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## Context-dependent effects of *Daphnia pulex* on pond ecosystem function: observational and experimental evidence

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**Abstract** Large-bodied zooplankton of the genus *Daphnia* are thought to be keystone species in freshwater pelagic habitats, potentially able to exert strong grazing effects and enhance phosphorus limitation of algae. I examined the degree to which *Daphnia pulex* differ from small-bodied zooplankton in their effects on algal biomass, seston C:P and N:P, total nitrogen and total phosphorus. This was done with both survey data from natural ponds and an in situ experiment in which *D. pulex* was compared to a small zooplankton assemblage under low and high nutrient conditions and in two different ponds. *D. pulex* effects on algae were only evident under high nutrient conditions. In natural ponds, *D. pulex* dominance resulted in a significantly weaker chlorophyll–total phosphorus relationship, with the divergence between *D. pulex* and small zooplankton-dominated systems being greatest in highly enriched ponds. In the experiment, *D. pulex* exerted stronger top-down control in enriched treatments only and tended to graze algae to lower levels in the more productive pond. Dynamics of C:P over the course of the experiment did not reveal strong effects of zooplankton composition. However, data on the final date of the experiment provided some evidence that *D. pulex* can enhance phosphorus limitation of algae; total phosphorus was lower and C:P higher in *D. pulex* treatments. Survey results revealed no effects of *D. pulex* on seston C:P or N:P, suggesting that this species may not be an important factor governing phosphorus limitation of algae in natural ponds.

**Keywords** Algae · Keystone species · Nutrient cycling · Stoichiometry · Zooplankton

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### Introduction

Recently the field of ecology has seen an upsurge of interest in the effects of species, species richness and functional groups on ecosystem function and stability (Schulze and Mooney 1993; Naeem et al. 1994; Tilman and Downing 1994; McGrady-Steed et al. 1997; Hulot et al. 2000). This research has been spurred in large part by practical needs, for gaining insight into such relationships undoubtedly holds important implications for how we manage natural resources as well as our ability to predict the outcome of environmental impacts and species losses. Traditionally, important or “keystone” species have been distinguished by their inordinately large effects on community properties relative to other species. Such key species may exact effects through their ability to dominate community biomass (a numerical effect) or through extraordinarily high per capita effects. While conventionally measured at the level of communities (e.g., effects on species composition or richness), the presence of such species could have important repercussions on ecosystem-level properties, causing the relationship between species richness and ecosystem function to be disjunct (Power et al. 1996). It is evident that our ability to comprehend and predict the consequences of variable species composition on ecosystem properties may depend vitally on identification and experimental validation of potential keystone species (Power et al. 1996).

In temperate freshwater systems, zooplankton body size is often considered to be an important determinant of planktonic community and ecosystem properties (e.g., Pace 1984; Vanni 1987; Elser et al. 1988; Mazumder 1994a, b; Schindler et al. 1997; Cottingham 1999; Tessier et al. 2001). Commonly, large-bodied zooplankton of the genus *Daphnia* are thought to control total primary production more effectively than other zooplankton taxa, such as small-bodied cladocera or copepods (Leibold 1989; Mazumder 1994a; Cottingham and Schindler 2000), earmarking *Daphnia* as key components of trophic cascades, successful bio-control of algal blooms and system stability (by buffering nutrient per-

turbations). Recently, attention has also focused on the carbon, phosphorus and nitrogen composition (C:N:P) of zooplankton and the implications of among species variation in elemental stoichiometry on nutrient recycling. With few exceptions, *Daphnia* tissues appear to have the lowest C:P and N:P ratios among freshwater zooplankton (Andersen and Hessen 1991; Sterner et al. 1992). Consequently, *Daphnia* may sequester greater amounts of phosphorus relative to nitrogen, leading to higher nitrogen to phosphorus excretion rates compared to less phosphorus demanding taxa (Sterner et al. 1992; Elser and Urabe 1999).

Taken together, these attributes single out *Daphnia* as potential keystone species whose presence can have significant impacts on the larger aquatic community and its ecosystem-level properties. While suspected, the reality of this assertion has seldom been tested via direct, long-term experimental manipulation of *Daphnia* in the field; rarer still are studies that compare monocultures of *Daphnia* with alternate taxa. A number of lake studies have compared nitrogen and phosphorus excretion by *Daphnia* versus zooplankton species with higher N:P ratios (e.g., copepods). These studies have largely confirmed theorized predictions; *Daphnia* recycle nitrogen at greater rates than phosphorus (reviewed in Elser and Urabe 1999). However, experimental elucidation of the biological relevance at the ecosystem level of such differential recycling (i.e., actual effects on algal assemblages in regards to elemental composition or nutrient limitation) is still in its formative stages (Elser and Urabe 1999). Furthermore, there have been remarkably few long-term investigations that have directly manipulated zooplankton composition with the intent of examining differential effects on algal production. Frequently, studies that have “manipulated” *Daphnia* presence or zooplankton size-structure have done so indirectly by manipulating the presence of planktivorous fish (e.g., Mazumder 1994b; Cottingham and Schindler 2000 and the studies analyzed in Sarnelle 1992a and Cottingham 1999), thus introducing potential confounding effects and experimental artifacts (e.g., nutrient recycling by fish; Vanni and Layne 1997). Those investigations that have directly manipulated zooplankton composition have found highly variable effects of *Daphnia* presence on algal standing crop, with some experiments finding small or insignificant differences (Turner and Mittelbach 1992; Brett et al. 1994), some revealing strong effects (Schoenberg and Carlson 1984; Sarnelle 1993; Tessier et al. 2001), and some finding effects that were highly variable or dependent on ecological setting (Vanni 1984; Leibold and Wilbur 1992; Steiner 2001a; reviewed in Leibold et al. 1997). The specific circumstances that mediate the strength of *Daphnia* grazing effects are poorly known. Yet, a number of studies, both experimental and observational, suggest that *Daphnia* may exert stronger effects under nutrient-enriched conditions (Vanni 1984; Sarnelle 1992a; Mazumder 1994a; Steiner 2001a). Moreover, our understanding of *Daphnia* impacts has been largely limited to studies of lakes. The

functional role that these species play in shallow ponds is generally unknown.

In the following paper I report on an experiment in which I assessed the effects of *Daphnia pulex* on pond ecosystem function – specifically algal standing crop, nitrogen and phosphorus content at the scale of the entire water column (total nitrogen, TN, and total phosphorus, TP), and seston C:P ratios as indicators of phosphorus supply rates and phosphorus limitation of algal growth (Healey and Hendzel 1980). *D. pulex* alone was compared to a diverse assemblage of taxa composed of small-bodied cladocera, copepods, and rotifers. The experiment was performed in two fishless ponds that varied naturally in production, and under experimentally enriched and unenriched conditions. To further investigate the effects of zooplankton composition and *Daphnia* dominance, I also surveyed natural ponds to examine the natural relationships between *D. pulex* abundance and algal standing crop and seston C:N:P.

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## Materials and methods

### Field experiment

The experiment was performed in August of 1998, in two fishless ponds (P12 and P14) at the Kellogg Biological Station (hereafter K.B.S.), experimental pond facility (Hickory Corners, Mich.). Both ponds are 1.6 m deep at their centers, with a surface area of approximately 700 m<sup>2</sup>. Though both ponds were fishless, zooplankton composition in the two differed greatly in the year of the study. In P12, *D. pulex* were present and dominant for the majority of the summer, whereas the P14 zooplankton community exhibited a seasonal loss of *D. pulex* and was dominated by calanoid copepods and small-bodied cladocera (primarily *Diaphanosoma brachyurum*) during the entire growing season. P12 was also the more productive of the two, harboring higher chlorophyll *a* (6.52 vs 2.62 µg l<sup>-1</sup>), zooplankton biomass (0.342 vs 0.048 mg l<sup>-1</sup>, dry weight) and TP (25.7 vs 16.3 µg l<sup>-1</sup>) at the time of the experiment.

The experiment was performed using 1,200-l polyethylene “bag” enclosures, sealed at their bottoms to exclude sediments, and suspended in the water column using floating frames. Bags were 1 m in diameter, extended to pond bottoms, and were screened with gray fiberglass window screening on their tops to exclude insects. Though screening may have reduced sun light in the bags (potentially reducing seston C:P), effects were assumed equal among all enclosures. To explore effects of zooplankton composition, two treatments were employed: *Daphnia pulex* alone and an assemblage of copepods, rotifers, and small-bodied cladocera alone (hereafter collectively referred to as “small” or “small-bodied” zooplankton for simplicity). *D. pulex* was chosen since it is the dominant *Daphnia* species found in permanent, fishless ponds in southwest Michigan (Steiner 2001b). The design was executed in both P12 and P14 concurrently. To explore the interaction of enrichment and zooplankton composition on algal biomass, “*D. pulex*” and “small zooplankton” treatments were further crossed with two nutrient treatments (“low” and “high”) in P14 only. Manipulations consisted of additions of phosphorus (as Na<sub>2</sub>HPO<sub>4</sub>) with high nutrient treatments receiving an initial concentration of 75 µg P l<sup>-1</sup> and low nutrient treatments receiving no additions for an initial concentration of 16 µg P l<sup>-1</sup>. Nitrogen (as NaNO<sub>3</sub>) was added with phosphorus in a 70:1 N to P molar ratio, matched to that of the ambient pond water (based on TN:TP measurements made earlier in the growing season). Nutrients were added as a single pulse at the start of the experiment. All treatments were replicated three times for a total of 18 enclosures. All bags were filled by pumping water from their respective ponds, at

mid-depth, through an 80- $\mu\text{m}$  zooplankton net to remove the ambient zooplankton community and invertebrate predators. Small-bodied zooplankton were collected with an 80- $\mu\text{m}$  zooplankton net from a pond at the experimental facility that contained no *Daphnia* and no invertebrate predators due to fish predators. The isolated assemblage contained all the major small-bodied taxa found in the experimental ponds during the summer season, including calanoid and cyclopoid copepods, *Diaphanosoma*, *Ceriodaphnia*, *Chydorus*, *Bosmina*, and numerous species of rotifers. To ensure their availability at the time of the experiment, *Daphnia pulex* were maintained in laboratory batch cultures. One day after bags were filled with water, enclosures were inoculated with the same total biomass of zooplankton (0.030 mg l<sup>-1</sup>, dry weight). Nutrients were then added 2 days after zooplankton additions and the experiment was allowed to run for 40 days.

All sampling was performed using integrated tube samplers (constructed of hard plastic tubing with a 7-cm diameter) that extended to enclosure bottoms. Zooplankton and phytoplankton were sampled weekly beginning on the 12th day of the experiment. Zooplankton samples were preserved in acid Lugol's solution for later enumeration. For each sample, 50 randomly chosen individuals of each species were also measured to obtain dry weight estimates (McCauley 1984). Following zooplankton collection, water samples were collected and placed on ice, in the dark. These were later filtered onto Gelman A/E glass fiber filters and frozen for subsequent measurement of chlorophyll *a* as a measure of algal biomass using narrowband fluorometry (sensu Welschmeyer 1994). Seston C:P was analyzed for the first, third and fifth sample periods only (corresponding to days 12, 26, and 40). Water samples were filtered through a 60- $\mu\text{m}$  mesh to remove zooplankton, divided in two, and filtered onto two separate, precombusted A/E glass fiber filters. One filter was immediately frozen for analysis of particulate phosphorus. The other filter was first fumed with concentrated HCl to remove carbon contributions from CaCO<sub>3</sub> and then frozen for later analysis of carbon content using a Carlo-Erba CHN analyzer. Note that Gelman A/E filters have an approximate pore size of 1.0  $\mu\text{m}$ , minimizing bacterial contributions to C and P estimates. Water samples (250 ml total) for TP and TN analyses were collected on days 12 and 40 and frozen for later analysis. TP and particulate phosphorus were analyzed using the ammonium molybdate method following persulfate digestion. TN was analyzed using second-derivative, scanning spectroscopy (Bachmann and Canfield 1996). Only algal biomass (chlorophyll *a*) and zooplankton were sampled from high nutrient treatments.

In the following analyses, pond identity is treated as a treatment factor or fixed effect. Thus, in order to examine zooplankton and pond effects, the experiment is treated as a 2 $\times$ 2 factorial design (i.e., zooplankton manipulations crossed with pond identity). To explore the interaction between nutrient enrichment and zooplankton composition on algal biomass, P14 enclosures (zooplankton composition crossed with nutrient manipulations) were analyzed as a separate 2 $\times$ 2 factorial experiment. Chlorophyll, zooplankton, TP, TN, and seston C:P responses through time were analyzed using univariate repeated measures ANOVA (rm-ANOVA), excluding initial (day 0) values. Due to potential violations of the assumption of circularity, Greenhouse-Geisser adjusted probabilities are presented (Von Ende 1993). TP and TN were only sampled on two dates; therefore adjusted *P*-values were not generated for these analyses. Examination of treatment effects for single sample dates was performed using ANOVA. All values were log<sub>10</sub> transformed to conform to assumptions of homogeneity of variances and statistics were performed using Systat Version 8.0.

#### Field survey

During the same year of the experimental study (1998), a field survey of 15 natural ponds plus 3 ponds from the K.B.S. experimental facility (including P12 and P14) was performed to determine the natural relationships among zooplankton composition, zooplankton biomass, algal standing crop, and seston C:N:P. Only fishless ponds were included in the survey. All ponds were found

within a 100-km radius around K.B.S. and contained water year-round during 3 years of monitoring (1996–1999). Maximal depths measured in June 1998 ranged between 0.4 and 1.6 m.

Ponds were sampled monthly beginning in late April and ending in late August. Low water levels, due to drought conditions, made sampling unfeasible in six ponds during the August sample period. Zooplankton were collected using tube samplers along a qualitative transect extending from 1 m from pond edge to pond center. Two replicate samples were taken, each integrating spatial variation along the transect. Zooplankton were preserved using acid Lugol's and identified to the genus or species level, excepting copepods, which were categorized as calanoid or cyclopoid. Rotifers were generally identified to the genus or species level with the exception of members of the Notommatidae and Proalidae, which were identified to the family level, and the Bdelloidea which were identified to the level of order. Fifty randomly chosen individuals of each taxon were also measured to obtain dry mass estimates. During each sample period, water was collected along the transect, stored on ice, and later analyzed for chlorophyll *a*, TP, TN, and seston C:N:P content. Protocols matched those in the experiment except water for C:N:P analyses was filtered through a 35- $\mu\text{m}$  mesh. Seston nitrogen content was analyzed, in conjunction with carbon, using a CHN analyzer.

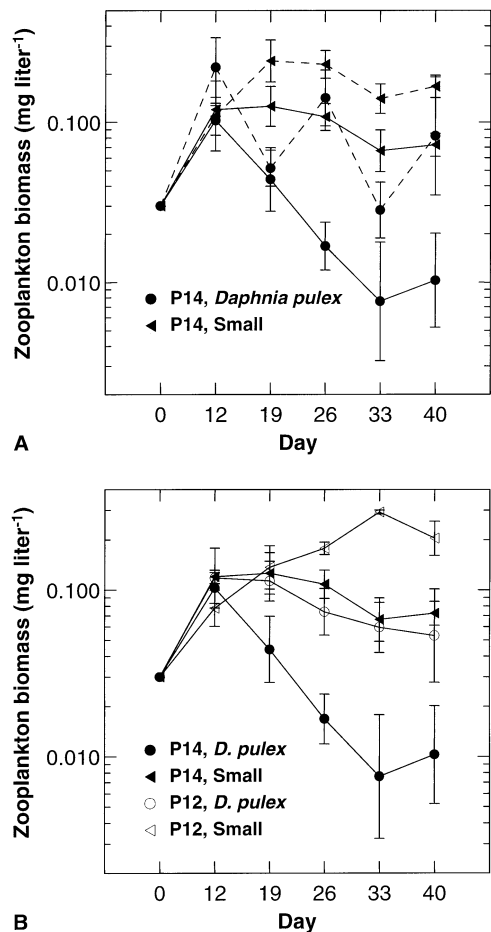
For descriptive analyses, measures from each sample period and pond were treated as separate data points. Though repeated measures in time within ponds may not be independent, treating the data in this manner permits inclusion of the considerable temporal variation in zooplankton composition and biotic/abiotic makeup that these systems exhibit over the growing season (variation that would be lost if a single grand mean was used for each pond). Zooplankton were first placed into four categories: copepods (including calanoids and cyclopoids), small-bodied cladocera (primarily *Ceriodaphnia*, *Diaphanosoma*, *Chydorus*, and *Bosmina*), rotifers, and *Daphnia pulex*. Excepting two sample dates in two ponds, in which *D. ambigua* and *D. dubia* were observed, *D. pulex* was the only *Daphnia* species encountered in the survey. When present, these two alternate species only comprised a minor fraction of zooplankton biomass (<5%) and were thus excluded from analyses (their inclusion does not alter results). The relationships between zooplankton composition, TP, TN, chlorophyll *a*, and C:N:P data were explored using least squares, linear regressions. All analyses were performed using Systat Version 8.0.

## Results

### Field experiment: nutrient and zooplankton effects

Nutrient enrichment had a significant positive effect on zooplankton biomass (Fig. 1A;  $P=0.001$ ,  $F_{1,8}=25.16$ , between-subjects effect, rm-ANOVA), as did zooplankton treatment; small-bodied zooplankton attained higher biomass levels regardless of nutrient level (Fig. 1A;  $P<0.0001$ ,  $F_{1,8}=54.18$ , between subjects effect, rm-ANOVA). When averaging over the course of the experiment, the small zooplankton assemblage in low nutrient treatments was dominated by *Diaphanosoma* (49.4 $\pm$ 4.1%, mean relative biomass $\pm$ SE), followed by *Bosmina* (31.6 $\pm$ 5.3%) and copepods (18.2 $\pm$ 1.6%). In high nutrient treatments, *Bosmina* dominated (46.2 $\pm$ 11.1%), followed by *Diaphanosoma* (28.4 $\pm$ 10.4%) and copepods (14.6 $\pm$ 2.8%).

As in previous investigations, nutrient enrichment and zooplankton composition had an interactive effect on algal biomass. A significant zooplankton $\times$ nutrient interaction was detected using rm-ANOVA (Fig. 2A;  $P=0.003$ ,  $F_{1,8}=17.82$ , between-subjects effect). When

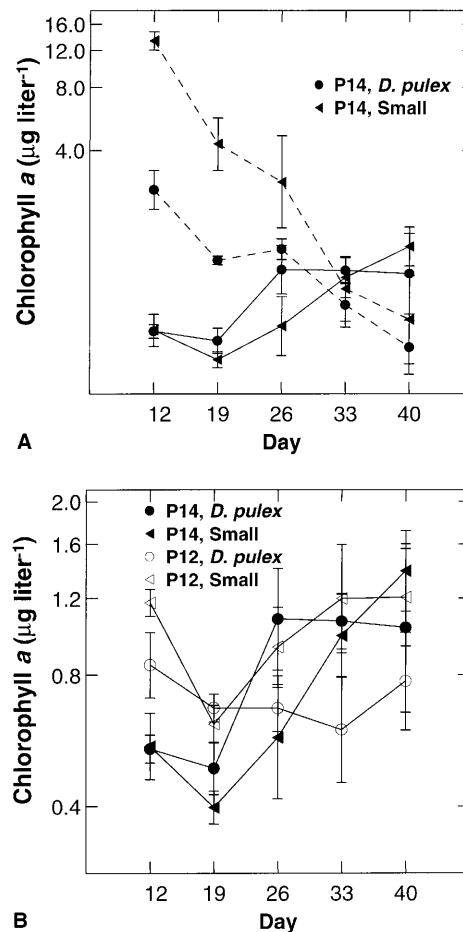


**Fig. 1** Responses of *Daphnia pulex* and small-bodied zooplankton (all taxa combined) to **A** low nutrient (solid lines) and high nutrient treatments (dashed lines) and **B** pond identity. Nutrient manipulations were only performed in P14. Shown are means ( $\pm 1$  SE). Initial (day 0) values are based on estimated biomass from enclosure inocula

analyzing low and high nutrient treatments separately, *D. pulex* and small-bodied zooplankton did not differ in their effects on chlorophyll *a* at low nutrient levels (Fig. 2A;  $P=0.532$ ,  $F_{1,4}=0.468$ , between-subjects effect, rm-ANOVA), but chlorophyll *a* was lower in the presence of *D. pulex* in high nutrient treatments (Fig. 2A;  $P=0.004$ ,  $F_{1,4}=34.04$ , between-subjects effect). However, effects were time dependent, a time $\times$ nutrient $\times$ zooplankton interaction was detected ( $P=0.034$ ,  $F_{4,32}=3.86$ , rm-ANOVA). The interactive effect of enrichment and *D. pulex* presence was evident only on the first three sample dates ( $P<0.03$  for all contrasts, ANOVA). By days 33 and 40, zooplankton effects and zooplankton $\times$ nutrient interactions were no longer significant ( $P>0.30$  for all contrasts, ANOVA).

#### Field experiment: pond and zooplankton effects

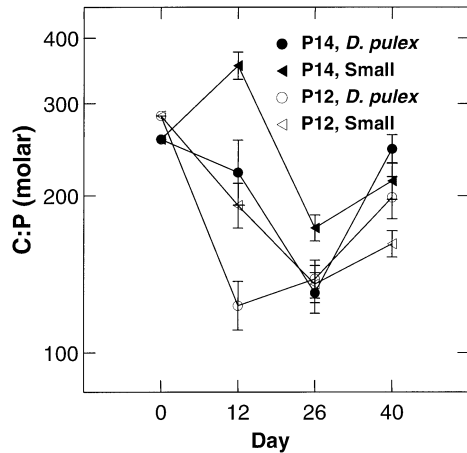
When comparing zooplankton responses in the two ponds, biomass was higher in P12 compared to P14



**Fig. 2** **A** Effects of zooplankton composition (*D. pulex* vs small-bodied zooplankton) and nutrient enrichment (high, dashed lines, versus low, solid lines) on mean chlorophyll *a* ( $\pm 1$  SE). **B** Responses of mean chlorophyll *a* ( $\pm 1$  SE) to zooplankton composition and pond identity (P12 versus P14)

(Fig. 1B;  $P<0.001$ ,  $F_{1,8}=48.83$ , between-subjects pond effect, rm-ANOVA). After day 12, biomass was generally higher in treatments with small-bodied zooplankton, compared to *D. pulex* treatments (Fig. 1B), with the difference between zooplankton treatments being greater in P14 ( $P=0.006$ ,  $F_{1,8}=8.12$ , between-subjects zooplankton $\times$ pond effect, rm-ANOVA). Averaging percent relative biomass over the experiment, the P12 small zooplankton treatment was primarily composed of *Diaphanosoma* ( $38.1\pm 3.4\%$ ), followed by copepods ( $33.8\pm 1.9\%$ ), and *Bosmina* ( $27.9\pm 2.0\%$ ).

Focusing on zooplankton and pond effects on algae, there were no significant between-subjects effects of pond identity on chlorophyll *a* ( $P>0.05$ ), nor was a significant main effect of zooplankton detected ( $P>0.10$ , rm-ANOVA). However, there was some indication of an interaction between zooplankton composition and pond identity, though not significant at the 0.05 level ( $P=0.064$ ,  $F_{1,8}=4.62$ , between-subjects effect). To further explore this potential interaction, I performed separate rm-ANOVA's for each pond separately. This



**Fig. 3** Effects of zooplankton composition (*D. pulex* versus small-bodied zooplankton) and pond identity (P12 versus P14) on mean seston C:P ( $\pm 1$  SE)

provided some evidence for a zooplankton effect. Examining P12 first, algal biomass tended to be lower in the presence of *D. pulex* (Fig. 2B), though the effect was only significant at the  $P=0.052$  level ( $F_{1,4}=7.53$ , between-subjects effect). In contrast, zooplankton composition had no effect on chlorophyll in P14 (Fig. 2B;  $P=0.53$ ,  $F_{1,4}=0.47$ , between-subjects effect).

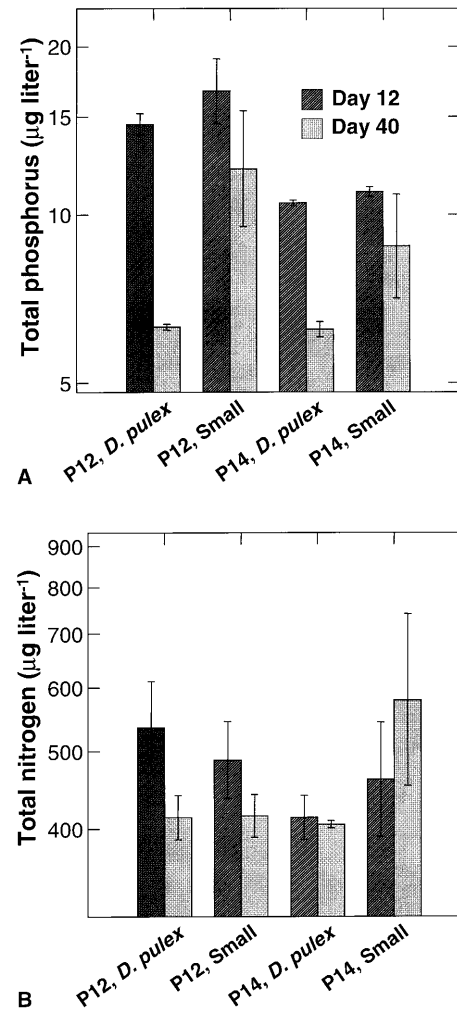
Zooplankton effects on seston C:P changed over the course of the experiment (Fig. 3); a time $\times$ zooplankton interaction was detected (G-G  $P<0.001$ ,  $F_{2,16}=22.03$ ). C:P ratios were lower in the presence of *D. pulex* on day 12 ( $P<0.001$ ,  $F_{1,8}=32.00$ , ANOVA). Yet, this trend had reversed by the final date of the experiment (Fig. 3); C:P ratios on day 40 were higher in the presence of *D. pulex* ( $P=0.016$ ,  $F_{1,8}=9.25$ , ANOVA).

TP decreased in all enclosures over time. However, declines were dependent on zooplankton treatment, with enclosures displaying greater decreases in the presence of *D. pulex* ( $P=0.021$ ,  $F_{1,8}=8.22$ , time $\times$ zooplankton effect). On day 12, TP did not differ between zooplankton treatments ( $P=0.13$ ,  $F_{1,8}=2.92$ , ANOVA), but TP was higher in P12 ( $P=0.0001$ ,  $F_{1,8}=47.85$ , ANOVA). By the end of the experiment (day 40), pond effects had disappeared ( $P=0.21$ ), but TP was lower in *D. pulex* treatments compared to small-bodied zooplankton (Fig. 4A;  $P=0.004$ ,  $F_{1,8}=16.61$ , ANOVA).

There were no effects of zooplankton composition on TN concentrations ( $P>0.10$ , rm-ANOVA). However, responses over time differed between ponds (Fig. 4B). A significant decrease was only detected in P12 ( $P=0.018$ ,  $F_{1,8}=8.75$ , time $\times$ pond effect, rm-ANOVA).

#### Field survey of fishless ponds

Field surveys revealed a striking amount of variation in zooplankton community structure and *D. pulex* dominance. Of the 18 ponds examined, half showed a complete loss of *D. pulex* by mid-season and only a minority

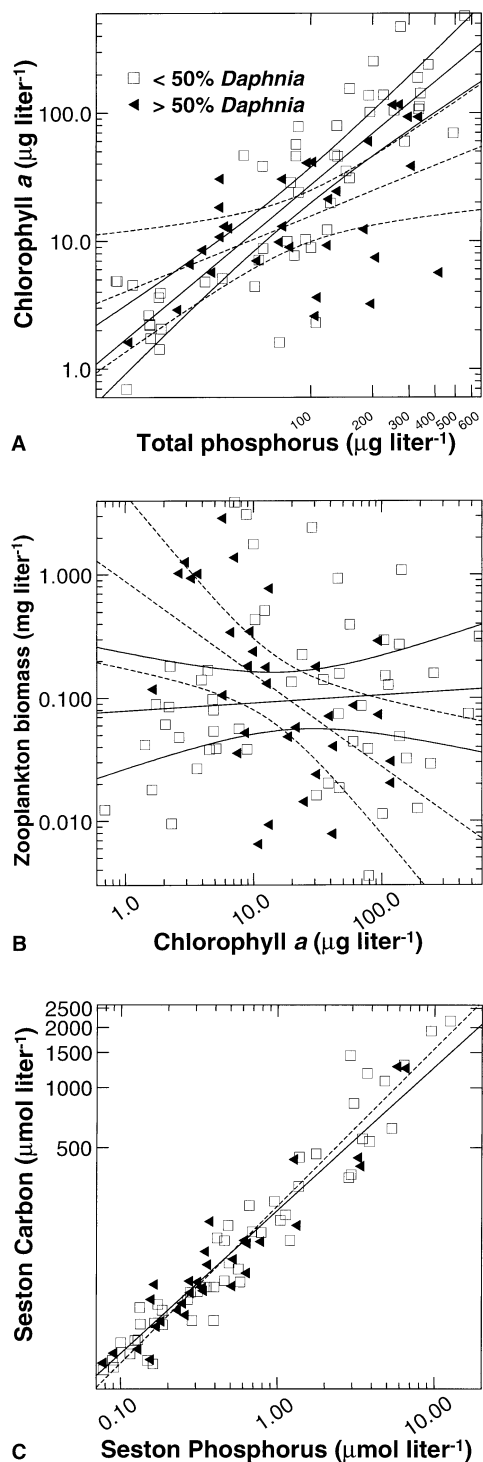


**Fig. 4** Effects of zooplankton composition and pond identity on **A** total phosphorus and **B** total nitrogen. Shown are means ( $\pm 1$  SE) for days 12 and 40 of the experiment

(5 ponds) exhibited *D. pulex* relative abundances greater than 50%, when averaging biomass over the growing season. Hence, a large amount of temporal and spatial variation in *D. pulex* incidence and relative biomass appears to be the norm in fishless ponds, despite the absence of planktivorous fish.

$\log_{10}(\text{chlorophyll } a)$  was regressed against the  $\log_{10}$  of TN, TP, and the biomass of each zooplankton category (see Materials and methods) using multiple linear regression. Chlorophyll *a* was strongly and positively correlated with  $\log_{10}(\text{TP})$ , a general indicator of productivity ( $P<0.0001$ ,  $r^2=0.741$ ,  $n=84$ ). However, chlorophyll *a* was also negatively related to *D. pulex* biomass ( $P=0.005$ ). A potential concern is that cyclopoid copepods can be predaceous and thus lumping them with calanoid copepods may not be strictly valid. However, excluding cyclopoids did not qualitatively alter regression results.

To further explore effects of *D. pulex* on algal standing crop, I first classed data as originating from a



**Fig. 5A–C** Results from the field survey of permanent fishless ponds. Dashed lines are *D. pulex* dominant samples, solid lines are *D. pulex* subdominant samples. **A** The relationship between chlorophyll *a* and total phosphorus (shown are 95% confidence intervals). **B** The relationship between zooplankton biomass and chlorophyll *a* (shown are 95% confidence intervals). **C** The relationship between seston carbon and phosphorus concentrations

“*D. pulex* dominant” sample if *D. pulex* relative biomass equaled or exceeded 50% of total zooplankton biomass or “*D. pulex* subdominant” if less than 50%. *D. pulex* dominance clearly altered the chlorophyll-TP relationship (Fig. 5A). When using log linear regressions, relationships were significant for both dominant and subdominant samples [dominant:  $P=0.0024$ ,  $r^2=0.268$ ,  $\log_{10}(\text{chl-}a)=0.655\log_{10}(\text{TP})-0.117$ ; subdominant:  $P<0.0001$ ,  $r^2=0.736$ ,  $\log_{10}(\text{chl-}a)=1.341\log_{10}(\text{TP})-1.248$ ]. However, the scaling factor (slope) from *D. pulex* dominant samples was significantly lower than the relationship from subdominant samples ( $P<0.05$ , *t*-test), though intercepts were not significantly different ( $P>0.10$ , *t*-test). A point of concern is that *D. pulex* relative abundance varied greatly over the growing season in several of the ponds, potentially confounding *D. pulex* dominance with time of year. I attempted to remove time effects by setting the first sample date as day zero and regressing  $\log_{10}(\text{chlorophyll } a)$  against sample day. A weak but significant positive relationship was detected ( $P=0.005$ ,  $r^2=0.092$ ). Residuals from this regression were then regressed against  $\log_{10}(\text{TP})$  for both *D. pulex* dominant and subdominant samples (as above). Results were similar to those depicted in Fig. 5A. Positive relationships were significant for both *D. pulex* dominant ( $P=0.004$ ,  $r^2=0.242$ ) and subdominant samples ( $P<0.0001$ ,  $r^2=0.740$ ). The slope from *D. pulex* dominant samples was significantly lower than from subdominant samples ( $P<0.05$ ), and intercepts were not significantly different ( $P>0.05$ ).

The relationship between  $\log_{10}(\text{zooplankton biomass})$  and  $\log_{10}(\text{chlorophyll } a)$  was also strongly affected by *D. pulex* dominance (Fig. 5B). When taken as a whole, there was no relationship between total zooplankton biomass and chlorophyll *a* ( $P=0.306$ , linear regression), nor was there a relationship between zooplankton and chlorophyll *a* in *D. pulex* subdominant samples (Fig. 5B;  $P=0.609$ ). In contrast, zooplankton biomass was significantly and negatively related to chlorophyll *a* when *D. pulex* was a dominant component of the zooplankton community (Fig. 5B;  $P=0.002$ ,  $r^2=0.284$ ,  $n=32$ ).

Regressions revealed no effects of zooplankton composition (absolute or relative biomass) on seston N:P or C:P, nor did *D. pulex* dominance affect the relationship between seston carbon and phosphorus or nitrogen and phosphorus (Fig. 5C; N:P patterns are not depicted for brevity). Because *Ceriodaphnia* may have high phosphorus requirements much like *Daphnia* (Hessen and Lyche 1991), I combined these two taxa and reran analyses with C:N:P data. This revealed no new patterns.

## Discussion

Interest in the size structure of zooplankton communities and variable dominance by *Daphnia* has a long and well-known history in aquatic ecology. Early research focused heavily on the determinants of this variation, elucidating the importance of size-selective predation (reviewed in

Gliwicz and Pijanowska 1989) and interspecific competition (reviewed in DeMott 1989). However, a separate body of work has also developed centered on the flipside of this question – the community and ecosystem-level consequences of variation in *Daphnia* incidence and dominance (e.g., Leibold 1989; Sarnelle 1992a, b; Schindler et al. 1997; Elser and Urabe 1999; Cottingham and Schindler 2000; Steiner 2001a; Tessier et al. 2001).

Much evidence indicates that *Daphnia* can have large impacts on algal standing crop. As suggested by the present study, the magnitude of these effects in ponds may hinge on the specific context in which zooplankton (*Daphnia* versus small-bodied taxa) effects are measured. Strong effects of *D. pulex* on algae were only apparent in high nutrient manipulations and in the more productive pond. Survey data also revealed significant effects of *D. pulex* dominance on trophic structure (Fig. 5B) and the chlorophyll-phosphorus relationship, with stronger effects at higher levels of TP (Fig. 5A). Nutrient enrichment appears to mediate the strength of *D. pulex*-algal interactions in ponds and consequently the degree of divergence between this large-bodied taxon and its smaller cohabitants. This insight complements previous investigations that have shown that the effects of *Daphnia* on algal production may only be evident under high nutrient conditions (e.g., Vanni 1984; Mazumder 1994a; Steiner 2001a). Nutrient effects may also explain disparate findings in other studies as well. For example, Turner and Mittelbach (1992) and Brett et al. (1994), studies both conducted in oligo-mesotrophic systems, found weak effects of *Daphnia* compared to alternate taxa. In contrast, Schoenberg and Carlson (1984) and Sarnelle (1993) found large *Daphnia* effects in their studies of highly productive lakes.

While these results are compelling they must be reconciled with the clear temporal component to *D. pulex*'s ability to control algal standing crop in the enrichment experiment. The rate and degree of algal accrual in response to the nutrient pulse were lower in *D. pulex* treatments, supporting the general contention that *Daphnia* enhance ecosystem stability by buffering the effects of nutrient pulse perturbations (Cottingham and Schindler 2000). However, chlorophyll *a* levels between zooplankton treatments were similar by the end of the experiment. This suggests that under some conditions small-bodied zooplankton can control algae as effectively as *Daphnia* if given time to respond numerically. Cottingham et al. (1997) drew similar conclusions. However, my pond survey results (Fig. 5A) suggest that there are TP levels above which *Daphnia* effects are consistently stronger than those of small zooplankton. When considering the natural range found in the survey, TP levels in my experiment (in both high and low nutrient treatments) were low and well within the range at which *D. pulex* effects were comparable to small zooplankton (Fig. 5A).

Mechanisms underlying zooplankton×nutrient interactions were likely two-fold. It is possible that *D. pulex* were better able to graze the algal assemblage initially present in high nutrient manipulations and in P12. Com-

pared to most small-bodied taxa, large *Daphnia* can achieve higher per capita filtration rates on small edible algae (Knoechel and Holtby 1986) and are known to feed on a much broader size spectrum of resources (Burns 1968). There is also evidence that large daphniids may be better able to digest “digestion-resistant” algae (Tessier et al. 2001). Hence, large *Daphnia* can access algal particles which are effectively resistant to grazing by small taxa – the very same algae generally favored under the combined action of grazing pressure and enrichment (Vanni 1987; Leibold 1989; Steiner 2001a). It is also possible that *D. pulex* were able to exact effects through a more rapid numerical/biomass response under nutrient enriched conditions; by the first sample period *D. pulex* biomass was higher than total small zooplankton biomass (Fig. 1A). This rapid response may have resulted from a greater capacity of *D. pulex* to consume and convert the large spike in algal production following the initial nutrient pulse (an  $r_{\max}$  strategy). Algal assemblages following enrichment events will also likely be of greater nutritional quality (i.e., possess lower C:P and N:P ratios) – an attribute that should benefit potentially phosphorus-limited *Daphnia* species.

This experiment also explored the effect of zooplankton identity on nutrient pools and seston stoichiometry. The role of zooplankton composition in pelagic nutrient cycling is at present a central area of research in aquatic ecosystem ecology (Anderson 1997; Elser and Urabe 1999). The potential for different species of zooplankton to differentially excrete nitrogen and phosphorus is largely accepted. Yet, the biological significance of this phenomenon, i.e., the ability of planktonic grazers to alter elemental limitation of primary producers, is an important aspect to this dynamic whose widespread applicability awaits thorough experimental validation.

My experiment provided some support for predicted effects of zooplankton composition. When examining nutrient data on the final date of the experiment, TP was lower in the presence of *D. pulex* while TN levels were unaffected, indicative of higher sedimentation rates and/or a shunting of nutrients to an organic component not included in a sample of the water column (e.g., algal growth on enclosure walls). *D. pulex* did depress phytoplankton in P12 to a greater extent than small zooplankton, which could have resulted in enhanced wall growth due to competitive release. However, phytoplankton levels in the two zooplankton treatments were similar in P14, making differential wall production in *D. pulex* versus small zooplankton treatments unlikely. It is also possible that *D. pulex* densities fell below the limits of detection. By day 40, densities in P12 were approximately 6 individuals per liter and 1 individual per liter in P14, compared to small zooplankton densities of 385 and 78 individuals per liter. Hence, a 250 ml water sample may have been inadequate to sample this “pool” of nutrients. Using an estimate of 1.58% phosphorus per unit zooplankton mass (the maximal level for a *Daphnia* species from Table 1 of Sterner et al. 1992), *D. pulex* at the end of the experiment could only have comprised a

mean of  $1.085 \mu\text{g P l}^{-1}$  in P12 and  $0.206 \mu\text{g P l}^{-1}$  in P14 – levels far too low to fully account for differences in TP among zooplankton treatments. Therefore, sedimentation seems a more probable explanatory mechanism. TP may have been lost as phosphorus-rich *D. pulex* senesced and sank to enclosure bottoms over the course of the experiment. The lack of extensive mixing in the enclosures may have made much of this nutrient pool inaccessible to algae in the water column. Though stoichiometric theory commonly focuses on effects of differential excretion rates among zooplankton (see Elser and Urabe 1999), other studies have also proposed post-mortem sedimentation as an additional mechanism by which *Daphnia* can decrease phosphorus availability to algae (e.g., Sarnelle 1992b; Sterner et al. 1992). Sedimentation should have also resulted in losses of TN over time. This was observed in P12 but not in P14. Sustained TN levels in P14 are rather puzzling. Nitrogen in detrital pools may have been recycled more quickly in P14, but why this would be is unknown.

Depressed TP levels at the end of the experiment did translate into higher seston C:P in *D. pulex* treatments, symptomatic of enhanced phosphorus limitation of algae. However, effects were modest in magnitude and only manifest on a single sample date. Furthermore, basing conclusions on ratios from the final sample date alone is only justified if these data are representative of steady-state conditions. Though zooplankton dynamics and chlorophyll *a* levels appeared to stabilize by the end of the experiment, C:P data do not allow us to infer whether nutrient ratios had stabilized or were still in flux at the termination of the experiment. Thus, caution should be exercised when interpreting these data.

The apparent weak effects on algal stoichiometry are somewhat perplexing since the enclosure environment should have accentuated zooplankton compositional effects. Mixing and sediment resuspension were likely minimal, potentially enhancing effects of resource recycling within the water column. However, theory predicts that divergent P excretion among zooplankton is only probable under a limited range of extant seston C:P ratios. Strong differences among zooplankton in P excretion, relative to C ingestion, are only expected for seston C:P ratios less than the C:P of the grazers (Hessen and Andersen 1992; Elser and Urabe 1999). This may explain, in part, the apparent absence of strong zooplankton effects over the course of the experiment. At the initiation of the study, seston C:P in P12 and P14 were 256 and 290. Zooplankton C:P likely ranged from approximately 80:1 for *D. pulex* to approximately 200:1 for copepods, with *Diaphanosoma* and *Bosmina* falling midway between these extremes (Hessen and Andersen 1992; Sterner and Hessen 1994). Hence, near the start of the experiment ambient seston C:P was at or above grazer C:P and within the range at which P excretion differences among taxa may have been small. As the experiment progressed, seston C:P began to drop, perhaps allowing some expression of grazer composition on phosphorus release.

Survey results provided further indication that *D. pulex* presence may be of little consequence to algal nutrient limitation in natural pond settings; no effects of zooplankton composition on seston C:P or N:P were detected. In the majority (67%) of pond samples collected, seston C:P was greater than 250, levels at which differences among zooplankton taxa in phosphorus excretion may have been weak. However, removing these samples from analyses did not expose any heretofore-unseen zooplankton effects on C:P. Furthermore, in 94% of samples, seston N:P was greater than 15 (65% were greater than 25), levels at which strong differences in N:P excretion should have been expressed among taxa (Sterner et al. 1992). Yet, no effect on seston N:P was evident. Given that nutrient supply within ponds integrate numerous potential inputs, both autochthonous and allochthonous, it is perhaps not surprising that zooplankton had weak or non-existent effects on seston stoichiometry in these habitats. More importantly, these systems are shallow (less than 2 m deep) and thus the photic zone and mixing events extend to nutrient rich sediments. Hence, zooplankton effects on nutrient supply may easily be overwhelmed by inputs from this source. Finally, a large body of research has focused on the impacts of algal stoichiometry on zooplankton community structure (see Sterner and Hessen 1994; Hassett et al. 1997; DeMott and Gulati 1999). The absence of correlations between pond seston C:N:P and zooplankton composition further suggests that stoichiometry may not be an important constraint on zooplankton composition in these systems.

Is *D. pulex* a keystone species? A truly rigorous test of this question is only afforded by comparing effects of all zooplankton taxa either in isolation or by experimentally removing each species in turn and observing changes in community/ecosystem attributes, a daunting task and one that is truly intractable if one considers the copious species pool of planktonic grazers found within any given region. A more amenable course of inquiry will likely be comparisons of the sort used here, in which a suspected key species is experimentally contrasted with one or several alternate species. Such data can be complemented with observational evidence to enhance the robustness of conclusions. True and indisputable determination of the “keystoneness” of such focal species will lie in the synthesis of many such experiments and surveys. In itself, the present study imparts a rather indeterminate conclusion. Grazer control of algae may depend critically on *Daphnia* presence in pond ecosystems, but only under a subset of natural conditions (e.g., high productivity systems, following nutrient perturbations, or early in the growing season when spring mixing events create spikes in algal production). Both survey and experimental evidence point to this, in addition to previously published work. However, the capacity for *Daphnia* to alter algal nutrient limitation in ponds may be limited. My study provided some evidence that *D. pulex* can enhance phosphorus limitation beyond the effects imposed by small-bodied taxa (specifically within artificial enclosures that preclude mixing and sediment input). In

more realistic settings (i.e., natural ponds), nutrient loading and ambient C:P ratios may relegate the possibility of any *Daphnia* effect to a small subset of water-bodies. Alternate nutrient supply sources (e.g., sediments and mixing events) may render strong zooplankton effects moot in even these ponds. Far-reaching generalities about *Daphnia*'s central position in pond ecosystem functioning then must be tempered by considerations of the interplay of context and the function in question.

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