Toxicon
May 2021, Volume 195, Pages 37-47
https://doi.org/10.1016/j.toxicon.2021.03.009
https://archimer.ifremer.fr/doc/00684/79572/

Archimer
https://archimer.ifremer.fr

Cell free *Microcystis aeruginosa* spent medium affects *Daphnia magna* survival and stress response

Bojadzija Savic Gorenka [1, *], Colinet Hervé [1], Bormans Myriam [1], Edwards Christine [2], Lawton Linda A. [2], Briand Enora [3], Wiegand Claudia [1]

[1] UMR ECOBIO 6553 CNRS, Université de Rennes 1, Campus de Beaulieu, Rennes, France
[2] School of Pharmacy and Life Sciences, Robert Gordon University, Aberdeen, United Kingdom
[3] IFREMER, Phycotoxins Laboratory, F-44311 Nantes, France

* Corresponding author : Gorenka Bojadzija Savic, email address : gorenka.bojadzija@gmail.com

Abstract :

Primary consumers in freshwater ecosystems, such as the zooplankton organism Daphnia magna, are highly affected by cyanobacteria, both as they may use it as a food source but also by cyanobacterial metabolites present in the water. Here, we investigate the impacts of cyanobacterial metabolites focussing on the environmental realistic scenario of the naturally released mixture without crushing cyanobacterial cells or their uptake as food. Therefore, D. magna were exposed to two concentrations of cell free cyanobacterial spent medium from Microcystis aeruginosa PCC 7806 to represent higher and lower ecologically-relevant concentrations of cyanobacterial metabolites. Including microcystin-LR, 11 metabolites have been detected of which 5 were quantified. Hypothesising concentration and time dependent negative impact, survival, gene expression marking digestion and metabolism, oxidative stress response, cell cycle and molting as well as activities of detoxification and antioxidant enzymes were followed for 7 days. D. magna suffered from oxidative stress as both catalase and glutathione S-transferase enzyme activities significantly decreased, suggesting enzyme exhaustibility after 3 and 7 days. Moreover, gene-expressions of the 4 stress markers (glutathione S-transferase, glutathione peroxidase, catalase and thioredoxin) were merely downregulated after 7 days of exposure. Energy allocation (expression of Glyceraldehyde-3-phosphate dehydrogenase) was increased after 3 days but decreased as well after 7 days exposure. Cell cycle was impacted time dependently but differently by the two concentrations, along with an increasing downregulation of myosin heavy chain responsible for cell arrangement and muscular movements. Deregulation of nuclear hormone receptor genes indicate that D. magna hormonal steering including molting seemed impaired despite no detection of microviridin J in the extracts. As a consequence of all those responses and presumably of more than investigated molecular and physiological changes, D. magna survival was impaired over time, in a concentration dependent manner. Our results confirm that besides microcystin-LR, other secondary metabolites contribute to negative impact on D. magna survival and stress response.

Please note that this is an author-produced PDF of an article accepted for publication following peer review. The definitive publisher-authenticated version is available on the publisher Web site.

Highlights

► Cell free *M. aeruginosa* spent medium affected *Daphnia* survival and physiology ► Medium with higher concentration of cyanobacterial metabolites was more detrimental ► Other secondary metabolites, besides microcystin-LR highly likely had negative impact on *Daphnia*

Keywords : zooplankton, cyanobacteria, secondary metabolites, PCC7806, oxidative stress, transcriptomics

Please note that this is an author-produced PDF of an article accepted for publication following peer review. The definitive publisher-authenticated version is available on the publisher Web site.

Journal Pre-proof

44 **Abbreviations**

45 MC-LR: microcystin-LR
46 des-MC-LR: des-microcystin-LR
47 CP: cyanopeptolin
48 AC: aerucyclamide
49 CP-A: cyanopeptolin A
50 AC-A: aerucyclamide A
51 AC-D: aerucyclamide D
52 LC: cyanobacterial spent medium diluted with the BG11 in the ratio 1:400
53 HC: cyanobacterial spent medium diluted with the BG11 in the ratio 1:40
54
55 **Acknowledgements**
56
57 We are grateful to D. Azam and M. Coke from the PEARL INRA 1036 U3E system (The
58 National Infrastructure in Biology and Health, France) for providing the *Daphnia*
59 *magna* strain (originating from l'INERIS) and Bertrand Le Rouzic (University of Rennes 1)
60 for providing us with the *Scenedesmus communis* culture (originating from the lake of Grand
61 Lieu). We are thankful to Sophie Michon-Coudouel and Virginie Daburon who performed
62 genetic analyses at the molecular ecology platform (UMR 6553 Ecobio, Rennes,
63 CNRS/UR1), as well as Marion Chorin from Plateforme EcoChimie (EcoChim, UMS OSUR
64 3343) for help with enzyme analysis. We would like to thank Len Montgomery (Robert
65 Gordon University) for proofreading the manuscript.
66
67 **Funding**
68
69 Gorenka Bojadzija Savic was supported by a Presidential scholarship from the University of
70 Rennes 1. The project benefited from funding from the Centre National de la Recherche
71 Scientifique (CNRS) Initiative Structurante Ecosphère continentale et côtière (EC2CO)
72 under the Interactions métaboliques entre cyanobactéries et daphnies (MICYDA) project.
73 Gorenka Bojadzija Savic was granted a mobility grant from the Doctoral School EGAAL
74 (Bretagne-Loire, France) to visit the Robert Gordon University in Aberdeen, Scotland for
75 metabolites analyses.
76
77 **Declaration of interests**
78
79 ☒ The authors declare that they have no known competing financial interests or personal
80 relationships that could have appeared to influence the work reported in this paper.
81
82
83

Journal Pre-proof

Journal Pre-proof

84 # 1. INTRODUCTION
85

86 Due to eutrophication in freshwater ecosystems, cyanobacterial proliferation frequently
87 results in blooms that potentially produce bioactive or toxic metabolites that harm the
88 environment and humans (Heisler *et al*., 2008). *Microcystis* is one of the most widespread
89 cyanobacterial genus in freshwaters, with *Microcystis aeruginosa* being one of the most
90 commonly detected and investigated (Svirčev *et al*., 2019). Like other cyanobacteria,
91 *Microcystis* produces a diverse range of secondary metabolites that have been shown to
92 impact life traits and physiology of aquatic organisms including zooplankton such as
93 *Daphnia* (Lürling and van der Grinten, 2003; Merwe and Sebbag, 2012). One of the most
94 detected toxins produced by cyanobacteria is microcystin (MC), with more than 250 variants
95 described so far (Mowe *et al*., 2015; Svirčev *et al*., 2019). Microcystins change the
96 phosphorylation state of proteins by inhibiting 1 and 2A protein phosphatases, thus
97 disrupting pathways involving phosphorylation (MacKintosh *et al*., 1990; Trinkle-Mulcahy
98 and Lamond, 2006; Zurawell *et al*., 2005). Furthermore, microcystins trigger oxidative stress
99 in aquatic organisms (Amado and Monserrat, 2010).
100 Besides microcystins, *Microcystis sp.* produces a wide range of intracellular and
101 extracellular secondary metabolites such as aeruginosins, cyanopeptolins, cyclamides,
102 microginins and microviridins (Welker and Von Döhren, 2006), that have various negative
103 effects on *Daphnia* physiology (Rohrlack *et al*. 2001, Bister *et al*. 2004, Ishida *et al*. 2007,
104 von Elert *et al*. 2012). Similar to microcystins, cyanopeptolins (CP) are synthesized through
105 non-ribosomal metabolic pathways, by non-ribosomal peptide synthetases (NRPSs) and
106 polyketide synthase (PKS) (Welker and Von Döhren, 2006). CP are widely distributed and
107 diverse compounds with more than 82 variants described so far (Gademann *et al.* 2010).
108 Aeruginosins are synthesized non-ribosomally and they can inhibit trypsin-type serine proteases
109 (Ishida *et al.* 2000, 2007). CPs and aeruginosins are potent inhibitors of the serine proteases
110 trypsin and chymotrypsin, that are among the main digestive enzymes in *Daphnia* (von Elert
111 *et al*. 2005; Gademann and Portmann 2008; Elkobi-Peer *et al*. 2013). Microviridins are one of
112 the largest oligopeptides produced in cyanobacteria, with 13 to 14 amino acids (1600-1900 Da),
113 contrasting to the other groups, they are ribosomally synthesized tricyclic depsipeptides. They
114 are produced by different cyanobacterial genera, including *Microcystis* sp., however, the natural

Journal Pre-proof

116 Microviridin J has been identified as causing fatal molting disruption in *Daphnia pulicaria*
117 via inhibition of *Daphnia* proteases, thus leading to the death of the animals (Kaebernick *et*
118 *al*., 2001; Rohrlack *et al*., 2004). Cyclamides are cyclic hexapeptides with cytotoxic
119 properties to crustacea (Ishida *et al*., 2000; Portmann *et al*., 2008). Cyanopeptides can occur
120 in high frequency comparable to MC concentrations in aquatic environments and thus may
121 pose problems for drinking water purification from surface water reservoirs (Beversdorf *et*
122 *al*., 2018, 2017; Janssen, 2019; Natumi and Janssen, 2020). Concentrations of
123 cyanopeptolines (< 7 ug/L^{-1}), anabaenopeptins (< 1 ug/L^{-1}) and microginins (< 1 ug/L^{-1}) have
124 been reported in surface waters (Beversdorf *et al*., 2017), however, more research is needed
125 regarding their diversity and concentration in aquatic environments. Furthermore, their effect
126 on organisms as a single compound or in a mixture are not investigated in detail.

127 Nevertheless, it has been suggested that in natural conditions some oligopeptides may be
128 even more harmful than MCs and other cyanotoxins to certain zooplankton species, in
129 particular as digestion inhibitors are active at much lower concentrations than the classical
130 cyanotoxins (Von Elert *et al*., 2004). Digestive enzyme inhibition would cause starvation,
131 impair growth and reproduction, eventually leading to slow death (Von Elert *et al*., 2004).

132 Among the freshwater zooplankton species, *Daphnia* graze on phytoplankton including
133 cyanobacteria, thereby connecting the primary production to the consumers within the
134 aquatic food web (Ger *et al*., 2016). Dominance of nutritionally inadequate phytoplankton,
135 such as cyanobacteria, which lack important sterols and fatty acids necessary for *Daphnia*
136 growth and development, can represent an obstacle in efficient carbon transfer to higher
137 trophic levels (Martin-Creuzburg *et al*., 2008). During their lifetime, however, *Daphnia* are
138 able to develop tolerance to cyanobacterial metabolites, as a physiological response to
139 bioactive compounds (Gustafsson and Hansson, 2004; Ortiz-Rodríguez *et al*., 2012; Sarnelle
140 and Wilson, 2005). Several mechanisms have been described so far, such as *Daphnia's*
141 ability to remodel their digestive enzymes (Schwarzenberger *et al*. 2012, von Elert *et al*.
142 2012), or increase of antioxidant and biotransformation enzyme activity (Dao *et al*., 2013;
143 Ortiz-Rodríguez and Wiegand, 2010; Sadler and von Elert, 2014a). When exposed to
144 cyanobacterial metabolites that are chymotrypsin and trypsin inhibitors, *Daphnia* switch to

Journal Pre-proof

Journal Pre-proof

145 [illegible]

146 (Schwarzenberger *et al*. 2012, von Elert *et al*. 2012). The antioxidative enzymatic defenses
147 include catalase (CAT), superoxide dismutase (SOD), glutathione-peroxidase (GPx), while
148 glutathione S-transferase (GST) is the detoxification mechanism (Ighodaro and Akinloye,
149 2018; Pflugmacher *et al*., 1998). *Daphnia* can reduce oxidative stress caused by
150 cyanobacterial metabolites by increasing CAT activity (Ortiz- Rodríguez and Wiegand,
151 2010; Wojtal-Frankiewicz *et al*. 2014). Similar increased activity of antioxidant SOD and
152 CAT enzyme providing cellular protection against ROS is found in a study where *Daphnia*
153 *magna* was fed with *Microcystis* diet (Lyu *et al*., 2016b). *Daphnia* can reduce the toxicity of
154 microcystin by increasing the activity of GST catalysing the biotransformation via
155 conjugation to glutathione (Dao *et al*. 2010; Ortiz-Rodríguez *et al*., 2012; Miles *et al*. 2016).
156 Furthermore, studies in *Daphnia* have shown that cyanobacterial metabolites can affect
157 expression of genes involved in digestive system (Asselman *et al*., 2014; Drugă *et al*., 2016;
158 Schwarzenberger *et al*., 2012), cell cycle (De Coninck *et al*., 2014b; Giraudo *et al*., 2017;
159 Tong *et al*., 2017), and oxidative stress (De Coninck et al., 2014b; Lyu et al., 2016a;
160 Rhiannon et al., 2011). However, characterizing transcriptional responses of *Daphnia* to
161 cyanobacterial toxins so far focussed merely on effect of microcystin or cyanobacterial media
162 without considering other bioactive molecules that might be present apart from microcystin
163 (Asselman *et al*., 2012; De Coninck *et al*., 2014a; Drugă *et al*., 2016; Lyu *et al*., 2016b).
164 Besides being affected by grazing on cyanobacteria, zooplankton can also be affected by
165 naturally produced cyanobacterial metabolites released in water (Barrios *et al*., 2015;
166 Ferrão-Filho *et al*., 2014; Smutná *et al*., 2014). Nevertheless, most of the studies investigate
167 impact of cyanobacteria on *Daphnia* grazing on them, or exposure to extracts obtained by
168 crushing cyanobacterial cells (from a culture or from a field sample), or to purified
169 compounds (Dao *et al*., 2013; Esterhuizen-Londt *et al*., 2016; Peng *et al*., 2018). Hence, it is
170 of interest to investigate if *Daphnia* are impaired by the presence of cyanobacterial
171 metabolites naturally released during cyanobacterial normal growth, without necessarily
172 feeding on them, or crushing cells. Furthermore, knowing the composition and concentration
173 of metabolites present in the medium, naturally released as a product of cyanobacterial

174 [illegible]

175 cyanobacterial metabolites.

176 Therefore, the aim and novelty of this study is *Daphnia* exposure to cell free cyanobacterial
177 spent medium obtained from a two weeks culture of exponentially growing *M. aeruginosa*,
178 containing metabolites naturally released during that time. In addition, we detected and
179 quantified cyanobacterial metabolites present in the medium, beside commonly investigated
180 microcystin. We monitored markers of stress response in order to predict *Daphnia*
181 physiological phenotypes as a response to cyanobacterial spent medium. We analyzed the
182 effects of cell free cyanobacterial spent medium that contained a quantified mixture of
183 compounds on i) *Daphnia* survival, ii) activity of enzymes involved in oxidative stress and
184 detoxification and iii) expression of candidate genes involved in digestion, growth and
185 development, detoxification and oxidative stress response. We hypothesize a negative impact
186 on *Daphnia* survival, a trigger of CAT and GST activities, as well as expression of genes
187 involved in *Daphnia* growth and development, digestion, detoxification and oxidative stress.
188
189

Journal Pre-proof

Journal Pre-proof

190 # 2. MATERIALS AND METHODS
191

192 ## 2.1. Culture conditions
193

194 ### *2.1.1. Microcystis aeruginosa PCC 7806*
195

196 An axenic microcystin (MC) producing *M. aeruginosa* strain PCC 7806 was obtained
197 from the Pasteur Culture collection of Cyanobacteria in Paris, France
198 (https://research.pasteur.fr/en/team/collection-of-cyanobacteria/). The strain was grown in
199 100% cyanobacterial BG11 medium (SIGMA), under a 14h:10h light:dark regime using
200 daylight white fluorescent tubes (Toshiba, 15 W, FL15D) with 20 μmol photons m^{-2} s^{-1}
201 illumination at a constant temperature of 20±1°C (Sanyo incubator). The culture was
202 maintained in exponential growth phase, while the axenicity was regularly evaluated as
203 described in (Briand et al., 2012).
204

205 ### *2.1.2. Daphnia magna*
206

207 The *D. magna* clone was obtained from the PEARL INRA 1036 U3E. As the exposure to
208 cyanobacteral metabolites was realized via *M. aeruginosa* PCC 7806 spent medium, the *D.*
209 *magna* clone was acclimated to BG11 medium (SIGMA). Before performing experiments we
210 compared osmolarity of BG 11 medium with the osmolarity of commonly used Artificial
211 *Daphnia* medium, Elendt M4 and Elendt M7. Osmolarity of BG 11 medium was similar to
212 that of these *Daphnia* media, thus not affecting *Daphnia* survival. During the first three
213 weeks *D. magna* were slowly acclimated and adjusted to the cyanobacterial BG 11 medium
214 that was used in all the experiments, by gradually increasing the % of BG11 medium until it
215 reached 100% in the end of the acclimation period. After the acclimation period *D. magna*
216 were grown in 100% of BG11 medium for a month, before neonates (< 36h old) were used in
217 the experiment. During the acclimation and cultivation period no irregularities in *Daphnia*
218 survival, eating, movement and growth were noticed, suggesting that *D. magna* were
219 successfully acclimated to the medium (Bojadzija Savic *et al*., 2020). *D. magna* were grown
220 and cultivated in the aquarium at a constant temperature of 20°C, light intensity of 15 μmol
221 photons m^{-2} s^{-1} having a day/night cycle of 14h:10h (Sanyo MIR 154) and daily renewal of

Journal Pre-proof

Scenedesmus communis originating from lake Grand Lieu, France, that was isolated in our laboratory (University of Rennes 1).

Sufficient food that 100 *Daphnia* (According to OECD guidelines for testing of chemicals 202) that should be available daily in the medium is equivalent to 2.8 - 5.6x10^8 cells/mL of *S. communis*. *Daphnia* are usually maintained in medium that is not appropriate for algal growth (Elendt M4, Elendt M7- OECD guidelines for testing of chemicals), therefore when *S. communis* are introduced to *Daphnia* medium, they are consumed before growing in high densities. However, in our cultures *Daphnia* were adjusted to BG11 medium that was also used for *S. communis* culture. Hence, it allowed *S. communis* to grow and reach high densities (10x higher than the initial one, within one week) while being consumed by *Daphnia*. As high density of *S. communis* disrupts normal *Daphnia* functioning; its concentration had to be adjusted to 2.8 - 5.6x10^7 cells/mL to maintain equilibrium between growth and consumption. In order to keep the *S. communis* culture in more accessible form for consumption, in particular by neonates (i.e. unicellular /in pairs instead of four cells having long spikes), *Scenedesmus* culture was diluted every few days before feeding.

2.2. Experimental design

All experiments were performed in 5 replicates in 2 L aquariums and lasted for 3 and 7 days. All *Daphnia* were fed daily with *S. communis*, whose density was monitored and adjusted daily. For the control, 150 *D. magna* neonates (< 36h old) per aquarium were raised in BG11.

For the treatment, the cyanobacterial spent media was prepared in the following way: from an exponentially growing *M. aeruginosa* PCC 7806 culture, centrifuged cells were transferred in fresh sterile BG 11 medium to remove extracellular metabolites. From the pellet, a cyanobacterial culture of 2x10^5 cells/mL initial density was grown for two weeks, reaching a cell density of 1x10^6 cell/mL. From that culture, cell free cyanobacterial spent medium was collected by filtering through 0.2 µm sterile cellulose nitrate filter. *D. magna* were exposed to two concentrations of cyanobacterial spent medium: a) diluted with the BG11 in the ratio 1:40 (thereafter called HC for high concentration) and b) 1:400 (thereafter called LC for low

Journal Pre-proof

Journal Pre-proof
254 MC-LR in our experiments (final concentration of MC-LR: LC: 0.5 µg/L, HC: 5 µg/L), as
255 dissolved MC ranges in the environment between 0.2 and 11 µg/L (Lahti *et al*., 1997;
256 Pawlik-Skowrońska *et al*., 2008; Rastogi *et al*., 2015; Su *et al*., 2015).
257 *D. magna* neonates (150 per treatment) were exposed to 2 L of these two concentrations.
258 At the end of the exposure (3 days and respectively 7 days), *D. magna* samples
259 (biotransformation, oxidative stress, energetic profiles) were taken by collecting them over a
260 mesh, briefly rinsed with fresh BG11 media, and after removal of the excess media, snap
261 frozen in liquid nitrogen and stored at -80°C until analysis.
262
263 **2.3. Cyanobacterial secondary metabolites analysis**
264
265 Cyanobacterial secondary metabolites previously known to be produced by this
266 particular strain PCC7806 (Briand *et al*., 2016; Rohrlack *et al*., 2004; Sadler and von Elert,
267 2014b) were monitored after 2 weeks growth phase from initially $2x10^5$ cells/mL to $1x10^6$
268 cells/mL. Cyanobacterial cells were separated from the supernatant by centrifugation. Cell
269 free spent medium was filtered through 0.2 µm filter and 1 mL was lyophilized. Extraction of
270 lyophilized material was done in 0.5 ml 50% methanol and processed as described in
271 Bojadzija Savic *et al*., 2019. Waters Acquity Ultra-High Performance Liquid
272 Chromatography coupled to a Xevo quadrupole time of flight mass spectrometer was used
273 for the metabolites analysis. Cyanobacterial peptides were detected using extracted ion
274 chromatograms for the respective specific masses of the different compounds (Bojadzija
275 Savic *et al*., 2019). Microcystin-LR (MC-LR), Microcystin-des-LR (des-MC-LR),
276 cyanopeptolin A (CP-A), and aerucyclamide A (AC-A) and D (AC-D) were quantified using
277 linear relationship between peak area (MC-LR and des-MC-LR at 238 nm, CP-A at 220 nm,
278 and AC-A at 237 nm and AC-D at 240 nm) and known concentrations of the toxin standards.
279 The microcystin-LR standard was purified as previously described (Edwards *et al*., 1996).
280 CP-A standard and AC-A and AC-D standard were purified using preparative HPLC
281 (Biotage Parallex Flex, Cardiff, UK) and Flex V3 software for instrument control and data
282 acquisition as described in (Bojadzija Savic *et al*., 2020). Detection and quantification of the
283 cyanobacterial peptides was done by MassLynx v4.1 software.
284
285 **2.3. *D.magna* survival**
286

Journal Pre-proof

Journal Pre-proof

(identified as not moving and decaying *Daphnia* on the bottom of the aquarium) with results expressed in percentages of total individuals at the start of the exposure.

2.4. Enzyme extraction and measurement

Fifty *Daphnia* were resuspended and homogenized in 1 mL of ice cold extraction buffer (0.1 M phosphate buffer pH 6.5, glycerol, 1 mmol EDTA, and 1.4 mmol dithioerythritol) using Lysing Beads-Matrix E (MPbio) in the Vibro-mill MM200, (RETSCH) for 3 min at the frequency of 25 Hz to break the cells, followed by centrifugation (10,000 g, 10 min, 4°C, Sigma 3K18C). The supernatant was used for enzyme measurements using a spectrofluorometer (SAFAS Monaco Xenius XC, Monaco). Catalase (CAT) activity was assayed by measuring the rate of disappearance of H_2O_2 at 240 nm (Chang and Kao, 1997). GST was assayed at 340 nm using 1-chloro-2, 4-dinitrobenzene (CDNB) as substrate. SOD activity was determined using a photochemical assay based on the reduction of nitro blue tetrazolium (NBT) according to total Superoxide Dismutase (T-SOD) assay kit (Hydroxylamine method, SIGMA KIT), however SOD activity in our experiments remained below the level of detection. All enzyme activities were related to the protein content in the extract, measured according to Bradford (1976).

2.5. Candidate function gene expression

2.5.1. RNA extraction and reverse transcription

RNA extraction and reverse transcription was done according to (Colinet *et al.* 2010) from the unexposed control and from *Daphnia* exposed to LC and HC after 3 and 7 days. For each condition and sampling time point, five RNA samples (i.e. biological replicates), each consisting of a pool of 50 *Daphnia*, were used. *Daphnia* were ground to fine powder in 1.5 mL tubes placed in liquid nitrogen. Samples were mixed with lysis buffer (containing 1% β-mercaptoethanol) from RNA extraction kits (Qiagen) and crushed for 10 min to complete homogenization. RNA extraction and purification was performed following the manufacturer's instructions (Qiagen). Total RNA was eluted in 40 μL of DEPC-treated water. RNA was quantified and quality-checked with a Nanodrop 1000 (Thermo Scientific, Waltham, MA). Three hundred nanograms of total RNA was used in the reverse transcription to cDNA, using the SuperScript ® III First-Strand Synthesis System for RT-PCR

Journal Pre-proof

323 20°C until use.

324

325 **2.5.2. Real-time PCR**

326

327 We used primers of candidate genes involved in *Daphnia* energy
328 (glyceraldehyde-3-phosphate dehydrogenase: *gapdh*), digestive system (protease: *ct383*),
329 oxidative stress (glutathione S-transferase: *gst*, glutathione peroxidase: *gpx*, catalase: *cat*,
330 thioredoxin: *trx*,), cell cycle (histone: *h2a*, myosin heavy chain: *mhcrt*) and molting (nuclear
331 hormone receptor: *hr3*, nuclear hormone receptor: *ftz-f1*) that potentially could be affected by
332 cyanobacterial metabolites. We used ***β***-actin as the reference gene. Oligonucleotide primers
333 were obtained from the previously published references (see **Table 1.** for details), having
334 efficiency between 92% and 105% (Giraudo *et al*., 2017; Houde *et al*., 2013; Lyu *et al*., 2014;
335 Rhiannon *et al*., 2011; Schwarzenberger *et al*., 2010; Tong *et al*., 2017; Wang *et al*., 2016).
336 Oligonucleotide primers were made by Integrated DNA Technologies, BVBA.

337

Table 1. Oligonucleotide primers used in the experiments.

Gene name	Symbol	Primer sequence 5'-3'	Amplicon size	Reference
Glyceraldehyde-3-phosphate dehydrogenase	***gapdh***	F- TGCTGATGCCCCAATGTTTGTTGT R-GCAGTTATGGCGTGGACGGTTGT	132	(Giraudo *et al*., 2017)
Protease CT383	***ct383***	F- TTGGCACCTTCCACCGAAT R- TCATCAGGACTGGAGAAACGC	183	(Schwarzenberger *et al*., 2010)
Glutathione S-transferase (NCBI: No. EFX81634.1)	***gst***	F- GGGAGTCTTTTACCACCGTTTC R- TCGCCAGCAGCATACTTGTT	150	(Wang *et al*., 2016)
Glutathione peroxidase	***gpx***	F- AACGTTACGATGCCAGTTCC R- TCTTTCGAGCGGTTGAGATT	212	(Rhiannon *et al*., 2011)
Catalase	***cat***	F- AGGTGCCTTTGGATACTTTGA R- TTGCGTATTCCTTGGTCAGTC	495	(Lyu *et al*., 2014)
Thioredoxin	***trx***	F- GTATCCACGCCAGTCCTTGTT R- TCCTTCCACTTTTCCTCCCTTA	129	(Liu *et al*., 2019)
Histone 2A	***h2A***	F- CTGGTGCCCCTGTCTACCTA R- TAGGGAGGAGAACAGCCTGA	219	(Giraudo *et al*., 2017)
Myosin heavy chain	***mhcrt***	F - GATGCCGTTTCCGAGATGAG R - CTCGGCGGTCATGTGGTC	132	(Tong *et al*., 2017)
Nuclear	***hr3***	F- AAGGTCGAGGATGAAGTGCG	81	(Giraudo *et al*.,

hormone receptor HR3		R- AAAGACGCTACTATCGGGCG		2017)
Nuclear hormone receptor FTZ-F1	*ftz-f1*	F- TCTTACCGGACATTCACGCC R- ACAGCCGTTGAGATGCTTGA	71	(Giraudo *et al.*, 2017)
Beta-actin	***β-actin***	F- GCCCTCTTCCAGCCCTCATTCT R- TGGGGCAAGGGCGGTGATTT	189	(Houde *et al.*, 2013)

Journal Pre-proof

338

339 Real-time PCRs were performed on the LightCycler 480 system. Reactions were performed
340 in 384-well LightCycler plates, using LightCycler 480 High Resolution Melting Master Mix
341 and the crossing point (Cp), equivalent to the cycle threshold (Ct), estimates were obtained
342 using the absolute quantification module in the software package. The PCR reactions were
343 performed in four replicates , containing 4 µL of cDNA sample, 2 µm each primer, and 6 µL
344 of the High Resolution Melting Master Mix. After 10 min at 95°C, the cycling conditions
345 were as follows: 60 cycles at 95°C for 10 s, 60°C for 15 s, and 72°C for 15 s. To validate the
346 specificity of amplification, a post amplification melt curve analysis was performed.
347 Amplicons were first denatured at 95°C for 1 min, and then cooled to 65 °C, and the
348 temperature was then gradually raised to 95°C). Fluorescence data were recorded
349 continuously during this period, and subsequently analyzed using the Tm calling module in
350 the LightCycler 480 software.

$$R = \frac{(E_{target})^{\Delta CPtarget(control-treated\ sample)}}{(E_{reference})^{\Delta CPtarget(control-treated\ reference)}}$$

351 Relative expression ratios (R) (i.e. fold change) were calculated using the efficiency
352 calibrated model of (Colinet et al., 2010; Pfaffl, 2001). In the Pfaffl model, CP is the crossing
353 point (i.e. Ct) and E the efficiency of PCRs. qPCR CT values of all candidate genes have been
354 provided in **Supplementary 1**. The ratio of the target gene is expressed in treated samples
355 versus matched controls (calibrators), and normalized using the housekeeping reference
356 gene.
357

358 **2.7. Statistical analyses**

359

360 R Core Team (2013) was used to access statistical analysis of the obtained data. All data
361 are presented as mean ± standard deviation. Significant differences were determined at

Journal Pre-proof

Journal Pre-proof

362 $p<0.05$. We performed t-tests to determine the difference between CAT, GST activities, as
363 well as gene expression between the control and treatment. Repeated-measures analysis of
364 variance was done to determine the difference in *Daphnia* survival between Control/LC,
365 Control/HC and LC/HC). Repeated-measures analysis of variance is a mixed linear model
366 with day, treatment (Control vs Treatment) and interaction between day and treatment
367 considering the repeated measures on replicates (random effect). Normality of residuals was
368 tested via Shapiro test (residuals normaly distributed when $p>0.05$). Anova was performed to
369 test the effects of the model. Pairwise comparison with correction for multiple comparison
370 was performed to check significant differences between control and the two treatments,
371 differences between LC and HC and if there was time dependency effect on control and
372 treatment.
373
374

Journal Pre-proof

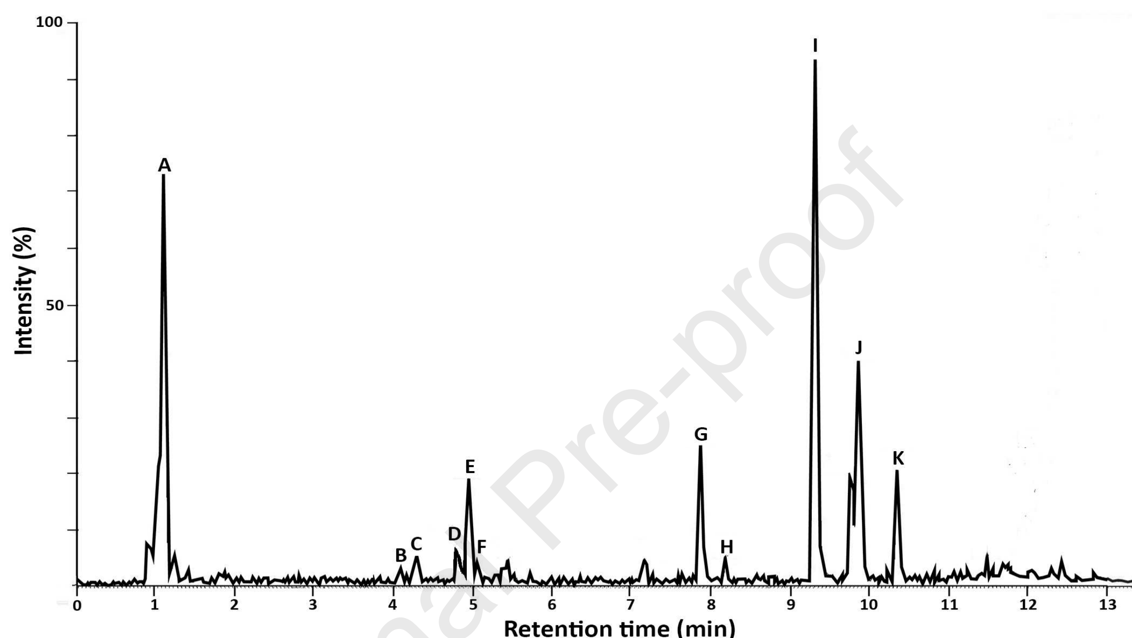
375 # 3. RESULTS

376

377 ## 3.1. Composition of the two weeks old *M. aeruginosa* PCC7806 medium

378

379 In the media produced by exponentially growing *M. aeruginosa* PCC7806, 11
380 metabolites were detected after 2 weeks of cultivation. *M. aeruginosa* PCC 7806 produced
381 MC-LR and des-MC-LR, cyanopeptolins, CP (963A, A and B), aerucyclamides, AC (A,B,C
382 and D) and aeruginosins (684 and 602) (**Figure 1.**).



383
384 **Figure 1.** Secondary metabolites detected in *M. aeruginosa* PCC 7806. (**A**) aeruginosin 684, (**B**)
385 cyanopeptolin B, (**C**) aeruginosin 602, (**D**) des-MCLR, (**E**) MC-LR, (**F**) CP A, (**G**) aerucyclamide D, (**H**)
386 cyanopeptolin 963A, (**I**) aerucyclamide A, (**J**) aerucyclamide C, (**K**) aerucyclamide B.

387

Journal Pre-proof

388 The concentrations of five extracellular metabolites (MC-LR, des-MC-LR, CP-A,
389 AC-D, AC-A) in *M. aeruginosa* PCC 7806 after 2 weeks growth phase from $2x10^5$ cells/mL
390 initially to $1x10^6$ cells/mL, and in dilutions HC and LC are shown in **Table 2.**

391

Table 2. Concentrations of extracellular metabolites in original medium of the 2 weeks *M. aeruginosa* PCC 7806 and its HC and LC dilutions

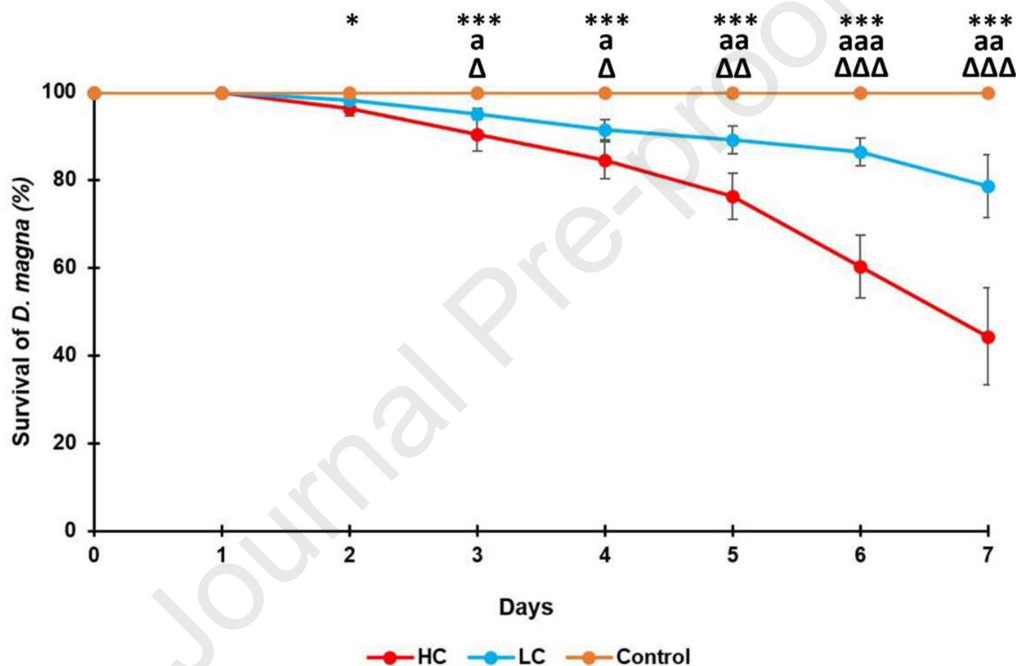
Extracellular metabolite	Original spent *M. aeruginosa* PCC 7806 medium (µg/L)	HC (µg/L)	LC (µg/L)
MC-LR	**218±21**	**5.45±0.53**	**0.55±0.05**
Des-MC-LR	**61±1**	**1.53±0.03**	**0.15±0.01**
CP-A	**135±11**	**3.38±0.28**	**0.34±0.03**
AC-D	**93±10**	**2.33±0.25**	**0.23±0.02**

Journal Pre-proof
392

393 ## 3.1. *Daphnia* survival
394

395 The high concentration of the cyanobacterial medium (HC) caused a significant decrease
396 in survival from day 2 onwards, compared with the control, while exposure to the low
397 concentration (LC) caused significant decrease after day 3. Both concentration levels
398 significantly decreased *Daphnia* survival over the course of the experiment. *Daphnia*
399 survival was significantly higher in LC exposure from day 3 onwards (80% survival after 7
400 days) compared with the HC exposure (40% survival after 7 days) (**Figure 2**.)

401



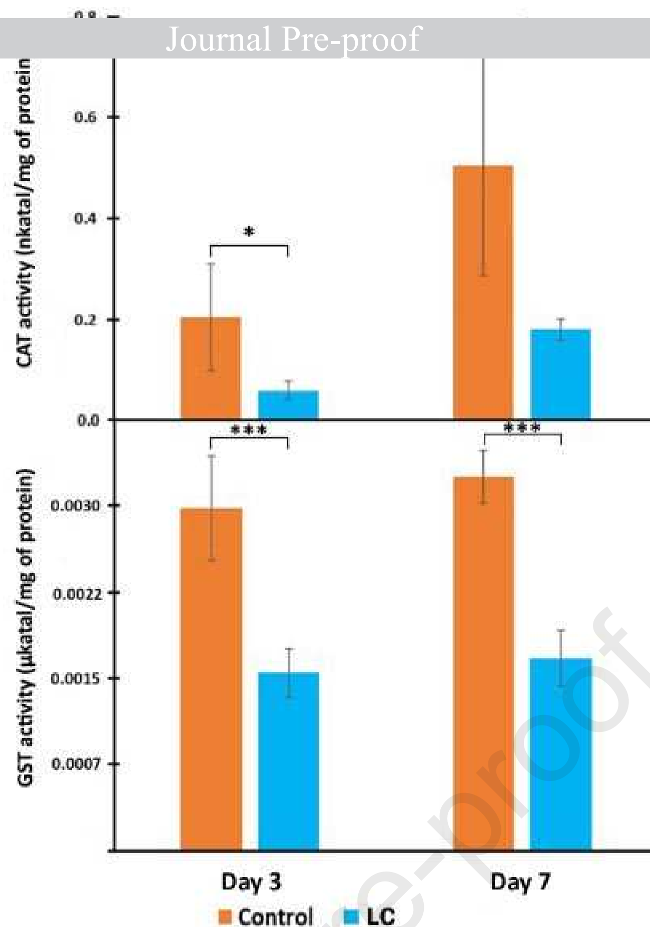
402
403 **Figure 2.** *D.magna* survival when exposed to HC and LC. Control vs HC: *; Control vs LC: a ; HC vs LC: Δ. *,
404 a, Δ ($p < 0.05$), **, aa, ΔΔ ($p < 0.01$), ***, aaa, ΔΔΔ ($p < 0.01$); repeated-measures analysis of variance
405

406 ## 3.2. Antioxidant and detoxification enzymes
407

408 CAT activity was significantly lower on day 3 and day 7 in the LC exposure, compared
409 to the control. Similarly GST activity was significantly lower on day 3 and 7 in the treatment
410 exposed to LC, compared to the control. Due to the high mortality in the HC exposure, the
411 biomass of the remaining *Daphnia* was insufficient for enzyme analysis on both days (**Figure
412 3.**).

413

Journal Pre-proof



414
415 **Figure 3.** CAT and GST activity in *D.magna* exposed to LC * (p < 0.05), ** (p < 0.01), *** (p < 0.01), t-test
416

417 ## 3.5. Selected genes expression

418

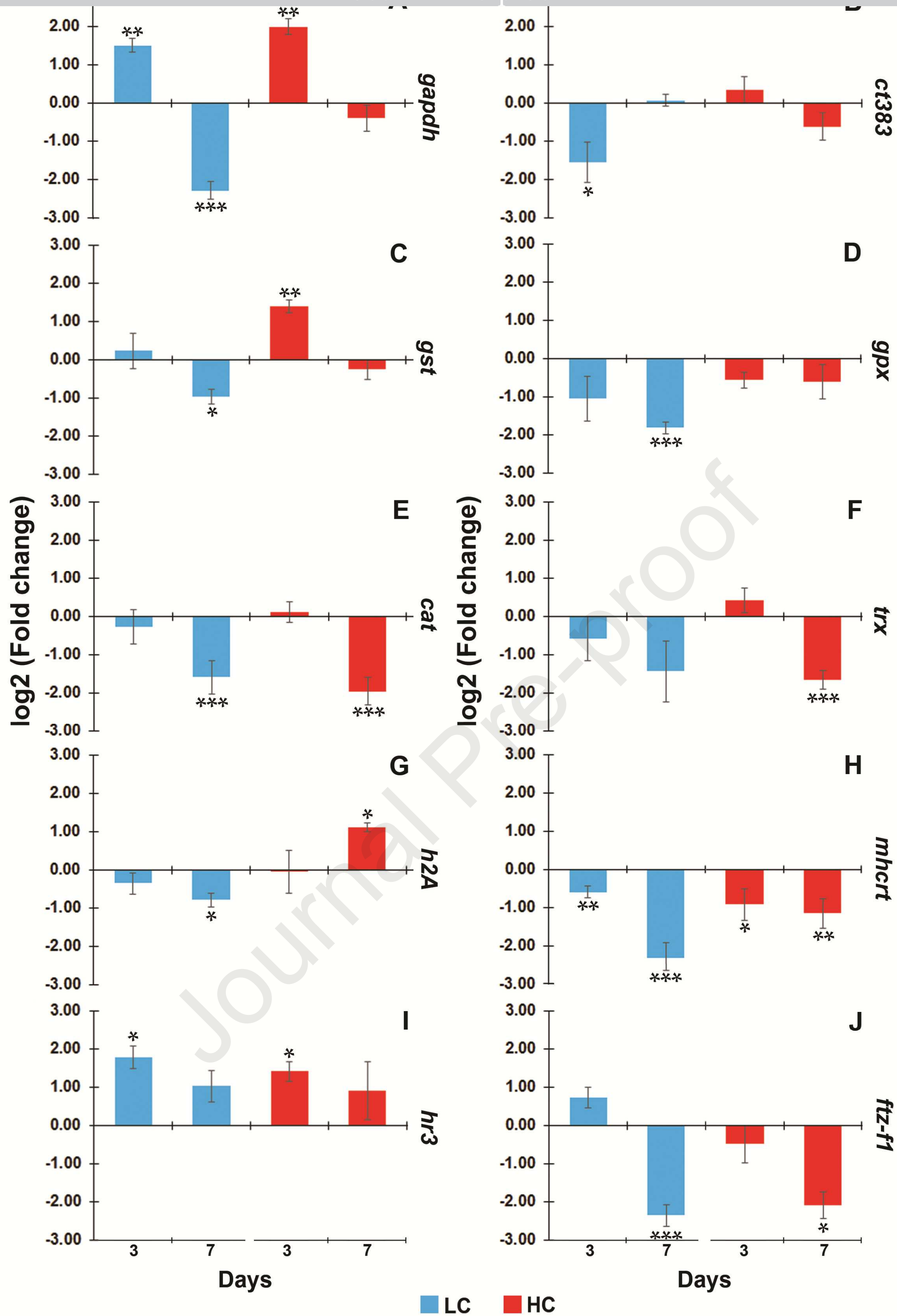
419 Glyceraldehyde 3-phosphate dehydrogenase (*gapdh*) was significantly overexpressed on
420 day 3 when *Daphnia* were exposed to both HC and LC, but downregulated after 7 days,
421 significant for LC (**Figure 4. A**). The gene coding for digestive enzyme Protease CT383
422 (*ct383*) was significantly downregulated after 3 days exposure to LC (day 3) of *Microcystis*
423 spent medium, but not in the other treatment (**Figure 4**. **B**). Concerning the oxidative stress
424 and detoxification related genes, merely downregulation was observed. The only exception
425 was the gene encoding for the detoxification enzyme Glutathione S-transferase (*gst*) which
426 was significantly upgulated on day 3 when exposed to HC, while on day 7, *gst* was
427 downregulated at LC treatment (**Figure 4. C**). Furthermore, Glutathione peroxidase (*gpx*)
428 expression was significantly downregulated on day 7 after *Daphnia* exposure to LC (**Figure**
429 **4. D**). Genes responsible for oxidative stress enzymes Catalase (*cat*) (**Figure 4. E**) and
430 Thioredoxin (*trx*) (**Figure 4. F**) were downregulated on day 3 (LC) and day 7 (HC and LC).
431 The response of the histone 2A (*h2A*) gene was the opposite of this, when exposed to HC and

Journal Pre-proof

Journal Pre-proof

433 exposed to HC or LC (**Figure 4. G**). HC and LC also had significant negative effect on gene
434 expression of Myosin heavy chain (*mhcrt*) that was significantly underregulated on day 3 and
435 7 (**Figure 4. H**). Cyanobacterial secondary metabolites affected two genes involved in the
436 molting cycle: the Nuclear hormone receptor HR3 (*hr3*) and FTZ-F1 (*ftz-f1*). While the *hr3*
437 was significantly upregulated on day 3 when exposed to both HC and LC (**Figure 4. I**)
438 *ftz*-was significantly downregulated on day 7 when exposed to both HC and LC (**Figure 4. J**).
439

Journal Pre-proof



440

441 **Figure 4.** Effect of cyanobacterial media (LC and HC) on the relative gene expressions expressed as log2(fold
442 change). Relative expressions in *D. magna* for: A) Glyceraldehyde 3-phosphate dehydrogenase (*gapdh*), B)
443 Protease CT383 (*ct383*), C) Glutathione S-transferase (*gst*), D) Glutathione peroxidase (*gpx*) E) Catalase (*cat*),
444 F) Thioredoxin (*trx*), G) histone 2A (*h2A*), H) Myosin heavy chain (*mhcrt*), I) Nuclear hormone receptor HR3
445 (*hr3*), J) Nuclear hormone receptor FTZ-F1 (*ftz-f1*). Gene expressions were normalized to the control condition
446 for each sampling time. Replicates of culture are represented as a mean and error bars represent the standard
447 deviations (n = 5). * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.01$), t-test

448

Journal Pre-proof

449 # 4. DISCUSSION
450

451 *Daphnia* are unselective phytoplankton filter feeders, and as such can be exposed to
452 cyanobacterial metabolites by direct grazing on cyanobacteria as well as by uptake of
453 metabolites released in the water. While previous studies have demonstrated the negative
454 impacts of cyanobacterial medium (Lanaras and Cook 1994, Neumann *et al.* 2000, Dao *et al.*
455 2010, 2013, Esterhuizen-Londt *et al*. 2016, Peng et al. 2018) on *Daphnia*, the novelty of this
456 study was to connect responses from selected markers on the gene-level to enzymatic
457 activities and to consequences for the survival of *D. magna* exposed to cyanobacterial
458 cell-free sprent medum. From the mixture of cyanobacterial metabolites in the medum we
459 identified 11 and quantified 5.

460 As *M. aeruginosa* was grown in BG11, *D. magna* had to be acclimatised to this medium
461 during several weeks before the experiment. Survival of the control group throughout the
462 experiment was always high (>98%). These results are in line with the literature where
463 non-treated *D. magna* survival was always high (close to 100%) in the first 8 days of
464 experiments, although specific *D. magna* cultivation medium was used (Dao *et al*. 2010,
465 Ortiz-Rodríguez *et al*. 2012), suggesting that BG 11 medium used in our study did not have
466 impact on the *D. magna* survival. Apart from increased CAT activities no other changes were
467 observed in the control group over the exposure time, we nevertheless suggest for further
468 studies to compromise between both media by adding necessary minerals, e.g. calcium, using
469 diluted BG 11, while keeping the osmolarity as we ensured in our exposures.

470 Cyanobacterial spent medium had a dose and time dependent negative impact on *D.*
471 *magna* survival, verifying our hypothesis. The concentrations used (Table 2.) were
472 detrimental thus preventing *D. magna* from acclimatisation during the exposure period.
473 These detrimental effects within 7 days were not expected as these concentrations were
474 chosen according to previous studies showing that survival of *D. magna* rapidly decreased
475 during 3 weeks exposure to crude extract containing 50 µg/L of dissolved microcystin, while
476 exposure to 5 µg/L did not impact *Daphnia* survival much (Dao *et al*., 2010). Similar results
477 were observed by Lürling and van der Grinten (2003) where exposure to 3.5 µg/L of
478 dissolved microcystin showed no significant decrease in survival over 7 days. When exposed
479 to crude extract or artificial mixtures of pure microcystins containing 60 µg/L of total MC

Journal Pre-proof

480 (MC-LR 21.1 ± 1.9 µg/L, MC-RR 37.2 ± 4.8 µg/L and MC-YR 6.1 ± 0.8 µg/L) it did not
481 appear to have an impact on *D. pulex* survival for 6 days (Esterhuizen-Londt *et al.*, 2016).
482 Our results are, however, in line with studies exposing *D. magna* to *M. aeruginosa* containing
483 1.4–9 µg/L of MC, where feeding was inhibited, followed by increase of mortality in the first
484 week of the treatment (DeMott, 1999; Demott *et al.*, 1991; Ghadouani *et al.*, 2004; Rohrlack
485 *et al.*, 2001). *D. laevis* (two clones) and *D. similis* exposed to five *Microcystis* extracts in
486 which microcystins were detected (434 - 538 mg/g of lyophilized sample) suffered decreased
487 survival, reproduction and disturbance in egg production after 48 h (Herrera *et al.* 2015).
488 Crude extract obtained from *Microcystis* spp. had lethal effect on *D. magna* neonates, with
489 LC50 (48 h) ranging from 168.3−442.7 mg microcystin (total) DW L^{-1} (Pham, 2018).
490 Susceptibility to microcystin is, however, not only species-specific, but *Daphnia* species can
491 also show different responses within their clones (DeMott, 1999; Hairston *et al.*, 1999;
492 Rohrlack *et al.*, 2001).
493 As both concentrations in our experiment caused mortality (20% for LC and 60% for
494 HC) within the 7 days exposure, we suggest a high *D. magna* sensitivity to the other
495 cyanobacterial metabolites present in the spent medium, as besides MC-LR and desmethyl
496 MC-LR, cyanopeptolin-A, aerucyclamides A and D were quantified, and aeruginosins (602
497 and 684), cyanopeptolins (B and 963) and aerucyclamides (B and C) detected. HC and LC of
498 *Microcystis* spent medium downregulated a gene encoding the digestive enzyme protease,
499 *ct383*, thus potentially interfered with *D. magna* digestion and as a consequence could
500 contribute to the increasing mortality in a concentration and time dependent manner.
501 Similarly, when *D. magna* were fed with MC-producing strain of PCC 7806 wild type, *ct383*
502 was downregulated after 6 days, leading to reduced total chymotrypsin activity
503 (Schwarzenberger *et al.*, 2010).
504 When fed with *Microcystis* strain UWOCC MRC (a non-microcystin producing strain) a
505 lethal molting disruption in *Daphnia* spp. has been observed, suggesting that cyanobacterial
506 proteases, other than microcystin, could interfere with the molting cycle (Kaebernick *et al.*,
507 2001). Despite the absence of microviridins J in our medium, known to impair the molting
508 cycle in *Daphnia* via protease inhibition (Rohrlack *et al.*, 2004), upregulation of a gene
509 coding for nuclear hormone receptor (*hr3*) followed by significant downregulation of *ftz-f1*

Journal Pre-proof

511 observed. Our results suggest that other metabolites have a role in impairment of these
512 pathways which may affect *Daphnia* molting.

513 Activity of anti-oxidative stress enzymes is a crucial defense mechanism against the
514 induction of oxidative stress by microcystins (Amado and Monserrat, 2010). Through
515 increased oxidative stress enzyme activities and detoxication, *D. magna* are able to enhance
516 acclimation to cyanobacterial metabolites (Ortiz-Rodríguez *et al*., 2012). CAT is a highly
517 efficient antioxidant enzyme, responsible for reduction of H_2O_2 concentrations in the cells
518 (Fridovich, 1998). Elevated CAT activity can prevent oxidative damage caused by
519 cyanotoxins thus provides oxidative protection for *Daphnia* (Wojtal-Frankiewicz *et al*.,
520 2013). Increased CAT activity was observed in adults and neonates after 24 h when exposed
521 to 100 µg/L pure MC-LR, while exposure to 5 µg/L MC-LR or less, CAT activity was similar
522 to control (Ortiz-Rodríguez *et al*., 2012). In our experiments, genes of antioxidant enzymes,
523 *cat* and *trx,* were downregulated, confirming the results of the enzyme activity. The
524 significant decrease in CAT activity on days 3 and 7, suggests that these enzymes were
525 exhausted due to the presence of the mixture of cyanobacterial compounds in the
526 concentrations applied. Similarly, CAT decreased in *D. magna* after being exposed to
527 cyanobacterial crude extract (containing 60 µg/L total MC) during the whole exposure period
528 of 72 h while the pure toxin MC-LR increased its activity (Esterhuizen-Londt *et al*. 2016).
529 Even when *D. magna* were exposed to extracts from non-microcystin and
530 non-cylindrospermopsin medium, their CAT decreased after day 1 and 7 (Dao *et al*., 2013).
531 In combination with the observed lethality, our results suggest that *D. magna* oxidative
532 defence response was repressed in such way that oxidative damages may have occurred
533 resulting in lethal effects.

534 In *D. magna and D. longispina,* increased GST activity can detoxify microcystin up to a
535 certain concentration or exposure duration, which can also be transferred to the next
536 generation (Ortiz-Rodrigues *et al*., 2012; Wojtal-Frankiewicz *et al*. 2013, 2014). Despite the
537 upregulation of *gst* within the first 3 days, our exposures, however, decreased GST activity,
538 compared with the control on day 3 and day 7, where expression of *gst* gene was
539 downregulated as well. Our GST results concern one or at maximum two out of on average 7

Journal Pre-proof

541 have had a different response. Asselman *et al*. (2012) showed that sigma-class *gst* genes in *D.*
542 *pulex* were upregulated when fed on MC-producing *M. aeruginosa* after 16 days. However,
543 our results are in line with previous studies where short term exposure (48 h) to
544 microcystin-producing *M. aeruginosa* PCC7806 increased *delta-gst* transcript levels, while
545 long term exposure (15 days) downregulated them (Lyu et al., 2016a). Similar decrease in
546 GST activities were observed in *D. magna* exposed to cyanobacterial medium (containing 60
547 µg/L total MC) after 24 h and 72 h (Esterhuizen-Londt *et al*., 2016). Furthermore, GST
548 activity in *D. magna* decreased with increased MC-LR concentrations from 10 to 2000 µg/L
549 (Chen *et al*., 2005), suggesting enzymes exhaustibility as also seen in (Dao *et al*., 2013).

550 Our results suggest that even lower concentrations of MC in the cyanobacterial medium,
551 along with the other cyanobacterial metabolites can affect enzyme activity in a similar way
552 like cyanobacterial medium containing higher microcystin concentrations. Besides
553 cyanobacterial metabolites that were detected, cyanobacterial medium could contain
554 undetected compounds that could potentially interfere with overall ecotoxicity (Smutná *et*
555 *al*., 2014) or inhibit enzymes activity, such as microcin SF608 that was shown to have
556 inhibitory impact on GST (Wiegand *et al*., 2002).

557 When *Daphnia* are in toxic environments, mobilization of detoxification and antioxidant
558 defense mechanisms, as well as growth and development, comes with energetic cost (Calow,
559 1991; McKee and Knowles, 1986; Pane *et al*., 2004). For instance, *D. magna* exposed to
560 nickel (Pane *et al*., 2004) or the fungicide tebuconazole (McKee and Knowles, 1986) showed
561 increased energy consumption, as glycogen and lipids levels were decreased. In our
562 experiments energetic resouces were instantly used, visible by the induction of *gapdh*
563 (involved in glycolysis) at day 3. Upregulation of *gapdh* has been observed in *D. magna* in
564 response to diet containing microcystins after 4 days of the experiment, suggesting that
565 ingestion of microcystin induces glycolysis and protein catabolism (Schwarzenberger *et al*.,
566 2009). Our results further suggest that severe depletion of energy affected also the muscular
567 activity, as the myosin heavy chain was immediately and significantly downregulated in both
568 experimental exposures (HC and LC). Depletion of energy in combination with
569 downregulation of the myosin heavy chain could provide a mechanistic explanation of the

Journal Pre-proof

570 [partially obscured line]

571 *al*., 2001; Ghadouani *et al*., 2004).

572 The response of the histone 2A gene (*h2A*) (involved in normal cell cycle progression)
573 may indicate two different cellular pathways, depending on the exposure concentration. The
574 downregulation at LC hints on apoptotic processes, while the upregulation at HC exposure
575 may imply cellular dysfunction occurring during necrotic processes, indicating a stronger
576 negative effect of HC medium on *Daphnia,* that is in line with the higher mortality in this
577 exposure. Activation of apoptotic pathways are positively correlated with the decline in
578 *mhcrt* (Tong *et al*., 2017) that was also observed in our study, suggesting apoptotic processes
579 in *D. magna*, caused by cyanobacterial metabolites. Used concentrations of cyanobacterial
580 metabolites in our exposures may have been too high for *Daphnia* to adapt, therefore use of
581 lower concentration in future studies would provide deeper understading of *Daphnia*'s
582 molecular response to the cyanobacterial metabolites.
583

584 # 5. CONCLUSION
585

586 In conclusion, *D. magna* survival was strongly affected by cyanobacterial spent medium
587 at both LC and HC, due to the combined effect of MC and the other secondary metabolites
588 present. *D. magna* were not able to acclimate during the short exposure period. Medium with
589 the highest tested concentration of cyanobacterial metabolites was the most detrimental as
590 expected. Exposure to cyanobacterial compounds in the *M. aeruginosa* spent medium
591 affected *D. magna* genes involved in i) digestion, thus mobilization of the limited internal
592 energetic resources, ii) oxidative stress and detoxification, iii) muscular activity and iv) cell
593 regulation including the molting process. We also observed consequences of cyanobacterial
594 medium on detoxification and antioxidant capacities which were dose and in particular
595 time-dependent indicating an exhaustion of the enzymes with high concentration or long
596 exposure duration. Despite this, the concentrations were chosen based on available data of
597 pure MCs or MCs in crude extracts causing low lethality, for following sublethal effects an
598 even lower concentration is recommended.
599
600

Journal Pre-proof

601 # 6. BIBLIOGRAPHY

Journal Pre-proof

602

603 Amado, L.L., Monserrat, J.M., 2010. Oxidative stress generation by microcystins in aquatic
604 animals: Why and how. Environ. Int. doi:10.1016/j.envint.2009.10.010

605 Asselman, J., De Coninck, D.I.M., Glaholt, S., Colbourne, J.K., Janssen, C.R., Shaw, J.R.,
606 De Schamphelaere, K.A.C., 2012. Identification of pathways, gene networks, and
607 paralogous gene families in daphnia pulex responding to exposure to the toxic
608 cyanobacterium Microcystis aeruginosa. Environ. Sci. Technol. 46, 8448–8457.
609 doi:10.1021/es301100j

610 Asselman, J., Hochmuth, J.D., De Schamphelaere, K.A.C., 2014. A comparison of the
611 sensitivities of Daphnia magna and Daphnia pulex to six different cyanobacteria.
612 Harmful Algae 39, 1–7. doi:10.1016/j.hal.2014.06.008

613 Barrios, C.A.Z., Nandini, S., Sarma, S.S.S., 2015. Effect of crude extracts of
614 Dolichospermum planctonicum on the demography of Plationus patulus (Rotifera) and
615 Ceriodaphnia cornuta (Cladocera). Ecotoxicology 24, 85–93.
616 doi:10.1007/s10646-014-1358-8

617 Beversdorf, L.J., Rude, K., Weirich, C.A., Bartlett, S.L., Seaman, M., Kozik, C., Biese, P.,
618 Gosz, T., Suha, M., Stempa, C., Shaw, C., Hedman, C., Piatt, J.J., Miller, T.R., 2018.
619 Analysis of cyanobacterial metabolites in surface and raw drinking waters reveals more
620 than microcystin. Water Res. 140, 280–290. doi:10.1016/j.watres.2018.04.032

621 Beversdorf, L.J., Weirich, C.A., Bartlett, S.L., Miller, T.R., 2017. Variable cyanobacterial
622 toxin and metabolite profiles across six eutrophic lakes of differing physiochemical
623 characteristics. Toxins (Basel). 9. doi:10.3390/toxins9020062

624 Bister, B., Keller, S., Baumann, H.I., Nicholson, G., Weist, S., Jung, G., Süssmuth, R.D.,
625 Jüttner, F., 2004. Cyanopeptolin 963A, a chymotrypsin inhibitor of Microcystis PCC
626 7806. J. Nat. Prod. 67, 1755–1757. doi:10.1021/np049828f

627 Bojadzija Savic, G., Bormans, M., Edwards, C., Lawton, L., Briand, E., Wiegand, C., 2020.
628 Cross talk: Two way allelopathic interactions between toxic Microcystis and Daphnia.
629 Harmful Algae 94, 101803. doi:10.1016/j.hal.2020.101803

630 Bojadzija Savic, G., Edwards, C., Briand, E., Lawton, L., Wiegand, C., Bormans, M., 2019.
631 Daphnia magna Exudates Impact Physiological and Metabolic Changes in Microcystis
632 aeruginosa. Toxins (Basel). 11, 421. doi:10.3390/toxins11070421

633 Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram
634 quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72,
635 248–254. doi:10.1016/0003-2697(76)90527-3

636 Briand, E., Bormans, M., Gugger, M., Dorrestein, P.C., Gerwick, W.H., 2016. Changes in
637 secondary metabolic profiles of Microcystis aeruginosa strains in response to
638 intraspecific interactions. Environ. Microbiol. 18, 384–400.
639 doi:10.1111/1462-2920.12904

640 Briand, E., Bormans, M., Quiblier, C., Salençon, M.J., Humbert, J.F., 2012. Evidence of the

Journal Pre-proof

Journal Pre-proof

641 cost of the production of microcystins by Microcystis aeruginosa under differing light
642 and nitrate environmental conditions. PLoS One 7, e29981.
643 doi:10.1371/journal.pone.0029981

644 Calow, P., 1991. Physiological costs of combating chemical toxicants: ecological
645 implications. Comp. Biochem. Physiol. C. 100, 3–6.

646 Chang, C.J., Kao, C.H., 1997. Plant growth regulation., Plant growth regulation. Kluwer
647 Academic Publishers.

648 Chen, W., Song, L., Ou, D., Gan, N., 2005. Chronic toxicity and responses of several
649 important enzymes in Daphnia magna on exposure to sublethal microcystin-LR, in:
650 Environmental Toxicology. pp. 323–330. doi:10.1002/tox.20108

651 Colinet, H., Lee, S.F., Hoffmann, A., 2010. Temporal expression of heat shock genes during
652 cold stress and recovery from chill coma in adult Drosophila melanogaster. FEBS J.
653 277, 174–185. doi:10.1111/j.1742-4658.2009.07470.x

654 Dao, T.S., Do-Hong, L.C., Wiegand, C., 2010. Chronic effects of cyanobacterial toxins on
655 Daphnia magna and their offspring. Toxicon 55, 1244–1254.
656 doi:10.1016/j.toxicon.2010.01.014

657 Dao, T.S., Ortiz-Rodríguez, R., Do-Hong, L.C., Wiegand, C., 2013. Non-microcystin and
658 non-cylindrospermopsin producing cyanobacteria affect the biochemical responses and
659 behavior of Daphnia magna. Int. Rev. Hydrobiol. 98, 235–244.
660 doi:10.1002/iroh.201301626

661 De Coninck, D.I.M., Asselman, J., Glaholt, S., Janssen, C.R., Colbourne, J.K., Shaw, J.R.,
662 De Schamphelaere, K.A.C., 2014a. Genome-wide transcription profiles reveal
663 genotype-dependent responses of biological pathways and gene-families in daphnia
664 exposed to single and mixed stressors. Environ. Sci. Technol. 48, 3513–3522.
665 doi:10.1021/es4053363

666 De Coninck, D.I.M., Asselman, J., Glaholt, S., Janssen, C.R., Colbourne, J.K., Shaw, J.R.,
667 De Schamphelaere, K.A.C., Pfrender, M.E., Lopez, J.A., De Coninck, D.I.M., Janssen,
668 C.R., Shaw, J.R., De Schamphelaere, K.A.C., 2014b. Conserved transcriptional
669 responses to cyanobacterial stressors are mediated by alternate regulation of paralogous
670 genes in Daphnia. Mol. Ecol. 48, 1844–1855. doi:10.1021/es4053363

671 DeMott, W.R., 1999. Foraging strategies and growth inhibition in five daphnids feeding on
672 mixtures of a toxic cyanobacterium and a green alga. Freshw. Biol. 42, 263–274.
673 doi:10.1046/j.1365-2427.1999.444494.x

674 Demott, W.R., Zhang, Q.-X., Carmichael, W.W., 1991. Effects of toxic cyanobacteria and
675 purified toxins on the survival and feeding of a copepod and three species of Daphnia.
676 Limnol. Ocean. 36, 1346–357. doi:10.4319/lo.1991.36.7.1346

677 Drugă, B., Turko, P., Spaak, P., Pomati, F., 2016a. Cyanobacteria Affect Fitness and Genetic
678 Structure of Experimental Daphnia Populations. Environ. Sci. Technol. 50, 3416–3424.
679 doi:10.1021/acs.est.5b05973

680 Drugă, B., Turko, P., Spaak, P., Pomati, F., 2016b. Cyanobacteria Affect Fitness and Genetic
681 Structure of Experimental *Daphnia* Populations. Environ. Sci. Technol. 50, 3416–3424.

Journal Pre-proof

682 doi:10.1021/acs.est.5b05973
Journal Pre-proof
683 Edwards, C., Lawton, L.A., Coyle, S.M., Ross, P., 1996. Laboratory-scale purification of
684 microcystins using flash chromatography and reversed-phase high-performance liquid
685 chromatography, in: Journal of Chromatography A. Elsevier B.V., pp. 163–173.
686 doi:10.1016/0021-9673(95)01004-1

687 Elkobi-Peer, S., Singh, R.K., Mohapatra, T.M., Tiwari, S.P., Carmeli, S., 2013. Aeruginosins
688 from a microcystis sp. bloom material collected in Varanasi, India. J. Nat. Prod. 76,
689 1187–1190. doi:10.1021/np4001152

690 Esterhuizen-Londt, M., von Schnehen, M., Kühn, S., Pflugmacher, S., 2016a. Oxidative
691 stress responses in the animal model, Daphnia pulex exposed to a natural bloom extract
692 versus artificial cyanotoxin mixtures. Aquat. Toxicol. 179, 151–157.
693 doi:10.1016/j.aquatox.2016.09.003

694 Esterhuizen-Londt, M., von Schnehen, M., Kühn, S., Pflugmacher, S., Oxidative stress
695 responses in the animal model, Daphnia pulex exposed to a natural bloom extract versus
696 artificial cyanotoxin mixturesEsterhuizen-Londt, M., von Schnehen, M., Kühn, S.,
697 Pflugmacher, S., 2016b. Oxidative stress responses in the animal model, Daphnia pulex
698 exposed to a natural bloom extract versus artificial cyanotoxin mixtures. Aquat.
699 Toxicol. 179, 151–157. doi:10.1016/j.aquatox.2016.09.003

700 Ferrão-Filho, A.S., Herrera, N.A., Echeverri, L.F., 2014. Microcystin accumulation in
701 cladocerans: First evidence of MC uptake from aqueous extracts of a natural bloom
702 sample. Toxicon 87, 26–31. doi:10.1016/j.toxicon.2014.05.015

703 Fridovich, I., 1998. Oxygen toxicity: a radical explanation. J. Exp. Biol. 201.

704 Gademann, K., Portmann, C., 2008. Secondary Metabolites from Cyanobacteria: Complex
705 Structures and Powerful Bioactivities. Curr. Org. Chem. 12, 326–341.
706 doi:10.2174/138527208783743750

707 Gatte-Picchi, D., Weiz, A., Ishida, K., Hertweck, C., Dittmann, E., 2014. Functional analysis
708 of environmental DNA-derived microviridins provides new insights into the diversity of
709 the tricyclic peptide family. Appl. Environ. Microbiol. 80, 1380–1387.
710 doi:10.1128/AEM.03502-13

711 Ger, K.A., Urrutia-Cordero, P., Frost, P.C., Hansson, L.A., Sarnelle, O., Wilson, A.E.,
712 Lürling, M., 2016. The interaction between cyanobacteria and zooplankton in a more
713 eutrophic world. Harmful Algae 54, 128–144. doi:10.1016/j.hal.2015.12.005

714 Ghadouani, A., Pinel-Alloul, B., Plath, K., Codd, G. a., Lampert, W., 2004. Effects of
715 Microcystis aeruginosa and purified microcystin-LR on the feeding behavior of
716 Daphnia pulicaria. Limnol. Oceanogr. 49, 666–679. doi:10.4319/lo.2004.49.3.0666

717 Giraudo, M., Douville, M., Cottin, G., Houde, M., 2017. Transcriptomic, cellular and
718 life-history responses of Daphnia magna chronically exposed to benzotriazoles:
719 Endocrinedisrupting potential and molting effects. PLoS One 12, e0171763.
720 doi:10.1371/journal.pone.0171763

721 Gustafsson, S., Hansson, L.-A., 2004. Development of tolerance against toxic cyanobacteria
722 in Daphnia. Aquat. Ecol. 38, 37–44. doi:10.1023/B:AECO.0000020985.47348.5e

Journal Pre-proof

723 Hairston, N.G., Lampert, W., Cáceres, C.E., Holtmeier, C.L., Weider, L.J., Gaedke, U.,
724 Fischer, J.M., Fox, J. a., Post, D.M., 1999. Lake ecosystems: Rapid evolution revealed
725 by dormant eggs. Nature 401, 446. doi:10.1038/46731

726 Hayes, J.D., Flanagan, J.U., Jowsey, I.R., 2005. Glutathione transferases. Annu. Rev.
727 Pharmacol. Toxicol. 45, 51–88. doi:10.1146/annurev.pharmtox.45.120403.095857

728 Heisler, J., Glibert, P.M., Burkholder, J.M., Anderson, D.M., Cochlan, W., Dennison, W.C.,
729 Dortch, Q., Gobler, C.J., Heil, C.A., Humphries, E., Lewitus, A., Magnien, R., Marshall,
730 H.G., Sellner, K., Stockwell, D.A., Stoecker, D.K., Suddleson, M., 2008. Eutrophication
731 and harmful algal blooms: A scientific consensus. Harmful Algae 8, 3–13.
732 doi:10.1016/j.hal.2008.08.006

733 Herrera, N.A., Echeverri, L.F., Ferrão-Filho, A.S., 2015. Effects of phytoplankton extracts
734 containing the toxin microcystin-LR on the survival and reproduction of cladocerans.
735 Toxicon 95, 38–45. doi:10.1016/j.toxicon.2014.12.016

736 Houde, M., Carter, B., Douville, M., 2013. Sublethal effects of the flame retardant
737 intermediate hexachlorocyclopentadiene (HCCPD) on the gene transcription and
738 protein activity of Daphnia magna. Aquat. Toxicol. 140–141, 213–219.
739 doi:10.1016/j.aquatox.2013.06.008

740 Ighodaro, O.M., Akinloye, O.A., 2018. First line defence antioxidants-superoxide dismutase
741 (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the
742 entire antioxidant defence grid. Alexandria J. Med. 54, 287–293.
743 doi:10.1016/j.ajme.2017.09.001

744 Ishida, K., Christiansen, G., Yoshida, W.Y., Kurmayer, R., Welker, M., Valls, N., Bonjoch,
745 J., Hertweck, C., Börner, T., Hemscheidt, T., Dittmann, E., 2007. Biosynthesis and
746 Structure of Aeruginoside 126A and 126B, Cyanobacterial Peptide Glycosides Bearing
747 a 2-Carboxy-6-Hydroxyoctahydroindole Moiety. Chem. Biol. 14, 565–576.
748 doi:10.1016/j.chembiol.2007.04.006

749 Ishida, K., Nakagawa, H., Murakami, M., 2000. Microcyclamide, a cytotoxic cyclic
750 hexapeptide from the cyanobacterium Microcystis aeruginosa. J. Nat. Prod. 63, 1315–
751 1317. doi:10.1021/np000159p

752 Janssen, E.M.-L., 2019. Cyanobacterial peptides beyond microcystins – A review on
753 co-occurrence, toxicity, and challenges for risk assessment. Water Res. 151, 488–499.
754 doi:10.1016/J.WATRES.2018.12.048

755 Kaebernick, M., Rohrlack, T., Christoffersen, K., Neilan, B.A., 2001. A spontaneous mutant
756 of microcystin biosynthesis: Genetic characterization and effect on Daphnia. Environ.
757 Microbiol. 3, 669–679. doi:10.1046/j.1462-2920.2001.00241.x

758 Lahti, K., Rapala, J., Färdig, M., Niemelä, M., Sivonen, K., 1997. Persistence of
759 cyanobacterial hepatotoxin, microcystin-LR in particulate material and dissolved in lake
760 water. Water Res. 31, 1005–1012. doi:10.1016/S0043-1354(96)00353-3

761 Lanaras, T., Cook, C.M., 1994. Toxin extraction from an Anabaenopsis milleri — dominated
762 bloom. Sci. Total Environ. 142, 163–169. doi:10.1016/0048-9697(94)90324-7

763 Liu, S., Ding, R., Nie, X., 2019. Assessment of oxidative stress of paracetamol to Daphnia

Journal Pre-proof

764 magna via determination of Nrf1 and genes related to antioxidant system. Aquat.
765 Toxicol. 211, 73–80. doi:10.1016/j.aquatox.2019.03.014
Journal Pre-proof
766 Lürling, M., van der Grinten, E., 2003. Life-history characteristics of *Daphnia* exposed to
767 dissolved microcystin-LR and to the cyanobacterium *Microcystis aeruginosa* with and
768 without microcystins. Environ. Toxicol. Chem. 22, 1281–1287.
769 doi:10.1002/etc.5620220614

770 Lyu, K., Gu, L., Li, B., Lu, Y., Wu, C., Guan, H., Yang, Z., 2016a. Stress-responsive
771 expression of a glutathione S-transferase (delta) gene in waterflea Daphnia magna
772 challenged by microcystin-producing and microcystin-free Microcystis aeruginosa.
773 Harmful Algae 56, 1–8. doi:10.1016/j.hal.2016.04.009

774 Lyu, K., Guan, H., Wu, C., Wang, X., Wilson, A.E., Yang, Z., 2016b. Maternal consumption
775 of non-toxic Microcystis by Daphnia magna induces tolerance to toxic Microcystis in
776 offspring. Freshw. Biol. 61, 219–228. doi:10.1111/fwb.12695

777 Lyu, K., Wang, Q., Li, Z., Chen, R., Zhu, C., Liu, J., Yang, Z., 2014. Age-dependent survival
778 and selected Gene expression in Daphnia magna after short-term exposure to low
779 dissolved oxygen. J. Plankton Res. 37, 66–74. doi:10.1093/plankt/fbu097

780 MacKintosh, C., Beattie, K.A., Klumpp, S., Cohen, P., Codd, G.A., 1990. Cyanobacterial
781 microcystin-LR is a potent and specific inhibitor of protein phosphatases 1 and 2A from
782 both mammals and higher plants. FEBS Lett. 264, 187–92.

783 Martin-Creuzburg, D., von Elert, E., Hoffmann, K.H., 2008. Nutritional constraints at the
784 cyanobacteria Daphnia magna interface: The role of sterols. Limnol. Oceanogr. 53,
785 456–468. doi:10.4319/lo.2008.53.2.0456

786 McKee, M.J., Knowles, C.O., 1986. Effects of fenvalerate on biochemical parameters,
787 survival, and reproduction of Daphnia magna. Ecotoxicol. Environ. Saf. 12, 70–84.
788 doi:10.1016/0147-6513(86)90007-2

789 Merwe, D. Van der, Sebbag, L., 2012. Investigation of a Microcystis aeruginosa
790 cyanobacterial freshwater harmful algal bloom associated with acute microcystin
791 toxicosis in a dog. J. Vet. ….

792 Miles, C.O., Sandvik, M., Nonga, H.E., Ballot, A., Wilkins, A.L., Rise, F., Jaabaek, J.A.H.,
793 Loader, J.I., 2016. Conjugation of Microcystins with Thiols Is Reversible:
794 Base-Catalyzed Deconjugation for Chemical Analysis. Chem. Res. Toxicol. 29, 860–
795 870. doi:10.1021/acs.chemrestox.6b00028

796 Mowe, M.A.D., Mitrovic, S.M., Lim, R.P., Furey, A., Yeo, D.C.J., 2015. Tropical
797 cyanobacterial blooms: A review of prevalence, problem taxa, toxins and influencing
798 environmental factors. J. Limnol. doi:10.4081/jlimnol.2014.1005

799 Natumi, R., Janssen, E.M.L., 2020. Cyanopeptide Co-Production Dynamics beyond
800 Mirocystins and Effects of Growth Stages and Nutrient Availability. Environ. Sci.
801 Technol. 54, 6063–6072. doi:10.1021/acs.est.9b07334

802 Neumann, U., Campos, V., Cantarero, S., Urrutia, H., Heinze, R., Weckesser, J., Erhard, M.,
803 2000. Co-occurrence of non-toxic (cyanopeptolin) and toxic (microcystin) peptides in a
804 bloom of Microcystis sp. from a Chilean lake. Syst. Appl. Microbiol. 23, 191–197.
Journal Pre-proof

805 doi:10.1016/S0723-2020(00)80004-1
Journal Pre-proof
806 Ortiz-Rodríguez, R., Dao, T.S., Wiegand, C., 2012. Transgenerational effects of
807 microcystin-LR on Daphnia magna. J. Exp. Biol. 215, 2795–2805.
808 doi:10.1242/jeb.069211

809 Ortiz-Rodríguez, R., Wiegand, C., 2010. Age related acute effects of microcystin-LR on
810 Daphnia magna biotransformation and oxidative stress. Toxicon 56, 1342–1349.
811 doi:10.1016/j.toxicon.2010.07.020

812 Pane, E.F., McGeer, J.C., Wood, C.M., 2004. Effects of chronic waterborne nickel exposure
813 on two successive generations of Daphnia magna. Environ. Toxicol. Chem. 23, 1051–6.

814 Pawlik-Skowrońska, B., Pirszel, J., Kornijów, R., 2008. Spatial and temporal variation in
815 microcystin concentrations during perennial bloom of Planktothrix agardhii in a
816 hypertrophic lake. Ann. Limnol. 44, 145–150. doi:10.1051/limn:2008015

817 Peng, S., Deng, D., He, P., Xu, X., Zhang, C., Cao, J., Liu, Q., Zhang, T., 2018. Effects of
818 Microcystis aeruginosa on the life history traits and SOD activity of Daphnia similoides
819 sinensis. Environ. Sci. Pollut. Res. 25, 30696–30707. doi:10.1007/s11356-018-3040-9

820 Pfaffl, M.W., 2001. A new mathematical model for relative quantification in real-time
821 RT-PCR, Nucleic Acids Research.

822 Pflugmacher, S., Wiegand, C., Oberemm, A., Beattie, K.A., Krause, E., Codd, G.A.,
823 Steinberg, C.E.W.W., 1998. Identification of an enzymatically formed glutathione
824 conjugate of the cyanobacterial hepatotoxin microcystin-LR: The first step of
825 detoxication. Biochim. Biophys. Acta - Gen. Subj. 1425, 527–533.
826 doi:10.1016/S0304-4165(98)00107-X

827 Pham, T.L., 2018. First report of microcystin-producing Microcystis (Chroococales,
828 Cyanobacteria) in a central highland Vietnam lake. Fundam. Appl. Limnol. 191, 189–
829 197. doi:10.1127/fal/2018/1124

830 Portmann, C., Blom, J.F., Gademann, K., Jüttner, F., Jüttner, F., Jüttner, F., 2008.
831 Aerucyclamides A and B: Isolation and synthesis of toxic ribosomal heterocyclic
832 peptides from the cyanobacterium Microcystis aeruginosa PCC 7806. J. Nat. Prod. 71,
833 1193–1196. doi:10.1021/np800118g

834 Rastogi, R.P., Madamwar, D., Incharoensakdi, A., 2015. Bloom dynamics of cyanobacteria
835 and their toxins: Environmental health impacts and mitigation strategies. Front.
836 Microbiol. doi:10.3389/fmicb.2015.01254

837 Rhiannon, D.M., Dakic, V., Williams, T.D., Winter, M.J., Chipman, J.K., 2011.
838 Transcriptional responses in neonate and adult Daphnia magna in relation to relative
839 susceptibility to genotoxicants. Aquat. Toxicol. 104, 192–204.
840 doi:10.1016/j.aquatox.2011.04.016

841 Rohrlack, T., Christoffersen, K., Kaebernick, M., Neilan, B.A., 2004. Cyanobacterial
842 protease inhibitor microviridin J causes a lethal molting disruption in Daphnia pulicaria.
843 Appl. Environ. Microbiol. 70, 5047–5050. doi:10.1128/AEM.70.8.5047-5050.2004

844 Rohrlack, T., Dittmann, E., Börner, T., Christoffersen, K., 2001. Effects of Cell-Bound
845 Microcystins on Survival and Feeding of Daphnia spp. Appl. Environ. Microbiol. 67,
Journal Pre-proof

846 3523–3529. doi:10.1128/AEM.67.8.3523-3529.2001
Journal Pre-proof
847 Sadler, T., von Elert, E., 2014a. Physiological interaction of Daphnia and Microcystis with
848 regard to cyanobacterial secondary metabolites. Aquat. Toxicol. 156, 96–105.
849 doi:10.1016/j.aquatox.2014.08.003

850 Sadler, T., von Elert, E., 2014b. Dietary exposure of Daphnia to microcystins: No in vivo
851 relevance of biotransformation. Aquat. Toxicol. 150, 73–82.
852 doi:10.1016/j.aquatox.2014.02.017

853 Sarnelle, O., Wilson, A.E., 2005. Local adaptation of Daphnia pulicaria to toxic
854 cyanobacteria. Limnol. Oceanogr. 50, 1565–1570. doi:10.4319/lo.2005.50.5.1565

855 Schwarzenberger, A., Courts, C., von Elert, E., 2009. Target gene approaches: Gene
856 expression in Daphnia magna exposed to predator-borne kairomones or to
857 microcystin-producing and microcystin-free Microcystis aeruginosa. BMC Genomics
858 10, 527. doi:10.1186/1471-2164-10-527

859 Schwarzenberger, A., Kuster, C.J., Von Elert, E., 2012. Molecular mechanisms of tolerance
860 to cyanobacterial protease inhibitors revealed by clonal differences in Daphnia magna.
861 Mol. Ecol. 21, 4898–4911. doi:10.1111/j.1365-294X.2012.05753.x

862 Schwarzenberger, A., Zitt, A., Kroth, P., Mueller, S., Von Elert, E., 2010. Gene expression
863 and activity of digestive proteases in Daphnia: Effects of cyanobacterial protease
864 inhibitors. BMC Physiol. 10, 6. doi:10.1186/1472-6793-10-6

865 Smutná, M., Babica, P., Jarque, S., Hilscherová, K., Maršálek, B., Haeba, M., Bláha, L.,
866 2014. Acute, chronic and reproductive toxicity of complex cyanobacterial blooms in
867 Daphnia magna and the role of microcystins. Toxicon 79.
868 doi:10.1016/j.toxicon.2013.12.009

869 Street, S.M., Eytcheson, S.A., LeBlanc, G.A., 2019. The role of nuclear receptor E75 in
870 regulating the molt cycle of Daphnia magna and consequences of its disruption. PLoS
871 One 14, e0221642. doi:10.1371/journal.pone.0221642

872 Su, X., Xue, Q., Steinman, A.D., Zhao, Y., Xie, L., 2015. Spatiotemporal dynamics of
873 microcystin variants and relationships with environmental parameters in lake Taihu,
874 China. Toxins (Basel). 7, 3224–3244. doi:10.3390/toxins7083224

875 Svirčev, Z., Lalić, D., Bojadžija Savić, G., Tokodi, N., Drobac Backović, D., Chen, L.,
876 Meriluoto, J., Codd, G.A., 2019. Global geographical and historical overview of
877 cyanotoxin distribution and cyanobacterial poisonings. Arch. Toxicol. 1–53.
878 doi:10.1007/s00204-019-02524-4

879 Tong, Q., Zhang, M., Cao, X., Xu, S., Wang, D., Zhao, Y., 2017. Expression and activation
880 of Daphnia pulex Caspase-3 are involved in regulation of aging. Gene 634, 37–46.
881 doi:10.1016/j.gene.2017.08.035

882 Trinkle-Mulcahy, L., Lamond, A.I., 2006. Mitotic phosphatases: no longer silent partners.
883 Curr. Opin. Cell Biol. doi:10.1016/j.ceb.2006.09.001

884 Von Elert, E., Agrawal, M.K., Gebauer, C., Jaensch, H., Bauer, U., Zitt, A., 2004. Protease
885 activity in gut of Daphnia magna: Evidence for trypsin and chymotrypsin enzymes.
886 Comp. Biochem. Physiol. - B Biochem. Mol. Biol. 137, 287–296.
Journal Pre-proof

887 doi:10.1016/j.cbpc.2003.11.008

888 Von Elert, E., Oberer, L., Merkel, P., Huhn, T., Blom, J.F., 2005. Cyanopeptolin 954, a
889 chlorine-containing chymotrypsin inhibitor of Microcystis aeruginosa NIVA Cya 43. J.
890 Nat. Prod. 68, 1324–1327. doi:10.1021/np050079r

891 von Elert, E., Zitt, A., Schwarzenberger, A., 2012. Inducible tolerance to dietary protease
892 inhibitors in Daphnia magna. J. Exp. Biol. 215, 2051–2059. doi:10.1242/jeb.068742

893 von Elert, Eric, Zitt, A., Schwarzenberger, A., 2012. Inducible tolerance to dietary protease
894 inhibitors in *Daphnia magna*. J. Exp. Biol. 215, 2051–2059. doi:10.1242/jeb.068742

895 Wang, L., Peng, Y., Nie, X., Pan, B., Ku, P., Bao, S., 2016. Gene response of CYP360A,
896 CYP314, and GST and whole-organism changes in Daphnia magna exposed to
897 ibuprofen. Comp. Biochem. Physiol. Part - C Toxicol. Pharmacol. 179, 49–56.
898 doi:10.1016/j.cbpc.2015.08.010

899 Welker, M., Von Döhren, H., 2006. Cyanobacterial peptides - Nature's own combinatorial
900 biosynthesis. FEMS Microbiol. Rev. doi:10.1111/j.1574-6976.2006.00022.x

901 Wiegand, C., Peuthert, A., Pflugmacher, S., Carmeli, S., 2002. Effects of microcin SF608
902 and microcystin-LR two cyanotobacterial compounds produced by Microcystis sp., on
903 aquatic organisms. Environ. Toxicol. 17, 400–406. doi:10.1002/tox.10065

904 Wiegand, C., Pflugmacher, S., Oberemm, A., Steinberg, C., 2000. Activity development of
905 selected detoxication enzymes during the ontogenesis of the zebrafish (Danio rerio). Int.
906 Rev. Hydrobiol. 85, 413–422.
907 doi:10.1002/1522-2632(200008)85:4<413::AID-IROH413>3.0.CO;2-3

908 Wojtal-Frankiewicz, A., Bernasi??ska, J., Jurczak, T., Gwo??dzi??ski, K., Frankiewicz, P.,
909 Wielanek, M., 2013. Microcystin assimilation and detoxification by Daphnia spp. in
910 two ecosystems of different cyanotoxin concentrations. J. Limnol. 72, 154–171.
911 doi:10.4081/jlimnol.2013.e13

912 Wojtal-Frankiewicz, A., Bernasińska, J., Frankiewicz, P., Gwoździński, K., Jurczak, T.,
913 2014. Response of daphnia' s antioxidant system to spatial heterogeneity in
914 cyanobacteria concentrations in a lowland reservoir. PLoS One.
915 doi:10.1371/journal.pone.0112597

916 Ziemert, N., Ishida, K., Weiz, A., Hertweck, C., Dittmann, E., 2010. Exploiting the natural
917 diversity of microviridin gene clusters for discovery of novel tricyclic depsipeptides.
918 Appl. Environ. Microbiol. 76, 3568–3574. doi:10.1128/AEM.02858-09

919 Zurawell, R.W., Chen, H., Burke, J.M., Prepas, E.E., 2005. Hepatotoxic cyanobacteria: a
920 review of the biological importance of microcystins in freshwater environments. J.
921 Toxicol. Environ. Health. B. Crit. Rev. 8, 1–37. doi:10.1080/10937400590889412

922

Journal Pre-proof

Journal Pre-proof

- Cell free *M. aeruginosa* spent medium affected *Daphnia* survival and physiology
- Medium with higher concentration of cyanobacterial metabolites was more detrimental
- Other secondary metabolites, besides microcystin-LR highly likely had negative impact on *Daphnia*

Journal Pre-proof

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Journal Pre-proof