The ecological interactions between a microsporidian parasite and its host *Daphnia magna*

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Summary

1. Freshwater plankton populations suffer frequent epidemics of microparasitic diseases. The mechanisms which lead to outbreak and spread of these parasites are poorly understood. A set of experiments was carried out to distinguish between hypotheses explaining the introduction, spread and persistence of a microparasite in *Daphnia magna*.

2. Transmission of the microsporidian parasite *Pleistophora intestinalis* is horizontal through ingestion of free-floating parasite spores, or from spores taken up from pond sediments by the host. At 4 °C parasite spores remained infectious after 3 months, explaining how the parasite persists through periods of host diapause.

3. Parasite transmission probability was inversely related to the water volume in which infected and uninfected hosts were kept. The same density effect was found for the intensity of infections.

4. Host nutritional conditions did not influence parasite multiplication inside the host. However, well-fed hosts became infected more often than poorly fed daphnids of the same age, which can best be explained by their larger size, and consequently their higher filtering rates.

5. Both sexes and all life stages tested of the host were susceptible to infection. Growth and transmission of the parasite was greatly impaired at 6 °C, but no differences in parasite growth were found between 12, 16, 20 and 23 °C.

6. The impact of *P. intestinalis* on host fecundity was inversely correlated with initial spore dose.

7. Long spore survival outside the host, reduced transmission at low temperatures and density-dependent transmission were the main factors in the interaction of *P. intestinalis* and its host *D. magna*. The results are consistent with field studies of other horizontally transmitted microsporidian parasites in cladoceran and rotifer populations.

Key-words: Cladocera, density dependence, spore survival, temperature dependence, transmission.

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Introduction

Pronounced density fluctuations over several orders of magnitude are typical for most short-lived planktonic organisms such as algae, rotifers and planktonic crustaceans. In these populations, abundance might drop temporarily even to zero during periods of diapause. Microparasite prevalences in plankton populations are usually zero or very low during the spring, followed by epidemic disease outbreaks when host density increases in late spring or summer (Canter & Lund 1951; Green 1974; Miracle 1977; Sayre, Adams & Wergin 1979; Brambilla 1983; Yan & Larsson 1988; Bruning, Lingeman & Ringelberg 1992; Vidtmann 1993). We found this seasonal pattern in a field study of three *Daphnia magna* Straus populations, with summer prevalences of the microsporidian parasite *Pleistophora intestinalis* Chatton reaching up to 100% in adult hosts (H. Stirnadel and D. Ebert, unpublished). Two questions arise from the apparently widespread seasonal prevalence pattern.

First, how does a parasite persist through low host densities and start an epidemic once host density has recovered? Density-dependent transmission often

creates severe population bottlenecks for parasites when host densities are below the threshold density necessary for parasite persistence (i.e. parasite $R_0 < 1$; Hassell & May 1973; Anderson & May 1978, 1979, 1986; Beddington, Free & Lawton 1978; Blower & Roughgarden 1989; Onstad *et al.* 1990). To escape extinction, a horizontally transmitted parasite needs special adaptations to survive periods of low host density. As a possible mechanism for the initial outbreak of *Spirobacillus* epidemics, Green (1974) speculated that parasite spores are taken up from pond sediment by benthic-feeding cladocerans in early summer, and the parasite then spreads through the host population. This hypothesis requires long-lived infective parasite stages (see Anderson & May 1981 for a discussion), and a mechanism for the uptake of resting parasite spores after the host density has recovered. Neither has been investigated in zooplankton.

Secondly, what factors limit the spread of the parasite over the season? Following the successful introduction of the parasite, several hypotheses have been proposed to explain its subsequent spread through zooplankton populations. (i) Parasite transmission is density dependent (Canter & Lund 1951; Green 1974; Miracle 1977; Brambilla 1983; for epibionts: Chiavelli, Mills & Threlkeld 1993). (ii) Parasite transmission is limited at low temperatures (Miracle 1977; Ruttner-Kolisko 1977; Sayre *et al.* 1979). (iii) Host stress facilitates the spread of the parasite (Seymour *et al.* 1984; France & Graham 1985). And (iv) differences in resistance between host sex or size classes may influence parasite spread (Breed & Olsen 1977; Andreadis 1988). Testing for these hypotheses under natural conditions is difficult, because the hypotheses are not mutually exclusive and combinations of several factors could play a role. Furthermore, under natural conditions, some of the relevant factors often coincide with each other and do not allow for separation of their effects. For example, periods of high zooplankton density are often followed by declining food conditions, and plankton densities are usually low during low temperatures in winter (Wetzel 1975). In addition to these problems, difficulties in the identification of microparasites from field samples sometimes results in the pooling of different parasite species into 'convenience groups' (Yan & Larsson 1988). Only experiments in which all these factors can be controlled can help to distinguish between hypotheses and to understand the epidemiology of plankton parasites.

Here I describe a series of laboratory experiments, conducted to understand the ecological interactions of the microsporidian parasite *P. intestinalis* with its host *D. magna*. I tested the above mentioned hypotheses in order to understand how *P. intestinalis* enters a host population and which factors limit its subsequent spread. Furthermore, since this parasite is known to reduce fecundity and survival of its host (Ebert 1994b), I tested for the relation between parasite intensity and host reproduction to evaluate the potential of the parasite to regulate its host population. This point is of considerable interest with respect to the current debate on whether plankton dynamics are driven by external or internal factors (McCauley & Murdoch 1987; McCauley 1993).

Materials and methods

HOST AND PARASITE

Daphnia magna is a planktonic crustacean common to many ponds in Europe. Clonal offspring of a single female *D. magna* isolated in July 1992 from a pond in southern Oxfordshire, UK were used for the experiments. This female was infected with the parasite *P. intestinalis*. Since vertical transmission does not occur in this parasite it is possible to isolate some new-born of an infected host within a few hours after they are released from the brood pouch and use them to establish a parasite-free clonal line. The other new-born left with the mother can be used to establish a parasitized culture.

The microsporidium *P. intestinalis* is a cytoplasmic parasite of the gut epithelium of *D. magna* (Green 1974; Larsson 1981; Ebert 1994a). The parasite can be identified by its sporophorous vesicles (SV) of 8–15 μm diameter found in the epithelium gut cells of its host, and by the pyriform 2×3 μm spores. Parasite infestation can be quantified by counting the SV in the dissected gut under a microscope (Ebert 1994a,b). Electron microscopic investigations revealed that the laboratory strain of *P. intestinalis* used here represented only one parasite species. This parasite has been found in five of seven sampled *D. magna* populations in south-east UK and was also found in 4% formaldehyde-preserved samples from North Germany and Poland (D. Ebert, unpublished data).

The parasite was kept in the laboratory in a monoclonal *D. magna* culture for several host generations by replacing half of the infected hosts with new-born from an uninfected stock culture at about 2-week intervals. Infective parasite spores are expelled from the host with the faeces and disperse in the water, infecting the introduced *D. magna*.

EXPERIMENTAL CONDITIONS

Experiments were conducted under a constant light/dark cycle (16/8 h) and a temperature of 20 °C if not described otherwise. The artificial *Daphnia* medium ADaM (Klüttgen *et al.* 1994) was used for the cultures. A suspension of the monocellular algae *Chlamydomonas reinhardii* Dang was used as food. If not stated otherwise, 10^5 algae cells ml^{-1} were added daily as a standard food ration. All experiments are summarized in Table 1.

Table 1. Description of experiments and summary of result

Number	Tested factor	Experiment	Result
1	Transmission	Infectiousness of mud samples	Mud samples are infective
2	Transmission	Infectiousness after 3 month at 4 °C	High spore survival
3	Transmission	Susceptibility of hosts in relation to size and sex	All size and sex classes are susceptible
4	Transmission	Various densities (beaker sizes)	Strong density dependence
5	Parasite growth and reinfection	Various densities (beaker sizes)	Strong density dependence
6	Parasite growth	Two host-feeding regimes	No effect
7	Transmission	Two host-feeding regimes	Well-fed hosts become more often infected
8	Transmission	6, 12, 16, 20 and 23 °C	Impaired transmission at 6 °C
9	Parasite growth	12, 16, 20, 23 °C and two densities	No temperature effect, but density effect
10	Host fecundity Parasite growth Parasite vesicle size	Three different levels of parasite dose	Fecundity decreases with parasite dose No effect No effect

SPORE UPTAKE AND SURVIVAL

Experiment 1

To test if *D. magna* becomes infected through the uptake of spores from pond sediment, about 100 ml of mud from each of four ponds harbouring parasitized populations of *D. magna* were collected. The mud was washed and sieved (0·2 mm pore width) and left for 3 days to allow for sedimentation. About 10 ml of these sediments were put into 400 ml of fresh medium (two replicates for each of the four mud samples and two controls with sediment heated for 2 h in a waterbath at 95 °C). After allowing sedimentation for 3 days, five uninfected juvenile and five uninfected adult *D. magna* were transferred into each beaker. Standard food ration was added every other day. After 24 days all *Daphnia* were dissected to see if infection had taken place.

Experiment 2

To test if parasite spores could survive host diapause, about 500 infected daphnids from a field sample were starved, in 1 litre of medium. The remains of these daphnids were used either directly, or after they had been kept for 3 months at 4 °C, to infect daphnids from the stock culture. Single new-born females were kept in 100 ml medium containing about 1 ml of the *Daphnia* remains. When these females were 12 days old they were dissected to look for parasite infections. At this age parasite spore density was high enough to give a clear indication of the presence of the parasite and allow quantification of spore load (see below).

FACTORS INFLUENCING PARASITE GROWTH AND TRANSMISSION

Experiment 3: host size and sex

Both sexes and different host size classes were tested for susceptibility to the parasite. Single uninfected new-born males or females (0·7–1·0 mm total body length), pre-adult females (1·6–2·1 mm), large adult females (>3·5 mm) and adult males (>2 mm) were kept together with infected (uninfected in the controls) females (2·5–3·5 mm) for 3 days in 100-ml beakers. Eight replicates and two controls per group were used. After 3 days, experimental animals, which were distinguishable by body size, were isolated by placing them in fresh beakers. After 12 days all animals were dissected for parasite infections.

Experiment 4: host density

To test for density effects on transmission single new-borns (<24 h old) were placed together with single infected non-egg-bearing adult females in beakers containing 8, 25, 120, 260 or 600 ml medium. After 2 days, during which no food was given, new-borns were isolated by placing them in 25 ml beakers and dissected and examined for the presence of infections at age 12 days. Fifteen replicates were used per beaker size (total $n = 75$).

Experiment 5

To test for a density effect on the spore load found in the host gut, about 200 stock new-borns (<24 h old) were parasitized by keeping them for 1 day together with about 100 large non-egg-bearing, infected females. The freshly infected new-borns were placed in groups of five in beakers containing 8, 25, 120 (seven replicates each), 260 or 600 ml (three replicates) medium. 10^7 cells of algae were added daily to each beaker. After 12 days daphnids were dissected and SV quantified.

Experiment 6: host nutritional condition

To test if host nutritional condition has an impact on parasite spore load found in the host gut, a cohort of

60 new-born females which were infected as described in experiment 5 and then kept together in 400 ml of medium, was used. At age 12 days, 20 of these females were dissected and SV counted. The remaining females were separated into beakers with 25 ml of medium and randomly assigned to two treatment groups, fed with either 10^6 cell ml^{-1} every day or with 10^5 cells ml^{-1} every other day. After a further 8 days, the SV in these females were counted.

Experiment 7

To test if animals in poor nutritional conditions acquire the parasite more readily then well-fed animals, 80 new-born daphnids from the stock clone were placed individually in 100-ml beakers. Forty were fed with 5×10^6 cells algae ml^{-1} daily and 40 with 10^4 cells algae ml^{-1} every other day. After 5 days all 80 females were placed in fresh medium without food and kept together with one infected adult female for 36 h. The body-length of all test animals was measured and they were transferred into fresh medium. Animals were fed further with standard food ration. At day 12 the 80 animals were dissected for the presence of infections. Three well-fed females were lost due to handling error.

Experiment 8: temperature

Single infected adults were kept with three uninfected new-borns in 25-ml beakers at 6, 12, 16, 20 or 23 °C. When at least one of the three new-borns per beaker carried eggs, all three were dissected for parasite spores. This experiment was done twice. Since the results were the same, the data were pooled (two times $n = 5$ at each temperature). In this and the following experiments all animals were acclimatized to the experimental temperature conditions for at least 1 week prior to the beginning of the experiment.

Experiment 9

To quantify the combined effects of host density and temperature on parasite transmission and growth, I kept three uninfected new-borns together with one infected adult host in beakers with 25 or 120 ml medium at 12, 16, 20 or 23 °C. A 6 °C treatment was omitted, because in the earlier temperature experiment transmission at this temperature was very low. The infected hosts were 21 days old, and had become infected shortly after birth. After 5 days the infected adults were removed from the beakers. When half of the females at each temperature regime reached maturity, all of them (two beaker sizes × 3 replicates × three females per beaker; $n = 18$) were dissected and SV quantified. The mean of the three females per replicate was used in the analysis.

Experiment 10

Four new-borns (<24 h) were placed in each of 64 100-ml beakers. To each beaker, nine adult non-egg-bearing females (2·5–3·5 mm body length) were added. Zero, 1, 3 or 9 infected adults were placed together with 9, 8, 6 or 0 uninfected females, representing the controls, low, intermediate and high initial parasite dose, respectively ($n = 6$ per treatment). After 3 days, four new-borns from each beaker were placed individually in fresh medium and each (four new-borns × four treatments × 16 replicates; $n = 256$) given standard food ration daily. From each replicate one animal at each of 6, 11 and 16 days of age was dissected. The number of SV in the gut was counted, and the diameter of 10 SV each measured at a magnification of $640 \times$ using phase contrast microscopy, unless all SV had burst during preparation. The spore load of the animals dissected at age 16 days could not be counted reliably due to very high spore density. The mean SV diameter of each dissected female was used in the analysis. The parasite multiplication rate was calculated as [log (spore load day 11) − log (spore load day 6)]/(11 − 6). The fourth daphnids in each replicate were used to estimate the impact of the parasite on host fecundity. For 25 days the number of offspring produced by each of these females was recorded.

Results

SPORE UPTAKE AND SURVIVAL

The partially decomposed remains from previously infected daphnids were infectious (100%), indicating that the parasite does not die immediately with its host. The mud samples from four ponds were infectious in all replicates, indicating that this is a source of infection after a period of low host density (experiment 1; Table 1). Besides *P. intestinalis*, the fungus *Metschnikowiella biscuspidata* Metchnikoff was found to parasitize *Daphnia* in three of these replicates and the parasitic bacterium *Pasteuria ramosa* Metchnikoff was found in one replicate.

Fourteen of 24 replicates (= 58%) became infected from the decomposed remains after they had been kept for 3 months at 4 °C (experiment 2; Table 1). In these and in all the following experiments controls were not infected.

FACTORS INFLUENCING PARASITE GROWTH AND TRANSMISSION

Host size and sex

All replicates of the pre-adult and adult females and of the adult males became infected (experiment 3).

Seven of the eight male new-borns and seven of the eight female new-borns were infected. This suggests that all size and sex classes were susceptible to *P. intestinalis*.

Host density

The probability that an infected host infected an uninfected new-born decreased linearly with the logarithm of beaker size [Fig. 1; logistic regression of being infected or not on beaker size (PROC CATMOD; SAS Institute 1990) $\chi^2 = 11\cdot01$, $P = 0\cdot0009$, $n = 75$]. Beaker size also had a strong effect on the spore load found in females, which were infected before they had been placed into beakers of different sizes (Fig. 2). A linear regression of the log–log transformed data had a significant negative slope ($n = 27$, $r^2 = 0\cdot74$, $P < 0\cdot01$), indicating that females in small beakers developed higher spore loads then those in larger beakers. Since females were infected before being placed into the different beakers, higher spore loads were a consequence of multiple infections with previously expelled spores floating in the medium.

Host nutritional condition

The results of experiment 5 (Fig. 2) might have been confounded by a feeding effect, since beaker size
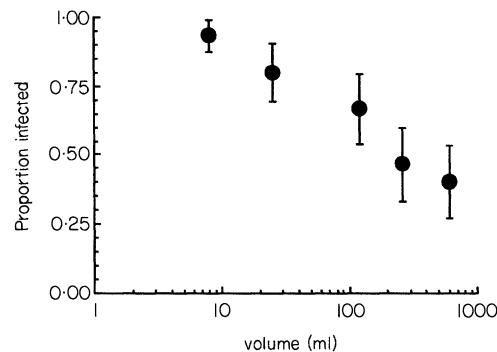
Fig. 1. Relation between beaker size and parasite transmission ($\pm$ SE). The proportion of infected hosts per beaker size is shown ($n = 15$ per beaker size). The effect of beaker size was significant ($P = 0\cdot4469$).
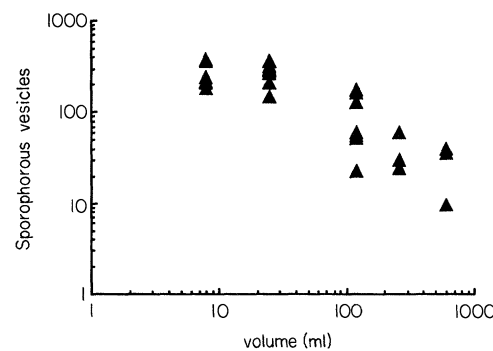
Fig. 2. The impact of beaker size on spore loads found within the host. The mean number of sporophorous vesicles of five females per replicate is shown (beaker size effect: $r^2 = 0\cdot74$, $P < 0\cdot01$).

influenced food uptake of the host. However, SV counts of females kept in either high or low food conditions were not significantly different (well-fed: 419·1, STD = 195·8; poorly fed: 328·0, STD = 107·02; $t = 1\cdot209$, $P > 0\cdot3$), indicating that the results of experiment 5 were unlikely to have been influenced by a food effect. The mean SV count before the food treatment in experiment 6 was 48·36 (STD = 28, $n = 20$).

Transmission experiments from infected to either previously well-fed or previously poorly fed 5-day-old hosts showed that 86·5% ($n = 37$) of the well-fed, but only 60% ($n = 40$) of the poorly fed, females became infected ($\chi^2 = 6\cdot8$, $P = 0\cdot009$). The well-fed hosts were larger than the poorly fed hosts at age 7 days (mean body length well-fed: 2·82 mm; poorly fed: 1·74 mm; $t = 13\cdot8$, $P < 0\cdot001$).
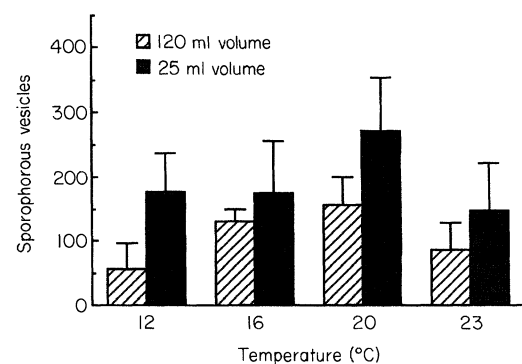
Temperature

All test animals became intensely infected (spore load >100) when exposed to infected hosts at 12, 16, 20 and 23 °C. However, at 6 °C eight replicates stayed uninfected, while two replicates showed very weak infections (only two and four SV were found in the entire gut, respectively), suggesting that the spread of the parasite is impaired at temperatures below 12 °C (experiment 8).

Daphnids infected with *P. intestinalis* at 12, 16, 20 and 23 °C showed no difference in parasite spore load by the time the first eggs were deposited into the brood pouch (Fig. 3; $F_{3,23} = 1\cdot17$, $P = 0\cdot35$; experiment 9). Higher parasite loads were found in the 25-ml beakers than in the 120-ml beakers (Fig. 3; $F_{1,23} = 4\cdot12$, $P = 0\cdot05$). The temperature by density interaction was not significant ($F_{3,23} = 0\cdot21$, $P = 0\cdot9$).

INFECTIVE DOSE, PARASITE MULTIPLICATION AND VIRULENCE

A significant reduction of host fecundity with increasing parasite spore dose was observed (Fig. 4; $F_{3,63} = 25\cdot39$, $P < 0\cdot0001$). The spore load increased

Fig. 3. Number of sporophorous vesicles ($\pm$ SE) of *P. intestinalis* found in female *D. magna* kept in 120- (hatched bars) and 25-ml medium (dark bars) at four temperatures.
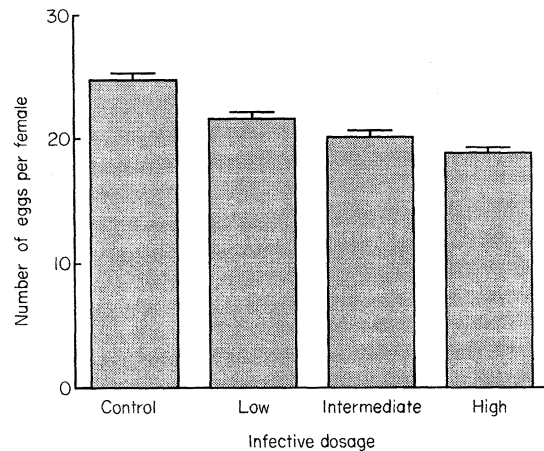
Fig. 4. The effect of three different initial parasite doses on host fecundity. The number of viable eggs produced during the first four clutches is shown. The reduction in fecundity is significant ($P < 0.4461$).
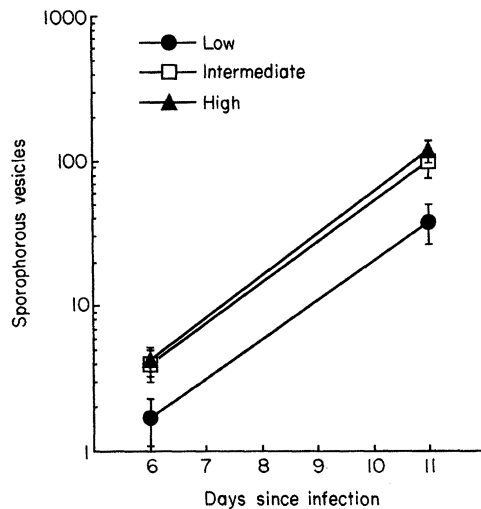

Fig. 5. The increase in spore load (= number of sporophorous vesicles) from day 6 to day 11 in three parasite dose treatments. Note spore load is $\log_{10}$ transformed.

from day 6 to 11 and reflected the initial infective dose (Fig. 5; $F_{day} = 149.02$, $P < 0.0001$, $F_{dose} = 3.45$, $P < 0.03$, $F_{dose \times day} = 0.32$, $P = 0.7$), although the difference between intermediate and high dose was small. The parasite multiplication rate (the slope in Fig. 5) did not increase with treatments ($F_{2,47} = 0.58$, $P = 0.5$). Size of sporophorous vesicles did not differ between doses ($F_{treatment} = 2.04$, $P = 0.13$, $F_{day} = 0.03$, $P = 0.9$, $F_{treat \times day} 2.04$, $P = 0.13$).

Discussion

PARASITE PERSISTENCE AND SPREAD

This study intended to clarify the ecological factors that are important for uptake, spread and persistence of the microsporidian parasite *P. intestinalis* in its planktonic host *D. magna*. Three factors emerged explaining the persistence of this microparasite: (i)

spores are able to survive outside the host; (ii) density dependent transmission; and (iii) impaired transmission at low temperatures. These findings are consistent with reports from many other horizontally transmitted microsporidians and with field studies on planktonic cladocerans and rotifers (for references see below).

The demonstrated infectiousness of mud samples collected in natural ponds suggests a mechanism by which an epidemic starts after the parasite has temporarily disappeared from a host population. The uptake of two other microparasites from these mud samples (*Metschnikowiella biscuspidata* and *Pasteuria ramosa*) suggests that pond sediments might act as spore banks and are important for the start of epidemics. The uptake of substrate particles by *Daphnia* has been described in connection with low densities of planktonic food (Horton *et al.* 1979; Freyer 1991), and was first suggested by Green (1974) as the mechanism by which microparasites enter host populations. This behaviourial switch in mode of feeding appears to be an essential component for the start of an epidemic. Although host nutritional condition itself seems to play no role in spread and growth of the parasite (experiments 6 and 7), its impact on the mode of host feeding makes it an important epidemiological factor.

The observation that *D. magna* and *D. pulex* have the richest parasite fauna of all *Daphnia* species (Green 1974; Larsson 1981; Bendt 1988) might be related to the tendency of these species to switch more easily to browsing near the bottom of the pond than smaller *Daphnia* species (Horton *et al.* 1979; Lampert 1987). Freyer (1991) speculated that browsing behaviour in *Daphnia* is phyletically primitive. Its costs in terms of parasitism might be the reason why some *Daphnia* species evolved to not browse on pond sediments anymore.

After the parasite had entered the host population, transmission probability increased with decreasing distance to the nearest infected neighbour (Fig. 1). The lack of effects of poor host nutritional conditions indicated that parasite spread during crowding conditions was mainly a density effect, rather than a feeding condition effect. Yan & Larsson (1988) came to the same conclusion by comparing microsporidian prevalence and food stress across two field seasons. In this study, previously well-fed hosts became infected more often than starved hosts (experiment 7). This is likely to be a feeding rate effect, since the well-fed animals were larger and had therefore a higher filtration rate (Lampert 1987) and an increased encounter rate with free floating spores (Vidtmann 1993). This would also concur with the observation that microsporidian infected-cladocerans in natural populations are often larger than uninfected hosts (Brambilla 1983; Yan & Larsson 1988; Vidtmann 1993).

Density dependence of transmission was favoured by Brambilla (1983), Yan & Larsson (1988) and Vidt-

mann (1993) as the most likely explanation of the seasonal occurrence of microsporidiosis in cladoceran populations. Since *P. intestinalis* remains infectious over a temperature range of at least 12–23 °C (Fig. 3), changes in temperature appear to play a minor role for parasite spread for most of the year. Miracle (1977) and Ruttner-Kolisko (1977) speculated that autumn declines in microsporidiosis in rotifer populations were caused by low temperatures. Microsporidians are generally known for their pronounced temperature sensitivity (Hurpin 1968; Fowler & Reeves 1975; Becnel & Undeen 1992), which was confirmed by the present results. The decline of microsporidian prevalence in the winter, observed in all reported zooplankton studies, might therefore be a result of temperature decline, rather than a consequence of the simultaneous decline in host density. However, since temperature decline and density decline in autumn are not strictly related to each other, one or the other factor might be more important for the decline of the parasite in any particular year.

Reduced feeding rates at low temperatures might also contribute to the autumn decline of parasite prevalence. However, during experiment 8 (Table 1) infected and uninfected animals were kept together until at least one of the uninfected females produced eggs. Since time until maturity was inversely related to temperature, lower feeding rates at low temperatures were at least partially compensated for by the longer time period of contact between infected and uninfected hosts. Impaired transmission at lower temperatures was also found for a bacterial endoparasite (*Pasteuria ramosa*) in the cladoceran *Moina rectirostris* (Sayre *et al.* 1979). However, the minimum temperature necessary for the development of this disease was 26 °C, and therefore is unlikely to be related to a reduced feeding rate.

The seasonal cycle of microsporidiosis in the cladoceran populations ends with spore deposition at the bottom of the pond in autumn and winter. After three months at 4 °C many spores were still infective, suggesting that spores are able to persist through periods when host density is low or when hosts diapause. Microsporidian spores have often been reported to have long durability outside their hosts (Hurpin 1968; Burges, Canning & Hurst 1971; Milner 1972; Henry & Oma 1974; Fuxa & Brooks 1979), presumably to survive periods of low host density (Anderson & May 1981).

Differential susceptibility between size classes or sexes of the host appears not to play a significant role in the spread of *P. intestinalis*, a finding which agrees with field observations of microsporidiosis in other cladocerans (Brambilla 1983; Yan & Larsson 1988; Vidtmann 1993). In contrast to cladocerans, resistance to microsporidian parasites with respect to life stage or sex was reported for sand shrimps and copepods (Breed & Olsen 1977; Andreadis 1988). This difference will certainly be reflected in the epidemiology of

microsporidiosis in these host groups. However, not enough is know to draw conclusions here.

Transmission probability of waterborne parasites might be expected to correlate directly and negatively with the dilution factor (mass action assumption; Anderson & May 1991). However, density dependence of transmission of *P. intestinalis* did not follow a simple dilution principle. Transmission decreased with the logarithm of volume (Fig. 1), indicating better transmission in large beakers than a dilution model would predict. It is likely that transmission probability increased due to local aggregation of hosts. The shorter the mean distance between an infected and an uninfected host, the more likely it is that transmission will occur (Blower & Roughgarden 1989). Swarming behaviour of *Daphnia* (Young 1978; Freyer 1991) could cause such an effect. A parasitized host might even be manipulated by the parasite to stay closer to other hosts in order to facilitate transmission (Moore & Gotelli 1990).

PARASITE GROWTH INSIDE THE HOST

The number of spores found in the host increased with time after infection and with the initial spore dose (Fig. 5). As a consequence, under conditions of high parasite prevalence and high host density, spore counts will increase as a consequence of both parasite multiplication and the accumulation of new infections. Hosts kept in small beakers had indeed larger spore counts then hosts kept in larger beakers (Fig. 2). The increase in repeated infections with host density allowed the parasite population to increase after its prevalence approached 100%. This pattern is more typical for macroparasites than it is for microparasites and needs to be considered in the epidemiology of this parasite (Anderson & May 1991). Multiplication rate and the size of the sporophorous vesicles were independent from the initial spore dose. This indicates that host exploitation per multiplying spore does not accelerate with spore density, which might be expected when parasites compete with each other for resources.

DOES *P. INTESTINALIS* REGULATE *DAPHNIA* POPULATIONS?

McCauley and Murdoch (Murdoch & McCauley 1985; McCauley & Murdoch 1987; McCauley 1993) concluded that *Daphnia* population dynamics appear to be internally driven rather than resulting from external forcing factors. Microparasitic diseases could provide such an internal driving force. Density-dependent transmission and disease-induced reduction in host fecundity suggest that *P. intestinalis* might be able to regulate host density (Anderson & May 1978; Scott & Dobson 1989). Decreasing host fecundity with increasing parasite intensity (Fig. 4) and parasite-induced host mortality (Ebert 1994b) could enhance this regulatory effect. Regulation of

plankton populations by parasites or epibionts has been suggested for phytoplankton (Canter & Lund 1951; Bruning *et al.* 1992) and zooplankton (Green 1974; Redfield & Vincent 1979; Xu & Burns 1990; Allen, De Stasio & Ramcharan 1993). In contrast, Brambilla (1983) speculated that although the microsporidium *Thelohania* sp. reduces population growth of *D. pulex*, it is unlikely that it regulates the growth of its host population. He argues that the strong impact of food level on fecundity and thus on population growth rate is likely to be the single most important factor in determining zooplankton dynamics. Laboratory studies in controlled microcosms might help to disentangle the effects of different density-limiting and density-regulating (*sensu* Sinclair 1989) factors. However, even if it turns out that parasites are not able to regulate plankton density, they are strong candidates in altering the outcome of species and clonal competition or promoting coexistence (Canter & Lund 1951; Holt & Pickering 1985).

Acknowledgements

References

Allen, Y.C., De Stasio, B.T. & Ramcharan, C.W. (1993) Individual and population level consequences of an algal epibiont on *Daphnia*. *Limnology and Oceanography*, **38**, 592–601.

Anderson, R.M. & May, R.M. (1978) Regulation and stability of host–parasite population interactions. I. Regulatory processes. *Journal of Animal Ecology*, **47**, 219–247.

Anderson, R.M. & May, R.M. (1979) Population biology of infectious diseases. Part I. *Nature*, **280**, 361–367.

Anderson, R.M. & May, R.M. (1981). The population dynamics of microparasites and their invertebrate hosts. *Philosophical Transactions of the Royal Society, B*, **291**, 451–524.

Anderson, R.M. & May R.M. (1986) The invasion, persistence and spread of infectious disease within animal and plant communities. *Philosophical Transactions of the Royal Society, B*, **314**, 533–570.

Anderson, R.M. & May, R.M. (1991) *Infectious Diseases of Humans*. Oxford, Oxford University Press.

Andreadis, T.G. (1988) Comparative susceptibility of the copepod *Acanthocyclops vernalis* to a microsporidian parasite, *Amblyospora connecticus*, from mosquito *Aedes cantator*. *Journal of Invertebrate Pathology*, **52**, 73–77.

Becnel, J.J. & Undeen, A.H. (1992) Influence of temperature on developmental parameters of the parasite host system *Edhazardia aedis* (Microsporida; Amblyosporidae) and *Aedes aegypti* (Diptera; Culicidae). *Journal of Invertebrate Pathology*, **60**, 299–303.

Beddington, J.R., Free, C.A. & Lawton, J.H. (1978) Model-
ling biological control: on the characteristics of successful natural enemies. *Nature*, **273**, 513–519.

Bendt, T. (1988) Parasiten und Epibionten der Blattfusskrebse II. Befall durch ein- und mehrzellige Tiere. *Mikrokosmos*, **77**, 83–91.

Blower, S.M. & Roughgarden, J. (1989) Parasites detect host spatial pattern and density: a field experimental analysis. *Oecologia*, **78**, 138–141.

Brambilla, D.J. (1983) Microsporidiosis in a *Daphnia pulex* population. *Hydrobiologia*, **99**, 175–188.

Breed, G.M. & Olsen, R.E. (1977) Biology of the microsporidian parasite *Pleistophora crangoni* n.sp. in three species of crangonid sand shrimps. *Journal of Invertebrate Pathology*, **30**, 387–405.

Bruning, K., Lingeman, R. & Ringelberg, J. (1992) Estimating the impact of fungal parasites on phytoplankton populations. *Limnology and Oceanography*, **37**, 252–260.

Burges, H.D., Canning, E.U. & Hurst, J.A. (1971) Morphology, development and pathogenicity of *Nosema oryzaephili* n. sp. in *Oryzaephilus surinamensis* and its host range among granivorous insects. *Journal of Invertebrate Pathology*, **17**, 419–432.

Canter, H.M. & Lund, J.W.G. (1951) Studies on plankton parasites. III. Examples of the interaction between parasitism and other factors determining the growth of diatoms. *Annals in Botany*, **15**, 359–372.

Chiavelli, D.A., Mills, E.L. & Threlkeld, S.T. (1993) Host preference, seasonality, and community interactions of zooplankton epibionts. *Limnology and Oceanography*, **38**, 574–583.

Ebert, D. (1994a) Genetic differences in the interactions of a microsporidian parasite and four clones of its cyclically parthenogenetic host. *Parasitology*, **108**, 11–16.

Ebert, D. (1994b) Virulence and local adaptation of a horizontally transmitted parasite. *Science*, **265**, 1084–1086.

Fowler, J.L. & Reeves, E.L. (1975) *In vivo* propagation of a microsporidian pathogenic to insects. *Journal of Invertebrate Pathology*, **25**, 349–353.

France, R.L. & Graham, L. (1985) Increased microsporidian parasitism of the crayfish *Orconectes virilis* in an experimentally acidified lake. *Water Air Soil Pollution*, **26**, 129–136.

Freyer, G. (1991) Functional morphology and the adaptive radiation of the Daphniidae (Branchiopoda: Anomopoda). *Philosophical Transactions of the Royal Society, B*, **331**, 1–99.

Fuxa, J.R. & Brooks, W.M. (1979) Mass production and storage of *Vairimorpha necatrix* (Protozoa: Microsporida). *Journal of Invertebrate Pathology*, **33**, 86–94.

Green, J. (1974) Parasites and epibionts of Cladocera. *Transactions of the Zoological Society of London*, **32**, 417–515.

Hassell, M.P. & May, R.M. (1973) Stability in insect host–parasite models. *Journal of Animal Ecology*, **42**, 693–726.

Henry, J.E. & Oma, E.A. (1974) Effect of prolonged storage of spores on field applications of *Nosema locustae* (Microsporida: Nosematidae) against grasshoppers. *Journal of Invertebrate Pathology*, **23**, 371–377.

Holt, R.D. & Pickering, J. (1985) Infectious diseases and species coexistence: a model in Lotka–Volterra form. *American Naturalist*, **126**, 196–221.

Horton, P.A., Rowan, M., Webster, K.E. & Peters, R.H. (1979) Browsing and grazing by cladoceran filter feeders. *Canadian Journal of Zoology*, **57**, 206–212.

Hurpin, B. (1968) The influence of temperature and larval stage on certain diseases of *Melolontha melolontha*. *Journal of Invertebrate Pathology*, **10**, 252–262.

Klüttgen, B., Dülmer, U., Engels, M. & Ratte, H.T. (1994) ADaM, an artificial freshwater for the culture of zooplankton. *Water Research*, **28**, 743–746.

Lampert, W. (1987) Feeding and nutrition in *Daphnia*.

Memorie Dell'Istituto Italiano di Idrobiologia Dott.Marco De Marchi, **45**, 143–192.

Larsson, R. (1981) A new microsporidium *Berwaldia singularis* gen. et sp. nov. from *Daphnia pulex* and a survey of microsporidia described from Cladocera. *Parasitology*, **83**, 325–342.

McCauley, E. (1993) Internal versus external causes of dynamics in a freshwater plant–herbivore system. *American Naturalist*, **141**, 428–439.

McCauley, E. & Murdoch, W.W. (1987) Cyclic and stable populations: plankton as paradigm. *American Naturalist*, **129**, 97–121.

Milner, R.J. (1972) The survival of *Nosema whiteri* spores stored at 4 °C. *Journal of Invertebrate Pathology*, **20**, 356–357.

Miracle, M.R. (1977) Epidemiology in rotifers. *Archiv für Hydrobiologie, Beiheft Ergebnisse der Limnologie*, **8**, 138–141.

Moore, J. & Gotelli, N.J. (1990) A phylogenetic perspective on the evolution of altered host behaviour: a critical look at the manipulation hypothesis. *Parasitism and Host Behaviour* (eds C.J. Barnard & J.M. Behnke), pp. 193–216. Taylor & Francis, London.

Murdoch, W.W. & McCauley, E. (1985) Three distinct types of dynamic behaviour shown by a single planktonic system. *Nature*, **316**, 628–630.

Onstad, D.W., Maddox, J.V., Cox, D.J. & Kornkven, E.A. (1990) Spatial and temporal dynamics of animals and the host-density threshold in epizootiology. *Journal of Invertebrate Pathology*, **55**, 76–84.

Redfield, G.W. & Vincent, W.F. (1979) Stages of infection and ecological effects of a fungal epidemic on the eggs of a limnetic copepod. *Freshwater Biology*, **9**, 503–510.

Ruttner-Kolisko, A. (1977) The effect of the microsporid *Plistophora asperospora* on *Conochilus unicornis* in Lunzer Untersee (LUS). *Archiv für Hydrobiologie, Beihefte Ergebnisse Limnologie*, **8**, 135–137.

SAS Institute (1990) *SAS/STAT, Version 6·06. SAS Institute Inc.*, cary, NC.

Sayre, R.M., Adams, J.R. & Wergin, W.P. (1979) Bacterial parasite of a cladoceran: morphology, development *in vivo* and taxonomic relationship with *Pasteuria ramosa* Metchnikoff 1988. *International Journal of Systematic Bacteriology*, **29**, 252–262.

Scott, M.E. & Dobson, A. (1989) The role of parasites in regulating host abundance. *Parasitology Today*, **5**, 176–183.

Seymour, R., Cowgill, U.M., Klecka, G.M., Gersich, F.M. & Mayes, M.A. (1984) Occurence of *Aphanomyces daphniae* infection in laboratory cultures of *Daphnia magna*. *Journal of Invertebrate Pathology*, **43**, 109–113.

Sinclair, A.R.E. (1989) Population regulation in animals. *Ecological Concepts* (ed. J.M. Cherrett), pp. 197–242. Blackwell Scientific Publications, Oxford.

Vidtmann, S. (1993) The peculiarities of prevalence of microsporidium *Larssonia daphniae* in the natural *Daphnia pulex* population. *Ekologija*, **1**, 61–69.

Wetzel, R.G. (1975) *Limnology*. Saunders College Publishing, Philadelphia.

Xu, Z. & Burns C.W. (1990) Effects of the epizotic ciliate, *Epistylis daphniae*, on growth reproduction and mortality of *Boeckella triarticulata* (Thomson) (Copepoda: Calanoida). *Hydrobiologia*, **209**, 183–189.

Yan, N.D. & Larsson, J.I.R. (1988) Prevalence and inferred effects of microsporidia of *Holopedium gibberum* (Crustacea: Cladocera) in a Canadian shield lake. *Journal of Plankton Research*, **10**, 875–886.

Young, J.P.W. (1978) Sexual swarms in *Daphnia magna*, a cyclic parthenogen. *Freshwater Ecology*, **8**, 279–281.