Ecological implications of parasites in natural *Daphnia* populations

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Abstract In natural host populations, parasitism is considered to be omnipresent and to play an important role in shaping host life history and population dynamics. Here, we study parasitism in natural populations of the zooplankton host *Daphnia magna* investigating their individual and population level effects during a 2-year field study. Our results revealed a rich and highly prevalent community of parasites, with eight endoparasite species (four microsporidia, one amoeba, two bacteria and one nematode) and six epibionts (belonging to five different taxa: Chlorophyta, Bacillariophyceae, Ciliata, Fungi and Rotifera). Several of the endoparasites were associated with a severe overall fecundity reduction of the hosts, while such effects were not seen for epibionts. In particular, infections by *Pasteuria ramosa*, White Fat Cell Disease and *Flabelliforma magnivora* were strongly associated with a reduction in overall *D. magna* fecundity. Across the sampling period, average population fecundity of *D. magna* was negatively associated with overall infection intensity and total endoparasite richness. Population density of *D. magna* was negatively correlated to overall endoparasite prevalence and positively correlated with epibiont richness. Finally, the reduction in host fecundity caused by different parasite species was negatively correlated to both parasite prevalence and the length of the time period during which the parasite persisted in the host population. Consistent with epidemiological models, these results indicate that parasite mediated host damages influence the population dynamics of both hosts and parasites.

Keywords *Daphnia magna* · Epibionts · Endoparasites · Fecundity reduction · Population effects

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Introduction

In natural host populations, parasitism is considered to be omnipresent (McCallum and Dobson 1995). Moreover, the specificity of host–parasite interactions, their density-dependent transmission and the negative influence of parasites on the reproduction and survival of the host imply that parasites may influence the population dynamics of their host (Sheldon and Verhulst 1996; Ebert et al. 1997; De Leo and Dobson 2002). In the simplest scenario, parasite-induced reduction in host survival and fecundity is expected to result in a decreased host population growth and density (Dobson and Crawly 1994). This has been suggested by several theoretical studies (Anderson and May 1979; McCallum and Dobson 1995; Tompkins et al. 2002), but has rarely been shown in natural host populations (Gulland 1995; Hudson et al. 1998; Hochachka and Dhondt 2000). Finding evidence for such negative relationships in field populations may be hampered by the fact that most natural host populations are infected by multiple parasite species (Thompson 1994) and that other environmental factors influence the host population as well, creating a complex picture of host population dynamics. Furthermore, the effect of parasites in natural populations is difficult to assess due to the lack of proper controls and practical complexities (Tompkins and Begon 1999). Nevertheless, empirical studies investigating the relationship between the individual level and population level effects of natural infections are needed, particularly to understand how to manage virulence in wildlife populations (De Leo and Dobson 2002).

The more virulent a parasite, the more negative will be its effect on host fecundity and/or survival. Consequently, virulent parasites have a stronger potential to suppress host population growth and to drive host populations to extinction than less virulent parasites (Anderson and May 1979; McCallum and Dobson 1995; Tompkins et al. 2002; Boots and Sasaki 2002). Moreover, parasites with a strong effect on host survival and/or fecundity are expected to be less often observed, because the infected hosts disappear quickly from the population (short infection times, Ebert et al. 2000a). Therefore, everything else being equal, the prevalence of virulent parasites is expected to be lower than those of harmless parasites (Anderson 1982; McCallum and Dobson 1995; De Leo and Dobson 2002; Gandon et al. 2002). Virulent parasites are also expected to show more dynamic changes in prevalence (Ebert et al. 2000a). In contrast, avirulent infections typically reach higher and more stable prevalences and are expected to persist for longer time spans (Anderson and May 1979).

Parasites show strong variation in their effect on hosts, offering the possibility to judge the effects of parasites on their hosts by comparing species with different levels of virulence. One such difference is found between endo- and ectoparasites (= epibionts). Micro- and endoparasites are small, unicellular parasites that complete their life cycle within the body of the host and that exploit the host tissues in a direct and often destructive way (Anderson and May 1979; Clayton and Moore 1997). Many micro-parasites have been shown to reduce fecundity, growth and survival of their hosts (Anderson and May 1991; Dobson and Grenfell 1995). In contrast, ectoparasites or epibionts are located on the body surface of their host. They may feed directly on the host or use the host only as substrate. In general, they are expected to have a smaller negative virulence effect on their hosts than endoparasites (Clayton and Moore 1997). On the other hand, ectoparasites may be more easily transmitted among hosts and thus can spread more rapidly (Ebert et al. 2001). Thus one would expect that epibionts reach higher prevalences than endoparasites, but are less able to influence their host population dynamics.

Fluctuations in population density of zooplankton organisms have traditionally been ascribed to predation, food limitation, or abiotic conditions (Sommer et al. 1989). However, for large zooplankton, like members of the genus Daphnia, there is increasing evidence that parasites are common and that they have the potential to influence their host population (Bengtsson and Ebert 1998; Green 1974; Ebert et al. 1997; Stirnadel and Ebert 1997; Ebert et al. 2000a, 2001; Bareo-Arco et al. 2001; Bittner et al. 2002). Laboratory studies have shown that endoparasites cause severe virulence effects in the host (Ebert et al. 2000a; Haag et al. 2003; Decaestecker et al. 2003) and that epibionts may reduce zooplankton fitness as well (Allen et al. 1993; Willey et al. 1990; Threlkeld and Willey 1993). A comparative analysis of the individual and population level effects of different parasites on their shared natural zooplankton host populations has so far not been explored. Neither did any field study rapport the relationship between virulence and either prevalence or the duration of the time period during which different zooplankton parasites are observed in their host population.

Here, we explored the potential effects of parasites on natural populations of *D. magna*. During a period of 2 years, we determined prevalence, infection intensity and species richness of parasites in two *D. magna* pond populations, as well as some key population characteristics of the host. This study has three aims: (1) We wanted to test whether, in natural host populations, fecundity and population density of the *D. magna* host are negatively associated with parasites, and (2) whether such negative associations are more pronounced in endoparasites than in epibionts. (3) Finally, we aimed to test whether the overall fecundity reduction caused by different parasites is related to population level aspects such as prevalence and persistence time in the host population.

Materials and methods

Study sites and sampling

We studied the parasites of the aquatic crustacean *D. magna* in two adjacent, shallow and eutrophic ponds (OM2 and OM3, Heverlee, Belgium). The ponds have a surface area of approximately 2.5 ha and are on average 1–2 m deep. In the past, these man made ponds functioned as fish culture units for carp. At present, a planktivorous fish community is still present: mainly carp—*Cyprinus carpio*, Prussian carp—*Carassius gibelio*, perch—*Perca fluviatilis*, and tench—*Tinca tinca*.

From April to December in both 1999 and 2000, we collected zooplankton samples at weekly to two-weekly intervals with the interval length being inversely related to water temperature. To determine the presence of parasites, we took samples with a 200-μm plankton net in the littoral zone of the ponds. Samples were kept at 4°C until analysis (maximum 5 days, in most cases within 36 h). To determine density of the zooplankton populations, we took quantitative samples in each pond. At three different locations in the vicinity of a fixed sample station, we took five samples of 1 l volume. To exclude the influence of genotype-dependent depth selection (De Meester et al. 1994), these samples covered the whole water column. To avoid contamination among ponds, we used different sampling equipment for each pond. Equipment was sterilized between sampling dates. Chlorophyll-a concentration was assessed from a 250 ml sample (methanol extraction) according to the protocol of Talling and Driver (1963).

Parasite and epibiont richness, infection intensity and prevalence

We investigated the live samples for the presence of parasites. In the entire study, a total of 2158 *D. magna* individuals were examined. From each sample, approximately 50 adult females were screened (adulthood of the females was assessed on the size of the abdominal processi, Edmondson and Litt 1982). We only determined the parasites in adult females, as parasites in juveniles are difficult to detect (Brambilla 1983; Stirnadel and Ebert 1997). We did not include males, since they only occur sporadically in the studied populations. Males might have been infected with parasites that are difficult to detect in females, such as parasites that are vertically transmitted through females but horizontally through males (Hurst 1993). The exclusion of males and juveniles in our analyses might thus have affected our prevalence and richness estimates. Yet, it is likely that this effect is minor, given the overall low abundance of males and the fact that transmission of spores is probably largely mediated by heavily infected, large females.

Signs of infection were first microscopically investigated with both light from the top and light from below. First, we focused on infections of the epidermal tissues, the ovaries and the haemocoel. Most types of infections were clearly visible as an alteration of color and/or transparency of the host. Body length (top of the head to the base of the tail spine) was measured and eggs/embryos in the clutch chamber counted. Secondly, all individuals were dissected to examine the gut and tissue contents for the presence of endoparasite spores under 400–1000 magnification using a phase-contrast microscope. We used Green (1974) and Stirnadel and Ebert (1997) to identify the parasites. Two microsporidia species could not be identified to species or genus level and were determined as Microsporidium 1 and 2. Both of these are most likely species new to science.

We determined several parasite variables. On each sampling date, 'prevalence' of a certain parasite species was calculated as the percentage infected adult females. 'Overall prevalence' of a sample is the average of the prevalence of all parasite species. 'Infection intensity' of the epibionts *Colacium* and *Protoderma* and the endoparasites Microsporidium 2 and White Fat Cell Disease were estimated as the percentage of host carapax or tissue infected. For all other species, the number of individuals or spores was estimated to determine infection intensity. 'Average infection intensity' of a sample is the intensity of a given parasite averaged over all host individuals in a sample (including NONinfected hosts). Because parasite species differ in the way they infect their host, we calculated a combined measure of infection intensity (= 'Overall infection intensity') of a sample as the sum of the centered and standardized intensities of all parasite species. Richness was determined as the number of parasite species in a *Daphnia* individual. 'Total richness' is the total number of parasite species observed in the sample.

Parasite induced overall fecundity reduction

The effect of parasites on host fecundity can be easily studied, because *Daphnia* carry their eggs for several days in a special brood pouch before the offspring are released. Therefore, for each sample, parasite induced overall fecundity reduction could be assessed as the difference between fecundity of infected to uninfected adult females. We included only those samples in which both females infected with the particular investigated parasite and NONinfected females were present. For each parasite, the statistical significance of the overall fecundity reduction was assessed by testing for differences between infected and NONinfected females in a sample with Wilcoxon Matched pairs-tests across all samples. When assessing the overall fecundity reduction of epibionts, we excluded endo-parasitized females from the data set. The effect of the epibiont *B. rubens* on reproductive characters could not be tested due to the low number of samples including *B. rubens* infections.

Data analysis

Daphnia magna population fecundity and density were analyzed with multiple regression analysis. *Daphnia magna* population fecundity was calculated for each sample as the mean clutch size, including zero-values for adults without eggs. We explored for associations of *D. magna* population density with temperature, Chlorophyll-a, and the different measures of prevalence, infection intensity and richness (treated as independent variables). In a multiple regression analysis with *D. magna* population fecundity as the dependent variable, the same independent variables were used, but Chlorophyll-a and average adult body size were specified as covariables. Year and pond identity were in all tests included as factors to account for interdependence of data within year and pond combinations. Time (day number in the year) was also specified as covariable, as suggested by ter Braak and Smilauer (1998).

Multiple regression analyses were performed in CANOCO 4.5 and statistical tests were based on Monte-Carlo permutations (999 random permutations; ter Braak and Smilauer 1998). Prior to each multiple regression analysis, explanatory variables were subjected to the manual forward selection procedure provided by CANOCO. Only variables that contributed significantly to the variation in the dependent variable were retained in the model. Permutations were restricted for time-series and performed within blocks of pond/year combinations (ter Braak and Smilauer 1998).

Results

In total, eight endoparasite species (four microsporidia, one amoeba, two bacteria and one nematode) and six epibionts (belonging to five different taxa: Chlorophyta,

Table 1 Characteristics and average prevalence of parasites across all samples

	Size of infective stages (μm)	Site of infection	Transmission mode	Average prevalence
Endoparasites				
Microsporidia				
Microsporidium 1	1.1–1.7×2.4–2.7	Epithelial cells gut	Horizontal	58.13%
Ordospora colligata	1.3–2.3×2.3–3.7	Epithelial cells gut	Horizontal	39.21%
Flabelliforma magnivora	5.5–6.0×2.8–3.0	Ovaries and fat cells	Vertical	0.61%
Microsporidium 2	3.0–3.5×1.5–1.8	Epidermal cells	Horizontal	36.08%
Amoeba				
Pansporella perplexa	<90	Gut	Horizontal	36.92%
Bacteria				
'White Fat Cell Disease'	<1	Fat cells	Horizontal	4.47%
Pasteuria ramosa	5–6	Hemacoel	Horizontal	5.70%
Nematoda				
Echinura unicata		Body cavity	Horizontal via vertebrate host	<0.5%
Epibionts				
Chlorophyta				
Colacium + *Protoderma* sp.	16–19×20–30	Carapax and filter apparatus	Horizontal	78.62%
Korshikoviella sp.			Horizontal	34.32%
Bacillariophyceae		Carapax	Horizontal	13.56%
Ciliata				
Vorticella sp.	20–25×70–150	Carapax/filter apparatus	Horizontal	49.43%
Fungi				
Amoebidium parasiticum	6–8×500	Carapax/filter apparatus	Horizontal	40.21%
Rotifera				
Brachionus rubens		Carapax	Horizontal	2.93%

Bacillariophyceae, Ciliata, Fungi and Rotifera) were recorded (Table 1). More than 50% of the host females were at least double infected (Fig. 1, Table 1).

Average prevalence (averaged across all samples) of different parasite species differed strongly, but was similar across years and ponds. Prevalences of the parasite species in 1999 were positively correlated with those in 2000 (Fig. 2; endoparasites in OM2: Pearson $r=0.91$, $P=0.004$, in OM3: Pearson $r=0.78$, $P=0.04$; epibionts in OM2: Pearson $r=0.91$, $P=0.01$, in OM3: Pearson $r=0.98$, $P<0.001$). There was a trend that most parasite prevalences were higher in OM2 than in OM3 (Fig. 2), but the rank order of parasite prevalences in the two ponds corresponded well with each other (endoparasites in 1999: Pearson $r=0.91$, $P=0.002$, in 2000: Pearson $r=0.53$, $P>0.05$; epibionts in 1999: Pearson $r=0.91$, $P<0.05$, in 2000: Pearson $r=0.9$, $P=0.041$). Thus, parasite prevalences were rather consistent across years and ponds, indicating that species identity is an important factor in determining prevalence.

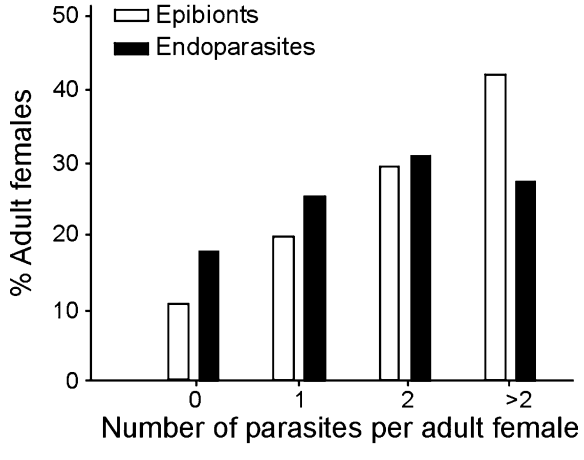

Fig. 1 Percentage of adult females categorized into NONinfected (0) and multiply infected (2 and >2) hosts. The graph represents the averages over the two ponds and the entire study period (2 years)

Pasteuria ramosa, White Fat Cell Disease, *Flabelliforma magnivora* and *Ordospora colligata* induced significant overall fecundity reductions in *D. magna* (Fig. 3). Microsporidium 2 and the Bacillariophyceae had a weakly significant negative effect on overall *D. magna* fecundity, but significance was lost after correction for multiple tests.

The results of the multiple regression analysis show that the population fecundity of *D. magna* was negatively associated with the overall infection intensity of the endoparasites (Fig. 4a, Table 2) as well as with total endoparasite richness (Fig. 4b, Table 2). None of the epibiont related variables contributed significantly to the multiple regression analysis of *D. magna* population fecundity (Table 2).

Daphnia density was found to be negatively related with overall endoparasite prevalence (Fig. 5a, Table 2).

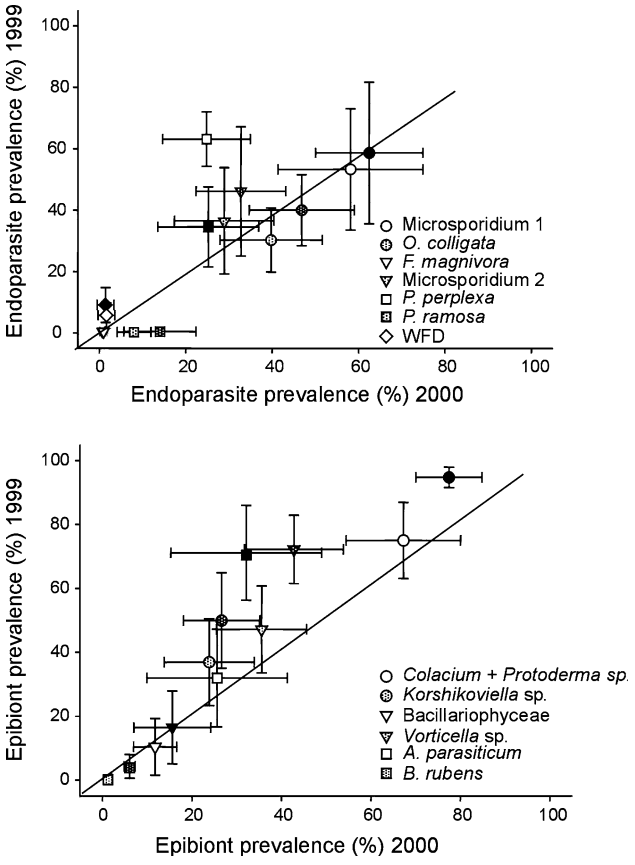

Fig. 2 Average (per sample) prevalence of endoparasites and epibionts in OM2 (*black symbols*) and OM3 (*empty symbols*) in 1999 and 2000. *Error bars* are twice the standard error. Line is line with slope 1. 'WFD' corresponds with White Fat Cell Disease

In contrast, epibiont richness (average per sample) was significantly positively correlated with *D. magna* population density (Fig. 5b, Table 2). The same correlations using time lags did not produce significant results (analysis not shown).

Overall *D. magna* fecundity reduction was significantly negatively associated with both average prevalence across all samples (with at least one infected individual) and the time period during which the parasites were observed in the host population (Fig. 6, Spearman Rank correlation between overall fecundity reduction and average prevalence: $r = -0.6$, $P = 0.03$; and average persistence time: $r = -0.76$, $P = 0.002$).

Discussion

During our 2-year study period, we found a rich and highly prevalent community of parasites in the two *D. magna* populations. We show for the first time high parasite prevalence in zooplankton populations inhabiting habitats with fish. Earlier analyses reporting high parasite prevalences involved fishless habitats (Brambilla 1983; Vidtman 1993; Stirnadel and Ebert 1997), which is in agreement with the expectation that levels of parasitism in *Daphnia* populations with vertebrate predation are lower than without (Ebert et al. 1997). This hypothesis is based on the finding that pathogens are rarely distributed randomly in their host populations, but aggregate in certain hosts (Hudson and Dobson 1995) and that the ability of a parasite to spread and persist in a host population depends on predators (Hall et al. 2005; Packer et al. 2003). In *Daphnia*, there is a higher uptake rate of infectious stages by large daphnids (due to their higher filtration rate) and an accumulation of parasite spores with age (Mangin et al. 1995; Stirnadel and Ebert 1997). Further, large zooplankton is preferred as prey by fish (Brooks and Dodson 1965). Furthermore, parasite infections have been observed to make *Daphnia* more conspicuous and make them a preferred prey for visually hunting predators (Threlkeld et al. 1993; Fels et al. 2004). Therefore, it is expected that size selective fish predation has the potential to constrain the occurrence and persistence of parasites in *Daphnia* popula-

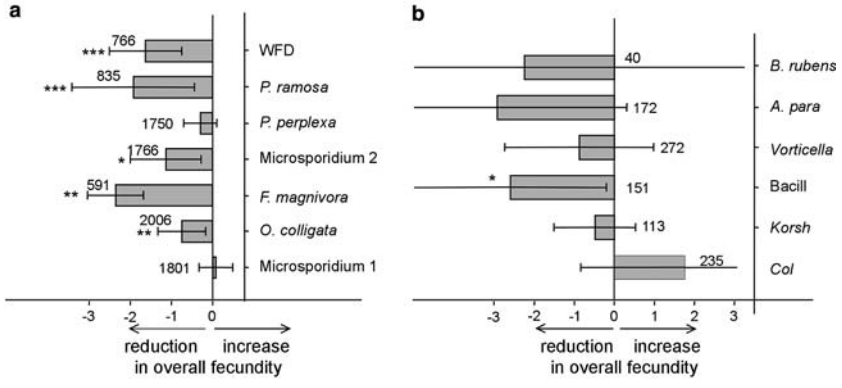

Fig. 3 Overall fecundity reduction of endoparasites (**a**) and epibionts (**b**). For each parasite, the bar gives the mean difference of the fraction of females with clutches times clutch size between infected and NONinfected females (mean taken over all samples). *Numbers on the horizontal bars* refer to the total number of *D. magna* adults on which the fraction calculations were based. *WFD* White Fat Cell Disease, *Col Colacium + Protoderma* sp., *Korsh Korshikoviella* sp., *Bacill* Bacillariophyceae, *A. para A. parasiticum*. Significance levels were determined by Wilcoxon Matched pairs tests: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. P values < 0.01 remained

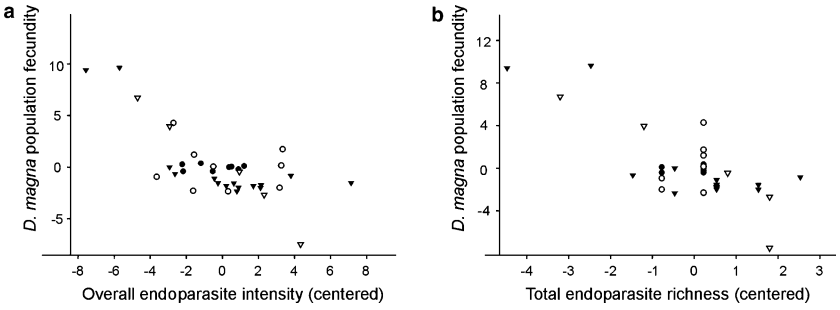

Fig. 4 *Daphnia magna* population fecundity (mean clutch size per sample, centered over all samples) in function of (**a**) overall endoparasite intensity (sum of centered and standardized intensities of all endoparasite species) and (**b**) total endoparasite richness (sum of all endoparasite species in a sample). Symbols present all samples at which parasites were found in 1999 (*solid symbols*) and

Table 2 Results of multiple regression on *Daphnia magna* population fecundity and density

	D. magna fecundity	*D. magna* density
Overall endoparasite prevalence		−0.66**
Overall endoparasite infection intensity	−0.54*	
Total endoparasite richness	−0.33*	
Average epibiont richness		0.84*
F	22.37	7.513
R^2	0.248	0.239
P	0.001	0.013

Standardized regression co-efficients and significance levels are given for the independent variables that were retained by the manual forward selection procedure
F: *F*-value; R^2: co-efficient of determination* $P < 0.05$; ** $P < 0.01$

tions (Bittner et al. 2002). In an experimental study (K. Pulkinnen and D. Ebert, in preparation), it has indeed been shown that the removal of larger *Daphnia* drives parasite population into extinction. Moreover, Duffy et al. (2005) showed that seasonal reductions in the intensity of fish predation on *Daphnia* accounted for the seasonal occurrence of parasite epidemics in natural *D. denitifera* lake populations. Therefore, it is surprising that we find high parasite prevalences in this study system. However, it is very likely that the fish community in our two ponds is not very efficient in feeding on *D. magna*, explaining why *D. magna*, as well as other cladocerans can persist in these ponds. This lower efficiency of fish predation may be related to the species composition of the fish community (cf. carp and crucian carp, for which *Daphnia* is only a small fraction of their diet, as they are generally benthic feeders, Callan and Sanderson 2003) and the fact that the two ponds have quite turbid water. In turbid ponds, visual hunting predators, such as fish, will not be efficient in feeding on zooplankton. The latter will be even more pronounced when the zooplankton performs diel vertical migration (DVM) behavior, by which it resides at greater depths during the day (De Meester et al. 1999). Moreover, it is shown that this DVM behavior can increase parasitic infection risks when *Daphnia* are exposed the pond sediments that contain parasite spores, as in this study system (Decaestecker et al. 2002, 2004). This may further enhance the spread of parasites into the *D. magna* population.

Our results showed that endoparasites can have a strong negative impact on the studied host populations. Certain endoparasite species induced a significant reduction in *D. magna* overall fecundity. More specific, the presence of White Fat Cell Disease, *P. ramosa*, Microsporidium 2, *F. magnivora* and *O. colligata* was

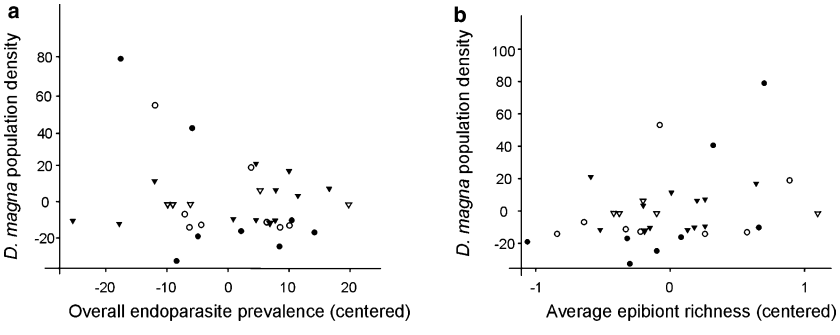

Fig. 5 *Daphnia magna* population density in function of (**a**) overall endoparasite prevalence (average of all endoparasite species) and (**b**) average epibiont richness (average richness per sample). Symbols present all the samples at which parasites were found in 1999 (*black symbols*) and in 2000 (*open symbols*), in OM2 (*circles*) and in OM3 (*triangles*). Data are centered with respect to ponds and years

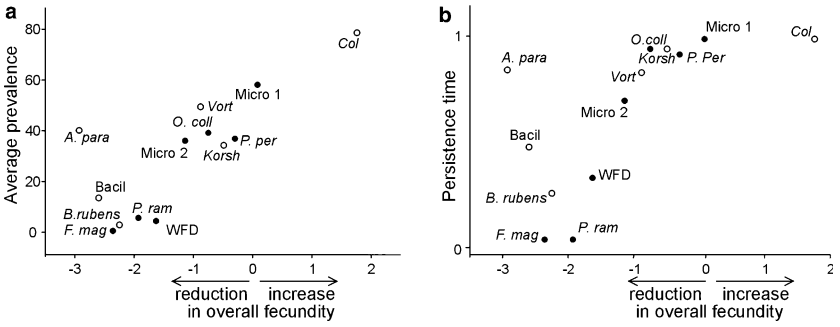

Fig. 6 Relationships between average prevalence (**a** average prevalence per sample with at least one infected individual), persistence time (**b**) and overall fecundity reduction of endoparasites (*solid symbols*) and epibionts (*open symbols*). Each data point represents the average for a given endoparasite or epibiont species. *F. mag F. magnivora*, *P. ram P. ramosa*, *A. para A. parasiticum*, *P. per P. perplexa*, *WFD* White Fat Cell Disease, *Micro 2* Microsporidium 2, *Micro 1* Microsporidium 1, *Col Colacium* + *Protoderma* sp., *Korsh Korshikoviella* sp., *Bacil* Bacillariophyceae

associated with overall fecundity reduction. Compared to other parasites, like Microsporidium 1, Microsporidium 2, *O. colligata* and *P. perplexa*, the parasite species *F. magnivora*, *P. ramosa* and White Fat Cell Disease can even be considered as highly virulent because overall fecundity was considerably lower in infected than in NONinfected individuals. For some of these endoparasites it is also shown that they reduce fecundity and/or survival of *Daphnia* under laboratory (Ebert et al. 2000a; Carius et al. 2001; Decaestecker et al. 2003) and field conditions (Stirnadel and Ebert 1997). Furthermore, castration of the host has been suggested to be a good strategy of the parasite in its competition with the host for resources (Baudoin 1975; Hurd 2001; Ebert et al. 2004). The negative effect of the endoparasites on individual host fecundity was also seen across samples. The average population fecundity was negatively associated with both the overall infection intensity and the total richness of the endoparasites. This effect is further reflected on the population structure of the host. *D. magna* population density was negatively correlated with average prevalence across all endoparasite species.

In contrast to the endoparasites, we observed no evidence for a negative impact of epibionts on the *D. magna* host populations. None of the epibiont species induced a significant overall fecundity reduction. Furthermore, there was no significantly negative effect of epibionts on host population fecundity. In contrast, epibiont richness even showed a positive relation with host population density. The difference in the effect on host performance between endoparasites and epibionts may be explained by smaller competition for resources between hosts and epibionts than between hosts and endoparasites, as none of the observed epibionts relies directly on host resources, as is the case for the endoparasites. In other studies, epibionts have been shown to negatively affect reproduction of their zooplankton substrate organisms (Willey et al. 1990; Threlkeld and Willey 1993). However, this has so far only been shown under laboratory conditions and when food levels were low.

One could argue that the negative relationship between host population parameters and parasite population parameters can equally well be explained by food-limitation cycles, associated with parasite density-dependent transmission. Such a relationship is to be expected if the interplay of stress and disease is based on the perspective of an individual host. In vertebrate animals it is indeed observed that overcrowding and malnutrition are often coupled with high burdens of parasites and high mortality of infected animals. The explanation is that stress impairs the defense mechanisms of individual hosts and as such leads to an increase in parasite within-host growth and transmission (Dobson and Bawden 1974; Slater and Keymer 1986). However, in invertebrates and in a population dynamic context of infection, other predictions emerge. When an outside stressor decreases host density and as such contact rates between infected and uninfected individuals, a decrease in the likelihood and impact of disease is expected. Moreover, if outside stress, such as food depletion, increases parasite removal and decreases parasite within-host rates, further decreases in the epidemic are to be expected (Lafferty and Holt 2003; Pulkkinen and Ebert 2004).

In *Daphnia*, food level has been shown to have no effect on individual host susceptibility to a microsporidian parasite (Ebert 1995). Moreover, experimental studies have shown that host starvation decreases parasite spore production. For a haplosporidian species (*Caullerya mesnili*) and a bacterial parasite (*P. ramosa*) it has been shown that parasite growth inside the host is higher in well-fed hosts than in poorly fed hosts (Ebert et al. 2000b; Bittner et al. 2002, Ebert et al. 2004). Further, under food stress, strongly infected individuals with a microsporidium species (*G. intestinalis*) were removed from the host population, resulting in (temporary) parasite removal. Food stress decreased the basic reproductive rate of a disease (R_0), however it increased the impact of disease on the host population (Pulkkinen and Ebert 2004). Consequently, in *Daphnia* parasites, one would expect a positive relationship between host density and parasite

transmission parameters when this relationship would be caused by food-limitation cycles, associated with parasite density-dependent transmission (the latter effect has been experimentally shown in *Daphnia* and its microparasites; Ebert 1995; Ebert et al. 2000b; Bittner et al. 2002; Regoes et al. 2003). However, in this study, there was a negative association between *Daphnia* population density and endoparasite transmission parameters. This finding suggests that the negative effect of endoparasites on *D. magna* density was stronger than the expected positive effect of parasite density-dependent transmission. It might be that at low food levels, the impact of endoparasites further reduced the density of the host population. Contrary to the endoparasites, epibiont richness was positively correlated with *D. magna* population density. Our observation of a positive relationship between increased epibiont richness and host population density is in agreement with earlier studies (Chiavelli et al. 1993; Threlkeld et al. 1993; Barea-Arco et al. 2001) and may be explained by an increased efficiency of horizontal transmission at high host population density.

Our results demonstrate a negative relationship between parasite induced overall fecundity reduction and prevalence. The least harmful parasites were the most prevalent, and this pattern was consistent across years. Further, the same pattern was seen within the epibionts and the endoparasites. Prevalence and persistence time of the most virulent parasite species were considerably lower than those of the less harmful species, which is in agreement with predictions from epidemiological models (Anderson and May 1979; Anderson 1982). Epidemic, highly virulent infections are characterized by periodic 'fade outs', whereas low virulent infections are more likely to be endemic, with relatively high prevalence (Anderson and May 1979).

In epidemiological models, virulence is mainly described as parasite-induced host mortality, whereas in our study we used parasite-induced fecundity reduction as measure of virulence. However, with the exception of castrating parasites like *P. ramosa*, the effects of parasites on host fecundity and host survival go hand in hand. Furthermore, the ecological consequence of both types of parasites will be equal: they both reduce host population densities, which may lead to host and parasite extinction (Ebert et al 2000a). Note, however, that parasites that increase host mortality have different evolutionary dynamics than parasites that reduce host fitness primarily through their effect on host fecundity. Virulence evolution in parasites that kill their hosts quickly is constrained by a trade-off between longevity and transmission, leading to an evolutionarily stable state of intermediate virulence. However, for parasites that do not affect host longevity, higher parasite transmission and virulence will be favored (O'Keefe and Antonovics 2002)

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