Oceanological and Hydrobiological Studies

International Journal of Oceanography and Hydrobiology

Volume 47, Issue 3, September 2018
pages (296-302)

ISSN 1730-413X
eISSN 1897-3191

Grazing of the copepod *Cyclops vicinus* on toxic *Microcystis aeruginosa*: potential for controlling cyanobacterial blooms and transfer of toxins

by

Zakaria A. Mohamed[1,*], Asmaa A. Bakr[1], Hamed A. Ghramh[2]

DOI: **10.1515/ohs-2018-0028**
Category: **Original research paper**
Received: **January 1, 2018**
Accepted: **February 9, 2018**

[1]*Department of Botany & Microbiology, Faculty of Science, Sohag University, Sohag 82524, Egypt*

[2]*King Khalid University, Abha 61413, P.O. Box 9004, Saudi Arabia*

Abstract

Grazing of zooplankton on phytoplankton may contribute to a reduction of harmful cyanobacteria in eutrophic waters. However, the feeding capacity and interaction between zooplankton and toxic cyanobacteria vary among grazer species. In this study, laboratory feeding experiments were designed to measure the grazing rate of the copepod *Cyclops vicinus* on *Microcystis aeruginosa* and the potential microcystin (MC) accumulation in the grazer. Copepods were fed a mixed diet of the edible green alga *Ankistrodesmus falcatus* and toxic *M. aeruginosa* for 10 days. The results showed that C. *vicinus* efficiently ingested toxic *Microcystis* cells with high grazing rates, varying during the feeding period (68.9–606.3 *Microcystis* cells animal^{-1} d^{-1}) along with *Microcystis* cell density. *Microcystis* cells exhibited a remarkable induction in MC production under grazing conditions with concentrations 1.67–12.5 times higher than those in control cultures. Furthermore, C. *vicinus* was found to accumulate MCs in its body with concentrations increasing during the experiment (0.05–3.21 µg MC animal^{-1}). Further in situ studies are needed to investigate the ability of *Cyclops* and other copepods to assimilate and detoxify MCs at environmentally relevant concentrations before deciding on the biocontrol of *Microcystis* blooms by copepods.

Key words: *Cyclops*, copepods, grazing, *Microcystis*, microcystin, accumulation

* Corresponding author: *mzakaria_99@yahoo.com*

DE GRUYTER

The *Oceanological and Hydrobiological Studies* is online at www.oandhs.pl

Introduction

Noxious cyanobacterial blooms have increased in freshwaters due to eutrophication caused by high nutrient loading from wastewater discharge and large-scale aquaculture (Paerl & Huisman 2008). Cyanobacteria, particularly the *Microcystis* genus, are widely distributed in lakes, rivers, ponds, brackish waters and even in harsh environments such as hot springs (Zurawell et al. 2005; Belykh et al. 2013). These species can produce a potent class of hepatotoxins called microcystins that may have serious impact on the functioning and health of aquatic ecosystems, as well as on wildlife and human health (Carmichael 1996). As they co-exist in the same environment, *Microcystis* sp. and zooplankton interact and affect each other. *Microcystis* sp. and herbivorous grazers interact in complex ways (Sarnelle et al. 2005) that may be attributed to genetic and/or physiological variation within and among prey and consumer populations (Wilson, Hay 2007). Cyanobacteria have defense mechanisms that reduce or inhibit herbivory, including aggregation into large growth forms (e.g. colonies and filaments) and/or production of secondary metabolites and allelochemicals, including toxins (Watanabe et al. 1996; Żak, Kosakowska 2016). As for the defense mechanism through production of toxins, the effect of these toxins on predators is not clear (Wilson et al. 2006). Some studies have demonstrated their negative effects on zooplankton (Zurawell et al. 2005), while others have shown no anti-herbivore effect of cyanotoxins (Wilken et al. 2010). Despite the defense of cyanobacteria against zooplankton grazing, some copepods show high ingestion rates on large forms of toxic cyanobacteria (Koski et al. 2002; Kozlowsky-Suzuki et al. 2003). This may be due to ability of these zooplankton species to capture such large forms (DeMott 1990) and to develop a physiological adaptation and resistance to cyanotoxins (Fulton, Paerl 1987). Nevertheless, the ingestion rates of *Microcystis* sp. by zooplankton differ significantly among copepod species (Ger et al. 2010), as copepods respond differently to anti-grazing defense of cyanobacteria (Engstrom et al. 2000). Such differences in cyanobacteria ingestion rates among copepods could affect the composition of the zooplankton community, and may be useful for controlling harmful cyanobacterial blooms in lakes (Wang et al. 2010). Therefore, ingestion rates for *Microcystis* sp. and other toxic cyanobacteria should be addressed for many copepod species to show how the latter tolerate *Microcystis* sp. and their toxins more than others (Lurling 2003; Ger et al. 2010). Limited studies, however, were performed on copepods grazing on *Microcystis* sp. (Work & Havens 2003; Wilson et al. 2006; Ger et al. 2010). One of the most important copepods is *C. vicinus*, which is regularly found in the zooplankton communities of many lakes and reservoirs, and is often referred to as a predator of many zooplankton and phytoplankton species (Devetter, Seda 2006). In Egyptian fishponds, the copepod *Cyclops* genus is abundant in freshwaters and has been observed to frequently co-occur with *Microcystis* blooms (Zakaria A. Mohamed, personal communication). Therefore, the objective of the present study was to investigate the grazing effects of *Cyclops* on toxic *M. aeruginosa* as a potential bioagent in the biocontrol of harmful cyanobacterial blooms in freshwater sources.

Materials and methods

Organisms

The cyanobacterium *M. aeruginosa* was obtained from the microalgal culture collection of the Botany & Microbiology Department, Faculty of Science, Sohag University, Egypt. This strain was originally isolated from fish ponds in the Sohag region, Egypt, and reported as a microcystin producer (Bakr 2016). The organisms were grown in 250-ml Erlenmeyer flasks for 21 days in a climatic chamber under controlled light (50 µmol photons m^{-2} s^{-1}), photoperiod (16:8 hours light-dark) and temperature (30 ± 0.5°C) in a BG-11 liquid medium. The green alga *Ankistrodesmus falcatus* was isolated from fish ponds and cultured under the same conditions as *M. aeruginosa*.

Cyclops vicinus individuals were collected from Egyptian fish ponds, where blooms of *Microcystis* are not known to occur. *Cyclops vicinus* were transferred individually using a pipette with a large tip opening to glass aquariums filled with GF/C Whatman (pore size = 1.2 µm) filtered water from the same fishpond. Before being used for culturing, the filtered fishpond water was sterilized in an autoclave at 1.05 kg cm^{-2} (15 psi) and 121°C. The species was verified under a *binocular microscope* (Olympus BH-2, Japan) at ×100 total magnification, using the identification key of freshwater crustacean zooplankton of Europe (Bledzki & Rybak 2016). Individual clones were not separated in order to study grazing at a population level. Prior to grazing experiments, *Cyclops vicinus* cultures were grown for one week at the same temperature and light cycle as *Ankistrodesmus falcatus* cultures, while feeding with *A. falcatus* every 2 days at an approximate concentration ranging from 5000 to 10 000 cells ml^{-1}.

Grazing experiments

Short-term grazing experiments were conducted to determine if *Cyclops* are able to feed on *M. aeruginosa* in the presence of a known edible prey (*A. falcatus*). These experiments were carried out in 6 one-liter glass bottles containing 500 ml filtered fishpond water and a known number of *M. aeruginosa* and the chlorophyte *A. falcatus*. Three bottles were dosed with 100 *Cyclops*, while the remaining bottles were not dosed with *Cyclops* and used as a control. The estimated average initial concentrations were approximately 12 000 cells ml^{-1} for *M. aeruginosa*, and 10 000 cells ml^{-1} for *A. falcatus*. Both treated and control bottles were incubated for 10 days under the same conditions for growing *M. aeruginosa* as described above. The number of *M. aeruginosa* cells in treated and control bottles was monitored daily by withdrawing an aliquot of culture under septic conditions. The cells were counted using a Sedgewick Rafter counter under a light microscope according to the method used by Hötzel and Croome (1999). The grazing rate of *Cyclops* on *M. aeruginosa* was estimated using the following equation according to Devetter and Seda (2006). The grazing rate (Gr) is given as the number of prey eaten by one predator per day:

$$Gr = (N_c - N_e)/C_e$$

where N_c is the number of prey in the control, N_e is the number of prey in experimental enclosures and C_e is the number of *C. vicinus* carnivorous stages in the enclosures.

In order to determine the effect of grazing by *Cyclops* on MC production in *M. aeruginosa*, 5 ml of algal suspension was removed daily from both control and treated cultures. The culture samples were filtered on Whatman GF/C filter paper, and filters with attached cells were extracted overnight in 80% aqueous methanol while grinding with 0.5 mm silica beads. The extracts were then centrifuged twice at 10 000 g for 10 min at 4°C, and the supernatants of each extract were pooled and subjected to a sterilized air stream to evaporate the organic solvent. The concentration of microcystins in the remaining aqueous fraction was determined by ELISA according to the method of Carmichael & An (1999) using a commercial Abraxis kit (Abraxis, USA). To detect microcystins potentially accumulated in the body of *Cyclops* during grazing, 10 individuals of *Cyclops* were sampled from each bottle daily. The animals were ground with a known volume of 80% methanol in a household mortar and left overnight for complete extraction of MCs. This step was repeated twice. The methanolic extracts were centrifuged at 10 000 rpm, and the relevant supernatants were combined together. The organic solvent was then evaporated through a sterilized air stream, and MC concentration in the remaining aqueous fraction was detected by ELISA as described above. The toxicity of MCs accumulated in the body of *Cyclops* was confirmed by a protein phosphatase inhibition assay (PPIA) according to Carmichael & An (1999).

Statistical analysis

Data analysis was performed using SPSS for Windows (SPSS Inc., V17). The one-way ANOVA test was used to compare significant differences ($p < 0.05$) in *M. aeruginosa* cell density, the ingestion rate of *Cyclops*, and microcystin concentrations between the treatment and control groups.

Results

The results of the present study have revealed that *Cyclops* caused a significant reduction in the growth of *M. aeruginosa* as estimated by the number of cells ($p < 0.05$), compared to control cultures (Fig. 1).

The cell density of *M. aeruginosa* declined steeply in cultures containing cyclops and was reduced to zero after seven days of incubation, while the cell density in control cultures (i.e. not-containing cyclops) increased during the incubation period and reached its exponential phase at 4-day incubation (Fig. 1).

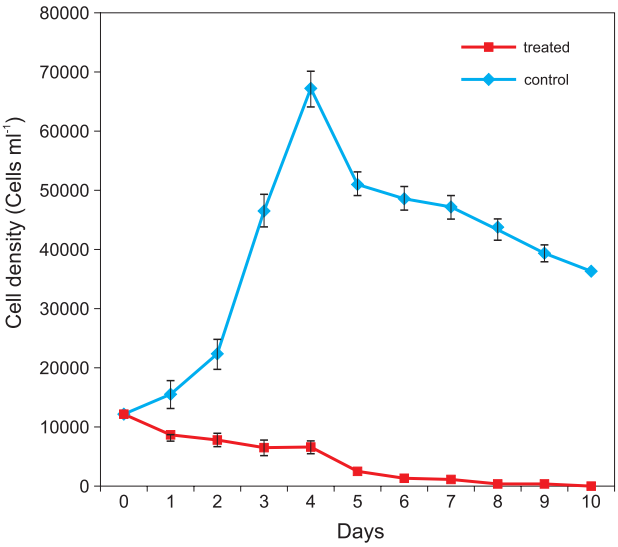

Figure 1

Changes in cell density of *M. aeruginosa* (cells ml^{-1}) over the course of the grazing experiment with *Cyclops vicinus*

The grazing rate of *Cyclops* on *Microcystis* significantly differed during the experiment and ranged from 68.9 to 606.3 *M. aeruginosa* cells (*Cyclops*)$^{-1}$ d^{-1} (Fig. 2). This ingestion rate seems to be correlated with the cell density of *M. aeruginosa* in the cultures (r = 75). The highest grazing rate was obtained on the fourth day of incubation, when the number of *Microcystis* cells reached its maximum (67 186 cells ml^{-1}) in the control cultures. Whereas, the lowest rate was determined after a 1-day incubation with cell density of 15 562 cells ml^{-1} (Figs 1, 2). Meanwhile, *Cyclops* also fed on edible food (*A. falcatus*) in addition to *M. aeruginosa*, but with lower grazing rates (71.83–385.43 *Anksitrodesmus* cells (*Cyclops*)$^{-1}$ d^{-1}) (Fig. 2). In addition to grazing on *M. aeruginosa*, the copepod *Cyclops* induced microcystin production within *Microcystis* cells during the feeding period, compared to control cultures (i.e. without *Cyclops*). MC concentrations in grazer-treated cultures were 1.67–12.5 times higher than those in control cultures (Fig. 3). These concentrations increased with the duration of cell exposure to a grazer, with the highest level obtained on the ninth day of incubation (189.5 pg MC cell^{-1}). Additionally, our results showed that *Cyclops* accumulated MCs at levels ranging from 0.05 to 3.21 µg *Cyclops* individual^{-1} in their body during the feeding on *M. aeruginosa*.

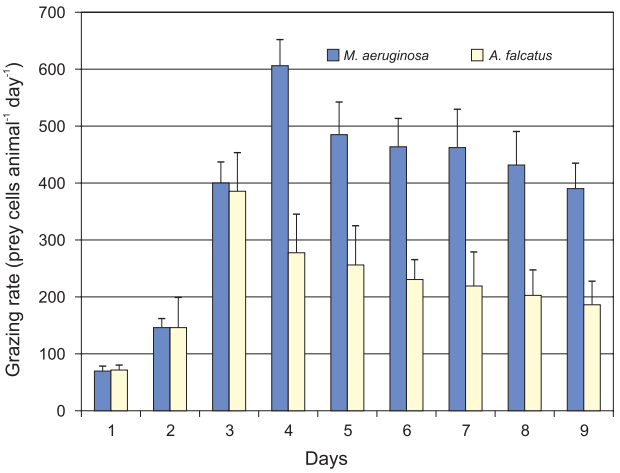

Figure 2

Grazing rate of *Cyclops vicinus* feeding on *M. aeruginosa* and *A. falcatus*

Discussion

Our results confirm the ability of the copepod *C. vicinus* to coexist with and feed on the toxic cyanobacterium *M. aeruginosa*. We fed *Cyclops* mixtures of *M. aeruginosa* with good food algae (*A. falcatus*) rather than pure diet (*M. aeruginosa*), as

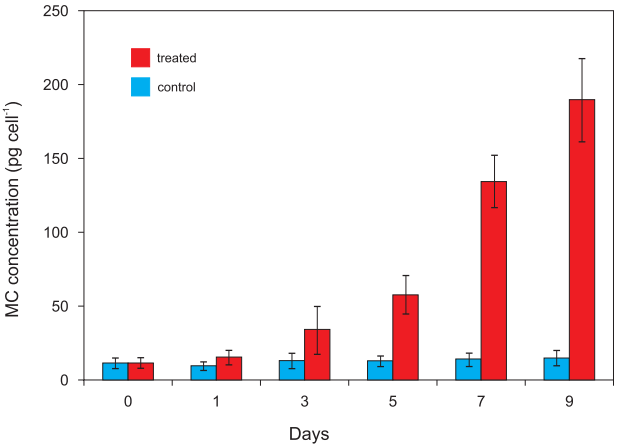

Figure 3

Changes in microcystin content in *M. aeruginosa* cells (pg cell^{-1}) in the presence of the grazer *Cyclops vicinus* and control cultures

pure diet of *Microcystis* reduces fitness and survival of zooplankton, and it is unlikely to occur in nature (Wilson et al. 2006; Ger et al. 2010). Despite the presence of edible food in the cultures, *Cyclops* were able to graze down toxic *M. aeruginosa* with grazing rates higher than those for the edible alga *A. falcatus*. These results are thus not consistent with previous studies showing that copepods can graze more on cyanobacteria when alternative food is scarce (Gorokhova, Engstrom-Ost 2009; Engström-Öst et al. 2011). Our results also contradict the hypothesis that cyanobacteria are generally not considered as preferred food for copepods (DeMott et al. 1991). However, these results are consistent with those obtained by Kozlowsky-Suzuki et al. (2003) reporting high rates of toxic cyanobacteria ingestion by copepods. Furthermore, Urrutia-Cordero et al. (2015) demonstrated the suppression of phytoplankton assemblages dominated by *Microcystis*, *Anabaena*, and *Planktothrix* by grazing of cyclopoid copepods. In response to grazing in our experiments, *M. aeruginosa* exhibited a several-fold increase in MC content (189.5 pg MC cell^{-1}) compared to that detected in control cultures (15.2 pg MC cell^{-1}). This indicates that *M. aeruginosa* induces toxin production as a way to combat or prevent grazing by copepods. These results are in line with those of previous studies reporting that toxic cyanobacteria increase toxin production as a defense strategy when they face the grazing pressure (Jang et al. 2003; Lurling 2003; Mohamed, Al-Shehri 2013; Selander et al. 2015). Interestingly, no mortality of *Cyclops* was observed during the feeding on *M. aeruginosa*. Also, we did not notice any reduction in the growth of the *Cyclops* body in this study, as the body size of *Cyclops* fed with *M. aeruginosa* did

not differ significantly from that of *Cyclops* fed with the edible alga *A. falcatus* in initial *Cyclops* cultures (data not presented in this paper). The results of our study are therefore not in line with those of previous studies that demonstrated a reduction in the growth of different zooplankton species during feeding on toxic cyanobacteria (Smutná et al. 2014; Herrera et al. 2015; Kaczkowski et al. 2017; Kosiba et al. 2018). However, our results are in agreement with those of Paes et al. (2016), who found that the biomass of calanoid copepods positively correlates with the microcystin concentrations in the environment. The absence of a negative effect of toxic *Microcystis* on *Cyclops* strongly supports the hypothesis that some herbivores are able to feed on toxic cyanobacteria and develop resistance to their cyanotoxins (Panosso et al. 2003). However, this resistance seems to be determined by the history of exposure to these toxins, as copepods that experience interaction with toxic cyanobacterial blooms could develop resistance to cyanotoxins and may be able to graze better on toxic cyanobacteria than unexposed copepods (Paes et al. 2016). Our data best fit this explanation, where *Cyclops* individuals used in the study were collected from eutrophic fishponds containing heavy blooms of toxic cyanobacteria. Otherwise, the discrepancy in the resistance of copepods to cyanotoxins could be attributed to species-specific differences through the capacity of some copepod species for detoxification of cyanotoxins (Ger et al. 2010).

The current study has also provided evidence for MC accumulation by *Cyclops* – the total MC concentrations detected in *Cyclops* tissues increased during the period of feeding on *M. aeruginosa* (0.05 to 3.21 µg *Cyclops* individual^{-1}). The exponential accumulation of MCs during feeding on toxic *M. aeruginosa* can be interpreted as an indirect indication of viability of *Cyclops* in this experiment as suggested by Shams et al. (2014). Despite several studies on MC accumulation in a variety of aquatic organisms, including bivalves, crustaceans, zooplankton and fish (Mohamed 2001; Mohamed et al. 2003; Zhang et al. 2009; Wojtal-Frankiewicz et al. 2013), to our knowledge this is the first study to address the accumulation of MCs by the copepod *Cyclops*. In this regard, Johnson et al. (1999) stated that cladocerans and copepods contain large lipid reserves, which could allow lipophilic MCs to accumulate in their bodies. Since *Cyclops* is omnivores with a high protein level, they occupy an important position in aquatic food webs and serve as food for certain fish (Piasecki et al. 2004). Therefore, the detection of MCs in the *Cyclops* body in our study suggests the transfer of such potent toxins to higher trophic levels. Many studies have provided evidence for the transfer of MCs accumulated in zooplankton grazers to higher trophic levels of the food web (Ibelings et al. 2005; Oberhaus et al. 2007; Lehman et al. 2010; Sotton et al. 2014). The presence of these toxins in the aquatic food web not only poses a risk to fish and other aquatic organisms, but also represents a threat to human health through the consumption of MC-contaminated fish and other aquatic organisms. However, MC concentrations in zooplankton, particularly *Daphnia*, may decrease despite a constant influx of toxins from the environment (Smith, Haney 2006; Shams et al. 2014). This decrease was attributed to the detoxification and elimination of MCs via excretion systems in these animals (Shams et al. 2014). Accordingly, our study indicates that the MC detoxification process may not be efficient in *Cyclops*, probably due to high MC concentrations in the ambient environment. This is consistent with the results of Chen et al. (2005) reporting that high MC concentrations cause a reduction in antioxidant enzyme activities, possibly resulting from overburdening of the detoxification system by MC metabolism in *Daphnia*. Similarly, Shams et al. (2014) demonstrated that higher proportions of toxic *Planktothix rubescens* could shift the balance between accumulation and excretion/detoxification toward greater accumulation of MC in grazers.

In conclusion, this study has clearly demonstrated the effective and significant grazing of the copepod *C. vicinus* on the toxic cyanobacterium *M. aruginosa* in the presence of the edible food alga, *A. falcatus*. The study also revealed that *M. aeruginosa* exhibited induction in MC production in response to grazing by *Cyclops* with 1.67–12.5 times higher toxin concentrations compared to control cultures. However, *Cyclops* survived such high toxin concentrations without any noticeable lethal effects. Our results not only have important implications for the understanding of grazer-prey interactions, but also provide important information on the future use of biomanipulation with *Cyclops* to control cyanobacterial blooms in eutrophic water sources. Nevertheless, the accumulation of MCs in the *Cyclops* body upon feeding on toxic *Microcystis* cells may hinder the use of *Cyclops* in the biomanipulation of *Microcystis* blooms, because it represents a major source of food for fish and consequently can act as a transfer vector of cyanotoxins to higher trophic levels. However, the balance between MC accumulation and its detoxification in copepods seems to be dependent on toxin concentrations in the diet (Shams et al. 2014), and MC detoxification pathways in grazers can be more efficient at low MC concentrations compared to high MC concentrations, which could cause partial

or full inhibition of detoxification pathways (Chen et al. 2005; Shams et al. 2014). Therefore, further in situ studies are needed to investigate the ability of *Cyclops* to assimilate and detoxify MCs at environmentally relevant concentrations. This will be the key determinant for the use of *Cyclops* as a potential biological control agent against *Microcystis* blooms.

Acknowledgements

The authors declare that there is no conflict of interest. The authors would like to express their gratitude to the Research Center of Advanced Materials – King Khalid University, Saudi Arabia for support.

References

Belykh, O.I., Dmitrieva, O.A., Gladkikh, A.S. & Sorokovikova, E.G. (2013). Identification of toxigenic cyanobacteria of the genus *Microcystis* in the Curonian Lagoon (Baltic Sea). *Oceanology* 53(1): 71–79.

Błędzki, L.A. & Rybak, J.I. (2016). *Freshwater Crustacean Zooplankton of Europe, Cladocera & Copepoda (Calanoida, Cyclopoida), Key to species identification, with notes on ecology, distribution, methods and introduction to data analysis*. Springer, XVI.

Carmichael, W.W. (1996). Toxic *Microcystis* and the environment. In M.F. Watanabe, K.-I. Harada, W.W. Carmichael & H. Fujiki (Eds.), *Toxic Microcystis* (pp. 1–11). CRC Press.

Carmichael, W.W. & An, J. (1999). Using of enzyme linked immunosobent assay (ELISA) and a protein phosphatase inhibition assay (PPIA) for the detection of microcystin and nodularin. *J. Nat. Toxins* 7(6): 377–385.

Chen, W., Song, L., Ou, D. & Gan, N. (2005). Chronic toxicity and responses of several important enzymes in *Daphnia magna* on exposure to sublethal microcystin-LR. *Environ. Toxicol.* 20(3): 323–330.

DeMott, W.R. (1990). Retention efficiency perceptual bias, and active choice as mechanisms of food selection by suspension-feeding zooplankton. In R.N. Hughes (Ed.), *Behavorial Mechanisms of Food Selection* (pp. 569–594). NATO ASI Series, Series G: Ecological Series. Springer, New York.

DeMott, W.R., Zhang, Q.X. & Carmichael, W.W. (1991). Effects of toxic cyanobacteria and purified toxins on the survival and feeding of a copepod and 3 species of *Daphnia*. *Limnol. Oceanogr.* 36(7): 1346–1357.

Devetter, M. & Seda, J, (2006). Regulation of rotifer community by predation of *Cyclops vicinus* (Copepoda) in the Rimov reservoir in spring. *Int. Rev. Hydrobiol*. 91(1): 101–111.

Engström, J., Koski, M., Viitasalo, M., Reinikainen, M., Repka, S. et al. (2000). Feeding interactions of the copepods *Eurytemora affinis* and *Acartia bifilosa* with the cyanobacteria *Nodularia* sp. *J. Plankton Res.* 22(7):1403–1409.

Engstrom-Ost, J., Hogfors, H., El-Shehawy, R., Stasio, B.D., Vehmaa, A. et al. (2011). Toxin producing cyanobacterium *Nodularia spumigena*, potential competitors and grazers: testing mechanisms of reciprocal interactions. *Aquat. Microb. Ecol.* 62(1): 39–48.

Fulton, R.S. & Paerl, H. (1987). Effects of colonial morphology on zooplankton utilization of algal resources during blue-green algal (*Microcystis aeruginosa*) blooms. *Limnol. Oceanogr.* 32(3): 634–644.

Ger, K.A., The, S.J., Baxa, DV, Lesmeister S. & Goldman, C.R. (2010). The effects of dietary *Microcystis aeruginosa* and microcystin on the copepods of the upper San Francisco estuary. *Freshw. Biol.* 55(7): 1548–1559.

Gorokhova, E. & Engstrom-Ost, J. (2009). Toxin concentration in *Nodularia spumigena* is modulated by mesozooplankton grazers. *J. Plankton Res.* 31(10): 1235–1247.

Herrera, N.A., Echeverri, L.F. & Ferrao-Filho, A.S. (2015). Effects of phytoplankton extracts containing the toxin microcystin-LR on the survival and reproduction of cladocerans. *Toxicon* 95(1): 38–45.

Hötzel, G. & Croome, R. (1999). A Phytoplankton Methods Manual for Australian Freshwaters, LWRRDC Occasional Paper 22/29, Land and Water Research and Development Corporation: Canberra.

Ibelings, B.W., Bruning, K., De Jonge, J., Wolfstein, K., Pires, L.M. et al. (2005). Distribution of microcystins in a lake foodweb: no evidence for biomagnification. *Microb. Ecol.* 49(4): 487–500.

Jang, M.H., Ha, K., Joo, G.J. & Takamura, N. (2003). Toxin production of cyanobacteria is increased by exposure to zooplankton. *Freshw. Biol.* 48(9): 1540–1550.

Johnson, K.M. (1999). *Microcystins in New Hampshire lakes and bioaccumulation in zooplankton*. Unpublished Master dissertation. University of New Hampshire, Durham, NH, USA.

Kaczkowski, Z., Wojtal-Frankiewicz, A., Gągała. I., Mankiewicz-Boczek. J., Jaskulska. A. et al. (2017). Relationships between cyanobacteria, zooplankton and fish in sub-bloom conditions in the Sulejów Reservoir. *J. Limnol.* 76(2): 380–396.

Kosiba, J., Krztoń, W. & Wilk-Woźniak, E. (2018). Effect of microcystins on proto- and metazooplankton is more evident in artificial than in natural waterbodies. *Microb. Ecol.* 75(2): 293–302. DOI: 10.1007/s00248-017-1058-z.

Koski, M., Schmidt, K., Engström-Öst, J., Viitasalo, M., Jónasdóttir. S.H. et al. (2002). Calanoid copepods feed and produce eggs in the presence of toxic cyanobacteria *Nodularia spumigena*. *Limnol. Oceanogr.* 47(3): 878–885.

Kozlowski-Suzuki B., Karjalainen M., Lehtiniemi M., Engstrom-Ost J., Koski M. et al. (2003). Feeding, reproduction and toxin accumulation by the copepods *Acartia bifilosa*

and *Eurytemora affinis* in the presence of the toxic cyanobacterium *Nodularia spumigena*. *Marine Ecol. Prog. Series* 249: 237–249.

Lehman, P.W., The, S.J., Boyer, G.L., Nobriga, M.L., Bass, E. et al. (2010). Initial impacts of *Microcystis aeruginosa* blooms on the aquatic food web in the San Francisco Estuary. *Hydrobiol.* 637(1): 229–248.

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(6): 2214–2220.

Mohamed, Z.A. (2001). Accumulation of cyanobacterial hepatotoxins by *Daphnia* in some Egyptian irrigation canals. *Ecotoxicol. Environ. Safe.* 50(1): 4–8.

Mohamed, Z.A., Carmichael, W.W. & Hussein, A.A. (2003). Estimation of microcystins in the freshwater fish *Oreochromis niloticus* in an Egyptian fish farm containing a *Microcystis* bloom. *Environ. Toxicol.* 18(2): 137–141.

Mohamed, Z.A. & Al-Shehri AM. (2013). *Grazing on Microcystis aeruginosa and degradation of microcystins by the heterotrophic flagellate Diphylleia rotans*. *Ecotoxicol. Environ. Safe.* 96: 48–52

Oberhaus, L., Gélinas, M., Pinel-Alloul, B., Ghadouani, A. & Humbert, J.F. (2007). Grazing of two toxic *Planktothrix* species by *Daphnia pulicaria*: potential for bloom control and transfer of microcystins. *J. Plankton Res.* 29(10): 827–838.

Paerl, H.W. & Huisman, J. (2008). Blooms like it hot. *Science* 320(5872): 57–58.

Paes, T.A.S.V., Costa, I.A.S., Silva, A.P.C. & Eskinazi-Sant'Anna, E.M. (2016). Can microcystins affect zooplankton structure community in tropical eutrophic reservoirs? *Brazil. J. Biol.* 76(2): 450–460.

Panosso, R., Carlson, P., Kozlowsky-Suzuki, B., Azevedo, S.M.F.O. & Granéli, E. (2003). Effect of grazing by a neotropical copepod, *Notodiaptomus*, on a natural cyanobacterial assemblage and on toxic and non-toxic cyanobacteria strains. *J. Plankton Res.* 25(9): 1169–1175.

Piasecki, W., Goodwin, A.E., Eiras, J.C. & Nowak, B.F. (2004). Importance of copepoda in freshwater in freshwater aquaculture. *Zool. Stud.* 43(2): 193–205.

Sarnelle, O. & Wilson, A.E. (2005). Local *adaptation of Dap*hnia *pulicaria* to toxic c*yanobacteria*. *Limnol. Oceanogr.* 34(5): 673–687.

Selander, E., Kubanek, J., Hamberg, M., Andersson, M.X., Cervin, G. et al. (2015). Predator lipids induce paralytic shellfish toxins in bloom-forming algae. *Proc. Natl. Acad. Sci. USA* 112(20): 6395–6400.

Shams, S., Cerasino, L., Salmaso, N. & Dietrich, D.R. (2014). Experimental models of microcystin accumulation in *Daphnia magna* grazing on *Planktothrix rubescens*: implications for water management. *Aquat. Toxicol.* 148: 9–15.

Smutná, M., Babica, P., Jarque, S., Hilscherova, K., Marsalek, B. et al. (2014). Acute, chronic and reproductive toxicity of complex cyanobacterial blooms in *Daphnia magna* and the role of microcystins. *Toxicon* 79: 11–18.

Sotton, B., Guillard, J., Anneville, O., Maréchal, M., Savichtcheva, O. et al. (2014). Trophic transfer of microcystins through the lake pelagic food web: evidence for the role of zooplankton as a vector in fish contamination. *Sci. Total Environ.* 466–467: 152–163.

Urrutia-Cordero, P., Ekvall, M.K. & Hansson, L.-A. (2015). Response of cyanobacteria to herbivorous zooplankton across predator regimes: who mows the bloom? *Freshw. Biol.* 60(5): 960–972.

Wang, X.D., Qin, B.Q., Gao, G. & Paerl, H.W. (2010). Nutrient enrichment and selective predation by zooplankton promote *Microcystis* (Cyanobacteria) bloom formation. *J. Plankton Res.* 32(4): 457–470.

Watanabe, M.F.(1996). Production of microcystins. In M.F. Watanabe, K.-I. Harada, W.W. Carmichael & H. Fujiki (Eds.), *Toxic Microcystis* (pp. 35–56). CRC Press.

Wilson, A.E. & Hay, M.E. (2007). A direct test of cyanobacterial chemical defense: variable effects of microcyst in treated food on two *Daphnia pulicaria* clones. *Limnol. Oceanogr.* 52(4): 1467–1479.

Wilson, A.E., Sarnelle, O. & Tillmanns, A.R. (2006). Effects of cyanobacterial toxicity and morphology on the population growth of freshwater zooplankton: meta analyses of laboratory experiments. *Limnol. Oceanogr.* 51(4): 1915–1924.

Wojtal-Frankiewicz, A., Bernasinska, J., Jurczak, T., Gwozdzinski, K., Frankiewicz, P. et al. (2013). Microcystin assimilation and detoxification by *Daphnia* spp. in two ecosystems of different cyanotoxin concentrations. *J. Limnol.* 72(1): 154–171. DOI: 10.4081/jlimnol.2013.e13.

Work, K.A. & Havens, K.A. (2003). Zooplankton grazing on bacteria and cyanobacteria in a eutrophic lake. *J. Plankton Res.* 25: 1301–1307.

Żak, A. & Kosakowska, A. (2016). Cyanobacterial and microalgal bioactive compounds – the role of secondary metabolites in allelopathic interactions. *Oceanol. Hydrobiol. Stud.* 45(1): 131–143.

Zhang, D., Xie, P., Chen, J., Dai, M., Qiu, T. et al. (2009). Determination of microcystin-LR and its metabolites in snail (*Bellamya aeruginosa*), shrimp (*Macrobrachium nipponensis*) and silver carp (*Hypophthalmichthys molitrix*) from Lake Taihu, China. *Chemosphere* 76(7): 974–981.

Zurawell, R.W., Chen, H., Burke, J.M. & Prepas E.E. (2005). Hepatotoxic cyanobacteria: a review of the biological importance of microcystins in freshwater environments. *J. Toxicol. Environ. Health B* 8(1): 1–37.