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# No uniform associations between parasite prevalence and environmental nutrients

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**Abstract.** The resource quality of the host has been shown to affect parasite transmission success, prevalence, and virulence. Seasonal availability of environmental nutrients alters density and stoichiometric quality (carbon–nutrient ratios) of both producers and consumers, suggesting that nutrient availability may drive fluctuations in parasite prevalence patterns observed in nature. We examined the interactions between the population dynamics of a keystone herbivore, *Daphnia*, and its parasites, and their associations with water nutrient concentrations, resource quantity and quality, and other environmental variables (temperature, pH, oxygen concentration) in a small lake, using general linear models. We found that the prevalence of two gut endoparasites was positively related to food source and quality as well as nitrogen content of *Daphnia*, whereas the prevalence of an epibiont and overall parasite species richness was negatively related to phosphorus content of *Daphnia*. When only endoparasite species richness was considered, no connections to nutrients were found. *Daphnia* density was not connected to parasites, but we found interactions between *Daphnia* fecundity and parasite prevalence. Overall, our results suggest that environmental nutrient concentrations and stoichiometric quality of the host have the potential to affect seasonality in parasite epidemics, but the connections between environmental carbon : nutrient ratios and parasite prevalence patterns are diverse and species specific.

**Key words:** *Daphnia longispina*; epibionts; host–parasite interactions; *Larssonia obtusa*; microsporidia; *Pasteuria ramosa*; seasonal epidemics; stoichiometry.

## INTRODUCTION

Anthropogenic alterations of global nutrient cycles and eutrophication have been suggested to promote parasite epidemics globally (Lafferty 1997, Townsend et al. 2003, Johnson et al. 2007, 2010, McKenzie and Townsend 2007); indirectly by affecting host availability (Johnson et al. 2007), or directly by enhancing parasite growth and virulence (Bruno et al. 2003, Voss and Richardson 2006). While increases in nutrient input are often beneficial for parasites (Johnson et al. 2010), the effects of environmental nutrient enrichment on parasite epidemics might be ecologically complex and context dependent. The parasite prevalence patterns (the proportion of infected individuals in a host population) have been poorly studied under the prevailing nutrient regimes, making it hard to predict the species-specific responses of different parasites to anthropogenic alterations in nutrient cycles.

Generally, the incidence of parasites is tightly linked to host population dynamics, as host density and size distribution (Stirnadel and Ebert 1997, Bittner et al. 2002, Hall et al. 2007) or host genotype and genotypic

diversity (Little and Ebert 1999, Decaestecker et al. 2003, Altermatt and Ebert 2008) affect the establishment and development of parasite epidemics. Parasites, in turn, are capable of shaping host population dynamics (Hudson et al. 1998, Johnson et al. 2009, Hall et al. 2011), through modifying life-history traits of host individuals. However, recent experimental evidence suggests that in addition to host availability, nutritional status and especially the stoichiometric quality (i.e., carbon–nitrogen–phosphorus ratio [C:N:P] of the host) are connected to parasite transmission success, production of infective propagules within the host, and parasite virulence (Pulkinen and Ebert 2004, Frost et al. 2008a, b, Hall et al. 2009, Seppälä et al. 2011). Host population dynamics and nutritional status, in turn, are altered by environmental conditions. Thus, it could be assumed that changes in the environment could lead to changes in parasite infection patterns.

Indeed, in nature, seasonal changes in various environmental variables affect parasite prevalence patterns (Altizer et al. 2006): temperature, rainfall, predation pressure (Duffy et al. 2005), or habitat characteristics (Cáceres et al. 2006, Johnson et al. 2009) have been found to explain fluctuations in disease dynamics. Seasonal changes in the availability of the main limiting nutrients, N and P, drive fluctuations in the biomass and production of autotrophs and consum-

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ers in both terrestrial and aquatic ecosystems (Elser et al. 2007). In addition, changes in N and P concentrations also alter the resource quality of the consumers, and consequently their nutrient ratios (C:N:P), which may cascade further up to parasite epidemics. Thus, environmental N and P concentrations have high, yet undescribed, potential to drive parasite prevalence patterns. However, the interactions between host resource quality and parasite epidemics have rarely been described in natural environments.

In this study, we examined the role of environmental nutrient concentrations in explaining the dynamics of a highly abundant *Daphnia longispina* (O. F. Müller) population and its parasites in a lake lacking fish predation. *Daphnia* are P rich as compared to other zooplankton, and thus sensitive to nutrient ratios of food sources (Sterner and Hessen 1994). *Daphnia* are hosts to numerous endoparasites and epibionts, which reduce their growth, reproduction, and survival (e.g., Green 1974, Stirnadel and Ebert 1997, Ebert 2005). In freshwater ecosystems, *Daphnia* have a major impact on food-web structure, affecting the flow of energy and nutrients between producers and upper trophic levels (Jansson et al. 2007, Lampert 2011). In our tri-trophic system, the two upmost trophic levels, parasites and *Daphnia*, have a reciprocal relationship, which is possibly affected by bottom-up effects of resource quantity and quality. Specifically, we hypothesized that water inorganic N and P concentrations would influence parasite infection patterns by affecting the quantity and/or quality of *Daphnia* food sources and hence either availability or stoichiometric quality of *Daphnia* hosts. Based on the current view (e.g., Johnson et al. 2007, 2010), which suggests that eutrophication is often connected with increases in disease incidence, we expected to see parallel patterns in prevalence of different parasites in relation to nutrient concentrations.

## METHODS

### *Study site*

Lake Mekkojärvi is a shallow (mean depth 3 m) humic lake in southern Finland (61°13' N, 25°8' E; see Appendix A). During open-water season (May–November), the lake has a narrow oxic and euphotic epilimnion (0.5–1.0 m). The zooplankton community of the lake is dominated by *Daphnia longispina*, with biomass fluctuating between 1000 and 20 000 individuals/m<sup>2</sup> during the open water season (Salonen and Lehtovaara 1992). Both phytoplankton and bacteria (especially methanotrophic bacteria) are important food sources for *D. longispina* in Lake Mekkojärvi.

In spring and early summer, *Daphnia* feed mainly on abundant epilimnetic phytoplankton, but switch to a bacterial diet in late summer and autumn when phytoplankton biomass is low (Kankaala et al. 2006, Taipale et al. 2008). In order to feed on bacteria, *Daphnia* have to migrate to the oxic–anoxic boundary layer, where, e.g., methanotrophic bacteria and green

sulfur bacteria are most abundant (Taipale et al. 2009, Karhunen et al. 2013).

In 2007, we surveyed the parasite community and found four prevalent endoparasite species infecting *D. longispina* in the lake. Subsequently we identified one bacterium (*Pasteuria ramosa*) and one microsporidian (*Larssonia obtusa*) species. The smaller of the remaining two endoparasites (unidentified small gut parasite, hereafter referred to as USGP) is most likely a microsporidian. It forms clusters with varying numbers of spores (15–30 spores per cluster) inside the gut epithelial cells. The larger endoparasite (unidentified large gut parasite, hereafter referred to as ULGP) is oval and ~20 µm long. It can be found interstitially, or possibly within the epithelial cells. We also found one epibiont, *Vorticella* sp., colonizing *D. longispina*.

### *Sampling and environmental variables*

We sampled Lake Mekkojärvi 25 times during the ice-free period from May until September or November between 2008 and 2010. Sampling intervals ranged from two to four weeks throughout the ice-free periods. We took water samples from the epilimnion (0–0.6 m), metalimnion (0.6–1.2 m), and hypolimnion (1.2–3.0 m), using a Limnos tube sampler (height 60 cm, volume 4.25 L; Limnos, Turku, Finland). We passed samples through a 100-µm plankton net to collect *Daphnia* for calculating their densities. From the water passed through the net, we measured total N and P concentrations (TN and TP, respectively), chlorophyll *a* concentrations, and water inorganic N and P concentrations (NH<sub>4</sub>, NO<sub>2</sub> + NO<sub>3</sub>, PO<sub>4</sub>; see Appendix A). TN and TP were used as proxies of the stoichiometric quality of the food sources (Järvinen and Salonen 1998) and chlorophyll *a* as a proxy of food quantity. We took additional zooplankton samples as vertical hauls from the whole water column using a 100-µm plankton net. We either counted the parasites within these additional samples, or ran them for nutrient and stable isotope analyses. We measured oxygen (mg O<sub>2</sub>/L) and temperature (°C) at 0.2, 0.5, 1.0, 2.0, and 3.0 m using a YSI 55 probe (accuracy 0.3°C and 0.3 mg O<sub>2</sub>/L; Yellow Springs Instruments, Yellow Springs, Ohio, USA) and subsequently averaged these values (oxygen across the epilimnion and temperature across the whole water column) to generate a single value per sampling date. In order to estimate periods of ice cover and thermal stratification, temperature was also surveyed with data loggers at depths of 0.2, 0.5, 1.0, 2.0, and 3.0 m between March 2008 and September 2009. The sampling period was divided into three seasons based on the water surface temperature: spring (<10°C), summer (≥10°C), and autumn (<10°C).

### *Host stoichiometry and population dynamics*

We determined the stoichiometry (C [%], N [%], P [%], C:N, C:P, and N:P) and stable isotope values of carbon and nitrogen (δ<sup>13</sup>C and δ<sup>15</sup>N) of *Daphnia* (see Appendix

A). Stable carbon isotope values can be used as indicators of dietary carbon sources (Vander Zanden and Rasmussen 1999). In Lake Mekkajärvi, bacteria have more negative  $\delta^{13}\text{C}$  values than phytoplankton. Lower  $\delta^{13}\text{C}$  values of *Daphnia* would thus indicate *Daphnia* feeding proportionally more on methanotrophic and other bacteria, located in the oxic–anoxic boundary layer, than on phytoplankton located in the epilimnion (Taipale et al. 2008). For easier interpretation of the results, we multiplied the values of  $\delta^{13}\text{C}$  in our data by  $-1$  to make a new variable with higher values indicating a higher proportion of bacteria in the diet ( $_{\text{rev}}\delta^{13}\text{C}$ , or reverse  $\delta^{13}\text{C}$ ). Stable nitrogen values give information on seasonal variation in the nitrogen source of producers, which is further reflected in nitrogen values of *Daphnia*, or on the trophic position of *Daphnia* (Lehmann et al. 2004, Matthews and Mazumder 2007). In Lake Mekkajärvi, the  $\delta^{15}\text{N}$  values of the main nitrogen sources of producers differ,  $\delta^{15}\text{N}$  of  $\text{NH}_4$  being higher than  $\delta^{15}\text{N}$  of  $\text{NO}_2 + \text{NO}_3$  (Taipale et al. 2008).

For the *Daphnia* density calculation, zooplankton was preserved in 70% ethanol. We counted the total number of *D. longispina* and the number of eggs/embryos in the brood pouch. We also measured the lengths of 100 *Daphnia* individuals per sample from the top of the head to the base of the tail spine under a preparation microscope (40 $\times$  magnification; Olympus SZX9, Olympus, Hamburg, Germany). We estimated mean *Daphnia* density and length, and mean fecundity as mean number of gravid per adult female ( $>1.24$  mm), as in Johnson et al. (2009).

#### *Infection prevalence, endoparasite and epibiont richness*

To assess the presence of parasites, we screened 50–150 live adult *Daphnia* per sampling date (as in Stirnadel and Ebert 1997, Decaestecker et al. 2005, Ebert 2005). First, we screened *Daphnia* for visible signs of parasite infections under a preparation microscope with transmitted light, and for epibiont *Vorticella* sp. individuals attached to the carapace. Infections with *P. ramosa* or *L. obtusa* could be recognized as a whitish mass either in the body cavity or in the neck of hosts, and the presence was further verified by examination of the dissected animal under phase-contrast microscope (400 $\times$  magnification; Leitz Biomed, Leica Microsystems, Wetzlar, Germany). We dissected the gut of the animals from the body in order to verify USGP and ULGP infections. The species-specific prevalence of a parasite was calculated as the number of *Daphnia* infected with the parasite species per total females examined per sampling date, and overall endoparasite prevalence as the number of *Daphnia* infected with any of the endoparasites per total females examined per sampling date. The overall parasite/endoparasite species richness was estimated as the mean of the number of all parasite species/endoparasite species per infected *Daphnia* per sampling date. The parasite-induced overall fecundity reduction

for each parasite species was calculated as the difference between fecundity of infected to uninfected adult females (Decaestecker et al. 2005).

#### *Statistical analysis*

In our statistical analysis, we used epilimnetic values of TN, TP, and chlorophyll *a* concentration to represent algal food quality and quantity, since light and oxygen conditions do not allow significant algal production in lower water layers. Concomitantly, we used epilimnetic water values for following the connections between inorganic N and P concentrations, resource quality and quantity, and *Daphnia* stoichiometry. We conducted principal component analyses (PCA) for the seston and water nutrient concentrations (TN, TP,  $\text{NH}_4$ ,  $\text{NO}_2 + \text{NO}_3$ ,  $\text{PO}_4$ ), and for the elemental content of *Daphnia* (C [%], N [%], P [%], C:N, C:P, N:P) in order to reduce dimensionality of the data and to transform interdependent variables into significant and independent components, which facilitated fitting the subsequent general linear models due to the reduction of collinear variables. We assessed the significance of principal components by the broken-stick method (i.e., sharp decline in consecutive eigenvalues; Cattell 1966). We used general linear models (GLM) to explore the relationships of the prevalence of a certain parasite species, overall parasite species richness, or endoparasite species richness to biotic and environmental variables, including pH, mean epilimnetic oxygen concentration, mean water temperature, algal food quality (epilimnetic chlorophyll *a* concentrations), *Daphnia* density, mean length of *Daphnia*, stable carbon and nitrogen isotope values ( $_{\text{rev}}\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ) of *Daphnia*, and principal components of water and seston nutrient concentrations (PC1\_Nutrients, PC2\_Nutrients) and the stoichiometry of *Daphnia* (PC1\_ *Daphnia*, PC2\_ *Daphnia*). In addition, we studied the relationships of *Daphnia* density and *Daphnia* fecundity with overall endoparasite prevalence and *Vorticella* sp. prevalence. In these models, we included the variables listed and overall endoparasite and *Vorticella* sp. prevalence as predictor variables (excluding *Daphnia* density and *Daphnia* mean length as predictors when *Daphnia* density was dependent variable). Year and season were added to all models as categorical variables to control for the nonindependence of observations arising from the similarity of sampling conditions. To normalize the data, log-transformation was used for  $\text{PO}_4$  and  $\text{NO}_2 + \text{NO}_3$ , reciprocal transformation for  $\text{NH}_4$ , square-root-transformation for chlorophyll *a* concentration, *Vorticella* prevalence, *Daphnia* density, and fecundity, arcsine-square-root-transformation for USGP prevalence, and exponential transformation for overall endoparasite prevalence. Due to several predictor variables, we analyzed only the main effects. The Akaike information criterion (AIC) was used for selecting the minimum adequate model. Model selection was carried out using the stepAIC function from R package MASS (Venables and Ripley 2002).

This function is a stepwise algorithm that sequentially searches through all possible models for the one that minimizes the AIC. The stepwise search was performed both ways (i.e., backward and forward), as regular one-step approximations are considered less robust and less profitable (Venables and Ripley 2002). To test if the main effects of categorical variables (year and season) were significant, GLM models with and without categorical variables were compared with log-likelihood tests. To further ensure that time dependency of observations were correctly taken into account by including year and season effects, we confirmed that there was no autocorrelation between residuals in any of the presented models with autocorrelation function (acf) and partial autocorrelation function (pacf) plots. The overall fecundity reduction of each parasite was assessed by testing for differences in the clutch size of uninfected and infected *Daphnia* with Wilcoxon matched pairs tests. When assessing the overall fecundity reduction of *Vorticella* sp., we excluded endoparasitized *Daphnia* from the data set, as in Decaestecker et al. (2005). All statistical analyses were carried out using R version 3.0.1 (R Development Core Team 2013).

## RESULTS

### *Environmental variables*

All environmental variables showed both annual and seasonal dynamics (see Fig. B1 in Appendix B). Water inorganic N and P concentrations and algal food quality (TN and TP) varied significantly between seasons and years (Fig. 1A). For the PCA of the seston and water nutrient concentrations, the scree plot suggested two factors (Table 1), which collectively explained 79% of the variance. The first component (PC1\_Nutrients) described the concentrations of inorganic N and P (NH<sub>4</sub> in positive loading and NO<sub>2</sub> + NO<sub>3</sub> and PO<sub>4</sub> in negative loadings), and the second component (PC2\_Nutrients) described concentrations of epilimnetic TN and TP in positive loadings (proxy of algal quality in the epilimnion).

### *Host stoichiometry and population dynamics*

The elemental content of *Daphnia* varied considerably annually and seasonally (Fig. 1B, Fig. B2 in Appendix B). *Daphnia* C:N, C:P, and N:P ratios decreased with increases in algal food quality (increase in epilimnetic TN and TP), and were highest in midsummer and declined towards autumn (Fig. 1B). For the stoichiometry of *Daphnia*, two principal factors were retained (Table 1), with a total of 87% of the variance explained. The first component (PC1\_Daphnia) described P and C content of *Daphnia* (P [%] in positive loading, C [%], C:P, and N:P in negative loading), while the second component (PC2\_Daphnia) described N content (N [%] in positive loading and C:N in negative). Stable carbon and nitrogen isotope values of *Daphnia* showed seasonal variation (Fig. 1B), indicating seasonal changes in food sources of *Daphnia*.

We found *D. longispina* consistently throughout the open water season, but the density varied considerably from 420 to 48 200 individuals/m<sup>3</sup> (Fig. 2A). The *Daphnia* population showed two to three peaks during a sampling period. In May, immature *Daphnia* dominated the population, while a low number of females with a high number of eggs were also present. In midsummer, another density peak was observed. In 2008 and 2009, the highest *Daphnia* density was seen in late autumn. The proportion of adult females ranged between 0.05 and 0.98, being highest during the midsummer peak or in late autumn. Sexually reproducing females (bearing resting eggs) were observed from September onward each year.

### *Parasite dynamics*

The prevalence of the five parasite species varied highly between the species. Prevalence of both gut parasites, USGP and ULGP, showed one to two peaks during a sampling period. In addition, the prevalence of USGP showed annual and seasonal variation (Table 2). Prevalence of the epibiont *Vorticella* sp. showed high seasonal variation, but exhibited the highest peaks in midsummer (Fig. 2B, Table 2). Both *Pasteuria ramosa* and *Larssonia obtusa* occurred sporadically and showed rather low prevalence throughout the study period (Appendix B: Fig. B3), and were thus not included in statistical analyses, except for the overall parasite species richness/endoparasite species richness and overall endoparasite prevalence. *P. ramosa* was typically observed after mid-August while *L. obtusa* was found almost throughout the open water season, except in 2010. Multiple infections occurred in 24% ± 6% (mean ± SE) of infected *Daphnia*.

### *The interaction between environmental variables and host-parasite dynamics*

USGP prevalence increased with algal food quality (PC2\_Nutrients), pH, and with a decrease in  $_{\text{rev}}\delta^{13}\text{C}$  values, indicating a decrease in the proportion of bacteria and increase in the proportion of algae in diet of *Daphnia* (Table 2). In ULGP, prevalence was positively associated with the mean length of *Daphnia*, the proportion of bacteria in the diet of *Daphnia* ( $_{\text{rev}}\delta^{13}\text{C}$  values), and PC2\_Daphnia, indicating that ULGP prevalence increased with an increase in the N content of *Daphnia* (decrease in C:N). The prevalence of *Vorticella* sp., as well as the overall parasite species richness, increased with temperature, but were negatively related to PC1\_Daphnia describing the P content of the host (positively to C, C:P, N:P). The endoparasite species richness was best explained with a rather complicated model, but only a positive association with the mean length of *Daphnia* was statistically significant (Table 2).

*Daphnia* density decreased with algal food quantity, the proportion of bacteria in the diet of *Daphnia* (increase in the proportion of algae, decrease in  $_{\text{rev}}\delta^{13}\text{C}$

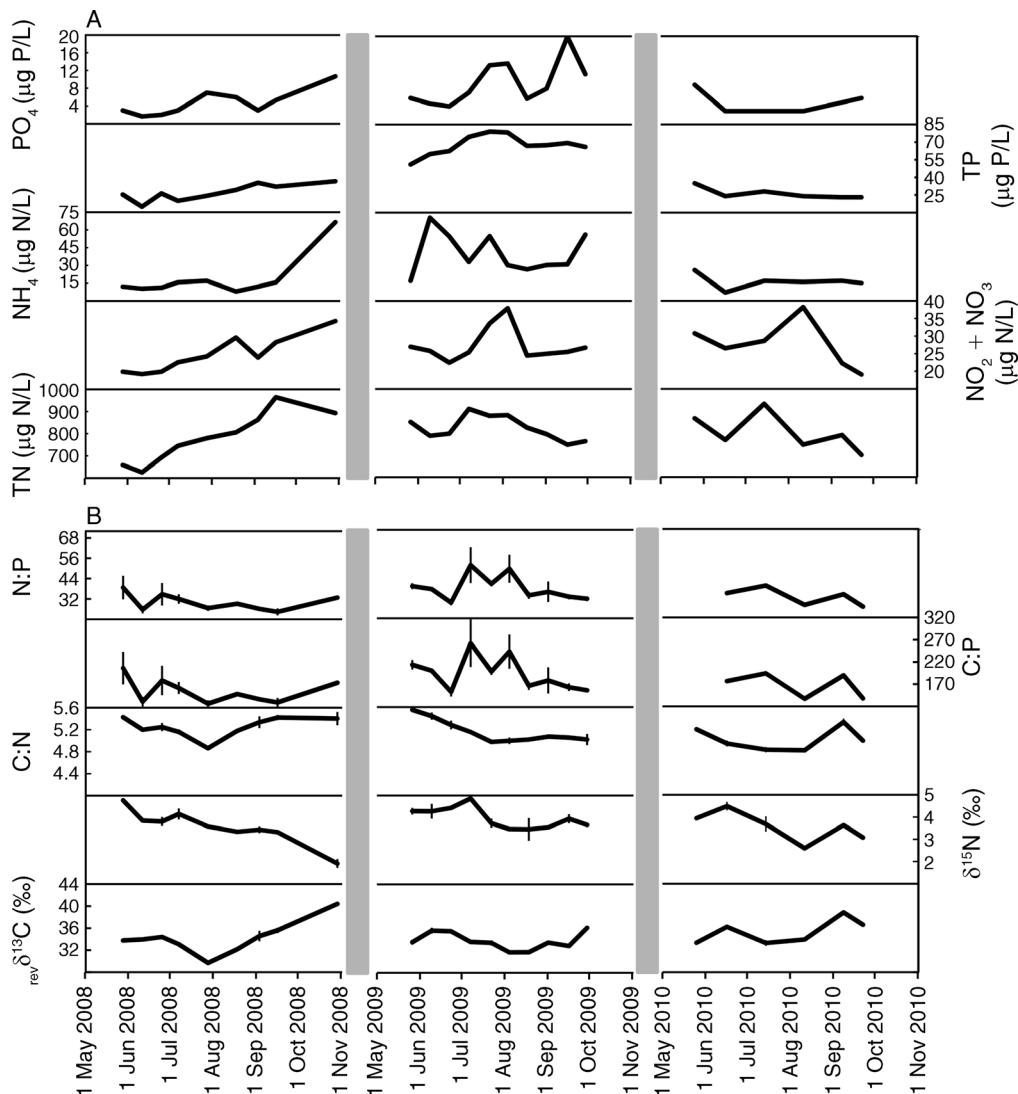


FIG. 1. (A) Inorganic and seston nutrient concentrations (PO<sub>4</sub>, NH<sub>4</sub>, NO<sub>2</sub> + NO<sub>3</sub>, total P, and total N) in the epilimnion of Lake Mekkojärvi during the open water season between years 2008 and 2010 on 25 sampling occasions. (B) Stable carbon and nitrogen isotope values (<sup>rev</sup>δ<sup>13</sup>C, δ<sup>15</sup>N) and C:N, C:P, and N:P of *Daphnia* for the same period (mean ± SE for two to three replicates). Reverse (rev) δ<sup>13</sup>C values were used in order to clarify their interpretation. Gray bars denote breaks between sampling seasons due to ice cover.

value of *Daphnia*; Table 2), and δ<sup>15</sup>N values. Fecundity increased with algal food quality (PC<sub>2</sub>\_Nutrients), temperature, oxygen, and *Daphnia* density, and decreased with overall endoparasite prevalence. *L. obtusa* and the epibiont *Vorticella* sp. induced significant overall fecundity reductions at the individual level (Fig. 3).

#### DISCUSSION

By combining environmental variables, host density, host stoichiometry, and parasite dynamics, our study revealed connections between prevailing environmental nutrient concentrations and parasite dynamics. We expected higher water inorganic N and P concentrations

to lead to higher *Daphnia* food quality (higher TN and TP concentrations), which would further result in higher availability of susceptible *Daphnia* hosts, or in higher N and P content (i.e., lower C:P and C:N) in *Daphnia*, increasing their quality as a resource for parasites. These were further expected to lead to higher parasite prevalence in the host population or higher overall parasite species richness within individual hosts (Frost et al. 2008a, Hall et al. 2009). When inspecting the prevalence patterns of each parasite species separately, we found that host food quality or host N content was positively associated with the prevalence of the two most common endoparasites, USGP and ULGP. The two other endoparasitic species, *L. obtusa* and *P. ramosa*,

TABLE 1. The loadings for principal component analysis of epilimnetic water and seston nutrient concentrations and of *Daphnia* stoichiometry.

A) Nutrient concentrations		
Nutrients	PC1_Nutrients	PC2_Nutrients
TN ( $\mu\text{g N/L}$ )	-0.406	0.513
TP ( $\mu\text{g P/L}$ )	-0.378	0.639
NH <sub>4</sub> ( $\mu\text{g N/L}$ )	0.457	0.429
NO <sub>2</sub> + NO <sub>3</sub> ( $\mu\text{g N/L}$ )	-0.492	-0.333
PO <sub>4</sub> ( $\mu\text{g P/L}$ )	-0.491	-0.183
Variance explained	60%	19%
B) <i>Daphnia</i> stoichiometry		
Stoichiometry	PC1_Daphnia	PC2_Daphnia
C (%)	-0.392	0.129
N (%)	-0.305	0.616
P (%)	0.470	0.278
C:N	0.054	-0.691
C:P	-0.507	-0.208
N:P	-0.522	-0.076
Variance explained	58%	29%

Notes: Components were associated with inorganic water N and P concentrations (NH<sub>4</sub>, NO<sub>2</sub> + NO<sub>3</sub>, PO<sub>4</sub>; PC1\_Nutrients), with algal quality in the epilimnion (total P [TP], total N [TN]; PC2\_Nutrients), with P and C content of *Daphnia* (P, C, C:P, N:P; PC1\_Daphnia), and with N content of *Daphnia* (N and C:N; PC2\_Daphnia), respectively.

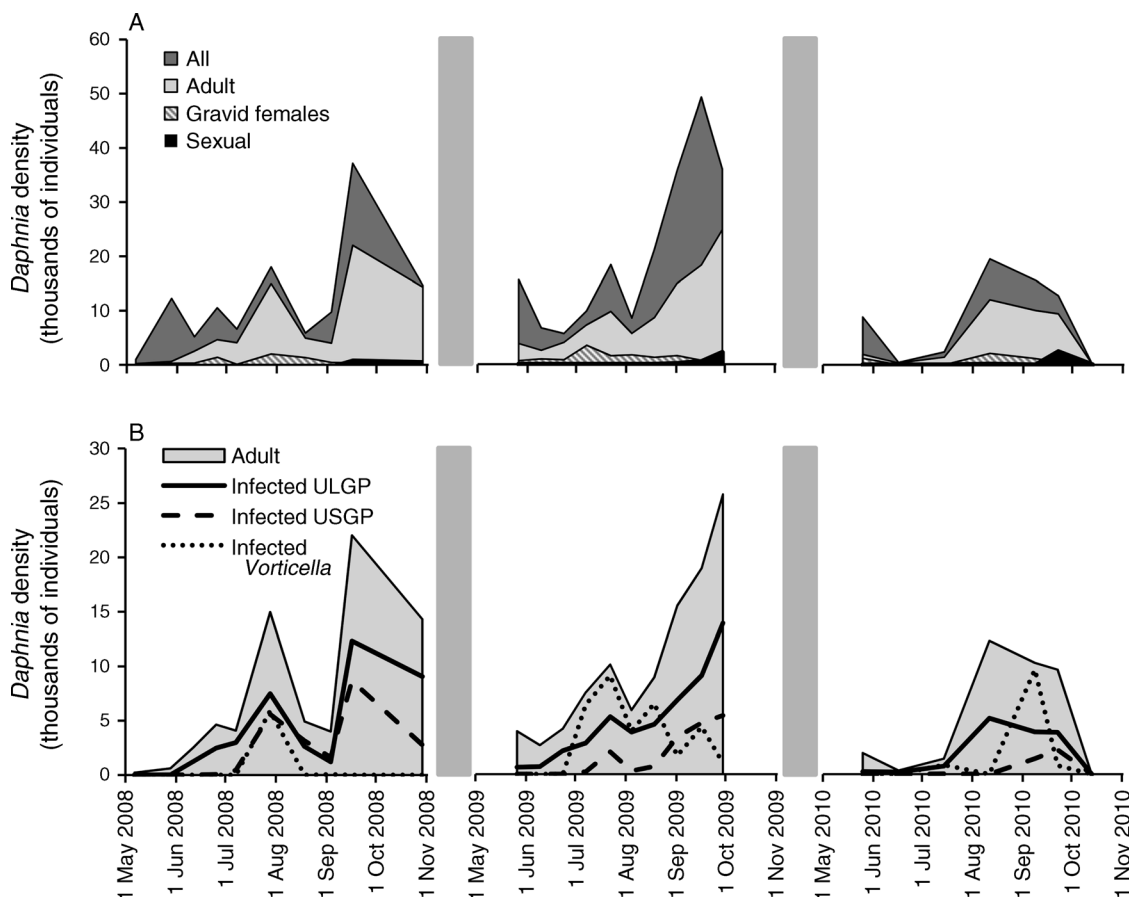


FIG. 2. (A) Density of all (dark gray shaded area), adult (light gray shaded area), gravid (hatched area), and sexually reproducing (black shaded area) *Daphnia longispina* in Lake Mekkojärvi during the open water season between years 2008 and 2010. (B) Density of *D. longispina* adults (light gray shaded area) and ones infected with unidentified large gut parasite (ULGP; solid line), unidentified small gut parasite (USGP; dashed line), or colonized with *Vorticella* sp. (dotted line) during the same period. Number of sampling occasions is 25. Gray bars denote breaks between sampling seasons due to ice cover.

TABLE 2. Results of the general linear models for parasite prevalence, overall parasite species richness, endoparasite species richness, *Daphnia* density, and fecundity.

Parameters	Estimate	Standard error	Test coefficient	<i>P</i>
<b>USGP prevalence†</b>				
Intercept	-1.48	1.32	-1.13	0.276
Year			-0.30	<0.001
Season			-0.29	<0.001
PC2_Nutrients	0.10	0.04	2.54	0.021
pH	0.69	0.21	3.22	0.005
rev $\delta^{13}\text{C}$	-0.05	0.02	-2.64	0.017
<b>ULGP prevalence</b>				
Intercept	-0.92	1.11	-0.83	0.420
pH	-0.39	0.19	-2.12	0.050
Temperature	0.03	0.02	1.37	0.189
Sqrt(chlorophyll <i>a</i> )	0.00	0.03	0.09	0.930
<i>Daphnia</i> mean length	0.52	0.23	2.22	0.041
PC1_Daphnia	-0.00	0.01	-0.11	0.910
PC2_Daphnia	0.06	0.02	3.67	0.002
rev $\delta^{13}\text{C}$	0.06	0.02	2.91	0.010
$\delta^{15}\text{N}$	0.04	0.05	0.77	0.454
<b><i>Vorticella</i> prevalence‡</b>				
Intercept	-0.58	0.43	-1.35	0.195
Season			-0.56	0.004
PC2_Nutrients	-0.10	0.06	-1.80	0.089
Temperature	0.11	0.04	2.71	0.014
Oxygen concentration	-0.08	0.05	-1.72	0.103
PC1_Daphnia	-0.12	0.03	-4.44	<0.001
<b>Overall parasite species richness</b>				
Intercept	-0.42	0.36	-1.16	0.262
Season			-1.92	<0.001
PC2_Nutrients	0.07	0.06	1.21	0.244
Temperature	1.13	0.04	2.98	0.008
Sqrt(chlorophyll <i>a</i> )	-0.08	0.06	-1.35	0.194
Sqrt( <i>Daphnia</i> density)	-0.00	0.00	-1.67	0.113
PC1_Daphnia	-0.11	0.03	-4.03	<0.001
<b>Endoparasite species richness</b>				
Intercept	-2.53	1.70	-1.48	0.160
Year			-0.26	0.004
PC2_Nutrients	0.09	0.06	1.50	0.155
pH	0.45	0.34	1.33	0.206
Temperature	-0.04	0.03	-1.65	0.122
Sqrt(chlorophyll <i>a</i> )	0.07	0.06	1.26	0.229
<i>Daphnia</i> mean length	0.87	0.37	2.37	0.033
PC1_Daphnia	0.04	0.02	1.65	0.121
PC2_Daphnia	0.07	0.03	2.00	0.066
$\delta^{15}\text{N}$	0.04	0.08	0.54	0.596
<b><i>Daphnia</i> density‡</b>				
Intercept	682.89	164.58	4.15	<0.001
Sqrt(chlorophyll <i>a</i> )	-27.49	6.17	-4.46	<0.001
rev $\delta^{13}\text{C}$	-10.01	3.58	-2.80	0.011
$\delta^{15}\text{N}$	-29.98	11.47	-2.61	0.016
<b>Fecundity‡ (proportion of gravid adult females)</b>				
Intercept	-3.21	1.72	-1.87	0.079
PC2_Nutrients	0.17	0.06	2.75	0.014
Temperature	0.10	0.03	2.92	0.010
Oxygen concentration	0.10	0.04	2.56	0.021
Sqrt( <i>Daphnia</i> density)	0.00	0.00	2.18	0.045
<i>Daphnia</i> mean length	0.56	0.40	1.40	0.181
Exp(overall endoparasite prevalence)	-0.62	0.17	-3.55	0.003
rev $\delta^{13}\text{C}$	0.04	0.03	1.51	0.150
$\delta^{15}\text{N}$	0.13	0.09	1.46	0.164

Notes: The models reported contain only those variables included in the Akaike information criterion (AIC) best-fitting model. Test coefficients are  $\chi^2$  for categorical variables (year and season) and *t* value for others. Significance was set at  $P < 0.05$ . The abbreviation sqrt stands for square root. The subscript rev stands for reverse; reverse (rev)  $\delta^{13}\text{C}$  values were used in order to clarify their interpretation.

† Arcsine-sqrt-transformed.

‡ Sqrt-transformed.



occurred too sporadically for separate analyses, but when inspecting endoparasite species richness (also including these two latter species) we found no connection between species richness and nutrients. The prevalence of the abundant epibiont *Vorticella* sp. and the overall parasite species richness, in which *Vorticella* was included, were found to be negatively associated with host P content. These results show that the connections between nutrients and host-parasite dynamics are complex and parasite species specific.

Our findings on the two most common endoparasite species are in agreement with the hypothesis that improvements in host food quality or stoichiometric content enhance parasite transmission. Furthermore, the positive association of the prevalence of USGP with host food quality (higher seston N and P concentrations) is in agreement with our earlier study, where we found a trend for increase in USGP prevalence under experimental nutrient enrichment (Aalto et al. 2013). High epilimnetic food quality might have increased the availability of susceptible *Daphnia* or their filtration rate in the epilimnion, and thus enhanced the uptake of infectious stages and their transmission. This is supported by the positive association of USGP prevalence with algal food quality and the time *Daphnia* spent in epilimnion, i.e., the proportion of algae in the diet (negative association with  $_{\text{rev}}\delta^{13}\text{C}$ ). Yet another potential mechanism explaining positive associations for USGP with food quality might be that infection increased the grazing pressure exerted by *Daphnia* towards epilimnetic phytoplankton. Increased grazing could have enhanced nutrient recycling and increased algal nutrient content, as suggested by Urabe et al. (2002). For ULGP, transmission and establishment of the infection might have been facilitated by high host N content, which was derived from bacteria rather than from epilimnetic algae. This is supported by the positive association of the ULGP with host N content and the proportion of bacteria in the diet of *Daphnia* ( $_{\text{rev}}\delta^{13}\text{C}$ ). In addition, in Lake Mekkojärvi, large *Daphnia* migrate down to the oxic-anoxic boundary layer to feed on bacterial food (Taipale et al. 2008, 2009). Parasite propagules might accumulate at the density gradient formed by the boundary, increasing the risk of exposure for migrating *Daphnia* (see Decaestecker et al. 2004), as suggested by the positive association of ULGP prevalence with the size of *Daphnia* and proportion of bacterial feeding.

Due to their sporadic occurrence, we did not gain information on the species-specific connections with environmental variables for the two other endoparasitic species, *L. obtusa* and *P. ramosa*. Connections to host quality or host resource quality could have been expected at least for *P. ramosa*, since it has been shown to decrease host P content in experimental infections (Frost et al. 2008b). For the epibiont *Vorticella* sp., we found a negative association with host P content. Since epibionts do not directly exploit host nutrient reserves,

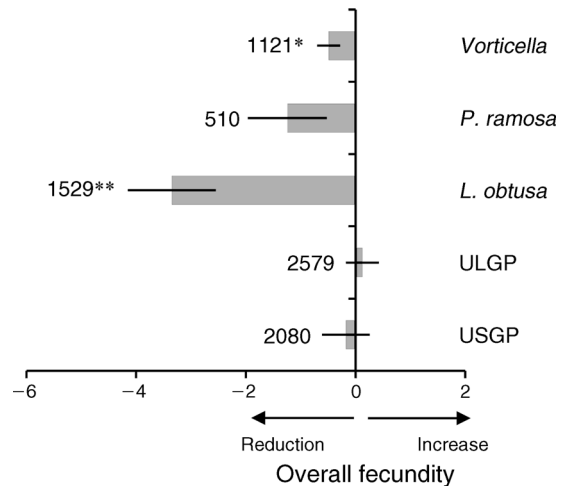


FIG. 3. Overall fecundity reduction in *Daphnia* induced by each parasite species (*Vorticella*, *Larsonia obtusa*, *Pasteuria ramosa*, ULGP, and USGP). For each parasite, the bar gives the mean difference of the clutch size between infected and uninfected females (mean taken over all samples). Numbers on the bars refer to the total number of *D. longispina* females on which the calculations were based.

\*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ .

we suggest that our finding is due to indirect parasite-induced changes in host stoichiometric content. *Vorticella* sp. is known to compete for food with *Daphnia* in Lake Mekkojärvi (Kankaala and Eloranta 1987), and strong resource competition might lead to lower P intake in *Daphnia*.

Opposite to our expectations, we found that the overall parasite species richness was negatively associated with P content of *Daphnia*, which could be explained by the prevalence of *Vorticella* sp. However, endoparasite species richness was not connected to nutrients, but only to *Daphnia* size. Possibly this reflects the contrasting stoichiometric demands of the different endoparasite species studied. In a previous study, *P. ramosa* infection was found to reduce P and N content of *Daphnia* (Frost et al. 2008b), while we found only positive associations between the endoparasites USGP and ULGP and host nutrient content/food quality. Our results indicate that using overall (endo)parasite species richness might result in a flawed interpretation of the connections between individual parasite species and nutrient dynamics.

In our survey, *Daphnia* density had inverse relationships with the proxies of algal quantity (chlorophyll *a*) and bacterial feeding ( $_{\text{rev}}\delta^{13}\text{C}$ ). This suggests that the *Daphnia* population increase was facilitated by algal production and not bacterial food, the quantity of algae being suppressed by *Daphnia* feeding. We found no association between *Daphnia* density and parasites, but in accordance with the results obtained from other natural *Daphnia* populations (Stirnadel and Ebert 1997, Ebert et al. 2001, Decaestecker et al. 2005, Johnson et al. 2009), host fecundity was associated with overall

endoparasite prevalence. These findings could be explained by the differences in the stoichiometric demands of the endoparasite species at Lake Mekkojärvi. We have previously suggested that parasites with high P demands show high virulence in P-rich *Daphnia* due to heavy exploitation of host P reserves (Aalto and Pulkkinen 2013). When prevalent, such parasites might thus have significant effects on *Daphnia* population dynamics by limiting P needed for host survival and reproduction. The two most prevalent species, USGP and ULGP, did not have negative associations with host P content and did not cause fecundity reductions in hosts, which suggests low or moderate virulence, with little impact on host population dynamics. This is further supported by the finding that both USGP prevalence and *Daphnia* fecundity increased when algal food quality was high. Thus the negative connection between host fecundity and endoparasite prevalence might have been caused by the virulent endoparasites *L. obtusa* and *P. ramosa*. While *L. obtusa* was found to have a high negative effect on the clutch size of *Daphnia* (this study, Bengtsson and Ebert [1998]) and previous laboratory experiments have demonstrated same with *P. ramosa* (Ebert et al. 2000, Frost et al. 2008a), they both occurred too sporadically to make an impact on the overall pattern between *Daphnia* population density and overall endoparasite prevalence. The negative effect of *Vorticella* sp. on clutch size contradicts the results of an earlier study (Decaestecker et al. 2005), but can be explained with resource competition between the host and the epibiont (Kankaala and Eloranta 1987), or with changed swimming behavior and increased energy consumption of the host when carrying a heavy epibiont burden (Chiavelli et al. 1993, Threlkeld and Willey 1993).

We acknowledge that our suggestions cannot be resolved without laboratory experiments and exact information on life cycles and transmission patterns of all the species (Aalto et al. 2013). In addition, other factors, e.g., predation or the genetic diversity of host population, may have driven parasite dynamics. Lake Mekkojärvi lacks zooplanktivorous fish, thus the impact of predation in controlling parasite epidemics is much lower than shown for some lakes with fish (e.g., Duffy et al. 2005), but we cannot totally rule out the effects of invertebrate predation, especially by *Chaoborus*. The seasonal variation in host genotype frequency may drive seasonality in parasite prevalence if some host clones are more susceptible to parasites than the others (Little and Ebert 1999). We have no data concerning the genetic structure of the *Daphnia* population in Lake Mekkojärvi. However, it seems that the seasonal genetic variation might also be driven by environmental nutrient cycles and stoichiometric food quality (Jeyasingh et al. 2009), and thus is implicitly included in our statistical models.

In conclusion, our study demonstrates that environmental nutrient levels have the potential to affect parasite prevalence patterns, particularly those of rather

benign parasite species, which are usually prevalent and persist in hosts throughout the open water season (Decaestecker et al. 2005, Wolinska et al. 2007). We found that the effect of nutrients on parasite dynamics was diverse, as some parasites showed positive and some showed negative associations with N and P of the host resource or of the host. Our findings agree with the ones gleaned from earlier studies, where host food quality has been associated with infection rates and/or parasite virulence (Frost et al. 2008a, Hall et al. 2009, Aalto and Pulkkinen 2013). As the loading of carbon and nutrients is increasing in aquatic systems, changes in the natural fluctuations of nutrient concentrations and in the stoichiometry of food webs may thus be expected (Monteith et al. 2007, Elser et al. 2009, 2010, Jeppesen et al. 2009). This has been suggested to increase disease risk and severity (Johnson et al. 2007, 2010, McKenzie and Townsend 2007). However, our study corroborates the view that the effect of nutrient enrichment on the prevailing parasite dynamics is strongly dependent on the host–parasite system studied, making it hard to develop robust global generalizations about the effects of increased nutrient load on disease dynamics.

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#### SUPPLEMENTAL MATERIAL

##### Appendix A

Description of the study site, sampling procedure, and chemical analysis ([Ecological Archives E095-223-A1](#)).

##### Appendix B

Supplementary results: description of stratification and ice cover, environmental variables, elemental composition of *Daphnia*, and proportion of *Daphnia* infected with *Larsonia obtusa* and *Pasteuria ramosa* during the study period ([Ecological Archives E095-223-A2](#)).