

- SCHINDLER, D. W., H. KLING, R. V. SCHMIDT, J. PROKOPOWICH, V. E. FROST, R. A. REID, AND M. CAPEL. 1973. Eutrophication of lake 227 by addition of phosphate and nitrate: The second, third and fourth years of enrichment, 1970, 1971 and 1972. *J. Fish. Res. Board Can.* **30**: 1415–1440.
- , R. HESSLEIN, AND G. KIPPHUT. 1977. Interactions between sediments and overlying waters in an experimentally eutrophied Precambria Shield lake, p. 235–243. *In* H. L. Golterman [ed.], *Interactions between sediments and fresh water*. Dr. W. Junk B. V. Publishers.
- SMOLDERS, A., AND J. G. M. ROELOFS. 1993. Sulphate-mediated iron limitation and eutrophication in aquatic ecosystems. *Aquat. Bot.* **46**: 247–253.
- TESSENOW, U. 1974. Lösungs- Diffusions- und Sorptionsprozesse in der Oberschicht von Seesedimenten. *Arch. Hydrobiol. Suppl.* **47**: 1–79.

Received: 10 September 2002

Accepted: 13 November 2002

Amended: 21 November 2002

*Limnol. Oceanogr.*, 48(2), 2003, 933–938  
© 2003, by the American Society of Limnology and Oceanography, Inc.

## Nutrient limitation on a planktonic rotifer: Life history consequences and starvation resistance

**Abstract**—Nutrient content of plants is low relative to that of herbivores. Dietary nutrients can limit the growth and reproduction of herbivores. We studied life history consequences and starvation resistance for a common planktonic rotifer, *Keratella cochlearis*, feeding on algae grown on nutrient-limited media. A strain of *Cryptomonas* was grown on three types of growth medium with nitrogen (N) and phosphorus (P) adjusted to produce N-limiting, P-limiting, and nutrient-sufficient conditions. The analysis of nutrient content of *Cryptomonas* cells grown on nutrient-limited conditions showed that those algae exhibited higher P content than algae growing on a nutrient-sufficient medium. However, *Cryptomonas* reached lower densities in nutrient-limited media. The life history responses of *Keratella* to food quality were examined with low and high food availability. Our study showed that the interaction of food quantity and quality had a significant effect on rotifer growth rates. We observed, at low food levels, that *Keratella* growth rates were highest when the algae were grown under P limitation. The lowest rotifer growth rates were obtained when the rotifer fed on *Cryptomonas* grown in nutrient-sufficient conditions. Our study also showed that starvation resistance was higher for those rotifers fed on N-limited *Cryptomonas*. In natural situations, rotifers could even grow better under nutrient-limited conditions if this group of algae is dominant in the phytoplankton community. Moreover, differences in starvation resistance could be critical in determining competitive outcome and community structure in nutrient-variable environments.

Like higher plants, algae have relatively high ratios of C : P and C : N, and under N or P limitation, the C : N : P ratio of phytoplankton varies considerably (Goldman et al. 1979; Moal et al. 1987). Numerous studies have shown that N limitation, and particularly P limitation, reduce the quality of algae as food for zooplankton (Sommer 1992; Sterner et al. 1993; Rothhaupt 1995; Lüring and Van Donk 1997; DeMott 1998; DeMott et al. 1998; Schulz and Sterner 1999; Conde-Porcuna 2000; DeMott et al. 2001; Plath and Boersma 2001; Villar-Argaiz and Sterner 2002). Traditionally in these experiments, N- or P-limited media have been used to alter the elemental ratios of algal food and to exploit this

feature to create food supplies of differing quality for zooplankton. Most of these studies have focused largely on crustacean zooplankton (usually *Daphnia*) feeding on a single species of green algae (usually *Scenedesmus*). The extrapolation of these results to actual field conditions is quite limited because natural populations are usually made up of a variety of crustacean and noncrustacean zooplankton that feed on an algal community made up of a frequently diverse array of algae. However, few experimental studies have examined nutrient limitation for rotifer species (Rothhaupt 1995; Conde-Porcuna 2000), and/or nutrient limitation for zooplankton using nongreen algal species as food resources (Lüring and Van Donk 1997; Boersma 2000). There is no a priori reason to assume that *Scenedesmus*, a relatively rare alga in the wild, is generally representative of all other algae that zooplankton are likely to feed on in natural lakes (Brett et al. 2000). Therefore, more studies that examine how nutrient availability affects the elemental composition of non-green algal food items and their quality as food for different zooplankton are clearly necessary.

Rotifer species are more susceptible than *Daphnia* or copepods to nutrient limitation, especially P limitation (Morales-Baquero and Conde-Porcuna 2000; Conde-Porcuna et al. 2002). Conde-Porcuna et al. (2002) observed that the abundances of some rotifer species were not correlated with food availability but showed a strong dependence on P availability in a reservoir. Different susceptibilities of zooplankton species to nutrient limitation could be important in explaining the dynamics of these organisms in natural situations. Rothhaupt (1995) and Conde-Porcuna (2000) showed that P limitation significantly reduces the growth rates of the rotifers *Brachionus* and *Anuraeopsis*. Rothhaupt (1995) also showed that N-limited *Scenedesmus* allowed similar rotifer growth rates at low to moderate concentrations of food, but maximum growth rates at high food concentration were significantly reduced. However, no attempt has been made on life history consequences for rotifers feeding on nutrient-limited phytoplankton.

Starvation resistance reflects the ability of a species to store energy and control its allocation during periods of extreme

resource limitation (Kirk 1997). Studies on the abilities of rotifers to function as storage specialists, or their abilities to withstand periods of starvation, are extremely rare (Kirk 1997; Yoshinaga et al. 2000). In addition, nothing is known about rotifer starvation resistance when they are fed on algae of different quality. Conde-Porcuna (2000) observed that *Anuraeopsis* was less susceptible to exploitative competition by *Daphnia* when growing on N-limited *Scenedesmus*, suggesting that rotifers feeding on N-limited algae could be less susceptible to starvation. This needs to be tested.

In the present study, life history experiments were performed with the rotifer *Keratella cochlearis* to test the food quality effects of the alga *Cryptomonas* sp. growing on nutrient-limited and nutrient-sufficient media. We also analyzed the relative importance of nutrient limitation on *Keratella* under different food concentrations. We test the hypothesis that *Keratella* individuals should have longer starvation times when feeding on algae growing on N-limited media.

**Rotifers and algae—***Keratella cochlearis* (mean  $\pm$  SE; body length,  $105 \pm 0.81 \mu\text{m}$ ; posterior spine length,  $48.5 \pm 1.33 \mu\text{m}$ ) was from zooplankton cultures at the Institute of Water Research (University of Granada). This strain, originally collected from Iznájar Reservoir (southern Spain) in spring 1997, was cultured in 500-ml glass vessels with mineral water at  $20 \pm 0.5^\circ\text{C}$ ; they were fed on *Cryptomonas* sp. cells ( $\sim 0.2 \text{ mg C L}^{-1}$ ) five times a week, and the medium was partially renewed ( $\sim 25\%$ ) every week.

The cryptophyte *Cryptomonas* sp. ( $777 \mu\text{m}^3$ ;  $16.44 \mu\text{m} \times 9.5 \mu\text{m}$ ) was also from the culture collection at the Institute of Water Research in Granada. This strain, also isolated from Iznájar Reservoir in summer 1996, was routinely cultured in a chemostat on standard culture medium-Z8 (Skulberg and Skulberg 1990) in a room at  $18 \pm 0.5^\circ\text{C}$ , with photosynthetically active radiation of  $\sim 90 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$  in a 14:10 light:dark (LD) regime.

For our study, this strain of *Cryptomonas* was grown in several batch cultures (0.8 liters) using three types of growth medium modified from the standard medium-Z8. N as  $\text{NaNO}_3$  and  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ , and P as  $\text{K}_2\text{HPO}_4$  were adjusted to produce N-limiting ( $120 \mu\text{mol L}^{-1} \text{ N}:40 \mu\text{mol L}^{-1} \text{ P}$ ; N:P = 3), P-limiting ( $640 \mu\text{mol L}^{-1} \text{ N}:6.4 \mu\text{mol L}^{-1} \text{ P}$ ; N:P = 100), and nutrient-sufficient ( $640 \mu\text{mol L}^{-1} \text{ N}:40 \mu\text{mol L}^{-1} \text{ P}$ ; N:P = 16) conditions. These ratios and nutrient concentrations should provide nutrient-limited algae for rotifers (Conde-Porcuna 2000). Moreover, Lürling and Van Donk (1997) obtained nutrient-limited cells of the cryptophyte *Cryptomonas pyrenoidifera* using similar P concentrations.

All experimental cultures were inoculated with algal cells from the stock culture of *Cryptomonas* to obtain an initial density of  $\sim 1,000 \text{ cells ml}^{-1}$ , and they were cultured at  $18 \pm 0.5^\circ\text{C}$  and illuminated at  $23 \pm 7 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$  (14:10 LD).

We employed two batch cultures per N:P ratio. Every 6 d, 25–50% of the volume of each batch culture was harvested (log-phase algae) and replaced with fresh medium. Rotifers were fed on a mixture of the algae harvested from both cultures of each N:P treatment.

Algal density was calculated by counting two subsamples using an Uthermöhler chamber.

**Chemical compositions of algal foods—**Algae were harvested on day 15 of the life history experiments and part of the volume was filtered on precombusted ( $550^\circ\text{C}$ ) GF/C filters. Particulate C and N were determined using a CNH analyzer (Perkin Elmer) and particulate P was analyzed as soluble reactive P (Murphy and Riley 1962) after a potassium persulfate digestion. All analyses were performed with two to four replicates. Internal cell concentration of N ( $Q_N$ ) and P ( $Q_P$ ) were calculated from these data.

**Life table experiments—***K. cochlearis* was grown on three types of *Cryptomonas* cultured under nutrient-sufficient (NS), N-limiting (NL), or P-limiting (PL) conditions at two food levels (low food,  $80 \text{ cells ml}^{-1}$  [ $0.016\text{--}0.025 \text{ mg C L}^{-1}$ ], and high food,  $500 \text{ cells ml}^{-1}$  [ $0.114\text{--}0.132 \text{ mg C L}^{-1}$ ]). We have previously estimated that the threshold food level for population growth of that clone of *K. cochlearis* fed on this *Cryptomonas* strain is (mean  $\pm$  SE)  $0.018 \pm 0.004 \text{ mg C L}^{-1}$  (Ramos-Rodríguez and Conde-Porcuna unpubl. data).

Only second-clutch neonates were used for the life history experiments. Their mothers and grandmothers were cultured under identical conditions, except that they were fed on *Cryptomonas* from the chemostat. Mothers carrying their second clutch were placed in the six treatments described previously. Consequently, second-clutch neonates were born on NS, PL, and NL algae. Life table experiments were started with these 24 newly hatched females per treatment. Animals were placed individually into 2-ml wells of 24-well, sterile, polystyrene tissue culture plates (IWAKI) containing mineral water with *Cryptomonas*. All the treatments were maintained at  $20 \pm 0.5^\circ\text{C}$  with fluorescent lighting ( $\sim 20 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ) on a 14:10 LD cycle. Every 24 h, *Keratella* individuals were checked and transferred to clean tissue culture plates containing fresh food suspension. Newly hatched individuals were counted and separated from their mothers, and the day of hatching was noted. The life table experiments were continued until all animals were dead. The intrinsic rate of increase was calculated by solving Euler's equation iteratively, with confidence intervals determined by the jackknife technique described in Meyer et al. (1986).

The algal cultures were not axenic, but bacterial contamination did not contribute significantly to rotifer nutrition. We estimated bacterial abundance in the food suspensions with  $80 \text{ cells ml}^{-1}$ , from the beginning to the end of this experiment, as colony-forming units following methodology proposed by Rodina (1972). Maximum bacterial biomass was  $<1\%$  of the threshold food concentration of our strain of *K. cochlearis*.

**Starvation resistance—**For 5 d, 24 female neonates were inoculated individually into 2-ml wells containing  $80 \text{ cells ml}^{-1}$  *Cryptomonas* ( $0.016\text{--}0.025 \text{ mg C L}^{-1}$ ) in varying states of nutrient limitation (NS, PL, and NL). All neonates were obtained from well-fed mothers proceeding from our *K. cochlearis* stock culture. After day 5, all surviving individuals were washed with sterile mineral water, then they were subjected to complete starvation ( $0.22\text{-}\mu\text{m}$  filtered mineral water). Survival was assessed every 24 h until all the animals were dead. The temperature, light intensity, and pho-

Table 1. Internal cell concentrations of P ( $Q_P$ ) and N ( $Q_N$ ) (pmol cell<sup>-1</sup> basis) and average elemental ratios on an atomic basis (mol/mol) in *Cryptomonas* cells growing on nutrient-sufficient (NS), P-limited (PL), and N-limited (NL) media. Final *Cryptomonas* densities in the cultures (cell ml<sup>-1</sup>) are also shown. Values are means  $\pm$  1 SE for both experiments: low food and high food.

<i>Cryptomonas</i>	$Q_P$	$Q_N$	C:N:P	Cells (ml <sup>-1</sup> )
NS	0.18 $\pm$ 0.00	5.13 $\pm$ 0.07	133:29:1	29,446 $\pm$ 1,322
PL	0.25 $\pm$ 0.02	4.00 $\pm$ 0.22	75:16:1	5,308 $\pm$ 1,113
NL	0.25 $\pm$ 0.03	4.07 $\pm$ 0.75	85:16:1	16,761 $\pm$ 2,609

toperiod conditions were as previously described for the life table experiments.

**Statistical analyses**—Statistical analyses were performed using the STATISTICA program (Statsoft). Normality and homogeneity of variances were checked by the Kolmogorov–Smirnov test and Bartlett’s test, respectively (Sokal and Rohlf 1995). No transformation made the data normally distributed. In consequence, a two-way analysis of variance design for ranked data (the Scheirer–Ray–Hare extension of the Kruskal–Wallis test) was performed (see p. 445–447 in Sokal and Rohlf 1995) to test the effects of nutrient limitation and food concentration on life history traits of *Keratella*. Starvation resistance of *Keratella* individuals was tested with the Kruskal–Wallis test (Sokal and Rohlf 1995). Mann–Whitney *U*-tests, corrected for multiple testing with the sequential Bonferroni test (Rice 1989), were used for examining differences in life history traits and starvation resistance between pairs of treatments.

**Effect of nutrient availability on algal elemental composition**—*Cryptomonas* growth on nutrient-limited media was not well characterized by expression of algal elemental composition on either a picomole per cell or a ratio of cellular C basis (Table 1). Internal cell concentrations of P ( $Q_P$ ) were highest for NL and PL algae. Internal cell concentration of N ( $Q_N$ ) was highest for NS algae, and it was similar for NL and PL algae. C:P and N:P ratios were highest for NS *Cryptomonas*, and PL *Cryptomonas* had the lowest C:P ratio. However, NL and PL *Cryptomonas* reached lower densities relative to NS algae (Table 1).

In contrast, previous studies have shown that algae growing on nutrient-limited media reflected that limitation in their atomic C:P ratios (Sterner et al. 1993; Rothhaupt 1995; Lüring and Van Donk 1997; Boersma 2000). In those studies, C:P ratios were highest when the algae were grown under P limitation. As *Cryptomonas* growing on nutrient-limited media showed lower growth rates in our study, they could show low maximum growth rates relative to maximum P uptake velocities. Our results might suggest that *Cryptomonas* cells seem to be selected for a strategy of P storage at low nutrient concentrations. They could utilize the periodic P pulses supplied to the nutrient-limited cultures for luxury consumption, which enables them to survive the following period of reduced nutrient availability (Sommer 1984).

**Effect of food quality on life history experiments**—*Keratella* reached, at low food concentration, higher growth rates with PL and NL *Cryptomonas* as a food resource (Fig. 1, above, left panel). A two-way analysis of variance for ranked data showed that the *Keratella* growth rates were significantly influenced by both main effects (the food concentration and the type of limitation of the algae) as well as the interaction term (Table 2). Pairwise comparisons (Mann–Whitney *U*-test) showed that *Keratella* growth rate was strongly influenced by the nutritional state of the algae at low food concentration (Fig. 1, above, left panel). *Keratella* growth rates were lowest when feeding on NS algae. *Keratella* fed on PL algae showed the highest growth rates. Food quality had no significant effect on *Keratella* growth rates at high food level.

Life span was affected by the nutritional state of *Cryptomonas*, although the total number of offspring produced by a female and the juvenile period of *Keratella* were not affected (Table 2). Nevertheless, at low food concentration, the total number of offspring per female was significantly lower, and the juvenile period significantly higher, in animals fed on NS *Cryptomonas* than in those fed on PL algae (Fig. 1, below, left and right panel). The life span of animals fed on NS algae was significantly lower than that of animals fed on PL algae in both food concentrations (Fig. 1, above, right panel). The fertile period, estimated as the difference between the life span and the juvenile period, showed similar results. Furthermore, the life span of animals fed on NL *Cryptomonas* was not significantly different between both food levels.

This study provides the first test showing that algae growing on nutrient-limited conditions can be of high quality as a food resource for zooplankton. *Cryptomonas* cells grown on a P-limited medium were the best food for *Keratella* at low food concentration (near the food quantity threshold for growth). Some studies suggest a direct zooplankton mineral limitation (Hessen 1992; Sterner 1993), whereas others emphasize the importance of biochemical constituents like unsaturated fatty acids (Ahlgren et al. 1990). Boersma (2000) showed that both mineral and biochemical limitations of algal food play a role in the growth and population dynamics of zooplankters but that mineral requirements need to be met first. Our experiments show that P content of algal cells can influence *Keratella* growth rates considerably. We observed that small variations in the *Cryptomonas* C:P ratios (75–133) strongly reduced the growth rates of *K. cochlearis* at low food level. The apparent *Cryptomonas* storage of P in the nutrient-limited media could make the difference between positive and negative *Keratella* population growth.

The difference between low- and high-quality food should be less at low food concentration but greater at high food concentration (Sterner and Robinson 1994; Rothhaupt 1995). In contrast to these previous studies using *Scenedesmus* as the food resource, in our study, *Cryptomonas* grown on nutrient-sufficient and nutrient-limited media were equal in quality at high food concentration. Differences between *Scenedesmus* and *Cryptomonas* might explain these results. Lüring and Van Donk (1997) observed, using five alga species as food resources for *Daphnia*, that animals feeding on several P-



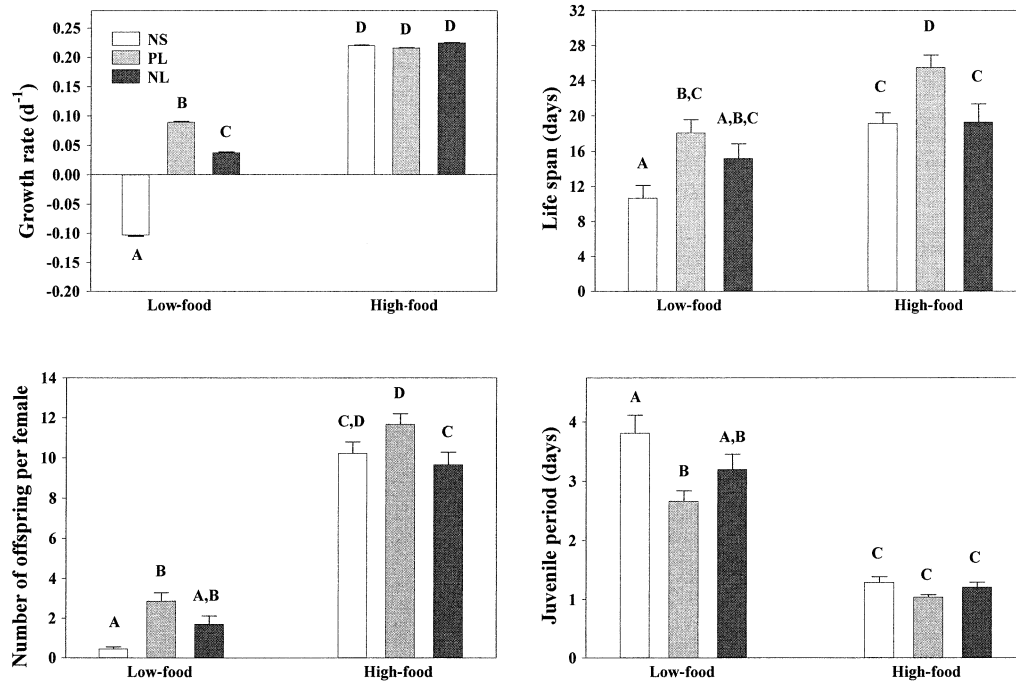


Fig. 1. Growth rates (above, left panel), life span (above, right panel), total number of offspring per female (below, left panel), and juvenile periods (below, right panel) of *Keratella cochlearis* fed on *Cryptomonas* growing on several media (NS, nutrient sufficient; PL, P limited; NL, N limited) at two different food concentrations. Error bars indicate standard errors; data points marked with identical characters are not significantly different (Mann–Whitney *U*-test). Probability values were adjusted for the number of simultaneous tests using the sequential Bonferroni test.

limited alga species produced fewer newborns, with the exception of feeding on P-limited *Cryptomonas pyrenoidifera*.

**Effect of food quality on starvation resistance**—A Kruskal–Wallis analysis of variance revealed a significant effect of nutrient treatment on longevity of *K. cochlearis* when animals starved after 5 d of feeding on *Cryptomonas* in varying states of nutrient limitation (Kruskal–Wallis ANOVA,  $H_{2,56} = 6.752$ ,  $p < 0.05$ ). Pairwise comparisons (Mann–Whitney *U*-test) showed that longevity was significantly higher in animals feeding on NL cells (mean  $\pm$  SE:  $3.58 \pm 0.28$ ,  $n = 19$ ) than in animals feeding on NS algae ( $2.59 \pm 0.23$ ,  $n = 17$ ). No significant differences were obtained between the longevities of animals feeding on PL ( $2.95 \pm 0.23$ ,  $n = 20$ ) and NL algae.

In consequence, *Keratella* individuals fed on NL algae live longer under starvation conditions. Those *Cryptomonas* cells had higher P content than NS algae. In addition, they had lower N content and higher C:N ratio than NS *Cryptomonas*. Groeger et al. (1991) showed that *Daphnia* fed on *Scenedesmus* grown on N-deficient media allocated more lipid to eggs, and their offspring lived longer under starvation conditions. Conde-Porcuna (2000) suggest that the rotifer *Anuraeopsis* lives longer when short of nitrogen.

Several studies show that organisms develop adaptations to fluctuating food concentrations (Gilbert and Schreiber 1998; Stelzer 2001). These adaptations include risky reproduction or the production of diapausing eggs when facing low food levels. Similar strategies could be developed by

zooplankton to deal with fluctuating food quality in natural ecosystems; consequently, to live longer under starvation conditions could be considered one of them. Kirk (1997) suggests that rotifer starvation times should allow survival of some species during short periods of low resource quantity or quality resulting, for example, in rapid fluctuations in the abundance of cryptomonads.

**Relevance to the natural situation**—Phytoplankton in the field can be nutrient limited at times. Several studies have shown that algae were limited by P in aquatic ecosystems in late spring (Sommer 1988, 1989; Conde-Porcuna et al. 2002). DeMott and Gulati (1999) suggest that *Daphnia* abundance in several lakes was strongly constrained by the seston C:P ratio. Brett et al. (2000) showed that differences in algal taxa food quality are substantially larger than one would expect to observe because of daphnid mineral P limitation across a range of natural lake seston C:P ratios. There are no studies showing natural lake seston C:P ratios and rotifer growth, abundance, or both. Conde-Porcuna et al. (2002) observed in a natural environment that the fecundity of several rotifer species can be affected by P limitation (estimated from N:P ratios). Unfortunately, data on seston C:P ratios are not available from that study.

Brett et al. (2000) suggest that zooplankton will be limited by the food quality of phytoplankton communities whenever these communities are not strongly dominated by diatoms, cryptophytes, or other highly nutritious phytoplankters. According to our results, low P or N concentration in the me-

Table 2. Two-way analysis of variance for ranked data (the Scheirer-Ray-Hare extension of the Kruskal-Wallis test) to test the effects of the type of limitation and food concentration of *Cryptomonas* on the population growth rate, life span, total number of offspring per female, and juvenile period of *K. cochlearis*.

Source	Growth rate (d <sup>-1</sup> )			Life span (days)			Offspring per female			Juvenile period (d)				
	df	SS	H	p	SS	H	p	SS	H	p	SS	H	p	
<b>Main effects</b>														
A: Limitation	2	11,476	7.08	0.029	25,341	15.63	<0.001	8,887	5.48	0.0646	2	4,577	3.43	0.18
B: Food concentration	1	166,076	102.41	<0.0001	28,464	17.55	<0.0001	159,633	98.44	<0.0001	1	114,268	85.69	<0.0001
A × B	2	15,854	9.77	<0.01	4,156	2.56	0.278	1,803	1.11	0.5741	2	470	0.352	0.8386
Error	133	9,696			163,291			48,563			120	29,960		

dium increases the quality of *Cryptomonas* as food resource for *Keratella*. Zooplankton populations could depend not only on the algal size as a determinant of edibility but also on the taxonomic features of the algal species and their specific nutrient requirements. Further studies on the interactions between several zooplankton species and several phytoplankton species under nutrient-limited conditions are needed to understand nutrient cycling and zooplankton strategies in aquatic ecosystems.

Eloisa Ramos-Rodríguez<sup>1</sup>

Departamento de Biología Animal y Ecología  
Facultad de Ciencias  
Universidad de Granada  
18071 Granada, Spain

José M. Conde-Porcuna

Instituto del Agua  
Universidad de Granada  
Ramón y Cajal 4  
18071 Granada, Spain

### References

- AHLGREN, G., L. LUNDSTEDT, M. BRETT, AND C. FORSBERG. 1990. Lipid composition and food quality of some freshwater phytoplankton for cladoceran zooplankters. *J. Plankton Res.* **12**: 809–818.
- BOERSMA, M. 2000. The nutritional quality of P-limited algae for *Daphnia*. *Limnol. Oceanogr.* **45**: 1157–1161.
- BRETT, M. T., D. C. MÜLLER-NAVARRA, AND S. PARK. 2000. Empirical analysis of the effect of phosphorus limitation on algal food quality for freshwater zooplankton. *Limnol. Oceanogr.* **45**: 1564–1575.
- CONDE-PORCUNA, J. M. 2000. Relative importance of competition with *Daphnia* (Cladocera) and nutrient limitation on *Anuraeopsis* (Rotifera) population dynamics in a laboratory study. *Freshw. Biol.* **44**: 423–430.
- , E. RAMOS-RODRÍGUEZ, AND C. PÉREZ-MARTÍNEZ. 2002. Correlations between nutrient limitation and zooplankton populations in a mesotrophic reservoir. *Freshw. Biol.* **47**: 1463–1473.
- DEMOTT, W. R. 1998. Utilization of a cyanobacterium and a phosphorus-deficient green alga as complementary resources by daphnids. *Ecology* **79**: 2463–2481.
- , AND R. D. GULATI. 1999. Phosphorus limitation in *Daphnia*: Evidence from a long term study of three hypereutrophic Dutch lakes. *Limnol. Oceanogr.* **44**: 1557–1564.
- , R. D. GULATI, AND K. SIEWERTSEN. 1998. Effects of phos-

<sup>1</sup> Corresponding author (eloisa@ugr.es).

### Acknowledgments

Financial support was provided from an FPI grant (Spanish Ministry of Education and Science) to E. Ramos-Rodríguez and from EU Project EVK2-CT-1999-00046 (European Union) and CICYT Project REN2001-2840/HID. We thank Aguas Lanjarón S.A. (Lanjarón, Spain) for providing the mineral water used in our study. P. Sánchez-Castillo is thanked for the isolate of the alga *Cryptomonas* sp. used in this study. C. Pérez-Martínez is thanked for technical assistance and valuable comments on algal cultures. We also thank R. Morales-Baquero, P. Carrillo, and two anonymous reviewers for making valuable suggestions to earlier drafts of this study.

- phorus-deficient diets on the carbon and phosphorus balance of *Daphnia magna*. *Limnol. Oceanogr.* **43**: 1147–1161.
- , ———, AND E. VAN DONK. 2001. Effects of dietary phosphorus deficiency on the abundance, phosphorus balance, and growth of *Daphnia cucullata* in three hypereutrophic Dutch lakes. *Limnol. Oceanogr.* **46**: 1871–1880.
- GILBERT, J. J., AND D. K. SCHREIBER. 1998. Asexual diapause induced by food limitation in the rotifer *Synchaeta pectinata*. *Ecology* **79**: 1371–1381.
- GOLDMAN, J. C., J. J. MCCARTHY, AND D. G. PEAVY. 1979. Growth rate influence on the chemical composition of phytoplankton in oceanic waters. *Nature* **279**: 210–215.
- GROEGER, A. W., M. D. SCHRAM, AND G. RICHARD. 1991. Influence of food quality on growth and reproduction in *Daphnia*. *Freshw. Biol.* **26**: 11–19.
- HESSEN, D. O. 1992. Nutrient element limitation of zooplankton production. *Am. Nat.* **140**: 799–814.
- KIRK, K. L. 1997. Life-history responses to variable environments: Starvation and reproduction in planktonic rotifers. *Ecology* **78**: 434–441.
- LÜRLING, M., AND E. VAN DONK. 1997. Life history consequences for *Daphnia pulex* feeding on nutrient-limited phytoplankton. *Freshw. Biol.* **38**: 693–709.
- MEYER, J. S., C. G. INGERSOLL, L. L. McDONALD, AND M. S. BOYCE. 1986. Estimating uncertainty in population growth rates: Jackknife vs. bootstrap techniques. *Ecology* **67**: 1156–1166.
- MOAL, J., V. MARTIN-JEZEQUEL, R. P. HARRIS, J. F. SAMAIN, AND S. A. POULET. 1987. Interspecific and intraspecific variability of the chemical composition of marine phytoplankton. *Oceanol. Acta* **10**: 339–346.
- MORALES-BAQUERO, R., AND J. M. CONDE-PORCUNA. 2000. Effect of the catchment areas on the abundance of zooplankton in high mountain lakes of the Sierra Nevada (Spain). *Int. Verh. Verein. Limnol.* **27**: 1804–1808.
- MURPHY, J., AND J. P. RILEY. 1962. A modified single solution method for the determination of phosphate in natural waters. *Anal. Chim. Acta* **27**: 31–36.
- PLATH, K., AND M. BOERSMA. 2001. Mineral limitation of zooplankton: Stoichiometric constraints and optimal foraging. *Ecology* **82**: 1260–1269.
- RICE, W. R. 1989. Analyzing tables of statistical tests. *Evolution* **43**: 223–225.
- RODINA, A. G. 1972. *Methods in aquatic microbiology*. University Park Press and Butterworth.
- ROTHHAUPT, K. O. 1995. Algal nutrient limitation affects rotifer growth rate but not ingestion rate. *Limnol. Oceanogr.* **40**: 1201–1208.
- SCHULZ, K. L., AND R. W. STERNER. 1999. Phytoplankton phosphorus limitation and food quality for *Bosmina*. *Limnol. Oceanogr.* **44**: 1549–1556.
- SKULBERG, O. M., AND R. SKULBERG. 1990. *Research with algal cultures*. NIVA's culture collection of algae. Norsk institut for vannforskning Rep. ISBN 82-551743-6.
- SOKAL, R. R., AND F. J. ROHLF. 1995. *Biometry*, 3rd ed. W. H. Freeman.
- SOMMER, U. 1984. The paradox of the plankton: Fluctuations of phosphorus availability maintain diversity of phytoplankton in flow-through cultures. *Limnol. Oceanogr.* **29**: 633–636.
- . 1988. Does nutrient competition among phytoplankton occur in situ? *Int. Verh. Verein. Limnol.* **23**: 707–712.
- . 1989. Nutrient status and nutrient competition of phytoplankton in a shallow, hypertrophic lake. *Limnol. Oceanogr.* **34**: 1162–1173.
- . 1992. Phosphorus-limited *Daphnia*: Intraspecific facilitation instead of competition. *Limnol. Oceanogr.* **37**: 966–973.
- STELZER, C. P. 2001. Resource limitation and reproductive effort in a planktonic rotifer. *Ecology* **82**: 2521–2533.
- STERNER, R. W. 1993. *Daphnia* growth on varying quality of *Scenedesmus*: Mineral limitation of zooplankton. *Ecology* **74**: 2351–2360.
- , AND J. L. ROBINSON. 1994. Thresholds for growth in *Daphnia magna* with high and low phosphorus. *Limnol. Oceanogr.* **39**: 1228–1232.
- , D. D. HAGEMEIERS, W. L. SMITH, AND R. F. SMITH. 1993. Phytoplankton nutrient limitation and food quality for *Daphnia*. *Limnol. Oceanogr.* **38**: 857–871.
- VILLAR-ARGAIZ, M., AND R. W. STERNER. 2002. Phosphorus limited algae induced life history bottlenecks in *Diaptomus clavipes*. *Limnol. Oceanogr.* **47**: 1229–1233.
- YOSHINAGA, T., A. HAGIWARA, AND K. TSUKAMOTO. 2000. Effect of periodical starvation on the life history of *Brachionus plicatilis* O.F. Müller (Rotifera): A possible strategy for population stability. *J. Exp. Mar. Biol. Ecol.* **253**: 253–260.

Received: 19 February 2002

Accepted: 29 September 2002

Amended: 28 October 2002