Ecology, 96(5), 2015, pp. 1421–1430

Parasite and nutrient enrichment effects on *Daphnia* interspecific competition

Ellen Decaestecker,[1,4] Dino Verreydt,[2] Luc De Meester,[2] and Steven A. J. Declerck[3]

[1]*Aquatic Biology, IRF Life Sciences, Kuleuven-Kulak, E. Sabbelaan 53, B-8500 Kortrijk, Belgium*
[2]*Laboratory of Aquatic Ecology, Evolution and Conservation, Kuleuven, Ch. De Beriotstraat 32, B-3000 Leuven, Belgium*
[3]*Netherlands Institute of Ecology (NIOO-KNAW), P.O. Box 50, 6700 AB Wageningen, The Netherlands*

Abstract. Increased productivity due to nutrient enrichment is hypothesized to affect density-dependent processes, such as transmission success of horizontally transmitting parasites. Changes in nutrient availability can also modify the stoichiometry and condition of individual hosts, which may affect their susceptibility for parasites as well as the growth conditions for parasites within the host. Consequently, if not balanced by increased host immuno-competence or life history responses, changes in the magnitude of parasite effects with increasing nutrient availability are expected. If these parasite effects are host-species specific, this may lead to shifts in the host community structure. We here used the *Daphnia*–parasite model system to study the effect of nutrient enrichment on parasite-mediated competition in experimental mesocosms. In the absence of parasites, *D. magna* was competitively dominant to *D. pulex* at both low and high nutrient levels. Introduction of parasites resulted in infections of *D. magna*, but not of *D. pulex* and, as such, reversed the competitive hierarchy between these two species. Nutrient addition resulted in an increased prevalence and infection intensity of some of the parasites on *D. magna*. However, there was no evidence that high nutrient levels enhanced negative effects of parasites on the hosts. Costs associated with parasite infections may have been compensated by better growth conditions for *D. magna* in the presence of high nutrient levels.

Key words: Binucleata daphniae; Daphnia; *nutrient enrichment;* Ordospora colligata; *parasite-mediated interspecific host competition; White Bacterial Disease.*

Introduction

There is an increasing awareness of the need to study host–parasite interactions in a wider ecological context (Burdon and Thrall 2009, Okamura and Feist 2011). Changes in (a)biotic conditions are suggested to affect host–parasite interactions (Laine and Tellier 2008, Wolinska and King 2009, Zhang and Buckling 2011, Aalto et al. 2014). A key feature of host–parasite interactions is density dependence, because transmission success of horizontally transmitted parasites is dependent on host density (Anderson and May 1978, Begon and Bowers 1995, Ebert et al. 2000). Given that nutrient input and system productivity are important factors that control host population densities, they are expected to determine the prevalence of their parasites (McKenzie and Townsend 2007). Infection intensity and virulence of parasites are often associated with their prevalence but difficult to predict. The strength of the overall negative impacts of parasites on host populations is, nevertheless, generally believed to increase with increasing productivity (Forde et al. 2004, Johnson et al. 2007).

Manuscript received 17 June 2014; revised 16 October 2014; accepted 22 October 2014. Corresponding Editor: D. M. Tompkins.

[4] E-mail: Ellen.Decaestecker@kuleuven-kulak.be

Productivity effects on host–parasite interactions may be particularly prominent in freshwater aquatic habitats, where micro-parasites are spread by means of free-living stages (Lafferty and Holt 2003, Ebert 2005). Eutrophication resulting from anthropogenic nutrient inputs is a well-known problem that has affected freshwater ecosystems worldwide (Scheffer et al. 2001, Elser et al. 2007, Frisch et al. 2014). Nutrient enrichment has the potential to alter competitive interactions among consumers and has been shown to have profound effects on the diversity and the composition of zooplankton communities (Declerck et al. 2007, 2011). In addition to density dependent effects, the relative availability of nutrients also determines food stoichiometry, which has been shown to affect zooplankton–parasite interactions (Frost et al. 2008, Aalto and Pulkkinen 2013).

An increased magnitude of parasite effects due to environmental changes often leads to important shifts in the structure of the community in which host–parasite interactions are embedded (Thrall et al. 2007, Fisher et al. 2012). Parasites alter the outcome of competition between hosts and therefore host community composition, if host species differ in their susceptibility and tolerance to the parasites (Combes 2001, Hatcher et al. 2006, Lafferty et al. 2006, Wood et al. 2007, Patot et al.

2012). Parasites are important drivers of biodiversity (Bradley et al. 2008) and even specialist parasites that are not so virulent provide an important, but overlooked, factor in determining species diversity (Fenton and Brockhurst 2008). In highly productive systems, parasites may lower the performance of competitively dominant host species and therefore preclude exclusion of weaker competitors. If the host is key to the realization of particular ecosystem functions, parasites may indirectly influence ecosystem functioning (keystone parasitism; Holt and Dobson 2006). Such parasite-mediated effects may also propagate throughout the whole ecosystem by changing food web interactions, thereby influencing production and energy flow (Hudson et al. 2006, Lafferty et al. 2008, Holdo et al. 2009).

So far, the extent to which extent nutrient availability affects the outcome of host competition in the presence of parasites is rarely investigated. The *Daphnia* model system has particular features to tackle this research question. Large *Daphnia* species are better competitors than smaller species (Lampert 2011) and parasitism has earlier been shown to induce different effects in different *Daphnia* species with larger species being more vulnerable to parasites than smaller species (Stirnadel and Ebert 1997). Parasitism is thus suggested to alter the outcome of competition between *Daphnia* species (Bittner et al. 2002). This has been confirmed for different taxa within a *Daphnia* hybrid system (Wolinska et al. 2007), but further proof for parasite-mediated interspecific host competition in zooplankton is lacking (Ebert 2005). We here performed an outdoor mesocosm experiment to investigate if parasite-mediated competition between *Daphnia* species is present and if this is contingent upon nutrient enrichment. We used *D. magna* and its parasites because it is one of the better-known zooplankton–parasite systems (Ebert 2005, Lampert 2011, Caceres et al. 2014). Compared to other zooplankton taxa, *D. magna* is a large-bodied, fast-growing, strong competitor, especially at high nutrient levels (Verreydt et al. 2012). In the absence of fish, it has the potential to outcompete most other pelagic zooplankton taxa and to exert a strong top-down control on phytoplankton biomass and productivity (Lampert 2011). In our experiment, we studied the effect of parasites on *D. magna* populations and the extent to which these effects depend on nutrient availability. The parasites we used have all been shown to cause pronounced reductions in *D. magna* population fecundity and density (Decaestecker et al. 2005). These parasites are also known to affect *D. magna* stronger than *D. pulex* (Stirnadel and Ebert 1997, Ebert 2005; E. Decaestecker, *personal observation*). We predicted that nutrient enrichment would intensify the effects of parasite infections on the *D. magna* populations and would reduce its competitive strength vis-a-vis *D. pulex*.

Methods

Experimental design and procedure

We performed an outdoor mesocosm experiment in which we exposed *Daphnia magna* populations to two nutrient levels in the presence or absence of *Daphnia* parasites (Fig. 1). In addition, we included a *Daphnia* community treatment by also inoculating *D. pulex* with *D. magna* in one-half of the mesocosms. There were three replicates per treatment combination, which resulted in 24 180-L mesocosms (90 cm diameter, 50 cm height) in total for the final experiment. The experiment was set up in four subsequent phases and ran from the start of June until the end of October in 2006 (Fig. 1). Preparatory phases were performed in the laboratory (always under a 16:8 day : night regime at 20°C with a food concentration of 2.5×10^5 *Scenedesmus obliquus* cells/mL in 500-mL jars, Fig. 1A and C) or in 800-L outdoor containers (120 cm in length, 70 cm in height, 100 cm in width; Fig. 1B).

The *Daphnia* species in our experiment are cyclical parthenogenetically reproducing organisms. Sexual eggs give rise to offspring that are genetically unique, which then reproduce parthenogenetically. As a result, *Daphnia* populations are composed of multiple clones. Micro-evolutionary responses of *Daphnia* populations to selection pressures, e.g., parasites, typically arise from clonal selection. To allow for such micro-evolutionary responses in our experiments, we created multiclonal populations of *D. magna* and *D. pulex*. These clones were extracted from the dormant egg bank of pond OM2 (Heverlee, Belgium, 50°51′47.67″ N; 4°43′16.36″ E). *Daphnia*–parasite coevolutionary interactions have been documented in this pond (Decaestecker et al. 2007, 2013). These *Daphnia* clones were reared as independent lines during two generations in the laboratory in order to have sufficient animals for further inoculations (Fig. 1A). Second, four 800-L containers were filled with water (two-thirds distilled water and one-third tap water) on 27 June 2006 and an inoculum of 26.67×10^9 *Scenedesmus obliquus* cells was added. Two containers received a high amount of nutrients (1 mg P/L and 16 mg N/L), corresponding to hypereutrophic conditions in lakes, whereas the other two containers received low nutrient additions (0.1 mg P/L and 1.6 mg N/L), corresponding to mesotrophic to eutrophic conditions (Fig. 1B). P and N were given under the form of KH_2PO_4 and $NaNO_3$, respectively. The molar ratio of P and N reflect the Redfield ratio. To increase algal biomass, we inoculated natural phytoplankton in addition to *S. obliquus* on 19 July 2006. As such, the phytoplankton species pool was enriched, which increased the probability of adding taxa that were able to grow well in the containers and contribute to a fast build-up of phytoplankton biomass. For this, we collected water from 16 regional ponds and lakes (13 L), filtered it three times over a 30-μm mesh, and distributed it in equal parts over the containers.

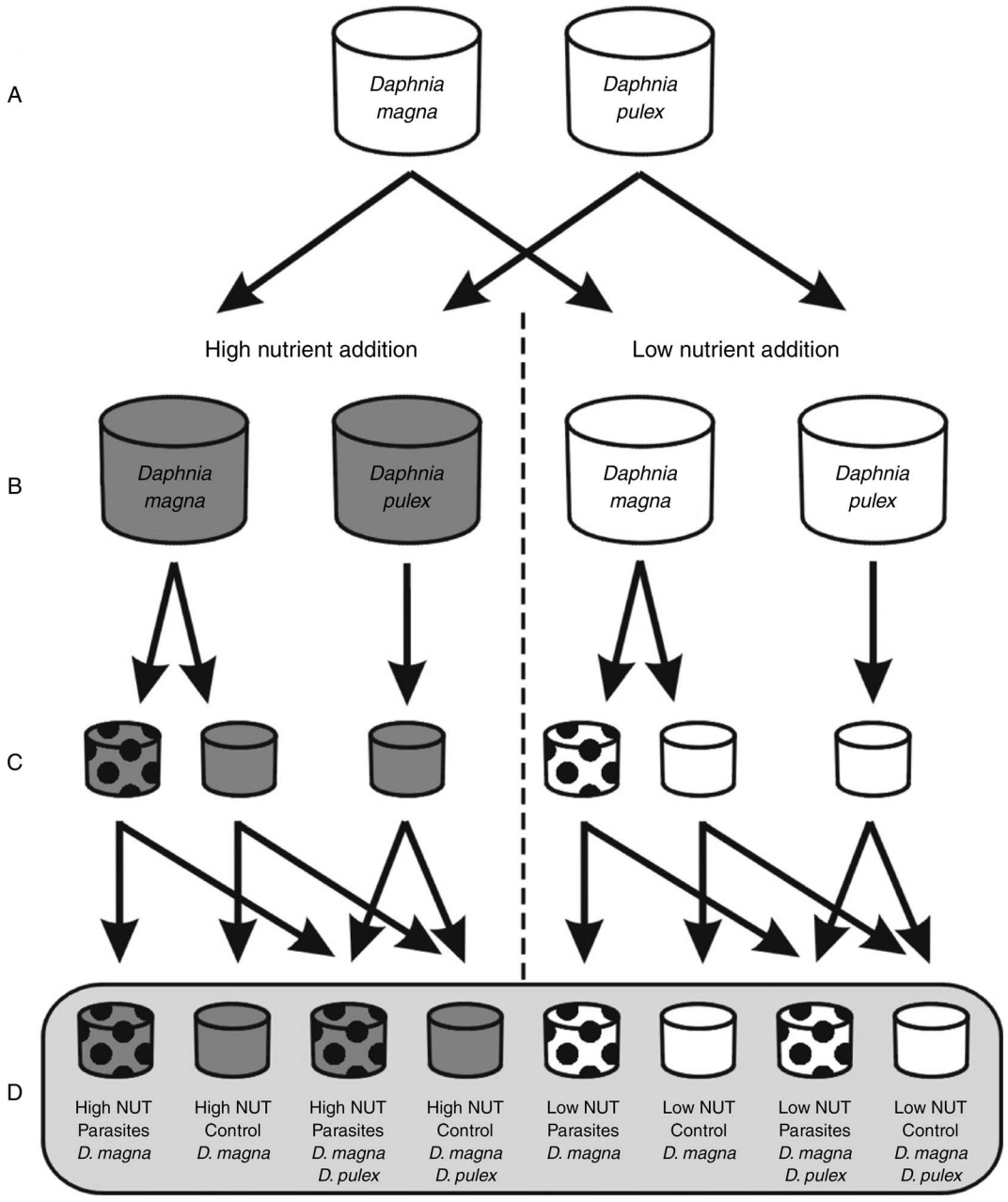

FIG. 1. Scheme of preparatory phases and final experimental design of the experiment. The experiment was performed in different phases. (A) We first cultured *Daphnia magna* and *Daphnia pulex* as single clonal lines in the laboratory during two generations (23 clones per *Daphnia* species). (B) Individuals from these cultures were then used to inoculate the 800-L outdoor containers at two different nutrient levels (C) *D. magna* and *D. pulex* individuals were isolated from these containers. *D. magna* individuals were exposed to a parasite treatment in the laboratory. Control *D. magna* and *D. pulex* individuals were also kept in the laboratory. Afterward, these *D. magna* (parasitized and control) and *D. pulex* (control) individuals were inoculated into the 180-L mesocosms (which were filled with a mixed volume of the filtered *D. magna* and *D. pulex* containers). Parasite presence is represented by dotted mesocosms and nutrient treatments as High NUT and Low NUT (gray and white mesocosms, respectively).

At the start of August, when a significant difference in the chlorophyll *a* concentration between the high- and low-nutrient-addition treatments had developed, we added *Daphnia* from the laboratory cultures to these 800-L containers (Fig. 1B). One high- and one low-nutrient container each received 1840 *D. magna* individuals composed of 23 clones (80 individuals per clone); the other high and low nutrient containers received a similar number of *D. pulex* clones and individuals. We performed this preparatory phase in order to obtain sufficient individuals to start up the final mesocosm populations because we wanted to avoid loss of

replication due to stochastic extinctions resulting from low initial population sizes. We also wanted to pre-adapt populations to the conditions in tanks and experimental nutrient levels, given that the introduction of laboratory grown *Daphnia* to low nutrient conditions in mesocosms right after exposure to parasites could substantially increase the risk of population extinctions.

On 11 August 2006, we randomly collected six sets of 150 *D. magna* juveniles from each of the 800-L *D. magna* containers and exposed them during three days to either a control or a parasite spore solution in 500-mL jars in the laboratory (Fig. 1C). To maximize parasite uptake, the parasite spores were kept in suspension by continuously rotating the jars during exposure. One-half of the jars received a homogenate of 100 infected *D. magna* individuals (parasite exposure), whereas the other half received a homogenate of 100 non-infected *D. magna* individuals (control exposure). Parasite spore solutions were made from infected *D. magna* individuals, collected from different localities in Flanders. The parasite spore solution consisted of a homogenate of individuals infected with *Ordospora colligata*, *Binucleata daphniae*, and the parasite causing White Bacterial Disease. Relative proportions of the parasites were not quantified. When setting up the mesocosm experiment, we randomly assigned the content of the jars to the mesocosms of the parasite addition treatments. *O. colligata* is a microsporidian species that infects the gut epithelium cells of *Daphnia*. *B. daphniae* is a microsporidian species that infects the integument cells of the hemocoel cavity of the carapax in *Daphnia*. White Bacterial Disease is caused by an infection of a so-far unknown agent, potentially a bacterial agent, infects the fat cells of *Daphnia*. Virulence levels induced by these parasites are variable, but transmission of all parasites is horizontal and occurs upon the death of the host. For a more detailed description of these parasites and their virulence effects, we refer to Refardt et al. (2008), Jansen et al. (2010), Coopman et al. (2013).

We started the experiment (Fig. 1D) on 14 August 2006 by filling 24 mesocosms (180 L) with water from the 800-L containers. Water from each of the four 800-L tanks was used to fill the mesocosms (not shown on Fig. 1). Low nutrient mesocosms of the experiment received one-half of their water from the 800-L low-nutrient tank with *D. magna* and one-half of their water from the 800-L low-nutrient tank with *D. pulex*. Accordingly, high-nutrient mesocosms were filled with water from both high-nutrient tanks. Before addition to the mesocosms, this water was first filtered three times over a 30-μm mesh to remove zooplankton. Subsequently, 300 *Daphnia* individuals were inoculated per mesocosm in four possible combinations: (1) 150 parasite-exposed *D. magna* + 150 non-exposed *D. magna*, (2) 150 *D. magna* from the control exposure (i.e., being exposed to a control solution of crushed, non-infected *D. magna*) + 150 non-exposed *D. magna*, (3) 150 parasite-exposed *D. magna* + 150 non-exposed *D. pulex*, and (4) 150 *D. magna* of the control exposure + 150 non-exposed *D. pulex* (Fig. 1D). Nutrient levels in the mesocosms were maintained through the weekly addition of 1/10th of the initial nutrient addition, given that nutrients tend to show a gradual decline in mesocosms with time (Declerck et al. 2007). Chlorophyll *a* was measured weekly throughout the whole experiment with a Trilogy Laboratory Fluorometer (Turner Designs, Sunnyvale, California, USA). Differences in chlorophyll *a* levels between nutrient addition treatment levels can be seen, indicating that the nutrient treatments have been effective (Appendix: Fig. A1).

To characterize the host and parasite populations, we collected 5-L of medium on 23 October 2006. After homogenizing the water column of each mesocosm, a sample was taken with a tube sampler and filtered over a 100-μm mesh. Immediately upon sample isolation, all WBD infected individuals in the sample were counted (otherwise phenotypic detection of this parasite is hampered, see also Decaestecker et al. [2005] and Ebert [2005]). Subsequently, the samples were frozen at −18°C. Upon thawing, we screened 20 adult *D. magna* and, where possible, 20 adult *D. pulex* for infections. *B. daphniae* parasite load was estimated by determining parasite coverage of the carapax and scored into classes ranging from class 0 with no infection to class 5 with completely covered carapax (as in Decaestecker et al. 2005). To detect *O. colligata* infections, the animals were dissected and the caecum was inspected for infection under a phase contrast microscope. Parasite load for this parasite was estimated by the degree of caecum occupancy and scored into classes ranging from 0 to 5 (determined based on the number of spore clusters present, as in Decaestecker et al. [2005]). The remaining individuals were fixed with acid lugol solution for later enumeration (*D. magna* and *D. pulex* density). To estimate host densities, we counted a minimum of 100 individuals in subsamples of a known volume. We calculated parasite prevalence (percentage infected *Daphnia* adults) and parasite infection intensity (average parasite load in infected individuals).

Data analysis

We tested the impact of nutrient addition, host community composition and exposure to parasites on $\log(x+1)$-transformed population densities of *D. magna and D. pulex* using three-way analysis of variance. For mesocosms with parasite addition treatments only, we tested the effects of nutrient addition and host community composition on logit-transformed prevalence data and untransformed infection intensities for each of the different parasite taxa. All these analyses were performed using the lm function of R 2.15.2 (R Core Team 2014).

Results

D. pulex was accidently introduced in the mesocosms of the pure *D. magna* treatment. As a result, we observed

TABLE 1. ANOVA results for the effects of the experimental treatments on *Daphnia* density.

Effects	df	SS	MS	*F*	*P*
D. magna					
Host community	1	0.0002	0.0002	0.0039	0.9507
Productivity	1	1.3524	1.3524	25.6347	0.0001
Parasite	1	1.0962	1.0962	20.7783	0.0003
Community × Productivity	1	0.0049	0.0049	0.0925	0.7649
Community × Parasite	1	0.0119	0.0119	0.2260	0.6409
Productivity × Parasite	1	1.2×10^{-6}	1.2×10^{-6}	2.3×10^{-5}	0.9962
Community × Productivity × Parasite	1	0.0002	0.0002	0.0038	0.9515
Residuals	16	0.8441	0.0528		
D. pulex					
Host community	1	0.6827	0.6827	3.4811	0.0805
Productivity	1	1.9745	1.9745	10.0684	0.0059
Parasite	1	2.7906	2.7906	14.2301	0.0017
Community × Productivity	1	0.3333	0.3333	1.6995	0.2108
Community × Parasite	1	0.3999	0.3999	2.0395	0.1725
Productivity × Parasite	1	0.0318	0.0318	0.1623	0.6924
Community × Productivity × Parasite	1	0.0026	0.0026	0.0131	0.9102
Residuals	16	3.1377	0.1961		

no effects of the host community treatment on final population densities of both species (Table 1). Irrespective from this, high nutrient levels invariably increased *D. magna* but reduced *D. pulex* densities (Table 1, Fig. 2A, B). The presence of parasites had a strong negative effect on the population densities of *D. magna* but enhanced *D. pulex* densities (Table 1, Fig. 2A, B). Consequently, the *D. pulex* to *D. magna* density ratio was highest under low nutrient-addition levels and in the presence of parasites (Fig. 2C; ANOVA result on the *D. pulex* to *D. magna* density ratio; nutrient addition treatment $F_{1,16} = 8.08$, $P = 0.0118$; parasite treatment $F_{1,16} = 17.94$, $P < 0.001$). We observed no parasite by nutrient interaction effect on the densities of any of the *Daphnia* species (Table 1).

All three parasites *O. colligata*, *B. daphniae*, and WBD infected *D. magna* (average prevalences 59.4%, 26%, and 4.7%, respectively), whereas no infections of these parasites were found on *D. pulex*. The prevalence and infection intensity of *B. daphniae* on *D. magna* was significantly higher at high- than at low-nutrient levels (Table 2, Fig. 3A). Both variables also tended to be higher in the mixed *Daphnia* inoculation treatments than in the *D. magna*-only treatments. In addition, we observed a significant interaction effect of nutrient addition and *Daphnia* host community composition on the prevalence of the parasite *O. colligata* (Table 2, Fig. 3B). In the pure *D. magna* treatment, prevalence of *O. colligata* was low at low nutrient levels (<20%) and very high at high nutrient levels (>80%). In the mesocosms that were initially stocked with both *D. magna* and *D. pulex*, the prevalence of this parasite was very high at both low and high nutrient concentrations (Table 2, Fig. 3B). WBD prevalence was always low (<5%) and treatments did not show significant effects (results not shown).

DISCUSSION

The focal species *D. magna* was more prone to infection by parasites than the weaker competitor *D. pulex*. *D. magna* is generally known as an efficient filter feeder that can strongly reduce the population growth of other zooplankton organisms through resource competition, provided that essential nutrients like phosphorus are not limiting (Lampert 2011, Hessen et al. 2013). In the absence of parasites, *D. magna* strongly dominated the experimental mesocosms at the cost of *D. pulex*, especially in high nutrient-level treatments. Addition of parasites, however, resulted in a shift in the species composition, favoring dominance by *D. pulex* over *D. magna*. Consequently, the presence of parasites resulted in a reversal of the competitive hierarchy of the two competing species. Our results suggest that parasitism can be an important driver of zooplankton community composition in natural ecosystems. The three parasite species used in our experiment (WBD, *O. colligata*, and *B. daphniae*) were previously observed in natural populations of both *D. magna* and *D. pulex*, but infections were always lower in *D. pulex* than in *D. magna* (E. Decaestecker, *personal observation*; see Stirnadel and Ebert 1997, Ebert 2005). In our experiment, we were unable to detect any parasites in *D. pulex*. Resistance of *D. pulex* to the parasites is probably the main reason for the observed parasite-induced dominance shift between *D. magna* and *D. pulex*. The reason for the inability of the parasites to infect *D. pulex* is likely the result of a high degree of host specialization of the parasite strains we used. Nevertheless, given that *D. pulex* in the field has repeatedly been shown to be less sensitive to infections than *D. magna*, we are confident that our experiment is representative for shallow freshwater ecosystems in Europe.

Average parasite prevalence in our experimental *D. magna* populations is comparable with what is found for

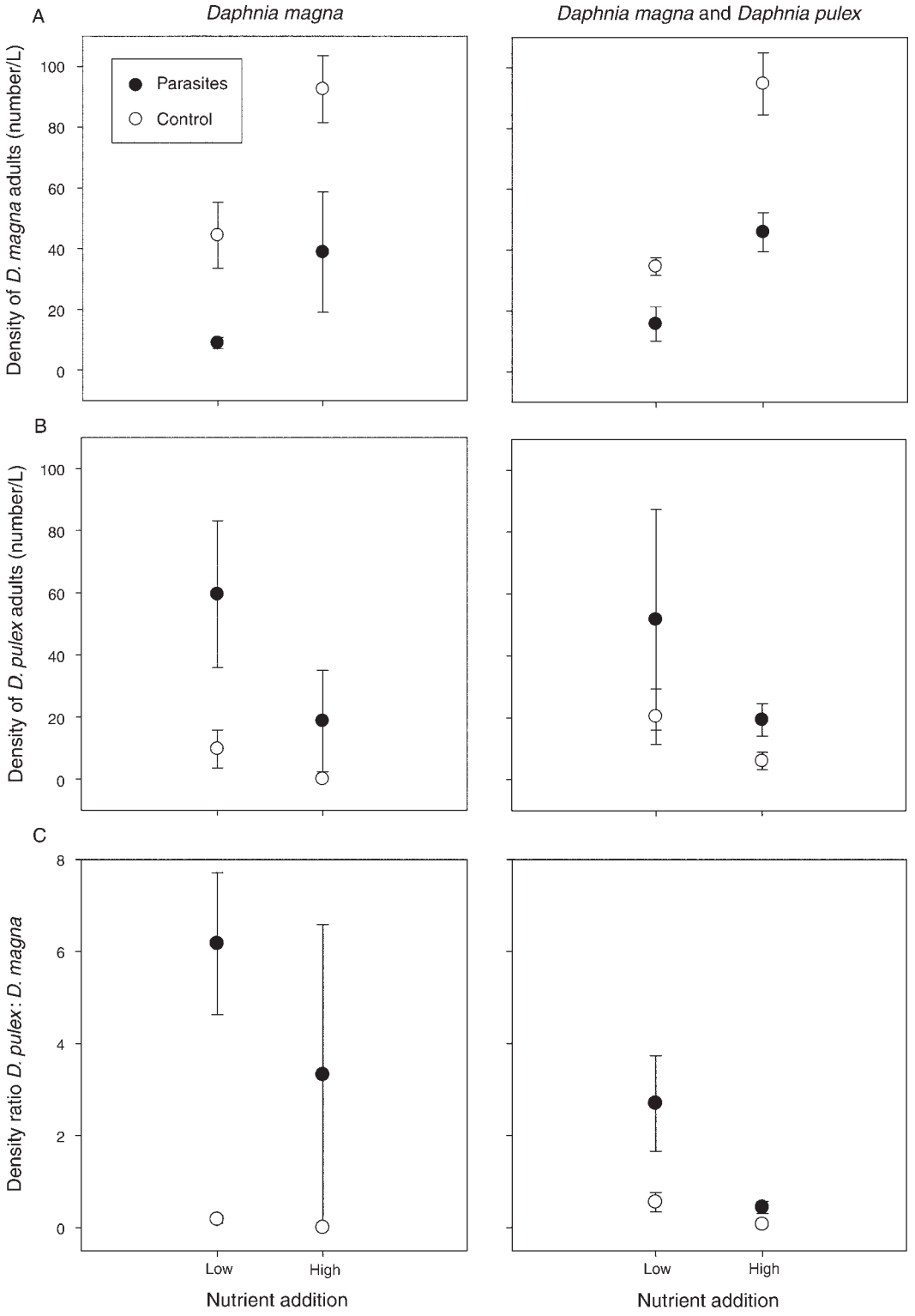

FIG. 2. Population densities of (A) *Daphnia magna* and (B) *Daphnia pulex* and (C) the *D. pulex* to *D. magna* ratio for each of the multi-factorial treatment combinations at the end of the experiment. Error bars denote ±SE. Left panel is the *D. magna* inoculation treatment, right panel is the *D. magna and D. pulex* inoculation treatment. Note that *D. pulex* individuals accidentally contaminated the containers of the *D. magna* inoculation treatment. Solid and open circles represent the parasite and control treatments, respectively.

TABLE 2. ANOVA results for the effects of the experimental treatments on the prevalence and infection intensity of *Daphnia magna* parasites.

Effects	df	SS	MS	*F*	*P*
Binucleata prevalence					
Productivity	1	32.816	32.816	19.062	0.002
Host community	1	21.210	21.210	12.320	0.008
Productivity × Community	1	0.741	0.741	0.430	0.530
Residuals	8	13.772	1.722		
Ordospora prevalence					
Productivity	1	29.698	29.698	16.443	0.004
Host community	1	12.283	12.283	6.800	0.031
Productivity × Community	1	22.680	22.680	12.557	0.008
Residuals	8	14.449	1.806		
Binucleata infection intensity					
Productivity	1	22.963	22.963	36.115	0.0003
Host community	1	3.203	3.203	5.038	0.055
Productivity × Community	1	0.403	0.403	0.634	0.449
Residuals	8	5.087	0.636		
Ordospora infection intensity					
Productivity	1	5.741	5.741	3.086	0.117
Host community	1	1.141	1.141	0.613	0.456
Productivity × Community	1	2.341	2.341	1.259	0.294
Residuals	8	14.880	1.860		

Note: We defined the prevalence as the percentage of infected *D. magna* individuals and infection intensity as the average parasite load in infected *D. magna*.

these parasites in a field study (Decaestecker et al. 2005). High nutrient levels resulted in higher population densities of *D. magna* and increased parasite prevalences (*B. daphniae* and *O. colligata*) and infection intensities (*O. colligata*) in this species. By increasing primary productivity, nutrient levels have resulted in higher population densities of *Daphnia*. Most likely, higher *Daphnia* densities have enhanced parasite transmission through increased host–parasite contact rates (Ebert et al. 2000, Pulkkinen 2007). Furthermore, a higher availability of nutrients, such as P or N, may have affected *Daphnia* body stoichiometry and, as such, improved the availability of resources to parasites (and parasite growth) in the *Daphnia* host (Frost et al. 2008, Hall et al. 2009, Aalto and Pulkkinen 2013). These effects may have resulted in the detected increased infection intensities, which are often associated with overdispersed spore loads and parasite aggregation in host populations (with a few individuals containing a high number of parasites [Regoes et al. 2002]). Abundant food may, therefore, have yield individuals causing potentially more transmission than the population average (Vale et al. 2013). This is likely if virulence effects on the *D. magna* population and associated density reductions are not too strong (Dallas and Drake 2014), as was the case in this study. The positive effect of nutrients on the prevalence and infection rate of *D. magna* parasites is in line with the current prevailing paradigm that host–parasite interactions are mediated by the environment and may be intensified by anthropogenic change. Human-induced environmental changes associated with land use have profoundly altered nutrient cycles and higher nutrient levels frequently lead to an increased risk of disease. This has been documented for a wide variety of parasites (including helminths, insect vectored diseases, myxozoa, and bacterial and fungal diseases [McKenzie and Townsend 2007, Bradley et al. 2008]).

Shifts in the magnitude of parasite effects with increasing nutrient availability are expected (Forde et al. 2004, Johnson et al. 2007), at least if these changes are not balanced by increased host immuno-competence or life history responses (e.g., reproduction shift [see Ebert et al. 2004, Vale and Little 2012]), often due to stoichiometric changes within the host (Aalto and Pulkkinen 2013, Hessen et al. 2013). Despite the strong positive effects of high nutrient concentrations on parasite prevalence and infection intensity, we observed no effect of nutrient addition on the degree to which parasites affected *D. magna* population densities. Indeed, negative parasite effects on *D. magna* population densities were found to be independent of the nutrient addition levels given that the proportional reduction of the population densities in the presence of parasites was similar at both nutrient levels (absence of parasite × nutrient addition effect on log-transformed data). A possible explanation for this discrepancy may be that mortality losses caused by parasite infections were compensated by stronger population growth rates of *Daphnia* in the presence of high nutrient levels. *Daphnia* is generally known to be a fast grower when P availability is high (Hessen et al. 2013). High growth rates may therefore have facilitated the *D. magna* populations to compensate parasite induced population losses. Furthermore, given that our experiment spanned several generations of the host populations, it is not

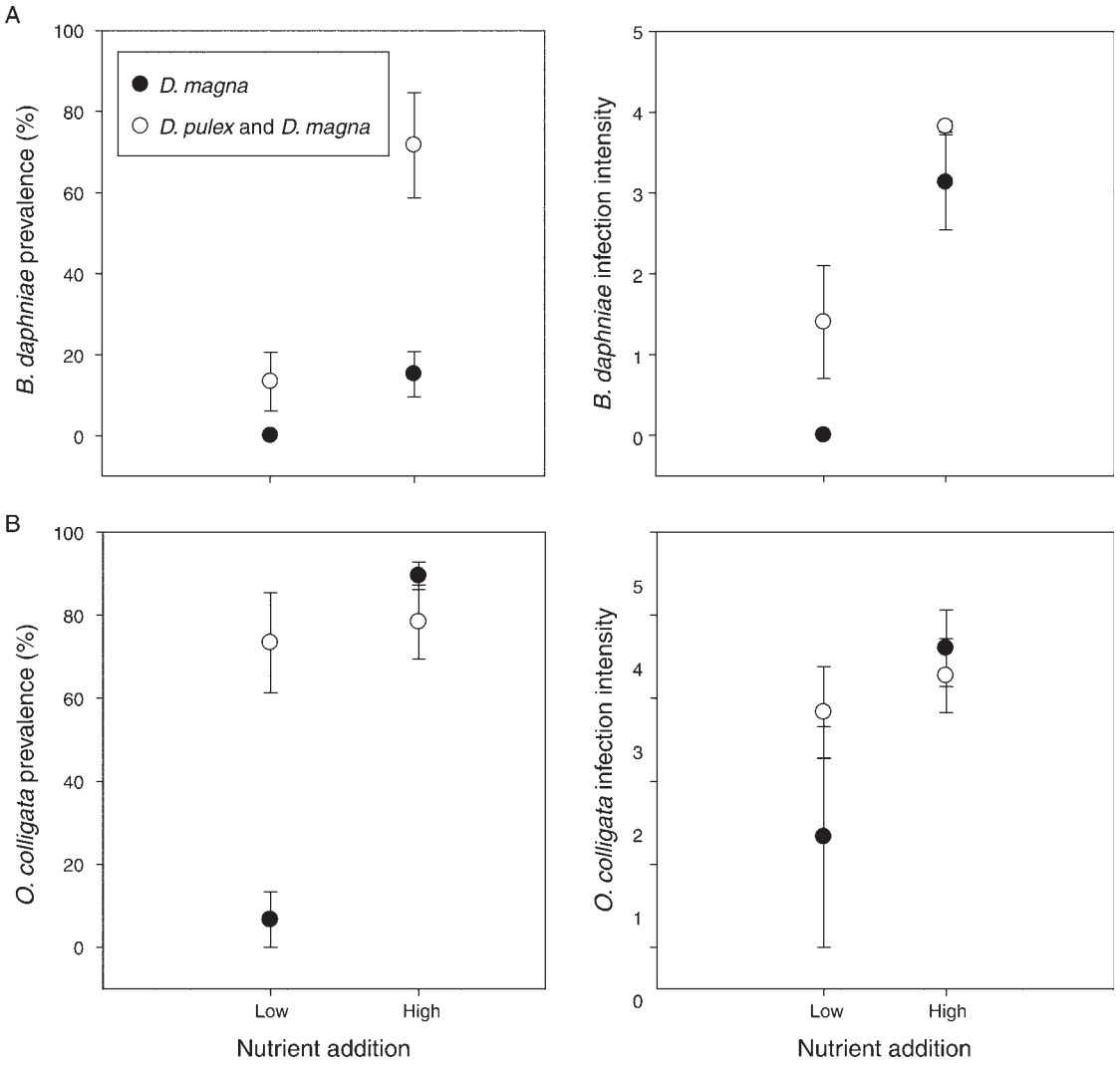

FIG. 3. (A) *Binucleata daphniae* prevalence and infection intensity and (B) *Ordospora colligata* prevalence and infection intensity in *D. magna* for each of the nutrient by host community treatment combinations. Error bars denote ±SE. Solid and open circles indicate the *D. magna* and the *D. magna* and *D. pulex* inoculation treatment, respectively. Only treatments with initial parasite exposure are shown.

unlikely that evolutionary responses in the host population have resulted in shifts toward a dominance of clones with higher tolerance for parasite infections in mesocosms with nutrient enhanced parasite impact at the end of the experiment. Evolutionary host population responses toward parasites are assumed to be strongest in highly productive systems (Duffy et al. 2012) or in dense host-population networks (Jousimo et al. 2014).

With our design, we originally also planned to evaluate the interaction effects of *Daphnia* and parasites in the presence and absence of *D. pulex*. Unfortunately, communities that were originally set up to contain only *D. magna* got contaminated with *D. pulex*. As a result, we found equal population densities of *D. magna* and *D. pulex* toward the end of the experiment, independent of the initial *Daphnia* inoculation treatments. Still, we observed some significant host composition treatment effects on parasite-related variables, such as *O. colligata* prevalence and *B. daphniae* prevalence and infection intensity. These effects are likely due to differences in the history of the community composition, given that the relative abundance of *D. pulex* in the pure *D. magna* treatment started at very low levels, whereas *D. pulex* in the *D. magna* and *D. pulex* treatment comprised one-half of the *Daphnia* biomass from the start of the experiment. The observed host community treatment effects on parasite-related variables therefore suggest that infection of *D. magna* has been mediated by the early presence of *D. pulex*, but lack of detailed data on the temporal dynamics of both *Daphnia* and parasite populations throughout the experiment hamper a more profound interpretation of the mechanisms that have resulted in

the observed host community effects on parasite-related variables. Apart from our inability to evaluate the dependency of parasite effects on *D. magna* in the absence of *D. pulex*, the contamination does not affect the main conclusion of our study, namely that parasites can reverse the competitive hierarchy between *Daphnia* species, and that increased nutrient availability can intensify the degree of parasitism on *D. magna*, although the latter does not necessarily need to result in a disproportional reduction of the population densities of this species as compared to low nutrient levels.

Acknowledgments

D. Verreydt has been supported by the Institute for the Promotion of Innovation through Science and Technology (IWT) in Flanders. This research was additional financially supported by Fund for Scientific Research (FWO, project G.0506.07) and KU Leuven Research Fund projects GOA/2008/006, STRT/1/08/019, the Excellence Center Financing PF/2010/007 and IAP/SPEEDY/P7/04. We thank two anonymous reviewers for comments on earlier versions of the manuscript.

Literature Cited

Aalto, S. L., T. Ketola, and K. Pulkkinen. 2014. No uniform associations between parasite prevalence and environmental nutrients. Ecology 95:2558–2568

Aalto, S. L., and K. Pulkkinen. 2013. Food stoichiometry affects the outcome of a *Daphnia*-parasite interaction. Ecology and Evolution 3:1266–1275.

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

Begon, M., and R. G. Bowers. 1995. Beyond host–pathogen dynamics. Pages 478–509 *in* B. T. Grenfell and A. P. Dobson, editors. Ecology of infectious diseases in natural populations. Cambridge University Press, Cambridge, UK.

Bittner, K., K.-O. Rothhaupt, and D. Ebert. 2002. Ecological interactions of the microparasite *Caullerya mesnili* and its host *Daphnia galeata*. Limnology and Oceanography 47:300–305.

Bradley, D. J., G. S. Gilbert, and J. B. G. Martiny. 2008. Pathogens promote plant diversity through a compensatory response. Ecology Letters 11:461–469.

Burdon, J. J., and P. H. Thrall. 2009. Coevolution of plants and their pathogens in natural habitats. Science 324:755–756.

Caceres, C., A. J. Tessier, M. A. Duffy, and S. R. Hall. 2014. Disease in freshwater zooplankton: what have we learned and where are we going? Journal of Plankton Research 36:326–333.

Combes, C. 2001. Parasitism: the ecology and evolution of intimate interactions. University of Chicago Press, Chicago, Illinois, USA.

Coopman, M., K. Muylaert, B. Lange, L. Reyserhove, and E. Decaestecker. 2013. Context dependency of infectious disease: the cyanobacterium *Microcystis aeruginosa* decreased White Bacterial Disease in *Daphnia magna*. Freshwater Biology 59:714–723.

Dallas, T., and J. M. Drake. 2014. Nitrate enrichment alters a *Daphnia*–microparasite interaction through multiple pathways. Ecology and Evolution 4:243–250.

Decaestecker, E., H. De Gersem, Y. Michalakis, and J. A. M. Raeymaekers. 2013. Damped long-term host-parasite Red Queen coevolutionary dynamics: a reflection of dilution effects? Ecology Letters 16:1455–1462.

Decaestecker, E., S. Declerck, L. De Meester, and D. Ebert. 2005. Ecological implications of parasites in natural *Daphnia* populations. Oecologia 144:382–390.

Decaestecker, E., S. Gaba, J. A. M. Raeymaekers, R. Stoks, L. Van Kerckhoven, D. Ebert, and L. De Meester. 2007. Host-parasite Red Queen dynamics archived in pond sediment. Nature 450:870–874.

Declerck, S. A. J., E. S. Bakker, B. van Lith, A. Kersbergen, and E. van Donk. 2011. Effects of nutrient additions and macrophyte composition on invertebrate community assembly and diversity in experimental ponds. Basic and Applied Ecology 12:466–475.

Declerck, S. A. J., M. Vanderstukken, A. Pals, K. Muylaert, and L. De Meester. 2007. Plankton biodiversity along a gradient of productivity and its mediation by macrophytes. Ecology 88:2199–2210.

Duffy, M. A., J. Housley, R. M. Ochs, D. J. Penczykowski, D. J. Civitello C. A. Klausmeier, and S. R. Hall. 2012. Ecological context influences epidemic size and parasite-driven evolution. Science 335:1636–1638.

Ebert, D. 2005. Ecology, epidemiology, and evolution of parasitism in *Daphnia*. National Library of Medicine, National Center for Biotechnology Information, Bethesda, Maryland, USA.

Ebert, D., H. J. Carius, T. J. Little, and E. Decaestecker. 2004. The evolution of virulence when parasites cause host castration and gigantism. American Naturalist 164:S19–S32.

Ebert, D., M. Lipsitch, and K. L. Mangin. 2000. The effect of parasites on host population density and extinction: experimental epidemiology with *Daphnia* and six microparasites. American Naturalist 156:459–477.

Elser, J. J., M. E. S. Bracken, E. E. Cleland, D. S. Gruner, W. S. Harpole, H. Hillebrand, J. T. Ngai, E. W. Seabloom, J. B. Shurin, and J. E. Smith. 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecology Letters 10:1135–1142.

Fenton, A., and M. A. Brockhurst. 2008. The role of specialist parasites in structuring host communities. Ecology Research 23:795–804.

Fisher, M. C., D. A. Henk, C. J. Briggs, J. S. Brownstein, L. C. Madoff, S. L. McCraw, and S. J. Gurr. 2012. Emerging fungal threats to animal, plant and ecosystem health. Nature 484:186–194.

Forde, S. E., J. N. Thompson, and B. J. M. Bohannan. 2004. Adaptation varies through space and time in a coevolving host–parasitoid interaction. Nature 431:841–844.

Frisch, D., P. K. Morton, P. R. Chowdhury, B. W. Culver, J. K. Colbourne, L. J. Weider, and P. D. Jeyasingh. 2014. A millennial-scale chronicle of evolutionary responses to cultural eutrophication in *Daphnia*. Ecology Letters 17:360–368.

Frost, P. C., D. Ebert, and V. H. Smith. 2008. Responses of a bacterial pathogen to phosphorus limitation of its aquatic invertebrate host. Ecology 89:313–318.

Hall, S. R., C. J. Knight, C. R. Becker, M. A. Duffy, A. J. Tessier, and C. E. Caceres. 2009. Quality matters: resource quality for hosts and the timing of epidemics. Ecology Letters 12:118–128.

Hatcher, M. J., J. T. A. Dick, and A. M. Dunn. 2006. How parasites affect interactions between competitors and predators. Ecology Letters 9:1253–1271.

Hessen, D. O., J. J. Elser, R. W. Sterner, and J. Urabe. 2013. Ecological stoichiometry: an elementary approach using basic principles. Limnology and Oceanography 58:2219–2236.

Holdo, R. M., A. R. E. Sinclair, A. P. Dobson, K. L. Metzger, B. M. Bolker, M. E. Ritchie, and R. D. Holt. 2009. A disease-mediated trophic cascade in the Serengeti and its implications for ecosystem C. PLoS Biology 7:e1000210.

Holt, R. D., and A. P. Dobson. 2006. Extending the principles of community ecology to address the epidemiology of host-pathogen systems. Pages 6–27 *in* S. K. Collinge and C.

Ray, editors. Disease ecology: community structure and pathogen dynamics. Oxford University Press, Oxford, UK.
Hudson, P. J., A. P. Dudson, and K. D. Lafferty. 2006. Is a healthy ecosystem one that is rich in parasites? Trends in Ecology and Evolution 21:381–385.
Jansen, M., R. Stoks, E. Decaestecker, A. Coors, F. Van De Meutter, and L. De Meester. 2010. Local exposure shapes spatial patterns in infectivity and community structure of *Daphnia* parasites. Journal of Animal Ecology 79:1023–1033.
Johnson, P. T. J., J. M. Chase, K. L. Dosch, R. B. Hartson, J. A. Gross, D. J. Larson, D. R. Sutherland, and S. R. Carpenter. 2007. Aquatic eutrophication promotes pathogenic infection in amphibians. Proceedings of the National Academy of Sciences USA 104:15781–15786.
Jousimo, J., A. J. M. Tack, O. Ovaskainen, T. Mononen, H. Susi, C. Tollenaere, and A. Laine. 2014. Ecological and evolutionary effects of fragmentation on infectious disease dynamics. Science 344:1289–1293.
Lafferty, K. D., et al. 2008. Parasites in food webs: the ultimate missing links. Ecology Letters 11:533–546.
Lafferty, K. D., A. P. Dobson, and A. M. Kuris. 2006. Parasites dominate food web links. Proceedings of the National Academy of Sciences USA 103:11211–11216.
Lafferty, K. D., and R. D. Holt. 2003. How should environmental stress affect the population dynamics of disease? Ecology Letters 6:654–664.
Laine, A. L., and A. Tellier. 2008. Heterogeneous selection promotes maintenance of polymorphism in host–parasite interactions. Oikos 117:1281–1288.
Lampert, W. 2011. *Daphnia*: development of a model organism in ecology and evolution. *In* O. Kinne, editor. Book 21. Excellence in ecology series. International Ecology Institute, Oldendorf/Luhe, Germany.
McKenzie, V. J., and A. R. Townsend. 2007. Parasitic and infectious disease responses to changing global nutrient cycles. EcoHealth 4:384–396.
Okamura, B., and S. W. Feist. 2011. Emerging diseases in freshwater systems. Freshwater Biology 56:627–637.
Patot, S., R. Allemand, F. Fleury, and J. Varaldi. 2012. An inherited virus influences the coexistence of parasitoid species through behaviour manipulation. Ecology Letters 15:603–610.
Pulkkinen, K. 2007. Microparasite transmission to *Daphnia magna* decreases in the presence of conspecifics. Oecologia 154:45–53.
R Core Team. 2014. R 2.15.2. R Project for Statistical Computing, Vienna, Austria. www.r-project.org
Refardt, D., E. Decaestecker, P. T. J. Johnson, and J. Vavra. 2008. Morphology, molecular phylogeny, and ecology of *Binucleata daphniae* ng, n. sp. (Fungi: Microsporidia), a parasite of *Daphnia magna* Straus, 1820 (Crustacea: Branchiopoda). Journal of Eukaryotic Microbiology 55:393–408.
Regoes, R. R., D. Ebert, and S. Bonhoeffer. 2002. Dose-dependent infection rates of parasites produce the Allee effect in epidemiology. Proceedings of the Royal Society B 269:271–279.
Scheffer, M., S. Carpenter, J. A. Foley, C. Folke, and B. Walker. 2001. Catastrophic shifts in ecosystems. Nature 413: 591–596.
Stirnadel, H. A., and D. Ebert. 1997. Prevalence, host specificity and impact on host fecundity of microparasites and epibionts in three sympatric *Daphnia* species. Journal of Animal Ecology 66:212–222.
Thrall, P., M. E. Hochberg, J. J. Burdon, and J. D. Beaver. 2007. Coevolution of symbiotic mutualists and parasites in a community context. Trends in Ecology and Evolution 22: 120–126.
Vale, P. F., M. Choisy, and T. J. Little. 2013. Host nutrition alters the variance in parasite transmission potential. Biology Letters 9:20121145.
Vale, P. F., and T. J. Little. 2012. Fecundity compensation and tolerance to a sterilizing pathogen in *Daphnia*. Journal of Evolutionary Biology 25:1888–1896.
Verreydt, D., L. De Meester, E. Decaestecker, M.-J. Villena, K. Van Der Gucht, P. Vannormelingen, W. Vyverman, and S. A. J. Declerck. 2012. Dispersal-mediated trophic interactions can generate apparent patterns of dispersal limitation in aquatic metacommunities. Ecology Letters 15:218–226.
Wolinska, J., B. Keller, M. Manca, and P. Spaak. 2007. Parasite survey of a *Daphnia* hybrid complex: host-specificity and environment determine infection. Journal of Animal Ecology 76:191–200.
Wolinska, J., and K. C. King. 2009. Environment can alter selection in host-parasite interactions. Trends in Parasitology 25:236–244.
Wood, C. L., J. E. Byers, K. L. Cottingham, I. Altman, M. J. Donahue, and A. M. H. Blakeslee. 2007. Parasites alter community structure. Proceedings of the National Academy of Sciences USA 104:9335–9339.
Zhang, Q.-G., and A. Buckling. 2011. Antagonistic coevolution limits population persistence of a virus in a thermally deteriorating environment. Ecology Letters 14:282–288.

Supplemental Material

Ecological Archives

The Appendix is available online: http://dx.doi.org/10.1890/14-1167.1.sm