

## DINING ON DISEASE: HOW INTERACTIONS BETWEEN INFECTION AND ENVIRONMENT AFFECT PREDATION RISK

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**Abstract.** Despite growing interest in ecological interactions between predators and pathogens, few studies have experimentally examined the consequences of infection for host predation risk or how environmental conditions affect this relationship. Here we combined mesocosm experiments, in situ foraging data, and broad-scale lake surveys to evaluate (1) the effects of chytrid infection (*Polycaryum laeve*) on susceptibility of *Daphnia* to fish predators and (2) how environmental characteristics moderate the strength of this interaction. In mesocosms, bluegill preferred infected *Daphnia* 2–5 times over uninfected individuals. Among infected *Daphnia*, infection intensity was a positive predictor of predation risk, whereas carapace size and fecundity increased predation on uninfected individuals. Wild-caught yellow perch and bluegill from in situ foraging trials exhibited strong selectivity for infected *Daphnia* (3–10 times over uninfected individuals). In mesocosms containing water high in dissolved organic carbon (DOC), however, selective predation on infected *Daphnia* was eliminated. Correspondingly, lakes that supported chytrid infections had higher DOC levels and lower light penetration. Our results emphasize the strength of interactions between parasitism and predation while highlighting the moderating influence of water color. *P. laeve* increases the conspicuousness and predation risk of *Daphnia*; as a result, infected *Daphnia* occur predominantly in environments with characteristics that conceal their elevated visibility.

**Key words:** bluegill and yellow perch; Chytridiomycota; *Daphnia pulex*; disease affects predator–prey relationship; interactions among parasites, prey, and predators; parasitism; predator–prey interactions; selective predation; Wisconsin (USA) lakes; zooplankton mortality.

### INTRODUCTION

Recent theoretical work has emphasized the potential importance of predation in controlling disease in prey populations (Packer et al. 2003, Ostfeld and Holt 2004, Hall et al. 2005). Although predators often acquire multi-host parasites from their prey (i.e., trophic transmission, Lafferty 1992), selective removal of infected prey can also reduce infection prevalence and pathogen transmission, especially for directly transmitted parasites. Thus, loss of predators could lead to increases in epidemic disease and a corresponding decline in prey population size (Packer et al. 2003). However, empirical data regarding the consequences of infection for host predation risk in natural environments have lagged behind theoretical developments, particularly for micro-parasitic diseases. Owing to the difficulties associated with manipulating predators, pathogens and their hosts, experimental investigations into the mechanistic interactions between disease and predation are uncommon (but see Hudson et al. 1992, Lafferty and Morris 1996).

Lake populations of *Daphnia* represent an ideal study system in which to explore predator–parasite interac-

tions and their importance for host populations and ecological communities. *Daphnia* are an important prey resource for many invertebrate and vertebrate predators (Mills and Forney 1983, Luecke et al. 1992). *Daphnia* also host numerous endoparasites, including protists, bacteria, viruses, fungi and helminths, many of which produce conspicuous infections (e.g., Green 1974). Finally, the ecological significance of large-bodied *Daphnia* in lake ecosystems, both as grazers on phytoplankton and as a preferred prey of planktivorous fishes (Lampert et al. 1986, Carpenter et al. 1987), suggest that interactions between the predators and pathogens of *Daphnia* could affect aquatic food webs. Although lake zooplankton communities are traditionally thought to be controlled by a combination of predation and resource availability, there is growing recognition of the important effects of parasites on zooplankton (e.g., Brambilla 1983, Yan and Larsson 1988, Ebert 2005).

Interactions between parasitism and predation are likely to have important consequences for zooplankton (Decaestecker et al. 2002). Predators that selectively remove infected zooplankton from a population can reduce transmission and suppress disease (Ostfeld and Holt 2004, Duffy et al. 2005). Alternatively, if predators avoid infected prey, mortality from disease and predation may be additive or synergistic, resulting in host population decline (Packer et al. 2003).

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Most zooplankton are transparent, and subtle changes in the appearance of zooplankton can elevate predation risk (Zaret 1980, Tucker and Woolpy 1984). Parasites of zooplankton frequently induce marked changes in the opacity and conspicuousness of infected hosts. While several studies have suggested that endoparasitic infections increase the vulnerability of zooplankton to predation (e.g., Duffy et al. 2005), none have tested this hypothesis experimentally, evaluated the strength of interactions between parasitism and predation, or examined factors that moderate this relationship.

Here we explored the influence of a chytrid pathogen (*Polycaryum laeve*) on predation risk for infected *Daphnia*. We used a combination of predation experiments and field studies to determine the influence of host size, fecundity, and infection status on susceptibility of *Daphnia* to fish predation. We tested the hypothesis that differences in predation risk were due to infection-induced changes in the visual appearance of *Daphnia* by evaluating the moderating influence of water color on fish selectivity. Dark, humic water (high in dissolved organic carbon, DOC) can limit the visual acuity of fish and thereby reduce predation on conspicuous zooplankton (e.g., Saegrov et al. 1996). Finally, to examine the landscape-scale importance of interactions among predators, parasites, and water color, we compared light absorbance and DOC concentrations among 25 lakes in Wisconsin (USA) with and without *P. laeve* infection. By addressing these questions at multiple scales and with a variety of approaches, we aimed to identify the mechanistic interactions between disease and predation and generate results robust against methodological artifacts.

## MATERIALS AND METHODS

### *Study system*

We focused on interactions between *Daphnia pulicaria* (hereafter "*Daphnia*"), a common, large-bodied cladoceran in North American lakes, and *Polycaryum laeve*, a recently described chytrid pathogen (Johnson et al. 2006). *P. laeve* causes sharp reductions in *Daphnia* survival, growth, and reproduction (Johnson et al. 2006). Severe infections markedly alter the appearance of infected individuals (Fig. 1), filling the body with conspicuous fungal sporangia. Following death of its host, *P. laeve* sporangia release flagellated zoospores that exit the *Daphnia* carcass. Although the full life cycle of *P. laeve* is not known, transmission is either direct, from infected to uninfected *Daphnia*, or indirect, involving an alternate host or free-living stage.

### *Mesocosm experiments*

Because our objective was to compare predation risk between infected and uninfected *Daphnia* under semi-realistic conditions, we used large (1250 L) outdoor mesocosms stocked with naturally occurring levels of *P. laeve* infection and multiple fish predators. Zooplankton from two lakes (Allequash and Tenderfoot) in Vilas County, Wisconsin, USA, known to support *P. laeve*

were acclimated to filtered (80  $\mu$ m) water from Trout Lake and allowed to distribute throughout mesocosms for one hour prior to fish addition. For each experimental trial, five bluegill (44–81 mm) that had been held without food for 24 h were added to a mesocosm. After a 2-h feeding period, fish were euthanized, measured, and contents of their stomachs preserved. Zooplankton remaining in the mesocosms were filtered through an 80- $\mu$ m net and preserved in 80% ethanol.

Six experimental trials from each lake were conducted in 2003: Allequash (24 and 26 June 2003) and Tenderfoot (21 and 24 June 2003) (two trials with fish and one without fish per date). In 2004 we conducted two additional trials to evaluate the effects of water color on fish selectivity. Mesocosms contained either clear water (DOC 2.75 mg/L, light absorbance at 280 nm = 0.279) or humic water (DOC 10.90 mg/L, absorbance at 280 nm = 2.49). Zooplankton were collected from Tenderfoot Lake and acclimated to mesocosms as above. Initial infection prevalence and stocked densities of *Daphnia* for all experimental trials are presented in Table 1.

### *Field sampling*

To evaluate whether fish selectively consumed infected *Daphnia* in situ, we collected age 0+ yellow perch (*Perca flavescens*) from Birch Lake using a purse seine and compared their stomach contents with vertical zooplankton tows from the same area. To ensure correspondence between collected zooplankton and fish foraging areas, we also conducted short-term foraging trials with bluegill (58–121 mm) maintained in modified minnow traps (6.4-mm mesh) in Allequash Lake. On three dates in June 2003 we suspended individually caged bluegill at 1.5 m and 3.5 m along buoyed anchor lines every 5–8 hours. Buoy lines were anchored in the deepest part of the lake (7 m) and were separated by 100 m. Zooplankton in the water column were sampled using a 80- $\mu$ m net.

### *Influence of water color on infection*

To understand the importance of water color in moderating interactions among *Daphnia*, *P. laeve* infection, and fish predation at the landscape scale, we compared dissolved organic carbon (DOC) levels and light absorbance between lakes that contained *P. laeve* ( $n = 13$  lakes) and lakes that were free of infection but supported *D. pulicaria* ( $n = 12$  lakes). Lakes were drawn from a set of 58 randomly selected systems in Vilas County sampled for zooplankton between 2 June and 5 July 2001–2004. Although each lake was sampled only once, *P. laeve* infection presence (but not prevalence) is fairly stable among years (P. Johnson, unpublished data). DOC and water color were measured using an OI total organic carbon analyzer and a spectrophotometer, respectively (see University of Wisconsin [USA] Center for Limnology methods, available online).<sup>4</sup>

<sup>4</sup> (<http://lter.limnology.wisc.edu/protocols.html>)

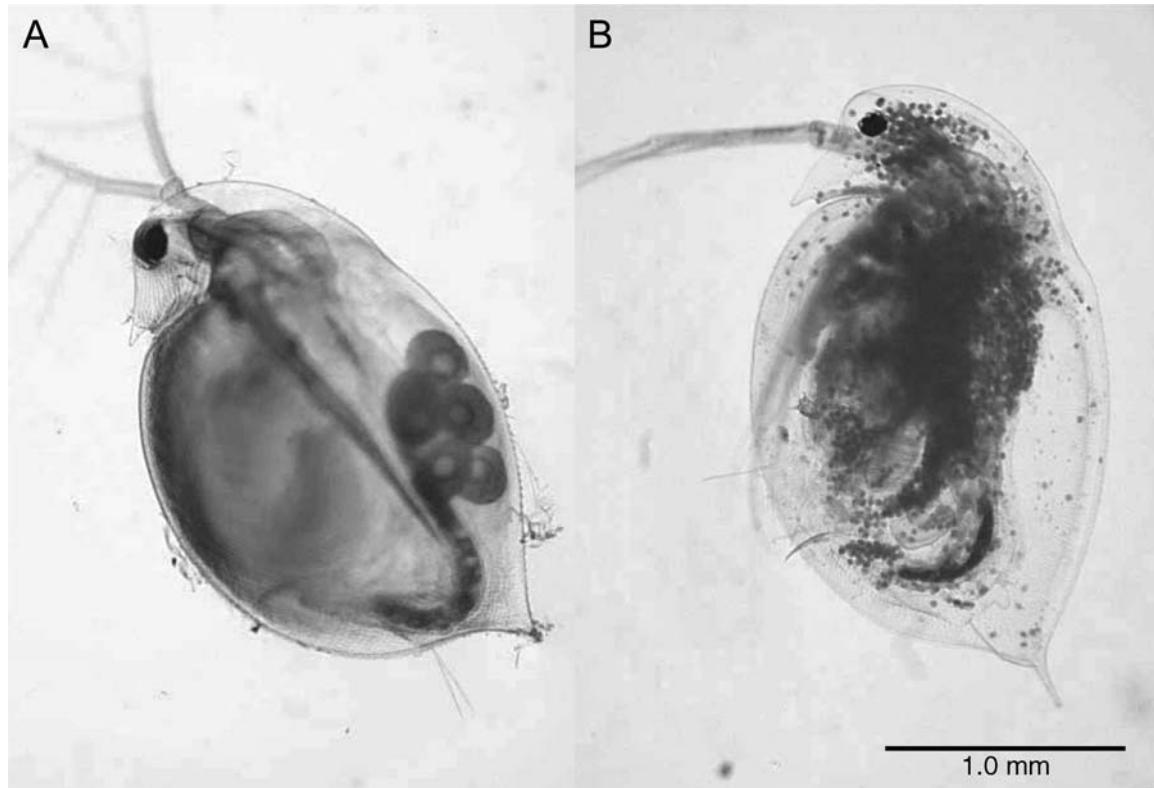


FIG. 1. (A). Uninfected adult *Daphnia pulicaria* with eggs in the brood chamber. (B) Heavily infected *D. pulicaria* filled with sporangia of the chytrid fungus, *Polycaryum laeve*.

#### Zooplankton and fish processing

We compared the contents of fish stomachs with zooplankton remaining in the corresponding mesocosm or with field-collected zooplankton. For each fish, we isolated and measured all *D. pulicaria* and recorded the presence of eggs, infection status, and infection intensity. Infection intensity is based on the number of chytrid sporangia per infected *Daphnia* and ranges from 1 (light infection, 1–500 sporangia) to 3 (heavy infection, >1000 sporangia) (Johnson et al. 2006). The intensity of sporangia within a host is the result of asexual reproduction and presumably increases with host size and time since exposure. For zooplankton remaining in the mesocosms, we examined >200 mature female *Daphnia* to determine infection prevalence and measured >150 individuals (infected and uninfected). We considered only mature *D. pulicaria*  $\geq 1.2$  mm in our analyses because smaller individuals rarely exhibited infection or eggs. Other cladocerans (e.g., *D. galeata mendotae*) were rare at the time of collection, and no other *Daphnia* endoparasites were noted.

We used two forms of analysis to evaluate the effects of infection on predation risk. Chesson's  $\alpha$  (Chesson 1983), which ranges from 0 (prey avoidance) to 1 (perfect selection), was used to measure the selectivity of each fish for infected *Daphnia*. The gut contents of field-collected fish were compared against available zoo-

plankton using Chesson's (1983) "Case 1" equation, which assumes no depletion of prey. To explicitly account for prey depletion during the mesocosm experiments, we used the "Case 2" form of  $\alpha$ :

$$\alpha_i = \frac{\ln[(n_{i0} - r_i)/n_{i0}]}{\sum_{j=1}^m \ln[(n_{j0} - r_j)/n_{j0}]} \quad i = 1, \dots, m$$

where  $n_{i0}$  is the number of prey type  $i$  at the beginning of the trial (calculated as the sum of prey type  $i$  recovered from all fish and individuals remaining in the mesocosm),  $r_i$  is the number of prey type  $i$  recovered from a particular fish, and  $m$  is the number of prey types (often infected and uninfected *Daphnia*). [Note: In parasitological models, another form of alpha is often used to denote the proportional increase in prey vulnerability as a function of parasitism (e.g., Lafferty 1992). An approximate conversion of Chesson's  $\alpha$  to this alternative metric is achieved by dividing Chesson's  $\alpha$  for infected prey by one minus the same value (or by Chesson's  $\alpha$  for uninfected prey).]

Values of Chesson's  $\alpha$  for each fish ( $n = 40$  fish) were compared against an expected  $\alpha$  based on the null hypothesis of nonselective foraging using a linear mixed model, treating mesocosm as a random effect nested within lake. An  $\alpha$  value of  $1/m$  indicates neutral selection

(e.g., 0.5 for  $m = 2$ ). Because we sought to create semi-realistic foraging conditions, experimental trials involved a large foraging arena (1250 L), a small number of fish (5), and a short feeding period (2 h). No schooling or agonistic behavior was observed among bluegill, and the feeding behavior of experimental fish likely mimics their behavior under natural conditions. Although the behavior and hence  $\alpha$  values of fish are not independent, they nonetheless represent  $\alpha$  values that should be expected under natural conditions where fish interact. The statistical analysis that incorporates a random effect of mesocosms addresses the possibility that the  $\alpha$  values for fish within a mesocosm are correlated by factoring out mesocosm-to-mesocosm variation in  $\alpha$ . We also performed a more conservative statistical test by treating each mesocosm as a sample unit ( $n = 8$  mesocosms), comparing the average alpha value from each tank against the expected alpha using a mixed model.

Because consumed and available *Daphnia* differed significantly in size, we also used logistic regression to simultaneously incorporate continuous (e.g., carapace size) and categorical (e.g., infection or fecundity status) input variables on predation likelihood (consumed vs. not consumed). This analysis used individual *Daphnia* as the unit of analysis and data from fish within a given mesocosm were combined. We evaluated the explanatory importance of *Daphnia* size, infection status, and their interaction on the probability a given *Daphnia* was consumed. Among infected *Daphnia*, we analyzed the importance of size and infection intensity, whereas among uninfected *Daphnia* the effects of size and fecundity were considered. The significance of each predictor variable was evaluated using the Wald statistic, and model fit was assessed using Nagelkerke  $R^2$ .

## RESULTS

### *Mesocosm experiments*

Across mesocosms, bluegill selectively consumed infected *Daphnia* over uninfected *Daphnia* (linear mixed model,  $\alpha \pm \text{SE} = 0.609 \pm 0.03$ ; 95% CI = 0.55–0.69; df = 7). Subsequent  $t$  tests of fish within each mesocosm indicated that infected *Daphnia* were consumed preferentially in 7 of 8 experimental trials (two-tailed  $t$  test,  $P < 0.05$ ; Fig. 2A). This pattern was consistent across scale, and a mixed model analysis using mesocosms, rather than fish, as the unit of replication revealed comparable evidence of selective predation on infected *Daphnia* ( $\alpha \pm \text{SE} = 0.640 \pm 0.05$ ; 95% CI = 0.52–0.76; df = 6).

To incorporate the effects of *Daphnia* size and infection status on predation risk simultaneously, we used logistic regression on the pooled experimental trials for a given lake and day (although results were similar if trials are analyzed individually). *Daphnia* size was a significant positive predictor of predation risk (see Table 1). Infection status also had a significant positive influence on predation in all experimental trials, causing a 1.5–4.9-fold increase in predation risk based on the

odds ratio ( $R_N^2$  [Nagelkerke  $R^2$ ] = 0.1–0.2). In all cases, the full model was significant based on a likelihood ratio test against the constant-only model ( $\chi^2 = 65.5$ –176; df = 2). However, size and infection status interacted significantly. While size was a strong positive predictor of predation risk for uninfected *Daphnia*, it was neutral or weakly significant for infected individuals (Table 1).

Among infected *Daphnia*, infection intensity was a positive predictor of predation risk only in the Allequash Lake trials. However, we found a significant size  $\times$  intensity interaction on predation risk. Infection intensity was a significant positive predictor of predation among small to moderate-sized *Daphnia*, but was a negative or neutral predictor for large *Daphnia*. Among uninfected *Daphnia*, fish exhibited selectivity for gravid *Daphnia* based on analyses of Chesson's alpha; however, some of this effect was due to the correlation between size and fecundity status. Larger uninfected *Daphnia* were more likely to contain eggs, and after including *Daphnia* size in the logistic-regression analysis, fecundity status was a positive predictor of predation risk in only two of four trials. Too few infected *Daphnia* (2% of the 2078 infected *Daphnia* examined) had eggs for inclusion in this analysis.

### *Effects of water color*

Results of the clear (low-DOC) water treatment in 2004 were comparable to mesocosm trials from 2003. Bluegill exhibited selection for infected *Daphnia* based on Chesson's alpha (two-tailed  $t$  test,  $t = 3.012$ ,  $P = 0.039$ ; Fig. 2B). In the logistic-regression analysis, both size and infection status positively affected predation likelihood ( $R_N^2 = 0.32$ ,  $\chi^2 = 151.6$ , df = 2,  $P < 0.001$ ). Again however, there was an interaction between these variables, with size influencing predation only among uninfected *Daphnia* (Table 1). Among infected individuals, infection intensity was a positive predictor of predation ( $R_N^2 = 0.4$ ,  $\chi^2 = 10.01$ , df = 2,  $P < 0.01$ ). Among uninfected *Daphnia*, larger size and the presence of eggs increased the likelihood of predation ( $R_N^2 = 0.32$ ,  $\chi^2 = 138.7$ , df = 2;  $P < 0.001$ ; Table 1).

In the high-DOC treatment, however, bluegill exhibited no selection for infected *Daphnia* (two-tailed  $t$  test for Chesson's  $\alpha$ ,  $t = -0.790$ ,  $P = 0.474$ ) (Fig. 2B). Although size remained a significant predictor of predation risk in the logistic regression, infection status explained no additional variation. Selection for gravid *Daphnia* was also eliminated (Fig. 2B). Among infected *Daphnia*, infection intensity had a positive influence on the likelihood of predation, whereas only size had a significant effect on predation risk for uninfected *Daphnia* (Table 1).

### *Field samples*

For yellow perch, 61 of 64 stomachs contained  $\geq 10$  *Daphnia* ( $28.3 \pm 2.69$  *Daphnia* [mean  $\pm 1$  SE]; range: 10–87 *Daphnia*). Infected *Daphnia* occurred in all but one of these and were often numerically dominant. Alpha

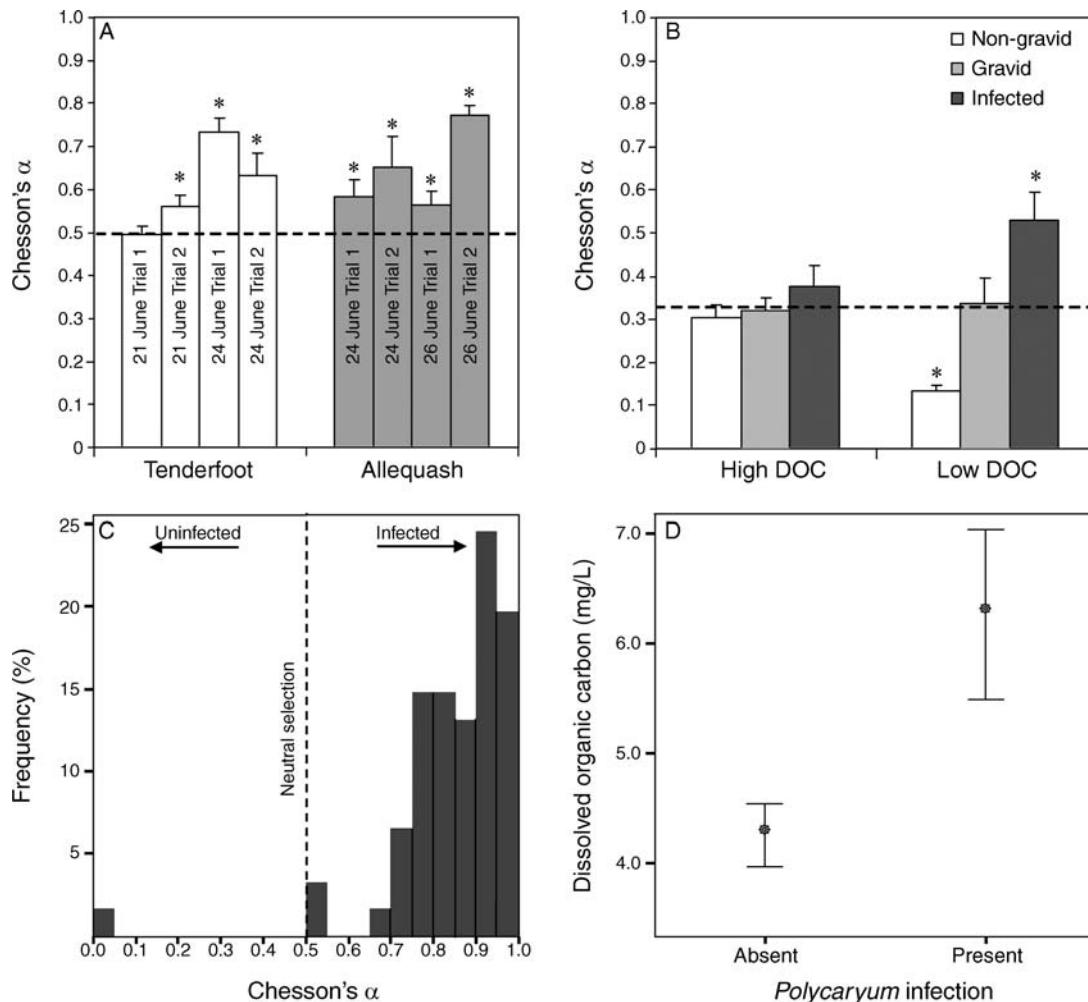


FIG. 2. Chesson's  $\alpha$  ( $\pm$ SE) for bluegill feeding on (A) infected and uninfected *Daphnia pulicaria* in mesocosm trials from Allequash and Tenderfoot lakes (Wisconsin, USA) and on (B) infected, uninfected gravid, and uninfected non-gravid *D. pulicaria* in mesocosms containing water with high or low concentrations of dissolved organic carbon (DOC). The dashed horizontal line indicates the expected value of  $\alpha$  for nonselectively foraging fish, calculated as 1.0 divided by the number of prey items, i.e., 1.0/2 = 0.5 for panel (A) and 1.0/3 = 0.33 for panel (B). Asterisks (\*) indicate a significant difference from the expected  $\alpha$  (two-tailed *t* test,  $P < 0.05$ ). (C) Frequency distribution of Chesson's  $\alpha$  values for 61 yellow perch (*Perca flavescens*) feeding on infected *D. pulicaria* in Birch Lake. The dashed line indicates expected value of  $\alpha$  for nonselectively feeding fish. (D) dissolved organic carbon (DOC) in 25 Vilas County (Wisconsin, USA) lakes that either supported *Polycaryum laeve* or were free of the infection. All lakes contained *Daphnia pulicaria*.

values averaged  $0.846 \pm 0.0195$  (mean  $\pm$  1 SE), and were significantly and substantially greater than the neutral selection value of 0.5 (two-tailed *t* test;  $t = 17.783$ ,  $P = 0.0001$ ,  $n = 61$ ; Fig. 2C). Correspondingly, we found significant positive effects of size and infection status on predation likelihood in logistic regression ( $R_N^2 = 0.18$ ;  $\chi^2 = 74.6$ ,  $df = 2$ ,  $P < 0.001$ ). However, size negatively influenced predation risk, possibly because of the small size (and smaller gape) of collected perch ( $35.6 \pm 0.28$  mm total length [mean  $\pm$  1 SE] (Hrabik et al. 2001).

Results from in situ foraging trials offered comparable results. Ten of the 36 fish had  $>10$  *Daphnia* in their stomachs (range: 16–252 *Daphnia*). Chesson's  $\alpha$  values for infected *Daphnia* averaged  $0.7319 \pm 0.0713$  and were significantly greater than the expected value of 0.5 (two-

tailed *t* test,  $t = 3.253$ ,  $P = 0.01$ ). Logistic regression revealed significant positive effects for *Daphnia* size and infection status on predation risk ( $R_N^2 = 0.41$ ;  $\chi^2 = 116.4$ ,  $df = 2$ ,  $P < 0.001$ ). However, infection status and *Daphnia* size interacted significantly, with size influencing predation likelihood for uninfected animals to a greater extent than for infected *Daphnia*.

#### Infection among lakes

DOC and light absorption were significantly greater in lakes with *Polycaryum laeve* infection (for DOC,  $F = 5.563$ ,  $P = 0.027$ ,  $df = 24$ ; for absorbance,  $F = 7.424$ ,  $P = 0.012$ ,  $df = 24$ ) (Fig. 2D). The levels of DOC and absorbance were highly correlated (Pearson  $r = 0.96$ ;  $n = 25$  lakes). It is possible that decreased predation in opaque

TABLE 1. Descriptive information and parameter estimates for field and mesocosm data for selective predation by fish on *Daphnia pulicaria*.

Source and sample of zooplankton and fish	Chesson's $\alpha$ †	<i>Daphnia pulicaria</i>			Unstandardized beta coefficients¶				
		$N_F$ ‡	Density (no./L)§	Initial infection prevalence (%)	All <i>Daphnia</i>		Infected <i>Daphnia</i>		
					Size (mm)	Infection	Size (mm)	Intensity	
Allequash Lake									
24 June 2003	0.618*	10	1.10	41.5	2.07 ± 0.25***	0.59 ± 0.14***	0.37 ± 0.39	0.84 ± 0.17***	
26 June 2003	0.597**	10	1.07	29.9	1.73 ± 0.29***	0.60 ± 0.17***	-1.26 ± 0.56*	0.50 ± 0.20*	
Tenderfoot Lake									
21 June 2003	0.526	10	0.99	25.9	2.93 ± 0.25***	0.51 ± 0.16*	1.83 ± 0.54**	0.31 ± 0.22	
24 June 2003	0.694**	10	1.60	15.4	1.03 ± 0.19***	1.30 ± 0.18***	-0.24 ± 0.41	-0.18 ± 0.23	
DOC									
Low	0.676*	5	0.82	4.9	4.58 ± 0.48***	1.58 ± 0.48**	-2.26 ± 2.3	2.86 ± 1.2*	
High	0.539	5	0.97	4.9	2.96 ± 0.36***	-0.21 ± 0.35	0.80 ± 1.8	3.15 ± 1.1**	
Yellow perch	0.846***	61	0.22	20.9	-1.09 ± 0.52*	2.37 ± 0.32***	-4.61 ± 0.85*	3.63 ± 0.42***	
Caged bluegill	0.732*	10	0.21	23.4	5.41 ± 0.69***	1.21 ± 0.35**	3.15 ± 1.1**	N/A	

Note: Yellow perch (*Perca flavescens*) were collected from Birch Lake (Vilas County, Wisconsin, USA); all other studies used bluegill (*Lepomis macrochirus*). Zooplankton were taken from two lakes (Allequash and Tenderfoot) in Vilas County.

\*  $P < 0.05$ ; \*\*  $P < 0.001$ ; \*\*\*  $P < 0.0001$ ; N/A denotes "not available."

† The average Chesson's  $\alpha$  values for infected *Daphnia*; Chesson's  $\alpha$  ranges from 0 to 1, with 1 indicating selection for infected *Daphnia*, 0 indicating selection for uninfected *Daphnia*, and 0.5 indicating neutral selection. Chesson's  $\alpha$  is easily converted into the proportional increase in predation risk (more commonly used in parasitological models; e.g., Lafferty 1992) by dividing  $\alpha$  by  $(1 - \alpha)$ .

‡ Number of fish examined.

§ Estimated from the "control" treatments in which no fish were added, or from field-collected zooplankton tows.

|| The percentage of infected *Daphnia pulicaria* among individuals  $> 1.2$  mm.

¶ Unstandardized beta coefficients from the logistic-regression analysis (without interaction terms). Data are means  $\pm$  SE.

# Number of *Daphnia* examined.

water would increase *Daphnia* abundance and that this would favor chytrid transmission. However, the influence of water color on disease was not mediated through *D. pulicaria* density or size, as we found no relationship between absorbance and the abundance of *Daphnia* (number per liter) ( $r = -0.189$ ,  $P = 0.366$ ) or mean *Daphnia* carapace length ( $r = 0.281$ ,  $P = 0.194$ ). We also found no relationship between DOC levels and effort-corrected fish catch ( $r = -0.042$ ,  $p = 0.0841$ ) or epilimnion depth ( $r = -0.19$ ,  $P = 0.14$ ). The prevalence of *P. laeve* infection correlated positively (but not significantly) with both DOC ( $r = 0.313$ ) and absorbance ( $r = 0.333$ ).

#### DISCUSSION

Predation has long been recognized as an important driver of zooplankton population dynamics. Few studies, however, have examined the influence of disease on zooplankton in lake ecosystems (e.g., Yan and Larsson 1988, Bittner et al. 2002), and fewer still have explored the interactions between these forces (e.g., Duffy et al. 2005). In our present study, multiple lines of evidence support the hypothesis that fish feed preferentially on *Daphnia* infected with the chytrid fungus, *Polycaryum laeve*. In experimental mesocosms, bluegill consumed infected *Daphnia* between 2 and 5 times more frequently than would be expected, even after accounting for *Daphnia* size. These patterns were corroborated by field results; yellow perch exhibited strong selection for infected *Daphnia* ( $> 10$  times over uninfected

individuals). Short-term foraging trials with caged bluegill yielded comparable data, indicating that these trends are not unique to a single fish species or lake.

The increased predation risk observed for infected *Daphnia* is most likely a consequence of their altered appearance. *Polycaryum laeve* infection fills the body cavity of *Daphnia* with thousands of darkly colored sporegania, increasing the animal's conspicuousness and reducing its transparency. Changes in zooplankton appearance resulting from hemoglobin production, melanization or even vital stains have caused similar increases in predation risk (e.g., Zaret 1980, Saegrov et al. 1996). Although the idea that endoparasites might increase the vulnerability of *Daphnia* to predators has been suggested previously (e.g., Duffy et al. 2005), this is the first study to combine mechanistic experiments with supporting field evidence to identify the relative importance of infection presence, infection intensity, and prey size and fecundity in determining predation risk. Willey et al. (1993) found that the prevalence of pigmented epibiont infestations in experimental tanks and small ponds declined following the addition of visual predators. Similarly, Duffy et al. (2005) reported that field-caught bluegill from four Michigan lakes contained significantly more *Daphnia dentifera* infected with *Spirobacillus cienkowskii* than were found in epilimnetic zooplankton tows.

Humic-stained water with decreased light penetration eliminated fish selectivity for infected *Daphnia*, support-

TABLE 1. EXTENDED

Unstandardized beta coefficients <sup>¶</sup>		
Uninfected <i>Daphnia</i>		
Size (mm)	Fecundity	$N_D$ #
2.86 ± 0.35***	0.22 ± 0.18	1101
2.50 ± 0.37***	1.05 ± 0.18***	1016
3.16 ± 0.31***	0.01 ± 0.18	1262
1.14 ± 0.24***	0.54 ± 0.19**	801
4.52 ± 0.51***	0.48 ± 0.21*	553
3.05 ± 0.38***	-0.26 ± 0.18	637
-0.35 ± 0.58	N/A	1101
6.57 ± 0.97***	N/A	438

ing the notion that changes in the visual appearance of infected animals are responsible for elevated predation. Although we cannot eliminate the possibility that parasite-induced changes in *Daphnia* behavior might also be involved, the humic-water treatment also reduced fish selection on gravid *Daphnia*, which are more conspicuous owing to pigmented eggs in the brood chamber (Tucker and Woolpy 1984). The loss of selectivity for infected *Daphnia* at higher DOC concentrations corroborates the work of Kitchell and Kitchell (1980), who suggested that humic water offers a visual refuge for conspicuous zooplankton.

Data from our survey of lakes expand the humic-refuge concept to parasite infection at the landscape scale. Lakes with *P. laeve* infection had significantly higher levels of DOC and light absorbance than lakes without the parasite. We suggest that this pattern is the direct result of interactions among fish predators, their *Daphnia* prey, and chytrid infections. Because of increased predation on infected *Daphnia* in clear-water systems, humic lakes offer a refuge for *Daphnia* with visually conspicuous infections. These patterns could not be explained by overall differences in fish abundance, *Daphnia* size or *Daphnia* density. We recognize, however, the possibility that other attributes of humic systems (e.g., acidity, microbial communities, productivity, UV-B protection) might additionally or alternatively be involved, especially as the complete life cycle of *P. laeve* is not known.

A combination of theoretical and empirical studies have suggested that selectively foraging predators can suppress disease epidemics in prey populations (Ostfeld and Holt 2004, Duffy et al. 2005, Hall et al. 2005), potentially leading to an increase in total prey density (Packer et al. 2003). This effect could be particularly favorable to *Daphnia* populations because infected individuals consume resources but do not reproduce, thereby restricting population growth. Although we

documented clear evidence of predator selectivity for *Daphnia* infected with *P. laeve*, the regulatory role of predators on disease in this system remains conjectural. Transmission of *P. laeve* requires death of its *Daphnia* host ("obligate killer," Ebert 2005), and preliminary evidence indicates that sporangia remain viable even following fish digestion. Thus, while fish predation can reduce the number of infected prey, it is unlikely to directly reduce transmission rates or disease incidence. Low levels of fish predation may even serve to redistribute the parasite throughout the lake. The combination of parasitism and predation could therefore have additive or synergistic negative consequence for *Daphnia* populations; infection by *P. laeve* decreases host birth rate (parasitic castration) and increases its death rate (Johnson et al. 2006), both directly (parasite-induced mortality) and indirectly (via elevated predation risk). At high levels of predation, however, fish are likely to reduce infection by depressing the host population below the parasite's transmission threshold.

Given the keystone importance of large-bodied *Daphnia* both as grazers and as food for fish (e.g., Carpenter et al. 1987), these results could have important implications for lake food webs. If infection, by making infected animals more conspicuous, substantially increases the foraging rate or total *Daphnia* consumption by young-of-the-year fish, epidemics could increase fish growth, survival, and overwintering success. If, however, the added effects of infection reduce *Daphnia* density or persistence, the opposite could occur. This situation could be exacerbated if infected *Daphnia* are lower in food quality owing to reduced digestibility of sporangia or the absence of nutrient-rich eggs and fatty acids. Resolution of these questions will require further and more long-term investigation.

In summary, our results highlight the importance of examining interactions among biological forces such as disease and predation. Previous studies have often focused on the direct influence of these forces in isolation, but our experimental and field results indicate that disease can significantly increase host predation risk. Moreover, interactions among disease, *Daphnia*, and fish predators may be strong enough to influence the landscape-scale distribution of parasite infection. Thus, while it has been suggested that diseases in zooplankton may be an artifact of predator-poor systems, we argue that disease has important direct and indirect influences on *Daphnia* populations and lake food webs. The strength of these effects is likely moderated through environmental characteristics (including water color), parasite life cycle or transmission mode, and food-web structure.

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## LITERATURE CITED

- 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.
- Brambilla, D. J. 1983. Microsporidiosis in a *Daphnia pulex* population. *Hydrobiologia* **99**:175–188.
- Carpenter, S. R., J. F. Kitchell, J. R. Hodgson, P. A. Cochran, J. J. Elser, M. M. Elser, D. M. Lodge, D. Kretchmer, X. He, and C. von Ende. 1987. Regulation of lake primary productivity by food-web structure. *Ecology* **68**:1863–1876.
- Chesson, J. 1983. The estimation and analysis of preference and its relationship to foraging models. *Ecology* **64**:1297–1304.
- Decaestecker, E., L. De Meester, and D. Ebert. 2002. In deep trouble: habitat selection constrained by multiple enemies in zooplankton. *Proceedings of the National Academy of Sciences (USA)* **99**:5481–5485.
- Duffy, M. A., S. R. Hall, A. J. Tessier, and M. Huebner. 2005. Selective predators and their parasitized prey: Are epidemics in zooplankton under top-down control? *Limnology and Oceanography* **50**:412–420.
- Ebert, D. 2005. *Ecology, epidemiology, and evolution of parasitism in Daphnia*. National Library of Medicine, National Center for Biotechnology Information, Bethesda, Maryland, USA. (<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Books>)
- Green, J. 1974. Parasites and epibionts of Cladocera. *Transactions of the Royal Society of London* **32**:417–515.
- Hall, S. R., M. A. Duffy, and C. E. Caceres. 2005. Selective predation and productivity jointly drive complex behavior in host–parasite systems. *American Naturalist* **165**:70–81.
- Hrabik, T. R., M. P. Carey, and M. S. Webster. 2001. Interactions between young-of-the-year exotic rainbow smelt and native yellow perch in a northern temperate lake. *Transactions of the American Fisheries Society* **130**:568–582.
- Hudson, P. J., A. P. Dobson, and D. Newborn. 1992. Do parasites make prey more vulnerable to predation? Red grouse and parasites. *Journal of Animal Ecology* **61**:681–692.
- Johnson, P. T. J., J. E. Longcore, D. E. Stanton, R. B. Carnegie, J. D. Shields, and E. R. Preu. 2006. Chytrid infections of *Daphnia pulex*: development, ecology, pathology and phylogeny of *Polycaryum laeve*. *Freshwater Biology* **51**:634–648.
- Kitchell, J. A., and J. F. Kitchell. 1980. Size-selective predation, light transmission, and oxygen stratification: evidence from the recent sediments of manipulated lakes. *Limnology and Oceanography* **25**:389–402.
- Lafferty, K. D. 1992. Foraging on prey that are modified by parasites. *American Naturalist* **140**:854–867.
- Lafferty, K. D., and A. K. Morris. 1996. Altered behavior of parasitized killifish increases host susceptibility to predation by bird final hosts. *Ecology* **77**:1390–1397.
- Lampert, W., W. Fleckner, R. Hakumat, and B. E. Taylor. 1986. Phytoplankton control by grazing zooplankton: a study on the spring clear-water phase. *Limnology and Oceanography* **31**:478–490.
- Luecke, C., L. G. Rudstam, and Y. Allen. 1992. Interannual patterns of planktivory 1987–89: an analysis of vertebrate and invertebrate planktivores. Chapter 14, pages 275–301 in J. F. Kitchell, editor. *Food web management: a case study of Lake Mendota*. Springer-Verlag, New York, New York, USA.
- Mills, E. L., and J. L. Forney. 1983. Impact on *Daphnia pulex* of predation by young yellow perch in Oneida Lake, New York. *Transactions of the American Fisheries Society* **112**:154–161.
- Ostfeld, R. S., and R. D. Holt. 2004. Are predators good for your health? Evaluating evidence for top-down regulation of zoonotic disease reservoirs. *Frontiers in Ecology and the Environment* **2**:13–20.
- Packer, C., R. D. Holt, P. J. Hudson, K. D. Lafferty, and A. P. Dobson. 2003. Keeping the herds healthy and alert: implications of predator control for infectious disease. *Ecology Letters* **6**:797–802.
- Saegrov, H., A. Hobaek, and J. H. Labeelund. 1996. Vulnerability of melanistic *Daphnia* to brown trout predation. *Journal of Plankton Research* **18**:2113–2118.
- Tucker, R. P., and S. P. Woolpy. 1984. The effect of parthenogenic eggs in *Daphnia magna* on prey location by the bluegill sunfish (*Lepomis macrochirus*). *Hydrobiologia* **109**:215–217.
- Willey, R. L., R. B. Willey, and S. T. Threlkeld. 1993. Planktivore effects on zooplankton epibiont communities: epibiont pigmentation effects. *Limnology and Oceanography* **38**:1818–1822.
- Yan, N. D., and J. I. R. Larsson. 1988. Prevalence and inferred effects of microsporidia of *Holopedium gibberum* (Crustacea: Cladocera) in a Canadian Shield lake. *Journal of Plankton Research* **10**:875–886.
- Zaret, T. M. 1980. *Predation and freshwater communities*. Yale University Press, New Haven, Connecticut, USA.