JOURNAL OF PLANKTON RESEARCH | VOLUME 31 | NUMBER 8 | PAGES 827–835 | 2009

Growth and survival responses of a tropical *Daphnia* (*Daphnia lumholtzi*) to cell-bound microcystins

RONALD SEMYALO[1]*, THOMAS ROHRLACK[2] AND PETTER LARSSON[1]

[1]DEPARTMENT OF BIOLOGY, UNIVERSITY OF BERGEN, ALLÉGATEN 41 5007, BERGEN, NORWAY AND [2]NORWEGIAN INSTITUTE OF WATER RESEARCH, GAUSTADALLÈEN 21, 0349 OSLO, NORWAY

*CORRESPONDING AUTHOR: ronald.semyalo@bio.uib.no

Received February 26, 2009; accepted in principle April 16, 2009; accepted for publication April 19, 2009; published online 11 May, 2009

Corresponding editor: Mark J. Gibbons

Eutrophic tropical freshwater bodies often have cyanobacterial blooms that last throughout the year and some of these blooms may produce compounds that are toxic to Daphnia. Nevertheless, tropical species like Daphnia lumholtzi continue to remain abundant in the presence of such blooms. We conducted an experiment on the growth and survival of D. lumholtzi. We fed a toxic cyanobacteria Microcystis aeruginosa PCC 7806 to study the response of D. lumholtzi to cell-bound microcystins. Daphnia lumholtzi showed poor growth and survival in the presence of M. aeruginosa PCC 7806 both with and without microcystins. Survival and growth improved significantly when M. aeruginosa comprised <50% of the food offered. However, regardless of the amounts of M. aeruginosa in the food, we did not find any significant difference in the growth or survival between the microcystin-containing and the microcystin-lacking treatments. We observed a significant difference in age at maturity between the microcystin-containing and the microcystin-lacking groups when the amount of M. aeruginosa in the food offered was <50%. The toxic cyanobacteria M. aeruginosa PCC 7806 contains substances other than microcystins that reduce the growth and survival of the tropical D. lumholtzi. However, under low cyanobacteria concentrations and in the absence of microcystins, D. lumholtzi shows improved reproduction rates.

INTRODUCTION

Cyanobacteria of the genus *Microcystis* often dominates in phytoplankton blooms that are characteristic of highly productive lakes (McDougall & Ho, 1991; Yin *et al.*, 1992; Vasconcelos *et al.*, 1993). In temperate regions, densities of *Microcystis* which tend to peak in the late summer and early autumn are often blamed for declining abundances of the zooplankton grazer *Daphnia* (Havel & Hebert, 1993; Ghadouani *et al.*, 1998; Jacoby *et al.*, 2000; Pattinson *et al.*, 2003).

Besides forming colonies that are sometimes too large to be ingested by zooplankton (Boing *et al.*, 1998), *Microcystis* cells may be nutritionally inadequate (Lampert, 1987) and lacking in essential unsaturated fatty acids (Demott & Mueller-Navarra, 1997; Martin-Creuzburg *et al.*, 2008). In some cases *Microcystis* may produce cyanotoxins like the hepatotoxic microcystins MCYST-LR (D-Asp^3), MCYST-LR (Dittmann *et al.*, 1997) and other secondary metabolites that are thought to be lethal to *Daphnia* (Demott *et al.*, 1991; Rohrlack *et al.*, 1999; Ghadouani *et al.*, 2004). Some *Microcystis* strains may also cause feeding inhibition in *Daphnia* (Demott, 1999) which has been attributed to a "bad taste factor" (Nizan *et al.*, 1986) or some other as yet unidentified non-mechanical feeding inhibitor (Jungmann, 1995; Rohrlack *et al.*, 2005). Laboratory studies that have investigated the specific effect of microcystins on the life history variables of temperate *Daphnia* species show that they cause an increase in mortality (Rohrlack *et al.*, 2001; Lürling, 2003) and may contribute

doi:10.1093/plankt/fbp032, available online at www.plankt.oxfordjournals.org

to lower reproduction (Lürling, 2003) and population growth rates (Tillmanns *et al.*, 2008). These effects are also observed at low concentrations of toxic *Microcystis* (Hietala *et al.*, 1997).

Daphnia, like most cladocerans, are relatively non-selective filter feeders (Demott, 1990), and may be unable to graze differentially on toxic and non-toxic cells based on the presence of toxic compounds like microcystins (e.g. Rohrlack *et al.*, 2001; Lürling, 2003). This is probably because the toxic effect of *Microcystis* cells may not be limited to a single compound like microcystins (Jungmann, 1995). It has also been hypothesized that the toxicity of *Microcystis* to *Daphnia* depends not only on the content of cellular microcystins but also on the rate at which it is ingested (Rohrlack *et al.*, 1999).

The effects of microcystins are both species- and clone-specific (Hietala *et al.*, 1997), suggesting that animals that have to survive longer bloom episodes and concentrations may either have higher levels of tolerance (Matveev *et al.*, 1994) or lower rates of ingestion feeding cyanobacteria (Rohrlack *et al.*, 2001). Experimental studies (Gustafsson & Hansson, 2004) have shown that *Daphnia* with repeated exposure to toxic algae in their natural habitat may have better population growth than those with little or no previous exposure. In the presence of higher temperatures, higher light intensities and absence of seasons in tropical lakes like Lake Victoria (Uganda), blooms of *Microcystis* often persist all year round (Haande, 2008); there the *Daphnia* community is exposed to longer episodes of microcystin-containing blooms and yet some populations of *Daphnia* like *D. lumholtzi* remain abundant (Mwebaza ndawula, 1994; Semyalo, 2003).

The invasion success of the tropical cladoceran *D. lumholtzi* in temperate and subtropical regions has been attributed to several factors, including having a long helmet and tail spine that function as a unique predator defence (Kolar & Wahl, 1998), higher production of resting eggs (Acharya *et al.*, 2006) and tolerance of higher temperatures and poorer food conditions (Havel & Hebert, 1993; Havens *et al.*, 2000; Pattinson *et al.*, 2003). In the United States, where most studies on the invasion success of *D. lumholtzi* have been done, densities of *D. lumholtzi* often peak in late summer conditions, which are associated with the occurrence of cyanobacteria blooms (Havens *et al.*, 2000; Pattinson *et al.*, 2003). The very few studies that have been conducted on the effects of microcystins on tropical *Daphnia* (Matveev *et al.*, 1994; Chen & Xie, 2004) suggest that tropical *Daphnia* may have a higher tolerance to toxic *Microcystis* than their temperate relatives, e.g. *D. carinata*, especially in the presence of other food sources like green algae.

In this study we investigate the null hypothesis that cell-bound microcystins do not affect the growth and survival of the tropical cladoceran, *D. lumholtzi*. We investigate the effects of cell-bound microcystins on life history variables of *D. lumholtzi* using the toxic microcystin-containing *M. aeruginosa* strain PCC 7806 and its microcystin-deficient mutant. We also use the green algae *Scenedesmus* spp. in mixtures with the wild-type and mutant type to compensate for nutritional inadequacy (e.g. Demott & Mueller-Navarra, 1997; Martin-Creuzburg *et al.*, 2008) associated with *Microcystis*.

METHOD

Algae and cyanobacteria

Microcystis aeruginosa

Stock cultures of the *wild-type* (microcystin-containing) and *mutant type* (non-microcystin-containing) cyanobacteria *M. aeruginosa* strain PCC 7806 were obtained from the Norwegian Institute of Water Research. The *M. aeruginosa* PCC 7806 strain was originally isolated from the Braackman Reservoir (The Netherlands) and is grown as single cells (Rohrlack *et al.*, 2001). It produces several bioactive compounds including the hepatotoxins, microcystins MCYST-LR and (D-Asp^3)MCYST-LR. Cells of this strain have a highly significant toxic effect on *Daphnia* (Rohrlack *et al.*, 1999). In order to obtain the mutant cell line of the strain PCC 7806, the peptide synthetase gene *mcyB* has been insertionally inactivated using a chloramphenicol-resistance cassette resulting in the total removal of microcystin synthesis while not affecting the production of other oligopeptides, such as cyanopeptolines (Dittmann *et al.*, 1997).

The wild-type and mutant type variants of *Microcystis* PCC 7806 were grown in O2 culture medium as axenic batch cultures. The cultures were maintained under continuous light (25 μmol of photons m^{-2} s^{-1}) supplied by white fluorescent lamps. They were shaken twice a day and grown at a temperature of 23 ± 1°C. The cultures were grown in 350 mL batches for a period of 3 days to a final concentration of 350 mm^3 L^{-1} (1 mm^3 L^{-1} of the wild-type and mutant type of *M. aeruginosa* PCC7806 is equivalent to 3.413×10^7 cells). The algal concentration was determined using a calibration curve of light absorbance at 800 nm and the algal dry weight per unit volume. In the laboratory, the growth rate of the wild-type and mutant type was linear and was not significantly different ($P > 0.05$). Because of the small size of the cells harvesting was more efficient by gentle centrifugation for 10 min at 500× *g*.

Scenedesmus spp.

The green alga *Scenedesmus* spp. is maintained in stock cultures at the Department of Biology, University of Bergen. Algae used for the experiment were grown separately in 500 mL batch cultures in O2 medium under constant illumination using two circular fluorescent tubes, at temperatures of 21−23°C. Cultures were harvested every third day, and left to sediment for 3 days to allow for removal of most of the algal medium. Each 500 mL batch culture was thus concentrated down to 50 mL and subsequently re-suspended in GFC-filtered lake water from Lake Myravann (Bergen, Norway) for use in the experiments. Algal concentration was determined using a calibration curve of light absorbance at 800 nm and dry weight per unit volume.

Daphnia lumholtzi

Daphnia lumholtzi was isolated from Lake Victoria (Uganda) and maintained in culture at the University of Bergen since 2003. The *Daphnia* cultures were maintained in 2 L jars of GFC-filtered lake water from Lake Myravann (Bergen, Norway) at temperatures of 21−23°C and a 16:8 h light:dark cycle. They were fed three times a week *ad libitum* on 2.0 mg DW L^{-1} of *Scenedesmus*. Animals used for the life history experiments were maintained in a separate climate chamber at a temperature of 25°C and a 12:12 h light:dark cycle for a period of 6 months prior to the experiment.

Experimental design

We conducted a laboratory experiment to investigate the effects of cell-bound microcystins on life history variables of growth, survival and reproduction on a single clone of *D. lumholtzi* grown on various mixtures of *Scenedesmus* spp. and a toxic *M. aeruginosa* strain PCC 7806 (wild-type) or its microcystin-deficient mutant (mutant type) at different food levels. The experiment consisted of the following food treatments—75% *Scenedesmus*:25% *M. aeruginosa*; 50% *Scenedesmus*:50% *M. aeruginosa*; 25% *Scenedesmus*:75% *M. aeruginosa*; 100% *M. aeruginosa*. Experimental controls of pure *Scenedesmus* spp. with food concentrations (100%, 75%, 50%, 25% and a starvation control—0%) similar to the amount of *Scenedesmus* spp. in the food mixtures were also included. The 100% algae concentration corresponded to 2.0 mg DW L^{-1}.

Daphnia were grown in flow-through systems (Lampert *et al.*, 1988) to ensure constant food conditions. The system consisted of a series of 250 mL flow-through chambers connected to larger 1.5 L glass jars (with a magnetic stirrer) by a peristaltic pump at a ratio of three chambers to one jar. Each chamber had an inlet at the top of the chamber and an outlet tube at the bottom to ensure a constant volume of media in the chamber and prevent overflow. The chambers were held in a waterbath that was maintained at a constant temperature of 25°C throughout the experiment. The reservoir glass jars were regularly filled with algae/lake water mixture to ensure that they did not dry up. Throughout the experiment, the 1.5 L food reservoirs contained algal suspension in lake water with a total concentration of 2.0 mg DW L^{-1}. Prior to the start of the experiment, 30 adult *Daphnia* were placed in three flow-through chambers, 10 in each chamber for a period of 12 h during which time, concentrations of food flowing out of the flow-through chambers were monitored and adjusted. This was done to ensure that the concentration of food in the flow-through chamber remained constant.

New-borns (<24 h-old) taken from a single mother in the stock culture were isolated and grown individually in 250 mL glass jars filled with GFC-filtered lake water, in a climate chamber at a temperature of 25°C and 12:12 h light:dark cycle and fed daily on high concentrations (2.0 mg DW L^{-1}) of *Scenedesmus* spp. These new-borns which served as mothers to the mothers of the experimental animals were daily transferred to new jars within the climate chamber, new-borns from the first and second clutches were removed regularly. The new-borns from the third clutch which served as experimental mothers were then transferred individually to 250 mL flow-through chambers in the flow-through system that was incubated in a waterbath at 25°C. New-borns from the third clutch of experimental mothers were used as experimental animals. Experimental animals were placed randomly in each of the 250 mL flow-through chambers. Each chamber received 10−11 animals, whereas each treatment and control group was made up of three chambers.

Length measurements were made from just above the eye to the base of the spine of three randomly selected individuals from each treatment taken daily. The chambers were also inspected for new-borns daily; these were counted and removed. Time to reach maturity and mortality in each chamber was recorded. The experiment was conducted for a period of 10 days or until total mortality of animals in all treatment groups.

Statistics

A linear mixed effects model of all treatment groups was used to test the effect of food type (*Scenedesmus* spp. and *Microcystis* spp.) and microcystins (mutant and wild) on growth. Tukey's *post hoc* test was used to identify

specific differences between the treatment groups (75% *Scenedesmus*:25% *M. aeruginosa*; 50% *Scenedesmus*:50% *M. aeruginosa*; 25% *Scenedesmus*:75% *M. aeruginosa* and 100% *M. aeruginosa*). The effect of food and microcystins on survivorship and prediction of age at death was analysed using survival analysis (failure-time analysis) with censoring. All statistics were performed using R version 2.7.1 (R Development Core Team, 2008).

By carrying out a survival analysis with censoring (failure-time analysis), we compared a null model (no predictors) of the survival data with a model explaining the data with predictors (treatment). We then contrasted the treatment groups against the no food (starvation) control using chambers as a random effect.

RESULTS

Survival

We found significantly lower survival among animals in treatment groups containing *M. aeruginosa* (wild and mutant types) than in the control groups with corresponding amounts of *Scenedesmus* spp. (Fig. 1; $P < 0.001$). Highest mortality was recorded in the 100% *M. aeruginosa* wild-type group, with the highest mortality by the second day of the experiment. There was no significant difference in survival between the various *Scenedesmus* food controls (100%, 75%, 50% and 25%; $P > 0.05$). Comparison of all treatment groups with the starvation control revealed no differences in survivorship ($P > 0.05$) except for when the proportion of *M. aeruginosa* was low in the food mixtures (25%; $P < 0.001$) regardless of the presence of microcystins. There were no chamber effects in our experiment ($P > 0.05$). The mean age at death for the no food control and all treatments containing over 75% *M. aeruginosa* was predicted to be less than 3 days.

Growth

Our results on comparison of all the treatment groups in the experiment after 3 days (Fig. 2) did not show any significant food ($P > 0.05$) or microcystins effect ($P > 0.05$) on growth. We also did not detect a significant interaction of food and microcystins on growth ($P > 0.05$).

For the treatment groups [75% *Scenedesmus*:25% *M. aeruginosa* (mutant and wild type)] that survived to maturity, we used an analysis of covariance to test for differences in growth slope between them and the experimental food control that had the same total amount of food offered (2 mg DW L^{-1}, 100% *Scenedesmus* only; Fig. 3). A contrast analysis between these three treatments showed a difference between the pure *Scenedesmus* treatment and the two mixed treatments [75% *Scenedesmus*:25% *M. aeruginosa* treatments (mutant, $P < 0.01$; wild, $P < 0.001$)], while the mutant and wild-type treatments did not differ from one another ($P > 0.05$). We also analysed for covariance in growth with time among the control groups (100%, 75%, 50% and 25%; *Scenedesmus* spp.) up to maturity, to test for an effect of reduction in amounts of *Scenedesmus* spp. in food offered on growth. We found a difference in growth slope only between the 100% *Scenedesmus* spp. control and the 25% control *Scenedesmus* spp. ($P < 0.01$), while there was no difference ($P > 0.05$) between 100% *Scenedesmus* spp. control and the 50% and 75% *Scenedesmus* spp. controls.

Reproduction

There were only two treatment groups in the experiment, with individuals that reached maturity [75% *Scenedesmus*:25% *M. aeruginosa* (mutant and wild-type)]. We used a one-way ANOVA to compare mean age at maturity between animals in the two treatment groups that survived until maturity and the 100% *Scenedesmus* control treatment. We found an effect of treatment on age at maturity ($P < 0.001$). Subsequent Tukey multiple comparisons test (Table I) showed that age at maturity for the *Scenedesmus* control group was lower than either of the treatment groups ($P < 0.01$) and that the age at maturity of the mutant type treatment group was lower than the wild-type treatment group ($P < 0.05$). There were no significant effects of food and microcystins on size at maturity ($P > 0.05$).

DISCUSSION

Daphnia lumholtzi growth and survival rate were not significantly influenced by the presence of microcystins in the cyanobacteria *M. aeruginosa* PCC 7806. Of all the life history variables investigated, microcystins only exhibited a significant effect on the age at maturity ($P < 0.05$) in treatment groups at the lowest levels (25%, 0.5 mg DW L^{-1}) of *M. aeruginosa* in the food.

A review of several experimental studies on the toxic effects of cyanobacteria to zooplankton (Wilson *et al.*, 2006) generally shows that though commonly assayed toxic compounds like microcystins may have no influence on grazer population growth they negatively affect survival. However, in this study all treatment groups with or without microcystins (except when *M. aeruginosa* was 25%) did not show any significant difference in

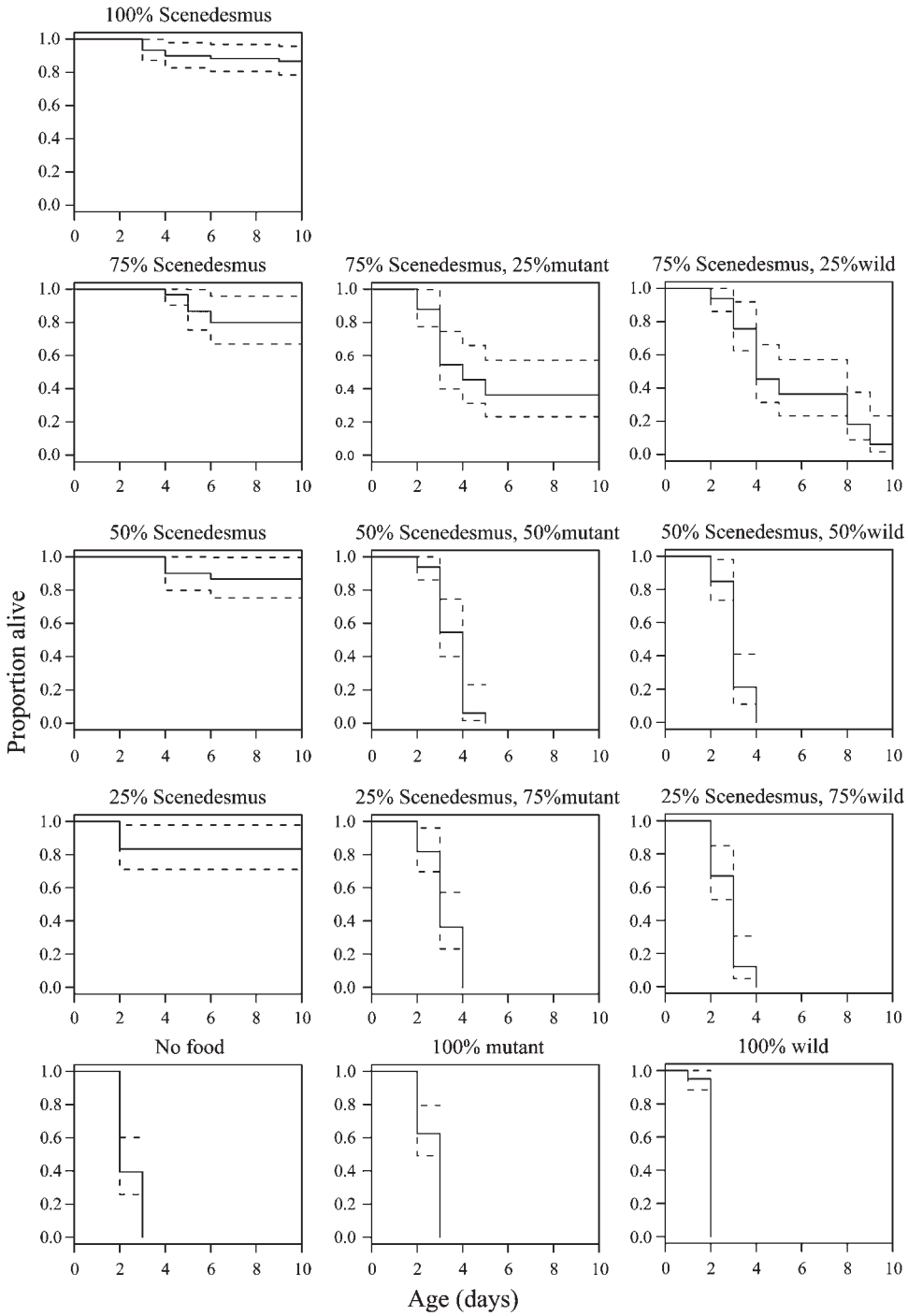

Fig. 1. Graphs showing percent survival with time of *Daphnia lumholtzi* fed with different amounts of *Scenedesmus* only, mixtures of *Scenedesmus* and mutant type or mixtures of *Scenedesmus* and wild-type. We use censoring (0 if animal is alive at termination of experiment, 1 if animal is dead at termination of experiment) to eliminate bias. Each treatment had three replicates.

survival when compared against each other and against the starvation control.

The low survival observed in these treatment groups is not linked to lowered amounts of *Scenedesmus* spp. offered but to the presence of *M. aeruginosa* in the food offered. However, we did not observe any linear relationship between amounts of *M. aeruginosa* in food offered and survivorship, instead the survivorship

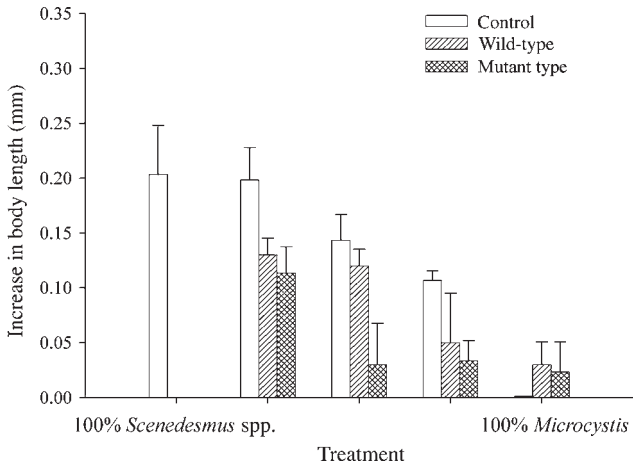

Fig. 2. Mean increase in body length of *Daphnia lumholtzi* juveniles (Day 1−Day 3) grown on different mixtures of *Scenedesmus* spp. and *M. aeruginosa* (100% *Scenedesmus*; 75% *Scenedesmus*:25% *M. aeruginosa*; 50% *Scenedesmus*:50% *M. aeruginosa*; 25% *Scenedesmus*:75% *M. aeruginosa* and 100% *M. aeruginosa* (light bars denote *Scenedesmus only*, light shaded denote mutant type and dark shaded denote wild-type).

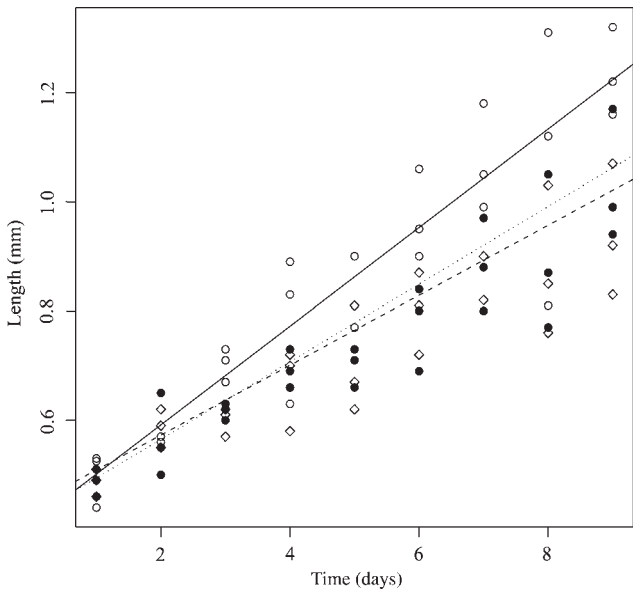

Fig. 3. Growth by length with time from Day 1 up to first clutch for *Daphnia lumholtzi* fed on different treatments of algae [*Scenedesmus sp.* only (solid line, ○) and 75% *Scenedesmus*: 25% *M.aeruginosa* [Mutant (short dash •) and Wild type(long dash ◇)] at 2 mg DW L^{-1}.

significantly improved when levels of *M. aeruginosa* in food offered were low (25%, 0.5 mg DW L^{-1}), similar findings have been observed with temperate *Daphnia* species *D. pulex* (Hietala *et al.*, 1997) and *D. magna* (Lürling, 2003). At these low *M. aeruginosa* levels, we did not observe a significant difference ($P > 0.05$) in survivorship between the microcystin-containing and the microcystin-lacking treatments. It is therefore likely that above a certain "threshold" level, which may be higher than 25% but lower than 50% of *M. aeruginosa* in food, we may find a positive linear relationship between survivorship of *D. lumholtzi* and a decrease in amounts of *M. aeruginosa* and possibly also a significant effect of microcystins.

A comparison of growth rates of *Daphnia* fed exclusively on *Scenedesmus* spp. and those fed on treatments with food mixtures that had low amounts of *M. aeruginosa* (25%) show that presence of cyanobacteria in the food had a significant negative effect on growth. However, we do not observe any significant effect of microcystins on growth (Fig. 3). The lowered growth rates here may have been a result of the presence of compounds like

protease inhibitors (Jungmann, 1995; Rohrlack *et al.*, 2005) or nutritional inadequacy (Lampert, 1987; Demott & Mueller-Navarra, 1997; Martin-Creuzburg *et al.*, 2008) and not presence of microcystins in the cyanobacteria used.

Although growth in the other (50%, 75% and 100% *M. aeruginosa*) treatment groups could only be followed for 3 days at the most (Fig. 1), we also observed a significant effect of increasing the percentage of *M. aeruginosa* on growth over time (Fig. 2; $P < 0.01$) but no significant effect when the amount of *Scenedesmus* was lowered by half (in controls) in corresponding amounts ($P > 0.05$). This finding underscores the role of protease inhibitors like microviridin J that cause a disruption of important protease-driven growth processes like moulting (Kaebernick *et al.*, 2001; Rohrlack *et al.*, 2004b) as well as causing a "hunger effect" through inducing lower filtration and ingestion rates (Lampert, 1981; Rohrlack *et al.*, 1999) that together with nutritional deficiency (Demott & Mueller-Navarra, 1997; Gragnani *et al.*, 1999) affect the growth rates.

Poor growth and survival exhibited by the *D. lumholtzi* in food treatments containing both wild-type (toxic) and mutant type (non-toxic) *M. aeruginosa* strain PCC 7806 could not be explained by the presence of microcystins. Our results reveal that observed differences in growth and survivorship of treatment groups could not be attributed to microcystins but rather to other factors that are not investigated here.

These may include factors such as protease inhibitors (Rohrlack *et al.*, 2004a) or ingestion inhibitors (Nizan *et al.*, 1986; Jungmann, 1995; Demott, 1999); both these factors are particularly important since they would mostly affect the early stages of growth, affecting growth processes like moulting. Other factors like the lack of polyunsaturated fatty acids and sterols may explain results obtained from treatment groups containing low amounts of *M. aeruginosa* (25%) that exhibited improved growth rate, which however was lower than growth in control groups. The effects of factors like nutritional deficiency (Lampert, 1987; Demott & Mueller-Navarra, 1997; Gragnani *et al.*, 1999) may be expressed more prominently later in the development process, as in this experiment the effect of microcystins was observed later in development of the *Daphnia* at the stage of reproduction on age at maturity (Table I).

Sensitivity to toxic cyanobacteria may be associated with differences in life history between cladoceran species rather than differences between tropical and temperate taxa (Ferrão-Filho *et al.*, 2000), yet it has also been shown that there are differences between life history strategies of temperate and tropical *Daphnia* species in dealing with environmental situations like toxic cyanobacteria (Sarma *et al.*, 2005). The general conclusion from studies with temperate *Daphnia* species is that toxic cyanobacteria pose a very serious threat to *Daphnia* populations (Tillmanns *et al.*, 2008). Field studies on the tropical cladocera *D. lumholtzi* have indicated that it is more tolerant to episodes of high cyanobacteria abundance than its temperate northern hemisphere relatives (Pattinson *et al.*, 2003). Some studies have even reported the ability of a tropical *Daphnia* species to graze on toxic cyanobacteria (Matveev *et al.*, 1994). The responses of *Daphnia* to cyanobacteria and its toxins are generally species and even clone-specific (Tillmanns *et al.*, 2008), since even some temperate species of *Daphnia* have also been shown to be tolerant to toxic cyanobacteria (Kurmayer & Juttner, 1999; Oberhaus *et al.*, 2007). This may be the case for the tropics, however information on tropical *Daphnia* remains limited.

Table I: Showing multiple comparisons of means by Tukey contrasts on a one-way analysis of variance conducted on the effect of different treatments [100% Scenedesmus spp. (Scen); 75% Scenedesmus:25% M. aeruginosa (mutant and wild-type)] on age at maturity for Daphnia lumholtzi

Linear hypotheses	Estimate	Standard error	*t*-value	*P*-value
Scen—mutant	−2.6667	0.3944	−6.761	0.00241
Wild—mutant	1.8333	0.4410	4.158	0.02004
Wild—Scen	4.5000	0.4410	10.205	<0.001

The eutrophic tropical Lake Victoria has high abundances of colony-forming and filamentous cyanobacteria that have been shown to produce cyanotoxins (Haande *et al.*, 2007). However, for this toxic effect to be expressed these toxic cells should be ingested (Lampert, 1987). This colonial and filamentous nature of cyanobacteria has been shown to be a deterrent to zooplankton grazing (Gliwicz, 1990; Nejstgaard *et al.*, 2007). By lowering their ingestion rates on these toxic cyanobacteria, especially at low concentrations (Lampert, 1981), as well as having a degree of tolerance to toxicity owing to repeated exposure to cyanobacteria toxins (Gustafsson & Hansson, 2004), *Daphnia* in Lake Victoria and other eutrophic tropical lakes are able to withstand long-term blooms of toxic cyanobacteria. In Lake Victoria microcystins have been detected during several studies (Krienitz *et al.*, 2002; Sekadende *et al.*, 2005; Haande *et al.*, 2007). However, the strains responsible for these microcystins are still not yet known, identification of strains responsible for toxicity in such tropical

lakes is also important to understanding how zooplankton responds to their toxicity.

ACKNOWLEDGEMENTS

We would like to thank Winfred Lampert for his advice with designing of the experiment. We thank Knut H. Jensen for his advice with statistical analysis and comments to the manuscript. We thank Josefin Titelman for her comments to the manuscript.

FUNDING

This study was supported by a grant from the Norwegian Agency for Development Cooperation (NORAD) under the Ecological and socio-economic implications of harmful algal blooms (HABs) in Ugandan lakes project (project number UGA-2027).

REFERENCES

Acharya, K., Jack, J. D. and Smith, A. S. (2006) Stoichiometry of *Daphnia lumholtzi* and their invasion success: are they linked?. *Arch. Hydrobiol.*, **165**, 433–453.

Boing, W. J., Wagner, A., Voigt, H. *et al.* (1998) Phytoplankton responses to grazing by *Daphnia galeata* in the biomanipulated Bautzen reservoir. *Hydrobiologia*, **389**, 101–114.

Chen, F. Z. and Xie, P. (2004) The toxicities of single-celled *Microcystis aeruginosa* PCC7820 and liberated *M. aeruginosa* to *Daphnia carinata* in the absence and presence of the green alga *Scenedesmus obliquus*. *J. Freshwat. Ecol.*, **19**, 539–545.

Demott, W. R. (1990) Retention efficiency, perceptual bias and active choice as mechanisms of food selection by suspension-feeding zooplankton. In Hughes, R. N. (ed.), *Behavioural Mechanisms of Food Selection*. Springer-Verlag, Berlin.

Demott, W. R. (1999) Foraging strategies and growth inhibition in five daphnids feeding on mixtures of a toxic cyanobacterium and green algae. *Freshwat. Biol.*, **42**, 263–274.

Demott, W. R. and Mueller-Navarra, D. C. (1997) The importance of highly unsaturated fatty acids in zooplankton nutrition: evidence from experiments with *Daphnia*, a cyanobacterium and lipid emulsions. *Freshwat. Biol.*, **38**, 649–664.

Demott, W. R., Zhang, Q. X. and Carmichael, W. W. (1991) Effects of toxic cyanobacteria and purified toxins on the survival and feeding of a copepod and 3 species of *Daphnia*. *Limnol. Oceanogr.*, **36**, 1346–1357.

Dittmann, E., Neilan, A., Erhard, H. *et al.* (1997) Insertional mutagenesis of a peptide synthetase gene that is responsible for hepatatoxin production in the cyanobacterium *Microcystis aeruginosa* PCC7806. *Mol. Microbiol.*, **26**, 779–787.

Ferrão-Filho, A. S., Azevedo, S. and DeMott, W. R. (2000) Effects of toxic and non-toxic cyanobacteria on the life history of tropical and temperate cladocerans. *Freshwat. Biol.*, **45**, 1–19.

Ghadouani, A., Alloul, B. P., Zhang, Y. *et al.* (1998) Relationships between zooplankton community structure and phytoplankton in two lime-treated eutrophic hardwater lakes. *Freshwat. Biol.*, **39**, 775–790.

Ghadouani, A., Pinel-Alloul, B., Plath, K. *et al.* (2004) Effects of *Microcystis aeruginosa* and purified microcystin-LR on the feeding behavior of *Daphnia pulicaria*. *Limnol. Oceanogr.*, **49**, 666–679.

Gliwicz, Z. M. (1990) Why do Cladocerans fail to control algal blooms? *Hydrobiologia*, **200**, 83–97.

Gragnani, A., Scheffer, M. and Rinaldi, S. (1999) Top-down control of cyanobacteria: a theoretical analysis. *Am. Nat.*, **153**, 59–72.

Gustafsson, S. and Hansson, L. A. (2004) Development of tolerance against toxic cyanobacteria in *Daphnia*. *Aquat. Ecol.*, **38**, 37–44.

Haande, S. (2008) On the ecology, toxicology, and phylogeny of cyanobacteria in Murchison Bay of Lake Victoria, Uganda. PhD Thesis. University of Bergen, Norway.

Haande, S., Ballot, A., Rohrlack, T. *et al.* (2007) Diversity of *Microcystis aeruginosa* isolates (Chroococcales, Cyanobacteria) from East-African water bodies. *Archiv. Microbiol.*, **188**, 15–25.

Havel, J. E. and Hebert, P. D. N. (1993) *Daphnia lumholtzi* in north-America—another exotic zooplankter. *Limnol. Oceanogr.*, **38**, 1823–1827.

Havens, K. E., East, T. L., Marcus, J. *et al.* (2000) Dynamics of the exotic *Daphnia lumholtzi* and native macro-zooplankton in a subtropical chain of lakes in Florida, USA. *Freshwat. Biol.*, **45**, 21–32.

Hietala, J., Laurén-Määttä, C. and Walls, M. (1997) Life history responses of *Daphnia* clones to *Microcystis* at different food levels. *J. Plankton Res.*, **19**, 917–926.

Jacoby, J. M., Collier, D. C., Welch, E. B. *et al.* (2000) Environmental factors associated with a toxic bloom of *Microcystis aeruginosa*. *Can. J. Fish. Aquat. Sci.*, **57**, 231–240.

Jungmann, D. (1995) Isolation, purification and characterisation of a new *Daphnia* toxic compound from axenic *Microcystis flos-aquea* strain PCC 7806. *J. Chem. Ecol.*, **21**, 1665–1676.

Kaebernick, M., Rohrlack, T., Christoffersen, K. *et al.* (2001) A spontaneous mutant of microcystin biosynthesis: genetic characterization and effect on *Daphnia*. *Environ. Microbiol.*, **3**, 669–679.

Kolar, C. S. and Wahl, D. H. (1998) Daphnid morphology deters fish predators. *Oecologia*, **116**, 556–564.

Krienitz, L., Ballot, A., Wiegand, C. *et al.* (2002) Cyanotoxin-producing bloom of *Anabaena flos-aquae*, *Anabaena discoidea* and *Microcystis aeruginosa* (Cyanobacteria) in Nyanza Gulf of Lake Victoria, Kenya. *J. Appl. Bot.*, **76**, 179–183.

Kurmayer, R. and Juttner, F. (1999) Strategies for the co-existence of zooplankton with the toxic cyanobacterium *Planktothrix rubescens* in Lake Zurich. *J. Plankton Res.*, **21**, 659–683.

Lampert, W. (1981) Inhibitory and toxic effects of Blue-Green-Algae on *Daphnia*. *Int. Rev. Gesamt. Hydrobiol.*, **66**, 285–298.

Lampert, W. (1987) Laboratory studies on zooplankton–cyanobacteria interactions. *N. Z. J. Mar. Freshwat. Res.*, **21**, 483–490.

Lampert, W., Schmitt, R. D. and Muck, P. (1988) Vertical migration of freshwater zooplankton: a test of some hypotheses predicting a metabolic advantage. *Bull. Mar. Sci.*, **43**, 620–640.

Lürling, M. (2003) *Daphnia* growth on microcystin-producing and microcystin-free *Microcystis aeruginosa* in different mixtures with the green alga *Scenedesmus obliquus*. *Limnol. Oceanogr.*, **48**, 2214–2220.

Martin-Creuzburg, D., von Elert, E. and Hoffmann, K. H. (2008) Nutritional constraints at the cyanobacteria–*Daphnia magna* interface: the role of sterols. *Limnol. Oceanogr.*, **53**, 456–468.

Matveev, V., Matveeva, L. and Jones, G. J. (1994) Study of the ability of *Daphnia carinata* king to control phytoplankton and resist cyanobacterial toxicity—implications for biomanipulation in Australia. *Aust. J. Mar. Freshwat. Res.*, **45**, 889–904.

McDougall, B. K. and Ho, G. E. (1991) A study of the eutrophication of North Lake, Western Australia. *Water Sci. Technol.*, **23**, 163–173.

Mwebaza ndawula, L. (1994) Changes in relative abundance of zooplankton in Northern Lake Victoria, East-Africa. *Hydrobiologia*, **272**, 259–264.

Nejstgaard, J. C., Tang, K. W., Steinke, M. *et al.* (2007) Zooplankton grazing on *Phaeocystis:* a quantitative review and future challenges. *Biogeochemistry*, **83**, 147–172.

Nizan, S., Dimentman, C. and Shilo, M. (1986) Acute toxic effects of the cyanobacterium *Microcystis aeruginosa* on *Daphnia magna*. *Limnol. Oceanogr.*, **31**, 497–502.

Oberhaus, L., Gelinas, M., Pinel-Alloul, B. *et al.* (2007) Grazing of two toxic *Planktothrix* species by *Daphnia pulicaria*: potential for bloom control and transfer of microcystins. *J. Plankton Res.*, **29**, 827–838.

Pattinson, K. R., Havel, J. E. and Rhodes, R. G. (2003) Invasibility of a reservoir to exotic *Daphnia lumholtzi*: experimental assessment of diet selection and life history responses to cyanobacteria. *Freshwat. Biol.*, **48**, 233–246.

R Development Core Team (2008) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Rohrlack, T., Henning, M. and Kohl, J. G. (1999) Mechanisms of the inhibitory effect of the cyanobacterium *Microcystis aeruginosa* on *Daphnia galeata's* ingestion rate. *J. Plankton Res.*, **21**, 1489–1500.

Rohrlack, T., Dittmann, E., Borner, T. *et al.* (2001) Effects of cell-bound microcystins on survival and feeding of *Daphnia* spp. *Appl. Environ. Microbiol.*, **67**, 3523–3529.

Rohrlack, T., Christoffersen, K., Hansen, P. *et al.* (2004a) Isolation, characterization, and quantitative analysis of Microviridin J: a new *Microcystis* metabolite toxic to *Daphnia*. *J. Chem. Ecol.*, **29**, 1573–1561.

Rohrlack, T., Christoffersen, K., Kaebernick, M. *et al.* (2004b) Cyanobacterial protease inhibitor microviridin J causes a lethal moulting disruption in *Daphnia pulicaria*. *Appl. Environ. Microbiol.*, **70**, 5047–5050.

Rohrlack, T., Christoffersen, K., Dittmann, E. *et al.* (2005) Ingestion of microcystins by *Daphnia*: intestinal uptake and toxic effects. *Limnol. Oceanogr.*, **50**, 440–448.

Sarma, S. S. S., Nandini, S. and Gulati, R. D. (2005) Life history strategies of cladocerans: comparisons of tropical and temperate taxa. *Hydrobiologia*, **542**, 315–333.

Sekadende, B. C., Lyimo, T. J. and Kurmayer, R. (2005) Microcystin production by cyanobacteria in the Mwanza Gulf (Lake Victoria, Tanzania). *Hydrobiologia*, **543**, 299–304.

Semyalo, R. P. D. (2003) Diel vertical migration of zooplankton in Murchison Bay Lake Victoria. Masters degree. Makerere University.

Tillmanns, A. R., Wilson, A. E., Pick, F. R. *et al.* (2008) Meta-analysis of cyanobacterial effects on zooplankton population growth rate: species specific responses. *Fundam. Appl. Limnol.*, **171**, 285–295.

Vasconcelos, V. M., Evans, W. R., Carmichael, W. W. *et al.* (1993) Isolation of Microcystin-Lr from a microcystis (cyanobacteria) waterbloom collected in the drinking-water reservoir for Porto, Portugal. *J. Environ. Sci. Health Part A*, **28**, 2081–2094.

Wilson, A. E., Sarnelle, O. and Tillmans, A. R. (2006) Effects of cyanobacterial toxicity and morphology on the population growth of freshwater zooplankton: Meta-analyses of laboratory experiments. *Limnol. Oceanogr*, **51**, 1915–1924.

Yin, C., Lan, Z., Zhao, M. *et al.* (1992) Determination of phosphorus concentration threshold for algal growth in eutrophic Chaohu Lake, China. *J. Environ. Sci. Health Part A—Environ. Sci. Eng. Toxic Hazard. Subst. Contr.*, **A27**, 433–443.