

Testing zooplankton food limitation across gradients of depth and productivity in small stratified lakes

William R. DeMott¹ and Jennifer R. Edington

Department of Biology, Indiana-Purdue University, Fort Wayne, Indiana 46805

Alan J. Tessier

W. K. Kellogg Biological Station and Department of Zoology, Michigan State University, Hickory Corners, Michigan 49060

Abstract

We examined the significance of vertical structure in lake plankton to grazer-resource coupling by quantifying how the intensity and mechanism of grazer food limitation varies with depth. We used *Daphnia* growth assays and also sampled resources (chlorophyll, particulate carbon, and phosphorus) and the day/night depth distributions of *Daphnia* spp. during summer in four depth strata of lakes that differ in trophic state. The growth assays included treatments with natural seston and seston supplemented with phosphate or *Synechococcus*, an energy- and P-rich cyanobacterium highly deficient in essential fatty acids and sterols. Results from growth assays and measurements of *Daphnia* P balance indicate strong energy limitation and weak and sporadic P limitation, despite seston C:P ratios in the upper depth strata above the predicted threshold for *Daphnia* P limitation. Growth improved with depth and was very high with resources collected from a deep layer of sulfur bacteria that was partially protected from grazing by low dissolved oxygen. Strong positive responses to *Synechococcus* supplements and high growth with resources from the sulfur bacteria layer are both contrary to limitation by essential lipids. The intensity of food limitation was independent of lake trophic state but was influenced by the quality of the deep-water refuge. Across depths and lakes, food limitation was dependent on *Daphnia* density; strong food limitation was associated with high *Daphnia* biomass ($>100 \mu\text{g L}^{-1}$) and *Daphnia* grazing $>20\%$ per day.

The coexistence of planktivorous fish and large-bodied *Daphnia* during summer is dependent on the presence of a deep-water refuge (Wright and Shapiro 1990; Tessier and Welser 1991). In such systems, *Daphnia* avoid fish predation by living in the deep-water refuge, or by migrating between surface waters and the deep refuge under cover of darkness. The strong grazing exerted by large-bodied *Daphnia* is well documented (Carpenter and Kitchell 1993; Tessier et al. 2001). However, we have little understanding of how food conditions for grazers vary with depth or trophic state in stratified lakes inhabited by large-bodied *Daphnia*.

Zooplankton migration into surface waters has usually been thought to reflect a trade-off between improved food conditions and increased risk of fish predation (Gliwicz and Pijanowska 1988). However, Williamson et al. (1996) showed that resources from a metalimnetic peak in chlorophyll supported better zooplankton reproduction than resources from surface waters. They also pointed out that subsurface maxima in algal abundance are common, especially in less productive waters. In contrast, Cole et al. (2002) found that resources from surface waters supported better *Daphnia* growth than resources from the metalimnetic chlorophyll maxima of two oligotrophic lakes. Accumulating evidence shows that food quality is often more important than

food quantity for grazers (reviews by Gulati and DeMott 1997; Sterner and Schulz 1998). Thus, an assessment of how food conditions vary with depth and trophic state needs to consider food quality.

Recently, most research on food quality for zooplankton has focused on deficiencies in elements and essential lipids. *Daphnia* and P have received much attention, in part because of their importance in lakes and in part because daphniids have high requirements for P compared with other crustacean zooplankton. Estimates of resource C:P (molar) thresholds for *Daphnia* usually range from 200 to 300 (Sterner and Hessen 1994). Resource C:P ratios >300 are common in lakes, leading to the prediction that *Daphnia* should often experience P limitation in nature (reviewed by Sterner and Schulz 1998; but see Brett et al. 2000). Studies of essential lipids have focused on highly unsaturated fatty acids in the omega-3 class (Brett and Müller-Navarra 1997). The fatty acid composition of the diet depends largely on resource taxonomy, with cyanobacteria typically being most deficient in essential fatty acids (e.g., Müller-Navarra et al. 2000). Cyanobacteria and other prokaryotes also lack essential sterols (Von Elert and Wolfram 2001).

Phytoplankton can also be poor-quality food because of digestion resistance, chemical deterrents, or constraints on ingestion due to particle size or shape (reviewed by Agrawal 1998). These algal defenses reduce carbon assimilation, leading to energy limitation of zooplankton growth. Limitation by elements or essential lipids is expected to be strongest when carbon assimilation is high. When energy availability is low, most assimilated energy is respired and nutrient-deficient and nutrient-sufficient resources are ex-

¹ Corresponding author (demott@ipfw.edu).

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pected to support similar, low growth rates (Sterner and Robinson 1994; Sterner 1997; Anderson and Pond 2000). Thus, low energy assimilation could reduce or even preclude limitation by elements or lipids [but see Boersma and Kreutzer (2002)]. Studies of hypereutrophic Dutch lakes (DeMott et al. 2001) and oligotrophic Canadian Shield lakes (Makino et al. 2002) provide evidence for the expected combined effects of P and energy limitation on *Daphnia* growth.

Tessier and Woodruff (2002a) showed that food limitation is much stronger in the surface waters of deep stratified lakes than in shallow lakes of similar productivity. This pattern was described as a cryptic trophic cascade because intense grazing by large *Daphnia* in the deep lakes caused a decline in food quality with no effect on chlorophyll concentration or size structure. Strong food limitation was associated with an increase in the percentage of digestion-resistant algae. DeMott and Tessier (2002) used growth assays and assimilation experiments to confirm that the food quality gradient between shallow and deep lakes was a gradient in food digestibility.

Predictions about how food quality should vary with depth are clearest for element limitation. Since nutrient limitation of phytoplankton is strongest in surface waters and declines with depth as light becomes more limiting (Sterner et al. 1997), the potential for element limitation of consumers should be highest in the upper water column. Vertically migrating consumers may be able to alleviate element limitation by spending part of the day feeding on nutrient-sufficient resources in deeper layers (Sterner and Schwalbach 2001). In support of the light:nutrient hypothesis, a field enclosure experiment in a Canadian Shield lake showed that strong shading can improve *Daphnia* growth by reducing the seston C:P ratio (Urabe et al. 2002).

The potential for limitation by essential lipids should be highest in depth strata with high concentrations of cyanobacteria or other prokaryotes relative to more nutritional food species. Finally, theory predicts that algal defenses against grazing should be favored in lakes and depth strata where grazing is most intense (Leibold 1989; Grover 1995) and this finding has been confirmed in field experiments (reviewed by Agrawal 1998).

How does the intensity of zooplankton food limitation vary along a gradient from oligotrophic to eutrophic stratified lakes? Field experiments often show that nutrient enrichment without significant predation simply leads to larger populations of food-limited grazers (reviewed by Leibold 1989). However, nutrient enrichment or shallow depth or both can cause more rapid oxygen depletion in the hypolimnion and thereby decrease or eliminate its use as a refuge from predation (Wright and Shapiro 1990; Tessier and Welser 1991). Furthermore, in highly eutrophic lakes, grazing intensity often declines as the phytoplankton becomes dominated by largely inedible taxa (Elser and Goldman 1991; DeMott et al. 2001b).

This study focuses on the summer period of stable thermal stratification when zooplankton communities can approach equilibrium with their food resources. We used a *Daphnia* juvenile growth assay to test how the intensity and mechanism of food limitation varied in four depth strata of four lakes that differ in productivity. In addition we measured the

Table 1. Characteristics of four study lakes from near Kellogg Biological Station in Michigan. Phosphorus data are from Tessier and Woodruff (2002) for the first three lakes and unpublished data for Baker. Phosphorus refers to total phosphorus sampled during spring turnover.

	Phosphorus ($\mu\text{g L}^{-1}$)	Max. depth (m)	Surface area (ha)
Lawrence	8.5	11.5	4.9
Warner	9.6	14.0	23.5
Little Mill	24.7	9.5	4.8
Baker	25.1	7.5	23.8

day/night depth distributions of *Daphnia* spp. and used empirical regressions to evaluate grazing intensity and biomass-dependent growth. Our overall goal was to test whether a cryptic trophic cascade can account for variation in *Daphnia* food limitation across gradients of depth and productivity in small, stratified lakes.

Methods

Study lakes, depth strata, and zooplankton communities—Each of four small, thermally stratified lakes was studied intensively for 5 d during June and July 2001. Phytoplankton productivity is P-limited in all four lakes during summer. We characterize two lakes as “oligotrophic” (Lawrence and Warner) and two lakes as “eutrophic” (Little Mill and Baker), on the basis of total P at spring overturn (Table 1). Centrarchiids are the dominant fish in all four lakes; bluegill (*Lepomis macrochirus*) and pumpkinseed (*L. gibbosus*) sunfish are the main planktivores and largemouth bass (*Micropterus salmoides*) is the major piscivore.

Field sampling was used to define and characterize four depth strata in each lake and to aid in the interpretation of laboratory growth assays with natural seston. All field samples were collected at a central station in the deepest part of each lake. Oxygen and temperature were measured at 1-m intervals with a Yellow Springs Instruments meter. Temperature measurements made during the week before the period of intensive study were used to define the epilimnion, metalimnion, and hypolimnion. Dissolved oxygen (DO) was used to subdivide the hypolimnion further on the basis of habitat quality for *Daphnia*. The upper hypolimnion (termed “hypolimnion”) with $\text{DO} > 2 \text{ mg L}^{-1}$ was considered a good habitat, whereas the lower hypolimnion with $\text{DO} < 1 \text{ mg L}^{-1}$ (the “anoxic boundary layer”) should be less suitable for grazers (Wright and Shapiro 1990). Because we wanted to avoid the reducing conditions and possible toxic effects, we discarded any samples that smelled of H_2S .

One set of zooplankton samples was collected during day (1100–1400 h) and night (2300–0100 h) from each lake with a 20-liter Schindler trap. Zooplankton samples were collected every meter in the two shallower lakes and at 2-m intervals in the two deeper lakes. Samples were preserved in 95% ethanol and all *Daphnia* in each sample were identified to species and counted under a dissecting microscope. Two *Daphnia* species (*D. pulicaria* and *D. dentifera*; the latter known in earlier studies as *D. galeata mendotae* or *D. rosea*)

dominated the zooplankton of each lake. To estimate biomass and filtering rates, 50 animals of each *Daphnia* species from a pooled sample for each lake were measured under a dissecting microscope. Biomass was estimated using a logarithmic length versus mass regression for each species. Live animals from Lawrence Lake were measured, dried, and weighed individually to the nearest 0.1 μg to calculate the biomass regressions. We estimated *Daphnia* community filtering rates for each layer and lake using an empirical body length versus filtering rate power function on the basis of in situ isotope feeding experiments with cladocerans in a stratified lake (Knoechel and Holtby 1986). Our estimates of biomass and grazing assume that *Daphnia* occupied the day depth for 16 h and the night depth for 8 h during each 24-h cycle.

Growth assays and food resources—Bioassays measured the juvenile growth of a cohort of a *D. pulex* \times *pulicaria* hybrid clone born within 24 h of the start of each experiment (Tessier et al. 2000). Bioassay results with this clone are representative of the growth and reproduction responses of common daphniid species in our region (Tessier and Woodruff 2002b). Our test clone also showed the greatest sensitivity to P limitation of 10 *Daphnia* taxa tested in laboratory experiments with P-sufficient and P-deficient green algae (DeMott and Pape pers. comm.). The animals were fed natural seston and seston plus supplements. We used a 2-liter horizontal Van Dorn bottle to collect seston from each depth stratum and filtered the water through a 62- μm nylon screen to remove zooplankton and large inedible algae. On two days during each experiment we filtered seston from each stratum through a 62- μm mesh screen and onto GF/F Whatman filters to estimate particulate organic carbon (Carlo-Erba C:H:N analyzer) and particulate P (persulfate digestion followed by molybdate reaction). Chlorophyll *a* was determined once for each lake by narrow band fluorometry (Welschmeyer 1994), using a set of samples collected at 1-m intervals with a 2-liter Van Dorn bottle and extracted overnight in cold 95% ethanol. We measured total chlorophyll *a* and the fraction passing through a 62- μm mesh screen.

In each bioassay experiment, each animal was placed in 100 ml of freshly collected seston (<62 μm) in a separate beaker each day. Before the animals were transferred, seston from the anoxic boundary layer was aerated and beakers with seston from each layer were kept on a lab bench for several hours to allow water temperature to equilibrate. Animals were kept in incubators at 20°C without light until age 4 d, when they were harvested for measurement of dry mass and P. The growth rate g (d^{-1}) was calculated as the difference between the natural logarithm of the final and initial dry mass, divided by the experiment duration (4 d). Final mass was based on the individual weights of six to eight animals per treatment, measured to the nearest 0.1 μg . Tessier and Woodruff (2002b) found a close 1:1 correspondence between *Daphnia* growth measured this way in beakers and simultaneous measurements in a flow-through system.

After weighing, all animals from a treatment were transferred to a 10-ml volumetric flask, ashed at 550°C and analyzed for P as described in DeMott et al. (1998).

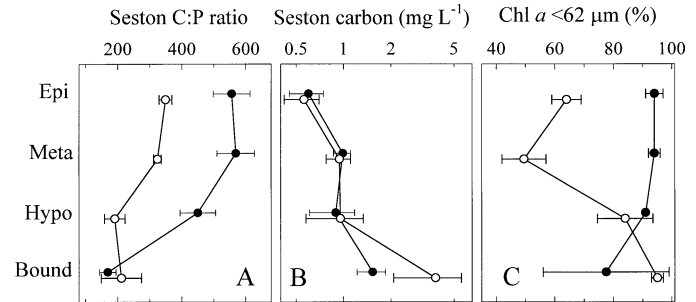


Fig. 1. Variation between lake classes and depth strata in (A) seston C:P ratio, (B) seston carbon (note log scale), and (C) the fraction of total chlorophyll passing through a 62- μm screen. Open circles represent eutrophic lakes; closed circles represent oligotrophic lakes. Each value is the mean \pm 1 SE for two lakes.

To test the mechanism(s) of food limitation we used three treatments after DeMott et al. (2001a): (1) a control with natural seston alone and seston supplemented with (2) phosphate (P) or (3) the energy- and P-rich cyanobacterium *S. elongatus* (*Syn.*). Phosphate was added as a 0.5 $\mu\text{mol L}^{-1}$ solution of KH_2PO_4 . Preliminary trials with epilimnetic seston showed that >80% of the phosphate was taken up within 3 h, reducing the seston C:P ratio to <200. *Synechococcus* was added at a concentration of 300 $\mu\text{g C L}^{-1}$, equivalent to 10–55% of ambient seston concentrations (see Results). The P and *Syn* supplements contained about the same amount of added P and thus were designed to reduce the dietary C:P ratio to about the same P-sufficient level. P-rich *Synechococcus* (C:P \sim 50) was cultured in 150-ml Erlenmeyer flasks after DeMott et al. (1998).

Responses to the supplements were quantified by subtracting the control growth rate (g , d^{-1}) from each supplement treatment value to give “growth differentials” (DeMott et al. 2001a). The difference in growth between seston supplemented with phosphate and seston alone (control) provides a measure of P limitation. Because the P and *Syn* treatments had the same P-sufficient C:P levels, we consider the difference in growth between the *Syn* and P treatments a measure of energy limitation once P requirements are satisfied. *Synechococcus* is a good source of P and energy but is highly deficient in essential fatty acids and sterols (DeMott and Müller-Navarra 1997; Von Elert and Wolfram 2001). Thus, strong positive responses to the *Syn* treatment are contrary to the essential lipid hypothesis.

Both laboratory (DeMott et al. 1998; Plath and Boersma 2001; DeMott 2003) and field (DeMott et al. 2001a) studies show that *Daphnia* experiences a decline in P balance during P-limited growth. We therefore calculated P balance differentials by subtracting the control *Daphnia* P balance (% mass) from the value for each supplement treatment.

Results

Seston stoichiometry and concentration—As expected, the seston C:P ratio declined with depth (Fig. 1A; two-way analysis of variance [ANOVA], with depth strata a subplot of lake; depth, $F_{3,6} = 39.28$, $P = 0.002$). The seston C:P ratio was above 300, the nominal value for *Daphnia* P lim-

itation, in the upper two layers of the eutrophic lakes and in the upper three layers of the less productive lakes (trophy, $F_{1,2} = 10.12$, $P = 0.086$; depth \times trophy interaction, $F_{3,6} = 12.96$, $P = 0.005$). Seston carbon tended to increase with depth ($F_{3,6} = 3.56$, $P = 0.087$) but no relation with trophic state was observed ($F_{1,2} = 2.72$, $P = 0.24$; Fig. 1B). One eutrophic lake, Little Mill, had lower seston carbon values in the upper three layers than one oligotrophic lake, Warner. The pattern for chlorophyll *a* was essentially the same as that for seston carbon in the same size range (data not shown).

Microscope observations revealed that the high carbon concentrations in the anoxic boundary layer largely comprised purple sulfur bacteria in Lawrence and Little Mill lakes, green sulfur bacteria in Baker, and filamentous cyanobacteria (mainly *Oscillatoria*) in Warner. Except for the boundary layer with filamentous cyanobacteria in Warner, >90% of the chlorophyll in samples from the oligotrophic lakes passed through a 62- μm screen (Fig. 1C). In contrast, only about half the chlorophyll from the upper two layers of the two eutrophic lakes passed through a 62- μm screen.

Temperature and oxygen—Temperature in the hypolimnion was 6–8°C in the three deeper lakes and 12–14°C in Baker. Temperature in the epilimnion was 23–25°C across all four lakes. The three deeper lakes had well-oxygenated upper hypolimnia (DO > 2 parts per million [ppm] about 2 m thick). In Baker, however, the hypolimnion with DO > 2 ppm was <1 m thick.

Daphnia biomass and vertical distribution—Two species of *Daphnia*, *D. dentifera* and *D. pulicaria*, dominated the zooplankton of all four lakes. *Daphnia* biomass was highest in one eutrophic lake, Little Mill, and lowest in the other

eutrophic lake, Baker (Table 2). This pattern was a consequence of both the low densities and small body sizes of the Baker populations. *D. dentifera* was more abundant in the epilimnetic and metalimnetic layers, whereas *D. pulicaria* was most abundant in the hypolimnion. As expected, the anoxic boundary layer contained the lowest *Daphnia* biomass in each lake. *D. dentifera* migrated into the surface waters at night, whereas *D. pulicaria* showed weaker upward migration with most individuals remaining below the epilimnion (migration data not shown). Differences between lakes in the depth distribution of total *Daphnia* biomass appear to reflect the relative abundance of the two species. For example, an increase in total *Daphnia* biomass from the surface to the hypolimnion in Lawrence is associated with a high abundance of *D. pulicaria*, the deep-water species. In contrast, a decline in total *Daphnia* biomass with depth in Warner reflects a low biomass of *D. pulicaria* in that lake.

The exponents of the length versus biomass power functions for *D. dentifera* (2.27) and *D. pulicaria* (2.36) are similar to the exponent of the length versus filtering rate power function (2.48; Knoechel and Holtby 1986). Thus, estimates of *Daphnia* biomass and filtering rates scale closely with each other [linear regression for 16 depth strata, community filtering rate (% per day) = $0.185 \times \text{Daphnia biomass } (\mu\text{g L}^{-1}) + 2.17$; $r^2 = 0.97$].

Daphnia growth and phosphorus balance—Growth in beakers with natural seston (controls) showed a tremendous range (0.00–0.58 d⁻¹; Fig. 2). Growth with natural seston differed between lakes and improved with depth (two-way ANOVA, lakes, $F_{3,109} = 105$, $P < 0.001$; depth, $F_{3,109} = 179$, $P < 0.001$; lake \times depth interaction, $F_{3,3} = 30.5$, $P < 0.001$). Mean growth in the upper three layers was highest in one eutrophic lake (Baker, 0.36 d⁻¹) and lowest in the

Table 2. The biomass and filtering rates of *Daphnia dentifera* (*D. d.*) and *D. pulicaria* (*D. p.*) in the epilimnion (Epi.), metalimnion (Meta.), hypolimnion (Hypo.), and anoxic boundary layer (Bound.) of the four study lakes. *Daphnia* biomass and filtering rates are the weighted means of day and night samples (see Methods). Length measurements of 50 individuals of each species from each lake were used to estimate biomass and filtering rate from power function regressions.

	Biomass ($\mu\text{g L}^{-1}$)				Length mm ind ⁻¹	Biomass $\mu\text{g ind}^{-1}$	Filtering rate ml ind ⁻¹ d ⁻¹
	Epi.	Meta.	Hypo.	Bound.			
Lawrence							
<i>D. d.</i>	81	78	23	10	1.19	8.4	19.6
<i>D. p.</i>	13	50	187	43	1.38	17.2	29.0
Total	94	128	210	53			
Warner							
<i>D. d.</i>	140	91	41	17	1.25	10.1	24.0
<i>D. p.</i>	1	6	44	17	1.77	33.9	59.6
Total	141	97	85	34			
Little Mill							
<i>D. d.</i>	178	83	27	15	1.37	12.1	33.0
<i>D. p.</i>	25	274	310	110	1.80	33.0	57.6
Total	214	358	337	125			
Baker							
<i>D. d.</i>	22	69	23	17	1.23	9.6	22.7
<i>D. p.</i>	1	6	2	1	0.84	6.5	10.6
Total	23	74	26	18			

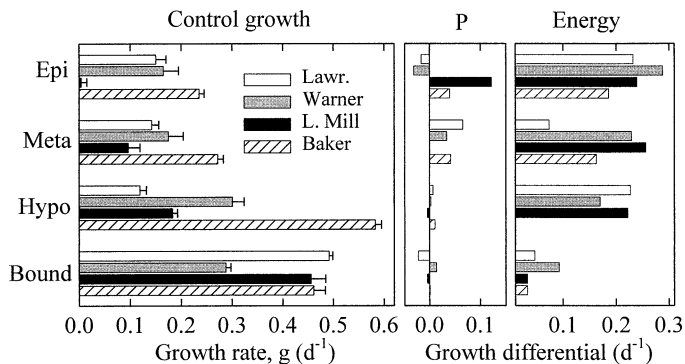


Fig. 2. *Daphnia* growth with seston from four depth strata of four lakes and responses to seston supplemented with phosphorus (P) or *Synechococcus*. Control growth rates are means \pm 1 SE for six to eight animals grown in separate beakers. The P growth differential is the difference between the phosphorus and control treatments. The energy growth differential is the difference between the *Synechococcus* and phosphorus treatments.

other eutrophic lake (Little Mill, 0.093 d⁻¹). Growth with Lawrence seston showed a small decline from the epilimnion to the hypolimnion. In the other three lakes, growth improved from the epilimnion to the hypolimnion. Growth was high with resources from the anoxic boundary layers of all four lakes.

Daphnia growth showed weak, sporadic responses to the P supplement, but strong responses to the *Synechococcus* supplement (Fig. 2). The energy differential declined with depth ($F_{3,6} = 6.95$, $P = 0.022$) but did not vary with trophic state ($F_{1,2} = 0.28$, $P = 0.65$). In contrast, the P growth differential was strongest in the eutrophic lakes ($F_{1,2} = 57.9$, $P = 0.017$) and did not vary consistently with depth (depth, $F_{3,6} = 1.73$, $P = 0.26$; depth \times trophy, $F_{3,6} = 3.56$, $P = 0.087$). The strongest response to P addition was observed with seston from the epilimnion of eutrophic Little Mill Lake (seston C:P = 336; P growth differential = 0.12). Across lakes and depth strata, the overall mean energy differential was 0.161, whereas the overall mean P growth differential was only 0.0148.

Daphnia P balance (as percent mass) in the control treatment increased with depth but did not vary with trophic state (Fig. 3: depth, $F_{3,6} = 6.95$, $P = 0.022$; trophy, $F_{1,2} = 0.03$, $P = 0.89$). *Daphnia* showed a similar response to the P and *Synechococcus* supplements by increasing P balance in the upper strata with P-deficient seston (paired *t*-tests for 10 depth strata with C:P ratio >300; P treatment, $t = 5.01$, $df = 9$, $P < 0.001$; *Syn* treatment, $t = 2.61$, $df = 9$, $P < 0.05$). The P-balance differential for the P treatment equaled or exceeded the P-balance differential for the *Syn* treatment (Fig. 3).

Relations between *Daphnia* growth, resource stoichiometry, and grazing intensity—As predicted by stoichiometric theory, we observed an inverse relation between growth in the control treatment and the seston C:P ratio across depth strata in the four lakes (Fig. 4A). However, the relation between the seston C:P ratio and the growth response to the P supplement was very weak (Fig. 4B).

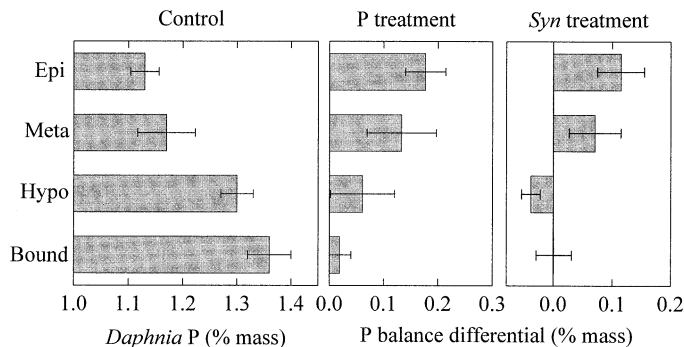


Fig. 3. *Daphnia* P balance (% mass) with seston from four depth strata of four lakes and responses to seston supplemented with phosphorus (P) or *Synechococcus* (*Syn*). The P balance differential is the difference between a supplement and control treatment. Data are means \pm 1 SE for four lakes.

Across depth strata and lakes, the control growth rate declined with increasing *Daphnia* biomass ($r_s = -0.76$) and grazing rate ($r_s = -0.77$; Spearman rank correlations, both $P < 0.001$). Grazing rates greater than about 20% per day (about 100 μg *Daphnia* L⁻¹) were usually associated with growth rates <0.2 d⁻¹ (Fig. 5). Figure 6 summarizes data for each lake, by comparing the mean growth rate response on resources from the upper three strata with total phosphorus and with grazing intensity in the same three strata. Resources from the two eutrophic lakes with very similar total P values supported growth rates at opposite extremes (Fig. 6A). Growth was inversely related to *Daphnia* grazing intensity (Fig. 6B).

Discussion

Trophic cascades occur in planktonic systems when release of zooplankton from predation results in strong grazing and high densities of food-limited grazers. A trophic cascade can be termed “cryptic” when grazing causes a shift to poor-quality resources, rather than a decline in resource abundance (Tessier and Woodruff 2002a). Our results support the hypothesis that a cryptic trophic cascade accounts for much of the variation in food limitation across gradients of depth and productivity in four small, stratified Michigan lakes during summer.

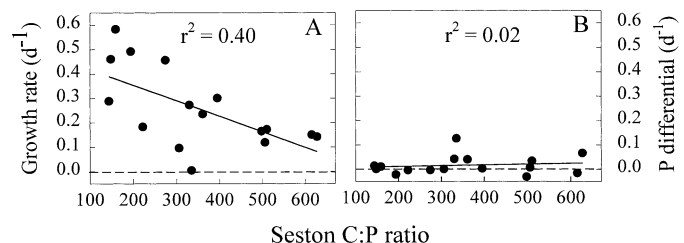


Fig. 4. Relation between the seston C:P ratio and (A) the control growth rate or (B) the P growth differential for four depth strata from four lakes. The P growth differential is the difference in growth between the phosphorus and control treatments. Data are fit to least-square linear regressions (solid lines).

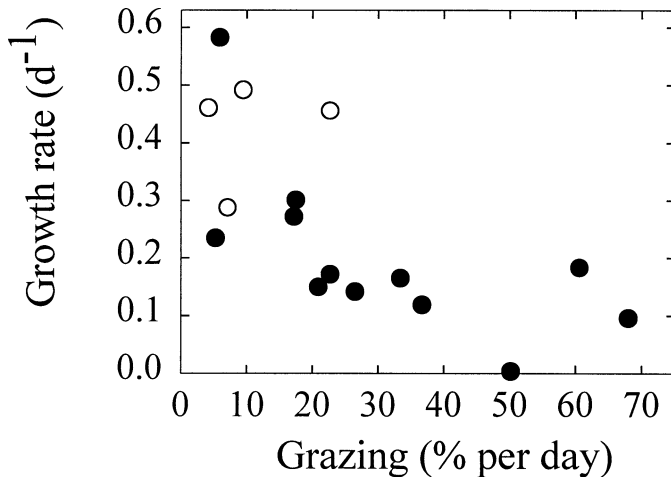


Fig. 5. Relation between the *Daphnia* grazing intensity and growth rate for four depth strata from four lakes. *Daphnia* community grazing was estimated from field samples of *Daphnia* size and abundance and an empirical length versus filtering rate regression after Knoechel and Holtby (1986). Open circles are data for the anoxic boundary layer; closed circles represent the upper three strata.

Variation in the intensity of food limitation—Among lakes and depth strata, the intensity of food limitation was *Daphnia* density dependent. We found three sources of variation in *Daphnia* density: (1) differences among lakes, (2) differences in the relative abundance of the two *Daphnia* species, and (3) a partial refuge from grazing associated with low dissolved oxygen. Among the four lakes, Baker, a eutrophic lake, had the thinnest deep-water refuge, the lowest *Daphnia* biomass, and supported the highest *Daphnia* growth rates. More productive lakes often experience stronger seasonal declines in dissolved oxygen in the hypolimnion, leading to decreases in refuge thickness and the loss of large *Daphnia*, especially *D. pulicaria*, during summer (Wright and Shapiro 1990; Tessier and Welser 1991). Sampling through the summer shows that *D. pulicaria* declines to undetectable densities in Baker but remains common in our three other lakes (Caceres and Tessier pers. comm.). Our growth assays show that the demise of *D. pulicaria* in Baker during summer is not due to poor food conditions, and therefore, support the deep-water refuge fish predation hypothesis.

In contrast to Baker, the other eutrophic lake, Little Mill, had both the highest *Daphnia* biomass and the poorest food resources. In this lake, high P loading is associated with dense populations of strongly food-limited *Daphnia*. This result supports the prediction that increased nutrients in a low predation environment leads to higher densities of food-limited grazers, rather than an improvement in food conditions (Leibold 1989, Grover 1995).

An effect of *Daphnia* species relative abundance is suggested by a comparison between the two oligotrophic lakes. Improved growth with depth in Warner is associated with low biomass of the deep-water species, *D. pulicaria*, whereas a slight decline in growth with depth in Lawrence is associated with high biomass of the same deep-water species. The relative abundance of *D. dentifera* and *D. pulicaria* is

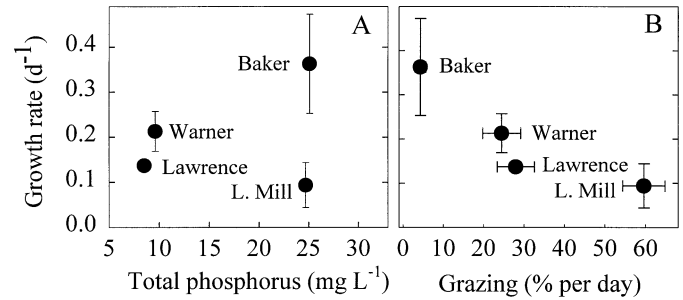


Fig. 6. Relation between growth rate and (A) total phosphorus or (B) *Daphnia* community grazing rate. Data are means \pm 1 SE for the upper three strata of each of four lakes.

influenced by both competition (Hu and Tessier 1995) and predation (González and Tessier 1997).

Finally, for all four lakes, *Daphnia* biomass was lowest in the anoxic boundary layer ($\text{DO} < 1$ ppm) and growth was highest with resources from this layer. Wright and Shapiro (1990) review evidence that low oxygen restricts *Daphnia* distributions. Low grazing on sulfur bacteria is also supported by a stable isotope study of the pelagic food chains of three meromictic lakes (Fry 1986).

Models of zooplankton diel migration and depth distributions usually assume that food resources are better in the well-lit surface waters than at greater depth (reviewed by Gliwicz and Pijanowska 1988). The migration patterns of *D. dentifera* and *D. pulicaria* are well documented for our three deepest study lakes (Haney and Hall 1975; Leibold 1990, 1991; Folt et al. 1993; González and Tessier 1997) and are verified by our sampling. Our results and those of Williamson et al. (1996) suggest, however, that animals migrating into the surface waters often experience a decline in resource conditions. The negative effects of declining resources at the surface are probably offset by the positive effects of higher temperature on growth and egg development (Loose and Dawidowicz 1994). In further support of the temperature hypothesis, Leibold (1989) found that the growth and reproduction of both *D. pulicaria* and *D. dentifera* was better for animals incubated with epilimnetic resources in the epilimnion than for animals incubated with hypolimnetic resources in the hypolimnion of Lawrence Lake. Our method of incubating growth assays at one temperature provides a means for separating responses to food resources from the confounding and potentially important effects of temperature.

Our estimates of *Daphnia* biomass and grazing were closely correlated. Knoechel and Holtby (1986) reported that their length versus filtering rate regression explained 93% of the variation in cladoceran filtering rates in a stratified lake, completely disregarding species identity, temperature, and time of day. However, since Haney and Hall (1975) reported higher night filtering rates in two of our study lakes, we tested the effect of doubling the night filtering rates on the growth versus grazing correlation. This scenario increases the relative grazing rate in the epilimnion, mainly at the expense of the metalimnion, and modestly improves the growth versus grazing correlation ($r_s = -0.81$ for higher night feeding vs. -0.77 for no time-of-day effect). The

length-based regression model almost certainly overestimates filtering rates for the anoxic boundary layer, because of the combination of low DO, cold temperature, and very high food concentration. However, since estimated grazing rates were already low in the anoxic boundary layer, corrections that lower them further would only strengthen the growth versus grazing correlation.

Mechanisms of food limitation—Our supplement treatments show that food limitation is largely a consequence of energy limitation. The strong contrast between the P and *Syn* treatments is especially revealing, as both treatments were designed to alleviate P limitation, although *Synechococcus* is also a source of readily assimilated energy. Since *Synechococcus* is highly deficient in essential fatty acids and sterols (DeMott and Müller-Navarra 1997; Von Elert and Wolfram 2001), the strong positive responses to the *Synechococcus* supplement are also contrary to limitation by essential lipids. Since prokaryotes are typically deficient in essential lipids, the high growth with resources from the boundary layer, where photosynthetic bacteria predominated, is also contrary to the essential lipid hypothesis.

Although the effects of P supplements on *Daphnia* growth were weak and sporadic, our *Daphnia* P-balance data show that animals feeding on P-deficient resources in the upper layers experienced a significant decline in P balance (as % mass) relative to animals feeding on P-sufficient resources in deeper layers. Moreover, both the P and *Syn* supplements largely alleviated the P deficits in the test animals' tissues. On the one hand, these results show that animals feeding on P-deficient resources experienced some form of "P stress" and were close to the edge between energy and P limitation when feeding on natural resources. On the other hand, the positive effects of the P supplements on *Daphnia* P balance give confidence that the supplements were able to overcome any P deficiency in the diet. Thus, the weak growth responses to the P supplements were not due to low quantity or poor assimilation of the added P.

Since our animals were constrained to feed on resources from one depth layer, our data do not show whether diel vertical migration would potentially alleviate P limitation and deficits in P balance (Sterner and Schwalbach 2001). Short-term (24 h) estimates of *Daphnia* growth show that ability to grow on P-deficient diets declines as *Daphnia* P balance declines (DeMott 2003). Such an effect could be important if animals migrate between strata with P-deficient and P-sufficient resources. Reducing P limitation by migration is more plausible in our eutrophic lakes, as even the upper hypolimnion of the oligotrophic lakes had seston C:P ratios >300. In any case, our results show little benefit to improving P balance unless energy assimilation also increases.

The potential for P limitation is strongest when energy assimilation is high (Sterner and Robinson 1994; Sterner 1997). Recently, Boersma and Kreutzer (2002) showed positive effects on *Daphnia* growth of adding phosphate to low concentrations of P-limited algae in laboratory experiments. They argue that stoichiometric models for zooplankton need to take into account departures from element homeostasis and low but nonzero excretion of scarce, limiting elements.

Although we detected P limitation, our results show that the balance between energy and P limitation can strongly favor energy limitation.

Previous studies that used *Daphnia* growth assays with P supplements found evidence for P-limited growth in both hypereutrophic (DeMott et al. 2001) and oligotrophic lakes (Elser et al. 2001) lakes. Furthermore, as predicted by stoichiometric theory, when seston C:P ratios were >300, *Daphnia* densities were very low and *Daphnia* only comprised a small fraction of the total zooplankton biomass in these lakes (DeMott and Gulati 1999; Urabe et al. 2002). However, the high *Daphnia* densities observed in our study, despite seston C:P ratios >300, are consistent with the finding that P was not a major factor limiting *Daphnia* growth.

The results presented here do not demonstrate the mechanism of energy limitation; however, a recent study including resources from the surface waters of six lakes, including Lawrence and Warner Lakes, provides strong support for the digestion resistance hypothesis and evidence contrary to effects of food abundance (DeMott and Tessier 2002). Growth assay results were closely correlated to assimilation efficiency, but unrelated to seston concentration. Moreover, concentrating natural seston twofold resulted in only a very weak improvement in growth, in contrast to very strong responses to *Synechococcus* supplements. The digestion resistance hypothesis gains further support from the observation that digestion-resistant algal taxa are abundant in the surface waters of stratified lakes in our region (Tessier and Woodruff 2002a). Boersma et al. (2001) also emphasize the importance of assimilation efficiency for seston food quality.

The high fraction of chlorophyll (>90%) passing through the 62- μ m screen in the oligotrophic lakes is contrary to an effect of algal size on edibility. Studies on surface waters of stratified lakes in our region show that about 70% of the chlorophyll passes through a 35- μ m screen and about 55% passes through a 15- μ m screen (Tessier and Woodruff 2002a). In contrast, about 50% of the chlorophyll from the upper two depth strata of our two eutrophic lakes was retained on a 62- μ m screen. However, our results suggest that the higher incidence of large algae is a characteristic of higher nutrient loading and independent of *Daphnia* growth or grazing intensity.

We sought to make our results more general by studying four lakes that differ in trophic state. However, our lakes span only a modest range of productivity and size and our experiments focused on a limited but crucial time of year. Thus, our results may not apply to ultraoligotrophic or highly eutrophic lakes, where zooplankton-phytoplankton coupling is expected to be weaker (Elser and Goldman 1991). Studies that emphasize seasonal dynamics provide a different perspective (e.g., Scheuerell et al. 2002). Moreover, deep-water refuges should be less important in lakes with more effective deep-water predators. Given these limitations, our results illustrate how a deep-water refuge from fish predation can mediate strong grazing intensity and zooplankton food limitation throughout the water column of small, stratified lakes.

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