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Strategies for the co-existence of zooplankton with the toxic cyanobacterium *Planktothrix rubescens* in Lake Zürich

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Abstract. Since the cyanobacterium *Planktothrix rubescens*, which dominates the phytoplankton community in Lake Zürich, is generally considered toxic to zooplankton, we addressed the question whether co-occurring zooplankton species have developed adaptive responses. Artificially shortened filaments (<30 μm in length) of *P.rubescens* significantly reduced survival of *Thamnocephalus platyurus* (Crustacea, Branchiopoda, Anostraca) naturally occurring in temporary ponds. In contrast to *Thamnocephalus*, the survival of co-existing zooplankton was unaffected (*Eudiaptomus gracilis*) or enhanced (*Daphnia hyalina* and *Cyclops abyssorum*). High sensitivity to the microcystins of *Planktothrix* was coupled to strict food avoidance in *Eudiaptomus*, but not in *Thamnocephalus*. *Daphnia* and *Cyclops* exhibited higher physiological resistance to cyanobacterial toxins, and ingested *Planktothrix*. For the lake zooplankton species, the feeding rates on high-quality algae were not significantly reduced in the presence of *Planktothrix*. In order to separate the effects of mechanical interference (filament length) versus toxins, clearance rates on *Planktothrix* filaments were compared to clearance rates on filaments subjected to toxin extraction. The results show that microcystins are important feeding deterrents against grazing by *Daphnia* since feeding rates on *Planktothrix* increased significantly after an aqueous–methanolic extraction of the major part of microcystins. On the other hand, copepods persisted in food avoidance, but exhibited high clearance rates on *Planktothrix* after a more lipophilic extraction was applied. Both microcystins and a lipophilic, unidentified toxin may contribute to the avoidance behaviour of copepods. For both *Daphnia* and copepods, the grazing resistance of *Planktothrix* is mediated by chemical defences rather than by the large size and the rigidity of the filaments.

Introduction

The filamentous, red-coloured, planktonic cyanobacterium *Planktothrix rubescens*, previously classified as *Oscillatoria rubescens* (Anagnostidis and Komárek, 1988), has dominated the phytoplankton community in Lake Zürich (Switzerland) for 100 years and probably occurred in the lake before its first detection in 1898 (Thomas, 1964). The cyanobacterium is toxic to zooplankton if ingested (Infante and Abella, 1985; DeMott and Moxter, 1991), but may also release toxins into the water after autolysis (at the lower edge of a metalimnetic layer, e.g. Feuillade, 1994a) or cell lysis after fungal or viral attack (Berg *et al.*, 1987). In addition, high densities of *Planktothrix* can inhibit the growth of other phytoplankton species and thus reduce the number of alternative food particles (Infante and Abella, 1985; Feuillade, 1994b). Since *Planktothrix* filaments reach high filament densities in the thermocline during summer stratification and in the epilimnion during autumn (Micheletti *et al.*, 1998; Walsby *et al.*, 1998), unfavourable conditions for zooplankton nutrition may occur. Several studies suggest negative effects of *Planktothrix* on zooplankton (Findenegg, 1953; Eberley, 1964; Faafeng and Nilssen, 1981; Edmondson and Litt, 1982; Nauwerck,

1988). However, despite the striking dominance of *Planktothrix* in Lake Zürich throughout the year, copepods (*Cyclops* spp., *Eudiaptomus gracilis*) as well as cladocerans (*Daphnia* spp.) co-occur regularly (Gammeter *et al*., 1997). Therefore, the question arises to what extent *Planktothrix* is detrimental to the zooplankton in Lake Zürich and the mechanisms by which zooplankton co-exist with the toxic cyanobacteria. It can be speculated that zooplankton have developed adaptive strategies to combat toxins and have played a crucial role in the evolution of toxin production in *Planktothrix* (cf. DeMott and Moxter, 1991). Consequently, the responses of zooplankton to toxic cyanobacteria need to be considered in the light of herbivore–phytoplankton interactions that have taken place in the past.

In order to co-exist with toxic cyanobacteria, zooplankton species may have evolved physiological resistance to cyanobacterial toxins (Starkweather and Kellar, 1983; Hanazato and Yasuno, 1987; Fulton, 1988) or behavioural adaptations to avoid the ingestion of toxic cells (Lampert, 1982; DeMott *et al.*, 1991). The latter requires the recognition and rejection of toxic cells either as a direct consequence of poisoning (e.g. by fast-acting neurotoxins) or by other signals associated with the toxic cells. Comparing the feeding behaviour of zooplankton on algae in the presence of toxic cells with feeding in the presence of purified toxins, DeMott *et al.* (1991) concluded that adaptive feeding responses of the zooplankton had occurred. The difficulty of such assays is that other toxic compounds, besides the well-known microcystins, have been isolated from *Microcystis* (Jungmann, 1992; Jungmann and Benndorf, 1994) and *Planktothrix* (Feuillade *et al.*, 1996), and the mechanism of toxin incorporation by the animals is unclear. Further, experiments which compared the feeding behaviour of *Daphnia* on hepatotoxic and non-hepatotoxic cells (Nizan *et al.*, 1986; Henning *et al.*, 1991; Jungmann *et al.*, 1991) demonstrated that different compounds are responsible for toxicity and inhibition of feeding. This difference is also known in terrestrial plant–herbivore interactions, for instance butterflies feeding on Cruciferae (Rodman and Chew, 1980).

In the second half of this century, *P.rubescens* was favoured by eutrophication in the alpine lakes as a consequence of intensive agriculture and urbanization of the countryside. Mass developments were reported from Austria (Findenegg, 1971), France (Feuillade, 1994c), Italy (Rovera and Vollenweider, 1968; Loizzo *et al.*, 1988), Norway (Skulberg and Skulberg, 1985) and North America (Edmondson and Litt, 1982). On the other hand, the relationship between the development of *Planktothrix* and the trophic state is unclear since *Planktothrix* can be found in Swiss lakes with different nutrient loadings (Schanz, 1994) and tends to persist in European lakes despite marked decreases in sewage load and phosphorus concentrations (Skulberg, 1978; Faafeng and Nilssen, 1981; Eckartz-Nolden and Nolden, 1992; Feuillade, 1994a). Zooplankton grazing may be one important factor in the re-oligotrophication of lakes and, in general, the grazing pressure on phytoplankton depends on the abundance of herbivorous zooplankton such as *Daphnia* spp. (Kerfoot, 1987). However, filamentous cyanobacteria are considered to be grazing resistant (e.g. Gliwicz, 1990) and *Daphnia* may be severely inhibited in feeding by mechanical interference with the large particles

(Gliwicz, 1977; Webster and Peters, 1978; Gliwicz and Siedlar, 1980; Porter and McDonough, 1984; Gliwicz and Lampert, 1990). Since *Daphnia* lacks an ability to discriminate effectively against individual toxic particles, the genus has often been found to be most susceptible to toxic cyanobacteria (Lampert, 1982; Fulton and Paerl, 1987; Burns *et al.*, 1989; Gilbert, 1990; Kirk and Gilbert, 1992). However, there are also observations showing no inhibitory effects of cyanobacterial filaments on *Daphnia* spp. (Knisely and Geller, 1986; Christoffersen, 1993; Schaffner *et al.*, 1994; Epp, 1996) and the question arises, therefore, to what extent adaptations to cyanobacterial toxicity could account for the discrepancies in the literature (cf. Gilbert, 1990; Matveev *et al.*, 1994). Surveys of *Planktothrix* blooms in European lakes have revealed that most of the blooms examined were hepatotoxic (Leeuwangh *et al.*, 1983; Berg *et al.*, 1986; Loizzo *et al.*, 1988; Sivonen *et al.*, 1990; Luukkainen *et al.*, 1993; Feuillade *et al.*, 1996); however, non-toxic blooms were observed in China (Quing-Xue *et al.*, 1991) and toxicity was not always caused by microcystins (Skulberg *et al.*, 1992; Reinikainen *et al.*, 1995; Feuillade *et al.*, 1996). Since copepods are also reported to handle and ingest filamentous cyanobacteria efficiently (DeMott and Moxter, 1991), it would be interesting to know whether some zooplankton species exist that readily graze on *P.rubescens*.

The toxic effects of *P.rubescens* on co-occurring copepods and cladocerans in Lake Zürich were investigated taking into account evolved adaptive responses to cyanobacterial toxins. First, the physiological responses to readily ingestible filaments and to extracts were compared to the survival of an anostracan crustacean. Freshwater Anostraca are generally considered ancient (Flößner, 1972) and typically inhabit temporary environments which dry out for most of the year, and thus do not develop an extensive phytoplankton community. Second, the feeding rates of lake zooplankton species on *P.rubescens* were compared to the feeding rates of the anostracan crustacean. If natural selection in response to toxic cyanobacteria on lake zooplankton has occurred, the anostracan crustacean should exhibit high physiological sensitivity paired with feeding on toxic cells and, therefore, should not be able to survive in the presence of the cyanobacteria. In contrast, the lake zooplankton species should exhibit mechanisms that reduce the negative impacts of the toxic cyanobacterium and demonstrate high survival in the presence of *Planktothrix*. In order to separate direct toxic effects versus behavioural effects on feeding reduction, short-term feeding trials were compared to the survival in the presence of toxic cyanobacteria. To find out whether food avoidance in lake zooplankton is a consequence of toxicity or related signals, ingestion rates on *Planktothrix* cells were compared to ingestion rates of cells subjected to toxin extraction. The extraction procedure removed the major part of the microcystins, but left the morphology of the filaments unchanged.

Method

Algae used in the experiments

Planktothrix rubescens (BC 9307, Bristol Collection) was isolated by A.E.Walsby from Lake Zürich in 1993 and grown in cyanobacterial medium as described by

Jüttner *et al.* (1983). The pH was adjusted to 9.0 with 1 M NaOH prior to autoclaving. Sterile Erlenmeyer flasks (3 l) were filled with 1 l of autoclaved medium, inoculated with *Planktothrix* and incubated under continuous illumination (6–10 μmol m^{-2} s^{-1}) at 20°C. For the experiments, growing cultures were taken ~1 month after transfer. Cyanobacteria were harvested by filtering onto a 25 μm nylon screen. The filaments were resuspended in 0.2-μm-filtered Lake Zürich water. *Planktothrix rubescens* typically grows as straight, rigid, long filaments. Under the culture conditions applied, the filaments (n = 141) were on average 550 (± 100) μm long and 3.4 (± 0.3) μm in diameter. The longest filament recorded was 3300 μm, the shortest 35 μm. Only 10% were shorter than 100 μm and 75% of the population did not exceed 650 μm. Assuming the filaments to be cylindrical and the carbon (C) content to be one-tenth of the wet weight, 1920 filaments ml^{-1} correspond to 1 mg C l^{-1}. A second cyanobacterium, *Anabaena* PCC 7120 (obtained from the Pasteur Culture Collection, Paris), was grown under the same culture conditions and formed comparatively soft and flexible filaments of similar length (370 ± 50 μm) and diameter (3.3 ± 0.2 μm). Eight per cent of the population were shorter than 100 μm and 90% did not exceed 650 μm.

The high-quality food algae used were *Cryptomonas ovata* (SAG 979.3) and *Scenedesmus acutus* Meyen (obtained from the Max-Planck Institute of Limnology in Plön, Germany). *Cryptomonas* were grown at 25°C in Erlenmeyer flasks (300 ml) filled with 100 ml of autoclaved cyanobacterial medium (pH ~7.0) in a controlled environmental shaker (120 r.p.m., 50 μmol m^{-2} s^{-1}). *Scenedesmus* were cultured in a medium according to Zehnder and Gorham (1960) at 19°C and 30 μmol m^{-2} s^{-1}.

Zooplankton used

Experiments were performed between October 1997 and April 1998. The dominant zooplankton species of Lake Zürich (*Cyclops abyssorum*, *Eudiaptomus gracilis*, *Daphnia hyalina*) were collected by vertical plankton tows in the northern part of Lake Zürich. Adult specimens were selected the same day, allowed to acclimate to the experimental temperature (19°C) for several days and fed low amounts of *Cryptomonas* (0.1 mg C l^{-1}). During the acclimation period, the mortality of copepods was generally low; however, that of *Daphnia* was high. For feeding experiments, adult *Daphnia* were taken from stock cultures grown in 1 l beakers filled with 0.2-μm-filtered lake water and the food level adjusted daily to 0.5 mg C l^{-1} (*Scenedesmus*). Zooplankton were transferred to fresh medium every week. To compensate for the lack of polyunsaturated fatty acids in *Scenedesmus* (e.g. Brett and Müller-Navarra, 1997), low amounts of *Cryptomonas* (0.1 mg C l^{-1}) were added. The freshwater anostracan *Thamnocephalus platyurus* is found in muddy, alkaline ponds of North America (Pennak, 1978). Resting eggs of *Thamnocephalus* were obtained from Bio International (Heitjesweg 1, 6085NJ HORN, The Netherlands) and hatched within 24–48 h in a Petri dish filled with EPA standard freshwater medium under continuous illumination (40 μmol m^{-2} s^{-1}) at 25°C (Crustacean Toxicity Screening Test, Thamnotoxkit F; see Centeno *et al.*, 1993). Larvae (instar II–III) were then transferred into filtered lake water

and used for toxicity experiments the same day. To conduct feeding experiments, larvae were raised in filtered lake water for a few days at 19°C and fed *Cryptomonas* (0.5 mg C l^{-1}) daily. The size ranges of all zooplankton species are given in Table I.

Toxicity experiments

Survival of zooplankton was measured in glass vials (25 ml, sealed with parafilm to prevent air bubbles) containing filaments of *Planktothrix* suspended in 0.2-μm-filtered lake water. In order to reduce morphological constraints on zooplankton feeding, the filaments were broken up into smaller pieces by a mixer (5 min), centrifuged at 5000 *g* for 10 min and resuspended in sterile filtered lake water. The short filaments obtained were still alive and continued growing under normal culture conditions. The fragmented filaments had a mean length of 26.3 (± 3.9) μm (n = 141) and 76% were shorter than 30 μm. The mean length of the *Anabaena* filaments treated in the same way was 10.6 (± 0.96) μm (n = 114). The food concentration was adjusted to 1.0 mg C l^{-1} by measuring absorbance at 550 nm and calculating cell concentrations from previously established calibration curves ($r^2 > 0.99$). Controls were run either with 0.2-μm-filtered lake water or suspensions of *Anabaena* at the same food concentration.

Survival was measured on 1-day-old *Thamnocephalus* larvae, 5-day-old individuals of *Daphnia* hatched from mothers which had been transferred from the lake into the laboratory, as well as on adult *Eudiaptomus* and *Cyclops* collected from the lake. Toxicity experiments for all species were carried out at the same time. Each treatment consisted of three parallels containing six adults of a species, placed on a roller apparatus (0.5 r.p.m.) in the dark. Animals were considered dead if they were lying on the bottom of the Petri dish without any escape movements from the pipette. Despite the use of sterile glassware and the daily counting and transfer of the animals to fresh medium, bacteria developed and probably served as an additional energy source. The results, consequently, represent a conservative estimate of the observed toxic effects. Survival data were analysed by the SPSS survival procedure (SPSS, 1993), which is a non-parametric multiple comparison test for censored data (i.e. data from experiments which are terminated before all animals die). Following the recommendations of Pyke

Table I. Mean (± SE) total lengths (μm) of zooplankton species used in experiments (n = number measured). Total lengths include rami, but not terminal setae for copepods and *Thamnocephalus*, and the distance of the top of the head to the base of the tail spine for *Daphnia*. One-day-old *Thamnocephalus* larvae (instar II–III) were used in toxicity experiments. Since 1-day-old larvae of *Thamnocephalus* did not feed during feeding experiments, larvae were raised for a few days on *Cryptomonas* prior to experiments

	Length	Min	Max	n
Thamnocephalus platyurus (1 day)	839 ± 18	512	1216	78
Thamnocephalus platyurus (4 days)	1120 ± 18	794	1331	48
Eudiaptomus gracilis	1148 ± 12	973	1306	45
Cyclops abyssorum	1461 ± 23	1216	1690	33
Daphnia hyalina	1596 ± 22	1408	1920	33

and Thompson (1986), statistical comparisons between survival curves are based on the log-rank test.

To remove the toxins from *Planktothrix*, cells were extracted with methanol–water (60:40, v:v) for 15 min. The suspension was centrifuged at 12 000 *g* for 3 min, the supernatant was separated and evaporated to dryness. The residue was dissolved in 3 ml of methanol and the solution evaporated again in a stream of N_2. Extracts were stored at –20°C. For chlorophyll (Chl) *a* determination, the pellet of the first extraction was subsequently extracted with methanol for 30 min, centrifuged and the absorbance of the supernatant was measured at 665 nm. The supernatant of the methanol extract was treated in the same way as the first extract. The concentration of the extracts was expressed in equivalents of cellular C calculated from the Chl *a* content (1 µg Chl *a* = 15.9 µg C). For the toxicity assay, 4–5 animals were pipetted into a well (3 ml volume) of a Corning multiwell test plate, composed of 24 wells, filled with 1 ml of filtered lake water and increasing concentrations (0–2.0 mg C ml^{-1}) of the cyanobacterial extracts (four replicates per concentration). Before starting an experiment, frozen extracts were dissolved in methanol and added at a maximum of 1% to the lake water (~10 µl). Compared to lake water, the addition of 1% of methanol alone did not adversely affect the survival of the animals. Since copepods survived in the control wells with filtered lake water for more than a week, the container size did not affect survival in the absence of food.

For comparisons among species, mortality proportions were transformed using logit transformation and regressed on log-transformed concentrations. The values of 24 h, 48 h and 72 h LC_{50} and their (95%) confidence intervals were estimated with the SPSS Probit procedure (SPSS, 1993) after correcting for mortality in the controls. LC_{50} values were calculated only if differences between treatments were significant (one-way ANOVA, $P < 0.05$) and mortality in controls did not exceed 15%.

Feeding experiments

Ingestion rate measurements were based on the amount of Chl *a* and phaeopigments accumulated by zooplankton from the cyanobacteria/algal suspensions over time. Following the method of Takatsuji *et al.* (1997), CO_2-narcotized specimens were transferred into 1.5 ml conical glass vials and extracted with 60 µl *N*,*N*-dimethylformamide (DMF) at 4°C overnight. Contrary to other solvents, DMF does not evaporate significantly and has a higher extraction ability. A 70 µl cuvette (Hellma, No. 105.251-QS) was used to increase the sensitivity of the fluorometric analysis of Chl *a* (Hitachi F-2000). Three individuals of *Daphnia* or *Cyclops*, five individuals of *Eudiaptomus* and 10 individuals of *Thamnocephalus* were sufficient to give reliable fluorescence readings. The excitation wavelengths were set at 430 and 450 nm, and the emission wavelengths at 670 and 636 nm for Chl *a* and Chl *c*, respectively. The relationship between Chl *a* concentration and fluorescence reading was determined by dissolving a Chl *a* standard (Sigma) in DMF and applying a specific extinction coefficient of 12.0 (µg ml^{-1}; Porra *et al.*, 1989). According to Takatsuji *et al.* (1997), the gut pigment contents were

expressed as the sum of Chl *a* and phaeopigments. Preliminary experiments showed that the gut passage time for pigments was ~1 h for each species.

There were five different feeding treatments: two controls with *Cryptomonas* at 0.1 and 0.2 mg C l^{-1}, respectively, a 1:1 mixture of *Cryptomonas* and fragmented *Planktothrix* at 0.1 mg C l^{-1}, fragmented *Planktothrix* at 0.1 mg C l^{-1} and fragmented *Planktothrix* at 0.1 mg C l^{-1} extracted with 60% methanol for 30 min (centrifuged at 5000 *g* for 10 min and resuspended in lake water). All food suspensions were prepared a few minutes prior to the start of the feeding experiment by pipetting an appropriate amount of food stock suspensions into 0.2-µm-filtered and aerated lake water. Since feeding experiments did not exceed 1 h and the Chl *a* content of the extracted *Planktothrix* did not decrease significantly, bacterial degradation was unlikely. Zooplankton species were adapted to *Cryptomonas* at 0.1 mg C l^{-1} 4 h prior to the experiment and then transferred into experimental food suspensions within a few minutes. Six to 10 individuals were either pipetted into completely filled 300 ml Erlenmeyer flasks or 250 ml Duran bottles and placed on a roller apparatus (0.5 r.p.m.) to compensate for different sinking velocities of food organisms. Results obtained in the different incubation vials did not differ and were combined in statistical analysis. At least two replicate experiments consisting of five parallels per treatment were performed for each species. Statistical comparisons between means were performed by one-way ANOVA followed by the Duncan multiple range test (SPSS, 1993). If the raw data of separate treatments were not normally distributed or not homogeneous in variance, they were either log transformed (*Eudiaptomus*, *Thamnocephalus*) or squared (*Daphnia*).

The Chl *c*/Chl *a* ratios were reciprocal square root transformed (*Daphnia*) or log transformed (*Eudiaptomus*) to equalize variances and to achieve normal distribution before ANOVA. The ratios of the two control groups were combined for *Thamnocephalus* since they differed significantly in variance and no transformation was possible to fulfil the pre-conditions for ANOVA (Bortz, 1989). An SPSS statistical package (Release 6.0 for Windows) was used for all statistical analyses (SPSS, 1993).

Grazing experiments

Grazing experiments were performed in order to investigate grazing impacts of the lake zooplankton on filaments of *P.rubescens*. The food concentration was set at 0.05 mg C l^{-1} and clearance rates were compared with the decrease in Chl *a* concentration in the controls with *Cryptomonas*, which should exhibit no resistance to grazing. *Planktothrix* was harvested by filtering 100 ml of a culture onto a 25-µm-mesh screen and resuspending in 0.45-µm-filtered lake water (treatment 1). To reduce grazing resistance in *Planktothrix*, microcystins were extracted with 60% methanol for 30 min, the filaments centrifuged at 5000 *g* for 10 min and resuspended in filtered lake water (treatment 2), and subsequently extracted in 100% methanol, centrifuged and resuspended in lake water (treatment 3). Each treatment was set up in triplicates and the experiment was repeated once. Fifteen adults of each species (*Eudiaptomus*, *Cyclops*, *Daphnia*) were pipetted into bottles (115 ml, without air bubbles), placed on a plankton wheel and allowed to

graze for 4 h. Clearance rates [CR; ml individual (ind.)$^{-1}$ h^{-1}] were calculated according to Gauld (1951) without any correction for algal and cyanobacterial growth during the experiment: CR = (ln C_c – ln C_g)/$t \times V/N$, where C_c is the Chl *a* concentration in controls, C_g is the Chl *a* concentration in grazing bottles, t is the duration of the experiment in hours, V is the volume of the bottle in millilitres and N is the number of individuals per bottle. Clearance rates were log (x + 1) transformed and compared by one-way ANOVA followed by the Duncan multiple range test (SPSS, 1993). Since the extracted filaments of *Planktothrix* contained relatively low amounts of Chl *a*, the clearance rates based on pigment data were verified by an independent estimation of biovolume via image analysis described by Walsby and Avery (1996). To control the condition of the filaments, samples were fixed with (1%) glutaraldehyde at the end of the experiment. Filaments of *Planktothrix* were collected on membrane filters (cellulose acetate, 0.8 μm pore size) by low-vacuum filtration, and images of autofluorescent filaments were analysed by epifluorescence microscopy at ×100 magnification (Reichert-Jung Polyvar, 546 nm excitation). The length of filaments was measured using a calibrated micrometer from images produced by a Sony video system equipped with a 3 CCD colour video camera (Model DXC-930P) and a Trinitron colour video monitor (Model PVM-1442QM).

Results

Toxicity of filaments

To measure the toxicity of *Planktothrix*, tests on zooplankton survival were performed. The zooplankton species studied exhibited different sensitivity when fed with toxic *Planktothrix* (Figure 1). Compared to the starvation treatment, the survival of *Thamnocephalus* was significantly reduced by *Planktothrix* ($P < 0.05$) and dead individuals were characterized by red-coloured guts. Though not significant, mortality was also enhanced for *Eudiaptomus* in the presence of *Planktothrix* ($P > 0.1$). In contrast, the survival of *Cyclops* and *Daphnia* was higher in the presence of *Planktothrix* as compared to controls without food ($P < 0.1$). Since the toxic cells have energetic value, the survival of zooplankton in the presence of *Planktothrix* was compared to survival in the presence of non-toxic *Anabaena* PCC 7120. In the presence of *Anabaena*, all species showed higher survival than in the controls without food. Compared to *Anabaena*, survival rates of zooplankton were significantly lower in the presence of *Planktothrix* ($P < 0.05$) for *Thamnocephalus* and *Eudiaptomus*, but not for *Daphnia* and *Cyclops* ($P > 0.1$). Filament numbers of *Planktothrix* decreased significantly in vials with *Thamnocephalus*: from 10 770 ± 3900 filaments ml^{-1} to 3730 ± 890 filaments ml^{-1} during the first 3 days of the experiment ($P < 0.05$, Mann–Whitney U-test). No significant decreases in filament densities were observed with other zooplankton.

Toxicity of extracts

The methanol–water extract of *P.rubescens* contained most of the microcystins (98.7%) detected by HPLC. The dominant microcystin has not yet been

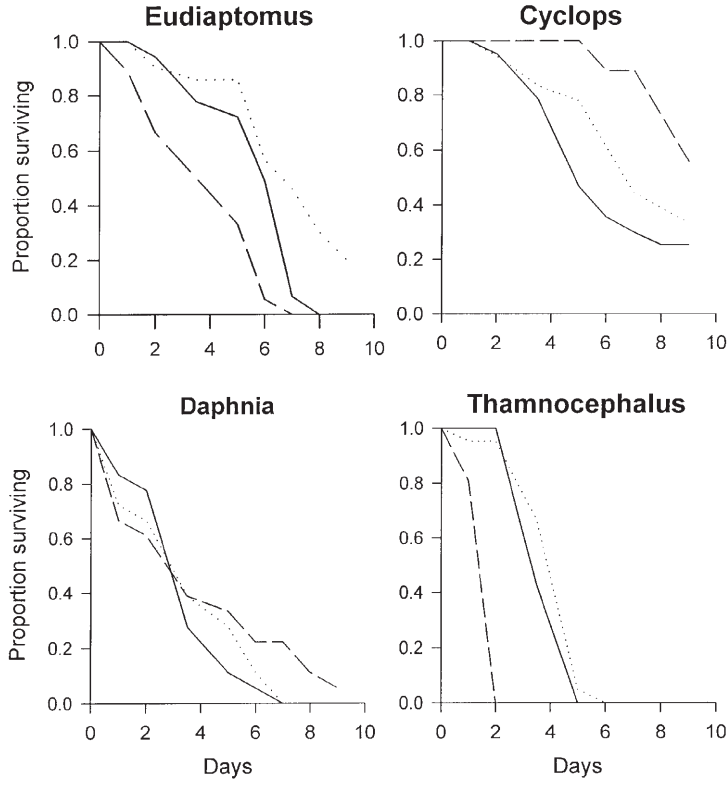

Fig. 1. Survival of *Eudiaptomus*, *Cyclops*, *Daphnia* and *Thamnocephalus* in the presence of fragmented *Planktothrix* (broken line) and fragmented *Anabaena* (dotted line) compared to controls without food (solid line). Survival was measured daily and each line represents the mean of triplicate treatments. For clarity, error bars have been omitted.

identified. Since the microcystins are all cyclic heptapeptides containing an unusual β-amino acid (Adda) and it is this chromophore which absorbs UV radiation at 240 nm (Cousins *et al.*, 1996), the concentration can be given in equivalents of purified microcystin-LR [1 μg C (cell extract) = 4.7 ng microcystin-LR]. The four zooplankton species demonstrated a wide range in sensitivity to extracts of toxic *Planktothrix* (Figure 2). In two independent experiments, specimens of *Eudiaptomus* died within 12 h at an approximate equivalent of 0.5 mg C ml^{-1} (cell extract). *Thamnocephalus* and *Daphnia* were intermediate in their sensitivity, and showed complete mortality only after a period of 36 h at an equivalent of 0.5 mg C ml^{-1} or more. In contrast, *Cyclops* exhibited no detectable dose-dependent mortality during the first 24 h and a comparatively weak response during the first 3 days of both experiments. The LC_{50} values (Table II) were highest for *Cyclops*, intermediate for *Daphnia*, lower for *Thamnocephalus* and lowest for *Eudiaptomus*. Since the methanolic extract contained only 1.3% as much microcystins as the methanol–water extract, toxicity was much reduced (Figure 3). The LC_{50}

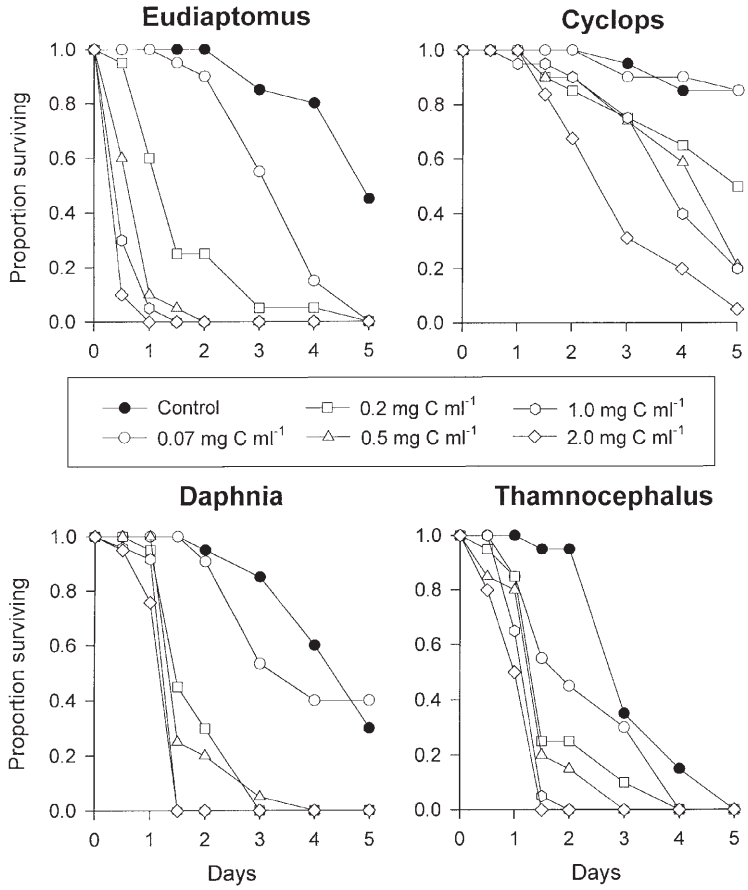

Fig. 2. Survival of *Eudiaptomus*, *Cyclops*, *Daphnia* and *Thamnocephalus* in the presence of varying concentrations (mg C ml^{-1}) of an aqueous–methanolic extract from *Planktothrix*. Data are means of four replicates. 1 μg C (cell extract) = 4.7 ng equivalents of microcystin-LR.

values of the first extract were considerably lower than those of the methanolic extract (Table II). The mortality of *Cyclops* was not affected by the methanolic extract. However, the toxicity observed for *Eudiaptomus*, *Thamnocephalus* and *Daphnia* was less reduced than would be expected if microcystins were the sole toxins.

Christoffersen (1996) suggested that body size, and thus the surface to volume ratio, is important for the sensitivity of an organism to toxins. The data in her review revealed a strong correlation between the size of an organism and its sensitivity to dissolved toxins. In this study, *Thamnocephalus*, which is smaller than *Eudiaptomus*, exhibited lower physiological sensitivity to dissolved microcystins compared to *Eudiaptomus*. Thus, a consistent pattern with size was not found.

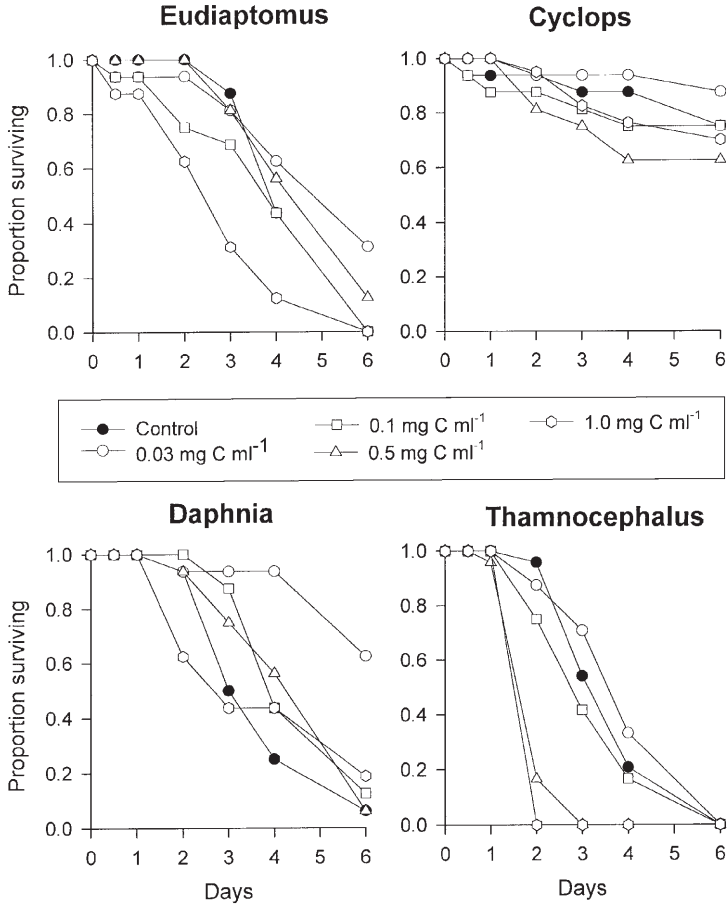

Fig. 3. Survival of *Eudiaptomus*, *Cyclops*, *Daphnia* and *Thamnocephalus* in the presence of varying concentrations (mg C ml^{-1}) of a methanolic extract from *Planktothrix*. Data are means of four replicates.

Feeding experiments

A comparison of Chl *a* concentrations between *Cryptomonas* at 0.1 mg C l^{-1} and the *Planktothrix* treatments revealed similar concentrations of Chl *a* (2 µg l^{-1}) and slightly higher numbers of food particles in the *Planktothrix* treatments [823 ± 77 (SE) particles ml^{-1} versus 777 ± 61 particles ml^{-1}]. There was no significant feeding inhibition of the zooplankton species when feeding on a 1:1 mixture of *Cryptomonas* and *Planktothrix* as compared to *Cryptomonas* controls (Figure 4, Table III). On the other hand, tendencies in feeding inhibition will be mentioned since they fit well to the overall picture of food avoidance. Although not significant, gut contents of both copepod species were lower when feeding on the mixture than in the two control groups containing only *Cryptomonas* (0.1 and 0.2 mg C l^{-1}).

Table II. LC_{50} (mg C ml^{-1}) values and their confidence limits (95%) for zooplankton exposed to cell extracts (aqueous–methanolic and methanolic) of toxic *P.rubescens*. LC_{50} values were not included if concentrations did not differ significantly from controls (overall significance $P > 0.1$, one-way ANOVA) or mortality in controls was high (>15%)

	24 h overall significance	LC_{50} (mg C ml^{-1})	48 h overall significance	LC_{50} (mg C ml^{-1})	72 h overall significance	LC_{50} (mg C ml^{-1})
Aqueous–methanolic extract						
Eudiaptomus	$P < 0.001$	0.17 (0.06–0.43)	$P < 0.001$	0.05 (0.04–0.08)	$P < 0.001$	0.04 (0.03–0.06)
	$P < 0.001$	0.25 (0.15–0.37)	$P < 0.001$	0.14 (0.1–0.2)	$P < 0.001$	0.08 (0.06–0.1)
Thamnocephalus	$P < 0.001$	0.37 (0.2–0.61)	$P < 0.001$	0.07 (0.02–0.15)	$P = 0.01$	–
	$P = 0.03$	2.0 (1.2–3.8)	$P < 0.001$	0.08 (0.01–0.19)	$P = 0.004$	–
Daphnia	$P = 0.08$	1.73	$P < 0.001$	0.32 (0.16–0.49)	$P < 0.001$	–
	$P = 0.009$	3.4	$P < 0.001$	0.19 (0.12–0.27)	$P < 0.001$	0.09 (0.04–0.14)
Cyclops	$P = 0.95$	No toxicity	$P = 0.7$	No toxicity	$P = 0.08$	–
	$P = 0.46$	No toxicity	$P = 0.01$	8.7	$P < 0.001$	1.84
Methanolic extract						
Eudiaptomus	$P = 0.66$	No toxicity	$P = 0.04$	1.13	$P = 0.004$	0.57
Thamnocephalus	$P = 0.45$	No toxicity	$P < 0.001$	0.22 (0.04–0.33)	$P < 0.001$	–
Daphnia	$P = 1$	No toxicity	$P = 0.007$	1.06	$P = 0.03$	0.87
Cyclops	$P = 0.16$	No toxicity	$P = 0.3$	No toxicity	$P = 0.85$	No toxicity

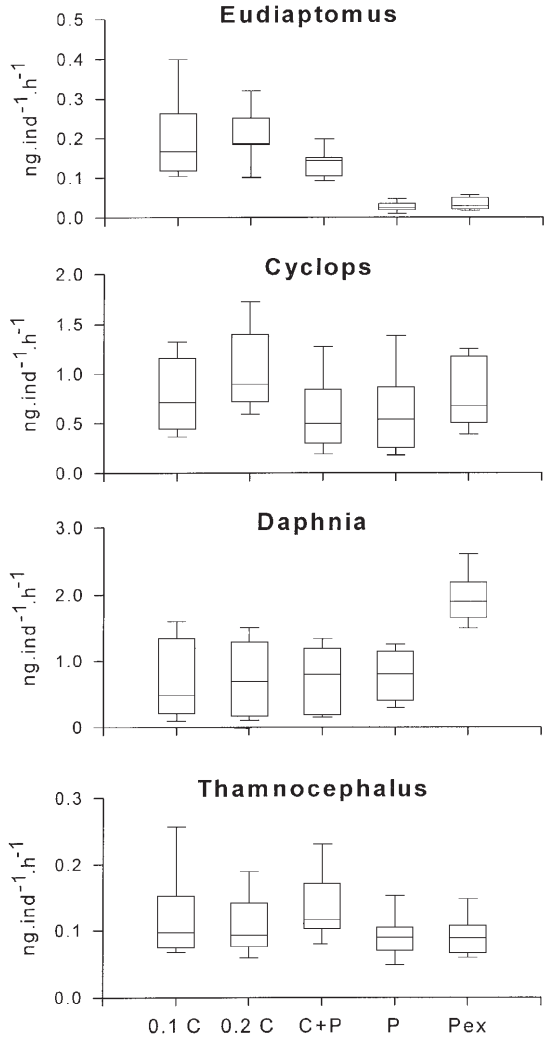

Fig. 4. Ingestion rates (ng Chl *a* plus phaeopigments ind.$^{-1}$ h^{-1}) at food densities of 0.1 mg C l^{-1} (0.1 C) and 0.2 mg C l^{-1} (0.2 C) of *Cryptomonas*, 0.2 mg C l^{-1} of a 1:1 mixture of *Cryptomonas* and fragmented *Planktothrix* (C + P), 0.1 mg C l^{-1} of fragmented *Planktothrix* (P) and 0.1 mg C l^{-1} of fragmented *Planktothrix* extracted with 60% methanol (Pex). The horizontal line of the box plots represents the median, the lower boundary the 25th percentile and the upper boundary the 75th percentile. Capped bars indicate the 10th and 90th percentiles. The range of ingestion values differs between species.

The ingestion rates of *Thamnocephalus*, however, tended to be higher in the food mixture.

There were consistent differences among the four investigated zooplankton species when feeding on pure suspensions of *Planktothrix*. Compared to controls (0.1 mg C l^{-1} *Cryptomonas*), *Eudiaptomus* was the only species avoiding the

Table III. Means ± confidence intervals (95%) of ingestion rates (ng ind.$^{-1}$ h^{-1}) of zooplankton species feeding in suspensions consisting of 0.1 mg C l^{-1} (C 0.1) and 0.2 mg C l^{-1} (C 0.2) of *Cryptomonas*, 0.2 mg C l^{-1} of a 1:1 mixture of *Cryptomonas* and *Planktothrix* (C + P), 0.1 mg C l^{-1} of *Planktothrix* (P 0.1) and 0.1 mg C l^{-1} of *Planktothrix* extracted with 60% methanol (Pex 0.1). The differences were tested using one-way ANOVA followed by the Duncan multiple range test. Symbols indicate homogeneous subsets whose highest and lowest means are not significantly different ($P > 0.05$). Numbers in parentheses indicate sample size

	C 0.1	C 0.2	C + P	P 0.1	Pex 0.1	Overall significance
Eudiaptomus	0.21 ± 0.03 (20)^{a}	0.21 ± 0.02 (15)^{a}	0.14 ± 0.01 (15)^{a}	0.03 ± 0.003 (20)^{b}	0.04 ± 0.006 (15)^{b}	$P < 0.001$
Cyclops	0.82 ± 0.09 (20)^{ab}	1.11 ± 0.13 (20)^{b}	0.63 ± 0.09 (20)^{a}	0.62 ± 0.12 (15)^{a}	0.79 ± 0.11 (10)^{ab}	$P = 0.013$
Daphnia	0.71 ± 0.15 (14)^{a}	0.75 ± 0.18 (10)^{a}	0.75 ± 0.16 (10)^{a}	0.79 ± 0.1 (13)^{a}	1.95 ± 0.13 (9)^{b}	$P < 0.001$
Thamnocephalus	0.12 ± 0.02 (18)	0.11 ± 0.01 (15)	0.15 ± 0.02 (15)	0.1 ± 0.01 (14)	0.1 ± 0.01 (14)	$P = 0.23$

ingestion of *Planktothrix* cells (Figure 4, Table III). The gut contents approximated to zero Chl *a* values after 1 h of incubation (ANOVA, $P < 0.001$). In contrast, *Daphnia* and *Thamnocephalus* did not exhibit significant decreases in gut pigment content compared to the controls. *Cyclops* was intermediate in food avoidance of *Planktothrix* and demonstrated a significant feeding inhibition only in comparison to the control with *Cryptomonas* at 0.2 mg C l^{-1} (Table III). Since *Thamnocephalus* died in the presence of *Planktothrix* much faster than the copepod species, the observed feeding inhibition in copepods must be attributed to food avoidance behaviour rather than to a direct consequence of poisoning.

The extraction of the microcystins of *Planktothrix* cells by 60% methanol did not alter the food avoidance behaviour of *Eudiaptomus* (Figure 4, Table III). Therefore, it can be assumed that food avoidance behaviour in *Eudiaptomus* is not a direct consequence of these toxins, but is caused by other factors. In contrast, feeding rates of *Daphnia* on *Planktothrix* cells increased significantly when methanol-extracted cells were offered as compared to the other food suspensions ($P < 0.001$). This increase was less significant in *Cyclops* as compared to the ingestion rates on *Planktothrix* ($P < 0.05$). Contrary to *Daphnia*, feeding rates of *Thamnocephalus* on *Planktothrix* were not altered by the extractions. This might indicate the presence of an unknown lipophilic toxic compound. The high mortality of *Thamnocephalus* exposed to the methanolic extract (Table II, Figure 3) is consistent with this result.

Gut pigment ratios

Since Chl *c* is restricted to cryptomonads and cyanobacteria contain only Chl *a*, the proportion of Chl *c* to Chl *a* in zooplankton body extracts can be taken as an indicator of selective feeding. Chl *a* fluorescence at 670 nm decreased much more than Chl *c* fluorescence at 636 nm when measured 60 s after the addition of 1 µl 2 M HCl to 60 µl DMF. Consequently, Chl *c* cannot be taken as a direct measure of feeding on *Cryptomonas*. An increase of the Chl *c*/Chl *a* ratio in zooplankton gut as compared to controls with *Cryptomonas* would clearly indicate the absence of feeding on the cyanobacteria. In contrast, a decrease of the Chl *c*/Chl *a* ratio compared to the controls will only be possible if individuals start feeding on *Planktothrix* that contains no Chl *c*.

Chl *c* comprised ~30% of the gut Chl *a* (plus phaeopigments) in the three lake zooplankton species, and the amount of Chl *c* tended to decrease in zooplankton species when ingesting *Planktothrix* (Table IV, Figure 5). The effect was particularly pronounced in *Daphnia* where Chl *c* approximated to zero in pure suspensions of *Planktothrix* ($P < 0.001$) and decreased in the 1:1 food mixtures. Similar results were observed with *Cyclops* ($P < 0.01$) and *Thamnocephalus* ($P < 0.001$), indicating that these species also feed on *Planktothrix*. In contrast, Chl *c*/Chl *a* ratios doubled in *Eudiaptomus* in the presence of pure suspensions of *Planktothrix* ($P < 0.001$). A significant negative relationship ($r = –0.76$, $P < 0.001$, Spearman rank correlation) between the Chl *c*/Chl *a* ratio and the Chl *a* content is consistent with the hypothesis that *Eudiaptomus* strictly avoided consuming *Planktothrix*. There was a positive relationship between the amount of Chl *c* and Chl *a* in the

Table IV. Means ± (95%) confidence intervals (sample size) of Chl *c*/Chl *a* ratios in zooplankton guts after 1 h of feeding in food suspensions described in Table III. The differences were tested by one-way ANOVA and the post hoc Duncan multiple range test. Symbols denote groups not significantly different at $P = 0.05$

	C 0.1	C 0.2	C + P	P 0.1	Pex 0.1	Overall significance
Eudiaptomus	0.3 ± 0.02 (20)^{a}	0.27 ± 0.02 (15)^{a}	0.25 ± 0.02 (15)^{a}	0.73 ± 0.07 (20)^{b}	0.66 ± 0.09 (15)^{b}	$P < 0.001$
Cyclops	0.27 ± 0.02 (20)^{a}	0.28 ± 0.02 (20)^{a}	0.21 ± 0.02 (20)^{ab}	0.18 ± 0.02 (15)^{b}	0.17 ± 0.04 (10)^{b}	$P = 0.006$
Daphnia	0.28 ± 0.06 (14)^{a}	0.29 ± 0.06 (10)^{a}	0.22 ± 0.04 (10)^{a}	0.07 ± 0.007 (14)^{b}	0.03 ± 0.003 (9)^{c}	$P < 0.001$
Thamnocephalus	0.42 ± 0.03 (17)^{a}	0.45 ± 0.01 (15)^{a}	0.45 ± 0.02 (15)^{a}	0.21 ± 0.03 (14)^{b}	0.18 ± 0.02 (14)^{b}	$P < 0.001$

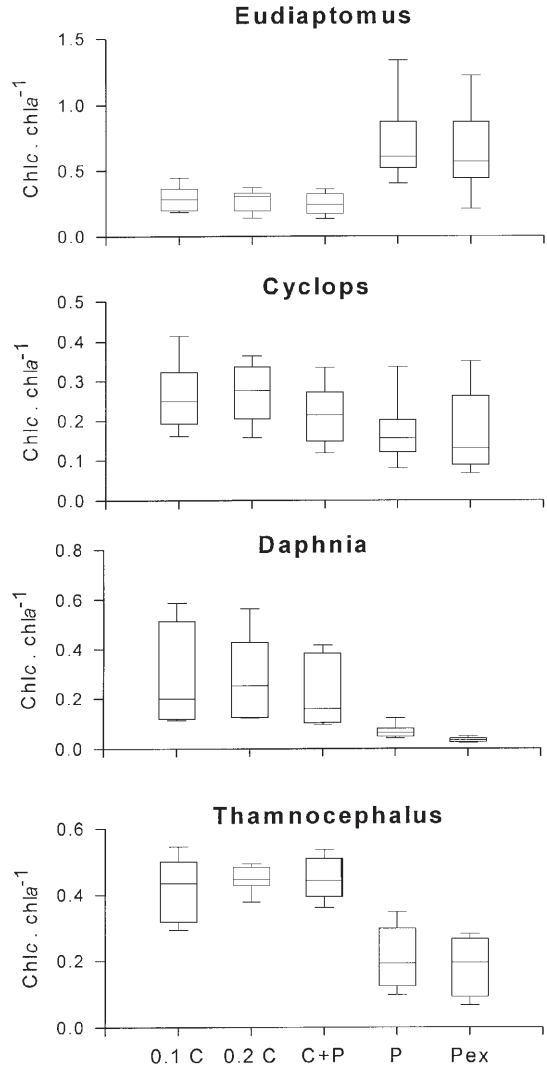

Fig. 5. Chl *c* to Chl *a* ratios in zooplankton guts from the same experiment as in Figure 4. The range given varies between species. For an explanation of box plots and symbols, see Figure 4.

gut of *Cyclops* (r = 0.22, P = 0.04, Spearman rank correlation), implying that *Cyclops* was feeding to a lesser extent on *Planktothrix* than on *Cryptomonas*. There were no correlations between the amounts of Chl *c* and Chl *a* in the gut of *Daphnia* and *Thamnocephalus*, indicating rather unselective feeding behaviour.

Grazing experiments

The strong autofluorescence of phycoerythrins in *Planktothrix* was taken as a measure for the integrity of filaments after methanol extraction. The

phycoerythrins are water-soluble chromoproteins assembled into phycobilisomes and do not dissolve in lipophilic solvents such as methanol. The cells of *Planktothrix* were permeabilized by methanol extraction, but the autofluorescence of the phycobilisomes indicated that the filaments maintained their original structure until the end of the grazing experiments. There were only a few filaments where single cells had lost their autofluorescence and thus most of their macromolecular constituents. In addition, length measurements of the filaments did not reveal significant changes compared to untreated *Planktothrix*. Mean lengths of the filaments decreased from 820 ± 150 μm ($n = 159$) in the untreated *Planktothrix* to 530 ± 100 μm ($n = 172$) after extraction with 60% methanol; in treatments in which the filaments were subsequently extracted with methanol, mean length was 580 ± 100 μm ($n = 171$). The differences between treatments were not significant (Kruskal–Wallis one-way ANOVA, $P = 0.15$) and were even smaller with regard to the median (360 μm versus 290 μm versus 330 μm for treatment groups 1, 2 and 3, respectively). Further support for low degradation of extracted filaments is provided by the Chl *a* to phaeopigment ratios which were similar for differently treated filaments (Kruskal–Wallis one-way ANOVA, $P = 0.75$). Consequently, any difference in Chl *a* concentration must be attributed to the grazing activity of the zooplankton. Clearance rates on *Planktothrix* were significantly lower in all grazers compared to *Cryptomonas* ($P < 0.05$, Duncan multiple range test; Figure 6). The clearance rates of copepods on *Planktothrix* were zero and the filtration rates of *Daphnia* ranged from 0.14 to 0.29 ml ind.$^{-1}$ h^{-1}. There was a distinct negative relationship between the clearance rate and the amount of phaeopigments present in *Cryptomonas* ($r = –0.95$, Spearman rank correlation), but not in *Planktothrix* ($r = 0.13$). After extraction with 60% methanol, clearance rates of *Daphnia* increased significantly ($P < 0.05$), but the filtration rates of copepods were not affected. The amount of phaeopigments was correlated with the clearance rates ($r = –0.77$). Although highly variable, clearance rates of all species increased significantly ($P < 0.05$) after subsequent extraction with methanol compared to untreated *Planktothrix*. The amount of phaeopigments was negatively related to the clearance rates ($r = –0.61$).

Discussion

The different chlorophylls present in cyanobacteria and cryptomonads can be used to quantify feeding on both groups of organisms. The results were performed over a period of 4 months (January–April), and two independent estimates of ingestion and clearance rates on *Planktothrix* revealed similar results. The use of chlorophylls as markers for the quantification of zooplankton diet selection can be obscured, however, for several reasons. Feuillade and Davies (1994) observed a lower Chl *a* content (per cent of fresh weight) in cyanobacteria compared to other algal taxa. In addition, the Chl *a* degradation in the gut of zooplankton may vary for cyanobacteria and algal groups (cf. Infante, 1973; Horn, 1981). It can be assumed to be faster in cryptomonads than in cyanobacteria consisting of membrane and mucilage layers that are not readily digestible. Degradation depends further on the ingestion rate and digestive acclimation of

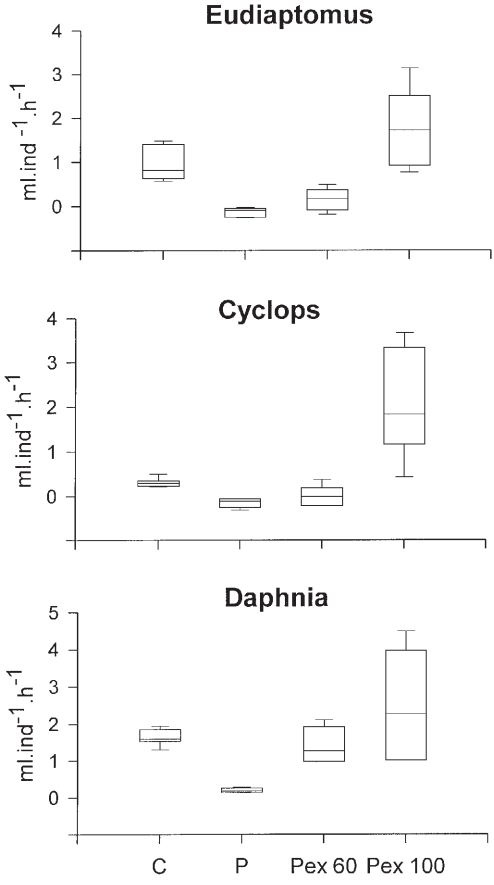

Fig. 6. Clearance rates (ml ind.$^{-1}$ h^{-1}) of the lake zooplankton species in food suspensions of *Cryptomonas* (C), unfragmented *Planktothrix* (P), unfragmented *Planktothrix* extracted with 60% methanol and resuspended in lake water (Pex 60), extracted with 60% methanol and methanol and resuspension in lake water (Pex 100).

zooplankton to food conditions (Penry and Frost, 1991; Head and Harris, 1994), as well as the amount of omnivory in the nutrition of a zooplankton species (Nelson, 1989). Finally, different chlorophylls may exhibit different resistance to acidic conditions and the attack of degradation enzymes in the gut (Head and Harris, 1992; this study). However, we do not think that any of these factors affect the major conclusions of this study since chlorophylls and their fluorescent derivatives are in general rather resistant to degradation in the gut (Pasternak and Drits, 1988; Head and Harris, 1992), and only minor variations in Chl content would be the result.

Although the feeding and grazing experiments gave similar results, there does seem to be some inconsistency for *Daphnia*. Contrary to expectation, the feeding rates on *Cryptomonas* were lower than those on methanol-extracted cells of

Planktothrix. The comparatively high feeding rates in the mixture of *Cryptomonas* and *Planktothrix* contradict the assumption that *Daphnia* feeds non-selectively. Infante (1973) observed much lower ingestion rates on *Cryptomonas ovata* compared to *Asterionella formosa* and *Scenedesmus acuminatus* for *Daphnia pulex* and *Daphnia longispina*. She was not able to explain the difference; however, it seems possible that large-sized cryptomonads may reduce ingestion due to their flagellate motility. Calculating the swimming velocity of *Cryptomonas* cells according to Sommer (1988; 300 μm s^{-1} for cells 6.5 μm in mean equivalent spherical diameter) and comparing with the average water flow across the filtering structures of *Daphnia pulicaria* (Brendelberger *et al.*, 1986; 1000 μm s^{-1}) indicates that both estimates have the same order of magnitude. The higher clearance rates on *Cryptomonas* in the grazing experiments may be due to the high animal density in the experimental bottles causing repeated filtration of the water with *Cryptomonas*. The finding that the ingestion rates of *Daphnia* were not affected by substituting 50% of the diet with *Planktothrix* seems inconsistent with other experiments performed with mixtures of good foods and toxic cyanobacteria (Lampert, 1981; Fulton and Paerl, 1987; DeMott *et al.*, 1991; Henning *et al.*, 1991; Jungmann *et al.*, 1991). However, these experiments have been carried out using *Scenedesmus* and *Chlamydomonas* as high-quality food sources, and are therefore not strictly comparable to the results obtained in this study.

The study differentiates between the effects of cyanobacterial toxins on zooplankton and the evolution of adaptive responses to cyanobacterial toxicity in zooplankton feeding. According to the traditional definition of toxicity (Lampert, 1987), a particular strain is considered toxic if the animals die more quickly in the presence of toxic food than in its absence. This definition, however, does not take into account the energy value of toxic cells as well as adaptive behavioural responses, e.g. reduced filtering rates of daphnids (cf. DeMott *et al.*, 1991). Biochemical analysis of toxins or the determination of acute toxicity by intraperitoneal injection into mice (DeMott and Moxter, 1991) would also not suffice to appraise toxicity in this study since strict food avoidance (*Eudiaptomus*) or physiological resistance (*Daphnia*, *Cyclops*) were responsible for the fact that toxicity of *Planktothrix* to lake zooplankton was not detected anymore. This does not mean that toxic effects on lake zooplankton are completely absent (see below). Corresponding to the definition of Lampert (1987), *Daphnia* spp. from two European lakes without dense populations of *P.rubescens* die much faster in the presence of *Planktothrix* than without any food (author's unpublished data). In this study, *Thamnocephalus*, as an example of a species not adapted to cyanobacterial toxins, was highly susceptible to *Planktothrix* and would be unable to co-exist with toxic cyanobacteria under natural conditions.

The current hypothesis suggests that smaller cladocerans or copepods are less susceptible to filamentous cyanobacteria than larger zooplankton (*Daphnia* spp.) because the latter are more likely to be mechanically inhibited during feeding, have a greater tendency to ingest toxic filaments and exhibit a greater sensitivity to cyanobacterial toxins (Lampert, 1982; Richman and Dodson, 1983; DeMott, 1989; Gilbert, 1990; Kirk and Gilbert, 1992). However, this hypothesis is not

supported by the observations from this study since *Eudiaptomus* was clearly more sensitive against *Planktothrix* than *Daphnia*. While the survival of *Daphnia* was unaffected by the filaments or even slightly enhanced, the survival of *Eudiaptomus* was generally reduced. These results are similar to the observations of DeMott *et al.* (1991) who found higher sensitivity of *Diaptomus* than *Daphnia* to cyanobacterial toxins. The resistance of *Daphnia* and *Cyclops* against cyanobacterial toxins probably enables both grazers to gain some nutritive advantage from *Planktothrix* feeding at least under food-limiting conditions. Several examples of zooplankton feeding on planktonic cyanobacteria exist (Moriarty *et al.*, 1973; DeBernardi *et al.*, 1981; Burns and Xu, 1990; Xu and Burns, 1991) and, despite the lack of essential fatty acids (Brett and Müller-Navarra, 1997), cyanobacteria may be sources of energy.

Daphnia exhibited much higher feeding rates on *Planktothrix* after the microcystins had been extracted. This experiment corroborates the assumption that the microcystins may be considered as an important factor for the observed reduced feeding rates of *Daphnia* on *Planktothrix*. The result that microcystins are the main factor inhibiting feeding in *Daphnia* seems to contradict the results from an earlier study with *Daphnia* and *Microcystis* spp. (Jungmann *et al*., 1991). These authors concluded that microcystins cannot be responsible for the inhibition of feeding in *Daphnia* as the toxicity, but not the feeding inhibition, disappeared after freeze–thawing and water extraction of the cells. Unfortunately, toxin analysis was not performed in parallel and the possibility persists that microcystins have been extracted from the dead cells into the lake water in the course of the survival experiment, but not during the much shorter feeding trials (cf. Penaloza *et al.*, 1990). It is not yet clear whether *Daphnia* exhibits lower feeding rates on *Planktothrix* cells as a direct consequence of poisoning by the incorporated microcystins or due to a behavioural response. W.R.DeMott (personal communication) argued that *Daphnia* may recognize the toxin within seconds or minutes of ingestion simply by feeling sick after ingestion and reducing the feeding rate. Thus, if the organism is still capable of feeding at a high rate when transferred into a non-toxic food suspension, a behavioural response can be assumed.

Eudiaptomus never ingested *Planktothrix* cells (not even the microcystin-extracted cells), indicating that this species probably uses other signals related to cyanobacterial toxicity. In general, highly selective grazers such as calanoid copepods are suggested as the evolutionary driving force for toxin production as a defence mechanism in cyanobacteria (DeMott *et al.*, 1991; Larsson and Dodson, 1993). Daphnids are less selective (DeMott, 1986) and poisonous algae/cyanobacteria should have no advantage compared to palatable ones when unselective grazing prevails. In this study, the high clearance rates of *Daphnia* on detoxified *Planktothrix* filaments indicate that *Daphnia* plays a persisting role in the evolution of microcystin production in *Planktothrix*. Thus, while the selective feeding behaviour of copepods is needed to explain the increase in the first cells to evolve toxic defences, the continuous grazing pressure on *Planktothrix* by *Daphnia* seems to be responsible for the further synthesis and accumulation of microcystins in *Planktothrix*. The dominance of *Planktothrix* in Lake Zürich (Gammeter *et al.*, 1997) is consistent with this hypothesis and toxin accumulation might

explain the current success of *Planktothrix* in the lake. Much more sophisticated examples of plant–herbivore biochemical co-evolution are reported from terrestrial ecology (Harborne, 1988).

The mechanism of food avoidance is different for copepods because grazing rates on *Planktothrix* filaments remained low after extraction with 60% methanol, but increased significantly after the subsequent methanol extraction. It is unlikely that the low clearance rates of copepods on *Planktothrix* extracted with 60% methanol were caused by morphological changes since both copepods are reported to handle and ingest filaments of similar cyanobacteria efficiently (DeMott and Moxter, 1991). It can be hypothesized that unknown, more lipophilic and less toxic compounds may cause the food avoidance of *Planktothrix* by copepods; however, the experiments do not rule out the possibility that both microcystins and the other, unidentified toxin cause feeding avoidance in copepods. The results demonstrate that: (i) chemical defences are more important than morphological features; (ii) microcystins probably cause food avoidance in *Daphnia*; (iii) the chemical stimuli for copepods may be related to some kind of toxin which is unknown or a lipophilic microcystin, or both.

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