

Mixotrophic versus photoautotrophic specialist algae as food for zooplankton: The light:nutrient hypothesis might not hold for mixotrophs

Alexis Katechakis,¹ Tanja Haseneder, Robert Kling, and Herwig Stibor

Ludwigs-Maximilians-University, Department Biology II—Aquatic Ecology, Großhadener Str. 2,
82152 Planegg-Martinsried, Germany

Abstract

We reared mixotrophic (*Ochromonas tuberculata* and *Cryptomonas* sp.) and photoautotrophic specialist algae (*Scenedesmus obliquus*) at different light:phosphorus supplies and compared their effects as food for zooplankton (*Daphnia magna*). According to the light:nutrient hypothesis (LNH), biomass and nutrient stoichiometry of phototrophic specialists depend strongly on light:phosphorus supplies. If this is true, herbivore growth and fecundity should be limited by food quantity at low light intensities and by stoichiometric food quality at high light intensities. In turn, phosphorus fertilization should cause a transition from limitation by food quality to limitation by food quantity. In contrast to the LNH, biomass and nutrient stoichiometry of mixotrophs were almost unaffected by alterations in the supply of light and dissolved nutrients. Bacterial counts indicate that mixotrophs compensated for light or phosphorus deficiency by heterotrophic nutrition. Compared to phototrophic specialists, a diet of *Cryptomonas* sp. therefore enabled a similar or higher and more stable secondary production at most light:nutrient supplies. *O. tuberculata*, however, appeared to be toxic. Our results indicate that mixotrophs might have a balancing effect on variations in transfer efficiency caused by perturbations to light and nutrient supplies.

Global perturbations to solar insolation and to biogeochemical cycles are altering the inputs of light and nutrients to ecosystems, thus influencing primary and secondary production (e.g., Lindroth et al. 1993; Schindler 1998). Studies in this context have traditionally focused on the role of food quantity and have suggested that high primary production and biomass should yield high secondary production and biomass and therefore potentially also sustain a higher biomass of top predators (e.g., Begon et al. 1996). More recently, however, it has become increasingly clear that food quality in terms of elemental nutrient composition may be a key determinant with regard to trophic efficiency in food webs (e.g., Hessen 1992; Gulati and DeMott 1997) and that food chain production varies with the degree of mismatch between the carbon:nutrient ratios of autotrophs and their consumers (e.g., Sterner et al. 1998; Hessen and Faafeng 2000).

A compilation of stoichiometric data in terrestrial and aquatic food webs indicates that carbon:nutrient ratios of autotrophs are suboptimal for herbivores in many ecosystems (Elser et al. 2000). In aquatic systems, for example, the mismatch between the cellular carbon:phosphorus (C:P) ratios of algae and their consumers can be very high. While the C:P ratios of phytoplankton may range from 100 to ~1,000 (e.g., Gächter and Bloesch 1985; Elser and Hassett 1994), C:P ratios of herbivorous zooplankton are typically much smaller and less variable. The total range of body C:P in crustacean zooplankton taxa studied to date varies from

50 to 200 (e.g., Andersen and Hessen 1991), showing limited intraspecific variability (strong physiological homeostasis), with most variation associated with differences among species. Hence, algae with low C:P ratios are rated a better food quality for herbivorous mesozooplankton than algae with high C:P ratios (e.g., Sterner et al. 1998; Hessen and Faafeng 2000; Makino et al. 2002).

High C:P ratios in autotrophs have been attributed to a joint effect of high light intensities and low P supplies. At high light:nutrient ratios, higher primary production may, therefore, paradoxically cause lower zooplankton production as a result of a reduction in transfer efficiency caused by low food quality. On the other hand, at low light supply, food quantity may limit secondary production. These relationships have been summarized in the light:nutrient hypothesis (LNH) by Sterner et al. (1997) and seem well supported by recent theoretical (Andersen 1997; Loladze et al. 2000) and empirical studies (e.g., Urabe and Sterner 1996; Hessen et al. 2002; Urabe et al. 2002a).

The LNH is based on the assumption that photoautotrophic specialists constitute the base of the food chain. To date, the role of mixotrophic organisms has been neglected within this context. Mixotrophic algae combine phototrophic and phagotrophic production dependent on the availability of light and nutrients (e.g., Sibbald and Albright 1991; Raven 1997) and have been found in several classes of phytoplankton (e.g., Jones 2000). Mixotrophic algae are widespread in pelagic ecosystems and, for the following reasons, mixotrophs can be expected to have different effects on the algae-herbivore interface than predicted by the LNH. First, the ability to use alternative production pathways indicates that the stoichiometric composition of mixotrophs might be less affected by alterations in the supply with light and dissolved nutrients than the stoichiometry of phototrophic specialists. Second, potentially limiting nutrients, particularly P, are often several orders of magnitude more concentrated in the biomass of food organisms of mixotrophs (bacteria and bac-

¹ Corresponding author (alexis.katechakis@gmx.net).

Acknowledgments

We thank Sebastian Diehl for helpful comments on an earlier draft of the manuscript and Angelika Wild and Achim Weigert for technical assistance. We also thank Dag Hessen and two anonymous reviewers for their most helpful comments toward the final version of the article. Experiments were supported by the Deutsche Forschungsgemeinschaft, DFG (project ST180/ 1-1, 1-2).

terial-sized particulate matter) than in the dissolved phase (e.g., Vadstein 2000). C:P ratios of bacteria are indeed relatively low and constrained (~ 10 –500) (e.g., Makino 2003). Heterotrophic nutrition might therefore entail low C:P ratios in mixotrophs, making them a nutrient-rich food source for herbivores at high environmental light:nutrient ratios as well. Third, mixotrophic organisms may dominate phytoplankton biomass under low light conditions and in low-nutrient environments (e.g., Riemann et al. 1995) (i.e., exactly in those environments where, according to the LNH, secondary production may be restricted by autotroph food quantity and quality, respectively).

Based on these expectations, we formulated the following two hypotheses and tested them experimentally in the laboratory: (1) The C:P ratios of mixotrophs are lower and much less dependent on external light:nutrient supply ratios than the C:P ratios of purely phototrophic algae, and (2) compared to photoautotrophic specialists, mixotrophs are a superior food source for herbivorous zooplankton at high light:nutrient supply ratios and in low light environments.

Material and methods

Experimental setup—Experiments were performed in semicontinuous two-stage chemostats consisting of 600-ml tissue culture flasks. We filled all chemostats with sterile-filtered, autoclaved water from oligotrophic Lake Langbür-gener See (South Bavaria, Germany). Chemostats were placed in a climate chamber at a temperature of $20^\circ \pm 1^\circ\text{C}$ and illuminated with fluorescent bulbs (Osram light code 77 and Osram cool-white 21–840, 36 W each, in equal parts, Osram) in a 16:8h light:dark rhythm.

In the first stages, we inoculated equivalent biovolumes (measured with a Casy 1 TTC particle counter, Schärfe Systems) of purely phototrophic *Scenedesmus obliquus* (SAG culture 276-3a, SAG culture collection, Göttingen, Germany), mixotrophic *Ochromonas tuberculata* (CCAP culture 933/27, CCAP culture collection, Ambleside, U.K.), mixotrophic *Cryptomonas* sp. (SAG 19.80), or a mixture of *S. obliquus* and *O. tuberculata*. Algal stock cultures were non-axenic. *O. tuberculata* and *Cryptomonas* sp. cover the two extremes of mixotrophic strategies documented in literature (e.g., Jones 2000). *Ochromonas* is a predominantly heterotrophic mixotroph (e.g., Sibbald and Albright 1991) that uses phototrophy only when prey concentrations limit heterotrophic growth (Rothhaupt 1996b). *Cryptomonas* is rated primarily as a phototroph, ingesting prey only at low rates, for example to meet requirements for cell maintenance during prolonged dark periods or for the uptake of essential organic carbon compounds, such as vitamins (e.g., Sanders and Porter 1988; Tranvik et al. 1989).

In the second-stage flasks, three neonates each of *Daphnia magna* were fed with material from the first-stage flasks. Neonates were born within 12 h before setting up the experiment, gathered from mothers established in stock cultures at our institute.

We conducted two series of experiments: One series in a light gradient (60 – $345 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$) at constant nutrient supply (N:P was 16:1 in molar units, with P = 0.5

$\mu\text{mol L}^{-1}$), and a second series in a P gradient (N:P = 16:1 to 16:20, with P = 0.5 to $10 \mu\text{mol L}^{-1}$) at constant light conditions ($345 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$). Light intensity in the first series was adjusted by shading the chemostats with layers of greaseproof paper. We established seven light intensities: 60, 90, 125, 160, 205, 265, and $345 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ (measured with a LI-1400 DataLogger, Li-Cor). P concentrations in the second series were adjusted by adding the desired concentration of P as NaH_2PO_4 . We used seven P concentrations: 0.5, 1, 2.5, 4, 5, 7.5, and $10 \mu\text{mol L}^{-1}$. N was added as NaNO_3 and NH_4Cl in equal parts to all treatments. All chemostats received a mixture of vitamins ($0.02 \mu\text{mol L}^{-1}$ vitamins H and B, $0.004 \mu\text{mol L}^{-1}$ vitamin B₁₂, final concentration) and supplementary nutrients (Na_2EDTA , FeSO_4 , MnCl_2 , $1 \mu\text{mol L}^{-1}$ final concentration each). To maintain a sufficient stock of bacterial biomass for potential phagotrophy by mixotrophs, chemostats received additionally $0.1 \mu\text{g glucose ml}^{-1}$. Every light:nutrient treatment was replicated in triplicate for every tested algal species.

After inoculation, first-stage chemostats with algal monocultures were allowed to grow for 7 d to attain a sufficient food concentration of at least 10^4 cells ml^{-1} (measured with a Casy 1 TTC particle counter) and to stabilize food quality before starting *Daphnia* performance experiments. Chemostats with mixtures of *S. obliquus* and *O. tuberculata* were run for 3 weeks before experiments started to enable equilibrium of both taxa. From the time of inoculation on, every 2 d, 200 ml of the culture suspension were replaced by fresh medium (sterile-filtered, autoclaved lake water, supplemented with nutrients as described above) and transferred to second-stage flasks, yielding an average dilution rate of the medium of $D = 0.17 \text{ d}^{-1}$. *D. magna* individuals remained undiluted. Experiments were terminated when all cladocerans in a chemostat had produced eggs or had reached adulthood (after 6–10 d). Adulthood was judged with regard to instar numbers and size of abdominal appendages (Stibor and Lampert 1993). During the experimental period, we inspected by eye whether grazers were intact several times a day by controlling their swimming behavior in the chemostats. To homogenize the culture suspension, we also mixed the vessels gently on this occasion.

Sample preparation and analysis—All parameters were determined once at the beginning and once at the end of the experiment.

Food quantity and quality—For the examination of algal biomass and C:P stoichiometry, we filtered known aliquots of the culture suspensions from first-stage chemostats on precombusted Schleicher and Schuell GF6 glass-fiber filters. Filters were dried in an oven at 60°C and stored in a desiccator (C) or freezer (P) until analysis. C content was determined with a C-Mat 500 carbon analyzer (Juwel). Algal P concentration was determined by spectrophotometric methods (acid molybdenum-blue technique) after oxidation by persulfate (APHA 1992).

To ascertain the proportions of *S. obliquus* and *O. tuberculata* in the mixed chemostats, we fixed samples with Lugol's iodine (5 g I₂ + 10 g KI in 100 ml distilled water, 1% final concentration), settled samples in Utermöhl cham-

bers (Hydrobios), and counted them in an inverted microscope (Leica DMIL, Leica) according to the method of Utermöhl (1958) and Lund et al. (1958).

To assess *Daphnia magna* performance, we determined the individuals' somatic growth rates and standardized egg numbers. Somatic growth rates, g (per day), were estimated by measuring the rate of change of body lengths, L (in mm), under a dissecting microscope and converting body lengths to body mass, ω (in μg), using conventional length–mass regressions, thus (Stibor 2002):

$$g = (\ln \omega_{\text{adult}} - \ln \omega_{\text{neonate}})/t,$$

where t is the experimental duration in days, and $\omega = 12.58 L^{2.41}$. Standardized egg number was calculated as the number of produced eggs per adult female divided by individual body length.

Bacterial net growth—To determine possible impacts on the bacterial guild in the chemostats, we preserved samples with 0.2- μm -filtered formaldehyde (2% final concentration), stained them with 4,6-diamidino-2-phenylindol (DAPI; 2 μg DAPI ml^{-1} , sample final concentration), after Porter and Feig (1980), and enumerated samples at $\times 1,000$ magnification using an epifluorescence microscope (Zeiss Axioplan, Carl Zeiss). Bacterial net growth rate, r (per day), was calculated as

$$r = (\ln C_1 - \ln C_0)/t$$

where C_1 and C_0 are the bacterial abundances (cells ml^{-1}) at the end and at the beginning of the experiment and where t is the experimental term in days.

Results

In the following, we will initially treat the monospecific cultures and subsequently the mixed treatments. Detailed results are presented first for light manipulations and then for P fertilization.

Food quantity and quality—Purely phototrophic algae reached higher maximum biomasses than mixotrophs in both the light and the P gradient, but generally provided a lower food quality in terms of C:P ratios. Moreover, both parameters were much more affected by changing light or nutrient supplies in phototrophic specialists than in mixotrophs.

The biomass and the C:P ratio of phototrophic specialist *Scenedesmus obliquus* increased considerably with increasing light supply. In contrast, mixotroph biomasses and C:P ratios remained largely constant across the light gradient (Fig. 1A,B, left panel; Table 1). Biomasses and C:P ratios of purely phototrophic algae were higher than values for mixotrophs throughout the light gradient. Only at the lowest light intensity of 60 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ did biomasses and C:P ratios not differ significantly between purely phototrophic and mixotrophic species (two-way analyses of variance [ANOVAs]; Table 2, and post-hoc Tukey-test analyses, $p < 0.01$).

While the biomass of *Cryptomonas* sp. increased slightly along the entire P supply range, P fertilization left biomasses of *S. obliquus* and *O. tuberculata* largely unaffected, except

for an initial increase in *S. obliquus* biomass, from the lowest to the second lowest P concentration (Fig. 1A, right panel; Table 1). Similarly, P fertilization influenced mixotroph C:P ratios only at the lowest P concentrations. In contrast, P fertilization severely affected C:P ratios of phototrophic specialists across the entire P gradient, leading to a more than sixfold decrease from ~ 800 to ~ 120 (Fig. 1B, right panel; Table 1). Similar to the light gradient, biomasses of phototrophic specialists were higher throughout the P gradient than values for mixotrophs (two-way ANOVAs, Table 2, and post-hoc Tukey-test analyses, $p < 0.01$). C:P ratios of phototrophic specialists approached mixotrophic C:P ratios with increasing P supply. At P supplies $\geq 4 \mu\text{mol L}^{-1}$ C:P ratios did not differ significantly between purely phototrophic and mixotrophic species (two-way ANOVAs, Table 2, and post-hoc Tukey-test analyses, $p < 0.01$).

Chemostats with mixtures of *S. obliquus* and *O. tuberculata* developed very similarly to chemostats containing only phototrophic specialists (Fig. 1A,B). Only at low light intensities of $\leq 120 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ did biomasses and C:P ratios of mixed cultures develop similarly to treatments with mixotrophs (Fig. 1A,B, left panel; two-way ANOVAs, Table 2, and post-hoc Tukey-test analyses, $p < 0.05$). This is because *S. obliquus* generally dominated mixed chemostats (Fig. 2), while *O. tuberculata* reached appreciable proportions of total biomass only at low light intensities of $\leq 120 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ (between $42.0\% \pm 7.9\%$ standard error [SE] of the means and $59.9\% \pm 6.4\%$). Its proportion of total biomass decreased continuously with increasing light supply. At light intensities $> 120 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ and throughout the P gradient, *O. tuberculata* was almost completely outcompeted by *S. obliquus* (relative biomass of *O. tuberculata* of $< 5\%$ and $< 3\%$, respectively).

Daphnia magna performance—ANOVAs indicate that growth and fecundity of *D. magna* differed for different food types, for different treatments (light supply or P supply), and for interactions between food type and treatments (Table 3). Animals feeding on *O. tuberculata* suffered high mortality during the course of the experiment and died out before they could reproduce. Therefore, they are not further treated here. Growth rates and reproduction were significantly affected by food quantity and quality (Fig. 1C,D).

In the light gradient, *D. magna* juveniles grew more rapidly and reproduced more robustly feeding on the mixotroph *Cryptomonas* sp. relative to the phototrophic specialist diet (two-way ANOVAs, Table 3, and post-hoc Tukey-test analyses, $p < 0.01$). At most light intensities, somatic growth and egg production were higher on *Cryptomonas* sp. than on *S. obliquus* (Fig. 1C,D, left panel), although purely phototrophs provided higher food quantities (Fig. 1A, left panel). This was due to a better food quality of *Cryptomonas* sp., gauged in terms of C:P ratios (Fig. 1B, left panel). Moreover, the constant mixotroph food characteristics across the light gradient (Fig. 1A,B, left panel) produced relatively stable *D. magna* responses. In contrast, somatic growth and egg production were more affected by changing light conditions feeding on *S. obliquus*, with highest performances at intermediate light intensities of between 90 and 160 $\mu\text{mol quanta}$

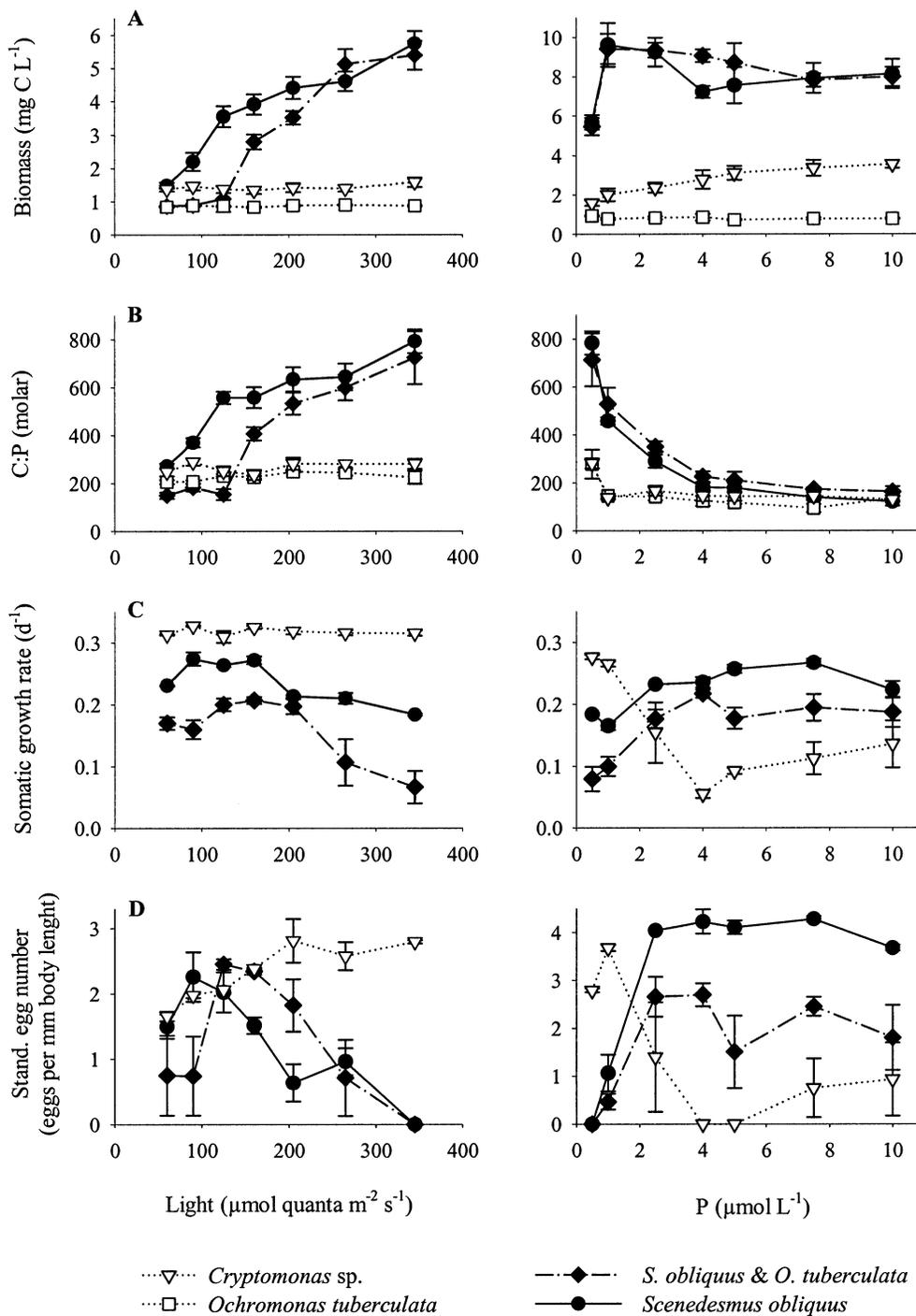


Fig. 1. (A and B) Biomasses (mg C L⁻¹) and C:P ratios (molar) of phototrophic specialist algae (*Scenedesmus obliquus*), mixotrophic algae (*Ochromonas tuberculata*, *Cryptomonas* sp.), and mixtures of phototrophic specialist and mixotrophic algae (*S. obliquus* and *O. tuberculata*) in a light gradient at constant nutrient supply (left panel) and in a P gradient at constant light conditions (right panel). Data points are means of 3×2 (replicates \times measurements) = 6 samples (see Materials and methods for details). Error bars indicate \pm SE of the means. (C and D) Average somatic growth rates (d⁻¹) and average standardized egg numbers (eggs per mm body length) of *Daphnia magna* feeding on phototrophic specialist algae, mixotrophic algae, or mixtures of phototrophic specialist and mixotrophic algae. Data points are means of three replicates. Error bars indicate \pm SE of the means.

Table 1. Regression coefficients (linear: $y = y_0 + ax$, or exponential decay: $y = y_0 + a \exp^{-bx}$) of biomass (mg C L^{-1}) and C:P ratio (molar) development for *Scenedesmus obliquus*, *Ochromonas tuberculata*, *Cryptomonas* sp., and a mixture of *S. obliquus* and *O. tuberculata* in a light gradient at constant nutrient supply and in a P gradient at constant light conditions (see Materials and methods for details). Sample size for each species in each gradient: $3 \times 2 \times 7$ (replicates \times measurements \times treatments) = 42. Values in brackets are \pm SE of the means.

Species	Light gradient				P gradient							
	y_0	a	r^2	$F_{1,40}$	p	df	y_0	a	b	r^2	F	p
<i>Scenedesmus obliquus</i>												
Biomass (mg C L^{-1})	1.3 (0.3)	0.01 (0.001)	0.70	93.2	<0.0001	1	7.8 (0.6)	0.02 (0.1)	—	0.001	0.1	0.83
C:P (molar)	256.3 (38.8)	1.6 (0.2)	0.64	71.1	<0.0001	2	157.7 (15.9)	1025.1 (104.9)	1.1 (0.2)	0.91	192.8	<0.0001
<i>Ochromonas tuberculata</i>												
Biomass (mg C L^{-1})	0.9 (0.02)	0.0001 (0.0001)	0.02	1.0	0.34	1	0.9 (0.1)	-0.01 (0.01)	—	0.03	1.1	0.30
C:P (molar)	209.7 (11.5)	0.1 (0.1)	0.06	0.3	0.58	1	140.3 (12.9)	-2.9 (2.4)	—	0.35	1.4	0.24
<i>Cryptomonas</i> sp.												
Biomass (mg C L^{-1})	1.3 (0.1)	0.001 (0.001)	0.03	1.1	0.30	1	2.0 (0.2)	0.2 (0.04)	—	0.31	18.0	0.0001
C:P (molar)	249.8 (17.5)	0.1 (0.1)	0.03	1.1	0.29	1	153.5 (12.6)	-1.8 (2.6)	—	0.02	0.7	0.41
<i>S. obliquus</i> + <i>O. tuberculata</i>												
Biomass (mg C L^{-1})	-0.9 (0.3)	0.03 (0.002)	0.87	273.3	<0.0001	1	13.9 (0.4)	-0.04 (0.1)	—	0.01	0.4	0.55
C:P (molar)	-18.5 (50.1)	2.8 (0.3)	0.76	126.1	<0.0001	2	245.7 (48.6)	716.2 (55.8)	0.4 (0.1)	0.81	82.3	<0.0001

$\text{m}^{-2} \text{s}^{-1}$ (Fig. 1C,D, left panel; two-way ANOVAs, Table 3, and post-hoc Tukey-test analyses, $p < 0.05$).

Results in the P gradient were not as clear. At low P concentrations of $\leq 1 \mu\text{mol L}^{-1}$, *D. magna* growth and fecundity were higher feeding on *Cryptomonas* sp. than on *S. obliquus* (Fig. 1C,D, right panel). With increasing P supply, phototrophic specialists provided the better food source (Fig. 1C,D, right panel; two-way ANOVAs, Table 3, and post-hoc Tukey-test analyses, $p < 0.01$). This is because at P supplies of $> 2.5 \mu\text{mol L}^{-1}$, *S. obliquus* attained food qualities similar to *Cryptomonas* sp. (Fig. 1B, right panel), but simultaneously provided higher food quantities (Fig. 1A, right panel). Although the investigated food characteristics of *Cryptomonas* sp. remained relatively constant across the P gradient (Fig. 1A, B, right panel), *D. magna* performance declined with rising P supply (Fig. 1C,D, right panel), a result that is different from the observations made in the light gradient. As was the case with the light gradient, somatic growth rates and egg production attained on *S. obliquus* were highest at intermediate P supplies from 5 to $7.5 \mu\text{mol L}^{-1}$ and from 2.5 to $7.5 \mu\text{mol L}^{-1}$, respectively (Fig. 1C,D, right panel; two-way ANOVAs, Table 3, and post-hoc Tukey-test analyses, $p < 0.05$).

As *S. obliquus* dominated almost all chemostats with mixtures of *S. obliquus* and *O. tuberculata* (Fig. 2), mixed diets and phototrophic specialist diets caused similar *D. magna* responses in both gradients (Fig. 1C,D). However, cladocerans usually grew and reproduced better on the pure *S. obliquus* diet, although the food characteristics of both kinds of diets were similar (Fig. 1A,B). Despite generally low relative biomasses, *O. tuberculata* may have had a detrimental effect on *D. magna* in the mixed diets.

Bacterial net growth—Two-way ANOVAs indicate that bacterial net growth rates differed for different alga species, but not for different treatments (light supply or P supply) (Table 4). Calculations of bacterial net growth rates are based on the bacterial abundances shown in Fig. 3B–D.

In the light gradient, bacterial net growth rates were influenced differently in the presence of phototrophic specialists or mixotrophic algae (two-way ANOVA, Table 4, and post-hoc Tukey-test analyses, $p < 0.05$). Mean bacterial net growth rates were negative in chemostats with *O. tuberculata* (-0.06 ± 0.02 SE of the means d^{-1}) or *Cryptomonas* sp. ($-0.05 \pm 0.01 \text{d}^{-1}$), but positive in chemostats with *S. obliquus* ($0.05 \pm 0.01 \text{d}^{-1}$). Linear regressions indicate that bacterial net growth rates increased slightly with light intensity in chemostats with *O. tuberculata* ($y = -0.15 + 0.0005x$, $r^2 = 0.91$, $F_{1,5} = 51.3$, $p < 0.001$), but remained constant throughout the light gradient in treatments with *Cryptomonas* sp. ($p = 0.75$) or *S. obliquus* ($p = 0.92$) (Fig. 3A).

In the P gradient, bacterial net growth rates were influenced differently in the presence of *O. tuberculata* than with *S. obliquus* or *Cryptomonas* sp. (two-way ANOVA, Table 4, and post-hoc Tukey-test analyses, $p < 0.05$). Mean bacterial net growth rates were highest under the influence of *O. tuberculata* ($0.24 \pm 0.04 \text{d}^{-1}$), $0.06 \pm 0.03 \text{d}^{-1}$ with *S. obliquus*, equivalent to the light gradient, and $0.01 \pm 0.01 \text{d}^{-1}$ in chemostats with *Cryptomonas* sp. Bacterial net growth rates

Table 2. Results of analyses of variance (ANOVAs) for biomass (mg C L⁻¹) and C:P ratio (molar) development for *Scenedesmus obliquus*, *Ochromonas tuberculata*, *Cryptomonas* sp., and a mixture of *S. obliquus* and *O. tuberculata* in a light gradient at constant nutrient supply and in a P gradient at constant light conditions (see Materials and methods for details). Sample size in each gradient: 3 × 2 × 7 × 4 (replicates × measurements × treatments × species) = 168.

	Light gradient					P gradient			
	df	Biomass (mg C L ⁻¹)		C:P ratio (molar)		Biomass (mg C L ⁻¹)		C:P ratio (molar)	
		F	p	F	p	F	p	F	p
Species	3	379.6	<0.001	117.8	<0.001	793.9	<0.001	123.7	<0.001
Treatment (light or P)	6	106.8	<0.001	53.3	<0.001	15.6	<0.001	62.5	<0.001
Species × treatment	18	41.8	<0.001	15.7	<0.001	5.9	<0.001	7.9	<0.001

were positively correlated to P supply under the influence of *O. tuberculata* ($y = 0.14 + 0.0002x$, $r^2 = 0.52$, $F_{1,5} = 5.5$, $p < 0.1$) and *Cryptomonas* sp. ($y = -0.03 + 0.01x$, $r^2 = 0.67$, $F_{1,5} = 10.0$, $p < 0.05$), but stable in chemostats with *S. obliquus* ($p = 0.89$) (Fig. 3A).

Discussion

Mixotrophs expressed low and remarkably stable C:P ratios between 100 and 300, despite huge variations in absolute and relative light and nutrient supplies. In contrast, C:P ratios of purely phototrophic algae varied between 100 and 800 (Fig. 1B). The response of phototrophic specialist C:P ratios to the manipulations accords well with the LNH (Sterner et al. 1997) and with previous studies, which showed that algal C fixation depends strongly on light intensity, while P acquisition is closely coupled to overall P

supply (e.g., Urabe and Sterner 1996; Makino et al. 2002; Urabe et al. 2002a).

In contrast to the LNH, changes in light or nutrient supply left the nutrient composition of mixotrophs almost unaffected. *O. tuberculata* and *Cryptomonas* sp. probably compensated for light or P deficiency by heterotrophic nutrition. We did not conduct specific grazing experiments with mixotrophs in the present study. Comparisons of bacterial net growth rates, however, indicate that *O. tuberculata* and *Cryptomonas* sp. ingested bacteria at low light and P supplies and that both mixotrophs became increasingly photoautotrophic at sufficiently high light and nutrient supplies (Fig. 3). As a consequence, at high light:nutrient ratios and at low light conditions, mixotrophs contained on average two to three times as much P per unit carbon as phototrophic specialists and fairly closely resembled the C:P ratios commonly found in bacteria (e.g., Makino et al. 2003). Similarly, algal C:P ratios were low and stable in mixed chemostats, as long as *O. tuberculata* contributed substantially to overall biomass. To the extent mixotrophs were suppressed, community C:P ratio increased rapidly, resembling the treatments containing only *Scenedesmus obliquus* (Figs. 1B, 2).

The observation that *O. tuberculata* was suppressed by *S. obliquus* at high light and nutrient levels (Fig. 2) and the finding that mixotrophs generally expressed lower biomasses than purely phototrophic algae (Fig. 1A) can be explained with the energetic costs mixotrophic organisms have to invest in the synthesis and maintenance of both a photosynthetic apparatus and in mechanisms for prey uptake and its subsequent digestion. These energetic costs may lower a mixotroph's resource use efficiency and may lower photosynthetic performance, resulting in a reduced maximum growth rate compared with a phototrophic or heterotrophic specialist. A mixotroph is therefore expected to be inferior if it competes with specialist phototrophs for light or with specialist phagotrophs for prey (e.g., Rothhaupt 1996a; Raven 1997; Jones 2000).

Another expectation that our results support is that at high light:nutrient ratios, as at low light conditions, the mixotroph *Cryptomonas* sp. provided the better food source for herbivore production compared to phototrophic specialists. At high light and nutrient supplies, phototrophic specialists presented the more favorable food (Fig. 1C,D).

In accordance with the LNH and numerous experimental studies (e.g., Urabe and Sterner 1996; Hessen et al. 2002;

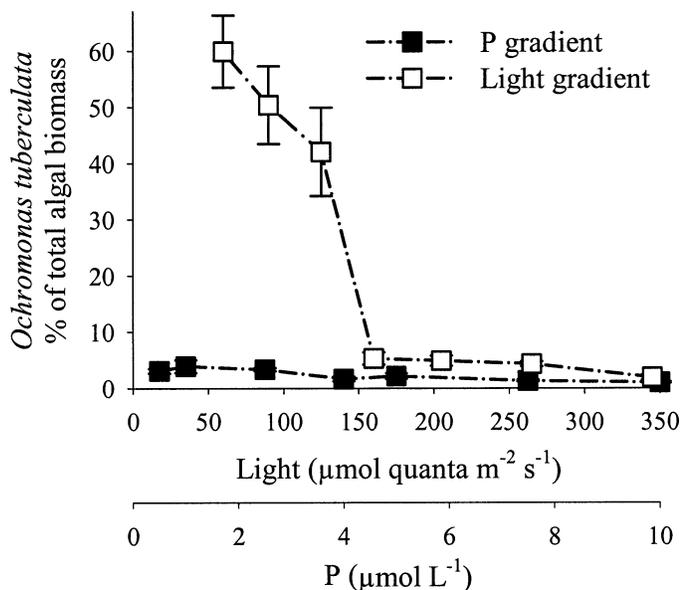


Fig. 2. Proportion of mixotrophic *Ochromonas tuberculata* biomass (%) of total biomass in competition with phototrophic specialist *Scenedesmus obliquus* in a light gradient at constant nutrient supply and in a P gradient at constant light conditions (see Materials and methods for details). Data points are means of 3 × 2 (replicates × measurements) = 6 samples. Error bars indicate ±SE of the means.

Table 3. Results of analyses of variance (ANOVAs) for growth rate and reproduction data of *Daphnia magna* feeding either on phototrophic specialist *Scenedesmus obliquus*, mixotrophic *Cryptomonas* sp., or a mixture of phototrophic specialist *S. obliquus* and mixotrophic *Ochromonas tuberculata* in a light gradient at constant nutrient supply and in a P gradient at constant light conditions (see Materials and methods for details). Sample size in each gradient: $3 \times 7 \times 3$ (replicates \times treatments \times species) = 63.

	Light gradient					P gradient			
	df	Somatic growth rate (d ⁻¹)		Stand and egg number (eggs per mm body length)		Somatic growth rate (d ⁻¹)		Stand and egg number (eggs per mm body length)	
		F	p	F	p	F	p	F	p
Food type	2	313.9	<0.001	20.0	<0.001	19.7	<0.001	22.3	<0.001
Treatment (light or P)	6	12.0	<0.001	4.4	0.001	0.3	0.909	3.8	0.004
Type \times treatment	12	3.8	<0.001	4.3	<0.001	10.7	<0.001	9.5	<0.001

Urabe et al. 2002a), the shifts in algal biomass and elemental stoichiometry of purely phototrophic *S. obliquus* caused a trade-off scenario for herbivores in both gradients. Juvenile somatic growth rates and adult fecundity of *D. magna* were limited by food quantity at low light intensities and were limited by food quality at high light intensities (Fig. 1C,D, left panel). In turn, P fertilization caused a transition from limitation by food quality to limitation by food quantity (Fig. 1C,D, right panel).

In contrast to the LNH, the steady food characteristics of mixotrophic *Cryptomonas* sp. enabled a constant herbivore production throughout the light gradient. It is remarkable that at most light:nutrient supply ratios, herbivore growth and fecundity were considerably higher on the mixotroph diet, although food quantity was on average 40% to 70% lower than in monocultures with purely phototrophs. Obviously, transfer efficiency was primarily triggered by food quality. This is consistent with the results received for specialist phototroph and for mixed treatments in the P gradient and sustains recent findings by other authors (e.g., Boersma and Kreutzer 2002; Urabe et al. 2002a; Acharya et al. 2004). However, the relative influences of food quality and quantity on zooplankton production are still controversial. Our results from pure and mixed cultures containing *S. obliquus* indicate that increases in food quantity stimulate secondary production only at low C:P ratios of <300, whereas improvements in food quality always enhance transfer efficiency (Fig. 1). This outcome is supported by various estimates of stoichiometric food quality thresholds, which show that the transition from C limitation to P limitation in the growth and

reproduction of *D. magna* takes place when food has a C:P ratio of about 250 to 300 (e.g., Hessen 1992; Hessen and Faafeng 2000; Urabe et al. 2002b).

In contrast to our expectations and the LNH, the performance of *D. magna* declined under P fertilization with *Cryptomonas* sp. as food, despite low C:P ratios of around 160 and an increasing food quantity (Fig. 1, right panel). The strongly decreasing responses in *D. magna* growth and fecundity indicate that other factors different from algal nutrient stoichiometry influenced food quality. Recent studies have shown, for example, that biochemical constraints like essential fatty acid deficiency may limit secondary production independent from the elemental nutrient composition of food organisms (e.g., Müller-Navarra et al. 2004), and especially at low C:P ratios of <300 (Boersma 2000; Elser et al. 2001; Urabe et al. 2002a,b). As explained above, we found indications for a decreasing contribution of phagotrophy to overall production at high light and P supplies in both mixotrophs. It is possible that a lowered intake of bacteria had an impairing effect on the food quality of *Cryptomonas* sp. beyond nutrient stoichiometry.

Despite all the positive effects described so far, our experiments imply that mixotrophs may also have detrimental impacts on the algae-herbivore interface. *O. tuberculata* gave some evidence for having a toxic impact on herbivores. *D. magna* suffered high mortality feeding on *O. tuberculata* shortly after the experiments started. We did not test for toxicity, but toxins have been isolated from ochromonads before (e.g., Spiegelstein et al. 1969), and their harmful effect on *D. magna* has been reported by Leeper and Porter (1995).

In conclusion, our study indicates that shifts in light:nutrient supply ratios must not necessarily be accompanied by shifts in seston nutrient stoichiometry as predicted by the LNH, if mixotrophs contribute substantially to overall seston biomass. Consequently, feeding on mixotrophs might also influence secondary production differently than proposed by the LNH. Our results strongly imply that mixotrophs should be considered in the further development of ecological theories that incorporate stoichiometric effects on food web dynamics. Various authors have suggested that variation in seston C:nutrient ratios may regulate the strength of trophic cascades in aquatic ecosystems, with strong cascades occurring at low particulate C:nutrient ratios (e.g., Elser et al. 1998; Hessen and Faafeng 2000). P limitation, supporting

Table 4. Results of analyses of variance (ANOVAs) for bacterial net growth in chemostats with *Scenedesmus obliquus*, *Ochromonas tuberculata*, or *Cryptomonas* sp. in a light gradient at constant nutrient supply and in a P gradient at constant light conditions (see Materials and methods for details). Sample size in each gradient: $1 \times 7 \times 2$ (replicates \times treatments \times species) for mixotrophs + $1 \times 3 \times 1$ for purely phototrophs = 17.

	Bacterial net growth rate (d ⁻¹)				
	df	Light gradient		P gradient	
		F	p	F	p
Species	2	6.5	<0.05	12.2	<0.01
Treatment (light or P)	6	2.3	0.13	0.6	0.71

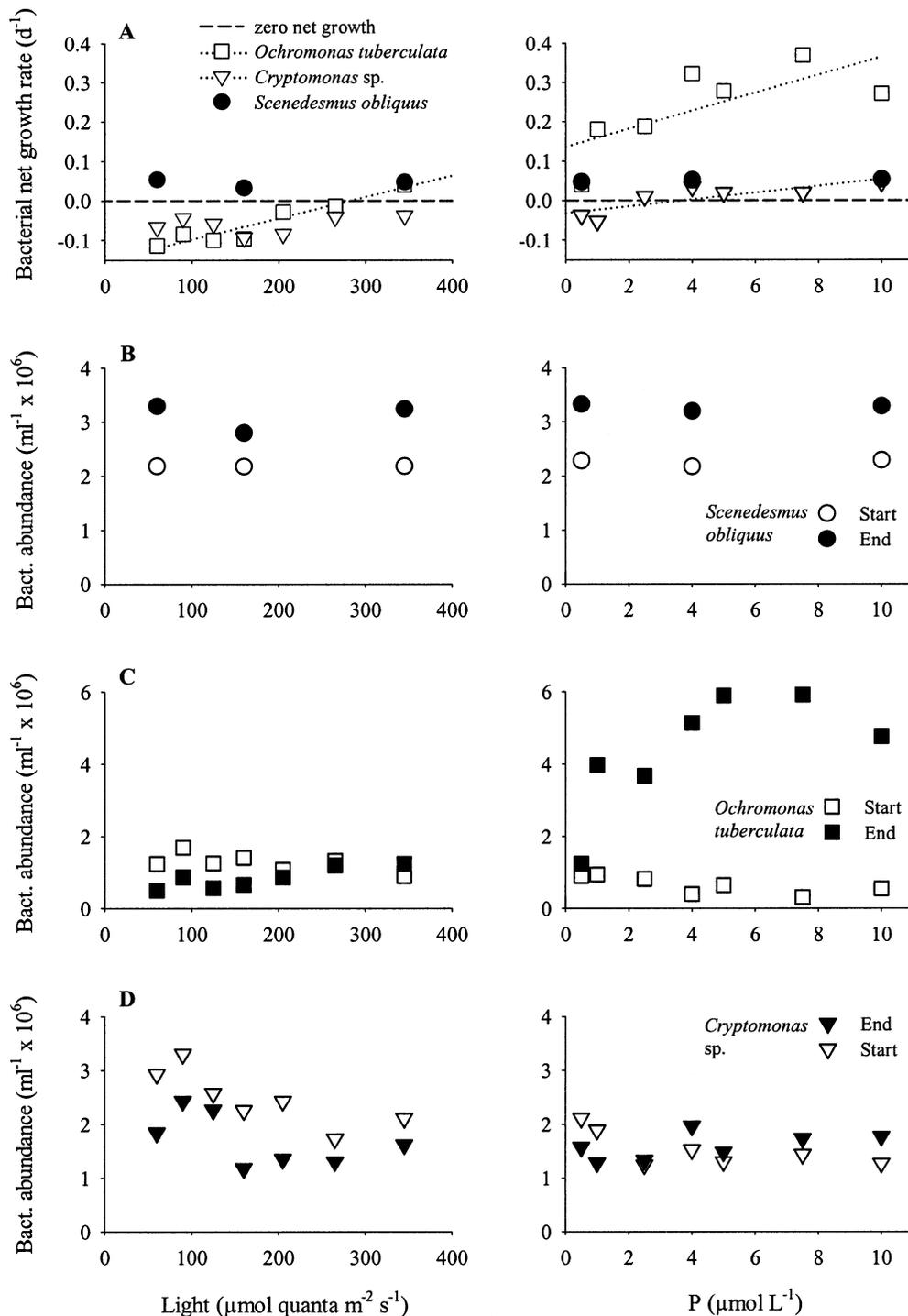


Fig. 3. (A) Bacterial net growth rates (d^{-1}) and (B to D) bacterial abundances ($\text{ml}^{-1} \times 10^6$) in the presence of phototrophic specialist algae (*Scenedesmus obliquus*) or mixotrophic algae (*Ochromonas tuberculata* or *Cryptomonas sp.*) in a light gradient at constant nutrient supply and in a P gradient at constant light conditions (see Materials and methods for details). Bacterial net growth rates were positively correlated to light supply ($y = -0.15 + 0.0005x$, $r^2 = 0.91$, $F_{1,5} = 51.3$, $p < 0.001$) under the influence of *O. tuberculata* and positively correlated to P supply under the influence of *O. tuberculata* ($y = 0.14 + 0.0002x$, $r^2 = 0.52$, $F_{1,5} = 5.5$, $p < 0.1$) and *Cryptomonas sp.* ($y = -0.03 + 0.01x$, $r^2 = 0.67$, $F_{1,5} = 10.0$, $p < 0.05$).

high C:P ratios, seems to be widespread in lakes today (Elser and Hassett 1994; Elser et al. 2000; Hessen and Faafeng 2000) and might be enhanced in the future (Schindler 1998). Sterner et al. (1997, 1998) predicted an increasing decoupling of higher and lower trophic levels in this case. Indeed, the trophic cascade appears to be muted in P-limited lakes (Carpenter and Kitchell 1993; Pace et al. 1999; Makino et al. 2002). Our results indicate that mixotrophs might act as a buffer within this context. Certainly the hypotheses put forward here merit further exploration in the laboratory and in the field.

References

- ACHARYA, K., M. KYLE, AND J. J. ELSER. 2004. Effects of stoichiometric dietary mixing on *Daphnia* growth and reproduction. *Oecologia* **138**: 330–340.
- ANDERSEN, T. 1997. Grazers as sources and sinks for nutrients. Springer.
- , AND D. O. HESSEN. 1991. Carbon, nitrogen, and phosphorus content of freshwater zooplankton. *Limnol. Oceanogr.* **36**: 807–814.
- APHA. 1992. Standard methods for the examination of water and wastewater, 18th ed. American Public Health Association.
- BEGON, M., J. HARPER, AND C. TOWNSEND. 1996. Ecology: Individuals, populations and communities, 3rd ed. Blackwell.
- BOERSMA, M. 2000. The nutritional quality of P-limited algae for *Daphnia*. *Limnol. Oceanogr.* **45**: 1157–1161.
- , AND C. KREUTZER. 2002. Life at the edges: Is food quality really of minor importance at low food quantities? *Ecology* **83**: 2552–2561.
- CARPENTER, S. R., AND J. F. KITCHELL. 1993. The trophic cascade in lakes. Cambridge Univ. Press.
- ELSER, J. J., T. H. CHRZANOWSKI, R. W. STERNER, AND K. H. MILLS. 1998. Stoichiometric constraints on food-web dynamics: A whole lake experiment on the Canadian Shield. *Ecosystems* **1**: 120–136.
- , AND R. P. HASSETT. 1994. A stoichiometric analysis of the zooplankton–phytoplankton interaction in marine and freshwater ecosystems. *Nature* **370**: 211–213.
- , K. HAYAKAWA, AND J. URABE. 2001. Nutrient limitation reduces food quality for zooplankton: *Daphnia* response to seston phosphorus enrichment. *Ecology* **82**: 898–903.
- , AND OTHERS. 2000. Nutritional constraints in terrestrial and freshwater food webs. *Nature* **408**: 578–580.
- GÄCHTER, R., AND J. BLOESCH. 1985. Seasonal and vertical variation in the C:P ratio of suspended and settling seston of lakes. *Hydrobiologia* **128**: 193–200.
- GULATI, R. D., AND W. R. DEMOTT. 1997. The role of food quality for zooplankton: Remarks on the state-of-the-art, perspectives and priorities. *Freshwat. Biol.* **38**: 753–768.
- HESSEN, D. O. 1992. Nutrient element limitation of zooplankton production. *Am. Nat.* **140**: 799–814.
- , AND B. O. FAAFENG. 2000. Elemental ratios in freshwater seston: Implications for community structure and energy transfer in food webs. *Arch. Hydrobiol. Special Issues Adv. Limnol.* **55**: 349–363.
- , P. FÆRØVIG, AND T. ANDERSEN. 2002. Light, nutrients and P:C-ratios in algae: Grazer performance related to food quality and quantity. *Ecology* **83**: 1886–1898.
- JONES, R. I. 2000. Mixotrophy in planktonic protists: An overview. *Freshwat. Biol.* **45**: 219–226.
- LEEPER, D. A., AND K. G. PORTER. 1995. Toxicity of the mixotrophic chrysophyte *Proterioochromonas malhamensis* to the cladoceran *Daphnia magna*. *Arch. Hydrobiol.* **134**: 207–222.
- LINDROTH, R. L., K. K. KINNEY, AND C. L. PLATZ. 1993. Responses of deciduous trees to elevated atmospheric CO₂-productivity, phytochemistry, and insect performance. *Ecology* **74**: 763–777.
- LOLADZE, I., Y. KUANG, AND J. J. ELSER. 2000. Stoichiometry in producer-grazer systems: Linking energy flow and element cycling. *Bull. Math. Biol.* **62**: 1137–1162.
- LUND, J. W. G., C. KIPLING, AND E. D. LECREN. 1958. The inverted microscope method of estimating algal numbers and the statistical basis of estimations by counting. *Hydrobiologia* **11**: 147–170.
- MAKINO, W., J. B. COTNER, R. W. STERNER, AND J. J. ELSER. 2003. Are bacteria more like plants or animals? Growth rate and resource dependence of bacterial C:N:P stoichiometry. *Funct. Ecol.* **17**: 121–130.
- , J. URABE, J. J. ELSER, AND C. YOSHIMIZU. 2002. Evidence of phosphorus-limited individual and population growth of *Daphnia* in a Canadian Shield lake. *Oikos* **96**: 197–205.
- MÜLLER-NAVARRA, D. C., M. T. BRETT, S. PARK, S. CHANDRA, A. P. BALLANTYNE, E. ZORTTA, AND C. R. GOLDMAN. 2004. Unsaturated fatty acid content in seston and tropho-dynamic coupling in lakes. *Nature* **427**: 69–72.
- PACE, M. L., J. J. COLE, AND S. R. CARPENTER. 1999. Trophic cascades revealed in diverse ecosystems. *TREE* **14**: 483–488.
- PORTER, K. G., AND Y. S. FEIG. 1980. The use of DAPI for identifying and counting aquatic microflora. *Limnol. Oceanogr.* **25**: 943–948.
- RAVEN, J. A. 1995. Comparative aspects of chrysophyte nutrition with emphasis on carbon, nitrogen and phosphorus, p. 95–118. *In* C. D. Sandgren, J. P. Smol, and J. Kristiansen [eds.], *Chrysophyte algae: Ecology, phylogeny and development*. Cambridge Univ. Press.
- . 1997. Phagotrophy in phototrophs. *Limnol. Oceanogr.* **42**: 198–205.
- RIEMANN, B., H. HAVSKUM, F. THINGSTAD, AND C. BERNARD. 1995. The role of mixotrophy in pelagic environments, p. 87–114. *In* I. Joint [ed.], *Molecular ecology of aquatic microbes*. NATO ASI Series G 38. Springer.
- ROTHHAUPT, K. O. 1996a. Laboratory experiments with a mixotrophic chrysophyte and obligately phagotrophic and phototrophic competitors. *Ecology* **77**: 716–724.
- . 1996b. Utilizations and substitutable carbon and phosphorus sources by the mixotrophic chrysophyte *Ochromonas* sp. *Ecology* **77**: 706–715.
- SANDERS, R. W., AND K. G. PORTER. 1988. Phagotrophic phytoflagellates. *Adv. Microb. Ecol.* **10**: 167–192.
- SCHINDLER, D. W. 1998. A dim future for boreal waters and landscapes: Cumulative effects of climate warming, stratospheric ozone depletion, acid precipitation and other human activities. *BioScience* **48**: 157–164.
- SIBBALD, M. J., AND L. J. ALBRIGHT. 1991. The influence of light and nutrients on phagotrophy by the mixotrophic nanoflagellate *Ochromonas* sp. *Mar. Microb. Food Webs* **5**: 39–47.
- SPIEGELSTEIN, M., K. REICH, AND F. BERGMANN. 1969. The toxic principals of *Ochromonas* and related chrysomonadina. *Verh. Int. Ver. Limnol.* **17**: 778–783.
- STERNER, R. W., J. CLASEN, W. LAMPERT, AND T. WEISSE. 1998. Carbon: phosphorus stoichiometry and food chain production. *Ecol. Lett.* **1**: 146–150.
- , J. J. ELSER, E. J. FEE, S. J. GUILFORD, AND T. H. CHRZANOWSKI. 1997. The light:nutrient ratio in lakes: The balance of energy and materials affects ecosystem structure and process. *Am. Nat.* **150**: 663–684.

- STIBOR, H. 2002. Yolk protein dynamics and predator released kairomones in *Daphnia magna*. *Ecology* **83**: 362–369.
- , AND W. LAMPERT. 1993. Estimating the size and maturity in field populations of *Daphnia* (Cladocera). *Freshwat. Biol.* **30**: 433–438.
- TRANVIK, L. J., K. G. PORTER, AND J. MCN. SIEBURTH. 1989. Occurrence of bacterivory in *Cryptomonas*, a common freshwater phytoplankter. *Oecologia* **78**: 473–476.
- URABE, J., M. KYLE, W. MAKINO, T. YOSHIDA, T. ANDERSEN, AND J. J. ELSER. 2002a. Reduced light increases herbivore production due to stoichiometric effects of light/nutrient balance. *Ecology* **83**: 619–627.
- , W. MAKINO, K. HAYAKAWA, AND J. J. ELSER. 2002b. Food quality determinants for *Daphnia* growth in P-limited lakes. *Verh. Int. Ver. Limnol.* **28**: 1–6.
- , AND R. W. STERNER. 1996. Regulation of herbivore growth by the balance of light and nutrients. *Proc. Natl. Acad. Sci. USA* **93**: 8465–8469.
- UTERMÖHL, H. 1958. Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. *Mitt. Int. Ver. Theor. Angew. Limnol.* **9**: 1–38.
- VADSTEIN, O. 2000. Heterotrophic planktonic bacteria and cycling phosphorus. Phosphorus requirements, competitive ability and food web interactions, p. 115–167. *In* B. Schink [ed.], *Advances in microbial ecology*. Kluwer.

Received: 11 November 2004

Accepted: 7 March 2005

Amended: 22 March 2005