parasites. Did the progress in molecular sciences have an impact on chytrid studies? Is the increased understanding of host-parasite coevolution reflected in work on algae and their fungal parasites? We mainly, but not exclusively, focus on the more recent literature. Of the older literature, the work by Canter and coworkers especially deserves to be mentioned (for a synopsis of their work see Canter Lund and Lund 1995). We discuss ecological and evolutionary aspects of chytrids that are parasitic on phytoplankton.

CHYTRIDS GENERAL DESCRIPTION

The aquatic fungi parasitic on phytoplankton mainly belong to the *Chytridiomycetes* (commonly known as chytrids). In the older literature (Sparrow 1960) parasitic fungi of phytoplankton were also found to belong to the *Oomycetes*. More recent studies show that *Oomycetes* do not belong to the Kingdom Fungi but are crown eukaryotes that are different from plants, animals, and fungi. Proposed Kingdoms for the Oomycetes are the Chromista and now also Stramenopila (Paquin et al. 1995, Tyler 2002, Schlegel 2003). The "fungi-like" *Oomycetes* have two types of flagella, whiplash and tinsel; chytrids have one posterior whiplash flagellum. *Oomycetes* have a cellulose cell wall and lack chitin, a characteristic thought to be important in separating *Oomycetes* from true fungi. Recent work questions the absence of chitin in *Oomycetes*, and Slusarenko and Schlaich (2003) concluded that we have not seen the last taxonomic revision of this group. The impact of parasitic *Oomycetes* on phytoplankton has been found to be much less than that of chytrids; hence, *Oomycetes* will not be considered further in this review.

The *Chytridiomycota* were reported for the first time by Braun (1886), who described them as follows (in German): "Das ganze Pflänzchen besteht aus einer einfachen blasenartigen Zelle, welche oft mit einer wurzelartigen Verlängerung in die Zellen des Nährorganismus eindringt" (the whole plant consists of a simple vesicle-like structure, which often penetrates the host cell with a root-like extension). The *Chytridiomycota* are thought to be one of the oldest and most basal groups within the fungi. Chytrids in their life cycle develop a structure called a sporangium that produces mobile spores equipped with a flagellum that aids in locomotion. Within the *Chytridiomycota*, there is a considerable amount of variation in morphology and ecology. Chytrids are common as saprobes, facultative and obligate parasites, and are found both in aquatic habitats (fresh water and marine) and in moist soils (the unwalled flagellated zoospores of chytrids require water for dispersal). Facultative and obligate parasitic *Chytridiomycetes* can be found on plants, animals (mostly insects and ruminants), protists (phytoplankton and charophytes), and other fungi. Morphological variation among the *Chytridiomycota* ranges from unicellular coenocytic to mycelium producing species. *Chytridiomycota*, like all other fungi, have chitin incorporated in their cell walls, although one species has been shown to have a cellulose-based

cell wall (Barr 1990, Longcore 1996, Van Donk and Bruning 1992, Powell 1993).

PHYLOGENETIC RELATIONSHIPS

The phylum *Chytridiomycota* contains a single class, the *Chytridiomycetes*, and is composed of five different orders: *Blastocladiales*, *Chytridialis*, *Monoblepharidales*, *Neocallimastigales*, and *Spizellomycetales*. The order *Chytridiales* is the largest (about 80 genera and 500 species) and least understood group within the *Chytridiomycetes*. *Chytridiomycota* are among a group of organisms sometimes referred to as "lower fungi" and at times also have been classified outside the fungi, a view opposed by others (Barr 1990). Molecular studies now firmly place the Chytridiomycota within the fungal kingdom (Bowman et al. 1992, Tehler et al. 2000). These studies also indicate that the phylum is genetically diverse and presumably polyphyletic (Paquin et al. 1997, James et al. 2000). Longcore (1996) described the history of chytridiomycete taxonomy since Sparrow (1960). The thalli of most chytrid species, however, possess insufficient morphological characters to construct an informative phylogenetic taxonomy (James et al. 2000). Similar thallus forms are commonly found in different phylogenetic groups. Barr (1990) gave an outline for chytridalean taxa on the basis of the sporangial development into families and on the basis of zoospore ultrastructure characters into genera. Patterns of sporangial development are better conserved within species than characteristics of the mature sporangium (with presence or absence of operculation as the prime characteristic). Doggett and Porter (1996b) put forward an ordinal classification supported by sexual characteristics such as plasmogamy and resting spore development. They discussed this as a basis for further taxonomic classification of the *Chytridiales*, which may complement the ultrastructural characteristics that are now predominantly used.

More recently, James et al. (2000) established further phylogenetic relationships among the orders of the *Chytridiomycota*, with special emphasis on the *Chytridiales*. Their work is based on sequence information of the small subunit rDNA gene in 54 chytrids. The data supported monophyletic origins for the *Blastocladiales*, *Monoblepharidales*, and *Neocallimastigales*. Monophyly of the *Chytridiales* and *Spizellomycetales* was not contradicted by the small subunit rDNA data. Hence, the molecular data underpin the taxonomy based on ultrastructure of the zoospores (Barr 1990). Four separate subordinal clades were identified within the *Chytridiales*: a *Chytridium* clade, a *Lacustromyces* clade, a *Nowakowskiella* clade, and a *Rhizophydium* clade. The molecular data did not support a classification based on the type of development of the sporangium; particular types of development were not specific for particular clades. The study by James et al. (2000) did not include species with some unique characters of their zoospores, including *Zygorhizidium* spp. and *Rhizophydium planktonicum* (Beakes et al. 1993), which are the main chytrids to be discussed in this review. Beakes et al. (1988) showed that distinctive ultrastructural characters of the zoospores in two *Zygorhizidium* species hindered their positioning in the orders proposed by Barr (1980). From their study it seems unlikely that the two species are closely related, although they have been placed in the same genus. Addition of sequences of these "atypical" taxa in molecular phylogenies will probably result in more clades than the four identified by James et al. (2000). More data, both molecular and ultrastructure, are needed for an informative identification of chytridialean genera and to update the monograph by Sparrow (1960).

LIFE CYCLE

The life cycle of a chytrid (arbitrarily) begins with the attachment of a motile zoospore to the surface of an algal host cell. Figure 1 shows zoospores of *Zygorhizidium planktonicum* Canter attached to their host *Asterionella formosa* Hassal. Zoospores settle on diatom cells. Encystment is the second step and is completed when a thickened wall is formed around the zoospore. In a successful infection process, the encysted zoospore will develop into a mature sporangium. The most common subsequent step after encystment is the formation of a germ tube, which enters the algal host cell via the girdle zone (Van Donk and Ringelberg 1983). The newly formed zoospores are released by a process called dehiscence. When the released zoospores infect another algal cell, the process is repeated (Canter Lund and Lund 1995). During dispersal the zoospores depend on internal energy storage (Holfeld 2000a). The attached zoospores completely depend on the host cell for nourishment and their development into mature sporangia.

The production of thick-walled sexually or asexually produced resting spores has been described for different chytrids infecting *Asterionella* (Beakes et al.

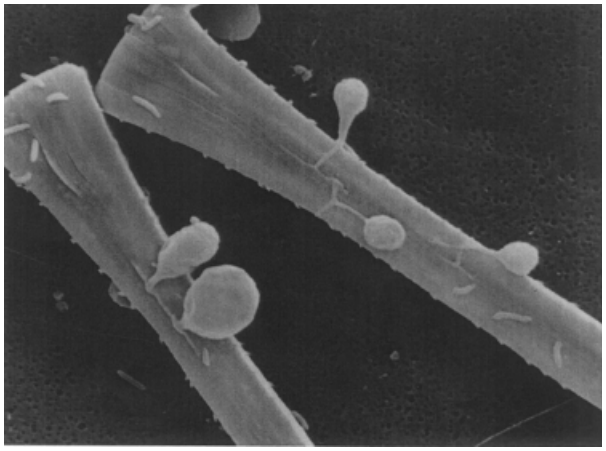

FIG. 1. Electron micrograph of zoospores of *Zygorhizidium planktonicum* attached to the surface of host cells of *Asterionella formosa*.

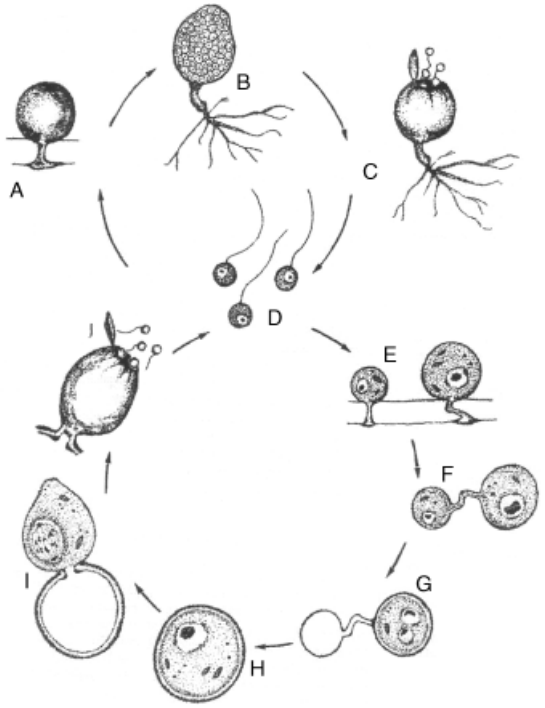

FIG. 2. Life cycle of *Zygorhizidium planktonicum* (Doggett and Porter 1996b; used with permission from the Mycological Society of America). The asexual infection phase (A–D) is characterized by uninucleate haploid thalli (A) that differentiate into operculate zoosporangia (B). Upon release (C), the uniflagellate zoospores (D) give rise to either asexual thalli or incipient gametangia (E). Sexual reproduction is evidenced by heterothallic gametangia (E–G), which fuse by means of a single epibiotic conjugation tube. Plasmogamy immediately follows wall fusion, and the donor protoplast migrates into the recipient gametangium, which in turn becomes the thick-walled resting spore (G). After wall deposition, the nuclei fuse and they zygotic nucleus remains undifferentiated throughout spore dormancy (H). Meiosis occurs within the newly formed meiosporangium after resting spore germination (I). The haploid zoospores are released by an operculate meiosporangium (J).

1993). Sexual spore production has been observed in *Zygorhizidium planktonicum* but not in *R. planktonicum*. The resting spores may serve to survive periods with adverse environmental conditions. Sexual reproduction takes place by gametangial copulation. The female thallus develops from a zoospore, in the same way as a sporangium does, but no zoospores are formed. Other zoospores remain small and behave as male thalli, emptying their contents into the female thallus through conjugation tubes (Van Donk and Ringelberg 1983). The recipient thallus becomes a thick-walled resting spore. The haplontic life cycle of *Zygorhizidium planktonicum* Canter with both the sexual and the asexual phase is shown in Figure 2.

HOST SPECIFICITY

The specific attachment (and encystment) of zoospores onto a particular host or group of algae indicates that specific signals are involved in the attraction of zoospores. Glycoproteins at the zoospore surface have been suggested to be the potential site involved in encystment or recognition of environmental cues, but the chemical composition of chytrids zoospores has not been established (Powell 1994). Motile cells of many chytrids have been shown to respond to a variety of environmental factors, including light, nutrients, pheromones, and host secreted compounds (Powell 1994). Muehlstein et al. (1988) found that strains of a marine *Rhizophydium* species were positively attracted to both amino acids and carbohydrates; amino acids were only weak stimulators.

Holfeld (2000a) postulated that the presence of nonhost algae interferes with the detection of the proper host by the fungal zoospores. The proportion of the host within the total phytoplankton community at which infection, more specifically an increase in the degree of infection, can be observed is a reasonable measure of the zoospores ability to detect and locate their hosts. Holfeld (2000a) related the proportion of the biovolume of seven different host species (mainly diatoms) to changes in the absolute density of infected cells of the particular host species (chytrids studied were several different *Rhizophydium* and *Zygorhizidium* spp. and *Hapalopera piriformis* Fott). In all host species an increase in infection was observed, even at low host proportions of the total biovolume. Parasitism at such dilute host concentrations seems contradictory to the poor specificity of parasite zoospores that emerges from the work by Canter and Jaworski (1981, 1982). They found that the attraction of chytrid zoospores to algal hosts was not very specific at all: Attraction of monospecific zoospores was recorded for a wide range of hosts, including pennate and centric diatoms, the green alga *Staurastrum*, and the cyanobacterium *Oscillatoria* (*Planktothrix*). Gromov et al. (1999) found that some fungal strains had very broad host ranges, whereas some were rather narrow. The extent to which chytrids are truly host specific has not been fully investigated. Holfeld (2000a) suggested that zoospore losses on the wrong host are prevented because the attraction to cells is reversible. Host specificity would occur during encystment of the zoospores rather than at the earlier stage of chemotaxis. Doggett and Porter (1995) found that zoospores of *Z. planktonicum* (in Lake Lanier a parasite of three different *Synedra* species) sometimes loosely adhered to *Asterionella* (even up to 10 zoospores per colony), but germ tube intrusion was never observed. Successful parasitism on *Asterionella* could not be induced by altered environmental conditions (decreased silica, altered irradiance, or modified temperature) or in mixed cultures containing the diatom *Synedra*.

ALGAE–CHYTRID INTERACTIONS IN THE FIELD

We now discuss recent studies that describe the epidemic development of chytrids in natural populations of their algal hosts. Most field studies stress the fact that parasitism is not restricted to weakened or

moribund cells. In contrast, the whole population of healthy dividing host cells seems susceptible to infection, and healthy cells may even be preferred by the parasites. Parasites can appear when growth conditions are favorable for the algal hosts. The population of the host and the parasite may even temporarily increase simultaneously. To describe the severity of fungal attacks, it is common to plot the proportion of infected host cells in their population, a parameter described as prevalence of infection, P_r (Bruning 1991c, Holfeld 2000b):

$$P_r = N_i \times 100/(N_i + N_u) \quad (1)$$

where N_i is the number of infected cells and N_u is the number of uninfected cells within the host population. Generally, P_r is referred to as the percentage of infected hosts. Bruning (1991c) warned against the use of P_r, because it not only depends on the severity of parasitism but also includes production and loss processes other than parasites of the phytoplankton. For instance, at high water temperatures and relatively low nutrient levels (that still allow sporangia of the chytrid *R. planktonicum* to develop relatively quickly, whereas its host *Asterionella* only grows slowly), populations of this diatom will already begin to decline at a prevalence of infection of 0.18. When other loss processes like grazing or sinking are active, diatom populations will decline even at lower prevalences. On the other hand, at low temperatures and growth saturating light and nutrient levels, increase of the diatom population is possible at prevalences up to 0.75 (Bruning et al. 1992). For an estimation of the impact of fungal phytoplankton parasites, cell counts of host- and parasite populations should at least be combined with measurements of the development time of the fungal sporangia (Bruning et al. 1992). Nevertheless, because of the frequent use of P_r in literature, we make references to these percentages. The mean intensity of infection (î) is a quantification of the mean number of parasites per individual host in a population:

$$\hat{I} = N_p \backslash N_h \quad (2)$$

where N_p is the number of attached parasites and N_h the number of host cells. Multiple infections (more than one parasite per host cell) have no special effect on P_r but do raise î. The dynamics of the infection of the diatom *Asterionella formosa* by the chytrid *Z. planktonicum* in Lake Maarsseveen, The Netherlands over a number of years are shown in Figure 3, which shows variation in both the host (infected and uninfected) *Asterionella* and the chytrid (expressed as P_r) populations. The prevalence of infection (host cells with any of the fungal stages attached) sometimes reached up to 90%, but the maximum value of P_r varied largely between (and within) years. Temperature seemed to be a major controlling variable; cold periods with extended ice cover suppressed fungal development and allowed *Asterionella* to reach a dominant position in the spring bloom of the phytoplankton. Freezing of the lake forced *Zygorhizidium* to form resting spores, and it required an extended period of elevated temperature (i.e. above freezing) to become infective again (Van Donk and Ringelberg 1983).

Sen (1987a) also addressed the occurrence of chytrid parasites of *Asterionella* in relation to limnological factors in Shearwater (UK). The main epidemics of *Z. planktonicum* coincided with low water temperature ($<5^\circ$ C); the highest prevalence of infection (63%) was even recorded under ice (i.e. in contrast to the epidemics in Lake Maarsseveen). Parasites were absent at elevated water temperatures. Lake level, pH, and irradiance also affected the epidemic development of the chytrids, although it is difficult to distinguish any direct effects of these factors on chytrids life history from their effects on host density. No single nonbiological factor seemed sufficient to explain the course of the epidemic. The occurrence of epidemics first and foremost required high host cell densities. Hence, parasites would show up whenever host cell densities were high enough, almost irrespective of the environmental conditions.

The studies of Sen on the occurrence of chytrids in Shearwater included other hosts besides *Asterionella* (Sen 1987a). A series of articles described the findings for the diatom *Fragilaria crotonensis* Kitton, for several centric diatoms (*Melosira*, *Cyclotella*, *Stephanodiscus*), for the cyanobacterium *Microcystis aeruginosa* Kuetz. Emend. Elenkin, and for several green algae (Sen 1987b,c, 1988a,b). Whereas the results for *Fragilaria* resembled those for *Asterionella* (for instance, up to 55% of the host cells were infected), infections of centric diatoms were low (e.g. $<1\%$ on *Melosira*), despite the presence of high host cell densities. The prevalence of infection was too low to exert any real effect on growth of the host populations. *Rhizidium microcystidis* Bülent Sen was described as a (novel) parasite of the nuisance cyanobacterium *Microcystis*. Maximum infection levels were high (90%), but in this case the occurrence of the parasites was correlated with two limnological factors: temperatures $>15^\circ$ C and an increase in pH. Clearly, these are also environmental factors correlated with the blooming of the host, *Microcystis*. As with *Asterionella*, host cell density and not environmental factors per se will have been the decisive factor in the occurrence of the *Rhizidium* parasitic on *Microcystis*. Green algae that were found to be infected by chytrids included *Coelastrum* (two different species), *Oocystis lacustris* Chodat, *Kirchneriella obesa* (W. West) Schmiddle, *Dictyosphaerium pulchellum* Wood, *Staurastrum* spp., and *Pandorina morum* (Muell.) Bory. The main chytrid involved resembled *Zygorhizidium parvum* Canter. Infection levels varied between hosts but could be relatively high, as in the case of *Staurastrum* (85%), *Coelastrum* (up to 36%), or *Kirchneriella* (26%). Nevertheless, Sen (1988b) concluded that parasitism showed no marked effect on the growth of the green algal host populations.

In a survey on the occurrence of fungal parasites and their seasonality, resource dependency, and specificity, Holfeld (1998) found a total of 27 different

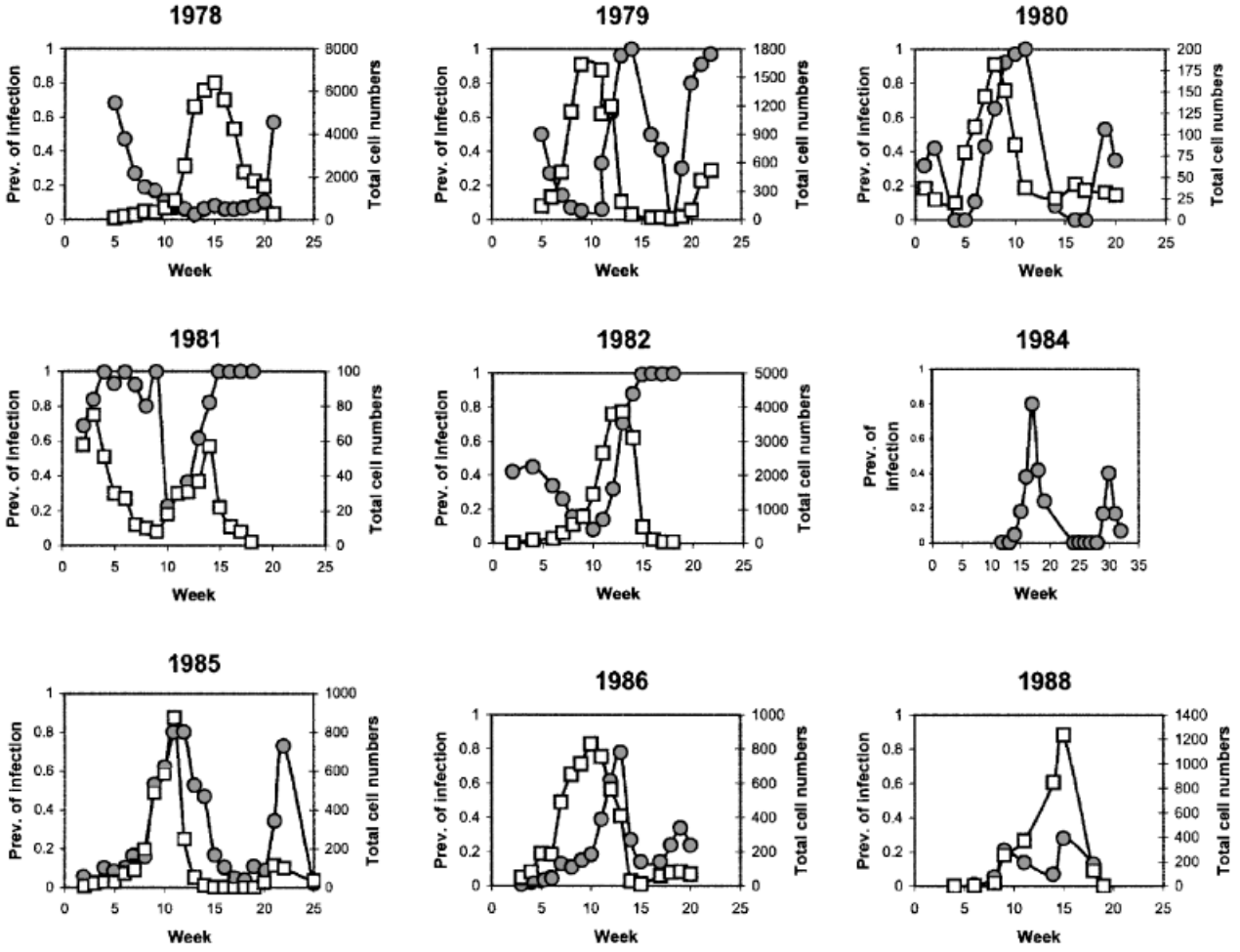

FIG. 3. Infection of *Asterionella* by chytrids in the field from 1978 to 1988. Abundance of the diatom *Asterionella formosa* (in cells per mL) observed in the upper 10 m of Lake Maarsseveen (closed symbols) and the prevalence of infection (open symbols). Please note that for 1984 the scale of the *x* axis is slightly different as is true for the right y-axis in most cases.

phytoplankton species in the Schösee (Germany) that were infected. The host species belonged to the *Cyanophyceae*, *Bacillariophceae* (diatoms), *Chlorophyceae* (green algae), *Chrysophyceae*, and *Dinomastigota*. Holfeld (1998) reported that almost all fungi were monocentric eucarpic chytrids, although holocarpic, endobiotic, biflagellate fungi were seen occasionally (this latter group, *Oomycetes*, is no longer classified as fungi). Seasonal peaks in host cell density commonly were accompanied by maximum intensities of parasitism. *Asterionella formosa* was infected by four different chytrids: *Rhizophydium planktonicum*, *R. tetrageneum* Canter, *Zygorhizidium affluens*, and *Z. planktonicum*. *Zygorhizidium* was the dominant genus, with epidemic development of *Z. planktonicum* in summer, of *Z. affluens* in February and March, and again of *Z. planktonicum* in late spring. Studies of the ultrastructure of the zoospores of these *Asterionella* parasites have shown that the zoospores are clearly different and may not be closely related (Beakes et al. 1993). It is suggested that the parasites have independently evolved a host-specific association with *Asterionella*.

The prevalence of infection of *Asterionella* in the Schösee (Holfeld 1998) peaked at more than 50%. Even higher percentages (80%) were observed for several *Stephanodiscus* species. Three different species of *Stephanodiscus* were infected but by three different chytrids, which did not transgress the species border. Fungal infections could be observed in almost any water sample. The proportion of infected cells within the total phytoplankton volume usually remained below 1%, but occasionally $>10\%$ was recorded. The primary factor determining the absence or presence of a particular chytrid was the availability of a suitable host. Epidemics, even with the same host–fungal system, were seen during different times of the year under strikingly different limnological conditions. Given the importance of host availability, a minimum threshold in host density for epidemic development of the parasite is to be expected. This threshold value differed between algal species: The relationship that emerges is that larger hosts (based on biovolume) can be infected at lower population densities. For instance, whereas for *Asterionella* 10–12 cells $\cdot$ mL^{-1} sufficed,

50 cells · mL^{-1} were needed for *Ankyra judayi* Fott. Other authors reported somewhat higher thresholds for *Asterionella* (e.g. 30 cells · mL^{-1} in Van Donk and Ringelberg 1983 and 90 cells · mL^{-1} in Sen 1987a), although lower values are mentioned as well (3–4 cells · mL^{-1} in Kudoh and Takahashi 1990). Kudoh and Takahashi (1992) hypothesized that the limited searching ability of a zoospore is (partially) responsible for host density depended parasitism. Based on data by Bruning (1991b), who determined that the infective lifetime of a zoospore was about 8 days and its searching efficiency was about 5 μL · cell^{-1} · d^{-1}, the authors concluded that an individual zoospore can only search 40 μL of water during its lifetime.

Holfeld (2000b) established the type of distribution (even, random, or aggregated) of the chytrid *Zygorhizidium* within their host population, the diatom *Stephanodiscus alpinus* Hustedt, during an 11-day epidemic. He also addressed the changes in host cell size during the epidemic and the effect of host cell size on size and fecundity of the parasite. No evidence for an aggregated type of distribution (which is common for parasites) was found, indicating no variation between hosts in their susceptibility to infection and that infections occurred independently of each other (Fig. 4). Holfeld (2000b) pointed out that the lack of aggregated parasite distribution also signifies that zoospores are dispersed singly, not in clumps. Also, no evidence for an even distribution was found. An even distribution would have implied density dependent mortality of algae, selective mortality of hosts with multiple infections, or induced resistance against infection. Thus, the random distribution of the parasites contradicts the occurrence of any of these phenomena in the field (Holfeld 2000b). *Zygorhizidium* seemed to prefer larger host cells to small ones: The median diameter of infected host cells always exceeded that of uninfected hosts. The relationship between host cell size and infection, however, has to be weak; otherwise, an aggregated distribution would be observed. Also, the volume of a dehisced sporangium was found to correlate positively with the volume of the (dead) host cell. The "quality" of the host cells decreased during the epidemic. Larger host cells were observed at the onset, providing a basis for high parasite fecundity and facilitating the establishment of the parasite population. Other studies that observed size selectivity, however, do not support this notion entirely: In *Asterionella* populations in Shearwater, intermediate-sized frustule colonies were more parasitized than larger or smaller ones (Sen 1987a).

Lund (reference in Doggett and Porter 1996a) originally proposed that germination of dormant resting spores of chytrids after a return of favorable conditions may provide the inoculum for recurring epidemics. Field studies relate epidemics of chytrids to periods with increased lake turbulence (Doggett and Porter 1996a). An increase in turbulence may resuspend dormant spores from the lake sediment, after which the different (e.g. increased light and/or temperature) conditions in the epilimnion may induce germination. Van Donk and Ringelberg (1983) observed that resting spores germinated at temperatures >4° C, coinciding with an increase in *Asterionella* host density in Lake Maarsseveen. It has been suggested that fungal periodicity may even be largely determined by resting spore germination and the concomitant effects of lake mixing (Doggett and Porter 1996a). These authors investigated the environmental conditions responsible for the onset of dormancy and germination in *Zygorhizidium planktonicum* f. sp. *Synedrae*. Environmental conditions that stimulated germi-

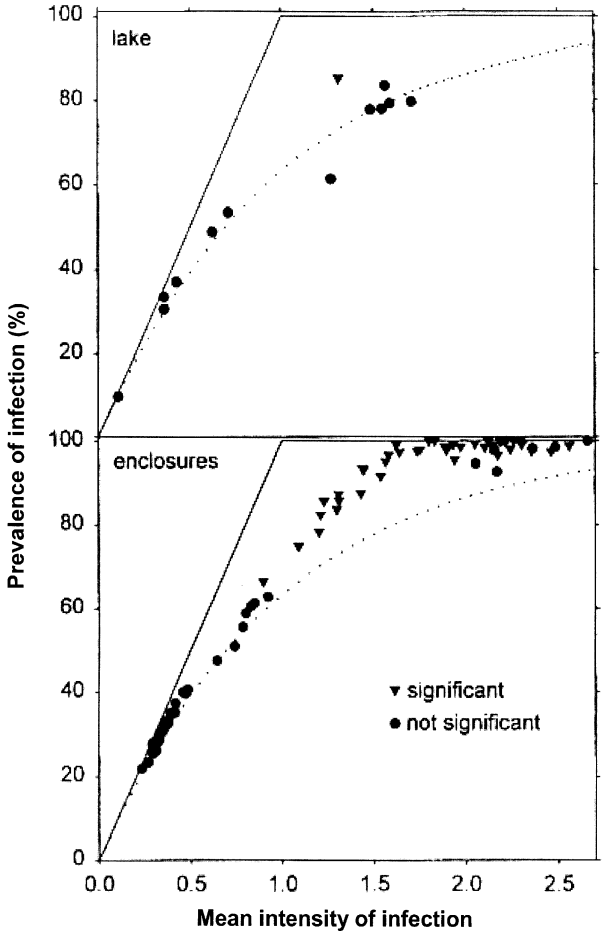

FIG. 4. Distribution of chytrids in their host population (Holfeld 2000b; used with permission from the American Society of Limnology and Oceanography). Distribution of *Zygorhizidium* sp. parasite individuals within their host population in the lake and in some enclosures within the lake. Solid line: theoretically perfect even-parasite distribution. Dotted line: theoretically perfect random-parasite distribution. Circles indicate which observations were not significantly different from values predicted by a perfectly random distribution (chi square test, $P > 0.05$). Triangles indicate significant differences between observations and corresponding values predicted by a perfect random distribution ($P < 0.05$). It is obvious that especially in the lake, the distribution of chytrids in their host population does not differ significantly from a random distribution. The absence of an even distribution contradicts the occurrence of density dependent mortality of algae, selective mortality of hosts with multiple infections, or induced resistance against infection in the lake.

nation, a water temperature between 10 and 13° C during isothermal conditions after the seasonal overturn of the previously stratified water column, are exactly those that favor diatom blooms. Diatoms prefer well-mixed conditions, because their large size and high buoyant density result in large sedimentation losses during periods of quiescence. The early stages of the infection may depend on the delivery of a synchronous cohort of infecting zoospores, as could result from the synchronous germination of resting spores. Experiments with resting spores of *Zygorhizidium* showed that this is a possible scenario: Spores survive long enough (at least 250 days) to wait for isothermal conditions, and germination may be triggered by relatively small increases in temperature (Doggett and Porter 1996a).

DEPENDENCE OF INFECTION ON ENVIRONMENTAL FACTORS

Field evidence seems to suggest that chytrid infectivity coincides with periods of reduced temperature and/or increased lake turbulence (Van Donk and Ringelberg 1983, Sen 1987a, Dogget and Porter 1996a). On the other hand, at very low water temperatures and extended periods of ice cover, epidemic development of chytrid parasites of *Asterionella* was halted (Van Donk and Ringelberg 1983). Obviously, light and turbulence in addition to temperature are affected by ice cover, and this would have had an impact on the epidemic development of chytrid fungi. It was exactly for these apparent contradictory observations in the field that Bruning (1991a–d) shifted his attention to controlled laboratory experiments and attempted to disentangle the relationship between environmental conditions and development of chytrids at different host densities. As stated by Van Donk and Bruning (1992), observations on natural populations did not clarify the circumstances under which the fungi multiply quickly enough to become epidemic. This in part may be because several environmental parameters commonly change simultaneously in aquatic systems. Environmental factors can affect the growth rate of an alga as well as its parasite, making it difficult to discern environmental effects from field observations (Van Donk and Ringelberg 1983, Sen 1987a–c, 1988a,b). Dogget and Porter (1996a) observed that the consequences of fungal parasitism could not be established beyond doubt, because other factors such as nutrient deficiency, temperature, and predation also influenced host populations. In Lake Maarsseveen, *Asterionella* often blooms twice a year, winter/early spring and summer (Fig. 3), although usually the winter bloom and fungal epidemic are larger. The fungus is able to become epidemic under both winter and summer conditions, namely under the combination of low temperature and low irradiance and high temperature and high irradiance (Bruning 1991c). Other combinations of light and temperature may be prohibitive for epidemic development, unless the host cell density is sufficiently high. Under some combinations of temperature and light, no epidemic development is possible. Even survival of the fungus may be in danger when low temperatures are combined with high light conditions.

After infection, a chytrid zoospore produces one sporangium that releases, after a development time of (d) days, (n) new zoospores, each with infectivity (i) and infective lifetime (q). These factors were determined in the experiments of Bruning (1991a–d). Growth and epidemic development of these parasites may be affected by environmental factors, because these factors alter the values of d, n, i, and q. As an example, Figure 5 shows the effect of light on the growth parameters of *R. planktonicum*, a common chytrid parasite of the freshwater diatom *Asterionella* (Bruning 1991a). The infectivity of the zoospore and the number of zoospores per sporangium decreased when the host became light limited, and the development time of the sporangia was also slightly reduced. No effect of light on the infective lifetime of the zoospores could be detected. Infection of *Asterionella* by *Rhizophydium* was halted completely in the dark, because zoospores cannot attach to the host in the absence of light. Reduced infectivity of chytrid zoospores at low light intensities has been reported by others (Canter and Jaworski 1986). Chytrid zoospores may use excretion products of the host (related to photosynthesis) to find or recognize host cells, so it is possible that light limitation interferes with chemotaxis.

A phytoplankton parasite can only become epidemic when the parasite population increases faster

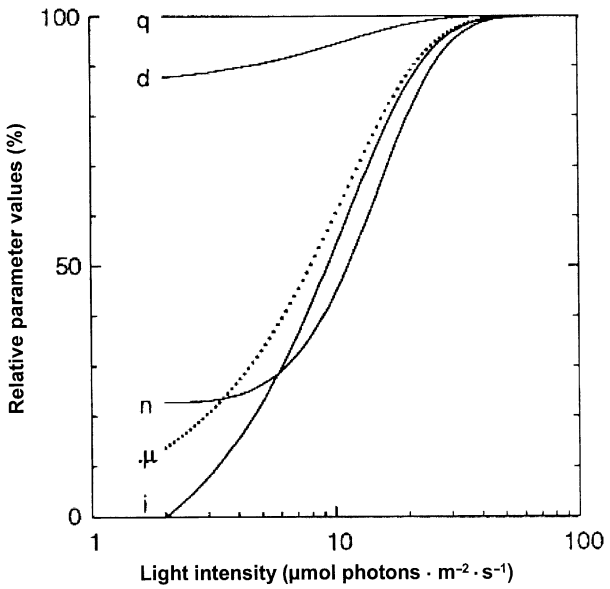

FIG. 5. Effects of light on fungal infection of *Asterionella* (Bruning 1991b; used with permission from Oxford University Press). The effects of light on the specific growth rate of *Asterionella* (μ) and on the growth parameters of the parasite *Rhizophydium:* the infective lifetime of the zoospores (q), the development time of the sporangia (d), the zoospore content of the mature sporangia (n), and the infectivity constant (i). Parameters are plotted as percentage of maximum value.

than the host population, because otherwise the prevalence of infection decreases. Therefore, epidemics in phytoplankton populations may arise more easily when the growth conditions for the host are unfavorable (Reynolds 1984, Kagami and Urabe 2002). Not all field observations support such a generalization, however, because several fungal epidemics have been reported in fast-growing phytoplankton populations during optimal external conditions for the host (Van Donk and Ringelberg 1983, Sen 1987a). The effects of light on the growth parameters of *Rhizophydium* may explain why unfavorable growth conditions for the host do not necessarily favor epidemic development, because the production and infectivity of the chytrid zoospores and therefore the growth rate of the parasite are affected by low irradiances (Fig. 5). When light limitation, or any other factor, depresses fungal growth more than algal growth, this factor will hamper rather than promote epidemic development.

To judge the effects of light limitation on growth and epidemic development, Bruning (1991b) calculated the minimum host cell density required for growth of the parasite (the survival threshold) and the host density at which the parasite can grow faster than the host (the threshold for epidemic development). The thresholds were calculated as functions of the growth rate of the host and the growth parameters d, n, i, and q of the parasite, which were all determined experimentally (Fig. 6). The survival threshold increased when *Asterionella* became light limited, which means that the parasite needed higher host densities to grow or grew slower at a given host density when the host was light limited. Thresholds for epidemic development, however, decreased at moderate light limitation and increased only at severe limitation. So it appears that a moderate light limitation of *Asterionella* will favor the development of an epidemic, despite the fact that growth of the parasite is reduced under such conditions.

Several investigators found that temperature may be an important factor in the occurrence of phytoplankton parasites (Van Donk and Ringelberg 1983, Sen 1987a,b). Temperature also affected the possibility for *Rhizophydium* to become epidemic in *Asterionella* populations (Bruning 1991d). At low temperatures, the development time of the sporangia of this parasite increased, an effect that was only partly counteracted by an increased number of zoospores per sporangium and an increased infective lifetime of the zoospores. As a consequence, the maximum growth rate of the parasite fell below the maximum growth rate of the host at temperatures below 5° C, which makes epidemic development at low temperatures only possible when the growth rate of *Asterionella* is severely reduced, for instance, by light limitation. Figure 7 describes the combined effects of light and temperature on the occurrence of the parasite. The contour plot for the threshold host density allowing epidemic development of the parasite shows a relatively flat plane at temperatures $>7°$ C and at irradiances down to 15 µmol photons $\cdot m^{-2} \cdot s^{-1}$. Here the effects of light and temperature are limited. However, under environmental conditions below these levels, epidemic development is halted rather abruptly (Bruning 1991d). Inhibition of epidemic development at very low temperatures has actually been observed under natural conditions by Van Donk and Ringelberg (1983) for *Z. planktonicum*. They found that in Lake Maarsseveen inhibition of the fungus coincided with ice periods without snow cover. Under these conditions, *Asterio-*

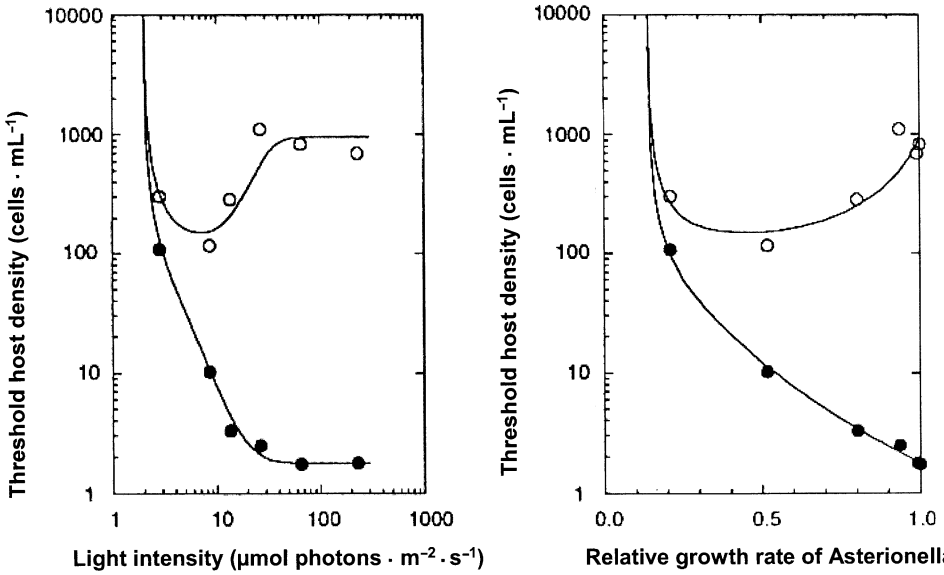

FIG. 6. Threshold host densities (Bruning 1991b; used with permission from Oxford University Press). Threshold host cell densities for survival (filled symbols) and epidemic development (open symbols) of the chytrids, plotted against the light intensity (left) and relative light-limited growth rate of the host *Asterionella* (right).

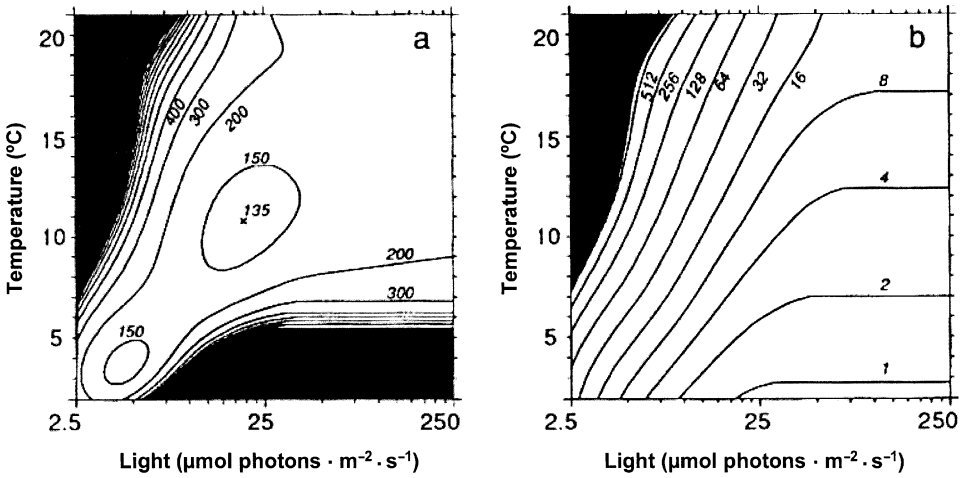

FIG. 7. Light–temperature interaction on *Rhizophydium* (Bruning 1991c; used with permission from Oxford University Press). Effects of light and temperature on the occurrence of *Rhizophydium*. Contour line labels represent the host threshold density values (cells · mL^{-1}) for epidemic development (a) and survival (b) of the parasitic chytrid.

nella still grew well and was able to outcompete other diatom species, because of the absence of parasitism and its higher affinity for phosphorus (Van Donk 1989).

Kühn and Hofmann (1999) studied the effect of turbulence on infection of a diatom by a parasitoid nanoflagellate that resembles chytrid zoospores. Turbulence enhanced the encounter rate between host cells and zoospores but reduced contact time. Turbulence can induce shear around diatom cells and potentially disturb the gradients in extracellularly released substances that act as attractant in chemotaxis, making it more difficult for chemosensory organisms to detect the algae. Kühn and Hofmann (1999) hypothesized that in addition to a minimum threshold density of host cells, there may be a critical level of turbulence above which epidemics fail to develop.

PHYTOPLANKTON SUCCESSION

Based on field observations, this section describes the impact of chytrids on their hosts, that is, on the phytoplankton community. Infection of one algal species may favor the development of other algal species; thus, parasitism can be an important factor controlling seasonal succession. Yet fungal parasitism as a controlling factor in algal populations still is a neglected area of research. Most aquatic studies, trying to quantify algal mortality, concentrate on physical–chemical factors and on grazing by the herbivorous zooplankton. The PEG (Plankton Ecology Group) model of phytoplankton succession (Sommer et al. 1986) summarizes the seasonal succession of phytoplankton (in deep stratifying lakes like Lake Constance) and the main steering factors of this succession in 17 discrete steps. Parasitism is not among those factors. Nonetheless, earlier work by Sommer (1984) showed the potential impact of parasitism on plankton dynamics in Lake Constance by causing high mortality of *Fragiliaria crotonensis* by *Rhizophydium fragelariae* Canter. *Fragiliaria* their populations collapsed despite high growth rates. Sommer (1987) stated that fungal parasites seemed to be most common on algae that are fairly resistant to grazing by zooplankton. Species eliminated by host-specific fungi can be replaced by ecologically similar species, provided that growth rate and inoculum are sufficient. Sommer (1987) gave an example where *Fragiliaria* was replaced by *Stephanodiscus binderanus* Krieger; however, he also gave an example where *Ceratium hirundinella* Muller could not be replaced, because the inoculum and growth rate of the only candidate to replace *Ceratium*, *Peridinium cinctum* Muller, were too low.

The first studies on fungal parasites of planktonic algae gave no evidence whether the fungi had any real effect on the algal population (De Wildeman 1931, Huber-Pestalozzi 1946). Weston (1941) was perhaps the first to review the role of aquatic fungi in limnology and pointed to the ability of fungal parasites to control the numbers of planktonic algae. Reynolds (1940) showed that a chytrid fungus reduced a population of *Staurastrum paradoxum* Meyer. However, the significance of the phenomenon was not quantitatively assessed until the studies of Canter and Lund (1951), focusing on the diatoms of the English Lake District (*Asterionella formosa*, *Fragilaria crotonensis*, *Tabellaria fenestrate* [Lyngb.] Kützing, and *Melosira italica* [Ehr.] Kütz) parasitized by chytrids. Canter and Lund stated that chytrids may delay the timing of maximum algal numbers or decrease the size of their maximal densities. One of the first examples of parasitism as a factor influencing seasonal succession was the replacement of a highly infected *A. formosa* population by *F. crotonensis* and *T. fenestrate* in Erstwhile Water in 1949 (Canter and Lund 1951). Canter and Lund (1969) found that fungal parasitism of desmid populations did not alter the overall seasonal patterns of the major groups of algae but that it did have a marked effect on the outcome of interspecific competition. Reynolds (1973) stated that one important effect of the epidemic of the chytrid fungus *Zygorhizidium affluens* on *Asterionella* in Crose Mere (UK) in 1968 was to permit the dominance of *Stephanodiscus astraea* (Ehr.) Grün, which was typically subdominant in nonepidemic years. Youngman et al. (1976) found that the growth of *Asterionella* in Farmoor Reservoir (UK) was interrupted

when 44% of the cells were parasitized by *Z. affluens*. This infection favored the development of *Stephanodiscus hantzschii* Grün and *S. astraea*. Sen (1987a) presented data to show that parasitism on *Asterionella* interrupted its vernal development in some years. Centric diatoms took over in the phytoplankton community. Van Donk and Ringelberg (1983) also showed that severe parasitism of *Asterionella* in Lake Maarsseveen favored the development of other diatoms, primarily *F. crotonensis*, *S. hantzschii*, and *S. astraea*.

FOOD-WEB ANALYSIS

In the previous section we saw that chytrids can change the succession of phytoplankton and can reduce algal bloom densities. Shifts in the phytoplankton community may affect the uptake of particles or alter the food quality for zooplankton. Marcogliese and Cone (1997) made a plea for the inclusion of parasites in food-web studies. Food-web analysis usually is structured around transfer of energy and carbon between different trophic levels. Parasites seem to have no place in this (despite their significant effects) because of their insignificant biomass. If included in a model, parasites are often considered to be top predators. Effects of parasites on their hosts include energetic demands, altered behavior, reduced growth, reduced fecundity, increased mortality, modification of interspecific competition, and enhanced susceptibility to predation. It is immediately clear that parasites can be extremely important in shaping communities, although they do so without consuming much of the food-web energy.

What is the fate of the parasitic fungi in the food web? A number of processes potentially contribute to population losses of the fungi. Dead fungi will be converted to dissolved organic matter, which in turn may be consumed by bacteria. Recently, another possible fate of one of the life stages of the chytrids was studied. It was found that fungal zoospores are grazed efficiently by *Daphnia galeata* (Fig. 8) The density of zoospores during an epidemic may be in the same order of magnitude as the density of (edible) phytoplankton cells (i.e. several thousands per milliliter). This implies that fungal zoospores may be a food source of some importance for *Daphnia* during fungal epidemics. Chytrids seem to be most common on algae that are fairly resistant to grazing by zooplankton, such as *Asterionella* and *Staurastrum* (Sommer 1987). Because of their large size these algae are believed to be lost mainly through sedimentation to the hypolimnion of lakes, without being consumed (Malone 1980, Legendre and Le Fèvre 1991, Kiørboe 1993). When those large algae are infected by fungi, however, materials (nutrients) within those algal cells are consumed by the parasitic fungus, allowing it to mature and reproduce. Some of the nutrients may now become available to *Daphnia* when the released zoospores are filtered and consumed. Even if fungal parasitism reduces phytoplankton populations, fungi may still support animal production through the grazing by

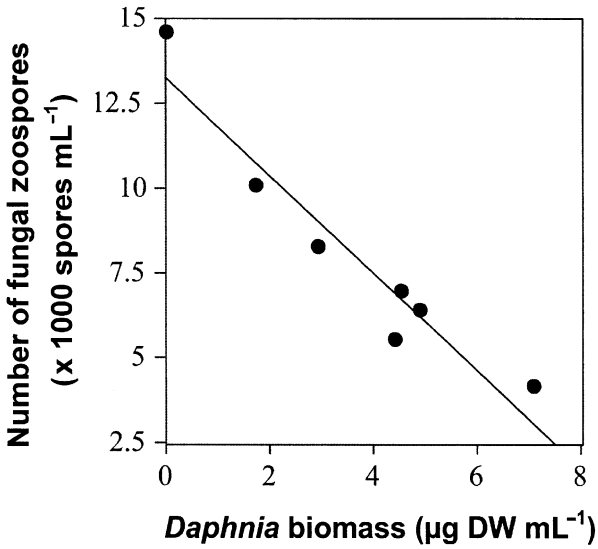

FIG. 8. Grazing on zoospores of *Zygorhizidium* by *Daphnia galeata*. An increase in *Daphnia* biomass reduces the number of zoospores that remains available for infection of *Asterionella formosa* (Kagami et al. 2004; used with permission from the American Society of Limnology and Oceanography).

Daphnia of fungal zoospores. Fungi may perhaps even facilitate animal production through transporting materials from large inedible algae to zooplankton. Overall, it is clear that parasites like the chytrid parasites of phytoplankton may alter flows of energy and matter. The inclusion of parasites in food-web analysis may perhaps invalidate elements of the trophic cascade model, which uses a hierarchy of feeding links based on body size to explain food-web structure. Parasites, being smaller than their hosts, cannot be incorporated into a size-based trophic cascade (Marcogliese and Cone 1997).

ALGAL DEFENSE

Despite all the evidence about the impact of fungal parasites on phytoplankton dynamics, algae are not defenseless. An alga may indeed be susceptible to a parasitic chytrid, in which case zoospore encystment and development of a sporangium will follow upon attachment of the zoospore, or an alga may be resistant (no observable response). A third response type is the so-called hypersensitivity response. In a hypersensitive reaction, the death of a host cell after attachment of a zoospore is so rapid that further development of the zoospore into a mature sporangium is halted and the infection process is curbed (Canter and Jaworski 1982). Hypersensitivity responses have been seen occasionally in samples from Lakes Maarsseveen and Vinkeveen in The Netherlands. The hypersensitivity response is a form of programmed cell death, a burst of superoxide production, and the expression of specific defense genes (White et al. 2000). Hypersensitivity has not been studied widely in algae, but more is known from higher plants. A hypersensitivity response

often is the consequence of a gene-for-gene type of interaction (Flor 1946). Resistance genes (R) in a host plant govern the specific recognition of avirulence genes (Avr) in the parasite. The recognition results in the induction of the plant's defenses, often resulting in the hypersensitivity reaction, preventing spread of the parasites (Bonas 1998). The outcome of the interaction depends on the genetic makeup of both the host and the parasite; a strain that is avirulent on a particular strain may still be virulent on another strain that lacks the matching resistance gene to the avirulence gene. Gromov et al. (1999) used 7 strains of *Rhizophydium* and 137 strains of chlorococalean algae in cross-inoculation experiments. Some fungal strains had very broad host ranges, whereas some were rather narrow. Strain X-34 CALU not only infected green algae from different genera but even infected the xanthophycean alga *Tribonema gayanum* Pasch. All parasites infected strains of different algal genera. The authors concluded that the sensitivity to a given parasite strain depends on the host strain but is not species specific. Paradoxical as these observations may seem, they seem to fit well with the gene-for-gene hypothesis. Algal strains with a resistance gene that matches the Avr gene in the fungus will not be susceptible to infection.

An alternative to the gene-for-gene model is the matching alleles model (Fig. 9). Theoretical models like the gene-for-gene and matching alleles models have in common that the outcome of the host–parasite association depends on the combination of host and parasite genotypes involved. In a gene-for-gene system, resistance occurs only when the parasite is avirulent *and* the host is resistant, with all other combinations yielding a susceptible response. Therefore, a susceptible host will be susceptible to all parasites, independent of their genotype (even when homozygous for avirulence). Similarly virulent parasites will infect all hosts, independent of their genotype. In the matching alleles model each parasite genotype is better at infecting a subset of host genotypes only (Fig. 9) (Agrawal and Lively 2002a,b). Which model fits best and which model is applicable to dynamics between *Asterionella* and *Zygorhizidium* is discussed below.

Some of the new techniques emerging in the genomics era could revolutionize our understanding of the genetic basis for infection of algae by chytrids and vice versa of the alga's defense. Kahmann and Basse (2001) reviewed genes expressed during fungal infection of plants. They discussed the early phases of infection (attachment, germination, appressorium development), the expression of genes involved in evading the plant's defenses, genes necessary for acquiring nutrition from the host, sporulation, and so on. Skinner et al. (2001) concluded that large-scale gene-finding exercises will provide many useful leads for the discovery of key events in the infection process. Tyler (2002) discussed the molecular basis for recognition of *Phytopthera* species (*Oomycetes*) by their host plants. Pathogen signals (elicitors) that are recognized by the host and trigger inducible plant defenses have been characterized at different levels. No such data have been collected (yet) for algae and their chytrid parasites.

A different aspect of algal defense comes from Pohnert (2000) concerning an induced chemical defense mechanism. Only seconds after diatom cells (e.g. *Asterionella* and *Thalassiossira*) were mechanically wounded, an enzymatic mechanism produced fatty acid derived metabolites, resulting in the release of α,β,γ,δ-unsaturated aldehydes. High concentrations of

A

		Parasite		
		avirulent AA	avirulent Aa	virulent aa
Host	susceptible rr	infection	infection	infection
	resistant Rr	no infection	no infection	infection
	resistant RR	no infection	no infection	infection

Gene For Gene

B

		Parasite		
		AA	Aa	aa
Host	rr	no infection	no infection	infection
	Rr	no infection	infection	no infection
	RR	infection	no infection	no infection

Matching allele

FIG. 9. (A) Gene for gene vs. allele models. *Gene-for-gene models* (modified after Clay and Kover 1996). Virulent parasites always infect their hosts, irrespective of the host genotype, i.e. even when the host carries a R(esistance) allele. Only the combination of an avirulent parasite, i.e. carrying an A(virulence) allele *and* a resistant host (carrying R), prevents infection of the host. (B) *Matching allele model*: All parasites are only able to infect a subclass of host genotypes. There are no overall infective parasites and no overall resistant hosts.

these defensive metabolites can be found locally 1–3 min after cell disruption. Preliminary data show that the enzymatically produced metabolites act as highly active fungicides against fungi like *Schizophyllum* and *Aspergillus nidulans*. Further tests should demonstrate whether there are any effects on parasitic fungi of *Asterionella*, which would give the metabolites a function under natural conditions, and is still a matter of speculation at the moment.

HOST-PARASITE COEVOLUTION

Antagonistic coevolution in hosts and parasites specifically involves evolution of host resistance and parasite infectivity with reciprocal selection on either characteristic (Carius et al. 2001). The nature of coevolution is described by the Red Queen hypothesis (Bell 1982). Red Queen dynamics are characterized by time-lagged, negative, frequency-dependent selection, resulting in cyclic abundance of host and parasite genotypes (Lively 2002). There are few unequivocal demonstrations of coevolution. Most evidence in the literature is based on certain preconditions that support coevolution. Coevolution is conditional upon clear reciprocal fitness costs between host and parasite, reciprocal host genotype × parasite genotype interactions (i.e. no universally infective parasites or universally resistant hosts should be present), and the existence of genetic variation for parasite infectivity and host resistance on which selection can act (Little 2002, Woolhouse et al. 2002). We now discuss the potential for coevolution between the diatom *Asterionella formosa* and the chytrid fungus *Zygorhizidium (Rhizophydium) planktonicum* by reviewing evidence for these preconditions.

Fitness costs are often discussed in terms of virulence. Virulence is defined here as the decrease in fitness of the host (damage done) caused by association with the parasite. Virulence evolves in response to host density: High host densities select for higher virulence (Bull 1994). Hosts evolve to minimize virulence, whereas parasites evolve to optimize virulence. In this coevolutionary arms race, parasites are believed to have the upper hand because of their short generation time, large population sizes, and (hence) high rate of evolution. In return, hosts would have evolved sexual reproduction that enables recombination. This is what has become known as the "sex against parasites" version of the Red Queen hypothesis. In a sexually reproducing population, every host constitutes a unique genotype, hindering parasite evolution to an optimal level of virulence and reducing the possibility for local adaptation (Ebert and Hamilton 1996).

Local adaptation occurs when parasites perform better on their own local population than on foreign populations (Kaltz and Shykoff 1998). In reciprocal infection experiments between *Asterionella* and *Zygorhizidium* isolated from different lakes in The Netherlands, we found (preliminary) support for local adaptation of the parasite on its host (unpublished results). The parasite seems locally adapted despite the fact that growth rate and effective population size of host and parasite are reasonably similar (Fig. 10). Under conditions where evolutionary rates of parasites and host are comparable, parasites are actually not expected to become locally adapted (Buckling and Rainey 2001).

Zygorhizidium is a highly virulent parasite of *Asterionella*, and host populations are decimated during epidemics, which usually occur twice a year. How can this parasite remain successful? The high density of hosts during a bloom may select for high virulence of the parasite because finding a new host after release of the zoospores is straightforward. Furthermore, it is not the host but the parasite for which sexual reproduction has been described. This would support the idea that the host population is genetically relatively uniform, allowing the parasite to optimize its virulence. Sexual reproduction, however, does occur in diatoms where it results in the production of auxospores. If cells do not undergo auxosporulation, they will continuously decrease in size and eventually die. Sexual reproduction is required to restore the diatoms to their original size and should occur at some regular interval. Models suggest that occasional sex should provide nearly the same advantages as obligate sex (West 2002). Mann (1993) argued that this may be the case in diatoms; cycles between sexual reproduction may vary between 2 and 40 years. However, even in the 45-year study of the *Asterionella* population in Windermere phenomena indicated sexual reproduction was not seen (Maberly et al. 1994). Canter Lund and Lund (1995) put it like this: "*Asterionella* is a very common diatom and has so often been studied that many millions of colonies must have been observed." Still, sexual reproduction has never been seen. Studies on amplified fragment length polymorphism (AFLP), however, show that *Asterionella* from Lake Maarsseveen is genetically diverse (unpublished results). The genetic structure of an asexual *Asterionella* population would depend on mutation rate, the effective population size (large for *Asterionella*), the frequency and intensity of periodic selection, and the rate of recombination through horizontal transfer of DNA. Alternatively, and perhaps more likely, it must be concluded that such a genetically diverse *Asterionella* population has sex after all. Experiments that are currently underway try to find support for this.

Infection of *Asterionella* in Lake Maarsseveen frequently approaches 100%. This would suggest that the diatom is universally susceptible, which would be prohibitive for coevolution. A random distribution of chytrids on their algal hosts, as found by Holfeld (2000a), would also seem to imply that a genetic basis for infectivity is lacking. Yet infection experiments between *Asterionella* and several isolates of *Zygorhizidium* indicated that no single strain of the fungus was able to infect all local host strains. Conversely, no strain of *Asterionella* was resistant to all parasite strains (Table 1). If there are no universally infective parasites, then what explains the extremely high levels of infection during epidemics? If we assume that every parasite

genotype can only infect a subset of host genotypes—predicted by the matching alleles model and supported by the infection experiments—we must assume that nearly every host allele for resistance is matched by a parasite allele for infectivity.

Asterionella appears to be unsuccessful in fending off parasites. How can the host survive (and even bloom regularly) despite a biannual epidemic of a highly virulent parasite? In most years, *Zygorhizidium* kills off the spring bloom of *Asterionella*. The spring bloom, however, would disappear anyway after the onset of stratification or depletion of silicon in the lake (Maberly et al. 1994). It nevertheless remains important to provide a large enough inoculum for the next bloom. There has to be part of the *Asterionella* population that escapes the fungal epidemic and restocks the lake with healthy cells. This is to be expected in any case, because the result of a fungal epidemic is to reduce host density to very low numbers, making it very hard for zoospores to find the last few remaining host cells. Moreover, for diatoms it has been shown that they form "seed banks" on the sediment, from where resuspended cells could provide the new inoculum (Jewson 1992, Itakura et al. 1997, McQuiod et al.

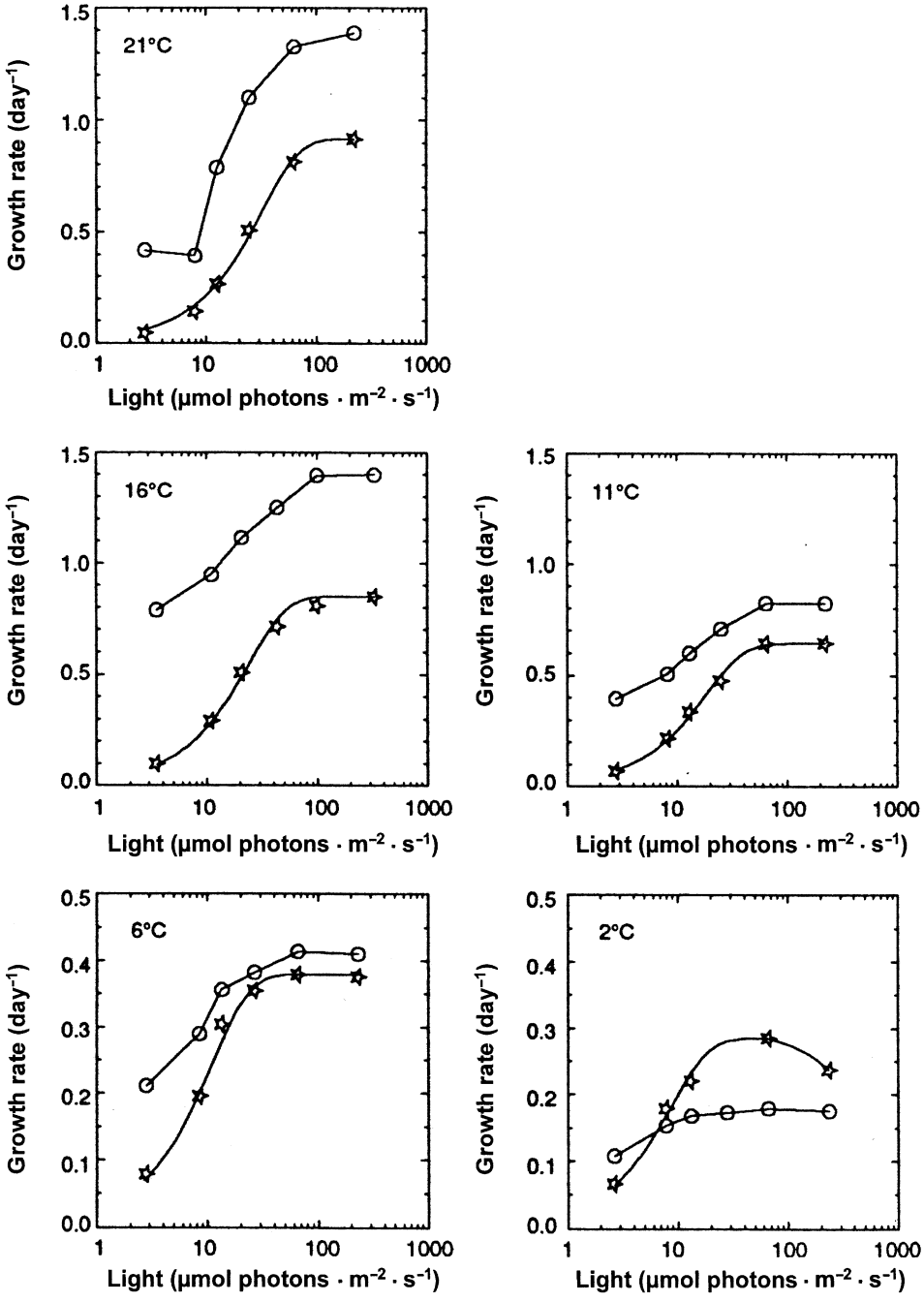

FIG. 10. Growth rates host vs. parasite (Bruning 1991c; used with permission from the American Society of Limnology and Oceanography). Comparison of the light-dependent growth rate of *Asterionella* (stars) with the maximum growth rate (at infinite host cell density) of *Rhizophydium* (circles) at five different temperatures. At 2 and 6° C the vertical scale is enlarged. Note that under certain conditions the generation time of the host is shorter than that of the parasite.

TABLE 1. Degree of infectivity of four different isolates of the chytrid parasite *Zygorhizidium planktonicum* on host isolates of *Asterionella formosa* from Lake Maarsseveen (hosts indicated with M) and Lake Vinkeveen (hosts indicated with V).

	Parasite isolates			
Host isolates	A	B	C	D
M1	2	3	3	3
M2	2	3	3	3
M3	1	3	3	3
M4	1	3	3	3
M5	2	3	3	0
M6	1	3	3	2
M7	2	2	3	3
M8	1	2	3	2
M9	2	3	0	2
M10	1	0	2	2
M11	2	3	3	2
M12	2	3	3	2
M13	2	1	3	0
M14	2	0	3	2
M15	2	2	3	0
M16	3	2	3	0
M17	1	3	0	2
V1	2	0	0	0
V2	1	3	2	3
V3	0	3	1	3
V4	2	3	3	3
V5	2	3	1	3
V6	2	3	0	3
V7	3	3	0	3
V8	2	1	0	3
V9	2	3	0	3
V10	2	1	0	3
V11	3	3	1	3
V12	2	2	1	3
V13	2	2	1	3
V14	2	3	2	3
V15	2	3	2	3
V16	2	3	2	3
V17	1	3	0	3

Parasite A was isolated from Lake Vinkeveen, B and C from Lake Maarsseveen, and D from Blelham Tarn in the English Lake District. A higher value in the table corresponds to higher degree of virulence, meaning that the number of infected host cells increases more quickly. A zero indicates that this particular host strain was resistant to this particular parasite strain, even in repeated infection experiments. No parasite strain is overall infective on all of its local host isolates. No host is overall resistant.

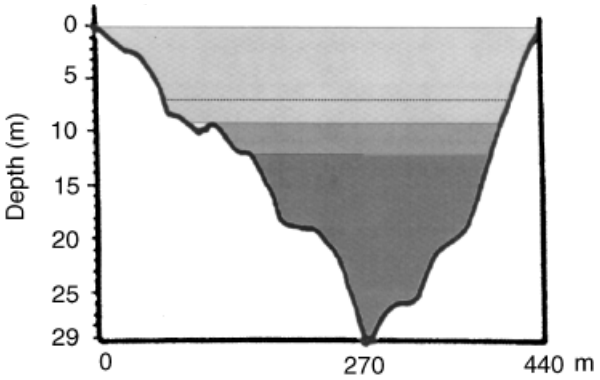

FIG. 11. A cross-section through Lake Maarsseveen (the *x* axis showing distance across the lake, the *y* axis showing depth of the lake). The three shades of gray indicate areas where, based on the experimental data by Bruning (1991c) shown in Fig. 7 and *in situ* data on light, temperature, and density of *Asterionella* in Lake Maarsseveen, the infection can become epidemic (light gray, top layer), where the fungus can survive (medium gray, middle layer), and where the fungus cannot become infective (dark gray, deep layer). The thin gray line indicates the depth of the thermocline. In spring only one layer with average conditions is present because the lake at that time is isothermal (not shown). Refuges for the host under the conditions near the sediment will affect the host–parasite interactions in the lake.

2002). From the experimental work of Bruning (1991d) we know that the conditions on the sediment and in deeper water layers (cold, dark) would indeed allow *Asterionella* to remain uninfected (Fig. 11). Refuges are believed to play an important role in shaping host–parasite coevolution (Little and Ebert 1999), and it seems plausible that this is true for *Asterionella* and *Zygorhizidium*.

When all three preconditions for coevolution (i.e. reciprocal fitness costs, reciprocal host genotype × parasite genotype interactions, and genetic variation for parasite infectivity and host resistance) are considered, it is clear that reciprocal fitness costs are present. The fungus is dependent on the host for reproduction; the host suffers massive population losses. The evidence for specific host genotype × parasite genotype interactions is not yet conclusive. Nothing is yet known about the genetic makeup of the chytrid fungus. The extremely high prevalence of infection seemed to indicate that an overall infective parasite was present, but infection experiments in the laboratory contradict this. Possibly every host allele in the population is matched by a parasite allele, aided by sexual reproduction of *Zygorhizidium*. The fact that the parasite seems locally adapted would support the evolutionary advantage of the fungus over the diatom (Lively 1999). There are areas in the lake where the host is free from infection, and these refuges seem important in the long-term survival of *Asterionella*. There is no information whatsoever on the third condition: the existence of genetic variation for resistance and infectivity in the *Asterionella–Zygorhizidium* system. It seems that individual clones that differ genetically also differ in their susceptibility for the parasite. It seems possible that coevolution has occurred between *Asterionella* and its parasitic chytrid fungus, but conclusive evidence is lacking. An experimental approach as taken by Buckling and Rainey (2001) may perhaps provide this evidence for *Asterionella* and its chytrid fungus *Zygorhizidium*.

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