EXPERIMENTAL EVIDENCE FOR DENSITY-DEPENDENT EFFECTS AND THE IMPORTANCE OF ALGAL PRODUCTION IN DETERMINING POPULATION GROWTH RATES OF RIVERINE ZOOPLANKTON

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ABSTRACT

Food limitation effects on life history traits of lake zooplankton have been well documented but few studies have examined linkages between population growth rates and food resources in riverine environments. In rivers, allochthonous inputs of particulate organic matter may mitigate food limitation effects allowing density-independent mechanisms associated with washout (discharge) and feeding interference (turbidity) to assume greater importance. We experimentally manipulated densities of commonly occurring riverine zooplankton (*Bosmina longirostris* and cyclopoid copepods) within 2000 l mesocosms containing ambient or algal-enriched food resources. The experiment was repeated through time (July, August, September) to represent the range of zooplankton densities and food resource levels observed in the Ohio River during warm-water, low-flow conditions. High growth rates and low sensitivity to density-dependent effects were observed during July when particulate organic carbon (POC) and chlorophyll concentrations were highest. Lower growth rates and stronger response to density-dependent effects were observed during August and September experiments when POC and chlorophyll concentrations were lower. Direct manipulations of algal abundance resulted in higher growth rates when gains in chlorophyll were accompanied by increases in the edible size fraction (September experiment). Algal C concentrations were found to be a significant predictor of variation in population growth rates for *Bosmina* but not cyclopoids. Algal C concentrations in the Ohio River rarely fell below experimentally derived minimum food thresholds but were often below saturation thresholds suggesting that population growth rates were constrained by autochthonous food resources despite the prevalence of allochthonous carbon. Copyright © 2005 John Wiley & Sons, Ltd.

KEY WORDS: rivers; zooplankton; food limitation; mesocosms; density-dependent effects; Bosmina; cyclopoids; Ohio River

INTRODUCTION

Linkages between zooplankton life history traits and the quantity and quality of their food resources have recently been the subject of much attention (see reviews by Gulati and DeMott, 1997; Sterner and Schulz, 1998). Food resources have been shown to influence zooplankton energy reserves (Tessier and Goulden, 1982), time of development (Hansen and Hairston, 1998), population growth rate (Rothhaupt, 1990), and community biomass and egg production (Lampert and Schober, 1980). Zooplankton species occurring in riverine environments are generally similar to those found in lakes but we know of no prior studies that have assessed population growth rates as a function of the quantity or quality of food resources. Hydrologic conditions in riverine environments impose stressful conditions on zooplankton arising from turbulent mixing, population dilution (from upstream and tributary inputs) and feeding interference (from suspended particulate matter). The net effect of these processes, coupled with predation by benthic and pelagic planktivores, may be to maintain low population densities and thereby mitigate food resource limitation.

Large rivers support diverse and at times abundant zooplankton communities (Basu and Pick, 1997; Gosselain *et al.*, 1998; Viroux, 1999) suggesting that resource levels may be adequate to sustain population growth in

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excess of mortality from predation and advection. Chlorophyll concentrations in rivers vary over a range that encompasses oligotrophic and eutrophic lakes with peaks in algal abundance typically occurring during low flow. Correlations between zooplankton abundance and chlorophyll have been reported for a number of rivers suggesting that autochthonous carbon may be an important food resource (De Ruyter van Steveninck *et al.*, 1992; Pace *et al.*, 1992; Admiraal *et al.*, 1994; Basu and Pick, 1997). During periods of elevated discharge, terrestrial organic matter dominates riverine seston. Allochthonous materials are predominantly detrital carbon of low food quality but their abundance may compensate for low quantities of more nutritious food resources (algae, bacteria). Alternatively, high concentrations of non-algal seston may have deleterious effects on riverine zooplankton, either directly by interfering with feeding mechanics (Saunders and Lewis, 1988; Pace *et al.*, 1992; Jack *et al.*, 1993) or indirectly, by diluting the intake of nutritionally important dietary elements (N, P, fatty acids and lipids). Therefore, while it is likely that riverine zooplankton exhibit greater reliance on allochthonous food resources, it is unclear whether this dependency promotes or offsets food limitation.

Food resources may be characterized as limiting or non-limiting based on species-specific delineations of minimum and saturating resource levels (Lampert and Schober, 1980; Osenburg and Mittelbach, 1996; Sterner and Schulz, 1998). Food resources above a minimum threshold are required for the growth and reproductive success of an individual and to maintain a population growth rate sufficient to offset losses. Increases in resource levels above the minimum threshold are accompanied by increases in individual and population growth rates until a saturating food threshold is achieved. Species-specific differences in minimum and saturating thresholds have been reported and these differences can in part explain zooplankton distribution patterns (DeMott and Kerfoot, 1982; Tessier and Goulden, 1987; Gliwicz, 1990; Santer and Lampert, 1995). Species exhibiting low minimum thresholds may be more successful in environments with poor or highly variable resource levels while species exhibiting high maximum growth rates are favoured by resource-rich environments (Gliwicz, 1990, 2001; Huggett, 2001). Characterizing food resources in the environment relative to minimum and saturating thresholds may therefore provide insight into the severity and frequency of food limitation and its importance in structuring zooplankton communities.

Phytoplankton are regarded as the predominant and nutritionally important food resource for many types of zooplankton and measures of algal biomass such as chlorophyll are widely used to characterize zooplankton food resources (Hanson and Peters, 1984; Santer and Lampert, 1995; DeStasio *et al.*, 1995). Delineation of minimum and saturating food thresholds from measures of algal abundance is complicated by two factors. First, algal food resources vary in quality depending on edibility and nutritional factors such as minerals (N, P), fatty acids and lipids (Urabe and Watanabe, 1992; Sterner *et al.*, 1993; Muller-Navarra, 1995a, 1995b; DeMott and Muller-Navarra, 1997). Poor food quality may constrain zooplankton growth rates even in environments where algal abundance is relatively high (Sterner and Schulz, 1998). Second, many zooplankton are capable of utilizing non-algal food resources that include allochthonous organic matter (Grey *et al.*, 2001), bacteria (King *et al.*, 1991) and, for predatory species, ciliates, heterotrophic nanoflagellates and small crustaceans (Jack and Gilbert, 1993; Stoecker and Capuzzo, 1990). Many species previously regarded as 'herbivores' or 'predators' have been shown to utilize a wide range of food resources, though their importance to growth and reproduction remain poorly understood (Sanders *et al.*, 1996; Makino and Ban, 2000).

We quantified population growth rates of two commonly occurring taxa from the Ohio River in 20001 mesocosms containing natural autochthonous and allochthonous food resources. To assess food limitation we compared population growth rates during varying resource conditions that arise from seasonal patterns in riverine particulate organic carbon (POC) and chlorophyll concentrations. Our approach was similar to that of other 'assay' experiments (e.g. Williamson *et al.*, 1996) in which zooplankton growth responses are used as a metric to characterize the quantity and quality of food resources. Typically, these experiments are conducted at zooplankton densities near or below ambient to ensure that density-dependent effects do not arise through food resource depletion. To determine whether food limitation could arise through resource depletion we simultaneously quantified population growth rates over a range of zooplankton densities. Zooplankton reliance on autochthonously derived food resources was tested in two of the three experiments by manipulating incident light levels to achieve variable algal abundance. We predicted that: (1) riverine zooplankton were food-limited and that variation in population growth rates at ambient densities would be correlated with food resource levels; (2) food limitation could arise through exploitative competition resulting in negative density-dependent effects; and (3) food limitation was dependent on auto-

chthonous production and therefore enhancements in algal C concentrations would result in higher population growth rates. Conversely, if riverine environments were food-saturated, or if zooplankton were reliant on allochthonous food resources, then variation in zooplankton and algal abundance would have little effect on zooplankton population growth rates.

METHODS

Mesocosm experiments were conducted at the Ohio River Experimental Station (ORES) located 40 km northeast of Louisville, Kentucky. The facility is situated along the banks of the Ohio River at a location near the midpoint of a navigation pool formed by the McAlpine Dam (30 km downriver). The Ohio River is regulated along its 1578 km length by 20 low-head dams that maintain a minimum navigable depth of 3 m. Water residence time within the McAlpine Pool is less than 2 days during high flow (typically January–April) but may be as long as 30 days when the river is at base flow (July–October). Hydrology, physical–chemical conditions and phytoplankton production within the pool are described in detail by Sellers and Bukaveckas (2003). Phytoplankton and zooplankton communities of the lower Ohio River are characterized in Thorp *et al.* (1994) and Wehr and Thorp (1997).

Experiments were conducted in July, August and September. Experimental units were opaque fibreglass tanks (2000 l) that were filled by pumping water from the Ohio River through a 63 μm net to remove macrozooplankton. Tanks were then inoculated with macrozooplankton using the contents of 63 μm net tows collected from the main channel of the river. Zooplankton were carefully transferred from a 120 l holding container into the experimental tanks to avoid injury to the animals. For each experiment, tanks were stocked in groups of three or four at low ($<5 \times$ ambient), moderate ($5–10 \times$ ambient) and high ($>10 \times$ ambient) densities. Realized starting densities were variable even among 'replicate' tanks resulting in semi-continuous gradients ranging from less than $1 \times$ ambient up to $36 \times$ (July), $20 \times$ (August) and $12 \times$ (September) ambient. We calculated the concentration factor (CF = ratio of initial stocking density to ambient river density) of each tank for use in statistical analyses of density-dependent effects. Variable algal abundance was achieved by manipulating incident light levels using shade cloth (August and September experiment only). Light levels in the river and the tanks were measured with a Li-Cor SA flat-plate quantum sensor (model 192) and Li-Cor 1000 data logger. Attenuation coefficients were used in combination with tank dimensions and river morphometry to derive estimates of daily mean irradiance (see Sellers and Bukaveckas (2003) for details). The High Light treatment approximated light conditions in the shallow (3–5 m) upper pool where positive algal growth rates are observed. The Low Light treatment simulated deeper depths (9–12 m) occurring in the lower pool where net phytoplankton production is near zero. Fourteen tanks were used in the July experiment and eighteen tanks were used in the August and September experiments (nine each for High and Low Light treatments).

Initial and final zooplankton densities were determined by collecting three replicate net tows (63 μm net; sample volume = 19 l for each tow) from each tank after mixing the tank to ensure uniform distribution of animals. Experiments lasted 10–14 days and population growth rates were calculated as the change in the number of individuals over time ($r = \ln N_t – \ln N_0$ day^{-1}, where N_t is the final density and N_0 is the initial density). Exponential growth was confirmed by selective samples taken at mid-experiment. Population growth rates were calculated for *Bosmina longirostris* and cyclopoid copepods, which together accounted for greater than 90% of macrozooplankton biomass in the river and the mesocosms. Analyses of a subset of initial and final samples from each experiment revealed that cyclopoids were predominantly ($>95\%$) *Diacyclops* sp. with *Mesocyclops* sp. accounting for a minor proportion of the total. Population growth rates of cyclopoids included adults and copepodites. Density-dependent effects were tested by univariate regression using the concentration factor as a predictor of population growth rates. The effects of variable shading levels were tested by deriving separate regressions for High and Low Light tanks and determining whether differences in slopes and intercepts were statistically significant (*t*-test).

Food conditions in the river and the mesocosms were quantified using measurements of chlorophyll and POC to estimate algal and non-algal C resources. Water samples were collected every 3 days and filtered through 0.45 μm and 25 μm filters. Filters were extracted in acetone for 12 hours and chlorophyll *a* was measured fluorometrically (Turner 10-AU fluorometer) with acid correction (Arar and Collins, 1997). The edible fraction was calculated as the difference between total (>0.45 μm) and non-edible (>25 μm) chlorophyll (Lampert, 1987). Chlorophyll concentrations were converted to algal C equivalents based on a 20:1 C:Chl ratio. This ratio was determined empirically

during mesocosm experiments in which varying shading levels were used to obtain variable yields of POC and chlorophyll in the absence of macrozooplankton grazers (Sellers and Bukaveckas, 2003). POC was regressed against chlorophyll ($R^2 = 0.90$; $p < 0.001$) and the slope of this line was used to estimate the C:Chl ratio. POC was collected on 0.5 μm filters, dried and analysed with a Perkin-Elmer 2400 series II CHNS/O analyser. Non-algal C was estimated by subtracting algal C from POC. Food resources within each tank were characterized based on average algal or non-algal C concentrations during the experiment. Variation in population growth rates as a function of food resources was modelled for the pooled dataset (three experiments) using a Michaelis–Menton formulation:

$$r = r_{max} \times \frac{(Food)}{(K + Food)}$$

where r_{max} (maximum growth rate at saturating food levels) and K (half saturation constant) were coefficients derived from least squares regression. Food (algal or non-algal C) and r (population growth rate) were measured variables comprising a single observation for each experimental unit (mesocosm). Solving the Michaelis–Menton model for the food concentration at which the population growth rate was zero yielded an estimate of the minimum food threshold. We defined the saturating food threshold as the food concentration at which the population growth rate was within 25% of r_{max}.

Physical and chemical data were collected every 3 days throughout the experiment to compare tank and river conditions. Comparable abiotic conditions in the tanks and river were maintained by replacing 20% of the water in the mesocosms with river water every 3 days. Macrozooplankton were captured by filtration of outflowing water through a 63 μm net and returned to the tanks. Filtration of inflowing water (63 μm net) prevented the introduction of new individuals. Temperature, dissolved oxygen, and pH were measured in the river and mesocosms using a Hydrolab SONDE IV. Turbidity was measured using a HACH 2100P turbidity meter. Three submersible pumps circulated water in the tanks with a combined strength sufficient to pump a volume of water equivalent to the tank volume every hour. To characterize zooplankton densities in the river, samples were collected monthly during May to October of 1998 and in July, August and September 1999 at the start of each experiment. Triplicate 25 l samples were taken from a depth of 1 m in the main channel and pumped through a 63 μm net. Tank and river samples were narcotized with CO_2, and preserved with sugared formalin (Haney and Hall, 1973). Zooplankton were identified using an Olympus SZX12 stereomicroscope. Body lengths were measured at the start and end of each experiment but no significant changes in mean length were detected in response to treatment effects. The incidence of egg-bearing individuals was too low to permit analyses of treatment effects on egg ratios.

RESULTS

River discharge for dates corresponding to the mesocosm experiments ranged from 100 to 600 $m^3 s^{-1}$ (Figure 1) and was below the long-term monthly average discharge for this period (*c.* 1000 $m^3 s^{-1}$; Sellers and Bukaveckas, 2003). River temperature and turbidity data were used to characterize seasonal variability and to allow comparisons with tank conditions. The July experiment was conducted when river temperatures rose from 26 to 30°C while the August experiment was conducted during a period of comparable temperatures anteceding the summer maximum (Figure 1). By the September experiment, river temperatures had declined to 20–22°C. Temperatures in the tanks were within 2°C of those measured in the river except during the August experiment when cool nights resulted in greater heat loss from the tanks. High Light tanks were warmer than Low Light tanks but differences were always less than 1°C. River turbidity levels were low (< 10 NTU) during all three experiments (Figure 1). Turbidity levels were somewhat higher during the July experiment (5–7 NTU) compared to the August and September experiments (2–4 NTU). Turbidity levels in the tanks were within 2 NTU of the river except during the July experiment when the tanks were 4–5 NTU lower.

In both the river and the mesocosms, highest average chlorophyll concentrations occurred in July (10–12 μg l^{-1}) with lower concentrations (<6 μg l^{-1}) observed in August and September (Figure 2). POC concentrations in the river and the mesocosms followed a similar pattern with highest average values in July (20 mg l^{-1}) and lower
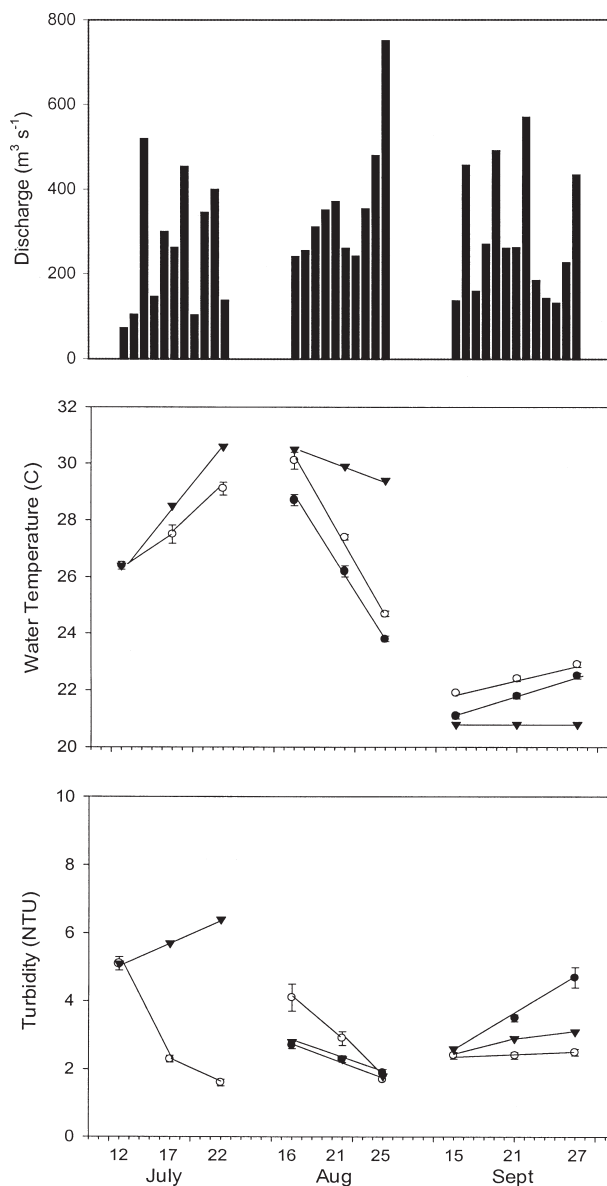
Figure 1. Discharge of the Ohio River near Louisville, KY (USGS no. 03294500) for dates corresponding to mesocosm experiments (upper panel). Temperature (middle panel) and turbidity (lower panel) of mesocosms and the Ohio River (at Westport, KY) during July, August and September (1999) experiments. ●, Low Light tanks; ○, High Light tanks; ▼, the river. Data shown are means and standard error of all tanks in each treatment (some error bars not visible)

values ($1 \, mg \, l^{-1}$) in August and September. Algal C expressed as a proportion of POC was low during July (1%) and higher (>5%) in August and September. Low Light tanks exhibited food conditions similar to those measured concurrently in the river as indicated by comparable levels of total chlorophyll, edible chlorophyll, POC and algal C:POC. Among High Light tanks, total chlorophyll concentrations averaged $3–4 \, \mu g \, l^{-1}$ higher than those observed in the river and the Low Light tanks ($p < 0.001$ excluding river versus High Light in September). The High Light treatment resulted in large increases in the amount of algal carbon relative to POC (August = 29%, September = 20%) due in part to low POC concentrations at this time. Increases in algal C were accompanied by increases in the edible chlorophyll fraction in September but not August. Overall, the edible fraction represented about one-third of total chlorophyll.
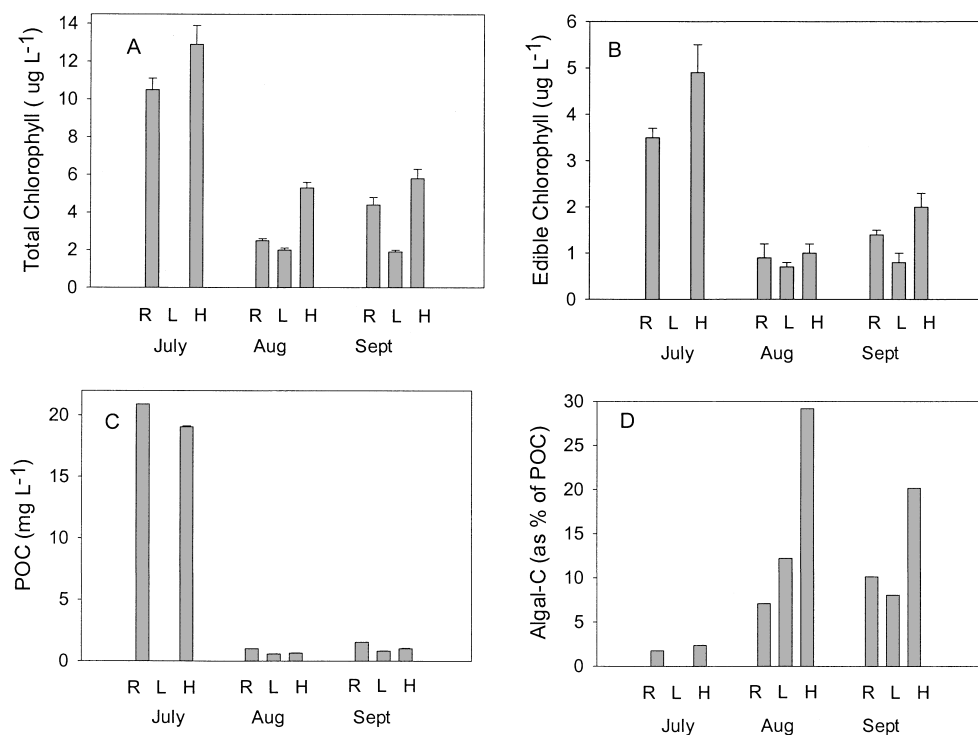
Figure 2.   Food resource conditions of mesocosms and the Ohio River based on total chlorophyll (A), edible chlorophyll (B), POC (C) and algal carbon as percentage of POC (D). *R* denotes average river conditions during each experiment (with standard error). *L* and *H* are average values and standard errors for Low Light and High Light tanks (no Low Light treatment in July)

Bosmina densities in the river ranged from <1 to 2 individuals (ind.) l^{-1} during 1998–1999 with no consistent seasonal trends (Figure 3). Cyclopoid densities were more variable ranging from 1 to 23 ind. l^{-1} with peak densities occurring in late-summer 1998 and low densities (<5 ind. l^{-1}) throughout 1999. Zooplankton densities in the mesocosms were within the range of those observed in the river with the exception of the September experiment during which the highest stocking levels exceeded densities observed in the river (Table I). Univariate regressions using concentration factors (CF) as a predictor of population growth rates were derived for each experiment and all models were found to be significant (Figure 4, Table II). Regression models accounted for 44–65% of the variation in *Bosmina* growth rates and 24–68% of the variation in cyclopoid growth rates. When starting densities were at or near river densities (CF = 1), positive population growth rates were observed in all three experiments for both *Bosmina* and cyclopoids. *Bosmina* growth rates at ambient densities were highest in July ($r = 0.35\,\mathrm{d}^{-1}$) and lower in August and September ($r = 0.15$ and $0.11\,\mathrm{d}^{-1}$, respectively). Growth rates of cyclopoids were highest in August ($r = 0.28\,\mathrm{d}^{-1}$) and lower in July and September ($r = 0.14$ and $0.18\,\mathrm{d}^{-1}$, respectively). Growth rates declined for both taxa and in all experiments at higher starting densities. For a range of concentration factors from 1 to 10, regression models predict modest reductions in *Bosmina* r (from 0.35 to $0.30\,\mathrm{d}^{-1}$) during July and large decreases during August (0.15 to $0.01\,\mathrm{d}^{-1}$) and September (0.11 to $-0.06\,\mathrm{d}^{-1}$). Negative growth rates among *Bosmina* populations were observed during the August experiment at starting densities exceeding $10 \times$ ambient (4 of 5 tanks) and during the September experiment at starting densities exceeding $6 \times$ ambient (6 of 8 tanks). Growth rates in July were always positive despite the broad range of concentration factors (up to $36 \times$). For cyclopoids, regression models predict small reductions in population growth rates with increasing density (CF = 1–10) during July (from 0.14 to $0.11\,\mathrm{d}^{-1}$) and August (0.28 to $0.21\,\mathrm{d}^{-1}$) and a large decrease during September (0.18 to $-0.03\,\mathrm{d}^{-1}$). Negative growth rates were observed during the July experiment at starting densities greater than $30 \times$ ambient (3 of 6 tanks) and during the September experiment at starting densities greater than $6 \times$ ambient (6 of 8 tanks).

During the September experiment, density had the greatest negative effect on r (slope = 12–15% of intercept) and accounted for the greatest proportion of variation in r (56–68%) for both *Bosmina* and cyclopoids. Density
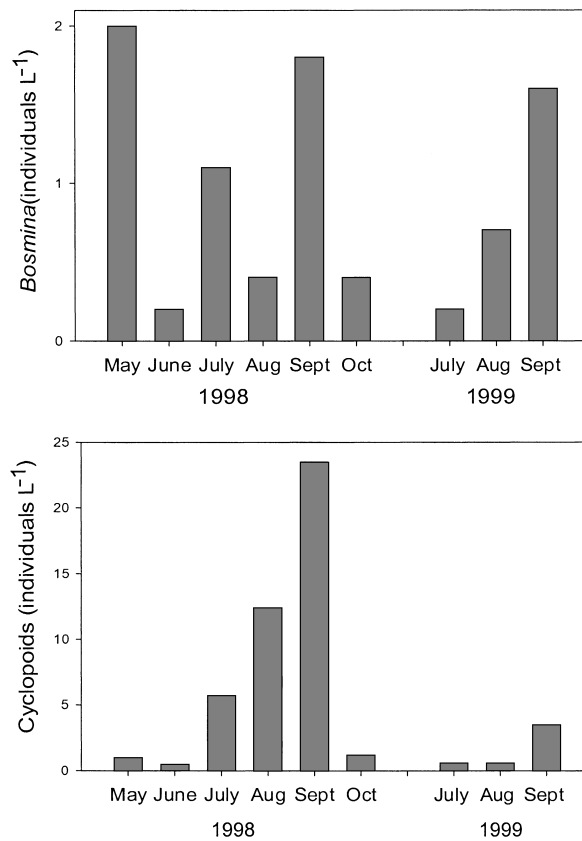
Figure 3. Densities of *Bosmina* and cyclopoid copepods (predominantly *Diacyclops*) in the Ohio River at Westport, KY during 1998 and 1999 (1999 samples correspond to mesocosm experiments)

effects were weakest (1–2% of intercept) and correlation coefficients were lower (0.26–0.44) during the July experiment. In August, cyclopoid growth rates exhibited little change over the range of stocking densities (slope = 3% of intercept; $R^2 = 0.24$) whereas *Bosmina* growth rates exhibited strong density-dependent responses (slope = 9% of intercept; $R^2 = 0.65$). Seasonal variation in the relative strength of the density effect reflected stronger treatment effects (slope c. -0.02) and lower growth rates (intercept $< 0.20 \, \mathrm{d}^{-1}$) during the September experiment. No significant effects from variable shading levels were detected for either *Bosmina* or cyclopoids during the August experiment since population growth rates in High and Low Light tanks were similar for corresponding

Table I. Average and range of initial densities for *Bosmina* and cyclopoid copepods in mesocosms during July, August and September experiments

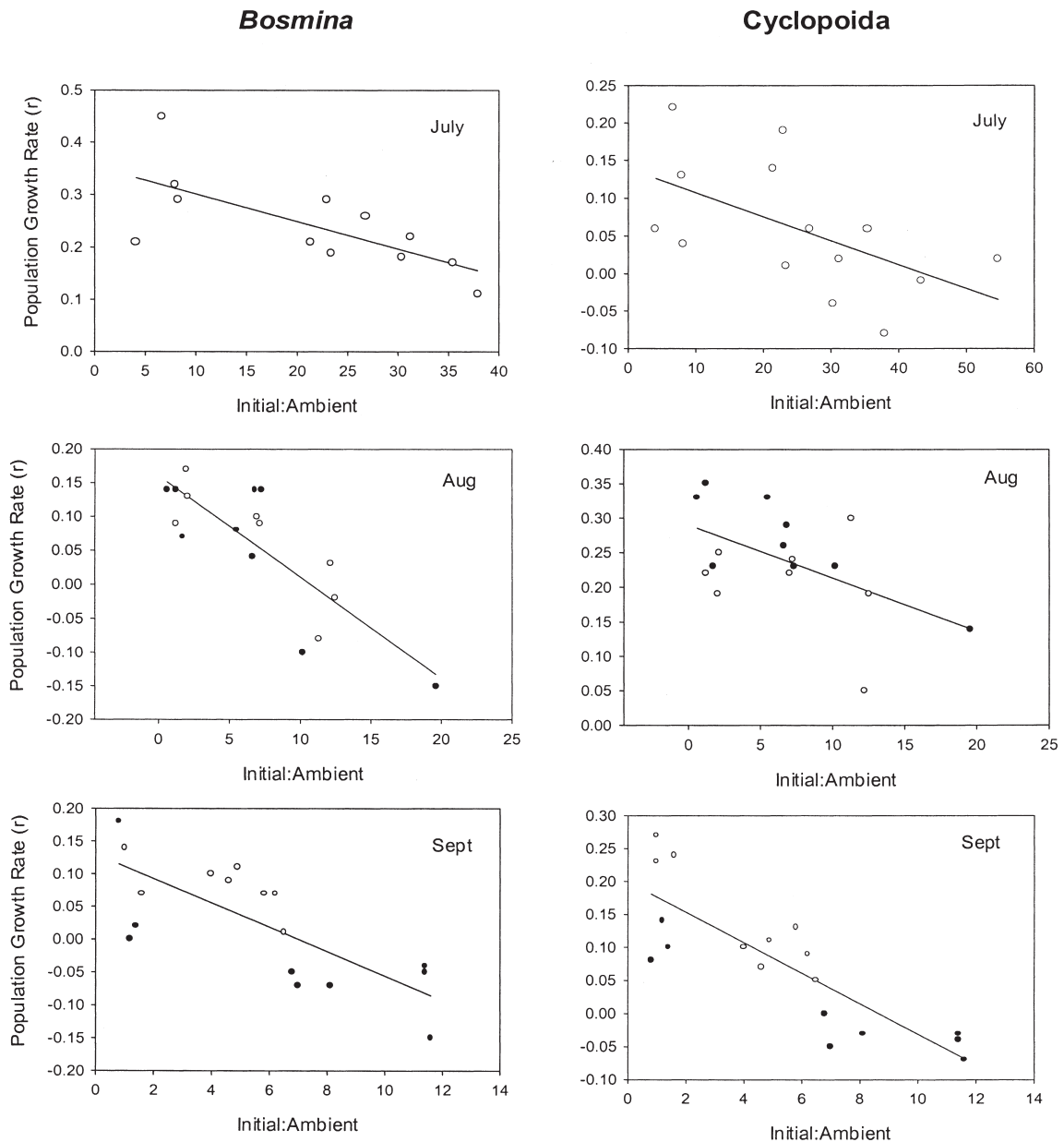| Organism	Month	Initial density (ind. l^{-1})	
		Average	Range
Bosmina			
	July	1	0.1–1.9
	August	2	0.3–4.4
	September	11	0.1–26
Cyclopoids			
	July	19	3–42
	August	7	0.5–21
	September	16	4–37

Bosmina

Cyclopoida

Figure 4. *Bosmina* and cyclopoid population growth rates (*r*) as a function of density in experimental mesocosms. Density is expressed as the ratio of initial stocking density to ambient river density. ●, Low Light tanks; ○, High Light tanks. Lines denote least squares regressions with associated statistics presented in Table II

densities (Figure 4). In September, *Bosmina* and cyclopoid growth rates in High Light tanks were greater than those observed in Low Light tanks and separate regressions revealed significant differences in model parameters. For both *Bosmina* and cyclopoids, *y*-intercepts were significantly different ($p < 0.03$ and $p < 0.001$, respectively) with higher values for regressions from High Light tanks (Table II). For cyclopoids, the slopes of the regression lines were also significantly different ($p < 0.001$) although direct comparisons were complicated by the smaller density gradient attained in the High Light treatment ($1–7 \times$) relative to the Low Light treatment ($1–12 \times$).

We tested the utility of algal and non-algal C concentrations as predictors of population growth rates using a Michaelis–Menton formulation and the pooled dataset for three experiments. Algal C concentrations were found to

Table II. Model equations predicting population growth rates of *Bosmina* and cyclopoid copepods in experimental mesocosms based on concentration factors (CF = ratio of initial stocking density to ambient river densities). Separate regressions were derived for High Light (HL) and Low Light (LL) treatments during the September experiment. Values in parentheses denote the standard error of the regression coefficients. N denotes the number of experimental units (mesocosms)

Organism	Month	Model	r^2	P	N
Bosmina	July	$r = -0.005(CF) + 0.353$ (0.001) (0.038)	0.44	<0.001	12
	August	$r = -0.015(CF) + 0.161$ (0.003) (0.023)	0.65	<0.001	17
	September	$r = -0.019(CF) + 0.130$ (0.004) (0.025)	0.56	<0.001	17
	HL	$r = -0.013(CF) + 0.141$ (0.005) (0.021)	0.45	<0.001	8
	LL	$r = -0.015(CF) + 0.070$ (0.005) (0.039)	0.50	0.019	9
Cyclopoids	July	$r = -0.003(CF) + 0.139$ (0.001) (0.039)	0.26	0.037	14
	August	$r = -0.008(CF) + 0.290$ (0.003) (0.026)	0.24	0.026	17
	September	$r = -0.023(CF) + 0.199$ (0.004) (0.024)	0.68	<0.001	18
	HL	$r = -0.034(CF) + 0.276$ (0.005) (0.025)	0.82	<0.001	9
	LL	$r = -0.015(CF) + 0.110$ (0.002) (0.020)	0.81	<0.001	9

be a significant predictor of variation in growth rates for *Bosmina* but not cyclopoids (Figure 5). The overall model was highly significant ($p < 0.001$) but accounted for a relatively small proportion of the variation in r ($R^2 = 0.33$). Growth rates generally increased over a range of algal C concentrations between 20 and 200 $\mu g\,l^{-1}$ and were consistently high at concentrations between 200 and 400 $\mu g\,l^{-1}$. Model parameters were significant ($r_{max} = 0.23 \pm 0.03\,d^{-1}$; $K = 9.2 \pm 0.7$; $p < 0.001$) and yielded estimates for minimum and saturating food thresholds at 40 and 180 $\mu g\,l^{-1}$ (respectively). Eleven mesocosms exhibited average food resource levels below the estimated minimum threshold and population growth rates were negative in four of these. The highest algal C concentration at which negative growth rates occurred was 83 $\mu g\,l^{-1}$. Twelve tanks contained algal C concentrations in excess of the estimated saturating threshold and positive growth rates were observed in all of these (average = 0.22 d^{-1}). Threshold values were compared to algal C concentrations measured in the upper, middle and lower reaches of the McAlpine Pool during 1998 and 1999 (Figure 5). Algal C concentrations in the river typically exceeded the minimum threshold for sustaining positive growth rates except during the early part of the growing season (May–June). Algal C in the river rarely exceeded the saturation threshold with the exception of an algal bloom occurring in the middle and lower pool during July 1999. Food resource models based on non-algal C did not account for a significant proportion of variation in growth rates for either *Bosmina* or cyclopoids.

DISCUSSION

Population growth rates of *Bosmina* and cyclopoid copepods were negatively correlated with initial stocking densities. Variation in growth rates along the density gradient may have arisen from a variety of mechanisms including competition, predation and food resource depletion. Interference competition has been shown to reduce population growth rates under conditions of severe crowding (Burns, 1995) but we regard this as unlikely given the densities and resource levels used in these experiments. Concentration factors for our study were comparable to those reported in various lake enclosure experiments (Vanni, 1987; Vanni and Temte, 1990; Spencer and Ellis, 1998) but resulting densities were lower due to sparse zooplankton abundance in the river at the time of our experiments.
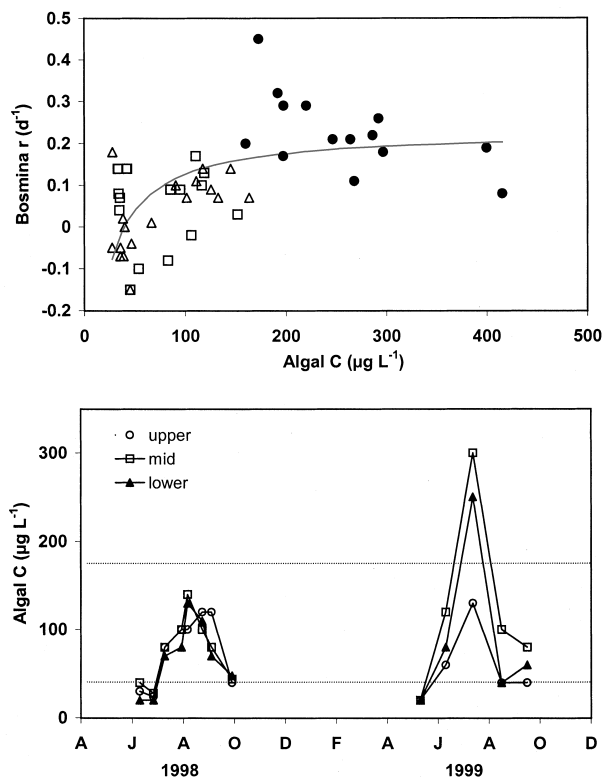
Figure 5.   Upper panel: *Bosmina* population growth rates (*r*) as a function of algal C concentrations during July (●), August (□) and September (△) experiments. Regression line derived from Michaelis–Menton model ($R^2 = 0.33$, $p < 0.001$). Lower panel: algal C concentrations in the upper, middle and lower reaches of the McAlpine Pool during 1998 and 1999. Dashed lines represent the minimum and saturating food thresholds derived from the Michaelis–Menton model

Average starting densities for *Bosmina* ranged from 1 to 11 ind. l^{-1} and were below peak densities previously observed in the Ohio (37 ind. l^{-1}; Thorp *et al.*, 1994) and other large rivers such as the Rideau (65 ind. l^{-1}; Basu and Pick, 1997). Average cyclopoid densities ranged from 7 to 19 ind. l^{-1} within the mesocosms. Ohio River concentrations typically do not exceed 25 ind. l^{-1} (Jack and Thorp, 2000; this study) although much higher values have been reported in other rivers (600 ind. l^{-1}; Marneffe *et al.*, 1996). Our estimates of population growth rates are based on enumeration of copepodites and adult stages and therefore reflect variable rates of recruitment from nauplii stages. The observed reduction in population growth rate with increasing density may in part be due to higher rates of predation by the carnivorous adults and older copepodites on nauplii (Williamson, 1980). We cannot discount the possibility that cyclopoids also preyed upon co-occurring macrozooplankton (principally *Bosmina*) but inter-experiment differences in growth rates did not follow patterns expected from a predator–prey interaction. Cyclopoids exhibited moderate growth rates when *Bosmina* were most abundant (September) and *Bosmina* exhibited their highest growth rates when cyclopoids were most abundant (July). Low predation rates on *Bosmina* may be due to the relatively small size of cyclopoids used in these experiments (length = 250–1500 μm).

Inter-experiment differences in *Bosmina* growth rates and their response to variable stocking densities were consistent with the hypothesis that food limitation and density-dependent effects play a role in regulating the abundance of riverine zooplankton. In July, high seston concentrations corresponded to high growth rates and low sensitivity to variation in stocking densities. Growth rates were lower and declined with increasing initial density during the August and September experiments when algal and non-algal C concentrations were low. We conclude that high seston concentrations during July were sufficient to offset food limitation despite apparently poor food quality (low proportion of algal C). Positive growth rates were observed over a broad range of concentration factors and suggest that July densities were well below the resource-based carrying capacity. Lower growth rates and greater sensitivity to density-dependent effects during August and September suggest that *Bosmina* populations

experienced greater food limitation at this time. Lower water temperature during the September experiment (c. 22°C) may have also contributed to lower growth rates. Modest increases in the proportion of algal C (10% of POC in August, September experiments) were insufficient to compensate for declines in seston concentration and resulted in growth rates less than half of those measured in July.

Direct manipulations of algal abundance (High Light tanks) resulted in higher zooplankton growth rates when gains in chlorophyll were accompanied by increases in the edible size fraction (September experiment). These findings suggest that changes in food quality associated with autochthonous production may be an important component of food limitation. Declines in chlorophyll and POC concentrations were observed in some tanks but these did not follow trends in stocking densities or growth rates as would be expected if resource depletion and exploitative competition had occurred. Increased grazing at higher stocking rates may have resulted in the depletion of particularly nutritious food resources that were not reflected in bulk measurements of chlorophyll and POC. For small cladocerans such as *Bosmina*, particle size may be as important a determinant of food quality as nutritional differences between algal and non-algal C. Prior work has shown that *Bosmina* specializes on larger particles than do co-occurring *Daphnia* species (DeMott and Kerfoot, 1982). Maximum growth rates during the September experiment (High Light tanks) were below minimum values for July suggesting that improvements in food quality alone were insufficient to compensate for reductions in food quantity following the late summer decline in seston concentrations.

Inter-experiment differences in cyclopoid growth rates did not follow seasonal changes in seston concentrations. Growth rates were higher in August and September than in July despite substantially lower algal and non-algal C concentrations. It is unlikely that low growth rates during the July experiment were due to negative effects arising from high concentrations of suspended particulate matter. High seston concentrations did not impact *Bosmina* populations (which exhibited maximum growth rates during the July experiment) and copepods are known to be tolerant of turbid conditions (Hart, 1988; DeMott, 1989). Laboratory and field studies have documented the importance of algal C for cyclopoid growth and reproduction (Hansen and Santer, 1995; Makino and Ban, 2000; Grey *et al.*, 2001) but we did not find algal C to be a significant predictor of variation in population growth rates. Shifting dietary preferences associated with changing stages in their life cycle complicates interpretation of cyclopoid responses to experimental manipulations. Adult copepods and older copepodites are largely carnivorous while young copepodites and nauplii are predominantly herbivorous. Early stages may benefit directly from increases in algal abundance while older stages benefit indirectly from associated increases in their prey. Protists and rotifers are often abundant in rivers but variation in their densities is poorly reflected in bulk measures such as POC. Small prey (<63 µm) including ciliates, heterotrophic nanoflagellates and some rotifers would be unaffected by pre-filtration and subsequent stocking such that predator:prey ratios should follow predator density gradients. This may account for the overall success of the univariate models in explaining variation in growth rate based on concentration factors.

Algal C concentrations accounted for a significant proportion of the variation in *Bosmina* growth rates suggesting that autochthonous production may be important despite the predominance of non-algal C in our system. The importance of algal C was apparent during the September experiment when increases in chlorophyll among High Light tanks were accompanied by significant increases in *Bosmina* growth rates. These results are consistent with correlative findings showing a positive relationship between *Bosmina* and algal biomass in the Hudson River (Pace *et al.*, 1992). Chlorophyll was also found to be a good predictor of *Daphnia* growth rates in a detritus-dominated tidal freshwater system (Muller-Solger *et al.*, 2002). Cyclopoid growth rates also increased in High Light tanks during the September experiment but our data do not allow us to determine whether this was a direct effect of cyclopoids feeding on algae or an indirect effect due to increased abundance of small prey. Our threshold algal C concentration for sustaining positive growth rates of *Bosmina* ($40 \, \mu g \, l^{-1}$) is somewhat lower than a previously reported minimum requirement for this species ($70 \, \mu g \, C \, l^{-1}$; Gliwicz, 1990). Our minimum thresholds are comparable to those reported for a variety of *Daphnia* species fed high quality algal food (20–$60 \, \mu g \, C \, l^{-1}$; Kreutzer and Lampert, 1999). Algal C concentrations in the Ohio River rarely fell below the minimum requirement to sustain positive growth rates of *Bosmina* indicating that the river provided a generally favourable environment supporting zooplankton production. This result is consistent with our concurrent finding that the mainstem Ohio River acts as a net source of zooplankton with outputs from the river exceeding inputs from tributary sources (D. L. Guelda and P. A. Bukaveckas, unpublished work). The saturation threshold concentration was fourfold higher ($180 \, \mu g \, C \, l^{-1}$)

and algal C concentrations in the river rarely exceeded resource-saturated levels. These findings suggest that realized population growth rates in the river are constrained by food resource availability. Studies of lake plankton have shown that high light environments can diminish food quality by increasing C:P ratios of algae (Sterner *et al.*, 1998). It is unclear whether similar processes operate in riverine environments but we cannot discount the possibility that increases in food quantity (measured as CHLa) in our High Light treatments may have been partly offset by reductions in food quality. Despite this, we observed significant population growth responses.

In summary, our findings support the hypothesis that riverine zooplankton experience food limitation but suggest that these effects are limited to late summer when seston concentrations decline below $1 \, \text{mg} \, l^{-1}$. During this time, *Bosmina* and cyclopoid growth rates were less than half of their resource-saturated growth rates. In our experimental setting, food limitation was offset by autochthonous production when algal C concentrations exceeded 20% of POC (September High Light tanks). In the river, however, variation in food resources was largely driven by allochthonous inputs since algal C concentrations were typically between 3 and 10% of POC. Allochthonous inputs to rivers and other advective systems (reservoirs, estuaries) may act to subsidize zooplankton production while decoupling its linkage to algal food resources. Predation may also play an important role in mitigating food limitation effects. Our previous mesocosm experiments have shown that the effects of planktivory by zebra mussels and larval fish reduce population growth rates of *Bosmina* and cyclopoids by a magnitude comparable to that observed in this food limitation study (Jack and Thorp, 2000, 2002).

REFERENCES

Admiraal W, Breebaart L, Tubbing GMJ, van Zanten B, de Ruijter van Steveninck ED, Bijkerk R. 1994. Seasonal variation in composition and production of planktonic communities in the lower River Rhine. *Freshwater Biology* **52**: 519–531.

Arar EJ, Collins GB. 1997. In vitro determination of chlorophyll a and phaeophyton a in marine and freshwater phytoplankton by fluorescence. *Methods for the Determination of Chemical Substances in Marine and Estuarine Environmental Samples*. USEPA: 1–12.

Basu BK, Pick FR. 1997. Phytoplankton and zooplankton development in a lowland, temperate river. *Journal of Plankton Research* **19**: 237–253.

Burns CW. 1995. Effects of crowding in different food levels on growth and reproductive investment of Daphnia. *Oecologia* **101**: 234–244.

DeMott WR. 1989. Optimal foraging theory as a predictor of chemically mediated food selection by suspension feeding copepods. *Limnology and Oceanography* **34**: 140–154.

DeMott WR, Kerfoot WC. 1982. Competition among cladocerans: nature of the interaction between *Bosmina* and *Daphnia*. *Ecology* **63**: 1949–1966.

DeMott WR, Muller-Navarra DC. 1997. The importance of highly unsaturated fatty acids in zooplankton nutrition: evidence from experiments with *Daphnia*, a cyanobacterium and lipid emulsions. *Freshwater Biology* **38**: 649–664.

De Ruyter van Steveninck ED, Admiraal W, Breebaart L, Tubbing GMJ, van Zanten B. 1992. Plankton in the River Rhine: structure and functional changes observed during downstream transport. *Journal of Plankton Research* **14**: 1351–1368.

DeStasio BT, Rudstam LG, Haning A, Soranno PA, Allen YC. 1995. An in situ test of the effects of food quality on *Daphnia* population growth. *Hydrobiologia* **307**: 221–230.

Gliwicz ZM. 1990. Food thresholds and body size in cladocerans. *Nature* **15**: 638–640.

Gliwicz ZM. 2001. Species-specific population-density thresholds in cladocerans? *Hydrobiologia* **442**: 291–300.

Gosselain V, Descy J-P, Viroux L, Joaquim-Justo C, Hammer A, Metens A, Schweitzer S. 1998. Grazing by large river zooplankton: a key to summer potamoplankton decline? The case of the Meuse and Moselle rivers in 1994 and 1995. *Hydrobiologia* **369**: 199–216.

Grey J, Jones RI, Sleep D. 2001. Seasonal changes in the importance of the source of organic matter to the diet of zooplankton in Loch Ness, as indicated by stable isotope analysis *Limnology and Oceanography* **46**: 505–513.

Gulati RD, DeMott WR. 1997. The role of food quality for zooplankton: remarks on state-of-the-art, perspectives and priorities. *Freshwater Biology* **38**: 753–768.

Haney JF, Hall DJ. 1973. Sugar-coated *Daphnia*: a preservation technique for Cladocera. *Limnology and Oceanography* **18**: 331–333.

Hansen AM, Hairston NG. 1998. Food limitation in a wild cyclopoid copepod population: direct and indirect life history responses. *Oecologia* **115**: 320–330.

Hansen AM, Santer B. 1995. The influence of food resources on the development, survival and reproduction of the two cyclopoid copepods: *Cyclops vicinus* and *Mesocyclops leuckarti*. *Journal of Plankton Research* **17**: 631–646.

Hanson JM, Peters RH. 1984. Empirical prediction of crustacean zooplankton biomass and profundal macrobenthos biomass in lakes. *Canadian Journal of Fisheries and Aquatic Sciences* **41**: 439–445.

Hart RC. 1988. Zooplankton feeding rates in relation to suspended sediment content: potential influences on community structure in a turbid reservoir. *Freshwater Biology* **19**: 123–139.

Huggett JA. 2001. Reproductive response of the copepods Calanoides carinatus and Calanus agulhensis to varying periods of starvation in the southern Benguela upwelling region. *Journal of Plankton Research* **23**: 1061–1071.

Jack JD, Gilbert JJ. 1993. Susceptibilities of different-sized ciliates to direct supression by small and large cladocerans. *Freshwater Biology* **29**: 19–29.

Jack JD, Thorp TH. 2000. Effects of the benthic suspension feeder *Dreissena polymorpha* on zooplankton in a large river. *Freshwater Biology* **44**: 569–579.

Jack JD, Thorp JH. 2002. Impacts of fish predation on an Ohio River zooplankton community. *Journal of Plankton Research* **24**: 119–127.

Jack JD, Toalson SA, Gilbert JJ. 1993. The effect of clays on a freshwater plankton community: an enclosure experiment. *Archiv fur Hydrobiologie* **127**: 257–270.

King CH, Sanders RW, Shotts EB, Porter KG. 1991. Differential survival of bacteria ingested by zooplankton from a stratified eutrophic lake. *Limnology and Oceanography* **36**: 829–845.

Kreutzer C, Lampert W. 1999. Exploitative competition in differently sized Daphnia species: a mechanistic explanation. *Ecology* **80**: 2348–2357.

Lampert W. 1987. Feeding and nutrition in Daphnia in Daphnia. *Memorie dell 'Instituto Italiano di Idrobiologia* **45**: 143–192.

Lampert W, Schober U. 1980. The importance of 'threshold' food concentrations. In *Evolution and Ecology of Zooplankton Communities*, Kerfoot WC (ed.). University Press of New England: Hanover, NH; 264–267.

Makino W, Ban S. 2000. Response of life history traits to food conditions in a cyclopoid copepod from an oligotrophic environment. *Limnology and Oceanography* **45**: 396–407.

Marneffe Y, Descy JP, Thome JP. 1996. The zooplankton of the lower river Meuse, Belgium: seasonal changes and impact of industrial and municipal discharges. *Hydrobiologia* **319**: 1–13.

Muller-Navarra DC. 1995a. Biochemical vs. mineral limitation in *Daphnia*. *Limnology and Oceanography* **40**: 1209–1214.

Muller-Navarra DC. 1995b. Evidence that a highly unsaturated fatty acid limits *Daphnia* growth in nature. *Archiv fur Hydrobiologie* **132**: 297–307.

Muller-Solger AB, Jassby AD, Muller-Navara DC. 2002. Nutritional quality of food resources for zooplankton (*Daphnia*) in a tidal freshwater system (Sacramento—San Joaquin River Delta). *Limnology and Oceanography* **47**: 1468–1476.

Osenberg CW, Mittelbach GG. 1996. The relative importance of resource limitation and predator limitation in food chains. In *Food Webs: Integration of Patterns and Dynamics*, Polis GA *et al.* (eds). Chapman and Hall: New York; 134–148.

Pace ML, Findlay SEG, Lints D. 1992. Zooplankton in advective environments: the Hudson River community and a comparative analysis. *Canadian Journal of Fisheries and Aquatic Sciences* **49**: 1060–1069.

Rothhaupt KO. 1990. Resource competition of herbivorous zooplankton: a review of approaches and perspectives. *Archiv fur Hydrobiologie* **118**: 1–29.

Sanders RW, Williamson CE, Stutzman PL, Moeller RE, Goulden CE, Aoki-Goldsmith R. 1996. Reproductive success of 'herbivorous' zooplankton fed algal and nonalgal food resources. *Limnology and Oceanography* **41**: 1295–1305.

Santer B, Lampert W. 1995. Summer diapause in cyclopoid copepods: adaptive response to a food bottleneck? *Journal of Animal Ecology* **64**: 600–613.

Saunders JF, Lewis WM. 1988. Zooplankton abundance in the Caura River, Venezuela. *Biotropica* **20**: 206–214.

Sellers T, Bukaveckas PA. 2003. Phytoplankton production in a large, regulated river: a modeling and mass balance assessment. *Limnology and Oceanography* **48**: 1476–1487.

Spencer CN, Ellis BK. 1998. Role of nutrients and zooplankton in regulation of phytoplankton in Flathead Lake (Montana, U.S.A.), a large oligotrophic lake. *Freshwater Biology* **39**: 755–763.

Sterner RW, Schulz KL. 1998. Zooplankton nutrition: recent progress and a reality check. *Aquatic Ecology* **32**: 261–279.

Sterner RW, Hagemeier DD, Smith WL, Smith RF. 1993. Phytoplankton nutrient limitation and food quality for *Daphnia Limnology and Oceanography* **38**: 857–871.

Sterner RW, Clasen J, Lampert W, Weisse T. 1998. Carbon: phosphorus stiochiometry and food chain production. *Ecology Letters* **1**: 146–150.

Stoecker DK, Capuzzo JM. 1990. Predation on protozoa: its importance to zooplankton. *Journal of Plankton Research* **12**: 891–908.

Tessier AJ, Goulden CE. 1982. Estimating food limitation in cladoceran populations. *Limnology and Oceanography* **27**: 707–717.

Tessier AJ, Goulden CE. 1987. Cladoceran juvenile growth. *Limnology and Oceanography* **32**: 680–686.

Thorp JH, Black AR, Haag KH, Wehr JD. 1994. Zooplankton assemblages in the Ohio River: seasonal, tributary, and navigation dam effects. *Canadian Journal of Fisheries and Aquatic Sciences* **51**: 1634–1643.

Urabe J, Watanabe Y. 1992. Possibility of N or P limitation for planktonic cladocerans: an experimental test. *Limnology and Oceanography* **37**: 244–251.

Vanni MJ. 1987. Effects of food availability and fish predation on a zooplankton community. *Ecological Monographs* **57**: 61–88.

Vanni MJ, Temte J. 1990. Seasonal patterns of grazing and nutrient limitation of phytoplankton in a eutrophic lake. *Limnology and Oceanography* **35**: 697–709.

Viroux L. 1999. Zooplankton distribution in flowing waters and its implications for sampling: case studies in the River Meuse (Belgium) and the River Moselle (France, Luxembourg). *Journal of Plankton Research* **21**: 1231–1248.

Wehr JD, Thorp JH. 1997. Effects of navigation dams, tributaries and littoral zones on phytoplankton of the Ohio River. *Canadian Journal of Fisheries and Aquatic Sciences* **54**: 378–395.

Williamson CW. 1980. The predatory behavior of Mesocyclops edax: predator preferences, prey defenses, and starvation-induced changes. *Limnology and Oceanography* **25**: 903–909.

Williamson CE, Sanders RW, Moeller RE, Stutzman PL. 1996. Utilization of subsurface food resources for zooplankton production: implications for diel vertical migration theory. *Limnology and Oceanography* **41**: 224–233.