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# Physiological interaction of *Daphnia* and *Microcystis* with regard to cyanobacterial secondary metabolites

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ABSTRACT

Cyanobacterial blooms in freshwater ecosystems are a matter of high concern with respect to human health and ecosystem services. Investigations on the role of cyanobacterial secondary metabolites have largely been confined to microcystins, although cyanobacteria produce a huge variety of toxic or inhibitory secondary metabolites. Mass occurrences of toxic cyanobacteria strongly impact freshwater zooplankton communities; especially the unselective filter feeder *Daphnia*. Daphnids have been shown to successfully suppress bloom formation. However, the opposite situation, i.e. the suppression of *Daphnia* populations by cyanobacteria can be observed as well. To understand these contradictory findings the elucidation of the underlying physiological mechanisms that help daphnids to cope with cyanotoxins is crucial.

We fed *Daphnia magna* with the cyanobacterium *Microcystis aeruginosa* PCC7806 for 24 h and used high-resolution LCMS analytics to analyze the *Microcystis* cells, the *Daphnia* tissue and the surrounding medium in order to investigate the fate of seven investigated cyanobacterial compounds (cyanopeptolins A–C, microcyclamide 7806A and aerucyclamides B–D). For none of these bioactive compounds evidence for biotransformation or biodegradation by *Daphnia* were found. Instead feeding and subsequent release experiments point at the importance of transport mechanisms in *Daphnia* with regard to the cyanopeptolins A and C and microcyclamide 7806A.

In addition we found hints for new inducible defense mechanism in *Microcystis* against predation by *Daphnia*. These putative defense mechanisms include the elevated production of toxic compounds other than microcystins, as could be demonstrated here for aerucyclamide B and D, cyanopoeptolin B and microcyclamide 7806A. Moreover, our data demonstrate the elevated active export of at least one cyanobacterial compound (microcyclamide 7806A) into the surrounding medium as a response to grazer presence, which might constitute an entirely new not yet described cyanobacterial defense mechanism.



## 1. Introduction

Anthropogenic influence on freshwater ecosystems, especially increased nutrient loading and global warming, has led to more frequently occurring cyanobacterial blooms (Dokulil and Teubner, 2000; Paerl and Huisman, 2008; O'Neil et al., 2012). Cyanobacteria in high abundances are known to have a strong impact on freshwater ecosystems; for instance by negatively affecting herbivorous zooplankton (Ghadouani et al., 2003; Wilson et al., 2006). Accordingly, the cyanobacteria-zooplankton interaction has become a matter of increasing importance (Ger et al., 2014). Two factors are regarded as the main cause for these detrimental effects. First, cyanobacteria are of very low food quality for grazers, for example due to mechanical interference (Porter and Mcdonough, 1984) or to low nutritional food quality (Von Elert et al., 2003; Von Elert, 2004). Secondly, cyanobacteria are typically rich in secondary metabolites. These compounds are very diverse, and a lot of them are known to have toxic effects on herbivorous zooplankton (Hansson et al., 2007; Paerl et al., 2001).

However, not only do cyanobacteria negatively influence the zooplankton community; they also provide a serious hazard for the usage of surface waters as drinking water or for recreational purposes (Stewart et al., 2006). Therefore, a lot of effort has been put into the control of cyanobacterial blooms. Among different strategies in water resource management aiming at the control of harmful cyanobacterial blooms are food chain manipulations in order to increase size and abundance of herbivorous zooplankton (Shapiro et al., 1975; Hansson et al., 1998). The success of such manipulative approaches is thereby strongly influenced by presence and abundance of the unselective filter feeder *Daphnia*

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(Leibold, 1989). However, while Chislock et al. (2013) could show that some daphnids are capable of efficiently reducing cyanobacterial biomass, the opposite situation, i.e. the decline of *Daphnia* abundance due to cyanobacteria, was also observed (Ghadouani et al., 2003). According to this, we argue that actually not only presence and abundance of *Daphnia* is of high importance, but also the question, which species or genotypes are present. Several studies revealed that *Daphnias*í sensitivity towards cyanobacterial diets differs strongly between species (Tillmanns et al., 2008; Kuster and Von Elert, 2013) and between clones within a species (Sarnelle and Wilson, 2005; Schwarzenberger et al., 2012; Jiang et al., 2013). For that reason, the elucidation of the physiological background explaining differences in the sensitivity of daphnids to cyanobacterial diets is crucial.

In an earlier study, we conducted an experiment focusing on the most prominent and best studied cyanobacterial toxin, i.e. microcystin-LR (MCLR) (Sadler and von Elert, 2014). In that study we could reject the hitherto assumed detoxification mechanism for MCLR via glutathione-S-transferase (GST) in *Daphnia* (Pflugmacher et al., 1998), and we proposed the possibility that transport mechanisms might be the key factor explaining differing sensitivities in *Daphnia* towards cyanobacteria.

Several studies showed pronounced negative effects of MCLR on *Daphnia* fitness parameters (Rohrlack et al., 1999; Lürling, 2003). On the other hand, Wilson et al. (2006) did not find a significant effect of MC on zooplankton growth rates, when correlating cyanobacterial toxicity and morphology with population growth rates of several zooplankton genera. With respect to *Daphnia magna*, another meta study could not detect significant differences between cyanobacterial diets with or without MCs (Tillmanns et al., 2008). In line with that, cyanobacterial strains that do not produce MCs also have pronounced detrimental effects on *Daphnia* fitness parameters (Lürling and van der Grinten, 2003; Tillmanns et al., 2008; Schwarzenberger et al., 2009). However, even in cases where a correlation between MCs and zooplankton fitness could be demonstrated, the question whether these correlations reflect causality remains unanswered.

In conclusion, it appears reasonable not only to focus on MCs in order to investigate the physiological background of *Daphnias*í differing sensitivity towards cyanobacterial diets, but to take into account further secondary metabolites, which also have putatively negative effects on *Daphnia* fitness.

We therefore re-examined an already existing dataset of mass spectrometry, derived from a 24 h feeding experiment performed with the unselective filter feeder *D. magna* and the MC containing cyanobacterium *Microcystis aeruginosa* PCC7806.

In that experiment we fed *D. magna* with saturating concentrations of the MC containing cyanobacterium for 24 h. In a subsequent chase experiment the daphnids were kept for another 54 h in the absence of cyanobacteria to account for released MCLR or MCLR conjugation products with a significant time delay. All experimental units (namely all incubation media, the cyanobacterial cells and the *Daphnia* tissue) from the 24 h feeding and the subsequent 54 h chase experiment were subjected to LCMS measurements in order to detect and quantify both MCLR and MCLR conjugation products. Based on these data, Sadler and von Elert (2014) rejected the idea of significant biotransformation of MCLR by *Daphnia*.

In the present study, we investigate the fate of further cyanobacterial secondary metabolites other than microcystins, after ingestion by *D. magna*. In particular, we try to answer the question, if any of these compounds are subjected to biotransformation or if *Daphnia* exports these compounds without prior biotransformation into the surrounding water.

For this purpose, we re-examined the already existing mass spectral dataset in order to detect and quantify relative amounts of seven different peptides (cyanopeptolins A–C (CP A, CP B, CP C; aerucyclamides B–D (AC B, AC C, AC D) and microcyclamide 7806A (MC 7806A)). We also calculated the relative amounts within all different experimental stages and parts and summed these up to obtain the overall relative amount.

We focused on two distinct classes of cyanobacterial peptides, namely the cyanopeptolins (CPs) and the cyclamides. The widely distributed cyanopeptolins act as protease inhibitors (Martin et al., 1993; Gademann and Portmann, 2008) and are produced in a non-ribosomal pathway similar to MCs (Welker and von Dohren, 2006). Their detrimental effects on zooplankton have been demonstrated repeatedly (Agrawal et al., 2005; Schwarzenberger et al., 2010; Von Elert et al., 2012). Cyclamides also constitute a widely distributed cyanobacterial peptide class, which is produced by a ribosomal pathway (Ziemert et al., 2008; Portmann et al., 2008a, 2008b). Only little is known about the impact of cyanobacterial cyclamides on zooplankton, but cyclamides have been shown to have cytotoxic properties with regard to the crustacean *Thamnocephalus platyurus* (Ishida et al., 2000; Portmann et al., 2008a, 2008b).

Besides the quantification of these compounds we also screened for putative conjugation products of the respective compounds with cysteine or glutathione. Finally, we determined the relative amounts of these compounds within the cyanobacterial cells in order to find out if the presence of *Daphnia* leads to changes of the compound composition within the cyanobacterium. Our findings revealed new insights into the question how daphnids deal with cyanobacterial compounds after ingestion and clearly highlight the possibility of efficient transport mechanisms not only for microcystins but also for other cyanobacterial compounds. Moreover, the presented data suggest the existence of further yet unknown inducible active anti grazer defense mechanisms in *Microcystis* against *Daphnia*.

## 2. Materials and methods

In the present study an already published mass spectrometry dataset has been re-examined. The experimental details are therefore already described in Sadler and von Elert (2014) in detail. Here, we only give a brief overview of the experimental setup.

### 2.1. Culture conditions

We cultured *D. magna* clone P (P132.85; derived from a pond (Driehoek) in Heusden (Netherlands), N51°44′01″, E5°08′17″) (De Meester, 1994) in membrane-filtered (0.2 μm), aged tap water at 20 °C. This water, in the following referred to as medium, was used for all experiments. *M. aeruginosa* PCC7806 was grown as chemostat culture in cyanobacteria medium (Von Elert and Jüttner, 1997) (constant light: 50 μE $m^{-2}$ $s^{-1}$; at 20 °C; dilution rate 0.23 per day). *Cryptomonas* sp. (Cryptophyceae, SAG 26.80; culture collection of algae at the University of Göttingen, Göttingen, Germany) was cultivated semi-continuously in cyanobacteria medium (constant light: 90–100 μE $m^{-2}$ $s^{-1}$; at 20 °C) with a 20% medium exchange every two days. Food concentrations in the experiment were set to 5 mg particulate organic carbon (POC) per liter and were estimated from photometric light extinction at 470 nm with previously determined carbon extinction equations.

### 2.2. Exposure experiments

#### 2.2.1. Short term exposure experiment (24 h)

200 adult *D. magna* individuals of various sizes in 500 mL of medium were fed with a 100% diet of *M. aeruginosa* PCC7806 for 24 h. Cyanobacterial cell density was determined with a Neubauer improved counting chamber at the beginning and the end of the experiment. Experiments were replicated threefold, and controls were carried out in absence of daphnids.