

Infection of the single-celled diatom *Stephanodiscus alpinus* by the chytrid *Zygorhizidium*: Parasite distribution within host population, changes in host cell size, and host–parasite size relationship

Abstract—An epidemic caused by a *Zygorhizidium* species infecting the single-celled planktonic centric diatom *Stephanodiscus alpinus* was analyzed for parasite distribution within the host population, final parasite size relative to host cell size, and size changes of infected and uninfected *S. alpinus* cells. Infections in the lake occurred at random within the whole host population. There was no evidence for aggregated or even distribution of the parasite individuals, indicating that the infections occur independently of each other. In enclosures in which light was enhanced compared to the lake, there tended to be an even parasite distribution within the host population, irrespective of whether plant nutrients were added. This suggests that infected host cells were negatively selected by the parasite zoospores under these conditions. Final parasite sporangium size and host cell size were positively correlated. Thus, parasite fecundity was limited by host cell size. Infected *S. alpinus* cells tended to be larger than uninfected cells, and the mean size of host cells within the population decreased during the epidemic. This might be due to selective infection of larger host cells or to the peculiar mode of cell division in diatoms.

A wide range of phytoplankton species is infected by microscopic fungi belonging to the zoosporic classes Chytridiomycetes and Oomycetes (Canter-Lund and Lund 1995). The infected algal cells usually die during the infection, causing nonphagotrophic losses in the host population. These parasites are host specific, mostly attacking only one or few closely related host species. Some are even host race specific (Canter and Jaworski 1982; Canter et al. 1992; Doggett and Porter 1995; Holfeld 1998). Therefore these parasites select for host taxon, and not particle size. Dispersal and infection is by flagellated, free-swimming zoospores that are posteriorly uniflagellate in the Chytridiomycetes and biflagellate in the Oomycetes.

Concerning the ecophysiology of these parasite infections, there have been experimental studies regarding the effect of light and darkness on the motility and infectivity of the zoospores of parasites of *Asterionella formosa* (Canter and Jaworski 1981; Bruning 1991a) and *Fragilaria crotonensis* (Canter and Jaworski 1982, 1983). The effects that various environmental parameters (temperature, light, nutrient limitation) had on the developmental time and sporangium size of the parasite *Rhizophyidium planktonicum* on its host *A. formosa* have been described by Bruning (1991b,c). On the ecosystem scale, the impact of fungal parasitism on the whole phytoplankton community has been addressed by Holfeld (1998).

However, nothing is known about changes in cell size of the infected host population during an epidemic or the effect of host cell size on the size of the mature parasite sporan-

gium and thus on the fecundity of the parasite. There is no information on the relative sizes of the host and parasite cells. Further, the type of distribution within the host population (aggregated, random, even) is not known for fungal phytoplankton parasites. The distribution of a parasite within its host population is an important ecological factor because it reflects the impact on the host population by a given number of parasite individuals.

An epidemic caused by a *Zygorhizidium* species infecting the single-celled planktonic diatom *Stephanodiscus alpinus* offered an opportunity to address these questions. This host alga and the parasite were especially suitable for the present study because of their simple shape that allowed the calculation of their volumes, and because even the early stages of the infection were clearly visible to the experienced eye. Further, the unicellular growth form of the host alga avoids the complications brought about by colony formation in diatoms such as *A. formosa* and *F. crotonensis*. The *S. alpinus* cell was always killed by the parasite, leaving a frustule almost devoid of cell contents.

This study was carried out at the Schöhsee, a stratified hardwater lake near Plön, North Germany (54°N) that has no permanent, surface-directional flow. The lake is holomictic and dimictic and rarely monomictic. The surface area is 83 ha; the mean depth is 13 m.

Daily sampling from the lake and enclosure experiments within the lake were performed from 27 January to 7 February 1989. An additional lake sample was taken on 23 January. During this period *Zygorhizidium* sp. caused an epidemic in the *S. alpinus* population. The population density of the host alga varied between 3 and 33 cells ml⁻¹ in the lake and 8 and 59 cells ml⁻¹ in the enclosures. A description and illustration of the parasite as well as the seasonal occurrence of host alga and parasite is given by Holfeld (1998).

Samples from the lake were taken with a 2-liter Ruttner bottle from the surface and at 2-m intervals down to a depth of 10 m. These samples were immediately pooled and mixed. A subsample was fixed with several drops of Lugol's solution (Utermöhl 1958) and examined using an inverted microscope (Utermöhl 1931). Numbers of *S. alpinus* cells that were infected and numbers of parasite individuals that were present on the infected hosts were noted. Empty *S. alpinus* frustules and *S. alpinus* cells bearing at least one dehisced sporangium of the parasite were considered to be dead and were not counted. In this way the relics of earlier infections that might have remained in suspension for some time and that might have obscured current processes were excluded. However, *S. alpinus* cells bearing just one dehisced parasite sporangium were used for the measurements of host cell size and final parasite size (see below).

During the experiment, the nutrient concentrations in the

lake were $15 \mu\text{g L}^{-1}$ soluble reactive phosphorus (SRP), $400 \mu\text{g L}^{-1}$ soluble reactive silicon (SRSi), and $140\text{--}165 \mu\text{g L}^{-1}$ soluble reactive nitrogen (SRN, sum of nitrate-, nitrite-, and ammonia-N). The temperature in the lake was $3.6\text{--}4.1^\circ\text{C}$, and the Secchi disk transparency was $8.60\text{--}9.20$ m. The nutrient concentrations were analyzed photometrically according to Strickland and Parsons (1972).

In addition to sampling directly from the lake, enclosure experiments were performed at the same time in a sheltered bay in the south of the lake. Two floats were fixed at a site where the water depth is about 6 m. In each of the floats four plastic bags (Trikoron, Alkor-Oerlikon Plastic) 1 m in diameter and 2 m in depth, with a volume of 1,600 liters were fixed with the opening 20 cm above the lake surface. The enclosures were filled by pumping lake water through a $100\text{-}\mu\text{m}$ mesh gauze to remove larger zooplankton. The bags separated the water inside the enclosures completely from the lake water. The enclosures were open at the top allowing free light penetration, gas exchange, and sampling. Two enclosures per float were subjected to one of the following treatments: (1) control: no additions; (2) nutrient treatment: increase in the natural nutrient concentration to $800 \mu\text{g L}^{-1}$ SRSi, $300 \mu\text{g L}^{-1}$ SRN, and $30 \mu\text{g L}^{-1}$ SRP. This high nutrient level was checked twice weekly and maintained by nutrient addition if necessary. The enclosures were filled 2 d before the first sampling, and the nutrients were added 1 d beforehand.

Samples were taken from the center of the enclosures with a 2-liter Ruttner bottle. Directly before sampling, the water in the enclosure was mixed by lifting a Secchi disk several times through the water column in rapid succession.

In lake samples, the diameter and length of *S. alpinus* cells bearing one single dehisced sporangium of the parasite were measured, along with the length and width of the dehisced sporangia. From these measurements the volumes of the host cells and the empty sporangia were calculated. The shape of the *S. alpinus* cells was considered to be a cylinder, and the shape of the parasite sporangia was considered to be a rotation ellipsoid. Dehisced sporangia had to be measured to obtain an estimate of the terminal parasite size. It would have been preferable to measure mature undehisced parasite sporangia that are distinguished by the regularly arranged, even-sized oil droplets of the unreleased zoospores. However, this stage seemed to be short in duration because only a few such sporangia were seen. Measurements were also taken from these rare specimens, but these were separated from the more numerous measurements of the cells carrying dehisced parasite sporangia.

Only those host cells were measured in which one valve was convex and the other concave. These convex-concave cells were by far the most common. The other two host cell types, convex-convex and concave-concave, were rarely seen and omitted from the measurements. Further, the valve diameter of infected and uninfected cells was measured in lake samples from 23, 27, and 30 January, and 01, 03, and 07 February 1989. The measurements of the host cells and of the parasite sporangia were performed microscopically at $500\times$ magnification using an eyepiece micrometer.

The prevalence of infection Pr , a measure of the numerical proportion of the infected individuals within the host

population, was calculated as percentage according to the formula

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

where N_i is the number of infected cells, and N_u is the number of uninfected cells within the host population.

The mean intensity of infection, \hat{I} , is a measure of the mean number of parasites per host in a population, and was calculated as the ratio of the number of individuals (here: attached fungal thalli) of a given parasite species, N_p , to the number of host individuals, N_h , which includes both infected and uninfected host individuals:

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

The probability P at which host cells with $k = 0, 1, 2, \dots$ parasites occur at a given mean intensity of infection \hat{I} can be predicted by the Poisson distribution if the infections take place independently of each other in a host population with uniform parasite susceptibility:

$$P(\hat{I}, k) = e^{-\hat{I}} (\hat{I}^k / k!) \quad (3)$$

Because the case of uninfected host cells (parasite number = 0) is included in the calculation, an expected prevalence of infection can be calculated from the frequency distribution and compared to the observed prevalence of infection.

Another distinct relationship arises if the parasites have a perfectly even distribution within the host population. In the present case, in which a given individual host can be infected by several individual parasites, but in which a given individual parasite might infect only one individual host, there are also impossible combinations of mean intensity and prevalence of infection. For example, it is impossible to find a prevalence of 80% at a mean intensity of 0.5. Furthermore, if the parasites have an aggregated distribution within the host population, the prevalence is lower than at random distribution given the same mean intensity of infection.

For the lake, the Poisson distribution describes the relationship well between mean intensity and prevalence of infection (Fig. 1). With one exception, the empirical results are not significantly different from the values predicted by random distribution. This is also true at the highest mean intensities exceeding 1.5. In contrast, in all enclosures mean intensities higher than 1.8 were observed. Here all empirical results differ, significantly in most cases, from the values predicted by random distribution as soon as the mean intensity of infection exceeds 1.0 (Fig. 1). This deviation indicates a more even parasite distribution than would be expected from perfect randomness of the infections.

The median of the diameter of the infected host cells was always larger than the median of the diameter of the uninfected cells (Fig. 2). With one exception, this difference is significant. Between the start and end of the period when measurements were taken, the reduction in size is significant for both infected and uninfected *S. alpinus* cells (Mann-Whitney U -test, $P \ll 0.01$).

The volume of the dehisced parasite sporangia is positively correlated with the volume of the host cell (Fig. 3). For a given host volume the parasite sporangia volume can vary by a factor of three. There were no empty parasite sporangia smaller than $60 \mu\text{m}^3$. The values for the mature

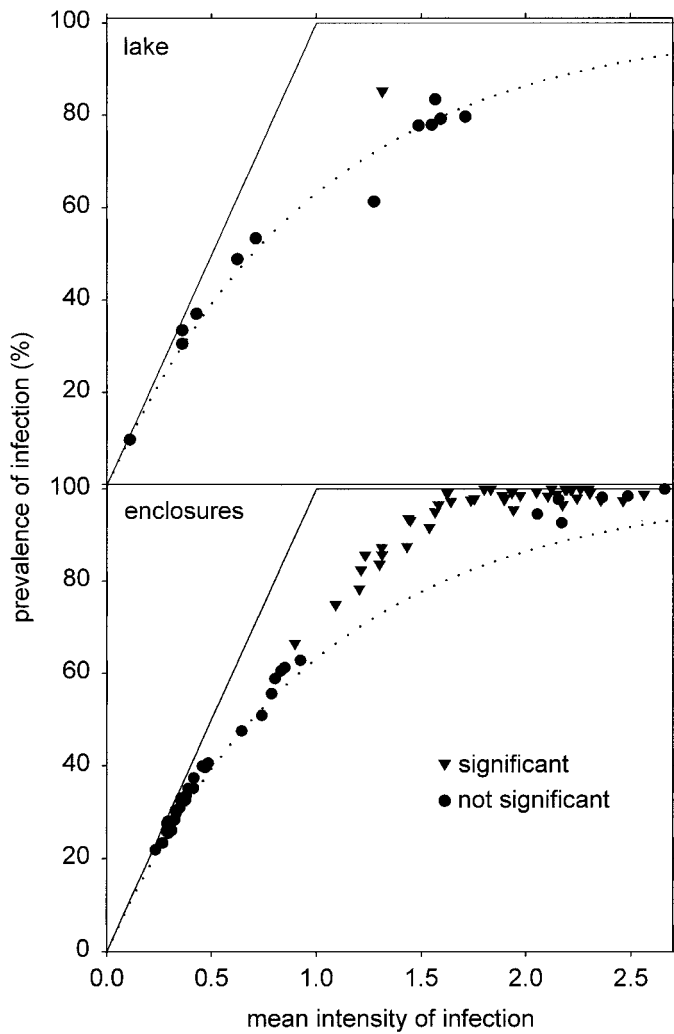


Fig. 1. Distribution of *Zygorhizidium* sp. parasite individuals within the host population in the lake and in the enclosures. Solid line: Theoretical, perfectly even parasite distribution. Dotted line: Theoretical, perfectly random parasite distribution. Even parasite distribution is represented by the area between dotted and solid line, aggregated parasite distribution by the area under the dotted line. Mean intensity/prevalence combinations above the solid line are impossible. Differences between empirical results and corresponding prevalence values predicted by perfect random distribution were significant (triangle; chi-square test, $P < 0.05$) or not significant (circle; chi-square test, $P > 0.05$).

undeveloped parasite sporangia, although not numerous and showing a wide size deviation, clearly show that these sporangia are much larger in relation to the host cell size. The ratio of parasite sporangium volume: host cell volume is on average 18% for the empty sporangia and 48% for the mature sporangia. In one extreme case the volume of the mature sporangium was 65% of the host cell volume.

In the lake and in the enclosures there was no indication of an aggregated parasite distribution. This is remarkable because aggregation is the most common form of parasite distribution (Anderson 1982; Begon et al. 1996). The lack of parasite aggregation indicates that there was no variation for susceptibility to infection within this host population of

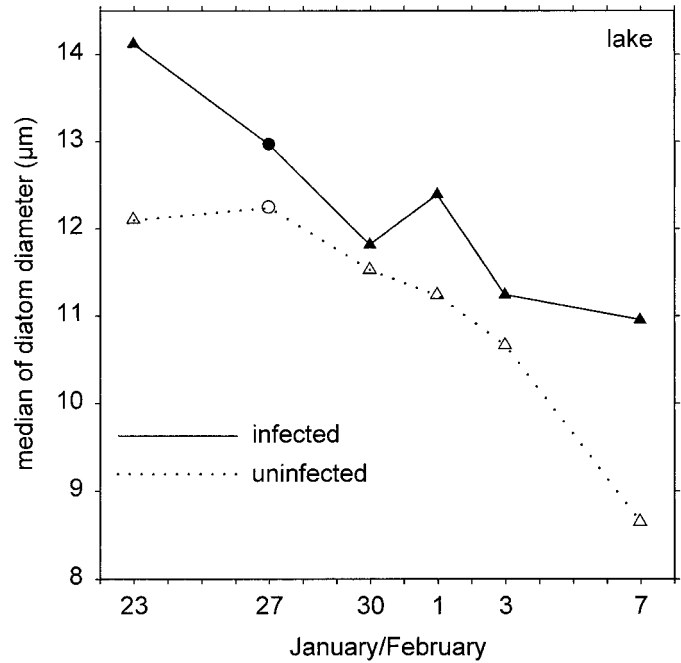


Fig. 2. Median of the valve diameter of infected (filled symbols, solid line) and uninfected (hollow symbols, dotted line) cells of *S. alpinus* in the lake. Differences between infected and uninfected cells were significant (triangle; Mann-Whitney U -test, $P < 0.05$) or not significant (circle; Mann-Whitney U -test, $P > 0.05$).

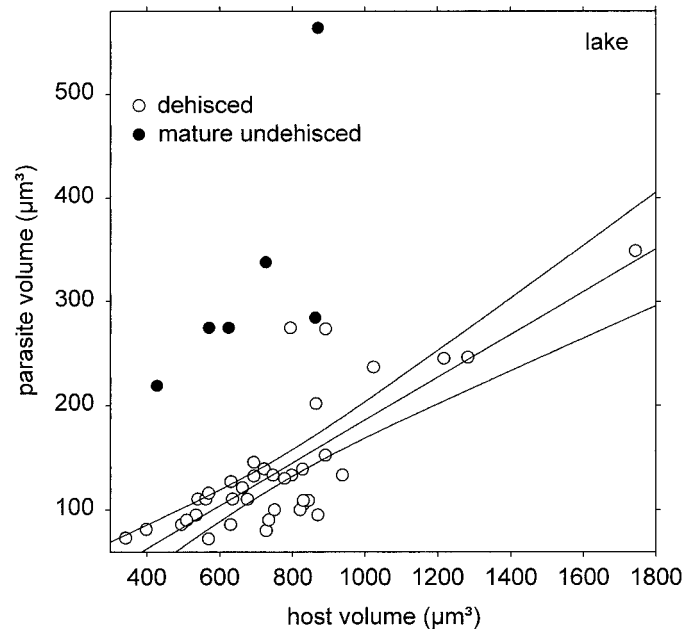


Fig. 3. Volume of the parasite sporangia plotted against the volume of the host cell in the lake. Pooled data from 27 and 30 January 1989. Only host cells bearing one single parasite sporangium are considered. Hollow circles: dehisced parasite sporangia. Filled circles: mature undeveloped parasite sporangia. Linear regression and 95% confidence intervals apply to dehisced parasite sporangia only ($y = -19.3488 + 0.205493x$; $N = 39$; $R^2 = 63.39\%$; $P < 0.001$).

S. alpinus. It also indicates independent dispersal of the parasite zoospores. This means that the zoospores are dispersed singly, not in clumps, and there is no attraction to each other or even swarm formation. This agrees with observations on zoospores of *Zygorhizidium planktonicum*, a parasite of *A. formosa* and *Synedra* (Canter and Jaworski 1986). In the lake, moreover, the parasite propagules apparently do not discriminate between infected and uninfected *S. alpinus* cells.

In the enclosures, irrespective of the treatment, the parasites tended to have an even distribution, which is also unusual for parasites. An even parasite distribution can be caused by density-dependent mortality of the parasites, selective mortality of host individuals suffering from high infection intensity, or induced resistance against new infections of the host (Begon et al. 1996). None of these mechanisms seems applicable in the present case. The infected host cell finally dies even if it is infected by only one parasite. This suggests, therefore, that there is no selective mortality of the host cells suffering multiple infections, and there is no evidence to support the occurrence of induced resistance.

Host cells with multiple infections might die faster than those with a single infection, or their parasite sporangia might dehisce earlier. An infected host cell was considered to be dead if it carried at least one dehisced parasite sporangium, and these cells were not counted. Multiple infected cells contribute equally to prevalence, as do cells with a single infection, but they make a larger contribution to mean intensity. Therefore excluding multiple infected host cells bearing at least one dehisced parasite sporangium moves the symbols in the diagram down a little (Fig. 1) but more to the left than if these cells were included. If this mechanism was at work, inclusion of these cells would result in a mean intensity higher than four for several of the observed prevalences. There is no indication that such high mean intensities existed. Dehiscence of the parasite sporangia, dispersal of the zoospores, and the resulting new random infections move the datapoints toward the dotted line rather than raising them above it.

A density-dependent mortality of the parasites is also unlikely because the chitin wall of a dead parasite remains visible for some days, and because no distinction was made between living and dead parasites. Therefore dead parasites, if they occurred at all, were included in the calculation anyway.

The host cells probably remain susceptible for additional parasites only for a limited time after the first infection. The parasite individual that caused the first infection probably influences the physiology of its host cell that might in turn obscure the recognition processes necessary for encystment of the parasite zoospores. This idea is supported by the observation that in those rare cases in which one dead host cell supported two or three dehisced parasite sporangia, they were always of almost identical size. Here the successful infections presumably took place within a short period of time. In laboratory cultures Canter and Jaworski (1981, 1986) observed that zoospores of *R. planktonicum* and *Z. planktonicum*, respectively, sometimes "buzzed" in a halo

around potential host cells. It is conceivable that in such situations almost synchronous multiple infections can occur.

According to this hypothesis the susceptible period of the host cell would be long enough in the lake to result in a random distribution of the parasite individuals in the host population. This susceptible period would be shorter in the enclosures, favoring a more even distribution of the parasite individuals in the host population. The question then arises as to how the difference between the lake and the enclosures occurs. Differences in nutrient supply are probably not the reason because an even distribution was observed in all enclosures. A more probable explanation is the difference in light supply. During the experiment, the lake was in full circulation, with a mean depth of 13 m compared to the 2-m depth of the enclosures. As an approximation, calculating the amount of light received by suspended particles according to Kirk (1983) and Sommer (1994) and assuming mixing, the availability of light in the enclosures was about twice as high as in the lake. It is possible that a physiologically more active host allows faster growth of the parasite, resulting in an earlier disorder of the host's physiology. This view is supported by Bruning (1991b), who found that the development time of the sporangia of *R. planktonicum* growing on light-saturated *A. formosa* was at the most only slightly longer, whereas the sporangium size was strongly increased compared to the sporangia growing on light-limited host cells.

Diatoms have a rigid frustule that determines the size and shape of the cell. Therefore, in a given sample, it is unlikely that swelling or hypertrophy of the infected diatoms takes place and can explain the observed size difference between infected and uninfected *S. alpinus* cells. More reasonable explanations are size selectivity of the parasite zoospores or the peculiar mode of cell division in diatoms.

Size selectivity of fungal phytoplankton parasites has been reported by Koob (1966). In the two *A. formosa* populations he examined only the second smallest of five size classes was parasitized. Further, Sen (1987) distinguished a micro- and a macropopulation in *A. formosa* according to the length of the frustules. The micropopulation was always much more parasitized, even if it was rarer than the macropopulation.

Compared to the results reported by Koob (1966) and Sen (1987), the host size effect is very weak in the present study (Fig. 2). There is no evidence for a resistant or peculiarly susceptible subpopulation in the *S. alpinus* population. The bias of the infected cells is just shifted toward a larger size. It is possible that larger host cells are more easily found or recognized by the parasite zoospores. Canter and Jaworski (1986) observed that potential host cells attract zoospores of *Z. planktonicum* only in the light and suggested that the alga releases attracting substances under these conditions. If this is true, large potential host cells could release more attracting substances than small ones. Continued selective killing of the larger *S. alpinus* cells might shift the host population toward a smaller average cell size.

Another explanation for the larger size of the infected cells is the peculiar mode of cell division in diatoms. After cell division only one of the two daughter cells has the same size as the mother cell. The second daughter cell is smaller (Round et al. 1990). Therefore, the average cell size of a

diatom population decreases in the course of successive cell divisions. Because infected host cells do not divide and finally die, their cell size is frozen at the moment of infection. In contrast, the uninfected cells continue dividing and successively decrease in average cell size. If this mechanism is at work in the present case, the size difference between infected and uninfected host cells as well as the general decrease in host cell size during the epidemic indicate that the parasite attacked a population of dividing host cells.

The positive correlation between host cell size and the parasite sporangium size is supported by the present study (Fig. 3). Because the zoospore size is roughly constant for a given chytrid species (Webster 1986), and the plasma in the sporangium is completely converted into zoospores, the larger sporangia are also more fecund. The fecundity of this parasite is therefore limited by the host cell size. Because of this limitation and because the host size decreases during the epidemic, the host quality for the parasite also decreases. Further, the inoculum of the parasite is expected to be higher than inferred from the mean cell size of the host population alone because infected host cells tend to be larger than average.

In conclusion, the present study indicates that the population of dividing host cells as a whole was susceptible to fatal parasite attack. The infections were not restricted to weakened or moribund host cells. Canter and Lund (1948) also noted that parasite zoospores can attack perfectly healthy *A. formosa* cells. The lack of parasite aggregation means that the impact on the host population is high because even a single parasite individual will kill its host cell. The host quality decreased during the epidemic. The large host cells at the beginning of the epidemic, causing high parasite fecundity, should facilitate the establishment of the parasite population.

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