

# Effects of toxic and non-toxic cyanobacteria on the life history of tropical and temperate cladocerans

ALOYSIO S. FERRÃO-FILHO,\* SANDRA M.F.O. AZEVEDO† and WILLIAM R. DEMOTT‡

\*Departamento de Biologia, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP 14040-901, Brazil

†Núcleo de Pesquisa de Produtos Naturais, Universidade Federal do Rio de Janeiro, Ilha do Fundão, RJ 21949-590, Brazil

‡Department of Biology, Indiana University–Purdue University, Fort Wayne, IN 46805, U.S.A.

## SUMMARY

1. This study compares the effects of four toxic strains of *Microcystis aeruginosa* on tropical and temperate Cladocera. Survival was tested in acute toxicity experiments using *Microcystis* alone or in mixtures with the edible green algae *Ankistrodesmus falcatus*. The effect of chronic exposure on population growth was estimated in life-table experiments by varying the proportion of *Microcystis* and the green algae. Nutritional deficiency was assessed using a non-toxic cyanobacterium in a zooplankton growth experiment. Feeding inhibition was tested using a <sup>14</sup>C-labelled green algae as a tracer in mixtures with toxic *Microcystis*.
2. Toxicity varied consistently between *Microcystis* strains, while sensitivity varied consistently between cladoceran species. However, no relationship was found between sensitivity and geographical origin or cladoceran body size. Two small-bodied cladocerans from the same tropical lake, *Ceriodaphnia cornuta* and *Moinodaphnia macleayi*, were the least sensitive and most sensitive species, respectively.
3. Surprisingly, two small tropical cladocerans survived longer without food than did three large *Daphnia* species and a third small tropical species.
4. Each of the three tropical *Microcystis* strains strongly reduced the population growth rate (little 'r') and reproductive output of each cladoceran, this reduction being proportional to the percentage of toxic cells in the diet.
5. As the sole food source, the non-toxic cyanobacterium, *Synechococcus elongatus*, supported poor growth in *M. macleayi*. The nutritional deficiency was overcome when *Synechococcus* was mixed with either *Ankistrodesmus* or an emulsion rich in omega-3 fatty acids.
6. *Microcystis* inhibited the feeding rate of two cladocerans, even when it comprised only 5% of a mixture with the green algae *A. falcatus*.
7. Differences in sensitivity to the toxic cyanobacterium appear to be associated with differences in life history between the cladoceran species rather than differences between tropical and temperate taxa. Slow-growing species that are resistant to starvation appear less sensitive to toxic *Microcystis* than fast-growing species, which also tend to die more quickly in the absence of food.

*Keywords:* cyanobacteria, filtering rate, growth rate, life-table experiments, zooplankton

---

Correspondence: Aloysio S. Ferrão-Filho, Departamento de Biologia, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP 14040-901, Brazil.

E-mail: aloysiosff@ffclrp.usp.br

## Introduction

Many cyanobacteria are toxic to zooplankton, causing increased mortality and reduced reproductive output (Porter & Orcutt, 1980; Hietala, Reinikainen & Walls, 1995). A given species of cyanobacteria may, however, include both toxic and non-toxic strains. For example, within the genus *Microcystis*, some strains cause high mortality rates and allow little or no reproduction (Nizan, Dimentman & Shilo, 1987; Smith & Gilbert, 1995), while other strains support high survival and fair to good rates of growth and reproduction (DeBernardi & Giussani, 1990). Moreover, resistance to cyanobacterial toxins can vary markedly among zooplankton species (Lampert, 1982; Fulton & Pearl, 1987; DeMott, Zhang & Carmichael, 1991; Gilbert, 1994; Smith & Gilbert, 1995).

Although several hypotheses have been proposed to explain cyanobacterial blooms in eutrophic lakes (Sterner, 1989; Vincent, 1989; Shapiro, 1990), the appearance of toxicity in such blooms is not yet understood (Carmichael, 1992). One hypothesis involves differential grazing on toxic and non-toxic cells by zooplankton (Lampert, 1981a). In this case, toxic strains would be favoured by the inhibition effect on the filtering rate of zooplankton and would have a selective advantage over non-toxic ones (Lampert, 1982). However, most cladocerans are relatively non-selective filter feeders (DeMott, 1990) and thus, in contrast to copepods, are not able to discriminate between toxic and non-toxic cells (DeMott & Moxter, 1991). Therefore, in lakes dominated by cladocerans, grazing would not favour toxic strains, because toxic and non-toxic clones would experience an equal benefit from the reduction in grazing and more readily grazed competitors would benefit even more.

A related hypothesis considers the evolutionary role of cyanobacterial toxins. Even though the effects of these toxins have been extensively investigated in vertebrates, especially in mammals, it is unlikely that they have evolved as a defence against warm-blooded animals (Lampert, 1981a). These toxins are more likely to have evolved as a defence against grazing by zooplankton, similar to the development of toxins by terrestrial plants against insect predation (Kirk & Gilbert, 1992). In fact, strong evidence from laboratory studies suggests that cyanobacteria toxins act as a defence against zooplankton predation by

inhibiting filtering rate (Lampert, 1981b, 1982; Nizan *et al.*, 1986; Fulton & Pearl, 1987; DeMott & Moxter, 1991; DeMott *et al.*, 1991; Haney, Sasner & Ikawa, 1995). The mechanism of feeding inhibition may occur after ingestion, resulting from the digestion of cyanobacterial cells and the delivery of toxic compounds, or as a result of chemical deterrence, causing behavioural avoidance of toxic cells before ingestion. Nevertheless, some evidence suggests that the compounds that cause acute toxic effects after the ingestion of toxic cells are different from the compounds that cause chemical deterrence of feeding rates (Jungmann, Henning & Jüttner, 1991).

Microcystins are the main cyanobacterial toxins associated with the toxicity to vertebrates and invertebrates (Carmichael, 1992). However, Jungmann & Benndorf (1994) proposed a controversial hypothesis that toxins other than microcystins are responsible for the toxicity of *Microcystis* to zooplankton. Also, Jungmann (1995) isolated and purified a new, but unidentified, compound from a strain of *Microcystis aeruginosa* Kützing (PCC7806) that proved to be more toxic to *Daphnia* than microcystins. However, his tests involved dissolved toxins rather than ingested toxins, which may influence the toxin absorption process. In addition, DeMott & Dhawale (1995) showed that microcystin-LR can inhibit the activity of protein phosphatase 1 and 2A from crude extracts of three zooplankton species. These results corroborate previous data obtained with the same zooplankton species, in which pure microcystin-LR and a strain of *Microcystis* (PCC7820) showed acute toxic effects to these species (DeMott *et al.*, 1991).

The nutritional value of non-toxic cyanobacteria to zooplankton has also been a matter of controversy. While many studies have shown that non-toxic cyanobacteria are a poor quality food for zooplankton (Porter & Orcutt, 1980; Infante & Abella, 1985; Hazanato & Yasuno, 1987; Matveev & Balseiro, 1990; Lundstedt & Brett, 1991; Smith & Gilbert, 1995), other studies have shown that some species of zooplankton exhibit good survival and growth when fed cyanobacteria (Burns & Xu, 1990; Gliwicz, 1990; Fulton & Jones, 1991). As DeBernardi & Giussani (1990) pointed out, the inadequacy of non-toxic cyanobacteria as food can be attributed to colony or filament size, which causes mechanical interference with feeding apparatus. Also, the low assimilation of cyanobacteria could be due to poor digestibility be-

cause, as in some gelatinous green alga (Porter, 1975), they are covered by a mucilaginous sheath that make digestion difficult (Lampert, 1987).

Nutritional deficiency in certain polyunsaturated fatty acids that are essential for zooplankton growth is another factor that may reduce the food quality of cyanobacteria for zooplankton (Ahlgren *et al.*, 1990; Müller-Navarra, 1995). In support of the fatty acid hypothesis, DeMott & Müller-Navarra (1997) found that the growth rates of three *Daphnia* species were markedly reduced when they were fed a non-toxic cyanobacterium, *Synechococcus elongatus* Nägeli, as a sole food source. Growth increased, however, when a green alga or an emulsion rich in omega-3 fatty acids was mixed with the cyanobacterium. This suggests that *Synechococcus* lack lipids that are important for *Daphnia*.

Most research on interactions between toxic cyanobacteria and zooplankton has focused on temperate taxa and no previous studies have directly compared tropical and temperate zooplankton. Because cyanobacteria are favoured by high temperature, tropical waters often experience frequent and long-lasting blooms of cyanobacteria. Thus, one can hypothesize that tropical zooplankton experience higher selection pressure for resistance to cyanobacterial toxins than temperate zooplankton. The major goal of this study is to test the hypothesis that tropical cladocerans are more resistant to toxic and non-toxic cyanobacteria than temperate cladocerans. To make a rigorous and general test, we used several experimental designs and four strains of toxic *Microcystis*, three isolated from tropical lakes. In order to examine further the applicability of research on temperate cladocerans to tropical species, we also studied

feeding inhibition by toxic cyanobacteria and nutritional deficiencies in a non-toxic cyanobacterium.

## Methods

### *Cultures of green algae, cyanobacteria and zooplankton*

Two green algae, four toxic *Microcystis* strains and a non-toxic cyanobacteria, *S. elongatus*, were obtained from several sources (Table 1). To avoid detrimental effects other than toxicity (e.g. colony size), only single-celled strains of *Microcystis* were used. Green algae were cultured in MBL medium (Stemberger, 1981), while cyanobacteria were cultured in ASM-1 medium (Gorham *et al.*, 1964), both in batch cultures and in the same temperature and light conditions as zooplankton. Daily, an appropriate volume of green algae culture was harvested, centrifuged and resuspended in zooplankton medium to feed the animals. For the experiments, cyanobacteria cultures were kept in exponential growth phase by harvesting about half of the culture volume every other day and replacing this volume with fresh ASM-1 medium. Calibration curves (absorbance versus particulate organic carbon) were used to estimate the carbon concentration of the algal suspensions.

Zooplankton species were collected from different lakes in tropical and temperate regions (Table 2). Zooplankton stock cultures were kept in 1 L glass jars with an artificial medium (modified from Tollrian, 1993) in a temperature-controlled chamber at  $20 \pm 1$  °C and 16:8 h light:dark cycle. These cultures were fed from stock cultures of the green algae *Ankistrodesmus falcatus* (Braun) and *Chlamydomonas reinhardtii* Dangeard at a total concentration of  $1.0 \text{ mg C L}^{-1}$ .

**Table 1** Green algae and cyanobacteria sizes and their sources

Species	Strain	Mean size ( $\mu\text{m}$ )	Source
<i>Ankistrodesmus falcatus</i>	NPIN-1	$3 \times 50$	A.J. Tessier, Michigan State University
<i>Chlamydomonas reinhardtii</i>	UTEX90	5.8*	University of Texas Culture Collection
<i>Microcystis aeruginosa</i>	NPLJ-2	5.0	Jacarepaguá Lagoon, RJ, Brazil
<i>Microcystis aeruginosa</i>	NPLJ-3	5.0	Jacarepaguá Lagoon, RJ, Brazil
<i>Microcystis aeruginosa</i>	NPLJ-6	5.0	Jacarepaguá Lagoon, RJ, Brazil
<i>Microcystis aeruginosa</i>	PCC7820	4.4†	Paris Culture Collection
<i>Synechococcus elongatus</i>	UTEX 563	1.5	University of Texas Culture Collection

\* DeMott (1988).

† Smith & Gilbert (1995).

RJ, Rio de Janeiro.

**Table 2** Zooplankton species, size ranges of adults and trophic status of their sources

Species	Size range ( $\mu\text{m}$ )	Source	Trophic status
<i>Ceriodaphnia cornuta</i>	0.4–0.6	Cabiúnas Lagoon, RJ, Brazil	Oligotrophic
<i>Daphnia pulex</i>	1.5–3.5	Carolina Biological Supply	—
<i>Daphnia pulicaria</i>	1.5–3.5	Crooked Lake, IN, U.S.A.	Mesotrophic
<i>Daphnia similis</i>	2.0–5.0	Cenpes-Petrobrás, RJ, Brazil	—
<i>Moina micrura</i>	0.5–1.2	Jacarepaguá Lagoon, RJ, Brazil	Hypereutrophic
<i>Moinodaphnia macleayi</i>	0.5–0.9	Cabiúnas Lagoon, RJ, Brazil	Oligotrophic

RJ, Rio de Janeiro.

This concentration is far above the incipient limiting level for daphnids (Lampert, 1977) and is sufficient for rapid growth and good reproduction. New zooplankton cultures were established once a week, when about 20–25 juveniles of each species were transferred to new medium.

#### Acute toxicity experiments

A series of acute toxicity experiments were carried out with the tropical and temperate cladocerans in order to compare their survival during exposure to toxic strains of *Microcystis*. The basic design for these experiments consisted of exposing 10 neonates (< 24 h old) of each cladoceran species in a glass tube with 30 mL of artificial medium and a toxic strain *Microcystis* at concentrations ranging from 0.1 to 1.0 mg C L<sup>-1</sup>. Survival was checked every day for 5 days. There were four replicate tubes per treatment. In some experiments, the animals were fed *Microcystis* alone and controls consisted of starved animals. In another series of survival experiments, animals were fed mixtures of *Microcystis* and *Ankistrodesmus* at a total concentration of 1.0 mg C L<sup>-1</sup>. Here, controls consisted of animals fed with 1.0 mg C L<sup>-1</sup> of the green algae. Mixed diets were used to eliminate possible effects of starvation and nutritional deficiency when cyanobacteria were added alone. Use of several experimental designs also increased the generality of our results. Median lethal time (LT<sub>50</sub>) for each strain, in each concentration, was estimated in hours by PROBIT regression analysis (SPSS Statistical Package, SSPS Inc., Chicago, IL, U.S.A.). We used LT<sub>50</sub> instead of median lethal concentration (LC<sub>50</sub>) because there were heterogeneous responses among the Cladocera to the *Microcystis* strains (i.e. cohorts of animals decreased to 50% on different days and we had no way to choose a specific day for calculating LC<sub>50</sub>). Also,

responses to toxic strains were compared to controls with starved or fed animals, for which the LC<sub>50</sub> cannot be calculated. Differences between treatments were tested statistically by analysis of variance (ANOVA) using the LT<sub>50</sub> data to test for the effects of cladoceran species, *Microcystis* strain and toxic cells concentration.

#### Chronic toxicity experiments (life-table experiments)

Two life-table experiments were carried out in order to assess the effects of chronic exposure to toxic strains of cyanobacteria on the intrinsic rate of population growth,  $r$ . These experiments started with cohorts of neonates (< 24 h old) which were fed toxic *Microcystis* mixed with different proportions of the green algae *Ankistrodesmus*, for a total concentration of 1.0 mg C L<sup>-1</sup>. Initially, 10–20 animals from each species were placed individually in glass tubes with 30 mL of artificial medium and the various algal suspensions. After 3 days, only *Daphnia* were transferred to 100 mL flasks with the respective algal suspensions to avoid food depletion due to their large size and higher filtering rates. Every day, the animals were transferred to new algal suspensions and checked for survival, the appearance of eggs and the number of newborns per female. These data were used to calculate the intrinsic rate of natural increase ( $r$ ). The experiments were run for 14–16 days, depending on the survival of the cohorts in the *Microcystis* treatments. Calculations showed that reproduction beyond this time had a negligible effect on  $r$ . The mean intrinsic rate of natural increase and its confidence interval were estimated using a computer bootstrap technique (Taberner *et al.*, 1993), with 500 replicates per run and a bias adjusted correction for small cohorts (Meyer *et al.*, 1986). Statistical differences between *Microcystis* treatments and controls were tested by  $t$ -tests.

### Growth with a non-toxic nutritionally deficient cyanobacterium

An experiment was designed to test the effects of a non-toxic but nutritionally deficient cyanobacterium (*S. elongatus*) on the growth (i.e. increase in mass) of a tropical cladoceran. The experiment started with a cohort of *Moinodaphnia macleayi* (King) < 24 h old. About 100 individuals were placed in 500 mL bottles in each treatment. There were four treatments with three replicate bottles per treatment: *Ankistrodesmus* alone (control; 0%); a 1:1 mixture of *Synechococcus* and *Ankistrodesmus* (50%); *Synechococcus* supplemented with a fish oil emulsion (+ FO); and *Synechococcus* alone (100%). The total concentration of algae in each treatment was 1.0 mg C L<sup>-1</sup>. Initially and after 2, 4 and 6 days, 10 animals from each bottle were transferred to a single small, pre-weighed aluminium container, dried at 60 °C overnight, and weighed on a Sartorius (Goettingen, Germany) electronic microbalance to the nearest microgram. Instantaneous net growth rates were calculated using the following equation:

$$g = [\ln(M_t) - \ln(M_o)]/t,$$

where  $M_o$  and  $M_t$  are the mean individual mass initially and after  $t$  days. At the end of the experiment, the number of eggs carried by each animal was also counted and significant differences between treatments were tested by ANOVA.

### Feeding experiment

Filtering rates were measured in order to evaluate the inhibitory effects of a toxic cyanobacterium on the feeding rates of two zooplankton species, *Daphnia pulex* De Geer and *Moina micrura* Kurz. In this experiment, we used <sup>14</sup>C (NaH<sup>14</sup>CO<sub>3</sub>) as a radiotracer to label the nutritious alga (*A. falcatus*), which was mixed with toxic *Microcystis* for a total concentration of 1.0 mg C L<sup>-1</sup>. The details of the algae labelling and radiotracer technique are described in DeMott (1988). Before the start of the experiment, two groups of animals from the same cohort were separated and acclimatized for 1 or 20 h, respectively, in 500 mL bottles with unlabelled food suspensions. Five animals from each species were then placed in a 100-mL beaker and allowed to feed for about 7 min on the same mixture with radioactive *Ankistrodesmus*. There

were three replicate beakers for each treatment and acclimation time. The animals were then anaesthetized in carbonated water, measured under a dissecting microscope and individually transferred to scintillation vials containing 0.5 mL of a tissue solubilizer (TS-2, Research International Corp., Mount Prospect, IL, U.S.A.). After 6 h, 10 mL of a scintillation cocktail (Ecolume, Research Products Int.) was added to each vial. Specific activities of algal suspensions were determined by filtering a 3.0-mL sample at the beginning of the experiment. Radioactivity was determined with a liquid scintillation spectrophotometer (Tracor Analytic, Delta 300, Model 6891) with internal standards for determining <sup>14</sup>C efficiencies. Feeding inhibition was tested by one-way ANOVA on log-transformed filtering rates.

### Toxin extraction and quantification

The toxin microcystin in the three tropical *Microcystis* strains was extracted and quantified using a method modified from Krishnamurthy, Carmichael & Sarver (1986). Freeze-dried cells from each strain were extracted three times with a mixture of buthanol:methanol:water (5:20:75), followed by centrifugation (4000 g, 10 min), evaporation of the supernatant to one-third of the original volume and passing this sample into a C18 cartridge. The sample in the cartridge was then eluted with 20 mL of 100% methanol and dried with hot air in an evaporator chamber. The sample was then resuspended in 1.0 mL of deionized water and kept in a freezer until the analysis. Analysis and quantification of the toxin was carried out using high performance liquid chromatography (HPLC) in a Shimadzu chromatographic apparatus with a diode array UV/Vis. SPDA-M10A. The analysis was carried out in isocratic conditions in a reverse phase column Supercosil LC18 (5 mm, 10 × 250 mm), using acetonitrile and ammonium acetate (20 mM, 28:72 v:v) as the mobile phase and absorbance at 238 nm. The spectrum of each chromatogram peak was analysed between 200 and 300 nm using a diode photodetector and was compared with a standard profile of microcystin-LR to confirm the presence of toxin in the sample. The toxin content was determined by comparing the area of the sample peaks with that of the microcystin-LR standard.

**Table 3** Microcystin contents in the *Microcystis* strains used in this study

Strain	Microcystin content ( $\mu\text{g mg}^{-1}$ )
NPLJ-2	4.08–4.90 ( $n = 2$ )
NPLJ-3	3.64 ( $n = 1$ )
NPLJ-6	3.40 ( $n = 1$ )
PCC7820	2.81–3.43*

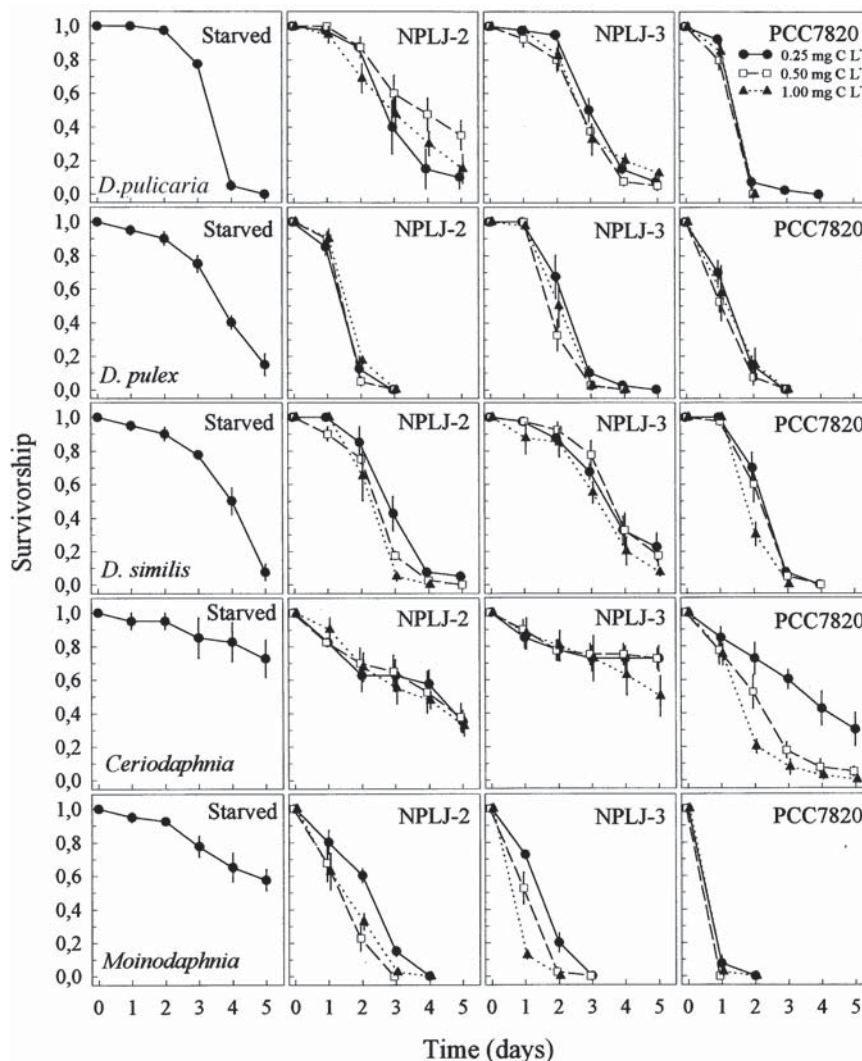
\* Arment & Carmichael (1996).

**Results**

*Acute experiments*

The *Microcystis* strains isolated from Brazilian waters contained high concentrations of the toxin micro-

cystin (Table 3). The three strains from Jacarepaguá Lagoon (Rio de Janeiro, Brazil) had a similar content of microcystins per dry weight, although strain NPLJ-2 showed a slightly higher concentration. According to Arment & Carmichael (1996), the microcystin-LR content of the *Microcystis* strain PCC7820 varied from 2.81 to 3.43  $\mu\text{g mg}^{-1}$  dry weight for exponentially and stationary growing cells, respectively. Microcystin-LR was the dominant form of microcystin in this strain. *Microcystis* strains from Jacarepaguá Lagoon had total microcystin contents that were about in the same range as the microcystin-LR content of the PCC7820 strain (Table 3). Our analysis of the Brazilian strains did not, however, identify different chemical forms of microcystins.



**Fig. 1** Acute toxicity experiment with five cladoceran species (rows) and three *Microcystis* strains (columns). Animals were exposed only to single diets of *Microcystis* in three different concentrations. Controls consisted of starved animals. There were ten animals per tube and four replicate tubes per treatment.

In the first acute experiment, all cladocerans were exposed to *Microcystis* alone in different concentrations and the controls consisted of starved animals (Fig. 1). Most cladocerans experienced more rapid mortality when exposed to *Microcystis* than in the starved controls. Except for *Daphnia pulex* (Forbes) in the NPLJ-2 and NPLJ-3 treatments and *Daphnia similis* Claus and *Ceriodaphnia cornuta* Sars in the NPLJ-3 treatment, cladocerans showed a significantly lower survival in the treatments with each *Microcystis* strain than in the controls without food (ANOVA,  $P < 0.05$ ).

ANOVA revealed significant differences in survival between cladoceran species ( $F = 72.6$ ;  $P < 0.0001$ ). Comparison of average  $LT_{50}$  values by the Tukey test ( $\alpha = 0.05$ ) showed a rank in the cladoceran species in their sensitivity to *Microcystis*, with *Moinodaphnia* being the most sensitive ( $LT_{50} = 24$  h) and *Ceriodaphnia* being the least sensitive ( $LT_{50} = 107$  h). Among *Daphnia* species, *D. pulex* was the most sensitive ( $LT_{50} = 36$  h), *D. pulex* was intermediate ( $LT_{50} = 59$  h) and *D. similis* was the least sensitive species ( $LT_{50} = 63$  h).

The cladoceran species also showed marked differences in survival under starvation. The three temperate *Daphnia* species showed similar average  $LT_{50}$  for starved animals in the control group (*D. pulex*: 76 h; *D. pulex*: 87 h; *D. similis*: 86 h). Surprisingly, the small-bodied tropical cladocerans survived longer under starvation than the large-bodied *Daphnia* ( $LT_{50}$  for *Ceriodaphnia*: 207 h; for *Moinodaphnia*: 151 h).

There were significant differences in cladoceran survival between *Microcystis* strains ( $F = 79.6$ ;  $P < 0.0001$ ) and also an interaction between cladoceran species and *Microcystis* strains ( $F = 19.3$ ;  $P < 0.0001$ ). Comparisons of average  $LT_{50}$  values for these strains by the Tukey test ( $\alpha = 0.05$ ) showed that strain PCC7820 was the most toxic ( $LT_{50} = 36$  h), NPLJ-2 was intermediate ( $LT_{50} = 56$  h) and NPLJ-3 was the least toxic strain ( $LT_{50} = 66$  h). In some *Microcystis* treatments, survival was not significantly different from the controls with starved animals. Thus, these trials alone do not provide convincing evidence of toxicity. For example, NPLJ-2 and NPLJ-3 treatments exhibited  $LT_{50}$  values for *D. pulex* that were not statistically different from the controls ( $P > 0.05$ ). The same occurred for *D. similis* and *Ceriodaphnia* in the NPLJ-3 treatment. For *D. pulex* and *Moinodaphnia*, however, each strain of *Microcystis* significantly reduced survival relative to the starvation controls ( $P <$

0.05). The tropical *Moinodaphnia* seemed to be the most adversely affected species, showing high sensitivity to all strains of *Microcystis*. For this species, mortality was pronounced during the first 2 days of the experiment. Moreover, in the PCC7820 treatment, most individuals of *Moinodaphnia* died during the first day of the experiment. This result further emphasizes the high toxicity of the PCC7820 strain, which was also observed in other studies (see 'Discussion' section).

The concentration of toxic *Microcystis* had little or no effect on cladoceran survival in the acute experiments with the toxic cyanobacterium alone ( $F = 1.4$ ;  $P = 0.2407$ ). Moreover, there was no significant interaction between concentration and cladoceran species ( $F = 1.3$ ;  $P = 0.2886$ ) or *Microcystis* strains ( $F = 0.9$ ;  $P = 0.4838$ ). A strong effect of *Microcystis* concentration was observed only for *Moinodaphnia* in the NPLJ-2 and NPLJ-3 treatments ( $P < 0.0001$ ) and for *Ceriodaphnia* in the PCC7820 treatment ( $P < 0.0001$ ). These results suggest a weak effect of toxic cell concentration when toxic *Microcystis* is offered as the only resource.

In Fig. 2, two tropical moinid species were exposed to *Microcystis* strains under two conditions. In the first, the moinids were given diets of *Microcystis* alone in three concentrations and controls consisted of starved animals. In the second condition, the moinids were given mixed diets of *Microcystis* and the green alga *A. falcatus*, and controls consisted of animals fed only on the green alga. In the mixed diets, there were also three concentrations of cyanobacteria mixed in different proportions with the green alga, making up a total food concentration of  $1.0 \text{ mg C L}^{-1}$ . ANOVA showed significant differences in survival between the moinid species ( $F = 95.3$ ;  $P < 0.0001$ ). Comparison of the average  $LT_{50}$  values by the Tukey test ( $\alpha = 0.05$ ) showed differences in sensitivity between the two moinids when exposed to *Microcystis* cells alone ( $P < 0.0001$ ) or in combination with the green algae ( $P < 0.0001$ ). In both cases, *Moinodaphnia* was more sensitive than *Moina micrura* to the toxic *Microcystis* strains. The overall mean  $LT_{50}$  for *Moinodaphnia* when exposed only to *Microcystis* cells was 37 h and for *Moina* was 53 h. When exposed to mixtures of cyanobacteria and green algae, the mean  $LT_{50}$  for *Moinodaphnia* increased to 54 h and for *M. micrura* to 97 h.

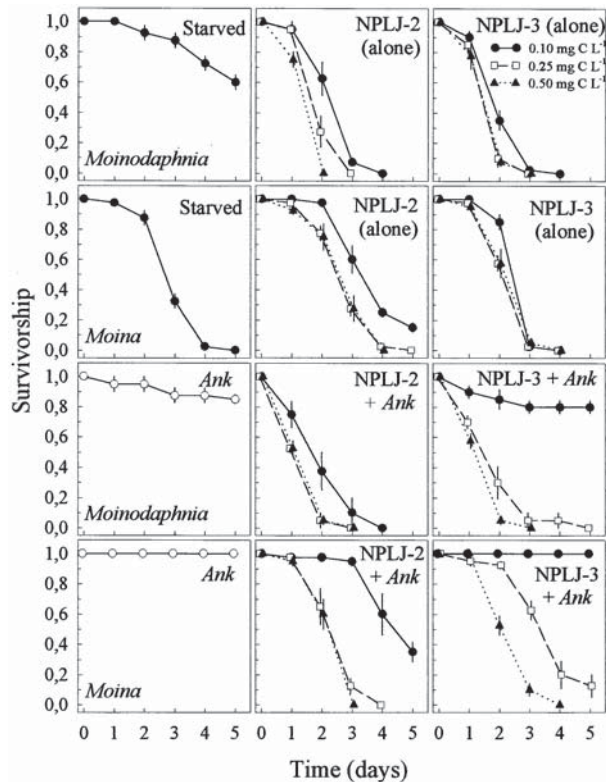


Fig. 2 Acute toxicity experiment with two moinids and two *Microcystis* strains. Two experimental designs were used: 1) animals fed diets of *Microcystis* alone with controls consisting of starved animals (upper two rows of panels); and 2) animals fed mixed diets of *Microcystis* and the green alga *A. falcatus* (*Ank*), and controls consisting of animals fed only the green alga (lower two rows of panels). In the mixed diets, food concentration was kept constant at  $1.0 \text{ mg C L}^{-1}$ . There were ten animals per tube and four replicate tubes per treatment.

Resistance to starvation also differed between these moinids (Fig. 2). While *Moinodaphnia* showed higher resistance to starvation, with an average  $LT_{50}$  of 151 h, *Moina* starved much faster (average  $LT_{50}$  of 61 h), with all individuals dying by the fourth day of the experiment.

The experiment with moinids also revealed significant differences between the *Microcystis* strains ( $F = 105.9$ ;  $P < 0.0001$ ) and an interaction between cladoceran species and strain of *Microcystis* ( $F = 7.9$ ;  $P < 0.0001$ ). Comparisons of the average  $LT_{50}$  values by the Tukey test ( $\alpha = 0.05$ ) showed that strain NPLJ-2 was less toxic than strain NPLJ-3 when offered as the only resource (mean  $LT_{50}$  for NPLJ-2: 56 h; mean  $LT_{50}$  for NPLJ-3: 45 h). In contrast, strain NPLJ-3 was less toxic than NPLJ-2 when offered in mixtures with

green algae (mean  $LT_{50}$  for NPLJ-2: 53 h; mean  $LT_{50}$  for NPLJ-3: 96 h).

The effect of diet type (*Microcystis* alone or in a mixed diet) on survival differed for the two moinids. Moreover, a strong interaction between diet type and *Microcystis* strain was observed ( $F = 74.2$ ;  $P < 0.0001$ ). This interaction means that the effect of the cyanobacteria strain depends on whether it is offered as a single resource or is mixed with good food. However, in the single food diet, *Moina* were not sensitive to NPLJ-2 strain (survival was not significantly different from the starved controls) and were only marginally sensitive to NPLJ-3 ( $P = 0.0424$ ). In the mixed food diet, however, *Moina* showed high sensitivity to both strains. In contrast, *Moinodaphnia* were sensitive to both strains in both single and mixed food diets.

In the acute experiment with moinids, there was a strong effect of concentration in the treatments with *Microcystis*, especially when mixed with the green algae ( $F = 197.4$ ;  $P < 0.0001$ ). Diet type also showed a significant effect, especially in the lower concentrations of cyanobacteria. For example, in contrast with the single diet treatment with only  $0.1 \text{ mg C L}^{-1}$  of the strain NPLJ-3 (Fig. 2, upper panels), *Moina* experienced no mortality with the same concentration of cyanobacteria when  $0.9 \text{ mg C L}^{-1}$  of the green alga was added to keep the food concentration constant at  $1.0 \text{ mg C L}^{-1}$  in the mixed diet (Fig. 2, lower panels).

Another acute experiment tested the response of a larger number of cladoceran species to the *Microcystis* strain NPLJ-6 (Fig. 3). As in the previous experiment, four cladoceran species were exposed to toxic cells in single or mixed diets. In the single diet treatments, animals were exposed only to three different concentrations of *Microcystis* with the controls consisting of starved animals (Fig. 3, left panels). In the mixed diet treatments, animals were exposed to *Microcystis* mixed in different proportions with the green alga *A. falcatus*, with the controls fed only the green alga (Fig. 3, right panels). The total food concentration of mixed diets was kept constant at  $1.0 \text{ mg C L}^{-1}$ , but the proportion of each food varied.

Again, *Moinodaphnia* was the most sensitive and *Ceriodaphnia* the least sensitive species (Fig. 3). The ANOVA showed significant differences between the cladocerans ( $F = 62.7$ ;  $P < 0.0001$ ). Comparing average  $LT_{50}$  values by the Tukey test ( $\alpha = 0.05$ ), the cladocerans can be ranked as follows, according to increasing sensitivity to this *Microcystis* strain: *Cerio-*



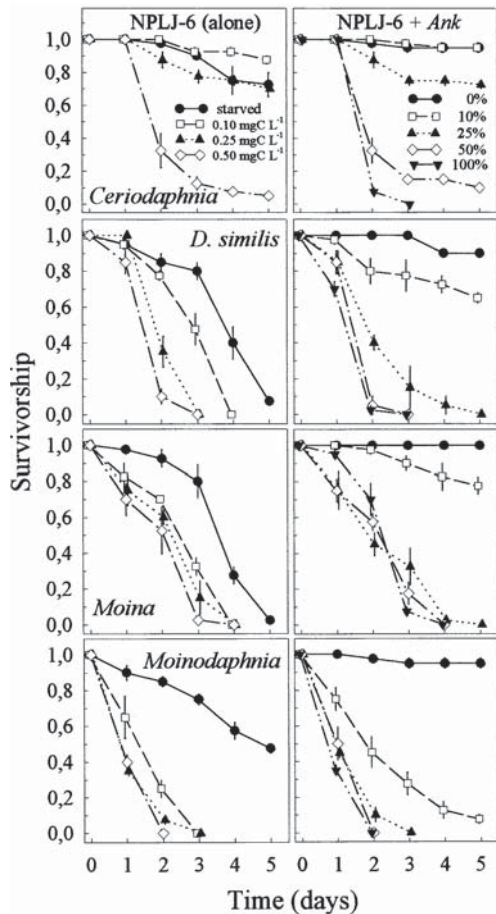


Fig. 3 Acute toxicity experiment with four cladoceran species exposed to the *Microcystis* strain NPLJ-6. There were two experimental conditions: 1) animals fed diets of *Microcystis* alone (left panels); and 2) animals fed mixed diets of *Microcystis* cells and the green alga *A. falcatus* (right panels). In the mixed diets, food concentration was kept constant at  $1.0 \text{ mg C L}^{-1}$ , with increasing percentage of *Microcystis* carbon in the diet. There were ten animals per tube and four replicate tubes per treatment.

*daphnia* ( $LT_{50} = 179 \text{ h}$ ); *Moina* ( $LT_{50} = 130 \text{ h}$ ); *D. similis* ( $LT_{50} = 100 \text{ h}$ ); and *Moinodaphnia* ( $LT_{50} = 77 \text{ h}$ ).

As in the previous experiments, diet type (single or mixed diets) strongly affected survival ( $F = 69.5$ ;  $P < 0.0001$ ). Addition of the green alga to the diet significantly increased survival ( $P < 0.0001$ ), especially in the lower concentrations of cyanobacteria (Fig. 3, right panels). The concentration of cyanobacteria in the food also had a significant effect on survival ( $F = 120.7$ ;  $P < 0.0001$ ). Increasing the proportion of *Microcystis* in the diet caused a significant reduction in survival of all cladocerans (Fig. 3, right panels).

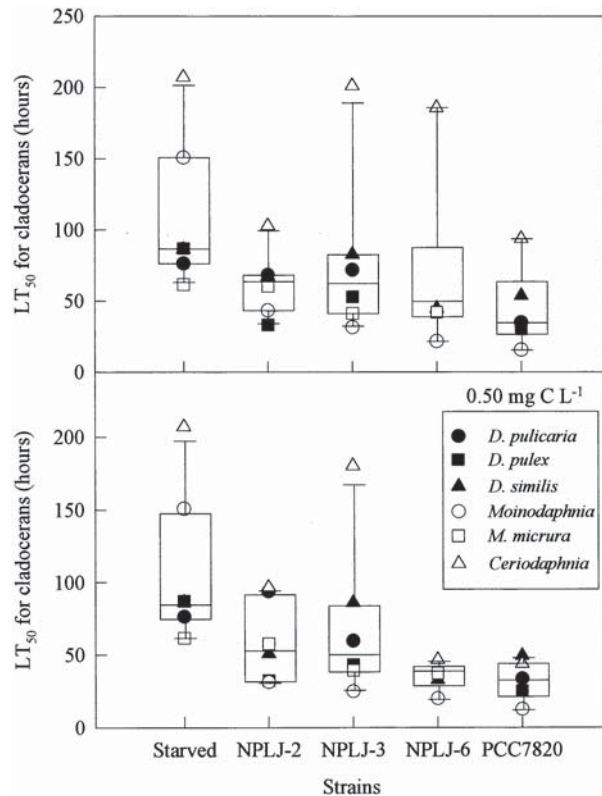


Fig. 4 Species ranking for all cladocerans according to the average  $LT_{50}$  values for intermediate concentrations of each *Microcystis* strain tested in the acute toxicity experiments with diets of *Microcystis* alone. The box plots indicate the means, error bars, 10th, 25th, 75th and 90th percentiles for all data in each strain.

In Fig. 4, we ranked all the results of the acute experiments according to  $LT_{50}$  values for each cladoceran species and for each strain of *Microcystis*, in the intermediate concentrations of single diets. This revealed consistent differences in cladoceran sensitivity and strain toxicity patterns. It is clear that cladocerans were, in general, more sensitive to the strain PCC7820, having lower mean values of  $LT_{50}$  for this strain, while strains NPLJ-2 and NPLJ-3 were less toxic. Strain NPLJ-6 showed toxicity similar to the PCC7820 strain. The tropical clone of the species *Moinodaphnia macleayi* was the most sensitive cladoceran, and the *C. cornuta* clone, from the same tropical lake, was the most resistant cladoceran to the presence of toxic cells. The tropical clone of *Moina micrura* and temperate *Daphnia* species were always intermediate in sensitivity to the *Microcystis* strains.

*Chronic exposure, life-table experiments*

The population growth rate of each cladoceran species was significantly reduced by an increasing concentration of toxic *Microcystis* (Table 4). Moreover, the intrinsic rate of increase ( $r$ ) was reduced in each treatment with a toxic strain of *Microcystis*, relative to the control with the green alga (Student's  $t$ -test,  $P < 0.05$ ). With one exception for *Moinodaphnia*, the

reduction in  $r$  was proportional to the percentage of *Microcystis* in the diet. At lower concentrations of toxic *Microcystis*, some individuals survived and reproduced, while others survived but did not reproduce. With higher concentrations of cyanobacteria, some cladocerans had their reproduction strongly inhibited, even to the point where all individuals died before reaching maturity.

**Table 4** Life-table data for cladocerans fed mixtures of toxic *Microcystis* and *Ankistrodesmus*, in a total concentration of 1.0 mg C L<sup>-1</sup>. Mean values of  $r$  were estimated by computer bootstrap resampling analysis (500 replicates per run) from survival and fecundity data. Treatments consisted of controls with 1.0 mg C L<sup>-1</sup> of the green alga only (0% toxic) and increasing percentages of toxic *Microcystis* in the diet

Zooplankton species	<i>Microcystis</i> strain	% toxic	$R_0$	$T$	$r$	CI (95%)	$r$ as % of control
<i>Daphnia pulex</i>	NPLJ-2 <sup>†</sup>	0.0	41.3	10.3	0.394	[0.365, 0.423]	100.0
		10.0	12.4	8.9	0.293	[0.257, 0.329]	74.4*
		20.0	2.5	7.8	0.110	[0.017, 0.202]	27.9*
		50.0	—	—	—	All animals died	—
<i>Moinodaphnia macleayi</i>	NPLJ-2 <sup>†</sup>	0.0	23.1	10.5	0.324	[0.301, 0.346]	100.0
		5.0	8.8	10.2	0.218	[0.178, 0.257]	67.3*
		10.0	—	—	—	No reproduction	—
		20.0	—	—	—	All animals died	—
	NPLJ-3 <sup>‡</sup>	0.0	28.0	11.2	0.341	[0.329, 0.354]	100.0
		5.0	19.1	11.2	0.290	[0.256, 0.325]	85.0*
		10.0	22.4	11.4	0.298	[0.284, 0.312]	87.4*
	NPLJ-6 <sup>‡</sup>	5.0	26.4	11.5	0.310	[0.299, 0.321]	90.9*
		10.0	16.4	10.8	0.276	[0.258, 0.293]	80.6*
		—	—	—	—	—	—
<i>Moina micrura</i>	NPLJ-2 <sup>†</sup>	0.0	43.3	10.7	0.475	[0.416, 0.535]	100.0
		5.0	6.6	9.2	0.235	[0.115, 0.355]	49.5*
		10.0	—	—	—	All animals died	—
	NPLJ-3 <sup>‡</sup>	5.0	20.4	9.6	0.393	[0.332, 0.453]	82.7*
		10.0	6.4	10.6	0.188	[0.132, 0.244]	39.6*
		20.0	2.3	11.1	0.109	[-0.003, 0.222]	22.9*
	NPLJ-6 <sup>‡</sup>	5.0	15.1	10.0	0.320	[0.266, 0.374]	67.4*
		10.0	4.6	9.9	0.169	[0.076, 0.262]	35.6*
		20.0	—	—	—	All animals died	—
		—	—	—	—	—	—
<i>Ceriodaphnia cornuta</i>	NPLJ-2 <sup>†</sup>	0.0	6.5	10.5	0.183	[0.157, 0.210]	100.0
		10.0	2.7	10.6	0.086	[0.062, 0.109]	47.0*
		20.0	0.9	10.6	-0.134	[-0.219, -0.049]	—
		50.0	—	—	—	No reproduction	—
	NPLJ-3 <sup>‡</sup>	0.0	12.7	11.8	0.238	[0.207, 0.269]	100.0
		5.0	9.9	12.6	0.193	[0.182, 0.204]	81.1*
		10.0	7.5	13.1	0.159	[0.138, 0.181]	66.8*
		20.0	5.1	12.4	0.131	[0.106, 0.157]	55.0*
		40.0	1.6	12.3	0.034	[-0.041, 0.108]	14.3*
	NPLJ-6 <sup>‡</sup>	5.0	8.8	12.5	0.194	[0.174, 0.213]	81.5*
		10.0	4.7	12.0	0.129	[0.104, 0.154]	54.2*
		20.0	2.5	11.5	0.086	[0.055, 0.116]	36.1*
		40.0	0.5	11.5	-0.044	[-0.136, 0.047]	—

$R_0$ : net reproductive rate;  $T$ : generation time;  $r$ : intrinsic rate of natural increase; CI: confidence intervals for  $r$  (95%).

\* Significant differences between controls and *Microcystis* treatments ( $t$ -test,  $P < 0.05$ ).

<sup>†</sup> Experiments run in November 1995.

<sup>‡</sup> Experiments run in February 1996.

The pattern of inhibition in the population growth rates of cladocerans was, in general, consistent with the acute toxicity experiments. The moinids were the most affected species, having their reproduction inhibited at a concentration as low as 5% of toxic *Microcystis* in the diet. *D. pulex* had decreased reproduction with increasing proportion of the strain NPLJ-2 in the diet, but was less affected than the moinids, showing positive population growth with 20% of toxic *Microcystis* cells. *Ceriodaphnia* was again the most resistant species, exhibiting positive growth in most of the treatments with toxic *Microcystis*. Despite the fact that *Ceriodaphnia* experienced negative population growth with 20% of the NPLJ-2 strain, most individuals reached maturity and reproduced at least once during the experiment.

The NPLJ-2 strain had the strongest adverse effect on cladoceran population growth rates. Each cladoceran exhibited its lowest  $r$  value with this strain. This strain also provided the strongest adverse effects on survival in the acute experiments with mixed diets. The strains NPLJ-3 and NPLJ-6 produced weaker adverse effects on the three cladoceran species. Although there was a consistent reduction in population growth rate for all cladoceran species with both strains,  $r$  values were almost always positive in the treatments with NPLJ-3 and NPLJ-6 strains.

#### Growth experiment with a nutritionally deficient, non-toxic cyanobacterium

Initially, animals in all treatments showed rapid growth rate, reaching more than double their initial

mass during the first 2 days of the experiment (Fig. 5). Mean growth rate during the first 2 days ( $\pm$  standard error (SE)) was not statistically different among the four treatments (Student–Newman–Keuls test,  $P = 0.29$ ). By the fourth day, however, animals in the *Synechococcus* treatment lost weight, whereas animals in the control with *Ankistrodesmus* and treatments with *Synechococcus* mixed either with the green alga (50% treatment) or a fish oil emulsion (+FO treatment) showed a high growth rate until the sixth and final day of the experiment (Table 5). Animals in the *Synechococcus* treatment started dying after the fourth

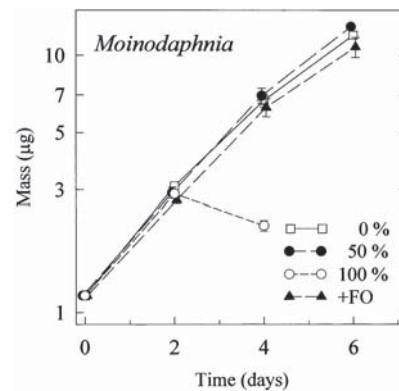


Fig. 5 Effects of *Synechococcus* on the growth rate ( $\text{day}^{-1}$ ) of *Moinodaphnia*. Animals fed on mixtures of *Synechococcus* and *Ankistrodesmus* at a total concentration of  $1.0 \text{ mg C L}^{-1}$  ('0%' = control = 100% *Ankistrodesmus*), or on mixtures of fish oil emulsions and *Synechococcus* (FO+). Data are means  $\pm$  SE for three replicate bottles per treatment.

Table 5 Results of one-way ANOVA for the net growth rate experiment. Data are mean growth rates ( $\text{day}^{-1}$ )  $\pm$  SE for 6 days of experiment

	Treatments				F	P
	Syn	Ank	50%	+FO		
0–2nd day	$0.454 \pm 0.023$	$0.489 \pm 0.031$	$0.457 \pm 0.049$	$0.427 \pm 0.036$	1.49	0.2889
0–4th day	$0.157 \pm 0.015$	$0.436 \pm 0.028$	$0.445 \pm 0.027$	$0.419 \pm 0.031$	84.96	<0.0001
0–6th day	—	$0.389 \pm 0.015$	$0.401 \pm 0.014$	$0.369 \pm 0.024$	2.33	0.1782

There were three replicates per treatment, which included: Syn = *Synechococcus*; Ank = *Ankistrodesmus*; +FO = Syn + fish oil emulsion; and a 1:1 mixture of *Ankistrodesmus* and *Synechococcus* (50%). Algal concentrations were  $1.0 \text{ mg C L}^{-1}$  and oil emulsion was  $0.2 \text{ mg C L}^{-1}$ . Treatments connected by a solid line are not significantly different (Student–Newman–Keuls test,  $P > 0.05$ )

day; therefore, we have data for only 4 days for this treatment. On the fourth day, mean growth rates in the treatment with 100% *Synechococcus* were significantly lower than that of the other treatments (Student–Newman–Keuls test,  $P < 0.0001$ ). By the sixth day, there were no significant differences in growth rate between animals fed *Ankistrodesmus*, mixtures of *Synechococcus* and *Ankistrodesmus* or *Synechococcus* and the fish oil emulsion (Student–Newman–Keuls test,  $P = 0.18$ ).

*Moinodaphnia* did not reproduce in the treatment with 100% *Synechococcus*. However, animals in the other treatments appeared healthy and most individuals produced eggs by the end of the experiment. Mean number of eggs ( $\pm$  SE) were as follows: *Ankistrodesmus*:  $5.23 \pm 0.36$ ; 50%:  $5.25 \pm 2.10$ ; + FO:  $4.21 \pm 2.90$  (not significant, Student–Newman–Keuls test,  $P > 0.05$ ).

#### Feeding inhibition experiments

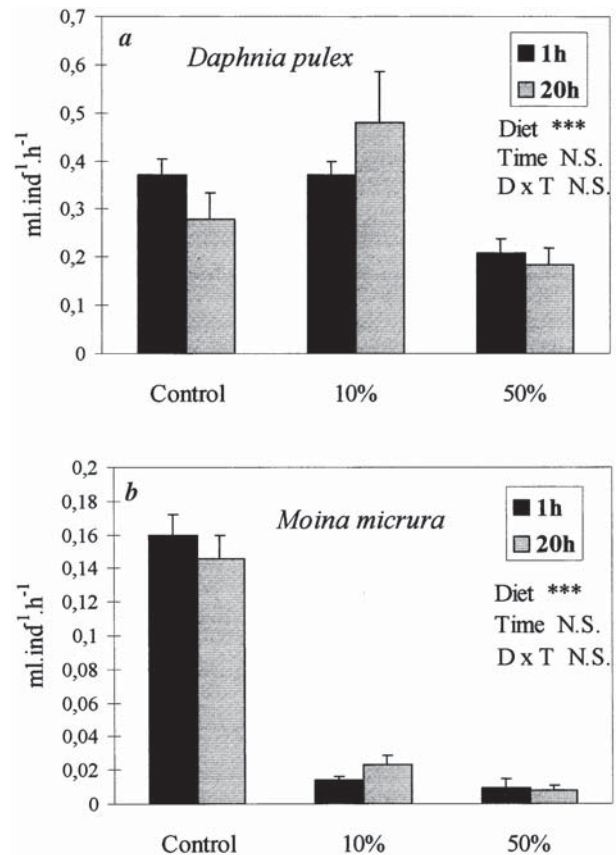
Both of the cladocerans tested exhibited feeding inhibition in mixtures of toxic *Microcystis* and *Ankistrodesmus*, but the magnitude of the inhibition differed (Fig. 6). The filtering rate of the temperate cladoceran, *D. pulex*, was more weakly inhibited than that of the tropical species, *Moina micrura*. In a 10% mixture of *Microcystis*, *D. pulex* did not show any reduction in its filtering rate after 1 or 20 h of acclimatization time (Fig. 6a). In contrast, in the 50% *Microcystis* treatment, *D. pulex* showed a significant reduction in filtering rate, which was, on average, only 44.0 and 34.3% of that of the control animals for 1 and 20 h acclimatization time, respectively ( $P = 0.002$ ).

For *Moina*, there was a strong and significant reduction in filtering rate in both mixtures (Fig. 6b). In the 10% *Microcystis* treatments, mean filtering rate for *Moina* was reduced to only 8.7 and 15.8% of controls in 1 and 20 h acclimatization times, respectively ( $P = 0.001$ ). In 50% *Microcystis* treatment, the reduction was even greater, reaching, on average, 5.8 and 5.3% of the control for 1 and 20 h acclimatization times, respectively ( $P = 0.001$ ). Neither cladoceran showed a significant effect of acclimatization time on filtering rate (Student–Newman–Keuls test,  $P > 0.05$ ).

## Discussion

### Acute toxic effects of *Microcystis*

Tropical and temperate cladocerans showed a wide



**Fig. 6** Effects of *Microcystis* strain NPLJ-2 on the feeding rates of a) *Daphnia pulex* and b) *Moina micrura*. Animals fed on mixtures of *Microcystis* and *Ankistrodesmus* at a total concentration of  $1.0 \text{ mg C L}^{-1}$ . Treatments indicate the percentage of *Microcystis* in the diet (control = 100% *Ankistrodesmus*). Animals were acclimatized for 1 or 20 h on the experimental food mixtures. Two-way ANOVA tested the effect of *Microcystis* ('diet') and acclimatization time ('time') on the feeding rates.

range of responses to toxic strains of *Microcystis*, depending on the cladoceran species, strain and diet type. Species ranking (Fig. 4) showed consistent differences among the cladoceran species but, contrary to our initial hypothesis, we did not find a consistent relationship between cladoceran origin and sensitivity to toxic cyanobacteria. There were both tropical and temperate more sensitive species (*Moinodaphnia*, *Moina* and *D. pulex*) and more resistant species (*Ceriodaphnia*, *D. pulicaria* and *D. similis*).

As *Microcystis* can inhibit filtering rates of cladocerans (Lampert, 1982; Nizan *et al.*, 1986; DeMott *et al.*, 1991) and is of poor nutritional value (Hazanato &

Yasuno, 1987; DeBernardi & Giussani, 1990), a diet of only toxic *Microcystis* can cause starvation and death. Typically, resistance to starvation is thought to scale with body size, with larger organisms respiring a lower percentage of their body mass per day (Threlkeld, 1976). Therefore, according to the model of Threlkeld (1976), large-bodied cladocerans should have higher resistance to starvation, surviving better when starved or when fed low quality food than small-bodied cladocerans. Surprisingly, our results contradicted these predictions. The smallest cladoceran used in our experiments, *C. cornuta*, was the most resistant whereas another small species from a tropical lake, *Moina micrura*, was the most sensitive to starvation (Fig. 4). The tropical *Moinodaphnia* was also more resistant to starvation than the much larger *Daphnia* species. There was also no consistent effect of body size in the sensitivity of cladocerans to toxic *Microcystis*. Two of the smallest species, *Ceriodaphnia* and *Moinodaphnia*, were the least and most sensitive species, respectively. Perhaps, the trophic status of the lakes of origin may help to explain these patterns in starvation resistance and sensitivity to toxic cyanobacteria among the cladocerans (Table 2). Both *Ceriodaphnia* and *Moinodaphnia* clones came from a tropical coastal lagoon with low primary productivity and may have been adapted to low food levels, whereas *Moina micrura* came from a tropical hyper-eutrophic coastal lagoon, in which *Microcystis* blooms heavily during most of the year. *Daphnia* species came from different sources, but they are probably well adapted to large fluctuations in resource levels. *D. pulicaria* came from Crooked Lake, a mesotrophic hard water lake, where some toxic *Oscillatoria* occurs. *D. pulex* is a common species in swamps, marshes and temporary ponds, where cyanobacteria are rare. Epp (1996) also showed that resistance to starvation can vary largely within different genotypes (clones) of *D. pulicaria*, which is also contrary to the body size–starvation resistance model proposed by Threlkeld (1976).

Our results suggest that the sensitivity of cladocerans to toxic *Microcystis* is associated with life histories rather than to geographic origin. In analogy with strategies in vascular plants (Ramensky, 1938; Grime, 1979), Romanovsky (1985) proposed three types of life history strategies in cladocerans. Small-bodied, fast-growing species ('explerents' or 'ruderals') are highly susceptible to starvation and to lower food

quality. These species have a short life span and allocate much energy to reproduction, as is shown by their high rate of population increase (high  $r$ ), and they can temporarily inhabit very productive environments, exploiting periods of high food availability. Large-bodied, fast-growing species ('violents' or 'competitors') have juveniles that are prone to starvation because of their relatively small size at birth and higher allocation of energy to somatic growth during the early instars. However, adults of large-bodied species, such as *Daphnia*, are more resistant to reduced food supply and are superior competitors in relatively undisturbed environments, with a potentially high rate of population growth (high  $r$ ). The third group of small-bodied, slow-growing species ('patients' or 'stress tolerators') is able to survive food depletion in disturbed environments due to a lower threshold food concentration, a low rate of juvenile growth and a slow population growth (low  $r$ ).

These three types of life history are well represented by the cladoceran species tested here. The genus *Moina* can be classified as 'explerent' because it has a high potential for population growth (high  $r$ ) and is highly sensitive to starvation. *Ceriodaphnia* fits well in the 'patient' type, having a high resistance to starvation and low population growth rate (low  $r$ ). The *Daphnia* species can be classified as 'violent', with a large body size at maturity and a relatively high population growth rate (high  $r$ ). In his hypothesis, Romanovsky (1985) mostly considered food quantity, although poor food quality and even toxic food could cause the same effects. Therefore, it seems plausible that species that are highly sensitive to starvation should also be more sensitive to toxic algae. Our results fit well with these expectations because two of the species most sensitive to starvation and with high potential for population growth (*Moina* and *D. pulex*) were also most sensitive to the toxic strains of *Microcystis*.

The toxicity of strain PCC7820 to temperate cladoceran species is well known (Nizan *et al.*, 1986; DeMott *et al.*, 1991; Reinikainen, Ketola & Walls, 1994; Hietala *et al.*, 1995; Smith & Gilbert, 1995), whereas no previous study has compared the toxicity of this strain with that of *Microcystis* strains from the tropics. The strain PCC7820 produces more than one kind of microcystin (LR, RR, YR and LA), all of them with similar toxic properties (Carmichael, 1992). Our analysis of the Brazilian strains did not, however, identify the different chemical forms of microcystins.

The cellular microcystin concentration varied little between Brazilian *Microcystis* strains and was within the same range found for other strains (DeMott *et al.*, 1991; Hietala *et al.*, 1995). Microcystin production, however, can vary with culture conditions, such as light intensity, nutrient medium composition and culture age (Sivonen, 1990; Utkilen & Gjørlme, 1992; Rapala *et al.*, 1997; Watanabe *et al.*, 1989). In this study, experiments were run at different times and toxin content was measured once or twice only for each strain of *Microcystis*. We did not confirm that microcystin content was the same during the experiments and, therefore, we did not test for a correlation between microcystin content and effects observed in the toxicity experiments. In addition, we cannot exclude the possibility that other toxins acted synergistically with microcystins. Recent studies have suggested that *Microcystis* toxins, other than microcystins, can exert acute toxic effects on *Daphnia* (Jungmann, 1992; Jungmann & Benndorf, 1994).

Differences in digestibility of the *Microcystis* cells could be another factor influencing toxicity for cladocerans. Microcystins are endotoxins (Carmichael, 1992), delivered only after digestion of the toxic cells during passage through the gut. Our strains showed differences in size and cell sheath production that may have caused differences in digestibility. The NPLJ-3 strain, which was one of the least toxic strains, produced large amounts of mucilage, even making toxin extraction difficult. The NPLJ-3 extracts looked like a gel, while the other strains had extracts with liquid consistency. However, differences in digestibility cannot explain the similarity in toxicity between NPLJ-2 and NPLJ-3 strains to some of the cladocerans. Porter (1975) showed that different zooplankton species have different abilities to digest the gelatinous sheaths of certain green algae. Also, Lampert (1982) suggested that zooplankton species differ in their capacity to digest algal cells and, therefore, are variously susceptible to toxic cyanobacteria. This may explain the higher sensitivity of *Ceriodaphnia* and *Daphnia* species to the NPLJ-2 strain, which produces less mucilage than the NPLJ-3 strain. The PCC7820 strain, which was the most toxic strain in the acute experiments, apparently has a thinner mucilaginous sheath than the other strains.

Diet composition also played an important role in the toxicity of *Microcystis* strains to the cladocerans. In nature, zooplankton generally feed on a mixed diet of

seston and it is unlikely that it will experience a diet purely of toxic cyanobacteria. Even in lakes dominated by cyanobacteria, the zooplankton diet will include good quality foods, such as green algae, bacteria and detritus, that may increase survival and reproduction.

The several experimental designs used in the acute toxicity experiments also showed that much more sensitivity was detected when we used mixed diets. In the experiments with single diets, there was a weak effect of *Microcystis* concentration on cladoceran survival, whereas in the mixed diets there were more statistically significant differences between survival curves in the different concentrations of cyanobacteria, suggesting a stronger effect of the *Microcystis* strains. These results also showed that the survival of cladocerans was significantly improved by adding a high quality alga. Therefore, the most suitable approach to estimate the toxicity of cyanobacteria seems to be one using mixed diets. Furthermore, the technical definition of toxic cyanobacteria, in which a strain is considered toxic only if it causes more deaths than the controls with starved animals, must to be viewed with caution. This is particularly important in the case of species that are very sensitive to starvation, such as *Moina micrura*.

#### *Effects of toxic Microcystis on population growth*

Both the tropical and the temperate cladocerans showed decreased growth and reproduction on the mixed diets including toxic *Microcystis*. Population growth rate ( $r$ ) decreased as the proportion of *Microcystis* in the diet increased, thus showing that sublethal mixtures of *Microcystis* in the diet can cause a population decline. Reproduction decreased in cladocerans with a *Microcystis* concentration as low as 5%, and even in higher concentrations of *Microcystis*, where good food (green algae) was still available above the threshold requirements of cladocerans. Therefore, these results are consistent with the chronic toxicity rather than the nutritional deficiency hypothesis. In the experiment with *Synechococcus*, however, as in other studies using mixtures of nutritionally deficient cyanobacteria (DeMott & Müller-Navarra, 1997; DeMott, 1998), growth and reproduction in a mixture of 50% cyanobacteria and green algae did not differ from the control with 0% cyanobacteria (100% *Ankistrodesmus*), not suggesting toxic effects in these cases.

The effects of *Microcystis* on cladoceran reproduction were of two kinds: 1) a delay of 1 or more days in age at first reproduction (data not shown); and 2) a reduction in reproductive output (eggs/female and clutch size; data not shown). As Allan (1976) pointed out, age at first reproduction and clutch size are the key factors in determining the intrinsic rate of natural increase. Furthermore, animals in the *Microcystis* treatments appeared smaller than control animals supplied with green algae and their ovaries were less developed. These effects were also observed in other studies with strain PCC7820 and non-toxic *Microcystis* strains (Hietala *et al.*, 1995; Smith & Gilbert, 1995).

Comparison of cladocerans in terms of absolute  $r$  values seems inappropriate, because the species differ in their life history traits and maximal population growth rates. Therefore, we compared  $r$  values as a percentage of the controls. In general, species differences in sensitivity to toxic algae, noted in the acute tests, were also reflected in the chronic tests. The cladocerans that had high  $r$  values in the controls and were more sensitive in the acute tests (e.g. *Moina*) had their reproduction more affected by *Microcystis* strains than those with low  $r$  values in the controls, which were also less sensitive to the toxic strains in the acute tests (e.g. *Ceriodaphnia*). These results are supported by Romanovsky's (1985) idea that cladocerans that invest heavily in reproduction when food conditions are suitable ('explerents') are more affected by starvation (or low food quality) than cladocerans that can resist longer periods of starvation ('patients').

Despite better survival, the population growth of *Moina* was more affected by strains NPLJ-3 and NPLJ-6 than *Moinodaphnia*, which was the most sensitive species in the acute toxicity experiments. Such differences in sensitivity to *Microcystis* between these two moinid species seems to reflect a trade-off between the allocation of energy to reproduction and to somatic maintenance in low quality food stress, because these two moinid species have different life histories in conditions of good food (controls with the green algae). *Moina* had a higher population growth rate in the controls, apparently investing more energy in reproduction than *Moinodaphnia* when food conditions were good. In the presence of low quality food (*Microcystis*), however, *Moina* survived better than *Moinodaphnia*, but at the expense of lowered reproduction.

For the only temperate cladoceran used in the life-table experiments, *D. pulex*, the rate of population increase was less affected by the NPLJ-2 strain than in the other cladocerans, despite being one of the most sensitive cladocerans in the acute toxicity experiments. This large-bodied cladoceran, which can be a superior competitor ('violent') in temperate zooplankton communities, typically has a large clutch size and a lower threshold food concentration for growth compared with small-bodied cladocerans, such as *Moina* (Romanovsky, 1985). Therefore, as in *Moinodaphnia*, *D. pulex* seems to invest more energy in maintaining a high rate of population increase despite lower survival in the presence of low quality food.

#### *Effects of nutritionally deficient cyanobacteria on growth and reproduction*

As observed with the toxic strains of *Microcystis*, the cyanobacterium *S. elongatus* caused reduced growth and reproduction in *Moinodaphnia* when offered as a single diet. When *Synechococcus* was complemented with high quality food (*Ankistrodesmus*) or fish oil emulsions, however, this cladoceran exhibited excellent growth and reproduction. In contrast, even small additions of *Microcystis* into the diet caused reductions in survival and reproduction in the acute and chronic experiments. These results suggest that *Synechococcus* is a non-toxic but nutritionally deficient resource. DeMott & Müller-Navarra (1997) found similar results for three temperate *Daphnia* species. As in this study, *D. pulicaria* failed to reproduce on a diet of *Synechococcus* alone. These authors attributed their results to the low content of polyunsaturated fatty acids (PUFAs) found in this cyanobacterium, such as linolenic acid, eicosapentatenoic acid (EPA) and docosahexaenoic acid (DHA), which are important dietary constituents (Ahlgren *et al.*, 1990; Müller-Navarra, 1995). In contrast, green algae like *Ankistrodesmus* and *Scenedesmus* are considered good foods and have a high contents of PUFAs, such as linolenic acid (Ahlgren, Gustafsson & Boberg, 1992), supporting good growth and reproduction for *Daphnia* (DeMott & Müller-Navarra, 1997; Kilham *et al.*, 1997; Lüring & Van Donk, 1997; Repka, 1997).

Although *Synechococcus* lacks some essential fatty acids, it is rich in phosphorus (P) and can support high growth rates in mixed diets (DeMott, 1998).

Complementing the diet of P-limited *Scenedesmus* with *Synechococcus*, DeMott (1998) found improved growth rates for five daphnid species. Therefore, in this case, a nutritional deficiency and not toxicity was the basis for poor growth and reproduction of the cladocerans. On the other hand, *Microcystis* has a high content of PUFAs (Krüger *et al.*, 1995) and non-toxic *Microcystis* can support good growth as a sole food resource (DeBernardi, Giussani & Pedretti, 1981; Lundstedt & Brett, 1991). Thus, *Synechococcus* cannot be considered a good model for non-toxic *Microcystis* because it has a different fatty acid composition. Also, Schimdt & Jónasdóttir (1997) found that when *Microcystis aeruginosa* was mixed in small proportions with the diatom *Thalassiosira weissflogii*, egg production of the copepod *Acartia tonsa* increased relative to single food diets of the diatom and cyanobacteria alone. In this case, the two foods were complementary resources and no toxic effect can be assumed.

#### Feeding inhibition by toxic *Microcystis*

The two cladocerans tested, *D. pulex* and *Moina micrura*, showed great differences in the strength of feeding inhibition. Although both were sensitive to the NPLJ-2 strain in the acute toxicity experiments, *M. micrura* showed much stronger feeding inhibition than *D. pulex* in the presence of that strain. *M. micrura* was also very sensitive to starvation, showing rapid mortality in the controls without food. As we mentioned before, some of the deaths in the acute toxicity experiment can be attributed not only to toxicity of the *Microcystis* but also to reduced food intake, caused by feeding inhibition.

According to Lampert (1982), the reduction in filtering rate can be a behavioural mechanism by which cladocerans can avoid ingesting toxic cells. This author showed that, after a short exposure (30 min) to toxic *Microcystis*, the filtering rate of *D. pulicaria* recovered immediately to the control level after they have received a pure suspension of *Scenedesmus*. DeMott *et al.* (1991) verified that *D. pulex* showed a lower reduction in the filtering rate and also that this species was more sensitive to dissolved microcystin-LR, than *D. pulicaria*; they considered this to be partly responsible for the higher mortality rate of *D. pulex* in the acute toxicity experiments. This agrees with our results and with Lampert's (1982) idea that rapid feeding inhibition could be a protec-

tive behaviour against the ingestion of toxic cells. Because *D. pulex* seems less inhibited by the presence of toxic cells, it probably ingests more of them than *D. pulicaria*.

Hunger is an important factor in the selection of algae by cladocerans and copepods (DeMott & Moxter, 1991; DeMott, 1993). If feeding inhibition protects cladocerans, feeding rate might increase after prolonged exposure to toxic algae. However, if feeding inhibition was caused mainly by direct toxicity and incapacity of feeding after a 1-h acclimatization time, we should expect that feeding rate would remain low, and might even decrease, after long-term continuous exposure to a diet containing toxic algae. In a study with five daphnids, DeMott (1999) found both patterns. Some species, including *D. pulex*, showed little or no recovery after long-term exposure to toxic *Microcystis*, while others, especially *Daphnia magna*, exhibited much higher filtering rates after 24 h of exposure to toxic *Microcystis* than after 1 h of exposure. In our experiments, however, neither cladoceran showed significant recovery after 20 h of acclimatization time.

Although we found a high content of microcystins in the *Microcystis* strains tested, we cannot necessarily attribute all toxic effects observed to the presence of these toxins. Other studies have found no relationship between the acute toxicity of *Microcystis* strains and the inhibition of filtering rates (Nizan *et al.*, 1986; Jungmann *et al.*, 1991). However, recent work by Rohrlak *et al.* (1999), using a mutant *Microcystis* clone from the PCC7806 strain that was not able to synthesize any variant of microcystin, proved that these toxins were the cause of mortality in *Daphnia galeata* but were not responsible for the inhibition of its filtering rates.

Previous studies of interactions between toxic cyanobacteria and zooplankton have focused on zooplankton from the temperate zone, with most studies testing various species of *Daphnia* (reviews by Lampert, 1987 and Christoffersen, 1996). In general, we found that results from temperate zooplankton concerning acute and chronic toxicity, nutritional deficiencies and feeding inhibition also apply to tropical zooplankton. Thus, toxic or nutritionally deficient cyanobacteria can be a selective mechanism in zooplankton communities, both in tropical and in temperate environments. Although zooplankton species vary markedly in their sensitivity to toxic *Micro-*



*cystis*, an understanding of this variation may be found in life history variation rather than in the geographic distribution.

### Acknowledgments

This research was supported by a fellowship from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brazil). The first two authors would like to thank Dr. DeMott for his collaboration, encouragement and advice during the first author's stay at the Crooked Lake Biological Station, Indiana, U.S.A.

### References

- Ahlgren G., Lundstedt L., Brett M.T. & Forsberg C. (1990) Lipid composition and food quality of some freshwater phytoplankton for cladoceran zooplankters. *Journal of Plankton Research*, **12**, 809–818.
- Ahlgren G., Gustafsson I.-B. & Boberg M. (1992) Fatty acid content and chemical composition of freshwater microalgae. *Journal of Phycology*, **28**, 37–50.
- Allan D. (1976) Life history patterns in zooplankton. *American Naturalist*, **110**, 165–180.
- Arment A.R. & Carmichael W.W. (1996) Evidence that microcystin is a thio-template product. *Journal of Phycology*, **32**, 591–597.
- Burns C.W. & Xu Z. (1990) Calanoid copepods feeding on algae and filamentous cyanobacteria: rates of ingestion, defecation and effects on trichome length. *Journal of Plankton Research*, **12**, 201–213.
- Carmichael W.W. (1992) Cyanobacteria secondary metabolites: the cyanotoxins. *Journal of Applied Bacteriology*, **72**, 445–459.
- Christoffersen K. (1996) Ecological implications of cyanobacterial toxins in aquatic food webs. *Phycologia*, **35** (Suppl 6), 42–50.
- DeBernardi R. & Giussani G. (1990) Are blue-green algae a suitable food for zooplankton? An overview. *Hydrobiologia*, **200/201**, 29–41.
- DeBernardi R., Giussani G. & Pedretti E. (1981) The significance for blue-green algae for filter-feeding zooplankton: experimental studies on *Daphnia* spp. fed *Microcystis aeruginosa*. *Verhandlungen der internationale Vereinigung für theoretische und angewandte Limnologie*, **20**, 1045–1048.
- DeMott W.R. (1988) Discrimination between algae and artificial particles by freshwater and marine copepods. *Limnology and Oceanography*, **33**, 397–408.
- DeMott W.R. (1990) Retention efficiency, perceptual bias and active choice as mechanisms of food selection by suspension-feeding zooplankton. In: *Behavioural Mechanisms of Food Selection* (ed. R.N. Hughes), pp. 569–593. Springer-Verlag, Berlin.
- DeMott W.R. (1993) Hunger-dependent diet selection in suspension-feeding zooplankton. In: *Diet Selection: An Interdisciplinary Approach to Foraging Behaviour* (ed. R.N. Huges), pp. 102–123. Blackwell Scientific, Oxford.
- DeMott W.R. (1998) Utilization of a cyanobacterium and a phosphorus-deficient green algae as complementary resources by daphnids. *Ecology*, **79**, 2463–2481.
- DeMott W.R. (1999) Foraging strategies and growth inhibition in five daphnids feeding on mixtures of a toxic cyanobacterium and green algae. *Freshwater Biology*, **42**, 263–274.
- DeMott W.R. & Dhawale S. (1995) Inhibition of *in vitro* protein phosphatase activity in three zooplankton species by microcystin-LR, a toxin from cyanobacteria. *Archiv für Hydrobiologie*, **134**, 417–424.
- DeMott W.R. & Moxter F. (1991) Foraging cyanobacteria by copepods: responses to chemical defences and resource abundance. *Ecology*, **72**, 1820–1834.
- DeMott W.R. & Müller-Navarra D.C. (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.
- DeMott W.R., Zhang Q.X. & Carmichael W.W. (1991) Effects of toxic cyanobacteria and purified toxins on the survival and feeding of a copepod and three species of *Daphnia*. *Limnology and Oceanography*, **36**, 1346–1357.
- Epp G.T. (1996) Clonal variation in the survival and reproduction of *Daphnia pulicaria* under low-food stress. *Freshwater Biology*, **35**, 1–10.
- Fulton R.S. & Jones C. (1991) Growth and reproductive responses of *Daphnia* to cyanobacterial blooms on the Potomac River. *Internationale Revue der gesamten Hydrobiologie*, **76**, 5–19.
- Fulton R.S. & Pearl H.W. (1987) Toxic and inhibitory effects of the blue-green alga *Microcystis aeruginosa* on herbivorous zooplankton. *Journal of Plankton Research*, **9**, 837–855.

- Gilbert J.J. (1994) Susceptibility of planktonic rotifers to a toxic strain of *Anabaena flos-aquae*. *Limnology and Oceanography*, **39**, 1286–1297.
- Gliwicz Z.M. (1990) *Daphnia* growth at different concentrations of blue–green filaments. *Archiv für Hydrobiologie*, **120**, 51–65.
- Gorham P.R., McLachlan L., Hammer U.T. & Kim W.K. (1964) Isolation and culture of toxic strains of *Anabaena flos-aquae* (Lyngb.) de Bréb. *Verhandlungen der internationale Vereinigung für theoretische und angewandte Limnologie*, **15**, 796–804.
- Grime J.P. (1979) *Plant Strategies and Vegetation Processes*. Wiley, New York.
- Haney J.F., Sasner J.J. & Ikawa M. (1995) Effects of products released by *Aphanizomenon flos-aquae* and purified saxitoxin on the movements of *Daphnia carinata* feeding appendages. *Limnology and Oceanography*, **40**, 263–272.
- Hazanato T. & Yasuno M. (1987) Evaluation of *Microcystis* as food for zooplankton in an eutrophic lake. *Hydrobiologia*, **144**, 251–259.
- Hietala J., Reinikainen M. & Walls M. (1995) Variation in life history responses of *Daphnia* to toxic *Microcystis aeruginosa*. *Journal of Plankton Research*, **17**, 2307–2318.
- Infante A. & Abella S.E.B. (1985) Inhibition of *Daphnia* by *Oscillatoria* in Lake Washington. *Limnology and Oceanography*, **30**, 1046–1052.
- Jungmann D. (1992) Toxic compounds isolated from *Microcystis* PCC7806 that are more active against *Daphnia* than two microcystins. *Limnology and Oceanography*, **37**, 1777–1783.
- Jungmann D. (1995) Isolation, purification and characterization of a new *Daphnia*-toxic compound from axenic *Microcystis flos-aquae* strain PCC7806. *Journal of Chemical Ecology*, **21**, 1665–1676.
- Jungmann D. & Benndorf J. (1994) Toxicity to *Daphnia* of a compound extracted from laboratory and natural *Microcystis* spp., and the role of microcystins. *Freshwater Biology*, **32**, 13–20.
- Jungmann D., Henning M. & Jüttner F. (1991) Are the same compounds in *Microcystis* responsible for its toxicity to *Daphnia* and inhibition of its filtering rate? *Internationale Revue der gesamten Hydrobiologie*, **76**, 47–56.
- Kilham S.S., Kreeger D.A., Goulden C.E. & Lynn S.G. (1997) Effects of algal food quality on fecundity and population growth rates of *Daphnia*. *Freshwater Biology*, **38**, 639–647.
- Kirk K.L. & Gilbert J.J. (1992) Variation in herbivore response to chemical defenses: zooplankton foraging on toxic cyanobacteria. *Ecology*, **73**, 2208–2217.
- Krishnamurthy T., Carmichael W.W. & Sarver E.W. (1986) Toxic peptides from freshwater cyanobacteria (blue–green algae). I. Isolation, purification and characterization of peptides from *Microcystis aeruginosa* and *Anabaena flos-aquae*. *Toxicon*, **24**, 865–873.
- Krüger G.H.J., De Wet H., Kock J.L.F. & Pieterse A.J.H. (1995) Fatty acid composition as a taxonomic characteristic for *Microcystis* and other coccoid cyanobacteria (blue–green algae) isolates. *Hydrobiologia*, **308**, 145–151.
- Lampert W. (1977) Studies on carbon balance of *Daphnia pulex* De Geer as related to environmental conditions. II. The dependence of carbon assimilation on animal size, temperature, food concentration and diet species. *Archiv für Hydrobiologie*, **48**, 310–335.
- Lampert W. (1981a) Toxicity of blue–green *Microcystis aeruginosa*: effective defence mechanism against grazing pressure by *Daphnia*. *Verhandlungen der internationale Vereinigung für theoretische und angewandte Limnologie*, **21**, 1436–1440.
- Lampert W. (1981b) Inhibitory and toxic effects of blue–green algae on *Daphnia*. *Internationale Revue der gesamten Hydrobiologie*, **66**, 285–298.
- Lampert W. (1982) Further studies on the inhibitory effect of the toxic blue–green *Microcystis aeruginosa* on the filtering rate of zooplankton. *Archiv für Hydrobiologie*, **95**, 207–220.
- Lampert W. (1987) Laboratory studies on zooplankton–cyanobacteria interactions. *New Zealand Journal of Marine and Freshwater Research*, **21**, 483–490.
- Lundstedt L. & Brett M.T. (1991) Differential growth rates of three cladoceran species in response to mono- and mixed-algal cultures. *Limnology and Oceanography*, **36**, 159–165.
- Lürling M. & Van Donk E. (1997) Life history consequences for *Daphnia pulex* feeding on nutrient-limited phytoplankton. *Freshwater Biology*, **38**, 693–709.
- Matveev V.F. & Balseiro E.G. (1990) Contrasting responses of two cladocerans in the nutritional value of nanoplankton. *Freshwater Biology*, **23**, 197–204.
- Meyer J.S., Ingersoll C.G., McDonald L.L. & Boyce M.S. (1986) Estimating uncertainty in population growth rates: jackknife vs. bootstrap techniques. *Ecology*, **67**, 1156–1166.

- Müller-Navarra D.C. (1995) Evidence that a highly unsaturated fatty acid limits *Daphnia* growth in nature. *Archiv für Hydrobiologie*, **132**, 297–307.
- Nizan S., Dimentman C. & Shilo M. (1986) Acute toxic effects of the cyanobacterium *Microcystis aeruginosa* on *Daphnia magna*. *Limnology and Oceanography*, **31**, 497–502.
- Porter K.G. (1975) Viable gut passage of gelatinous green algae ingested by *Daphnia*. *Verhandlungen der internationale Vereinigung für theoretische und angewandte Limnologie*, **19**, 2840–2850.
- Porter K.G. & Orcutt J.D. (1980) Nutritional adequacy, manageability and toxicity as factors that determine the food quality of green and blue-green algae for *Daphnia*. In: *Evolution and Ecology of Zooplankton Communities* (ed. W.C. Kerfoot), pp. 268–281. Hanover University Press, New England.
- Ramensky L.G. (1938) *Introduction to the Complex Soil-botanical Research of Regions*. Selkhozgiz Publications, Moscow.
- Rapala J., Sivonen K., Lyra C. & Niemelä S.I. (1997) Variation of microcystin, cyanobacterial hepatotoxins, in *Anabaena* spp. as a function of growth stimuli. *Applied and Environmental Microbiology*, **63**, 2206–2212.
- Reinikainen M., Ketola M. & Walls M. (1994) Effects of concentrations of toxic *Microcystis aeruginosa* and an alternative food on the survival of *Daphnia pulex*. *Limnology and Oceanography*, **39**, 424–432.
- Repka S. (1997) Effects of food type on the life history of *Daphnia* clones from lakes differing in trophic state. I. *Daphnia galeata* feeding on *Scenedesmus* and *Oscillatoria*. *Freshwater Biology*, **37**, 675–683.
- Rohrlak T., Dittman E., Henning M., Börner T. & Kohl J.G. (1999) Role of microcystins in poisoning and food ingestion inhibition of *Daphnia galeata* caused by the cyanobacterium *Microcystis aeruginosa*. *Applied and Environmental Microbiology*, **65**, 737–739.
- Romanovsky Y.E. (1985) Food limitation and life-history strategies in cladoceran crustaceans. *Archiv für Hydrobiologie*, **21**, 363–372.
- Shapiro J. (1990) Current beliefs regarding dominance by blue-greens: the case for the importance of CO<sub>2</sub> and pH. *Verhandlungen der internationale Vereinigung für theoretische und angewandte Limnologie*, **24**, 38–54.
- Sivonen K. (1990) Effects of light, temperature, nitrate, orthophosphate, and bacteria on growth of and hepatotoxin production by *Oscillatoria agardhii* strains. *Applied and Environmental Microbiology*, **56**, 2658–2666.
- Schimdt K. & Jónasdóttir S.H. (1997) Nutritional quality of two cyanobacteria: how rich is 'poor' food? *Marine Ecology Progress Series*, **151**, 1–10.
- Smith A.D. & Gilbert J.J. (1995) Relative susceptibilities of rotifers and cladocerans to *Microcystis aeruginosa*. *Archiv für Hydrobiologie*, **132**, 309–336.
- Stemberger R.S. (1981) A general approach to the culture of planktonic rotifers. *Canadian Journal of Fisheries and Aquatic Science*, **38**, 721–724.
- Sterner R.W. (1989) Resource competition during seasonal succession toward dominance by cyanobacteria. *Ecology*, **70**, 229–245.
- Taberner A., Castañera P., Silvestre E. & Dopazo J. (1993) Estimation of the intrinsic rate of natural increase and its error by both algebraic and resampling approaches. *Computed Applied Bioscience*, **9**, 535–540.
- Threlkeld S.T. (1976) Starvation and size structure of zooplankton communities. *Freshwater Biology*, **6**, 489–496.
- Tollrian R. (1993) Neckteeth formation in *Daphnia pulex* as an example of continuous phenotypic plasticity: morphological effects of *Chaoborus* kairomone concentration and their quantification. *Journal of Plankton Research*, **15**, 1309–1318.
- Utkilen H. & Gjørlme N. (1992) Toxin production by *Microcystis aeruginosa* as a function of light in continuous cultures and its ecological significance. *Applied and Environmental Microbiology*, **58**, 1321–1325.
- Vincent W.F. (1989) Cyanobacterial growth and dominance in two eutrophic lakes: review and synthesis. *Archiv für Hydrobiologie*, **32**, 239–254.
- Watanabe M.F., Harada K., Matsuura K., Watanabe M. & Suzuki M. (1989) Hepatopeptide toxin production during the batch culture of two *Microcystis* species (cyanobacteria). *Journal of Applied Phycology*, **1**, 161–165.

(Manuscript accepted 31 January 2000)