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Responses of *Daphnia pulex* populations to toxic cyanobacteria

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SUMMARY

1. We studied the effects of toxic cyanobacteria, *Microcystis aeruginosa*, on *Daphnia pulex* populations. The experiment was performed at room temperature in laboratory microcosms to which we added toxic *Microcystis* in two pulses. Green alga, *Scenedesmus obtusiusculus*, was provided in two concentrations.
2. *Microcystis* exposure resulted in a decreased population density of *Daphnia*. The proportion of adolescents was higher in the *Microcystis* treatment than in the control, while the proportion of newborns did not differ significantly from the control. This indicates delayed maturation of *Daphnia* exposed to *Microcystis*. We found no significant impacts of cyanobacteria, food level or clonal origin on population variability after correcting for differences in population size.
3. Juveniles of the two clones studied showed different sensitivities to toxic *Microcystis* in the acute test. However, in the population experiment we did not find clonal differences between *Microcystis* and control treatments in the relative proportion of juveniles.
4. The number of ephippia produced per adult was highest in the treatments with *Microcystis*. This indicates that cyanobacterial toxins may be one of several factors inducing ephippia formation, acting directly or through inhibition of feeding.

Introduction

Microcosm experiments provide a means to study the effects of abiotic or biotic factors on *Daphnia* populations (Neill, 1975; Kaitala & Maximov, 1984). Freshwater communities are regulated by both resources and predation (Carpenter, 1987). Thus, the quality of the food source, including the presence of toxins, might be a crucial factor affecting *Daphnia* populations.

Several species of cyanobacteria have a negative effect on the survival, growth and reproduction of *Daphnia* (Arnold, 1971; de Bernardi, Giussani & Pedretti, 1981; Nizan, Dimentman & Shilo, 1986; Lampert, 1987; Reinikainen, Ketola & Walls, 1994; Hietala, Reinikainen & Walls, 1995). The quality of cyanobacteria as food for daphnids may be poor because of filamentous or colonial structure, or because of toxic compounds (Lampert, 1987). In addition, nutritional deficiency may explain poor growth, survivorship and reproduction of *Daphnia* fed nontoxic, unicellular cyanobacteria (DeBiase, Sanders & Porter, 1990). Acute toxicity tests indicate age-specific differences in sensitivity. *Daphnia pulex* juveniles are more sensitive than adults to high levels of *Microcystis* (Reinikainen *et al.*, 1994). However, at low concentrations of additional food they survive better than adults, possibly since maternal lipids make them less sensitive to food shortage. Generally, the mortality of *D. pulex* exposed to a given level of toxic cyanobacteria

decreases with increasing concentrations of alternative foods (Lampert, 1981; Reinikainen *et al.*, 1994).

Various studies (Edmondson & Litt, 1982; Jarvis, Hart & Combrink, 1987; Moss, Stanfield & Irvine, 1991) report low abundances of *Daphnia* during blooms of cyanobacteria; but see Benndorf and Henning for differing results (1989). The presence of toxic cyanobacteria might increase the variability of the population size if it results in a sudden decline in the *Daphnia* population. Bengtsson & Milbrink (1995) found that interspecific competition (*D. magna–D. longispina*) resulted in lower population densities and higher population variabilities, while the effects of size-selective predation were less clear. High population variability may increase the probability of extinction (Pimm, Jones & Diamond, 1988).

Formation of resting eggs (ephippial eggs) is an important strategy of aquatic crustaceans for surviving unfavourable environmental conditions (DeStasio, 1989; Hairston *et al.*, 1995). The ephippial sheath protects the resting eggs, which can survive for many years (De Meester & De Jager, 1993; Hairston *et al.*, 1995) and hatch when environmental conditions are more favourable. The formation of resting eggs usually depends on several stimuli. Factors inducing development of ephippial eggs include short-day photoperiods, food limitation, crowding and low or high temperatures (Stross & Hill, 1965; Korpelainen, 1992; Kleiven, Larsson & Hobæk, 1992). The production of resting eggs is a potential strategy of *Daphnia* to survive toxic blooms of cyanobacteria.

In the present study, we examined the response of *D. pulex* De Geer populations to toxic cyanobacteria. We cultured *D. pulex* on two levels of the green alga *Scenedesmus obtusiusculus* Chod. with and without a toxic unicellular strain of *Microcystis aeruginosa* Kütz. We used two clones of *D. pulex*, as genotypes may differ in their life histories (Lynch, Spitze & Crease, 1989) as well as in their sensitivities to toxic cyanobacteria (Hietala *et al.*, 1995). Responses studied were population density and variability, age structure, and the production of ephippia. We tested the hypotheses: (i) that toxic cyanobacteria may decrease the population density; (ii) that toxic cyanobacteria may increase the population variability of *Daphnia*; (iii) whether cyanobacteria change the proportions of different developmental stages due to age-specific mortality, reduced reproduction and delayed maturation; (iv) whether exposure to cyanobacteria induces the production of resting eggs; (v) whether the effects of cyanobacteria exposure are less harmful at a higher concentration of additional food.

Materials and methods

Origin and culture of experimental species

We studied two laboratory clones of *D. pulex* that originated from ponds in SW Finland (clone 1) and Northern Germany (clone 2). Based on an acute toxicity test on ten *D. pulex* clones (Hietala, Laurén-Määttä & Walls, 1997), we know that these clones are moderately sensitive to toxic *Microcystis aeruginosa* (strain PCC 7820). Because the toxicity of the cyanobacterial culture may vary, the amount of the toxin microcystin-LR was analysed using high pressure liquid chromatography (HPLC; Meriluoto *et al.*, 1990). Samples were taken from the algal culture when the first pulse of cyanobacteria was added to the population experiment (see below). We also performed an acute toxicity test on the *Daphnia* clones the week before starting the population experiment. The two food organisms used in the experiment were maintained as unicellular batch cultures, *Microcystis* in Z8 medium (NIVA, 1990) and *Scenedesmus* in a green algal culture medium (Kylin, Sundberg & Tillberg, 1972).

The experimental clones were established from single females, which were cultured individually in 20-ml jars. The jars were filled with 25 µm sieved lake water and we added a high food level (80 000 cells ml^{-1} of *Scenedesmus*; 1.04 mg C l^{-1}). The lake water was collected from Lake Littoistenjärvi, SW Finland, (60.27°N,22.23°E) in November 1994 and contained no toxic algae. The second and later broods of the females were transferred to 0.5-l jars (thirty animals per jar) and cultured in similar conditions. After some generations we obtained sufficient animals to start the experiment.

Acute test

The combined effects of food level (low level 20 000 cells of *S. obtusiusculus* ml^{-1}, high level 80 000 cells ml^{-1}; 0.26 and 1.04 mg C l^{-1}, respectively) and six different levels of toxic *M. aeruginosa* (strain PCC 7820) (0, 20 000, 40 000, 80 000, 160 000 and 320 000 cells ml^{-1}; 0, 0.15, 0.30, 0.61, 1.22 and 2.43 mg C l^{-1}, respectively) were tested on egg-bearing

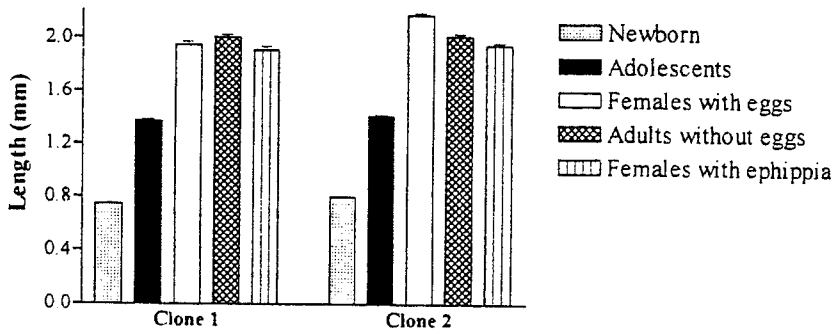

Fig. 1 Length distribution (mean ± SE) of developmental stages. The values are pooled from all treatments.

females and newborn animals of the two clones. The water was not changed during the experiment, nor were fresh algae added. There was a total of eight animals of each age-group and clone per food level; four 20 ml jars with two daphnids each. We checked for dead animals 24, 48 and 72 h after the start of the experiment.

Population experiment

The population experiment was performed at room temperature in 6-l covered semi-opaque plastic jars with sieved (25 μm) lake water under continuous fluorescent light at an intensity of 200 lux. Rippka (1988) recommended a light intensity of 200–500 lux for culturing cyanobacteria. Twenty mature and ten juvenile *D. pulex* females per jar were founders of the population cultures. After 1 week at a high food level (80 000 cells of *Scenedesmus* ml^{-1}), the jars were assigned to different food and cyanobacteria treatments (day 1 in the experiment). There were four replicates of each combination of three two-level treatment factors: clone (1 and 2), food level (20 000 and 80 000 cells of *Scenedesmus* ml^{-1}; 0.26 and 1.04 mg C l^{-1}, respectively) and cyanobacteria (0 and 80 000 cells of *Microcystis* ml^{-1}; 0 and 0.61 mg C l^{-1}, respectively), totalling thirty-two jars. The concentration of cyanobacteria cells corresponded to the lowest LC50 (72 h) value in the acute test.

During the experiment, we added *Scenedesmus* green algae every second day in low or high concentrations (20 000 or 80 000 cells ml^{-1}) to both the control and *Microcystis* treatments. *Microcystis* was added in two pulses (2 × 80 000 cells ml^{-1}) after the two first samplings on days 1 and 4. Since the algal culture media contained micro- and macronutrients, we corrected the nutrient levels by adding similar amounts of pure algal medium to each treatment.

Algal samples were taken, without stirring, from two replicate jars per treatment at days 0, 3 and 6. We added washing detergent to the samples to promote sedimentation of cyanobacteria cells. The samples were counted using the technique of Utermöhl (1958).

At the beginning of the experiment we sampled and counted the animals before we applied the different treatments. The same procedure was repeated on days 4, 7, 11, 15 and 19. First, we gently stirred the water and then sampled a volume of 200 ml. Each jar was sampled at least twice, or until 100 or more animals had been counted. If the animal number was very small, all animals were counted. We classified the animals into five developmental stages: newborn (approximately the two first instars); adolescents; females with eggs; adults without eggs; and females with ephippia. Since this classification was made without a microscope both females and males were included. To estimate size distributions within stages, we measured animals from two of four replicates from each treatment at day 1 and 7 to the nearest 25 μm under a microscope (40× magnification). A minimum number of 100 animals, and a minimum of twenty animals per stage, were measured from the upper edge of the compound eye to the ventral base of the spine. The lengths of the animals in different stages were similar for the two clones studied, except for females with eggs, which were larger in clone 2 (Fig. 1). Of the animals examined microscopically, 0–10% were males, depending on the sample. After measuring and counting, the animals were returned to the jars. On the last sampling date, all animals were preserved in 70% ethanol for later counting and classification.

On each sampling date, we counted the total number of free ephippia by eye without removing them. We also estimated the proportion of ephippia containing eggs at the end of the experiment by opening 10 ephippia from each replicate. We found eggs in 13%

of all ephippia opened. Treatment differences in the relative proportions of empty ephippia were negligible, and therefore we did not study this parameter further.

Data analysis

We calculated LC50 values (the concentration lethal to 50% of the animals) and their 95% confidence intervals from the acute toxicity test using logarithmically transformed doses (Probit analysis program vs. 2.3, National Swedish Environmental Protection Agency). The LC50 values were compared using the confidence intervals.

The model for the population experiment was a factorial analysis of variance (ANOVA) with three fixed treatments (food level, *Microcystis* exposure and clone), and the analyses were made using the general linear models (GLM) procedure in SAS for Windows (v. 6.08) (SAS Institute Inc., 1989).

We calculated mean animal densities for each sampling date and treatment in the mesocosm experiment. We also calculated the mean growth rate, r, for each treatment. Population growth rates were calculated from the formula $r = (\ln N_f - \ln N_i)/(t_f - t_i)$, where N_i is the population size at the initial date, t_i, and N_f is the population size at the final date, t_f. Population variability was measured for each replicate jar as the coefficient of variation (CV) of densities during the study period (McArdle, Gaston & Lawton, 1990). The relationship between the variance and mean of density is exponential (Taylor, 1961), so the CV is frequently dependent on the mean (McArdle *et al.*, 1990). Thus, we analysed the relation between logarithms of CV and density by linear regression. For the ANOVAs, we transformed both CV and density logarithmically to correct for heteroscedasticity. We analysed the treatment effects on mean densities by ANOVA and the effects on population variability by analysis of covariance (ANCOVA; density as covariate).

We counted the proportions of different developmental stages and applied compositional analysis of log-ratios (Aitchison, 1986; Aebischer, Robertson & Kenward, 1993). The sum of compositional data always equals 1 (or 100%). In the method described by Aitchison (1986), values are made independent by dividing each proportion by one of the other proportions. We chose to express each stage relative to the proportion of newborn animals, since newborns were usually present. If some stage was lacking, we set its proportion to 0.0001. The values were transformed into logarithms, as recommended by Aitchison (1986). Before analysing the data, we checked that the assumptions of multivariate analysis of variance (MANOVA) were satisfied (Scheiner, 1993). We first analysed whether the treatments had any effect on the distribution of developmental stages by applying a MANOVA on the mean distribution of developmental stages from all dates. After this, we made planned comparisons of the dependent variables in a repeated doubly multivariate design (SAS Institute Inc., 1989) to study which age group differed from each other. We analysed the effects of food level, *Microcystis* exposure and clonal origin on the proportions of newborns, adolescents and egg-bearing females against other age groups.

We calculated the numbers of released ephippia for

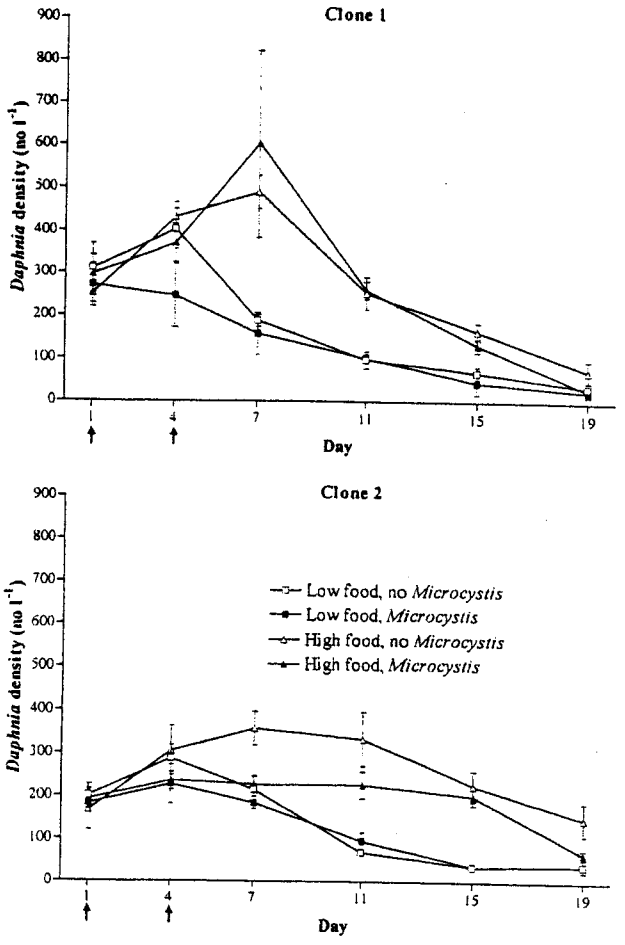

Fig. 2 Fluctuations in *Daphnia* population density in different treatments (mean ± SE). The arrows indicate the dates when the *Microcystis* pulses (80 000 cells ml^{-1}) were applied.

Table 1 LC_{50} values (72 h; cells ml^{-1}) of *Microcystis aeruginosa* for juveniles and adults of the two experimental *Daphnia pulex* clones at low and high food levels (*Scenedesmus obtusiusculus*; 20 000 and 80 000 cells ml^{-1}). Clone one juveniles suffered only slight mortality at high food level, and no values could be counted. Confidence intervals (95%) are given for data fitting the logistic dose–response curve. Groups with the same letters (*a*, *b*) have overlapping confidence intervals

	Clone 1		Clone 2	
	Juveniles	Adults	Juveniles	Adults
Low food	225 000 *b* (–)	149 000 *ab* (84 000–450 000)	77 000 *a* (49 000–118 000)	175 000 *b* (120 000–281 000)
High food	(high) *c*	180 000 *b* (–)	226 000 *b* (165 000–312 000)	194 000 *b* (128 000–377 000)

Table 2 *Daphnia* population densities (mean ± SE) and population growth rates, *r* (mean ± SE) in the different treatments

	Clone 1		Clone 2	
	control	*Microcystis*	control	*Microcystis*
Density				
Low food	184.2 ± 12.5	141.1 ± 19.2	141.1 ± 8.0	127.6 + 14.2
High food	277.4 ± 12.9	282.1 ± 50.2	256.0 ± 17.3	191.5 ± 11.7
r				
Low food	–0.122 ± 0.013	–0.151 ± 0.036	–0.089 ± 0.013	–0.093 ± 0.022
High food	–0.089 ± 0.034	–0.144 ± 0.030	0.005 ± 0.037	–0.060 ± 0.024

the periods between sampling dates, divided by the mean number of adult females during the time interval. Since only adult females are able to form resting eggs, newborn and adolescent animals were not taken into account. We applied a univariate repeated-measures analysis (ANOVAR) of treatment effects on ephippia production. Data were tested for normality, and special conditions for ANOVAR were controlled for as described by Potvin, Lechowicz & Tardif (1990).

Results

Acute test

The microcystin-LR content of our *Microcystis* culture was 8.9×10^{-5} ng $cell^{-1}$ (0.41% of the dry weight). This value is comparable with the toxicity of earlier cultures of the same strain (Reinikainen *et al.*, 1994; Laurén-Määttä *et al.*, 1995).

We calculated LC_{50} values separately for the two clones, food levels and age groups (Table 1). Clone 1 juveniles at the higher food level were very tolerant of the toxic algae. For this group, only one of eight animals died at the highest *Microcystis* levels, and no LC_{50} value could be estimated. The response of clone 1 juveniles at low food and clone 1 adults at high food did not fit the expected logistic model ($\chi^2 = 15.74$, d.f. = 3, $P = 0.0011$; $\chi^2 = 8.09$, d.f. = 3, $P = 0.044$), so the PROBIT program could not be used to calculate their confidence intervals. For the other groups, the estimated LC_{50} values ranged from 77 000 to 226 000 cells ml^{-1}. From the confidence intervals, two groups seemed to differ from the others. Juveniles of clone 2 at low food were the most sensitive, and juveniles of clone 1 at high food the most tolerant group.

Population experiment

Microcystis had virtually disappeared (0–10 cells ml^{-1}) within 2 days after being added to the microcosms, probably due to both ingestion by *Daphnia* and sedimentation of less viable cells. The concentration of *Scenedesmus* was also low (20–1590 cells ml^{-1}) in all treatments after 2 days.

Figure 2 describes fluctuations in population density during the study period. The mean population density over time was highest for the high food level, without *Microcystis* and for clone 1 (Tables 2 and 3). Population growth rates were negative, with the exception of clone 2 populations cultured at high food level without

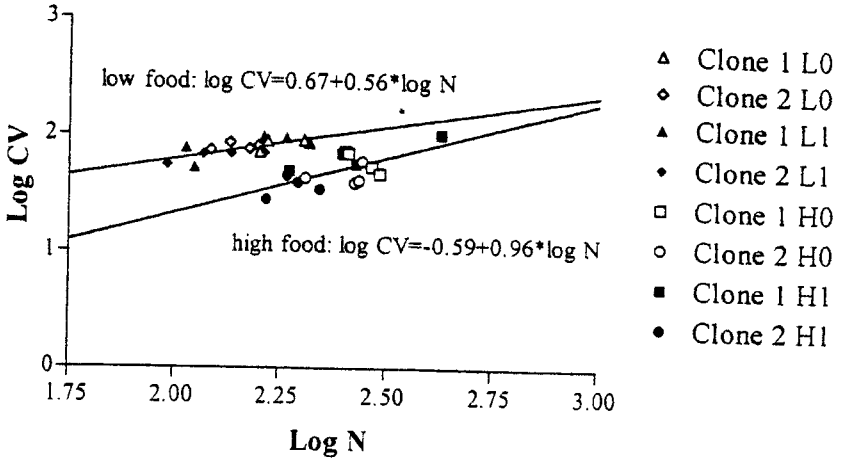

Fig. 3 Relationship between *Daphnia* population mean density (*N*) and variability over time (*CV*). The points represent replicate populations. The values are log-transformed. L = low food level, H = high food level, 0 = no *Microcystis*, 1 = *Microcystis* present. Regression lines are drawn separately for the low and the high food levels.

Table 3 ANOVA results for mean population density and variability (CV) over time. Population density is used as a covariate in the analysis of population variability. Treatment abbreviations: F = food level, M = *Microcystis*, C = clone, D = population density. Significance levels; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$

	Density				Population variability			
Source	MS	*F*	d.f.	*P*	MS	*F*	d.f.	*P*
F	2.210	55.82	1	0.0001***	0.30	0.92	1	0.35
M	0.260	6.45	1	0.018*	0.08	2.67	1	0.12
C	0.310	7.85	1	0.010**	0.01	0.47	1	0.50
F*M	0.004	0.10	1	0.75	0.00	0.00	1	0.95
F*C	0.003	0.08	1	0.78	0.10	3.58	1	0.071
M*C	0.004	0.10	1	0.75	0.08	2.77	1	0.11
F*M*C	0.100	2.41	1	0.13	0.00	0.03	1	0.86
D	–	–	–	–	0.34	11.78	1	0.002**
Error	0.040		24		0.66		23	

Microcystis. The mean growth rate was the highest in treatments without *Microcystis* and at the high food level (Table 2). Clone 2 had a higher growth rate than clone 1, in spite of lower mean densities. We did not find any significant regression between population variability and density when fitting all points to the same line ($r^2 = 0.018$, $P = 0.46$). However, analysing the low and high food levels separately resulted in significant positive regressions between log density and log CV (low food: $r^2 = 0.54$, $P = 0.0012$; high food: $r^2 = 0.49$, $P = 0.0027$) (Fig. 3). ANCOVA showed that differences in CV among treatments disappeared when correcting for population density (Table 3).

The *Microcystis* treatment and clonal origin had significant effects on the overall distribution of developmental stages (Table 4). We found differences for all the planned stage comparisons that we made (Table 4). The significant effect of *Microcystis* on the stage distribution was due to changes in the proportion of adolescents. The proportion of adolescents was higher in populations exposed to *Microcystis* than without *Microcystis*. This result suggests delayed maturation, as there were no significant differences in the proportion of newborns (Table 4, Fig. 4). Within-subject effects (the time effect and its interaction with the treatment effects) were found for time and for the interactions of time × *Microcystis* and time × clone (Table 4).

Clone 1 populations had a larger proportion of newborns than clone 2 populations. There was a significant interaction between clone and time (Table 4), as the main clonal differences regarding newborns were found on day 7 or later. During the experiment, there were proportionally fewer newborns at the high food level (Table 4, Fig. 4). However, food level did not have a significant effect on the overall distribution of stages ($P = 0.10$), so this result should be cautiously interpreted. The overall trend was a decrease in the proportion of newborns and an increase in the number of adolescents (Fig. 4, Table 4).

Table 4 MANOVA results for the distribution of *Daphnia* developmental stages (newborns, adolescents, females with eggs, adults without eggs and females with ephippia). In the MANOVA with repeated measurements, treatment effects are named 'between-subject effects'. The time effect and its interaction with the treatments are named 'within-subject effects'. Treatment abbreviations: F = food level, M = *Microcystis*, C = clone, T = time. Significance levels; + $P = 0.057$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Source	*F*	*P*	*F*	*P*	*F*	*P*
MANOVA on means for all dates; all stages						
F	2.20	0.10				
M	9.51	0.0002***				
C	9.83	0.0001***				
F*M	2.42	0.08				
F*C	0.98	0.44				
M*C	0.60	0.67				
F*M*C	0.91	0.48				
MANOVA with repeated measurements; planned comparisons						
Between subjects	Newborn vs. others*		Adolescents vs. others†		Females with eggs vs. other‡	
F	6.77	0.016*	1.13	0.30	1.49	0.23
M	0.06	0.80	11.25	0.003**	0.29	0.59
C	16.62	0.0004***	0.13	0.72	6.57	0.017*
F*M	0.61	0.44	0.07	0.79	1.25	0.28
F*C	0.31	0.58	0.34	0.56	1.46	0.24
M*C	0.79	0.38	1.73	0.20	0.14	0.71
F*M*C	0.43	0.52	0.09	0.77	0.90	0.35
Within subject	Newborn vs. others		Adolescents		Females with eggs vs. others	
T	13.33	0.0001***	40.34	0.0001***	15.61	0.0001***
T*F	0.80	0.56	0.75	0.60	1.03	0.43
T*M	1.56	0.22	5.33	0.0028**	1.11	0.38
T*C	4.58	0.006**	4.79	0.005**	2.02	0.12
T*F*M	0.76	0.59	1.18	0.35	0.49	0.78
T*F*C	1.49	0.24	1.55	0.22	1.19	0.35
T*M*C	1.39	0.27	0.26	0.93	0.79	0.57
T*F*M*C	0.74	0.60	1.17	0.36	1.01	0.44

*Error MS = 284.5, d.f. = 24; †error MS = 232.8, d.f. = 24; ‡error MS = 823.9, d.f. = 24

Clone 2 had relatively more females with eggs, but as the proportion of newborns was smaller, either clutch sizes must have been smaller or egg mortality higher (Table 4, Fig. 4). The overall trend was that the proportion of females with eggs decreased after day 1 and then increased towards the end of the experiment (Table 4, Fig. 4). This was probably a response to changes in population density.

Ephippia were produced only by a few individuals before day 1, so initial ephippia production did not differ between treatments. *Microcystis* exposure and clonal origin significantly affected the production of ephippia per adult (Table 5). Exposure to *Microcystis* resulted in a somewhat higher production of ephippia per adult compared to the control without *Microcystis*. Clone 2 produced more ephippia per adult than clone 1 (Fig. 5). The clones responded differently to *Microcystis*, as indicated by the significant *Microcystis**clone interaction ($P = 0.055$) (Fig. 5, Table 5). At low food, clone 2 produced more ephippia at *Microcystis* exposure, while clone 1 was indifferent (Fig. 5). The production of ephippia increased towards the end of the study period, particularly for clone 2 (Fig. 5). Accordingly, the time and time × clone effects were highly significant (Table 5).

Discussion

In this study, we found impacts of toxic *Microcystis* on population density and age structure (Table 6). In addition, adults exposed to *Microcystis* produced more ephippia than adults in the *Microcystis*-free control.

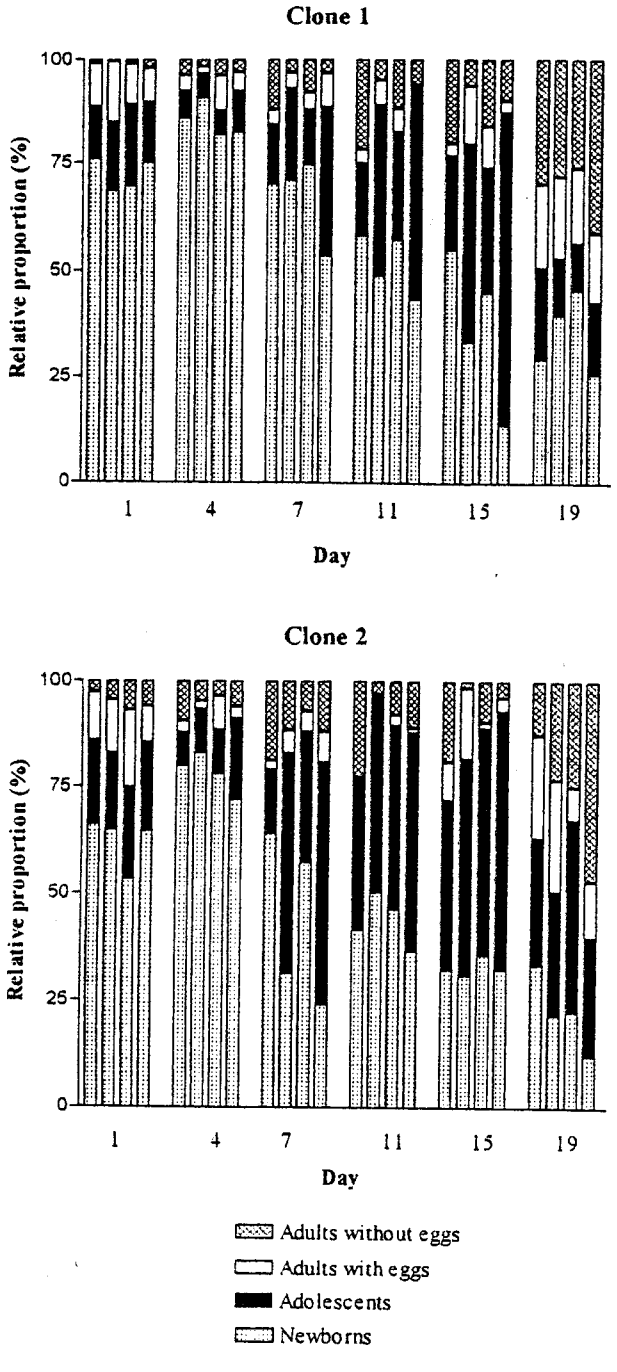

Fig. 4 Composition of *Daphnia* developmental stages in different treatments for the two clones. The proportions of females with ephippia varied between 0 and 2% of the *Daphnia* populations and are not included in this figure. Treatment bars appear in the following order (left to right) for each date: 1, low food without *Microcystis*; 2, low food with *Microcystis*; 3, high food without *Microcystis*; 4, high food with *Microcystis*.

Contrary to our original hypothesis, population variability was not affected by the presence of *Microcystis*. The two clones differed in mean density, age structure, and ephippial production. We also found some differences in their responses to toxic *Microcystis*.

One main impact of toxic cyanobacteria was a decrease in the mean density of *Daphnia* (Table 6). Also, many lake populations of *Daphnia* decrease during cyanobacterial blooms (Edmondson & Litt, 1982; Jarvis *et al.*, 1987; Moss *et al.*, 1991). However, few lake investigations have data on both toxicity of cyanobacterial blooms and *Daphnia* density. If the toxicity of the bloom is unknown or if the bloom is nontoxic, a decrease of *Daphnia* may be explained either by interference with the filtering activity or by poor nutritive value of cyanobacteria.

In our microcosms, most *Microcystis* cells existed as single cells or in small (2–3 cells) colonies, so mechanical disturbance of filtering is excluded. The animals in the *Microcystis* treatments were provided green algae in the same concentrations as the control animals. Consequently, the decline in population density is most probably due to the toxic effects of *Microcystis*. The HPLC analysis and acute toxicity test confirmed that the algal culture contained the toxin microcystin, and that it was lethal to *Daphnia* in moderate concentrations (LC50 72 h; 77 000–226 000 cells ml^{-1}). Studies by Berg, Skulberg & Skulberg (1987) show that the toxins may also leak into the water after decomposition of blooms. Thus, toxins may have been present even after the disappearance of added cyanobacteria. However, it has been shown that *Daphnia* is less sensitive to dissolved microcystins than to ingested cyanobacterial cells (DeMott, Zhang & Carmichael, 1991).

The relative biomass of cyanobacteria was high on days 1 and 4, immediately after the addition. At that time, cyanobacteria made up 70% of the algal biomass at the low food level and 37% of the biomass at the high food level. The observed effects on animal densities seemed to be quite short-lived, as *Microcystis* cells were present only for a short time. At the end of the experiment, animal densities were similar in the control and in the *Microcystis* treatments (Fig. 1). We found higher mortality in the acute test than in the population experiment. A *Microcystis* concentration of 80 000 cells ml^{-1} killed 50% of the clone 2 juveniles in the acute test, but we observed no such dramatic effect in the population experiment. Animal density at *Microcystis* exposure (one animal per 10 ml in the

Table 5 ANOVA results for univariate repeated time measurements of released ephippia. Treatment abbreviations: see Table 4. Significance levels: $+P = 0.055$; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$

	Between subjects*			Within subject†	
Source	*F*	*P*	Source	*F*	*P*
			T	29.58	0.0001***
F	0.64	0.43	T*F	0.87	0.48
M	4.63	0.042*	T*M	0.98	0.42
C	24.92	0.0001***	T*C	10.70	0.0001***
F*M	1.28	0.27	T*F*M	1.90	0.12
F*C	0.26	0.61	T*F*C	0.65	0.63
M*C	4.06	0.055+	T*M*C	1.24	0.30
F*M*C	0.08	0.78	T*F*M*C	0.06	0.99

*Error MS = 0.00013, d.f. = 24; †error MS = 0.012, d.f. = 96

Table 6 Summary of between-subject effects. Treatment abbreviations: F = food level, M = *Microcystis*, C = clone. When treatments differ regarding studied responses, the treatment with the higher value is given within the parentheses. Significance levels: $+P = 0.057$; $++P = 0.055$; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$

	Daphnia parameter			Age groups			
	Mean density	CV	Ephippia production	Newborn	Adolescents	Females with eggs	Young vs adults
F	***(high F)	NS	NS	*(low F)	NS	NS	+
M	*(no M)	NS	*(M)	NS	***(M)	NS	NS
C	**(C 1)	NS	***(C2)	***(C1)	NS	*(C1)	NS
F*M	NS	NS	NS	NS	NS	NS	*
F*C	NS	NS	NS	NS	NS	NS	NS
M*C	NS	NS	+ +	NS	NS	NS	NS
F*M*C	NS	NS	NS	NS	NS	NS	NS

acute test; 2–4 animals per 10 ml in the population experiment) may partly explain differences between the acute and the population experiments. Lower mortality in the population experiment may also be explained by spatial heterogeneity of *Daphnia* and cyanobacteria in larger systems, resulting in less efficient feeding of *Daphnia* on the toxic cyanobacteria.

The effects of toxic cyanobacteria are similar to effects of low food quality or abundance (Vanni & Lampert, 1992; Boersma & Vijverberg, 1994). *Daphnia* responds to toxic *Microcystis* by increased mortality, decreased growth, delayed maturation and decreased offspring production (Arnold, 1971; Lampert, 1981; Nizan *et al.*, 1986; Reinikainen *et al.*, 1994; Hietala *et al.*, 1995). One or several of these parameters may be responsible for the observed decline of the microcosm *Daphnia* populations exposed to *Microcystis*.

We decided not to count eggs and embryos, as we wanted to minimize the handling of the live animals. The number of eggs and embryos per female would have provided the best estimate of offspring production. However, the proportions of newborn and egg-bearing females together do give a fairly good indication of offspring production. Neither of these age groups decreased their relative numbers in the *Microcystis* treatment. However, the increased proportions of adolescents without increased reproduction in the *Microcystis* exposure indicate delayed maturation. Threlkeld (1986) also found delayed maturation in natural *Daphnia* populations in midsummer. The *in situ* study by Threlkeld (1979) indicated that an increasing amount of *Anabaena* was one of several factors resulting in the reduced reproduction of *Daphnia*. Mortality affecting both newborn and adult animals may be another reason for the decline of the *Daphnia* populations in our study.

We did not find interactions between food level and *Microcystis*. In *Daphnia*, the physiological condition of the mother can affect the fitness of her offspring (Tessier & Consolatti, 1991; Lampert, 1993). In our

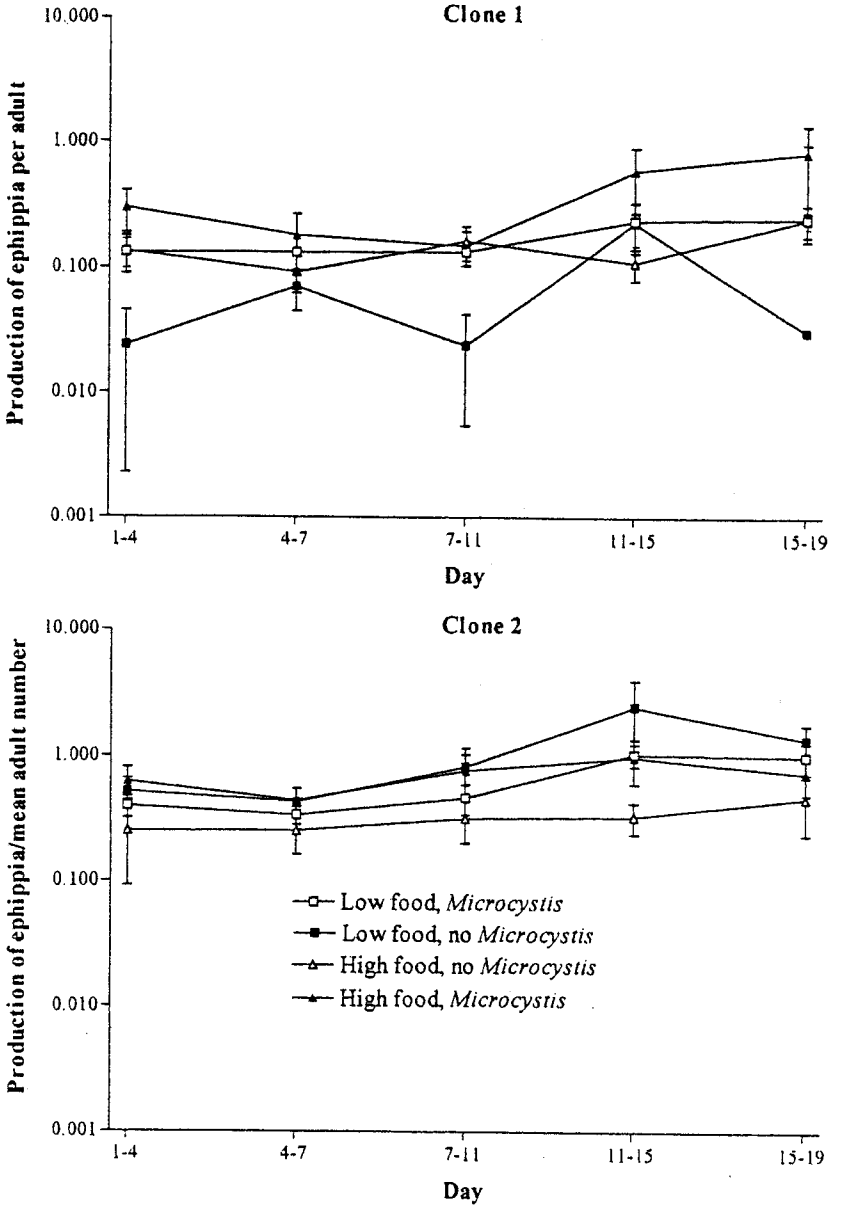

Fig. 5 Relative production of released ephippia (mean ± SE) at low and high food levels, calculated as the change in the observed number of ephippia between sampling dates divided by the total number of adults with or without eggs. The scale of the *y*-axis is logarithmic.

study, the founders of all populations were raised at a high food level. Consequently, the quality of animals at the low food level may be high at the beginning of the experiment due to maternal effects. The food level did fluctuate between the additions of food, although we added new food every second day. This problem is common for batch culture experiments. Another problem arising when one adds similar algal concentrations to several population cultures is that the amount of food per individual will differ depending on the population size. In our experiment, there were fewer animals in the *Microcystis* treatment than in the control. Thus, the animals exposed to *Microcystis* received somewhat more food per individual than the animals in the control. We might have found stronger effects from *Microcystis* if we had corrected the food level according to the number of animals. However, a constant food level per animal would have been difficult to maintain. Animal abundances change quickly, and animals consume different amounts during different life-history stages.

The results showing effects of food level on the distribution of developmental stages should be interpreted with some caution. The food level effects were

not evident when all stages were compared but only in the proportion of newborns. In our study, the added concentration of food was four times higher at the high than at the low food level, and there were less than twice as many animals (Table 2). At the high food level, we found higher population densities but a smaller proportion of newborns than at the low food level. One explanation is that the clutch sizes were smaller at high animal densities due to crowding. Burns (1995) proposed that the feeding rate of *Daphnia* is enhanced at densities lower than a certain threshold value, above which feeding is depressed. The reduced feeding results in depressed individual growth rates and smaller clutch sizes. An alternative explanation is that poorly assimilated food has accumulated and deterred the populations raised at the high food level.

Factors that increase the variability of *Daphnia* population size include interspecific competition (Bengtsson & Milbrink, 1995) and exposure to toxicants such as cadmium (Marshall, 1978). In our study, population variability was not increased by exposure to *Microcystis*. The toxic cyanobacteria should probably have been present for a longer time to affect population variability. Our experiment spans approximately only one population cycle. The only factor explaining population variability in our experiment was population size, but these two factors were positively correlated. Indeed, McArdle *et al.* (1990) recognized density-dependence as a major problem when comparing stabilities of animal populations. In contrast to our results, Bengtsson & Milbrink (1995) found that variability was higher at lower densities in laboratory populations of *Daphnia*. The difference may be explained by the different range of mean population densities (4–250 animals l^{-1} in the study by Bengtsson & Milbrink (1995); 100–400 animals l^{-1} in our study).

We found significant impacts of *Microcystis* exposure on ephippia production (Fig. 5). More resting eggs were produced towards the end of the experiment, which may reflect a timelag in the induction of ephippia formation. Delayed response to previous crowding or food limitation may affect the results, although ephippial production did not differ between food levels. Cyanobacteria toxins tend to inhibit feeding of *Daphnia* (Lampert, 1981). Thus, food limitation at *Microcystis* exposure may be a possible explanation for the enhanced induction of ephippia formation in this treatment. Production of resting eggs during blooms of toxic cyanobacteria may be a useful strategy to escape the harmful effects.

The two clones differed in mean density, ephippia production and in the distribution of developmental stages. Acute and life-history experiments (Hietala *et al.*, 1995, 1996) have revealed significant differences among *Daphnia* clones in their responses to toxic *Microcystis*, indicating that cyanobacteria may be a selective agent in clonal competition. Thus, it is helpful to use several clones when studying toxicity impacts on *Daphnia*, a fact frequently neglected in experimental studies. In the acute test, clone 1 juveniles were less sensitive to toxic *Microcystis* than clone 2 juveniles. However, there were no clonal differences in the distribution of developmental stages between *Microcystis* and control treatments in the population experiment. In the population study, a marginally significant interaction between *Microcystis* exposure and clone was present for the production of ephippia. However, clone 1 adults decreased their production of ephippia under *Microcystis* exposure when cultured at the lower food level. In conclusion, the clones showed slightly different responses to toxic cyanobacteria in both the acute test and in the population experiment. However, no consistent differences in sensitivity were found between the clones in these two experiments.

Using laboratory microcosms, we tested the impacts of toxic cyanobacteria together with different food levels on *Daphnia* populations. The applied pulses of toxic *Microcystis* resulted in lower population densities, probably because of increased mortality and delayed maturation. These effects have also been found, at lower concentrations, in life-table experiments. In contrast to the life-history experiments with *D. pulex* we did not find an effect of the toxic cyanobacteria on the offspring number. Our results indicate, however, that toxic cyanobacteria may be one factor inducing the formation of ephippial eggs.

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