

## FESTERING FOOD: CHYTRIDIOMYCETE PATHOGEN REDUCES QUALITY OF *DAPHNIA* HOST AS A FOOD RESOURCE

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**Abstract.** When parasitic infections are severe or highly prevalent among prey, a significant component of the predator's diet may consist of parasitized hosts. However, despite the ubiquity of parasites in most food webs, comparisons of the nutritional quality of prey as a function of infection status are largely absent. We measured the nutritional consequences of chytridiomycete infections in *Daphnia*, which achieve high prevalence in lake ecosystems (>80%), and tested the hypothesis that *Daphnia pulicaria* infected with *Polycaryum laeve* are diminished in food quality relative to uninfected hosts. Compared with uninfected adults, infected individuals were smaller, contained less nitrogen and phosphorus, and were lower in several important fatty acids. Infected zooplankton had significantly shorter carapace lengths (8%) and lower mass (8–20%) than uninfected individuals. Parasitized animals contained significantly less phosphorus (16–18% less by dry mass) and nitrogen (4–6% less) than did healthy individuals. Infected individuals also contained 26–34% less saturated fatty acid and 31–42% less docosahexaenoic acid, an essential fatty acid that is typically low in cladocera, but critical to fish growth. Our results suggest that naturally occurring levels of chytrid infections in *D. pulicaria* populations reduce the quality of food available to secondary consumers, including planktivorous fishes, with potentially important effects for lake food webs.

**Key words:** *Daphnia pulicaria*; disease ecology; fatty acid; food quality; food web; nitrogen; parasitism; phosphorus; *Polycaryum laeve*; stoichiometry; zooplankton.

### INTRODUCTION

Parasites are a ubiquitous yet understudied component of most food webs (Lafferty et al. 2006, 2008). Most organisms serve as host for one or more parasites and some infections comprise a substantial portion of total host biomass (Minchella and Scott 1991). As a result, actively feeding predators also consume the parasites of their prey. This arrangement may be beneficial to the parasite; many helminths, for example, depend on predation as a vehicle of transmission among hosts (trophic transmission). However, in many other cases, predators feeding on parasitized prey are not suitable hosts themselves, and ingested parasites are either digested as part of the meal or pass through the predator undigested (e.g., resistant stages; e.g., Kagami et al. 2004). Considering that infections often achieve a high prevalence or intensity among hosts (prey), and that changes in host appearance or behavior sometimes increase their susceptibility to predation (Duffy et al. 2005, Johnson et al. 2006b), many predators probably consume parasitized prey with high frequency. From an energetic standpoint, this raises the important question

of how parasitized and unparasitized resources differ in nutritional value. If parasites or parasitized host tissues vary in nutrient content or digestibility, infections among prey could have important consequences beyond simple changes in prey growth and abundance. However, because this information is lacking for most host-parasite-predator systems, the role of parasitism in energetic flows in food webs remains unknown.

*Daphnia* are an important prey species for many freshwater zooplanktivores (Larsson and Dodson 1993) and a keystone herbivore that conveys much of the secondary productivity in pelagic ecosystems (Dodson 2005). In addition to predators, however, *Daphnia* are vulnerable to a variety of parasitic infections that elevate mortality, reduce growth, lower reproductive output, and change migratory behavior (see Green 1974, Ebert 2005; Johnson et al., *in press*). Beyond their individual and population-level effects on *Daphnia*, parasites may have important food web effects. Differences in food quality between parasitized *Daphnia* and their uninfected counterparts have the potential to alter the flow of energy, nutrients, and lipids to organisms dependent on *Daphnia*, particularly during lake-wide epidemics with sustained high infection prevalence. Given the often high frequency of parasitism in *Daphnia* populations and the importance of *Daphnia* in the diet of planktivorous fishes, infected *Daphnia* may comprise a significant fraction of prey resources available to such predators.

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Moreover, consumer dependence on parasitized *Daphnia* can be enhanced because infected *Daphnia* are often more vulnerable to fish predation (Duffy et al. 2005, Johnson et al. 2006b).

In aquatic food webs, the ratios among elements such as carbon (C), nitrogen (N), and phosphorus (P) are valuable for predicting organism growth and reproduction and may contribute to ecosystem fluxes (Sterner and Elser 2002). In freshwater pelagic ecosystems, cladocerans such as *Daphnia* are often limited by the availability of P, as they require high concentrations of P to achieve maximum growth (Urabe et al. 1997). This requirement for P leads to higher levels of total body P in *Daphnia* (Sterner and Hessen 1994). Elemental stoichiometry of freshwater zooplankton is thought to vary primarily interspecifically rather than intraspecifically (Anderson and Hessen 1991), with relatively constant elemental ratios within species despite available food sources (Frost et al. 2005). This constancy suggests that elemental composition may be exploited by zooplanktivores for growth and development, and that ecosystem nutrient cycling may be sensitive to zooplankton community composition (e.g., Schindler et al. 1993), because excess nutrients are released (Anderson et al. 2005). Parasitism may alter these ratios and induce measurable effects on nutrient flow in aquatic food webs.

Parasitic infection may also influence host levels of fatty acids, which are key components of dietary nutrition in aquatic ecosystems (Arts and Wainman 1999). Lipids are energy sources for metabolism and certain fatty acids are essential to growth and development of consumers. Not only is the bulk quantity of these fatty acids vital to consumer energy acquisition, but the relative amounts of certain key fatty acids can be important (Brett and Mueller-Navarra 1997). Research on the nutritional requirements of several fish species suggests that  $\omega 3$  fatty acids, particularly docosahexaenoic acid (DHA), are critical to fish growth, reproduction, and survival (Adams 1999, Olsen 1999, Sargent et al. 1999a).

To determine the influence of parasitism on nutritional quality of *Daphnia*, we measured the nutritional content of individual *Daphnia* during naturally occurring lake-wide epidemics of a chytridiomycete pathogen. While the nutrient composition of zooplankton is important in driving consumer growth and the cycling of nutrients within lake ecosystems, the degree to which parasitic infections affect the overall elemental ratio and fatty acid composition of *Daphnia* is unknown. We focused on interactions between the freshwater cladoceran *Daphnia pulicaria* and a chytridiomycete parasite, *Polycaryum laeve* (Johnson et al. 2006a, b, 2008; Appendix A). *Polycaryum* undergoes seasonal epidemics in *Daphnia* with the greatest prevalence (up to 80%) in early spring, corresponding to an important period of growth for juvenile fish (Becker 1983). We tested the hypothesis that infected *Daphnia* are diminished as a

food resource relative to healthy individuals. Specifically, we measured the relative difference of whole-body N, P, and fatty acid content between infected and uninfected *D. pulicaria* from field-collected surveys.

## METHODS

### *Study site and sampling methods*

Allequash Lake is a 172-ha lake located in Vilas County, Wisconsin ( $46^{\circ}2'28''$  N,  $89^{\circ}37'41''$  W). It is one of 11 lakes in the North Temperate Lakes Long-Term Ecological Research program managed by the University of Wisconsin. Detailed information and limnological data of this lake are available online.<sup>5</sup> We sampled uninfected and infected *D. pulicaria* during two *P. laeve* infection epidemics, defined here as an infection prevalence in excess of 20% of mature hosts. These epidemics occurred immediately prior to ice-off in 2005 and 2006 during the extended period of ice cover when water temperatures vary between 0 and 5°C for several months prior to sampling. We collected vertical zooplankton tows on 2 April in 2005, and 4 March and 1 April 2006 from the deepest point (7 m) of Allequash Lake with an 80- $\mu$  mesh Wisconsin plankton net. All samples were placed on ice and immediately transported to the Trout Lake Research Station, inspected, and sorted by infection status and sex under a dissecting microscope. We selected only mature females (>1.2-mm carapace size) that were either uninfected or moderately to heavily infected (>500 sporangia within the carapace, see Johnson et al. 2006a). Uninfected individuals were further separated into gravid and non-gravid categories. *Daphnia* analyzed for inorganic C, N, and P were randomly selected, placed in 1-mL vials, and frozen at -4°C. The *Daphnia* were dried for 72 hours at 50°C and weighed to the nearest microgram on a Cahn model C-33 microbalance (Orion Research, Beverly, Massachusetts, USA). Zooplankton fatty acid samples were collected as stated previously, sorted and measured in the laboratory, and stored at -85°C.

Seston fatty acid, C, and N water samples were collected in 2006 using a 3.2-L Van Dorn bottle (Wildco, Buffalo, New York, USA) below the ice surface and 1 m above the bottom at a depth of 6 m. The seston was screened with a 243- $\mu$  mesh to remove *Daphnia* in the field and transported on ice in acid-washed polyethylene bottles, brought back to the laboratory, and immediately filtered onto precombusted (24 h at 450°C) 25-mm GF/F filters for C and N and 47-mm filters for fatty acids. C and N samples were dried immediately and stored in desiccators until processing. Particulate P was determined by subtracting soluble reactive P from total P from LTER data on the nearest sampling date. Seston fatty acid samples were filtered as stated previously, placed on dry ice, and stored at -85°C.

<sup>5</sup> (<http://lter.limnology.wisc.edu>)

### Chemical analyses

Elemental C and N samples of *Daphnia* were combined as groups of five or 10 individuals of each *Daphnia* type to achieve the mass required for analysis (uninfected gravid,  $n = 15$  replicate samples; non-gravid,  $n = 13$ , infected,  $n = 14$ ) for a total of 42 independent samples. Because *Daphnia* infected with *Polycaryum* rarely support eggs (Johnson et al. 2006a), we could not include an additional category for infected gravid individuals. Seston samples of 200 mL collected in 2006 from surface and 6 m in depth were prepared in triplicate ( $n = 6$ ). All C and N samples were wrapped in tin capsules and processed by the University of California at Davis stable isotope facility using a Europa Scientific Hydra 20/20 continuous-flow isotope ratio mass spectrometer (IRMS; PDZ Europa, Northwich, UK). Individual *Daphnia* were analyzed for P from each sampling date by placing individuals in acid-washed borosilicate glass scintillation vials. The contents were combusted at 500°C for two hours and then analyzed colorimetrically on a Beckman DU 640 spectrophotometer (Beckman Instruments, Fullerton, California, USA) following Solorzano and Sharp (1980). Results are reported as a dry mass percentage of total *Daphnia* dry mass (%DM).

For fatty acid analyses, five individual *Daphnia* of each group were combined (uninfected gravid, non-gravid, and infected) for four replicates of each group ( $n = 12$  individuals per group, or 60 individuals total). Samples were freeze-dried prior to extraction and weighed on an AD-6 microbalance (Perkin Elmer, San Jose, California, USA). Fatty acid quantity was calculated using the gas chromatograph peak area ratios of sample to a known (10 µL of 1 mg/mL) internal standard 21:0 added prior to extraction. Following extraction and methylation (Kattner and Fricke 1986), analysis was done by gas chromatograph (HP 6890, Agilent Technologies, Palo Alto, California, USA) with a programmable temperature vaporizer-injector, a fused silica DB-WAX (J and W Scientific, Folsom, California, USA) capillary column, and flame ionization detector at the University of California–Davis Limnology Laboratory. Individual fatty acid methyl esters (Sigma, St. Louis, Missouri, USA) in n-hexane were used as standards to determine retention times. Results are reported as percentage dry mass of fatty acid of total *Daphnia* dry mass (%DM). Following analysis we combined lipid fraction results based on similarity of function and evidence of their essential nature based on literature (e.g., Adams 1999, Olsen 1999, Sargent et al. 1999a, Ishizaki et al. 2001) generating composite fatty acid values to more clearly assess the role of parasitism in *Daphnia*. These groups are saturated fatty acids (SAFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), and highly unsaturated fatty acids (HUFA; Appendix B). We also included ratios of polyunsaturated fatty acids omega 3 to omega

6, DHA to eicosapentaenoic acid (EPA), and EPA to arachidonic acid (ARA; Table 1).

To compare the three groups of *Daphnia*, data were natural log-transformed to help equalize variance and tested for normality using Levene's test. Data were analyzed using one-way analysis of variance (ANOVA) or general linear model (GLM) ANOVA with type 3 sum of squares and Tukey's pairwise comparisons to determine individual differences ( $\alpha = 0.05$ ). To analyze seston P, we combined the three sampling dates and performed a paired *t* test between dates.

### RESULTS

Comparisons between the uninfected gravid, uninfected non-gravid, and infected *D. pulicaria* revealed statistically significant differences for all major response variables. Compared to uninfected gravid females, *Polycaryum*-infected *Daphnia* had significantly lower masses (ANOVA,  $P < 0.001$ ,  $F_{2,232} = 7.07$ ) ( $0.059 \pm 0.002$  mg,  $n = 77$  vs.  $0.074 \pm 0.003$ ,  $n = 69$ ) (mean  $\pm$  SE), were smaller in carapace length ( $P < 0.001$ ,  $F_{2,232} = 17.26$ ) ( $2.37 \pm 0.04$  mm vs.  $2.57 \pm 0.03$ ), and were greater in length-to-mass ratio ( $P < 0.015$ ,  $F_{2,232} = 4.28$ ). However, infected *Daphnia* were not significantly different in length, mass, or length-to-mass ratio from uninfected non-gravid *Daphnia* (Tukey's,  $P > 0.05$ , non-gravid,  $n = 89$ ) (Fig. 1).

Infected *Daphnia* contained significantly less P than uninfected gravid and non-gravid adults (GLM ANOVA,  $P < 0.001$ ,  $F_{2,81} = 9.62$ ; Tukey's,  $P < 0.05$ ; Fig. 1). P content of infected *Daphnia* was 16–18% lower than uninfected *Daphnia*, with infected exhibiting  $1.44\% \pm 0.04\%$  P (mean  $\pm$  SE,  $n = 30$  for each group), uninfected gravid exhibiting  $1.71\% \pm 0.04\%$  P, and uninfected non-gravid supporting  $1.76\% \pm 0.08\%$  P. The uninfected groups were not significantly different in P content from each other. Sample date was not a significant predictor of percentage P, and there were no significant interactions between date, infection status, or egg prevalence.

Total N of infected *Daphnia* was also significantly less than that of the uninfected individuals (GLM ANOVA,  $P < 0.004$ ,  $F_{2,36} = 6.61$ ; Tukey's,  $P < 0.05$ ). The N content was  $8.39\% \pm 0.18\%$  DM ( $n = 14$ ) for infected individuals,  $8.69\% \pm 0.18\%$  DM ( $n = 15$ ) for uninfected gravid individuals, and  $8.76\% \pm 0.17\%$  DM ( $n = 13$ ) for uninfected non-gravid *Daphnia*. However, with respect to N, sampling date was a significant predictor of N %DM (GLM ANOVA,  $P < 0.001$ ,  $F_{1,36} = 64.62$ ). Specimens from 5 March 2006 had the highest overall N ( $9.05\% \pm 0.09\%$ ,  $n = 29$  vs.  $7.94\% \pm 0.09\%$ ,  $n = 13$ ). There was no significant interaction between *Daphnia* status and sampling date. We did not find a significant difference in *Daphnia* C content between infected ( $41.7\% \pm 0.4\%$ ,  $n = 14$ ) and uninfected gravid ( $42.3\% \pm 0.3\%$ ,  $n = 15$ ) (Tukey's,  $P > 0.05$ ) (Fig. 1). However, C was significantly lower in uninfected non-gravid compared to both infected and gravid adult *Daphnia* (GLM ANOVA,  $P < 0.016$ ,  $F_{2,36} = 4.61$ ).

TABLE 1. Fatty acid composition of *Daphnia* and seston.

Fatty acids	Gravid	Infected	Uninfected	F	P	Seston, surface	Seston, 6 m
SAFA	26.01 ± 1.81	17.17 <sup>c</sup> ± 0.67	23.33 ± 1.94	19.05	<0.001	10.23	11.09
MUFA	30.37 ± 7.48	26.41 ± 4.28	34.61 ± 8.92	0.74	0.497	3.36	2.68
PUFA	47.2 ± 3.92	42.95 ± 2.9	38.45 ± 3.26	1.62	0.239	10.11	6.36
HUFA	26.93 ± 1.94	30.18 ± 2.09	22.38 ± 2.27	3.36	0.070	3.60	2.45
14:0	4.51 ± 0.69	1.87 <sup>c</sup> ± 0.27	3.96 ± 0.92	6.95	<b>0.010</b>	1.35	1.42
14:1	0.2 <sup>b</sup> ± 0.04	0.06 <sup>a</sup> ± 0.01	0.18 <sup>ab</sup> ± 0.06	5.40	<b>0.021</b>	0.01	0.02
15:0	1.58 ± 0.25	0.85 <sup>c</sup> ± 0.13	1.41 ± 0.07	5.67	<b>0.018</b>	0.30	0.36
16:0	15.66 <sup>b</sup> ± 1.14	11.48 <sup>a</sup> ± 0.51	13.79 <sup>ab</sup> ± 1.03	5.25	<b>0.023</b>	5.63	5.18
16:1	17.57 ± 7.23	13.51 ± 4.13	22.11 ± 8.86	0.17	0.848	1.16	1.14
17:0	1.04 ± 0.13	0.73 ± 0.09	1.03 ± 0.04	3.61	0.059	0.48	0.35
18:0	3.22 ± 0.11	2.24 <sup>c</sup> ± 0.05	3.16 ± 0.16	31.51	<0.001	2.48	3.78
18:1ω6&ω9	7.56 ± 0.34	6.61 ± 0.31	7.17 ± 0.54	1.51	0.261	1.56	1.02
18:1ω7	5.04 ± 0.07	6.23 <sup>c</sup> ± 0.34	5.15 ± 0.2	8.10	<b>0.006</b>	0.63	0.50
18:2ω6	3.72 ± 0.48	3.26 ± 0.45	3.3 ± 0.41	0.29	0.756	1.15	0.95
18:3ω6	1.05 ± 0.19	0.72 ± 0.12	0.84 ± 0.15	1.23	0.327	0.05	0.04
18:3ω3	6.36 ± 1.39	4.48 ± 0.93	5.24 ± 1.11	0.51	0.613	2.46	1.97
18:4ω3	9.13 <sup>b</sup> ± 1.32	4.31 <sup>a</sup> ± 0.49	6.7 <sup>ab</sup> ± 0.93	7.56	<b>0.008</b>	2.83	0.95
20:4ω6	3.46 ± 0.21	4.08 ± 0.28	3.3 ± 0.12	3.62	0.059	0.58	0.17
20:5ω3	23 ± 1.99	25.82 ± 2.05	18.67 ± 2.22	2.96	0.090	2.07	1.60
22:6ω3	0.48 ± 0.06	0.28 <sup>c</sup> ± 0.03	0.41 ± 0.02	7.45	<b>0.008</b>	0.95	0.68
ω3 PUFA	38.97 ± 3.32	34.88 ± 2.48	31.02 ± 2.8	1.80	0.207	8.39	5.25
ω6 PUFA	8.23 ± 0.66	8.06 ± 0.66	7.43 ± 0.5	0.44	0.656	1.79	1.16
DHA to EPA	0.021 ± 0.003	0.011 <sup>c</sup> ± 0.001	0.024 ± 0.004	8.46	<b>0.005</b>	0.46	0.43
EPA to ARA	6.81 ± 0.88	6.45 ± 0.73	5.63 ± 0.6	0.71	0.512	3.56	9.42
ω3 to ω6	4.74 ± 0.19	4.39 ± 0.35	4.16 ± 0.19	0.95	0.416	4.69	4.52

Notes: The superscripts a, b, and c indicate significant different groups in *Daphnia* based on Tukey's ( $P < 0.05$ ). There were five replicates of each group of *Daphnia* ( $n = 15$ ) with one replicate in 2005 and four replicates in 2006. Seston is composed of a single sample from surface water and 6 m in depth. Key to abbreviations: SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; HUFA, highly unsaturated fatty acids; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; ARA, arachidonic acid. Significant P values ( $P < 0.05$ ) are shown in boldface type.

Infected *Daphnia* exhibited significantly greater elemental ratios of C to N (ANOVA,  $P < 0.009$ ,  $F_{2,39} = 5.34$ , Tukey's,  $P < 0.05$ ) ( $4.99 \pm 0.08$  [DM to DM]) compared to uninfected non-gravid females ( $4.55 \pm 0.12$ ); uninfected gravid *Daphnia* were not significantly different from either group ( $4.75 \pm 0.08$ ; Fig. 1). Because we only had two independent dates for C and N analyses, we generated two statistically independent samples for N to P and C to P ratios; we present these as combined means. N to P ratios for infected, uninfected non-gravid, and uninfected gravid *Daphnia* were 5.81, 4.96, and 5.23, respectively. C to P was 28.9 in the infected group, 22.5 in the uninfected non-gravid, and 24.7 in the gravid group (Appendix C).

Infected *Daphnia* also contained significantly lower amounts of SAFA relative to uninfected *Daphnia* (GLM ANOVA,  $P < 0.01$ ,  $F_{2,12} = 19.0$ ; Fig. 2). There was 31–42% more of the essential fatty acid DHA among uninfected gravid and non-gravid *Daphnia* in comparison with infected individuals (GLM ANOVA,  $P = 0.01$ ,  $F_{2,12} = 7.45$ ). There were significantly lower levels of the DHA-to-EPA in the infected individuals (GLM ANOVA,  $P = 0.01$ ,  $F_{2,12} = 8.46$ ). Of the fatty acids screened in this study, the most abundant was 16:0, with mean concentration in *Daphnia* of 13.65 mg/g DM, while the most abundant essential fatty acid was EPA, at 16.4 mg/g (Table 1).

The average particulate P content in surface water seston ( $2.7 \pm 1.2$  mg/L) compared to 6 m in depth ( $5.7 \pm 0.9$  mg/L) was significantly greater (paired  $t$  test,  $P =$

0.04,  $n = 3$ , mean  $\pm$  SE) based on three sampling dates. The seston surface water samples collected on 4 March 2006 did not differ in C content ( $232.7 \pm 4.9$  µg/L,  $P = 0.32$ ,  $n = 3$ , mean  $\pm$  SE). There was significantly more N in surface water than in 6-m depth samples (ANOVA,  $P = 0.01$ ,  $F_{1,5} = 21.0$ ).

## DISCUSSION

Our results support the hypothesis that *D. pulicaria* infected with the chytridiomycete *P. laeve* are diminished in food quality relative to uninfected individuals. Our approach of using key elemental and fatty acid indicators revealed substantial reductions in P, N, and select fatty acids between infected *Daphnia* and uninfected conspecifics collected from the same lake on the same day. By comparing uninfected gravid, uninfected non-gravid, and infected *Daphnia* (which always lack eggs), we provide a life-history framework for the nutritional component analyses. Relative to gravid adults without infection, *Daphnia* infected with *Polyancistrum* were generally smaller, possibly owing to decreased growth and heightened mortality. Because infection is almost certainly transmitted horizontally (see Johnson et al. 2006a), infected *Daphnia* were larger and probably older than the smallest size classes of uninfected *Daphnia*, such that there was no overall size difference between the infected group and the uninfected non-gravid group. This observed overlap in size between groups further supports the notion that the nutritional differences we measured between infected and uninfected

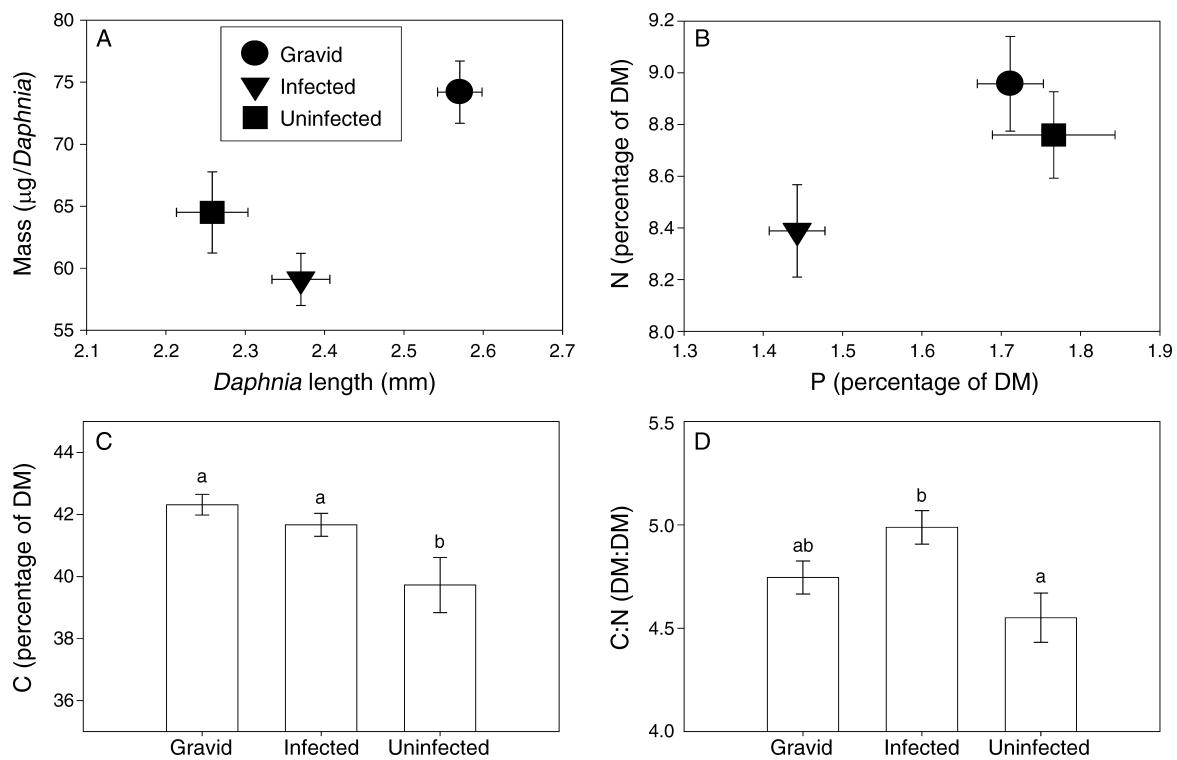


FIG. 1. (A) Mean length and mass bi-plot, (B) nitrogen and phosphorus (as a percentage of dry mass [DM]) bi-plot, (C) percentage carbon, and (D) carbon to nitrogen ratio (on a dry mass basis) of gravid, infected, and uninfected *Daphnia* collected during two epidemic events in Allequash Lake, Wisconsin, USA. Infected individuals that support eggs are incredibly rare because the parasite is a reproductive castrator (see Johnson et al. 2006). There are very few infected individuals with eggs during epidemics at any time of year. Error bars represent  $\pm \text{SE}$ , and the lowercase letters represent significant differences (GLM ANOVA and Tukey's,  $\alpha = 0.05$ ).

ed non-gravid *Daphnia* are due to the effects of parasitism, rather than to size-specific differences independent of parasitism. While we cannot eliminate the possibility that genetic variation or extremes in diet explain the differences in both infection and nutrient content (DeMott et al. 2004), we consider this unlikely because the nutrient composition of zooplankton is fairly stable within a population or species, even while it may vary among species (Frost et al. 2005).

In all key elemental ratios, infected *Daphnia* in this study exhibited lower N and P relative to the C content. This is notable given that infected *Daphnia* also exhibited lower overall fatty acid content, which is a large C source, indicating that the decrease in N and P associated with infection is greater than the observed decreases in C. Infected *Daphnia* did not retain the same elemental stoichiometric ratios among C, N, and P relative to their uninfected counterparts. *Polycaryum* infection in *Daphnia* was associated with an increase in C:P and an increase in C:N. This parasite effect contrasted with uninfected *Daphnia*, which tended to maintain constant C:P and C:N ratios even under varied food supplies and conditions (e.g., Hessen 1990, Andersen and Hessen 1991). Thus, a parasite may shift the stoichiometric ratio available to consumers due to

parasite biomass, metabolic requirements for growth, or there may be an alternate stoichiometric ratio caused by the onset of the infection in the tissue of the *Daphnia*. Further investigation into the influence of the parasite and its stoichiometric requirements may generate predictable changes in the whole body of zooplankton similar to those observed among zooplankton species, but at this point we do not know whether this is the case. In our study, *Daphnia*, as a whole, shifted toward decreased elemental N and P, suggesting that the overall food quality of infected organisms decreased.

We found lower saturated fatty acid content in infected *Daphnia*, indicating that infection causes catabolism of reserve energy. In addition to measured decreases in lipids, visual inspection of the tissues in infected individuals revealed little to none of the lipid globules commonly observed in healthy females. We found decreased levels of the essential fatty acid DHA in association with *Polycaryum* infection. DHA has been shown to limit some freshwater food webs (Ballantyne et al. 2003), such that limited DHA can retard functional brain and neural development in schooling fishes (Ishizaki et al. 2001), potentially constraining successful recruitment from larval to adult stages (Sargent et al. 1999b). *Daphnia* are low in DHA relative to other prey,

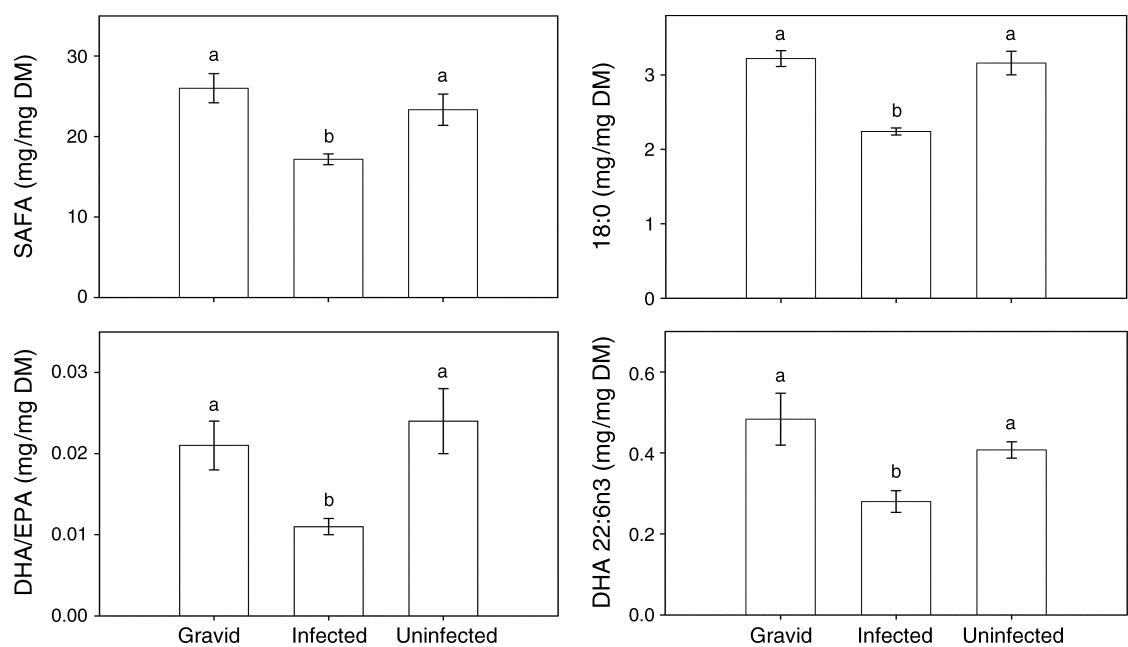


FIG. 2. Mean whole body fatty acids in *D. pulicaria* infected by *P. laeve*. Error bars indicate  $\pm$ SE, and letters indicate significant differences (GLM ANOVA and Tukey's,  $\alpha = 0.05$ ). Key to abbreviations: SAFA, saturated fatty acids; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.

such as copepods (Persson and Vrede 2006), but *Daphnia* are the primary food source that are preferentially preyed upon when infected (Johnson et al. 2006b), which suggests that parasite-induced or parasite-associated decreases in this essential fatty acid could have a substantial effect on the food web where *P. laeve* exists.

The mechanisms responsible for diminished food quality and altered body composition in the infected *Daphnia* most likely involve the combined influences of the (1) intrinsic stoichiometry of the parasite, which comprises as much as 17% of the total host biomass (P. Johnson, unpublished data), (2) altered metabolic demands of infected *Daphnia* relative to uninfected hosts, and the (3) alteration of feeding behavior among infected individuals. Seston fatty acids can influence grazer composition (Brett et al. 2006) and cooler temperatures can enhance accumulation of HUFAs, especially EPA in *Daphnia* (Schlechtriem et al. 2006), but these are unlikely to cause the differences we found because *Daphnia* were collected at the same time and location where water temperatures vary only a few degrees throughout the water column during ice cover. Because *P. laeve* infections have yet to be cultured and transmitted in vitro, we do not know the degree to which the altered composition observed here is due to feeding changes or elevated metabolic demands in parasitized *Daphnia*. Assuming that catabolism decreases elemental N, phosphorus, and lipids disproportionately relative to C, as observed here, the disease may literally be starving the *Daphnia*. While there is some degree of reallocation of nutrients from host to parasite, most of these changes would be incorporated into the chytridiomycete tissue

(minus conversion efficiency), which was included in the whole-body *Daphnia* nutrient measurements. Data on the specific nutrient composition of the isolated parasite tissue are currently lacking, but future investigations will help determine the extent to which nonparasitic tissues of the infected *Daphnia* differ from those of uninfected *Daphnia*. Furthermore, the decreased nutritive quality of the parasitized *Daphnia* may be exacerbated by the findings that the chitin-coated sporangia of *P. laeve* are relatively indigestible by fish, allowing the parasite to pass through the digestive system of fish predators undamaged (Johnson et al. 2006b). Consequently, any resources assimilated by the parasite from its host are likely rendered inaccessible to predators, substantially reducing both the total biomass and nutritional quality of *Daphnia* as a prey resource, especially if parasites preferentially occupy nutrients. In lakes that support *P. laeve* epidemics, infected *Daphnia* can be a dominant component of the zooplankton community (Appendix D) and of the diets of young-of-the-year yellow perch. Considering that this material is both lower in food quality and less digestible, the consequences on juvenile fish growth could be significant, particularly if a threshold body size must be achieved by overwintering fish (Carlander 1997, Post et al. 1998). Nevertheless, some of these costs could be offset by the increased ease with which infected individuals are detected and consumed by fish predators (see Johnson et al. 2006b), particularly when nutrients like P do not limit growth (Schindler and Eby 1997). Biochemical synthesis by predators of essential fatty acids such as DHA may also compensate fatty acid limitations (Agaba et al. 2005) or

even behavioral changes involving the transition to alternate prey, when available, that contain the lacking food quality constituent. A comprehensive evaluation of the net costs and benefits of *Polycaryum* infection in *Daphnia* on consumer growth and food web dynamics is beyond the scope of this study, but represents an important future area for investigation.

### CONCLUSION

Our results reveal that chytridiomycete infections of *Daphnia* have large negative effects on total body nutrition of *Daphnia*. The effects of *Polycaryum laeve* appear to induce diminished nutritional quality of the overall infected *Daphnia* compared to those of their uninfected counterparts, including those of similar size. There is a clear decrease in critical nutrient constituents in both the elemental and fatty acid composition of the *Daphnia*. We suggest that further investigation is needed to determine (1) how these nutritional changes manifest through the food web to influence zooplanktivorous consumers, (2) how these changes alter nutrient recycling in pelagic systems, and (3) by what mechanisms this nutritive degradation occurs in *Daphnia*. Nevertheless, by demonstrating that chytrid parasitism of *Daphnia* diminishes their nutrient quality, indirectly reducing the quality of food available to secondary consumers, our results have important implications for lake food webs and ecosystem nutrient dynamics.

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#### APPENDIX A

Photographs of *Daphnia pulicaria* showing uninfected gravid, uninfected non-gravid, and infected individuals (*Ecological Archives* E089-152-A1).

#### APPENDIX B

The nomenclature and structure of the fatty acids considered in this study (*Ecological Archives* E089-152-A2).

#### APPENDIX C

*Daphnia* elemental nutrient percentage composition and descriptive statistics of *Daphnia* length, mass, and elemental composition based on percentage dry mass for each sampling date (*Ecological Archives* E089-152-A3).

#### APPENDIX D

Table of observed copepods and cladocera with density in Allequash Lake, Wisconsin, on 19 April 2005 reported by the Northern Lakes LTER (*Ecological Archives* E089-152-A4).