**Title: Parasite and nutrient enrichment effects on *Daphnia* interspecific competition**

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**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.

Keywords:

Parasite-mediated interspecific host competition, nutrient enrichment, *Daphnia*, *Ordospora colligata*, *Binucleata daphniae*, 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 & 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 & 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).

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, Lafferty et al. 2006, Hatcher 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 ('key stone 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, it is rarely investigated to which extent nutrient availability affects the outcome of host competition in the presence of parasites. 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 and 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 and pers. observ.). 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 (Figure 1). In addition, we included a '*Daphnia* community' treatment by also inoculating *D. pulex* with *D. magna* in half of the mesocosms. There were three replicates per treatment combination, which resulted in 24 180 L mesocosms (90 cm diameter in width and 50 cm in 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 (Figure 1). Preparatory phases were performed in the laboratory (always under a 16:8 day:night regime at 20°C with a food concentration of $2.5 \times 10^5$ *Scenedesmus obliquus* cells per mL in 500 ml jars, Figure 1A and 1C) or in 800 L outdoor containers (120 cm in length, 70 cm in height and 100 cm in width, Figure 1B).

114 The *Daphnia* species in our experiment are cyclical parthenogenetically reproducing organisms.
115 Sexual eggs give rise to offspring that are genetically unique, which then reproduce
116 parthenogenetically. As a result, *Daphnia* populations are composed of multiple clones. Micro-
117 evolutionary responses of *Daphnia* populations to selection pressures, e.g. parasites, typically arise
118 from clonal selection. To allow for such micro-evolutionary responses in our experiments, we
119 created multiclonal populations of *D. magna* and *D. pulex*. These clones were extracted from the
120 dormant egg bank of pond OM2 (Heverlee, Belgium, 50°51'47.67"N; 4°43'16.36"E). *Daphnia*-
121 parasite coevolutionary interactions have been documented in this pond (Decaestecker et al. 2007,
122 2013). These *Daphnia* clones were reared as independent lines during two generations in the
123 laboratory in order to have sufficient animals for further inoculations (Figure 1A). Second, four 800
124 L containers were filled with water (2/3 distilled water and 1/3 tap water) on 27/06/2006 and an
125 inoculum of 26.67 x $10^9$ *Scenedesmus obliquus* cells was added. Two containers received a high
126 amount of nutrients (1 mg P $L^{-1}$ and 16 mg N $L^{-1}$), corresponding to hypereutrophic conditions in
127 lakes, whereas the other two containers received low nutrient additions (0.1 mg P $L^{-1}$ and 1.6 mg N
128 $L^{-1}$), corresponding to mesotrophic to eutrophic conditions (Figure 1B). P and N were given under
129 the form of $KH_2PO_4$ and $NaNO_3$, respectively. The molar ratio of P and N reflect the Redfield ratio.
130 To increase algal biomass, on 19/07/2006, we inoculated natural phytoplankton in addition to *S.*
131 *obliquus*. As such, the phytoplankton species pool was enriched, which increased the probability of
132 adding taxa that were able to grow well in the containers and contribute to a fast build-up of
133 phytoplankton biomass. For this, we collected water from 16 regional ponds and lakes (13 L),
134 filtered it three times over a 30 µm mesh and distributed it in equal parts over the containers.
135
136 At the start of August, when a significant difference in the chlorophyll *a* concentration between the
137 high and low nutrient addition treatments had developed, we added *Daphnia* from the laboratory
138 cultures to these 800 L containers (Figure 1B). One high and one low nutrient container each

139 received 1840 *D. magna* individuals composed of 23 clones (80 individuals per clone); the other
140 high and low nutrient containers received a similar number of *D. pulex* clones and individuals. We
141 performed this preparatory phase in order to obtain sufficient individuals to start up the final
142 mesocosm populations because we wanted to avoid loss of replication due to stochastic extinctions
143 resulting from low initial population sizes. We also wanted to pre-adapt populations to the
144 conditions in tanks and experimental nutrient levels, given that the introduction of laboratory grown
145 *Daphnia* to low nutrient conditions in mesocosms right after exposure to parasites could
146 substantially increase the risk of population extinctions.
147
148 On 11/08/2006, we randomly collected 6 sets of 150 *D. magna* juveniles from each of the 800 L *D.*
149 *magna* containers and exposed them during three days to either a control or a parasite spore solution
150 in 500 ml jars in the laboratory (Figure 1C). To maximize parasite uptake, the parasite spores were
151 kept in suspension by continuously rotating the jars during exposure. Half of the jars received a
152 homogenate of 100 infected *D. magna* individuals ('parasite exposure'), whereas the other half
153 received a homogenate of 100 non-infected *D. magna* individuals ('control exposure'). Parasite
154 spore solutions were made from infected *D. magna* individuals, collected from different localities in
155 Flanders. The parasite spore solution consisted of a homogenate of individuals infected with
156 *Ordospora colligata*, *Binucleata daphniae* and the parasite causing White Bacterial Disease.
157 Relative proportions of the parasites were not quantified. When setting up the mesocosm
158 experiment, we randomly assigned the content of the jars to the mesocosms of the parasite addition
159 treatments. *O. colligata* is a microsporidian species which infects the gut epithelium cells of
160 *Daphnia*. *B. daphniae* is a microsporidian species which infects the integument cells of the
161 hemocoel cavity of the carapax in *Daphnia*. White Bacterial Disease is caused by an infection of a
162 so far unknown agent, potentially a bacterial agent, which infects the fat cells of *Daphnia*.
163 Virulence levels induced by these parasites are variable, but transmission of all parasites is

164 horizontal and occurs upon the death of the host. For a more detailed description of these parasites
165 and their virulence effects, we refer to Refardt et al. (2008), Jansen et al. (2010), Coopman et al.
166 (2013).
167
168 We started the experiment (Figure 1D) on 14/08/2006 by filling 24 mesocosms (180 L) with water
169 from the 800 L containers. Water from each of the four 800 L tanks was used to fill the mesocosms
170 (not shown on Figure 1). Low nutrient mesocosms of the experiment received half of their water
171 from the 800 L low-nutrient tank with *D. magna* and half of their water from the 800 L low-nutrient
172 tank with *D. pulex*. Accordingly, high-nutrient mesocosms were filled with water from both high-
173 nutrient tanks. Before addition to the mesocosms, this water was first filtered three times over a
174 30µm mesh to remove zooplankton. Subsequently, 300 *Daphnia* individuals were inoculated per
175 mesocosm in four possible combinations: (1) 150 parasite-exposed *D. magna* + 150 non-exposed *D.*
176 *magna*, (2) 150 *D. magna* from the control exposure (*i.e.* being exposed to a control solution of
177 crushed, non-infected *D. magna*) + 150 non-exposed *D. magna*, (3) 150 parasite-exposed *D. magna*
178 + 150 non-exposed *D. pulex* and (4) 150 *D. magna* of the control exposure + 150 non-exposed *D.*
179 *pulex* (Figure 1D). Nutrient levels in the mesocosms were maintained through the weekly addition
180 of one tenth of the initial nutrient addition (see above), given that nutrients tend to show a gradual
181 decline in mesocosms with time (Declerck et al. 2007). Chlorophyll *a* was measured weekly
182 throughout the whole experiment with a Trilogy Laboratory Fluorometer, Turner Designs,
183 Sunnyvale, CA, USA. Differences in chlorophyll *a* levels between nutrient addition treatment levels
184 can be seen, indicating that the nutrient treatments have been effective (Figure S1).
185
186 To characterize the host and parasite populations, we collected 5 L medium at 23/10/2006. After
187 homogenizing the water column of each mesocosm, a sample was taken with a tube sampler and
188 filtered over a 100 µm mesh. Immediately upon sample isolation, all WBD infected individuals in

189 the sample were counted (otherwise phenotypic detection of this parasite is hampered, see also
190 Decaestecker et al. (2005) and Ebert (2005)). Subsequently, the samples were frozen at -18°C.
191 Upon thawing, we screened 20 adult *D. magna* and where possible 20 adult *D. pulex* for infections.
192 *B. daphniae* parasite load was estimated by determining parasite coverage of the carapax and scored
193 into classes ranging from class 0 with no infection to class 5 with completely covered carapax (as in
194 Decaestecker et al. 2005). To detect *O. colligata* infections, the animals were dissected and the
195 caecum was inspected for infection under a phase contrast microscope. Parasite load for this
196 parasite was estimated by the degree of caecum occupancy and scored into classes ranging from 0
197 to 5 (determined based on the number of spore clusters present as in Decaestecker et al. (2005)).
198 The remaining individuals were fixed with acid lugol solution for later enumeration (*D. magna* and
199 *D. pulex* density). To estimate host densities, we counted a minimum of 100 individuals in
200 subsamples of a known volume. We calculated parasite prevalence (percentage infected *Daphnia*
201 adults) and parasite infection intensity (average parasite load in infected individuals).
202
203 *Data analysis*
204 We tested the impact of nutrient addition, host community composition and exposure to parasites on
205 log(x+1)-transformed population densities of *D. magna and D. pulex* using three-way analysis of
206 variance. For mesocosms with parasite addition treatments only, we tested the effects of nutrient
207 addition and host community composition on logit-transformed prevalence data and untransformed
208 infection intensities for each of the different parasite taxa. All these analyses were performed using
209 the lm function of R 2.15.2 (R Core Team 2014).
210
211 **Results**

212 *D. pulex* was accidently introduced in the mesocosms of the pure *D. magna* treatment. As a result,
213 we observed no effects of the host community treatment on final population densities of both

214 species (Table 1). Irrespective from this, high nutrient levels invariably increased *D. magna* but
215 reduced *D. pulex* densities (Table 1, Figure 2A,B). The presence of parasites had a strong negative
216 effect on the population densities of *D. magna* but enhanced *D. pulex* densities (Table 1, Figure
217 2A,B). Consequently, the *D. pulex* to *D. magna* density ratio was highest under low nutrient
218 addition levels and in the presence of parasites (Figure 2C; ANOVA result on the *D. pulex* to *D.*
219 *magna* density ratio: nutrient addition treatment: $F(1,16) = 8.08$, $P = 0.0118$; parasite treatment:
220 $F(1,16) = 17.94$, $P < 0.001$). We observed no parasite by nutrient interaction effect on the densities
221 of any of the *Daphnia* species (Table 1).
222
223 All three parasites *O. colligata, B. daphniae* and WBD infected *D. magna* (average prevalences:
224 59.4%, 26% and 4.7%, respectively), whereas no infections of these parasites were found on *D.*
225 *pulex*. The prevalence and infection intensity of *B. daphniae* on *D. magna* was significantly higher
226 at high than at low nutrient levels (Table 2, Figure 3A). Both variables also tended to be higher in
227 the mixed *Daphnia* inoculation treatments than in the *D. magna* only treatments. In addition, we
228 observed a significant interaction effect of nutrient addition and *Daphnia* host community
229 composition on the prevalence of the parasite *O. colligata* (Table 2, Figure 3B). In the pure *D.*
230 *magna* treatment, prevalence of *O. colligata* was low at low nutrient levels (< 20 %) and very high
231 at high nutrient levels (>80 %). In the mesocosms that were initially stocked with both *D. magna*
232 and *D. pulex* the prevalence of this parasite was very high at both low and high nutrient
233 concentrations (Table 2, Figure 3 B). WBD prevalence was always low (<5%) and treatments did
234 not show significant effects (results not shown).
235
236 **Discussion**
237

238 The focal species *D. magna* was more prone to infection by parasites than the weaker competitor *D.*
239 *pulex*. *D. magna* is generally known as an efficient filter feeder that can strongly reduce the
240 population growth of other zooplankton organisms through resource competition, provided that
241 essential nutrients like phosphorus are not limiting (Lampert 2011, Hessen et al. 2013). In the
242 absence of parasites, *D. magna* strongly dominated the experimental mesocosms at the cost of *D.*
243 *pulex*, especially in high nutrient level treatments. Addition of parasites, however, resulted in a shift
244 in the species composition, favoring dominance by *D. pulex* over *D. magna*. Consequently, the
245 presence of parasites resulted in a reversal of the competitive hierarchy of the two competing
246 species. Our results suggest that parasitism can be an important driver of zooplankton community
247 composition in natural ecosystems. The three parasite species used in our experiment (WBD, *O.*
248 *colligata* and *B. daphniae*) were previously observed in natural populations of both *D. magna* and
249 *D. pulex*, but infections were always lower in *D. pulex* than in *D. magna* (personal observation and
250 other authors, see Stirnadel and Ebert (1997), Ebert (2005)). In our experiment, we were unable to
251 detect any parasites in *D. pulex*. Resistance of *D. pulex* to the parasites is probably the main reason
252 for the observed parasite-induced dominance shift between *D. magna* and *D. pulex*. The reason for
253 the unability of the parasites to infect *D. pulex* is likely the result of a high degree of host
254 specialization of the parasite strains we used. Nevertheless, given that *D. pulex* in the field has
255 repeatedly been shown to be less sensitive to infections than *D. magna*, we are confident that our
256 experiment is representative for shallow freshwater ecosystems in Europe.
257
258 Average parasite prevalence in our experimental *D. magna* populations is comparable with what is
259 found for these parasites in a field study (Decaestecker et al. (2005). High nutrient levels resulted in
260 higher population densities of *D. magna* and increased parasite prevalences (*B. daphniae* and *O.*
261 *colligata*) and infection intensities (*O. colligata*) in this species. By increasing primary productivity,
262 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 & 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 & Little 2012), often due to stoichiometric changes within the host (Aalto & 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 x 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 unlikely that evolutionary responses in the host population have resulted in shifts towards 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 towards 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* towards 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* & *D. pulex* treatment comprised half of the *Daphnia* biomass already from the start of the experiment. The observed host community treatment effects on parasite-related variables therefore suggest that infection of *D.*

313 *magna* has been mediated by the early presence of *D. pulex*, but lack of detailed data on the
314 temporal dynamics of both *Daphnia* and parasite populations throughout the experiment hamper a
315 more profound interpretation of the mechanisms that have resulted in the observed host community
316 effects on parasite related variables. Apart from our inability to evaluate the dependency of parasite
317 effects on *D. magna* in the absence of *D. pulex*, the contamination does not affect the main
318 conclusion of our study, namely that parasites can reverse the competitive hierarchy between
319 *Daphnia* species, and that increased nutrient availability can intensify the degree of parasitism on *D.*
320 *magna*, although the latter does not necessarily need to result in a disproportional reduction of the
321 population densities of this species as compared to low nutrient levels.
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**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.

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Zhang Q.-G., and A., Buckling. 2011. Antagonistic coevolution limits population persistence of a virus in a thermally deteriorating environment. Ecol. Lett. **14:** 282-288.

568 Table 1. ANOVA results testing for the effects of the experimental treatments on *Daphnia* density.

569

| *D. magna* | DF | Sum Sq | Mean Sq | F | p |
|---|---|---|---|---|---|
| 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 |
| CommunityxProductivity | 1 | 0.0049 | 0.0049 | 0.0925 | 0.7649 |
| CommunityxParasite | 1 | 0.0119 | 0.0119 | 0.2260 | 0.6409 |
| ProductivityxParasite | 1 | $1.2x10^{-6}$ | $1.2x10^{-6}$ | $2.3x10^{-5}$ | 0.9962 |
| CommunityxProductivityxParasite | 1 | 0.0002 | 0.0002 | 0.0038 | 0.9515 |
| Residuals | 16 | 0.8441 | 0.0528 | NA | NA |
| *D. pulex +1* | DF | Sum Sq | Mean Sq | F | p |
| 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 |
| CommunityxProductivity | 1 | 0.3333 | 0.3333 | 1.6995 | 0.2108 |
| CommunityxParasite | 1 | 0.3999 | 0.3999 | 2.0395 | 0.1725 |
| ProductivityxParasite | 1 | 0.0318 | 0.0318 | 0.1623 | 0.6924 |
| CommunityxProductivityxParasite | 1 | 0.0026 | 0.0026 | 0.0131 | 0.9102 |
| Residuals | 16 | 3.1377 | 0.1961 | NA | NA |

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573

574 Table 2. ANOVA results testing for the effects of the experimental treatments on the prevalence and
575 infection intensity of *D. magna* parasites. We defined the prevalence as the percentage of infected
576 *D. magna* individuals and infection intensity as the average parasite load in infected *D. magna*.

| *Binucleata* prevalence | DF | Sum Sq | Mean Sq | F | p |
|---|---|---|---|---|---|
| Productivity | 1 | 32.816 | 32.816 | 19.062 | 0.002 |
| Host community | 1 | 21.210 | 21.210 | 12.320 | 0.008 |
| ProductivityxCommunity | 1 | 0.741 | 0.741 | 0.430 | 0.530 |
| | 8 | 13.772 | 1.722 | NA | NA |

| *Ordospora* prevalence | DF | Sum Sq | Mean Sq | F | p |
|---|---|---|---|---|---|
| Productivity | 1 | 29.698 | 29.698 | 16.443 | 0.004 |
| Host community | 1 | 12.283 | 12.283 | 6.800 | 0.031 |
| ProductivityxCommunity | 1 | 22.680 | 22.680 | 12.557 | 0.008 |
| Residuals | 8 | 14.449 | 1.806 | NA | NA |

| *Binucleata* infection intensity | DF | Sum Sq | Mean Sq | F | p |
|---|---|---|---|---|---|
| Productivity | 1 | 22.963 | 22.963 | 36.115 | 0.0003 |
| Host community | 1 | 3.203 | 3.203 | 5.038 | 0.055 |
| ProductivityxCommunity | 1 | 0.403 | 0.403 | 0.634 | 0.449 |
| Residuals | 8 | 5.087 | 0.636 | NA | NA |

| *Ordospora* infection intensity | DF | Sum Sq | Mean Sq | F | P |
|---|---|---|---|---|---|
| Productivity | 1 | 5.741 | 5.741 | 3.086 | 0.117 |
| Host community | 1 | 1.141 | 1.141 | 0.613 | 0.456 |
| ProductivityxCommunity | 1 | 2.341 | 2.341 | 1.259 | 0.294 |
| Residuals | 8 | 14.880 | 1.860 | NA | NA |

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586 **Figure legends**

587

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

599

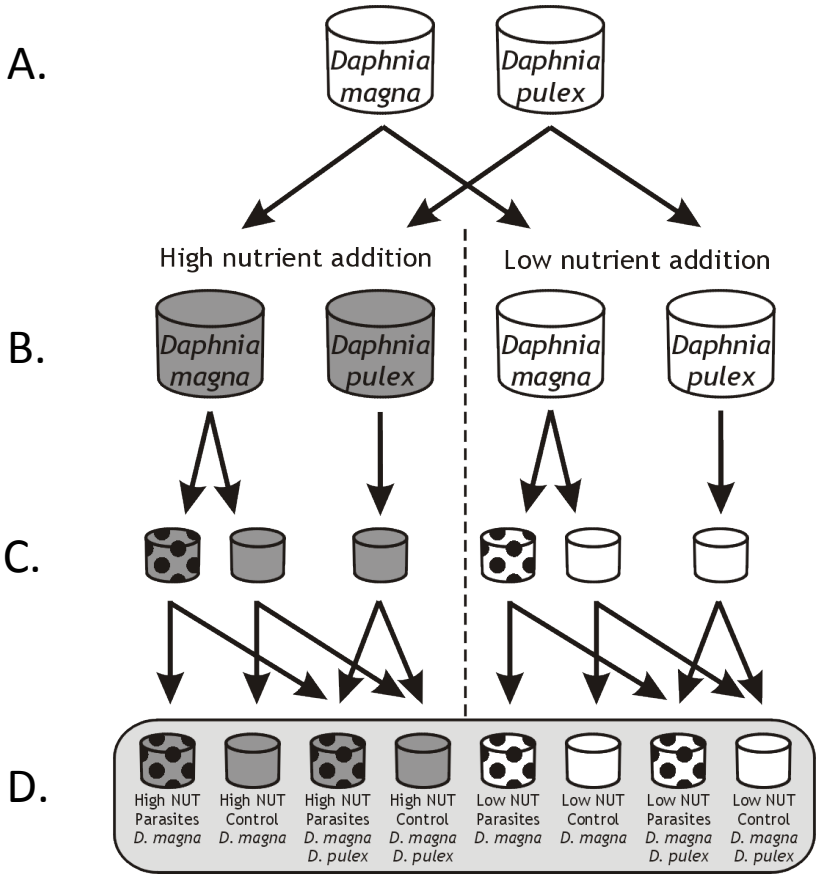
600 Figure 2. Population densities of *Daphnia magna* (A) and *Daphnia pulex* (B), and the *D. pulex* to *D.*
601 *magna* ratio (C) for each of the multi-factorial treatment combinations at the end of the experiment.
602 Error bars denote the standard error. Left panel is the *D. magna* inoculation treatment, right panel is
603 the *D. magna & D. pulex* inoculation treatment. Note that *D. pulex* indivduals accidentialy
604 contaminated the containers of the *D. magna* inoculation treatment. Black and white circles
605 represent the parasite and control treatments, respectively.

606

607 Figure 3. *B. daphniae* prevalence and infection intensity (A) and *O. colligata* prevalence and
608 infection intensity (B) in *D. magna* for each of the nutrient by host community treatment
609 combinations. Error bars denote the standard error. Black and white circles indicate the *D. magna*

610 and the *D. magna* & *D. pulex* inoculation treatment, respectively. Only treatments with initial
611 parasite exposure are shown.

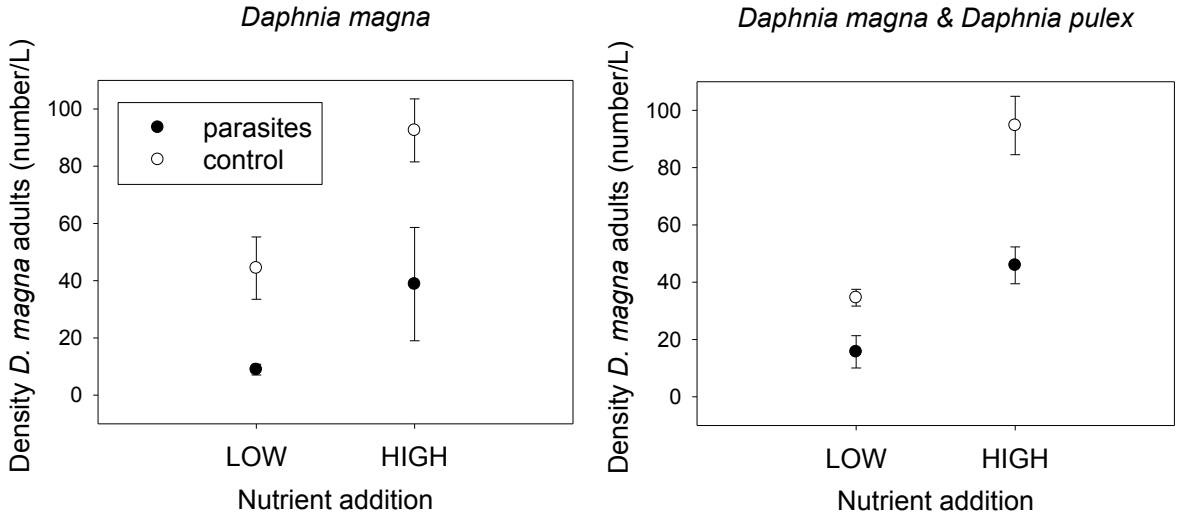
612 Figure 1



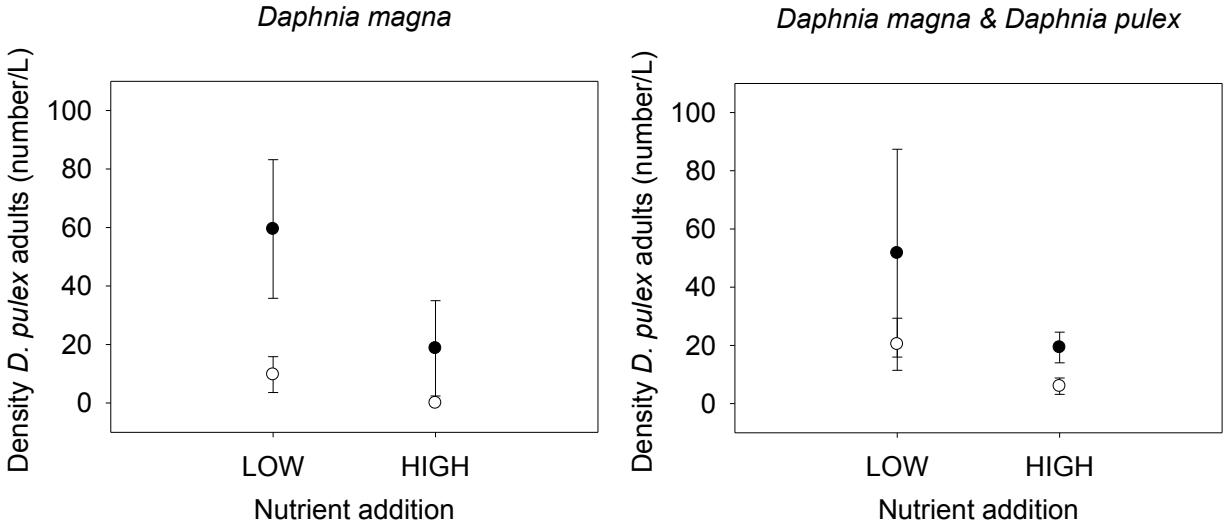

613

Figure 2

A


Daphnia magna
Daphnia magna & Daphnia pulex
parasites
control
Density D. magna adults (number/L)
0
20
40
60
80
100
LOW
HIGH
Nutrient addition

B


Daphnia magna
Daphnia magna & Daphnia pulex
Density D. pulex adults (number/L)
0
20
40
60
80
100
LOW
HIGH
Nutrient addition

C

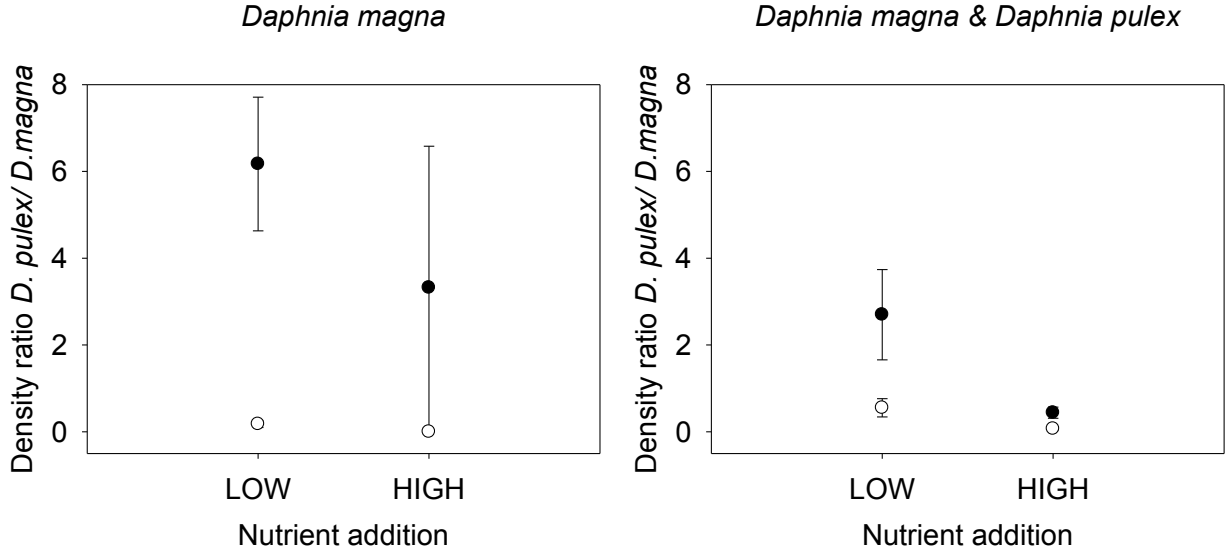
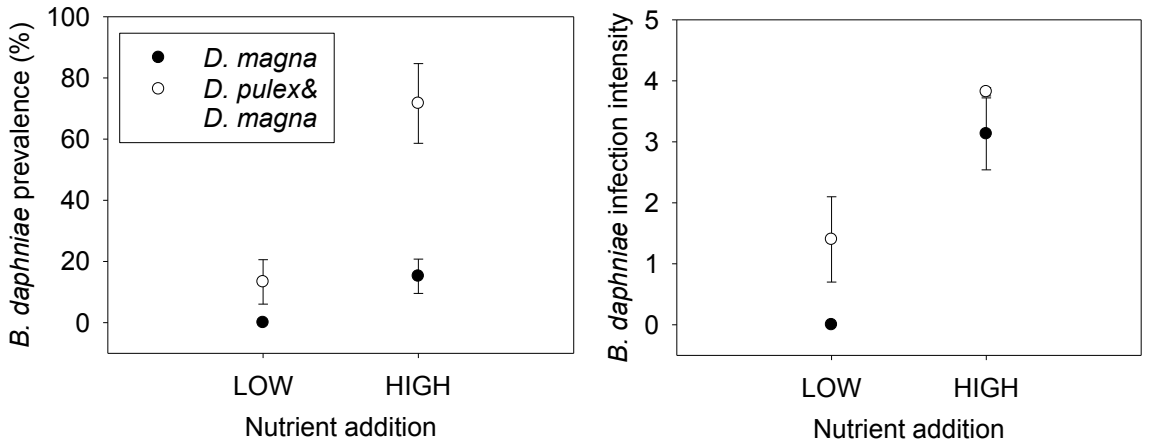
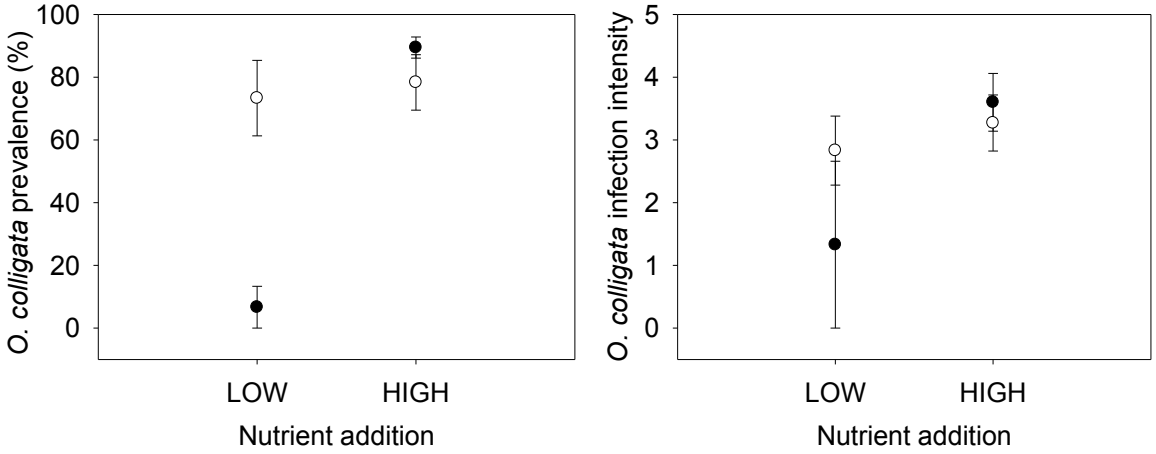

Daphnia magna
Daphnia magna & Daphnia pulex
Density ratio D. pulex/ D.magna
0
2
4
6
8
LOW
HIGH
Nutrient addition

Figure 3

A


B. daphniae prevalence (%)
100
80
60
40
20
0
D. magna
D. pulex&
D. magna
LOW
HIGH
Nutrient addition
B. daphniae infection intensity
5
4
3
2
1
0
LOW
HIGH
Nutrient addition

B


O. colligata prevalence (%)
100
80
60
40
20
0
LOW
HIGH
Nutrient addition
O. colligata infection intensity
5
4
3
2
1
0
LOW
HIGH
Nutrient addition