Chemosphere 287 (2022) 132028

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Fish tissue accumulation and proteomic response to microcystins is species-dependent

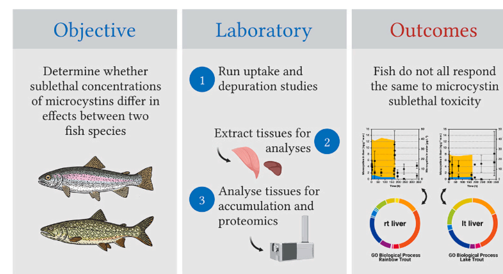
René S. Shahmohamadloo[a,b,*], Xavier Ortiz Almirall[c,d], Denina B.D. Simmons[e], David G. Poirier[c], Satyendra P. Bhavsar[c,f], Paul K. Sibley[a]

[a] School of Environmental Sciences, University of Guelph, Guelph, Ontario, Canada
[b] Department of Integrative Biology, University of Guelph, Guelph, Ontario, Canada
[c] Ministry of the Environment, Conservation and Parks, Toronto, Ontario, Canada
[d] School of Environmental Studies, Queen's University, Kingston, Ontario, Canada
[e] Faculty of Science, Ontario Tech University, Oshawa, Ontario, Canada
[f] Department of Physical & Environmental Sciences, University of Toronto, Toronto, Ontario, Canada

HIGHLIGHTS

- Microcystin uptake and depuration kinetics vary between fish species.
- Fish can retain microcystins in tissues after depuration phase in clean water.
- Livers accumulate microcystins and undergo sublethal molecular level toxicity.
- Proteomics reveal stress responses in fish after depuration phase in clean water.
- Fish may not all respond the same to cyanobacterial toxicity within and among species.

GRAPHICAL ABSTRACT



ARTICLE INFO

Handling Editor: James Lazorchak

Keywords:
Fish
Toxicology
Algal bloom
Microcystin
Cyanobacteria
Proteomics

ABSTRACT

Cyanotoxins including microcystins are increasing globally, escalating health risks to humans and wildlife. Freshwater fish can accumulate and retain microcystins in tissues; however, uptake and depuration studies thus far have not exposed fish to microcystins in its intracellular state (i.e., cell-bound or conserved within cyanobacteria), which is a primary route of exposure in the field, nor have they investigated sublethal molecular-level effects in tissues, limiting our knowledge of proteins responsible for microcystin toxicity pathways in pre-to-postsenescent stages of a harmful algal bloom. We address these gaps with a 2-wk study (1 wk of 'uptake' exposure to intracellular microcystins (0–40 μg L^{-1}) produced by *Microcystis aeruginosa* followed by 1 wk of 'depuration' in clean water) using Rainbow Trout (*Oncorhynchus mykiss*) and Lake Trout (*Salvelinus namaycush*). Liver and muscle samples were collected throughout uptake and depuration phases for targeted microcystin quantification and nontargeted proteomics. For both species, microcystins accumulated at a higher concentration in the liver than muscle, and activated cellular responses related to oxidative stress, apoptosis, DNA repair, and carcinogenicity. However, intraspecific proteomic effects between Rainbow Trout and Lake Trout differed, and interspecific accumulation and retention of microcystins in tissues within each species also differed. We demonstrate that fish do not respond the same to cyanobacterial toxicity within and among species despite being reared in the same environment and diet.

* Corresponding author. Department of Integrative Biology, University of Guelph, 50 Stone Road East, Guelph, Ontario, N1G 2W1, Canada.
E-mail address: rshahmoh@uoguelph.ca (R.S. Shahmohamadloo).

https://doi.org/10.1016/j.chemosphere.2021.132028
Received 18 April 2021; Received in revised form 20 August 2021; Accepted 24 August 2021
Available online 27 August 2021
0045-6535/© 2021 Elsevier Ltd. All rights reserved.

1. Introduction

Expansive blooms of the toxic cyanobacterium *Microcystis* are increasing globally (Harke et al., 2016; Wilhelm et al., 2020), threatening freshwater biodiversity (Reid et al., 2019) and ecosystem services (Smith et al., 2019; Guo et al., 2021), as well as increasing risks to the health of humans (Carmichael et al., 2001) and wildlife (Dyble et al., 2011; Poste et al., 2011; Gene et al., 2019; Shahmohamadloo et al., 2020a, 2020b). Microcystins are a family of over 250 congeners (Bouaïcha et al., 2019) that are produced primarily by *Microcystis* but also *Planktothrix*, *Dolichospermum*, *Oscillatoria*, and *Nostoc* (Chorus and Welker, 2021). In most cases, microcystin toxicity in humans and wildlife occurs in the liver through protein phosphatase (PP1, PP2A) inhibition, which causes a breakdown of the cytoskeleton in hepatocytes followed by cellular apoptosis and necrosis (Buratti et al., 2017; WHO, 2020). At a sufficient dose and period of exposure, microcystin hepatotoxicity can lead to tumor formation, haemorrhage, and organ failure (Pearson et al., 2010). Microcystin-LR and its desmethylated form, [D-Asp^3]-microcystin-LR, are cosmopolitan (Harke et al., 2016; Chorus and Welker, 2021) and identified as some of the most toxic congeners of this family (Shimizu et al., 2014). Based on growing evidence of their acute and chronic toxicity to organisms, the World Health Organization (WHO) recently updated provisional guideline values for microcystin-LR in drinking water (12 μg L^{-1} short-term, 1 μg L^{-1} lifetime), water for recreational use (24 μg L^{-1}), and tolerable daily intake (0.04 μg kg^{-1} bodyweight per day) (WHO, 2020).

Freshwater fish can accumulate microcystins, sometimes at lethal concentrations (Phillips et al., 1985; Tencalla et al., 1994; Kotak et al., 1996) but, in most cases, at sublethal levels where adverse effects in tissues can be observed (Tencalla and Dietrich, 1997; Adamovský et al., 2007; Bieczynski et al., 2013; Jiang et al., 2014; Chen et al., 2017; Sotton et al., 2017; Le Manach et al., 2018; Mohamed et al., 2020; Mehinto et al., 2021; Shahmohamadloo et al., 2021). Uptake studies ranging from laboratory to field exposures using diverse fish species have demonstrated that sublethal concentrations of microcystins can rapidly accumulate in the liver and alter PP1, catalase, and glutathione-S-transferase activity in Patagonian Pejerry (*Odontesthes hatcheri*) (Bieczynski et al., 2013); cause an excessive production of reactive oxygen species that further inhibit PP1 and PP2A in Common Carp (*Cyprinus carpio* L.) (Jiang et al., 2014); and, induce sex-dependent molecular changes in Medaka fish (*Oryzias latipes*) (Sotton et al., 2017) as well as biological disruptions in the liver of females and males (Le Manach et al., 2018). However, it is increasingly recognized that uptake and depuration studies (i.e., period of exposure to a toxin followed by a period of exposure in clean water) are more useful in understanding microcystin toxicokinetics in fish because they can help to explain the molecular initiating events and key events involved in the early stages of adverse outcome pathways in pre-to-postsenescent stages of a harmful algal bloom event. To our knowledge, uptake and depuration studies to date (Soares et al., 2004; Smith and Haney, 2006; Adamovský et al., 2007; Deblois et al., 2011; Dyble et al., 2011) have only exposed fish to microcystins in its extracellular state (i.e., dissolved or released from cyanobacteria) for the purpose of determining whether fish can effectively depurate these toxins from tissues, with little or no investigation into sublethal molecular-level effects. While these studies are consistent in their conclusion that fish across feeding guilds can rapidly uptake microcystins, results from the depuration phase are conflicting with some finding rapid elimination (Adamovský et al., 2007; Dyble et al., 2011) and others finding that fish can retain these toxins for extended periods of time (Soares et al., 2004; Smith and Haney, 2006; Schmidt et al., 2014). An implicit observation from these studies are the unexplored roles of inter- and intraspecific variation in response to cyanobacterial toxicity, and a call for further research was made to focus on these roles which can impact microcystin toxicokinetics in fish tissues (Adamovský et al., 2007). What's more, field observations show that no or only small concentrations of extracellular microcystins are found in freshwater ecosystems even after lysis of algal cells during the senescent stages of harmful algal blooms (Welker et al., 2001; Chorus and Welker, 2021). Intracellular microcystin concentrations (i.e., cell-bound or conserved within cyanobacteria) is therefore deemed an environmentally relevant route of aqueous exposure to fish that may feed on or passively uptake toxic cyanobacterial cells, and positively correlated with fish tissue microcystin concentrations detected by enzyme linked immunosorbent assay (ELISA), as summarized by Flores et al. (2018).

The use of ELISA, utilized in all the aforementioned studies, presents drawbacks in complex matrices such as fish tissues because it was designed for rapid water analysis (Geis-Asteggiante et al., 2011; Schmidt et al., 2014; Flores et al., 2018). If used for fish tissue analysis, ELISA is prone to false positive detections and underestimates the total amount of microcystins (i.e., free and bound fractions) because it only measures the free fraction (Geis-Asteggiante et al., 2011; Schmidt et al., 2014; Flores et al., 2018; Birbeck et al., 2019). An 2-methyl-3-methoxy-4-phenylbutyric acid (MMPB) method optimized for fish tissue analysis can resolve discrepancies in uptake and depuration studies since it targets both free and bound fractions of microcystins (Flores et al., 2018; Anaraki et al., 2020). This, in combination with aqueous exposure to intracellular microcystins, for the typical duration of a cyanobacterial bloom, while purposefully investigating sublethal molecular-level effects in tissues, can reveal a realistic response in fish.

We sought to answer three questions regarding the uptake and depuration kinetics of microcystins in fish. First, will microcystins intracellular to *Microcystis* accumulate and retain in fish tissues? Second, can we identify proteins responsible for microcystin toxicity pathways at sublethal levels? And third, what is the level of proteomic divergence between two fish species exposed under the same environmental conditions?

To address these questions, we: (1) measured and compared the temporal dynamics of microcystins in fish tissues using a targeted approach; and (2) measured proteome responses in fish to reveal microcystin toxicity pathways linked to toxin accumulation in tissues using a nontargeted approach. We designed this experiment to reflect the present mean number of days of a cyanobacterial harmful algal bloom in the field (Chapra et al., 2017) with 1 wk of exposure to toxic *Microcystis,* which was then followed by 1 wk in clean water. We selected Rainbow Trout (*Oncorhynchus mykiss*) and Lake Trout (*Salvelinus namaycush*) as our test organisms because they are ecologically and economically valuable to commercial fisheries and sports fishing in temperate (North American) lakes, and serve an important role as indicator species vulnerable to toxicity in freshwater ecosystems (Grabarkiewicz and Davis, 2008; Lynch et al., 2016; Smith et al., 2019). We hypothesized that intracellular microcystins will accumulate and retain in fish tissues, and will cause protein dysregulation at sublethal concentrations. To the best of our knowledge, this is the first uptake and depuration study exposing fish to intracellular microcystins, employing the MMPB method for tissue analysis, and investigating sublethal molecular-level effects on a temporal scale.

2. Material and methods

2.1. Culture and test solution preparation

Microcystis aeruginosa strain CPCC 300 was acquired from the Canadian Phycological Culture Centre (University of Waterloo, Waterloo, Canada). This strain was previously extracted from a culture in Pretzlaff Pond (Alberta, Canada) on August 7, 1990, and produces microcystin-LR (CAS: 101,043-37-2, $C_{49}H_{74}N_{10}O_{12}$) and [D-Asp^3]-microcystin-LR (CAS: 120,011-66-7, $C_{48}H_{72}N_{10}O_{12}$). Further details regarding our method for culturing *M. aeruginosa* CPCC 300 (Shahmohamadloo et al., 2019) and preparing the test solutions are provided in the Supporting Information (SI 1).

2.2. Test species

Rainbow Trout and Lake Trout were reared to juvenile life stages for each uptake and depuration study. Rainbow Trout (100 g) were cultured at the Aquatic Toxicology Unit (ATU) of the Ontario Ministry of the Environment, Conservation, and Parks (MECP; Etobicoke, ON, Canada). Lake Trout (70 g) were cultured at the Harwood Fish Culture Station of the Ontario Ministry of Northern Development, Mines, Natural Resources and Forestry (Harwood, ON, Canada) and were transported to the ATU. In this facility, fish were kept at 15 °C under a photoperiod of 16:8 h (light:dark) for two weeks to allow acclimatization prior to running the uptake and depuration experiments. There were no mortalities during the period of acclimatization. During this time, fish were fed daily with standard commercial pelleted food at a feeding rate of 1–5% of wet body weight. Fish were not fed 24 h prior to running the uptake and depuration experiments.

2.3. Microcystin experiments

Microcystin uptake and depuration experiments, 14 d in duration each, were conducted for Rainbow Trout and Lake Trout, separately, in the ATU following the MECP Laboratory Services Branch Animal Care Committee Animal Utilization Proposal (Laboratory Lab License #0053). A complete schematic of the experimental design is provided in Fig. 1. The experiments were designed so that on days 1–7 fish were exposed to a steady cell concentration of *M. aeruginosa* through aqueous exposure and static renewal (i.e., the test solution was renewed 3 × wk during each 14-d study), referred to as the “uptake phase”. On days 8–14 fish were exposed to clean water with no *M. aeruginosa* through aqueous exposure and static renewal, referred to as the “depuration phase”. Each experiment included one control (i.e., water-only), a low microcystins treatment (i.e., 1–5 μg total microcystins L^{-1}), and a high microcystins treatment (i.e., 20–40 μg total microcystins L^{-1}). These concentrations are based on the WHO’s guideline values for drinking and recreational waters (WHO, 2020) as well as concentrations typically measured in the environment (Cheng et al., 2017). The cell concentrations of *M. aeruginosa* were set at 2×10^5 cells mL^{-1} for the low treatment and 2×10^6 cells mL^{-1} for the high treatment, which from our previous work could guarantee the microcystin concentrations desired for this experiment. Notwithstanding, due to the nature of approximating microcystin concentrations from a live organism such as *M. aeruginosa*, some variability in final concentrations occurred between experimental units in each treatment. Each treatment had 10 experimental units (i.e., pails were 20 L to satisfy a loading density of <2.5 g fish L^{-1}). Each experimental unit had three fish (30 fish per treatment, 90 fish per test). Fish were randomly assigned to each experimental unit. Treatments were under constant aeration throughout the experiments. Fish were fed at the same time as solution changes with standard commercial pelleted food at a feeding rate of 1–5% of wet body weight.

Fish were collected at 10 time points during the 14-d period: 2, 4, 24, 72, 168, 170, 172, 192, 240, and 336 h. To maintain the same loading density throughout the experiments, at each time point one pail of 3 fish was randomly selected from each treatment and fish were sacrificed by a forceful blow to the head. Liver, kidney, and muscle were dissected, and growth parameters were measured (i.e., body length, body weight, liver weight, and liver somatic index (LSI)) (Table S2) prior to being quick-frozen at −80 °C for biochemical analysis.

2.4. Biochemical analysis

2.4.1. Water analysis

Water samples (5 mL) were collected at each time point (0, 2, 4, 24, 72, 168, 170, 172, 192, 240, and 336 h) to quantify the cell concentration, fluorescence, and standard water parameters (Table S3). The samples were stored in Corning® polypropylene centrifuge tubes, and quick-frozen at −80 °C before analysis for microcystin congeners at the MECP laboratories (Etobicoke, ON, Canada) using the method presented by Ortiz et al. (2017) and briefly described in the Supporting Information (SI 2).

2.4.2. Tissue analysis

Samples were analyzed at the MECP laboratories (Etobicoke, ON, Canada) for total microcystin concentration using an optimized MMPB method (Anaraki et al., 2020) that is described in the Supporting Information (SI 3). The calculated method detection limit (MDL) was 2.18 ng g^{-1} wet weight (w. w.). Tissue concentrations are presented on a wet weight basis throughout.

2.5. Proteomics analysis

Complete details of the proteomics analysis, including tissue preparation (Simmons et al., 2012), database searches and analysis of identified proteins are described in the Supporting Information (SI 4). Method variables used for separation and detection by LC-QTOF MS are described in Table S4.

2.6. Statistical analysis

Measured water concentrations of total microcystins were used in all statistical analyses. Normality and equality of variance were tested using the Shapiro-Wilk and Levene’s tests, respectively, and the data were transformed prior to statistical analysis, where necessary. If the

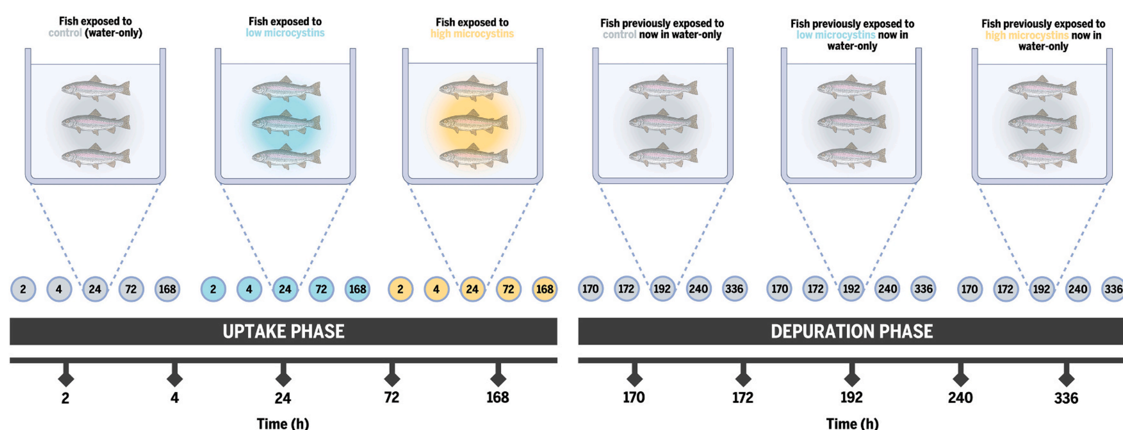


Fig. 1. Experimental design for the uptake and depuration studies. Each treatment had 10 experimental units, and each experimental unit had three fish (i.e., 30 fish per treatment, totalling 90 fish per test). One experimental unit per treatment was removed at each time point (h) to measure the uptake and depuration kinetics of microcystins at low and high levels of exposure. Experimental units that carried over into the depuration phase were placed in water-only.

assumptions of normal and equal variance passed, a two-way analysis of variance (ANOVA; $\alpha = 0.05$) was conducted to compare the main effects of time and treatment (independent variables) as well as their interaction effects on the liver somatic index, and microcystins in liver and muscle content (dependent variables) for Rainbow Trout and Lake Trout, respectively. If the interaction was not significant, and if a significant difference between the treatments was identified by ANOVA, a post hoc Tukey's test ($\alpha = 0.05$) was performed to compare all treatment means.

Correlations between total microcystins in water (MC_{water}) and total microcystins in liver (MC_{liver}) and muscle (MC_{muscle}), and liver somatic index (LSI) were calculated using Pearson's product moment correlation coefficient ($\alpha = 0.05$). Data was tested for normality using the Shapiro-Wilk's test ($\alpha = 0.05$). When normality failed ($p < 0.05$), a Kruskal-Wallis One Way Analysis of Variance (ANOVA) ($\alpha = 0.05$) was conducted. Linear regression was also performed to measure the relationship between the independent variable (MC_{water}) and dependent variables (MC_{liver}, MC_{muscle}, LSI). When the Shapiro-Wilk's test failed ($p < 0.05$), a post hoc Tukey's test ($\alpha = 0.05$) was performed to compare all treatment means. Statistical analyses were performed using Sigma Stat (Version 4.0, Systat Software, San Jose, CA, US).

Statistical analyses for label-free protein data (i.e., normalization, fold change, ANOVA, and tests of significance) were performed using Metaboanalyst 5.0, a comprehensive web-based tool for performing data analysis, visualization, and functional interpretation of proteins (Chong et al., 2018). Using this tool, we: (1) uploaded peak intensity tables; (2) normalized data using the median, log transformation, and pareto scaling to achieve a normal distribution; and (3) performed parametric statistics (i.e., volcano plots), to determine fold change compared to the control (water-only) for each treatment, with a fold-change threshold of 1.0 and a FDR-corrected p-value threshold of 0.05.

3. Results

3.1. Microcystins in water

For both experiments, MC_{water} was composed of 75 $\pm$ 3% microcystin-LR and 25 $\pm$ 3% [D-Asp^3]-microcystin-LR; no other microcystin variants or anatoxin-A were detected (Table 1). For Rainbow Trout, the starting MC_{water} was 4 μg L^{-1} and 40 μg L^{-1} in the low and high treatments, respectively. These concentrations were relatively consistent throughout the uptake phase (0–168 h) as the solutions were being renewed 3 × wk, after which the concentrations went down during the depuration phase (168–336 h) to approximately 0.01 μg L^{-1} and 0.1 μg L^{-1} in the low and high treatments, respectively, when the solutions were changed to water-only 3 × wk (Fig. 2, Table 1). For Lake Trout, the starting MC_{water} was 2 μg L^{-1} and 25 μg L^{-1} in the low and high treatments, respectively. These concentrations were also consistent throughout the uptake phase, after which the concentrations decreased during the depuration phase to approximately 0.04 μg L^{-1} and 0.2 μg L^{-1} in the low and high treatments, respectively (Fig. 2, Table 1). Fluorescence measurements showed no growth from *M. aeruginosa* during the two experiments (Table S3). We further note that due to the difficulty of approximating intracellular microcystin concentrations from a live organism such as *M. aeruginosa*, some variability (<5%) occurred in cell concentrations between time points in a treatment as well as the final concentration of microcystins.

3.2. Microcystins in tissues

3.2.1. Rainbow Trout

For Rainbow Trout liver and muscle, the pattern of uptake and depuration resembled a sinusoidal curve (Fig. 2, Table 1). As expected, livers accumulated more microcystins than muscles. Complete details of MMPB tissue measurements are provided in Table S5.

In livers across both treatments, microcystins were rapidly taken up within 2 h > MDL at 4.3 ± 1.8 ng g^{-1} w. w. and 4.9 ± 0.9 ng g^{-1} w. w. in low and high treatments, respectively, and maintained similar concentrations by the end of the uptake phase (168 h). In the depuration phase, maximum concentrations of 7.7 ± 2.8 ng g^{-1} w. w. and 11.1 ± 4.4 ng g^{-1} w. w. were observed between 170 h and 172 h in low and high treatments, respectively, after which steady levels were measured > MDL (2–4 ng g^{-1} w. w.) in both treatments by 336 h. For the uptake phase, time did not significantly affect MC_{liver} across treatments (F = 0.748, $p = 0.649$) (Table S6). However, for the depuration phase, time and treatment as independent variables significantly increased MC_{liver} (F = 5.263, $p = 0.002$ and F = 13.247, $p < 0.001$, respectively), and their joint effect significantly increased MC_{liver}, as shown by the interaction term (F = 2.735, $p = 0.022$) (Table S6). Both uptake and depuration phases showed that time and treatment did not significantly affect the LSI (Table S6).

In muscles across both treatments, microcystins were predominantly < MDL during the uptake phase with the exception of 2 h at 4.5 ± 2.5 ng g^{-1} w. w. in the low treatment and 24 h at 2.9 ± 1.7 ng g^{-1} w. w. in the high treatment. In the depuration phase, maximum concentrations of 6.6 ± 5.0 ng g^{-1} w. w. and 3.2 ± 0.8 ng g^{-1} w. w. were observed between 170 h and 172 h in low and high treatments, respectively, after which levels were measured < MDL in both treatments by 336 h. For uptake and depuration phases, time did not significantly affect MC_{muscle} in different treatments (F = 2.038, $p = 0.076$; F = 1.222, $p = 0.320$) (Table S6).

3.2.2. Lake Trout

For Lake Trout liver and muscle, the pattern of uptake and depuration resembled a u-shaped curve (Fig. 2, Table 1). As to be expected, livers accumulated more microcystins than muscles. Complete details of MMPB tissue measurements are provided in Table S5.

In livers across both treatments, microcystins were rapidly taken up within 4 h > MDL at 5.7 ± 5.7 ng g^{-1} w. w. and 1.7 ± 2.4 ng g^{-1} w. w. in low and high treatments, respectively, and decreased < MDL by the end of the uptake phase. In the depuration phase, maximum concentrations of 5.4 ± 4.4 ng g^{-1} w. w. and 8.2 ± 12.3 ng g^{-1} w. w. were observed by 336 h in low and high treatments, respectively. For uptake and depuration phases, time did not significantly affect MC_{liver} in different treatments (F = 1.462, $p = 0.213$; F = 0.915, $p = 0.518$), nor was there a significant interaction between time and treatment on the LSI (Table S6).

In muscles across both treatments, microcystins were predominantly < MDL during the uptake phase with the exception of 4 h at 3.7 ± 6.5 ng g^{-1} w. w. in the low treatment and 24 h at 2.4 ± 4.1 ng g^{-1} w. w. in the high treatment. In the depuration phase, a maximum concentration of 4.9 ± 8.4 ng g^{-1} w. w. in the high treatment was only observed by 336 h, while the low treatment was <MDL. For uptake and depuration phases, time did not significantly affect MC_{muscle} in different treatments (F = 1.056, $p = 0.419$; F = 0.728, $p = 0.666$) (Table S6).

3.3. Proteins detected with label-free proteomics

For Rainbow Trout liver and muscle, a total of 647 and 903 proteins were identified, respectively. After FDR-correction for each respective tissue (same order as above), 101 (15.6%) and 282 (31.2%) of these showed significant differential abundance from exposure to microcystins compared to the control ($p \leq 0.05$; Table S7-S8). GO bioinformatics identified major functions from these differentially expressed (DE) proteins and overlap between liver and muscle (Fig. 3). For biological processes, proteins were mainly involved in cellular (30–31% of DE proteins), metabolic (18–19%), and biological regulation (13–15%) (Fig. 3A). Molecular functions were mainly involved in binding (32–41% of DE proteins) and catalytic activity (28–40%) (Fig. 3B). Classification by cellular components revealed proteins were strictly located in the cellular anatomical entity (48% of DE proteins), intracellular (36%), and protein-containing complex (16%) (Fig. 3C). The top

Table 1
Measured concentrations for microcystin-LR and [D-Asp^3]-microcystin-LR in water (μg L^{-1}) and corresponding total microcystins in liver and muscle (ng g^{-1} w.w.), and liver somatic index (LSI) of Rainbow Trout and Lake Trout.

Time (h) [a]	Mortality (%)	Rainbow Trout					Lake Trout				
		Liver Somatic Index (%) ± SD (n = 3)	Microcystin-LR in water (μg L^{-1}) ± SD	[D-Asp^3]-microcystin-LR in water (μg L^{-1}) ± SD	Total microcystins in liver (ng g^{-1} w.w.) ± SD (n = 3) [b]	Total microcystins in muscle (ng g^{-1} w.w.) ± SD (n = 3) [b]	Liver Somatic Index (%) ± SD (n = 3)	Microcystin-LR in water (μg L^{-1}) ± SD	[D-Asp^3]-microcystin-LR in water (μg L^{-1}) ± SD	Total microcystins in liver (ng g^{-1} w.w.) ± SD (n = 3) [b]	Total microcystins in muscle (ng g^{-1} w.w.) ± SD (n = 3) [b]
CTRL 0	0	1.05 ± 0.22	0.0 ± 0.0	0.0 ± 0.0	<MDL	<MDL	0.92 ± 0.07	0.0 ± 0.0	0.0 ± 0.0	<MDL	<MDL
CTRL 2	0	0.99 ± 0.04	0.0 ± 0.0	0.0 ± 0.0	<MDL	<MDL	1.03 ± 0.16	0.0 ± 0.0	0.0 ± 0.0	<MDL	<MDL
CTRL 4	0	1.08 ± 0.16	0.0 ± 0.0	0.0 ± 0.0	<MDL	<MDL	1.05 ± 0.05	0.0 ± 0.0	0.0 ± 0.0	<MDL	<MDL
CTRL 24	0	0.94 ± 0.13	0.0 ± 0.0	0.0 ± 0.0	<MDL	<MDL	0.87 ± 0.15	0.0 ± 0.0	0.0 ± 0.0	<MDL	<MDL
CTRL 72	0	1.12 ± 0.09	0.0 ± 0.0	0.0 ± 0.0	<MDL	<MDL	0.88 ± 0.18	0.0 ± 0.0	0.0 ± 0.0	<MDL	<MDL
CTRL 168	0	1.05 ± 0.06	0.0 ± 0.0	0.0 ± 0.0	<MDL	<MDL	0.84 ± 0.18	0.0 ± 0.0	0.0 ± 0.0	<MDL	<MDL
CTRL 170	0	1.04 ± 0.08	0.0 ± 0.0	0.0 ± 0.0	<MDL	<MDL	0.92 ± 0.15	0.0 ± 0.0	0.0 ± 0.0	<MDL	<MDL
CTRL 172	0	1.04 ± 0.15	0.0 ± 0.0	0.0 ± 0.0	<MDL	<MDL	0.93 ± 0.25	0.0 ± 0.0	0.0 ± 0.0	<MDL	<MDL
CTRL 192	0	1.00 ± 0.24	0.0 ± 0.0	0.0 ± 0.0	<MDL	<MDL	0.83 ± 0.26	0.0 ± 0.0	0.0 ± 0.0	<MDL	<MDL
CTRL 240	0	1.02 ± 0.22	0.0 ± 0.0	0.0 ± 0.0	<MDL	<MDL	1.08 ± 0.10	0.0 ± 0.0	0.0 ± 0.0	<MDL	<MDL
CTRL 336	0	0.84 ± 0.10	0.0 ± 0.0	0.0 ± 0.0	<MDL	<MDL	0.88 ± 0.07	0.0 ± 0.0	0.0 ± 0.0	<MDL	<MDL
MC-LOW 0	0	1.05 ± 0.06	3.30 ± 1.15	0.31 ± 0.02	<MDL	<MDL	0.93 ± 0.17	1.10 ± 0.06	0.80 ± 0.05	<MDL	<MDL
MC-LOW 2	0	1.06 ± 0.09	0.80 ± 0.37	0.23 ± 0.10	4.0 ± 2.2	4.5 ± 2.5	0.87 ± 0.15	1.10 ± 0.06	0.79 ± 0.07	<MDL	0.8 ± 1.4
MC-LOW 4	0	1.24 ± 0.27	3.42 ± 3.01	0.39 ± 0.01	3.8 ± 2.2	<MDL	0.94 ± 0.08	1.10 ± 0.03	0.81 ± 0.03	5.1 ± 3.7	3.7 ± 6.5
MC-LOW 24	0	1.20 ± 0.17	3.33 ± 2.25	0.44 ± 0.04	2.0 ± 2.0	0.8 ± 0.8	0.89 ± 0.03	1.17 ± 0.03	0.84 ± 0.00	2.0 ± 2.0	<MDL
MC-LOW 72	0	0.94 ± 0.11	1.57 ± 0.04	0.40 ± 0.02	1.3 ± 1.3	<MDL	1.07 ± 0.21	1.19 ± 0.09	0.81 ± 0.04	4.2 ± 1.7	<MDL
MC-LOW 168	0	1.03 ± 0.01	1.58 ± 0.07	0.41 ± 0.03	2.7 ± 0.3	2.0 ± 1.0	0.82 ± 0.18	1.39 ± 0.03	0.94 ± 0.01	0.8 ± 0.8	<MDL
MC-LOW 170	0	1.18 ± 0.05	0.00 ± 0.00	0.01 ± 0.00	3.4 ± 0.2	0.7 ± 0.7	1.03 ± 0.29	0.03 ± 0.00	0.05 ± 0.00	<MDL	<MDL
MC-LOW 172	0	1.11 ± 0.02	0.00 ± 0.00	0.02 ± 0.00	7.7 ± 2.8	6.6 ± 5.0	0.95 ± 0.08	0.00 ± 0.00	0.05 ± 0.00	<MDL	<MDL
MC-LOW 192	0	1.05 ± 0.28	0.01 ± 0.00	0.02 ± 0.00	0.9 ± 0.9	<MDL	0.82 ± 0.14	0.02 ± 0.02	0.02 ± 0.03	<MDL	<MDL
MC-LOW 240	0	1.10 ± 0.10	0.01 ± 0.00	0.01 ± 0.00	<MDL	<MDL	0.94 ± 0.15	0.01 ± 0.02	0.02 ± 0.03	<MDL	<MDL
MC-LOW 336	0	1.02 ± 0.02	0.00 ± 0.00	0.06 ± 0.01	4.3 ± 0.9	2.1 ± 1.1	0.77 ± 0.09	0.01 ± 0.02	0.02 ± 0.03	5.3 ± 2.7	1.4 ± 1.4
MC-HIGH 0	0	1.07 ± 0.04	30.15 ± 0.06	8.12 ± 0.03	<MDL	<MDL	0.90 ± 0.06	13.51 ± 0.26	0.86 ± 0.17	<MDL	<MDL
MC-HIGH 2	0	1.23 ± 0.11	28.47 ± 7.82	7.81 ± 1.45	4.9 ± 0.9	<MDL	0.84 ± 0.18	13.13 ± 0.39	10.28 ± 0.11	<MDL	<MDL
MC-HIGH 4	0	1.15 ± 0.09	31.28 ± 1.06	8.44 ± 0.67	2.3 ± 1.8	<MDL	1.05 ± 0.04	13.99 ± 0.49	11.09 ± 0.31	1.5 ± 1.5	<MDL
MC-HIGH 24	0	1.13 ± 0.29	30.17 ± 0.07	8.21 ± 0.04	5.8 ± 3.2	2.9 ± 1.7	0.92 ± 0.18	13.24 ± 0.65	10.36 ± 0.43	4.6 ± 2.4	2.3 ± 2.3
MC-HIGH 72	0	1.06 ± 0.25	32.43 ± 0.81	8.86 ± 0.67	2.2 ± 1.1	<MDL	1.21 ± 0.25	12.68 ± 0.15	10.49 ± 0.01	0.8 ± 0.8	<MDL
MC-HIGH 168	0	0.95 ± 0.04	30.53 ± 0.98	8.13 ± 0.44	1.2 ± 1.2	<MDL	1.11 ± 0.24	13.69 ± 0.48	10.87 ± 0.45	<MDL	<MDL
MC-HIGH 170	0	1.01 ± 0.09	0.01 ± 0.01	0.11 ± 0.01	11.1 ± 4.4	2.7 ± 1.4	0.93 ± 0.14	0.06 ± 0.02	0.07 ± 0.01	<MDL	<MDL
MC-HIGH 172	0	1.16 ± 0.34	0.02 ± 0.03	0.12 ± 0.01	9.1 ± 2.8	2.9 ± 0.5	0.96 ± 0.06	0.11 ± 0.04	0.10 ± 0.02	<MDL	<MDL
MC-HIGH 192	0	1.03 ± 0.13	0.01 ± 0.02	0.16 ± 0.03	1.9 ± 1.0	<MDL	0.97 ± 0.18	0.04 ± 0.01	0.06 ± 0.01	4.7 ± 0.2	<MDL
MC-HIGH 240	0	0.97 ± 0.06	0.01 ± 0.01	0.15 ± 0.03	3.1 ± 1.7	0.8 ± 0.8	0.94 ± 0.17	0.01 ± 0.02	0.05 ± 0.00	4.9 ± 2.7	<MDL
MC-HIGH 336	0	1.32 ± 0.34	0.01 ± 0.01	0.13 ± 0.03	0.8 ± 0.8	<MDL	0.97 ± 0.07	0.01 ± 0.02	0.05 ± 0.00	8.2 ± 7.1	4.9 ± 4.9

[a] 2–168 h denote the uptake phase (exposure to microcystins); 170–336 h denote the depuration phase (exposure to clean water).
[b] The method detection limit (MDL) for microcystins in tissues is 2.18 ng g^{-1} w. w.; '<MDL' is reported where all triplicate measurements were <MDL.

protein classes identified were metabolite interconversion enzyme (15–21% of DE proteins), transporter (9–18%), and protein modifying enzyme (13–14%). GO enrichment analyses additionally revealed significant proteins were involved in ion binding ($p = 0.001$) in livers and organelles ($p = 0.03$) in muscles (Table S9). Across both tissues, proteins were significantly dysregulated in both low and high microcystin treatments after uptake and depuration phases.

For Lake Trout liver and muscle, a total of 374 and 1057 proteins were identified, respectively. After FDR-correction for each respective tissue (same order as above), 10 (2.7%) and 0 (0.0%) of these showed significant differential abundance from exposure to microcystins compared to the control ($p \leq 0.05$; Table S10-S11). GO bioinformatics identified major functions from these DE proteins in the liver (Fig. 3). For biological processes, proteins were mainly involved in cellular (26% of DE proteins), metabolic (19%), and biological regulation (19%) (Fig. 3A). Molecular functions were mainly involved in binding (36% of DE proteins) and catalytic activity (36%) (Fig. 3B). Classification by cellular components revealed proteins were strictly located in the cellular anatomical entity (50% of DE proteins) and intracellular (50%) (Fig. 3C). The top protein classes identified were metabolite interconversion enzyme (18% of DE proteins), transporter (18%), and protein modifying enzyme (18%). GO enrichment analyses additionally revealed significant proteins were involved in leukocyte proliferation ($p = 0.01$) in livers (Table S12). Across liver tissues, proteins were significantly dysregulated in both low and high microcystin treatments after uptake and depuration phases.

3.4. Correlation analysis

For Rainbow Trout, positive correlations were observed between MC_{liver} and MC_{muscle} in both low ($p < 0.001$) and high ($p < 0.001$) treatments. A significant correlation was additionally observed between MC_{water} and MC_{liver} in the depuration phase ($p < 0.001$) of the high treatment (Table S13). For Lake Trout, positive correlations were observed between MC_{liver} and MC_{muscle} in both low ($p = 0.004$) and high ($p = 0.002$) treatments. A moderate correlation was additionally observed between MC_{water} and MC_{liver} in the depuration phase ($p = 0.085$) of the high treatment (Table S13).

4. Discussion

4.1. Carnivorous species accumulate and retain intracellular microcystins

Microcystins consistently accumulate at higher concentrations in fish livers than muscles, but uptake and depuration kinetics vary across feeding guilds (Malbrouke and Kestemont, 2006; Zhang et al., 2009; Dyble et al., 2011; Poste et al., 2011). The present study exposed two carnivorous species, Rainbow Trout and Lake Trout, to microcystins in its intracellular state (i.e., cell-bound or conserved within cyanobacteria), which is deemed a realistic route of exposure in the field (Chorus and Welker, 2021), and at concentrations measured in the environment (Cheng et al., 2017). Using a targeted MMPB method, the present study found fish accumulated higher concentrations of intracellular microcystins in livers than muscles. Both species also revealed microcystins in tissues at the end of the experiments, with Rainbow Trout retaining highest levels at the end of the uptake phase (i.e., 1 wk of exposure to a steady cell concentration of *M. aeruginosa* through aqueous exposure) and Lake Trout retaining highest levels at the end of the depuration phase (i.e., 1 wk of exposure in clean water). Reasons for this may be that juvenile fish, as used in the present study, have not fully developed the ability to depurate microcystins compared to adult fish (Jiang et al., 2017), will accumulate more microcystins than at adult life stage (Poste et al., 2011), and will depurate microcystins from the liver at a slower rate than muscles (12 and 4 days, respectively) (Wood et al., 2006). Feeding guilds also differ in routes of uptake and metabolism of microcystins, with the transport of microcystins into internal organs in planktivores and omnivores effectively restricted to movement across the intestinal wall, and carnivores potentially relying on routes other than the gastrointestinal tract (e.g., gills) to uptake microcystins (Zhang et al., 2009). We further note rapid accumulation of microcystins within 2–4 h of exposure in Rainbow Trout and Lake Trout in both low and high treatments. Rapid accumulation in tissues was previously observed in Yellow Perch that were administered oral doses of 5 μg and 20 μg microcystin-LR, which saw elevated levels in livers and muscles by 4–6 h and peaked at 8–10 h (Dyble et al., 2011). A recent study on adult and juvenile Rainbow Trout similarly observed rapid accumulation of microcystins through aqueous exposure, regardless of whether it was in its intracellular and extracellular state, within 24 h in livers and muscles (Shahmohamadloo et al., 2021). The analytical method used is also an important determinant in revealing the concentration of microcystins in fish, with preference towards the MMPB method which provides the specificity and sensitivity necessary to capture all microcystins in tissues (Flores et al., 2018). Previous uptake and depuration studies used the ELISA method instead, which underestimates the total amount of microcystins by only measuring the free fractions; bound fractions contribute up to 90% of total microcystins in an organism's tissues (Lance et al., 2010).

The present study also observed, in most cases, weak temporal correlations between intracellular MC_{water} and microcystins in fish tissues, yet observed strong and positive temporal correlations between MC_{liver} and MC_{muscle} in both species. Strong ($p < 0.001$) and moderate ($p = 0.085$) correlations were, respectively, observed in the depuration phases of Rainbow Trout and Lake Trout in the high microcystin treatments. These findings somewhat contrast with the recent finding of a positive relationship between intracellular MC_{water} and microcystins in fish tissues that were analyzed by ELISA (Flores et al., 2018). We anticipated our results since carnivores may accumulate microcystins at a much lower rate than omnivores and planktivores (Flores et al., 2018) due to differences in diet (Wood et al., 2014). The present study also measured low residual MC_{water} (0.01–0.1 μg L^{-1}) in the depuration phase when juvenile fish were placed in clean water. This suggests juvenile fish ingested intracellular microcystins in the uptake phase and excreted low amounts during the study, with preferential accumulation in the liver for detoxification after being ingested. Drinking microcystin-contaminated water may have been a more influential route of uptake in the present study due to having a stress response to cyanotoxins during the experiment, which is known for juvenile fish (as shown by Best et al., 2003). In general, juvenile fish are more sensitive to stress since they invest more energy into growth and regulation than do adults (Wendelaar Bonga, 1997), which may reduce their capacity to depurate toxins from the body.

4.2. Sublethal effects observed in proteomes after depuration phase

At sublethal doses used in the present study (1–40 μg total microcystins L^{-1}), suites of proteins linked to microcystin toxicity pathways (Campos and Vasconcelos, 2010; WHO, 2020; Welten et al., 2020; Chorus and Welker, 2021) were significantly dysregulated in Rainbow Trout and Lake Trout during uptake and depuration phases (Table 2). Liver from both species saw significant increases in solute carrier family proteins (SLCs), which mediate transport of endogenous and exogenous compounds into cells (Hagenbuch and Meier, 2004) and facilitate the diffusion of microcystins through the plasma membrane by organic transporting polypeptides (Campos and Vasconcelos, 2010; Fischer et al., 2010; WHO, 2020; Chorus and Welker, 2021). It is postulated that after diffusion through the plasma membrane, microcystins bind with strong affinity to protein phosphatases which are responsible for cytoskeletal structure, cell replication, DNA repair and stress responses (Sun et al., 2014; Buratti et al., 2017; Welten et al., 2020; WHO, 2020). To prevent destabilization of the cytoskeleton and avert necrosis and

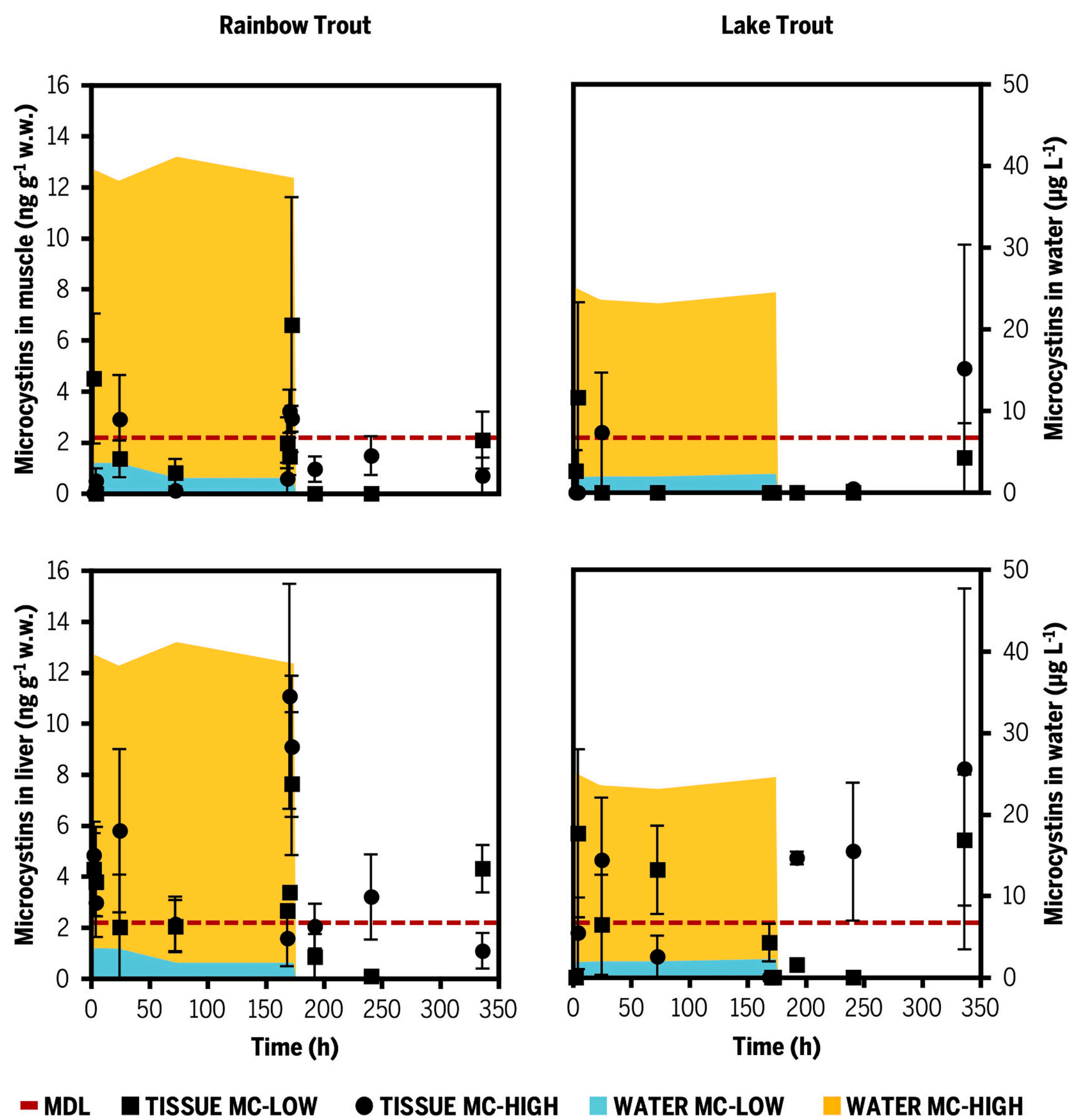


Fig. 2. Total microcystins (ng g^{-1} w. w.) measured in muscles (top panel) and livers (bottom panel) of Rainbow Trout and Lake Trout, respectively, exposed to microcystins measured in water (μg L^{-1}) at high and low concentrations. Left axis is associated with "TISSUE MC-HIGH" and "TISSUE MC-LOW", which denotes the two treatment groups that were exposed to "WATER MC-HIGH" and "WATER MC-LOW", respectively, which are associated with the right axis.

apoptosis, additional proteins are activated to protect cells from microcystin toxicity (Campos and Vasconcelos, 2010; Welten et al., 2020; Chorus and Welker, 2021).

For the present study, Rainbow Trout liver expressed a greater share of these proteins than Lake Trout, which corresponds with the frequent detection of MC_{liver} > MDL for Rainbow Trout in both uptake and depuration phases. For instance, the nuclear inhibitor of protein phosphatase 1 (PP1) significantly increased in abundance across low and high treatments in both phases. This demonstrates the rapid ability for microcystins to inhibit protein phosphatases and impact cell homeostasis (Campos and Vasconcelos, 2010). In response, several proteins were activated to protect Rainbow Trout hepatocytes from oxidative stress and carcinogenicity: DNA-dependent protein kinases (PRKDCs) that are responsible for DNA repair and regulated by protein phosphatases (Douglas et al., 2001); calcium-calmodulin-dependent multifunctional protein kinases (CaMKs) that are responsible for cell signaling and postulated to play a role in the late events of microcystin-induced cell death (Krakstad et al., 2006); mitogen-activated protein kinases (MAPKs) that are responsible for cell proliferation and differentiation (Gehringer, 2004) as well as regulating tumor-formation from the inhibition of protein phosphatases caused by microcystins (Komatsu et al., 2007); cytochrome P450s (CYPs) that are responsible for the detoxification of xenobiotic compounds such as microcystins and can lead to the production of reactive oxygen species (Nong et al., 2007); glutathione S-transferases (GSTs) that are responsible for the metabolism and detoxification of xenobiotic compounds such as microcystins (Fu and Xie, 2006); and P-glycoproteins (ABCBs) that are responsible for cellular excretion of cytotoxic compounds and can influence the susceptibility of fish to microcystins (Amé et al., 2009). GO enrichment analysis in Rainbow Trout further revealed the largest group of significantly dysregulated proteins linked to ion binding in livers. Ion binding, and more broadly ionic regulation, is a well-documented sublethal toxicity indicator since it is needed to maintain high ion levels in the blood relative to the environment (Marshall, 2002). Chronic exposure to lysed *M. aeruginosa* cells containing 41–68 μg microcystin-LR L^{-1} has resulted in ionic imbalances and reduced growth in Brown Trout (*Salmo trutta*) (Bury et al., 1995). Given the liver is a major contributor to iono/osmoregulatory processes in fish (Hwang et al., 2011), the present study's proteomic findings suggest intracellular microcystins can significantly dysregulate proteins related to ionic regulation in Rainbow Trout. For Lake Trout liver, fewer proteins linked to microcystin toxicity pathways were significantly dysregulated: B-cell lymphomas (BCLs) that

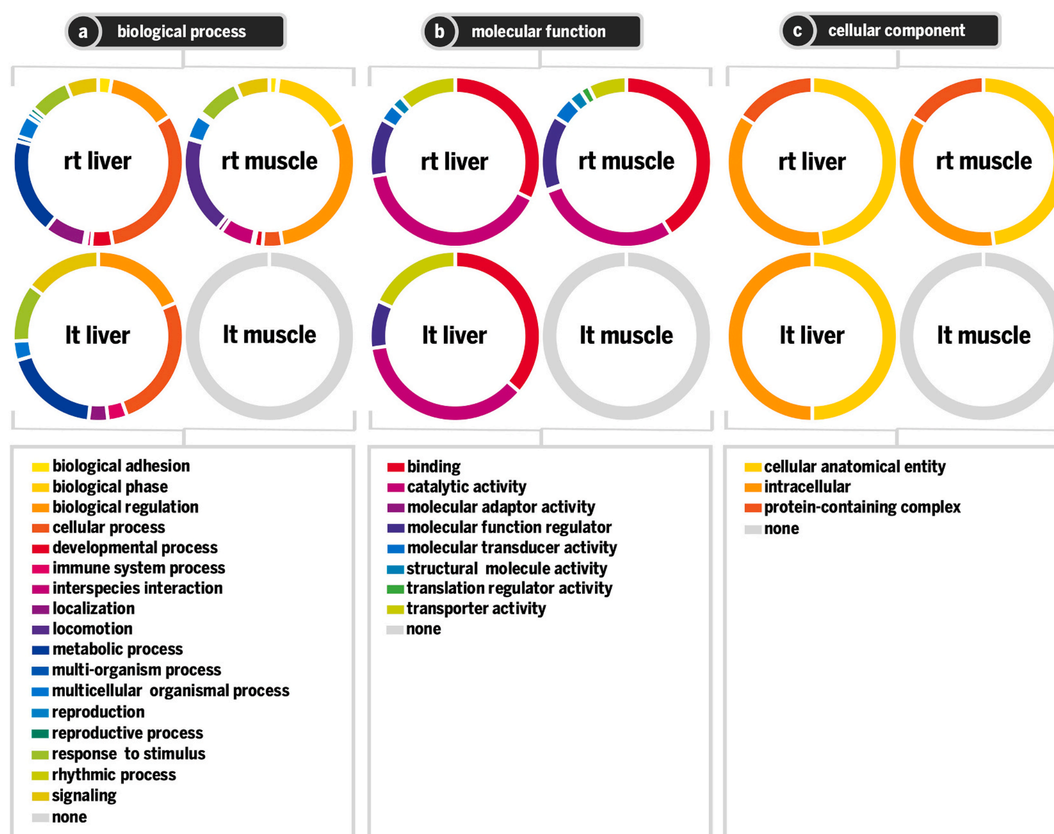


Fig. 3. Gene ontology (GO) classification of significant differentially abundant proteins with functional annotation in the liver and muscle of adult Rainbow Trout (rt) and Lake Trout (lt) exposed to microcystins after uptake and depuration phases.

are responsible for regulating mitochondrial apoptosis (Fu et al., 2005); integrins (ITGs) that are responsible for chemotaxis and inflammatory reactions in the presence of microcystins (Kujbida et al., 2009); and MAPKs that were also expressed in Rainbow Trout. GO enrichment analysis in Lake Trout further revealed the largest group of significantly dysregulated proteins linked to leukocyte proliferation in livers. Leukocyte profiles are frequently measured to assess for stress in vertebrates (Davis et al., 2008). Acute intraperitoneal injections of 400 μg microcystin-LR kg^{-1} body mass in Carp and Silver Carp (*Hypophthalmichthys molitrix*) saw a decrease in total leukocytes, which suggests a stress-induced response to cyanobacterial toxicity (Palikova et al., 1998). For the present study, this suggests intracellular microcystins can dysregulate leukocyte activity in livers of Lake Trout.

Interestingly, muscles in Rainbow Trout revealed the same cluster of proteins that were expressed in the liver, with the exception that they were only significantly dysregulated in the high treatment after the depuration phase. Conversely, no proteins were significantly dysregulated in Lake Trout muscles across both treatments and phases. These findings corroborate with the targeted approach for measuring microcystins in tissues, which revealed $MC_{muscle} > MDL$ in Rainbow Trout and almost none in Lake Trout except at the beginning and end of the experiment. GO enrichment analysis in Rainbow Trout further revealed the largest cluster of significantly dysregulated proteins linked to organelles in muscles. Organelle proteins are structurally and functionally important to cell signaling pathways, and exposure to 5 μg microcystin-LR^{-1} dysregulated proteins associated with oxidative stress in Medaka fish (Malécot et al., 2009). For the present study, this suggests intracellular microcystins can significantly dysregulate organelle proteins that are responsible for mitigating oxidative stress. Altogether, this suggests the possibility that molecular initiating events and key events involved in the early stages of adverse outcome pathways may manifest more quickly in Rainbow Trout and can carry on after exposure to a toxic cyanobacterial event, yet manifest more slowly in Lake Trout.

4.3. Environmental implications

An important and novel finding from this study is that Rainbow Trout and Lake Trout differed in their tissue accumulation and proteomic response to cyanobacterial toxins. This study therefore addresses an outstanding knowledge gap rendered possible by applying an optimized MMPB method to better understand the accumulation of cyanobacterial toxins from multiple freshwater fish species. This information is necessary to understand the extent of risk and avert exposure to higher trophic level species including humans and wildlife (Peng et al., 2010; Flores et al., 2018). Bioaccumulation of cyanobacterial toxins through aquatic food chains can be an elusive process and remains a pressing concern, as was recently evidenced by a linkage between microcystins and bald eagle mass death events in the United States that took nearly 30 years to uncover (Breinlinger et al., 2021).

5. Conclusion

By purposefully designing an experiment that mimics the mean duration of a harmful algal bloom in the field (Chapra et al., 2017) while exposing two ecologically and economically valuable fish species to cyanobacterial toxins in its intracellular state, this study reveals that, as a first step, microcystins will readily utilize organic transporting polypeptides to diffuse through the plasma membrane of cells and activate cellular responses related to oxidative stress, apoptosis, DNA repair, and carcinogenicity. The extent of intraspecific sublethal molecular-level effects between Rainbow Trout and Lake Trout differed, and the extent of interspecific accumulation and retention of microcystins in

Table 2
Differentially expressed proteins that were significantly dysregulated (FDR-corrected p-value ≤ 0.05) in liver and muscle of Rainbow Trout and Lake Trout exposed to microcystin treatments during uptake (0–168 h) and depuration (168–336 h) phases.

Symbol	Protein description	Low microcystins [a]		High microcystins [a]	
		Fold change [b]		Fold change [b]	
		168 h	336 h	168 h	336 h
Rainbow Trout (liver)					
Ppp1r8	Nuclear inhibitor of protein phosphatase 1	5.14*	5.06*	6.09*	7.09
Prkdc	DNA-dependent protein kinase catalytic subunit	−5.76*	−5.37*	−5.89*	−5.55*
Camkv	CaM kinase-like vesicle-associated protein	−2.13*	−1.74*	−2.24*	−1.92*
Mapk3	Mitogen-activated protein kinase 3	6.48*	5.91*	6.04*	5.50*
Cyp7a1	Cytochrome P450 7A1	−2.13*	−1.74*	−2.24*	−1.92*
Slc6a5	Solute carrier family 6 member 5	3.09*	2.31	2.62*	2.46*
Gsta3	Glutathione S-transferase A3	−2.13*	−1.74*	−2.24*	−1.92*
Abcb9	ATP-binding cassette sub-family B member 9	4.58*	3.84*	4.07*	4.48*
Rainbow Trout (muscle)					
Ppp3ca	Serine/threonine-protein phosphatase 2 B catalytic subunit alpha isoform	2.23*	2.07	2.22	2.24*
Camk1	Calcium/calmodulin-dependent protein kinase type 1	2.78	3.06	4.57	4.91*
Bcl6	B-cell lymphoma 6 protein	8.64*	8.45*	8.19*	8.69*
Map3k11	Mitogen-activated protein kinase kinase kinase 11	6.64*	6.40	6.50	6.50*
Cyp46a1	Cytochrome P450 Family 46 Subfamily A Member 1	6.41*	6.28	6.32	5.84*
Eif2b5	Translation initiation factor eIF-2B subunit epsilon	−2.80*	−2.47	−2.48	−2.76*
Il8	Interleukin-8	1.89*	1.62	1.95	1.72*
Itgb3	Integrin beta-3	−2.74*	−2.40	−2.40	−3.10
Slc12a5	Solute carrier family 12 member 5	5.35*	4.96	5.31	5.03*
Lake Trout (liver)					
Bcl6	B-cell lymphoma 6 protein	6.20	4.90*	4.84	4.73*
Mapk3	Mitogen-activated protein kinase 3	3.80*	4.13*	4.16*	4.06*
Itga6	Integrin alpha-6	6.27*	6.62*	6.78*	6.88*
Slc12a5	Solute carrier family 12 member 5	4.90*	5.40*	5.04*	5.38*
Lake Trout (muscle)					
–	–	–	–	–	–

[a] For the complete list of proteins along with significance and fold change values, please refer to Tables S7 – 10.
[b] Proteins that were significantly dysregulated (FDR-corrected p-value ≤ 0.05) are indicated by an asterisk (*).

tissues within each species also differed. This suggests that fish do not respond the same to cyanobacterial toxicity within and among species despite being reared under the same environmental conditions and diet. Interspecific variability in fish tissue microcystin concentrations can be linked to feeding habits and dietary exposure (Wood et al., 2014), while intraspecific variability may be a result of differences in mechanistic responses to the toxicity, biotransformation and excretion of microcystins (Adamovský et al., 2007). On the latter point, intraspecific variation, which can evolve rapidly in multiple species within an ecosystem, is an important driver of community structure and ecosystem function (Rudman et al., 2015, 2021). Understanding the role of intraspecific variation is critical to unraveling evolutionary responses in fish populations who encounter *Microcystis* blooms; likewise, understanding the role of intraspecific variation is also critical to unraveling evolutionary responses naturally occurring within *Microcystis* blooms, whose development, toxicity, and cosmopolitan nature continue to increase globally (Dick et al., 2021).

Certainly, we cannot discount the need for a longer depuration phase as a limitation in our study, which sets the stage for future work and may answer whether fish can completely eliminate cyanobacterial toxins from their bodies. Even so, climate change is projected to increase the mean number of days of a cyanobacterial harmful algal bloom to 16–23 days in 2050 and 18–39 days in 2090 (Chapra et al., 2017). Aware toxic *Microcystis* are increasing globally (Harke et al., 2016) and possess advanced mechanisms to outcompete other plankton in freshwater ecosystems (Wilhelm et al., 2020), future concerns are that fish populations may interact with cyanobacteria for extended periods. Longer periods of exposure, accumulation, and retention of cyanobacterial toxins in fish tissues can have economic impacts to commercial fisheries and recreational fishers, as well as health impacts to higher trophic level species including humans and wildlife.

Credit author statement

R.S.S., D.G.P., S.P.B. and P.K.S. conceived and designed the experiment. R.S.S. collected the data. R.S.S., X.O., and D.B.D.S. analyzed the data. R.S.S. wrote the paper. All authors read, amended, and approved the final manuscript.

Declaration of competing interest

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.

Acknowledgements

The authors adhere to a publication policy of submitting only their finest work to peer review journals and in the interest of the essentialness of science. Funding was provided for R.S. Shahmohamadloo and this project through an NSERC CREATE (2013–432269) program in Multiple Stressors and Cumulative Effects in the Great Lakes, and a Canada-Ontario Agreement (2218) through the Ontario Ministry of the Environment, Conservation and Park. We thank K. Stevack for assistance with raising the fish used for these experiments, and BioRender for providing an online platform to create the graphical abstract.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2021.132028.

References

Adamovský, O., Kopp, R., Hilscherová, K., Babica, P., Palíková, M., Pašková, V., Navrátil, S., Maršálek, B., Bláha, L., 2007. Microcystin kinetics (bioaccumulation and elimination) and biochemical responses in common carp (Cyprinus carpio) and silver carp (Hypophthalmichthys molitrix) exposed to toxic cyanobacterial blooms. Environ. Toxicol. Chem.: Int. J. 26 (12), 2687–2693. https://doi.org/10.1897/07-213.1.

Amé, M.V., Baroni, M.V., Galanti, L.N., Bocco, J.L., Wunderlin, D.A., 2009. Effects of microcystin–LR on the expression of P-glycoprotein in Jenynsia multidentata. Chemosphere 74 (9), 1179–1186. https://doi.org/10.1016/j.chemosphere.2008.11.068.

Anaraki, M.T., Shahmohamadloo, R.S., Sibley, P.K., MacPherson, K.A., Bhavsar, S.P., Simpson, A.J., Ortiz Almirall, X., 2020. Optimization of an MMPB Lemieux Oxidation method for the quantitative analysis of microcystins in fish tissue by LC-QTOF MS. Sci. Total Environ. 737, 140209. https://doi.org/10.1016/j.scitotenv.2020.140209.

Best, J.H., Eddy, F.B., Codd, G.A., 2003. Effects of Microcystis cells, cell extracts and lipopolysaccharide on drinking and liver function in rainbow trout Oncorhynchus mykiss Walbaum. Aquat. Toxicol. 64 (4), 419–426. https://doi.org/10.1016/S0166-445X(03)00105-X.

Bieczynski, F., Bianchi, V.A., Luquet, C.M., 2013. Accumulation and biochemical effects of microcystin-LR on the Patagonian pejerrey (Odontesthes hatcheri) fed with the toxic cyanobacteria Microcystis aeruginosa. Fish Physiol. Biochem. 39 (5), 1309–1321. https://doi.org/10.1007/s10695-013-9785-7.

Birbeck, J.A., Westrick, J.A., O'Neill, G.M., Spies, B., Szlag, D.C., 2019. Comparative analysis of microcystin prevalence in Michigan lakes by online concentration LC/MS/MS and ELISA. Toxins 11 (1), 13. https://doi.org/10.3390/toxins11010013.

Bouaïcha, N., Miles, C.O., Beach, D.G., Labidi, Z., Djabri, A., Benayache, N.Y., Nguyen-Quang, T., 2019. Structural diversity, characterization and toxicology of microcystins. Toxins 11 (12), 714. https://doi.org/10.3390/toxins11120714.

Breinlinger, S., Phillips, T.J., Haram, B.N., Mareš, J., Martínez Yerena, J.A., Hrouzek, P., Sobotka, R., Henderson, W.M., Schmieder, P., Williams, S.M., Lauderdale, J.D., Wilde, H.D., Gerrin, W., Kust, A., Washington, J.W., Wagner, C., Geier, B., Liebeke, M., Enke, H., Niedermeyer, T.H.J., Wilde, S.B., 2021. Hunting the eagle killer: a cyanobacterial neurotoxin causes vacuolar myelinopathy. Science 371 (6536), eaax9050. https://doi.org/10.1126/science.aax9050.

Buratti, F.M., Manganelli, M., Vichi, S., Stefanelli, M., Scardala, S., Testai, E., Funari, E., 2017. Cyanotoxins: producing organisms, occurrence, toxicity, mechanism of action and human health toxicological risk evaluation. Arch. Toxicol. 91 (3), 1049–1130. https://doi.org/10.1007/s00204-016-1913-6.

Bury, N.R., Eddy, F.B., Codd, G.A., 1995. The effects of the cyanobacterium Microcystis aeruginosa, the cyanobacterial hepatotoxin microcystin–LR, and ammonia on growth rate and ionic regulation of brown trout. J. Fish. Biol. 46 (6), 1042–1054. https://doi.org/10.1111/j.1095-8649.1995.tb01408.x.

Campos, A., Vasconcelos, V., 2010. Molecular mechanisms of microcystin toxicity in animal cells. Int. J. Mol. Sci. 11 (1), 268–287. https://doi.org/10.3390/ijms11010268.

Carmichael, W.W., Azevedo, S.M., An, J.S., Molica, R.J., Jochimsen, E.M., Lau, S., Rinehart, K.L., Shaw, G.R., Eaglesham, G.K., 2001. Human fatalities from cyanobacteria: chemical and biological evidence for cyanotoxins. Environ. Health Perspect. 109 (7), 663–668. https://doi.org/10.1289/ehp.01109663.

Chapra, S.C., Boehlert, B., Fant, C., Bierman Jr., V.J., Henderson, J., Mills, D., Mas, D.M.L., Rennels, L., Jantarasami, L., Martinich, J., Strzepek, K.M., Paerl, H.W., 2017. Climate change impacts on harmful algal blooms in US freshwaters: a screening-level assessment. Environ. Sci. Technol. 51 (16), 8933–8943. https://doi.org/10.1021/acs.est.7b01498.

Chen, L., Hu, Y., He, J., Chen, J., Giesy, J.P., Xie, P., 2017. Responses of the proteome and metabolome in livers of zebrafish exposed chronically to environmentally relevant concentrations of microcystin-LR. Environ. Sci. Technol. 51 (1), 596–607. https://doi.org/10.1021/acs.est.6b03990.

Cheng, H., Yan, W., Wu, Q., Liu, C., Gong, X., Hung, T.C., Li, G., 2017. Parental exposure to microcystin-LR induced thyroid endocrine disruption in zebrafish offspring, a transgenerational toxicity. Environ. Pollut. 230, 981–988. https://doi.org/10.1016/j.envpol.2017.07.061.

Chong, J., Soufan, O., Li, C., Caraus, I., Li, S., Bourque, G., Wishart, D.S., Xia, J., 2018. MetaboAnalyst 4.0: towards more transparent and integrative metabolomics analysis. Nucleic Acids Res. 46, 486–494. https://doi.org/10.1093/nar/gky310.

Chorus, I., Welker, M. (Eds.), 2021. Toxic Cyanobacteria in Water: a Guide to Their Public Health Consequences, Monitoring and Management. CRC Press, Boca Raton (FL), on behalf of the World Health Organization, Geneva, CH.

Davis, A.K., Maney, D.L., Maerz, J.C., 2008. The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists. Funct. Ecol. 22 (5), 760–772. https://doi.org/10.1111/j.1365-2435.2008.01467.x.

Deblois, C.P., Giani, A., Bird, D.F., 2011. Experimental model of microcystin accumulation in the liver of Oreochromis niloticus exposed subchronically to a toxic bloom of Microcystis sp. Aquat. Toxicol. 103 (1–2), 63–70. https://doi.org/10.1016/j.aquatox.2011.02.006.

Dick, G.J., Duhaime, M.B., Evans, J.T., Errera, R.M., Godwin, C., Kharbush, J.J., Nitschky, H.S., Powers, M.A., Vanderploeg, H.A., Schmidt, K.C., Smith, D.J., Yancey, C.E., Zwiers, C.C., Denef, V.J., 2021. The genetic and ecophysiological diversity of Microcystis. Environ. Microbiol. https://doi.org/10.1111/1462-2920.15615.

Douglas, P., Moorhead, G.B., Ye, R., Lees-Miller, S.P., 2001. Protein phosphatases regulate DNA-dependent protein kinase activity. J. Biol. Chem. 276 (22), 18992–18998. https://doi.org/10.1074/jbc.M011703200.

Dyble, J., Gossiaux, D., Landrum, P., Kashian, D.R., Pothoven, S., 2011. A kinetic study of accumulation and elimination of microcystin-LR in yellow perch (*Perca flavescens*) tissue and implications for human fish consumption. Mar. Drugs 9 (12), 2553–2571. https://doi.org/10.3390/md9122553.

Fischer, A., Höger, S.J., Stemmer, K., Feurstein, D.J., Knobeloch, D., Nussler, A., Dietrich, D.R., 2010. The role of organic anion transporting polypeptides (OATPs/SLCOs) in the toxicity of different microcystin congeners in vitro: a comparison of primary human hepatocytes and OATP-transfected HEK293 cells. Toxicol. Appl. Pharmacol. 245 (1), 9–20. https://doi.org/10.1016/j.taap.2010.02.006.

Flores, N.M., Miller, T.R., Stockwell, J.D., 2018. A global analysis of the relationship between concentrations of microcystins in water and fish. Front. Marine Sci. 5, 30. https://doi.org/10.3389/fmars.2018.00030.

Fu, W.Y., Chen, J.P., Wang, X.M., Xu, L.H., 2005. Altered expression of p53, Bcl-2 and Bax induced by microcystin-LR in vivo and in vitro. Toxicon 46 (2), 171–177. https://doi.org/10.1016/j.toxicon.2005.03.021.

Fu, J., Xie, P., 2006. The acute effects of microcystin LR on the transcription of nine glutathione S-transferase genes in common carp Cyprinus carpio L. Aquat. Toxicol. 80 (3), 261–266. https://doi.org/10.1016/j.aquatox.2006.09.003.

Gehringer, M.M., 2004. Microcystin-LR and okadaic acid-induced cellular effects: a dualistic response. FEBS Lett. 557 (1–3), 1–8. https://doi.org/10.1016/S0014-5793(03)01447-9.

Geis-Asteggiante, L., Lehotay, S.J., Fortis, L.L., Paoli, G., Wijey, C., Heinzen, H., 2011. Development and validation of a rapid method for microcystins in fish and comparing LC-MS/MS results with ELISA. Anal. Bioanal. Chem. 401 (8), 2617–2630. https://doi.org/10.1007/s00216-011-5345-0.

Gene, S., Shahmohamadloo, R., Ortiz, X., Prosser, R., 2019. Effect of *Microcystis aeruginosa*–associated microcystin-LR on the survival of 2 life stages of freshwater mussel (*Lampsilis siliquoidea*). Environ. Toxicol. Chem. 38 (10), 2137–2144. https://doi.org/10.1002/etc.4527.

Grabarkiewicz, J.D., Davis, W.S., 2008. An Introduction to Freshwater Fishes as Biological Indicators, an Introduction to Freshwater Fishes as Biological Indicators. U.S. Environmental Protection Agency, Office of Environmental, Washington, D.C.

Guo, Y., O'Brien, A.M., Lins, T.F., Shahmohamadloo, R.S., Almirall, X.O., Rochman, C.M., Sinton, D., 2021. Effects of hydrogen peroxide on cyanobacterium Microcystis aeruginosa in the presence of nanoplastics. ACS ES&T Water 1 (7), 1596–1607. https://doi.org/10.1021/acsestwater.1c00090.

Hagenbuch, B., Meier, P.J., 2004. Organic anion transporting polypeptides of the OATP/SLC21 family: phylogenetic classification as OATP/SLCO superfamily, new nomenclature and molecular/functional properties. Pflügers Archiv 447 (5), 653–665. https://doi.org/10.1007/s00424-003-1168-y.

Harke, M.J., Steffen, M.M., Gobler, C.J., Otten, T.G., Wilhelm, S.W., Wood, S.A., Paerl, H.W., 2016. A review of the global ecology, genomics, and biogeography of the toxic cyanobacterium, *Microcystis spp*. Harmful Algae 54, 4–20. https://doi.org/10.1016/j.hal.2015.12.007.

Hwang, P.P., Lee, T.H., Lin, L.Y., 2011. Ion regulation in fish gills: recent progress in the cellular and molecular mechanisms. Am. J. Physiol. Regul. Integr. Comp. Physiol. 301 (1), R28–R47. https://doi.org/10.1152/ajpregu.00047.2011.

Jiang, J., Wang, X., Shan, Z., Yang, L., Zhou, J., Bu, Y., 2014. Proteomic analysis of hepatic tissue of Cyprinus carpio L. exposed to cyanobacterial blooms in Lake Taihu, China. PloS One 9 (2), e88211. https://doi.org/10.1371/journal.pone.0088211.

Jiang, Y., Yang, Y., Wu, Y., Tao, J., Cheng, B., 2017. Microcystin bioaccumulation in freshwater fish at different trophic levels from the eutrophic Lake Chaohu, China. (Report). Bull. Environ. Contam. Toxicol. 99 (1), 69–74. https://doi.org/10.1007/s00128-017-2047-0.

Komatsu, M., Furukawa, T., Ikeda, R., Takumi, S., Nong, Q., Aoyama, K., Akiyama, S., Keppler, D., Takeuchi, T., 2007. Involvement of mitogen-activated protein kinase signaling pathways in microcystin-LR–induced apoptosis after its selective uptake mediated by OATP1B1 and OATP1B3. Toxicol. Sci. 97 (2), 407–416. https://doi.org/10.1093/toxsci/kfm054.

Kotak, B.G., Semalulu, S., Fritz, D.L., Prepas, E.E., Hrudey, S.E., Coppock, R.W., 1996. Hepatic and renal pathology of intraperitoneally administered microcystin-LR in rainbow trout (*Oncorhynchus mykiss*). Toxicon 34 (5), 517–525. https://doi.org/10.1016/0041-0101(96)00009-8.

Krakstad, C., Herfindal, L., Gjertsen, B.T., Bøe, R., Vintermyr, O.K., Fladmark, K.E., Døskeland, S.O., 2006. CaM-kinaseII-dependent commitment to microcystin-induced apoptosis is coupled to cell budding, but not to shrinkage or chromatin hypercondensation. Cell Death Differ. 13 (7), 1191–1202. https://doi.org/10.1038/sj.cdd.4401798.

Kujbida, P., Hatanaka, E., Vinolo, M.A.R., Waismam, K., de Holanda Cavalcanti, D.M., Curi, R., Poliselli Farsky, S.H., Pinto, E., 2009. Microcystins-LA,-YR, and-LR action on neutrophil migration. Biochem. Biophys. Res. Commun. 382 (1), 9–14. https://doi.org/10.1016/j.bbrc.2009.02.009.

Lance, E., Neffling, M.R., Gerard, C., Meriluoto, J., Bormans, M., 2010. Accumulation of free and covalently bound microcystins in tissues of Lymnaea stagnalis (Gastropoda) following toxic cyanobacteria or dissolved microcystin-LR exposure. Environ. Pollut. 158 (3), 674–680. https://doi.org/10.1016/j.envpol.2009.10.025.

Le Manach, S., Sotton, B., Huet, H., Duval, C., Paris, A., Marie, A., Yépremian, C., Catherine, A., Mathéron, L., Vinh, J., Edery, M., Marie, B., 2018. Physiological effects caused by microcystin-producing and non-microcystin producing Microcystis aeruginosa on medaka fish: a proteomic and metabolomic study on liver. Environ. Pollut. 234, 523–537. https://doi.org/10.1016/j.envpol.2017.11.011.

Lynch, A.J., Cooke, S.J., Deines, A.M., Bower, S.D., Bunnell, D.B., Cowx, I.G., Nguyen, V.M., Nohner, J., Phouthavong, K., Riley, B., Rogers, M.W., Taylor, W.W., Woelmer, W., Youn, S., Douglas Jr., B.T., 2016. The social, economic, and environmental importance of inland fish and fisheries. Environ. Rev. 24 (2), 115–121. https://doi.org/10.1139/er-2015-0064.

Malbrouck, C., Kestemont, P., 2006. Effects of microcystins on fish. Environ. Toxicol. Chem. 25 (1), 72–86. https://doi.org/10.1897/05-029R.1.
Malécot, M., Mezhoud, K., Marie, A., Praseuth, D., Puiseux-Dao, S., Edery, M., 2009. Proteomic study of the effects of microcystin-LR on organelle and membrane proteins in medaka fish liver. Aquat. Toxicol. 94 (2), 153–161. https://doi.org/10.1016/j.aquatox.2009.06.012.
Marshall, W.S., 2002. Na+, Cl−, Ca2+ and Zn2+ transport by fish gills: retrospective review and prospective synthesis. J. Exp. Zool. 293 (3), 264–283. https://doi.org/10.1002/jez.10127.
Mehinto, A.C., Smith, J., Wenger, E., Stanton, B., Linville, R., Brooks, B.W., Howard, M. D., 2021. Synthesis of ecotoxicological studies on cyanotoxins in freshwater habitats–Evaluating the basis for developing thresholds protective of aquatic life in the United States. Sci. Total Environ. 148864. https://doi.org/10.1016/j.scitotenv.2021.148864.
Mohamed, Z., Ahmed, Z., Bakr, A., Hashem, M., Alamri, S., 2020. Detection of free and bound microcystins in tilapia fish from Egyptian fishpond farms and its related public health risk assessment. J. Consum. Protect. Food Saf. 15 (1), 37–47. https://doi.org/10.1007/s00003-019-01254-0.
Nong, Q., Komatsu, M., Izumo, K., Indo, H.P., Xu, B., Aoyama, K., Majima, H.J., Horiuchi, M., Morimoto, K., Takeuchi, T., 2007. Involvement of reactive oxygen species in Microcystin-LR-induced cytogenotoxicity. Free Radic. Res. 41 (12), 1326–1337. https://doi.org/10.1080/10715760701704599.
Ortiz, X., Korenkova, E., Jobst, K.J., MacPherson, K.A., Reiner, E.J., 2017. A high throughput targeted and non-targeted method for the analysis of microcystins and anatoxin-A using on-line solid phase extraction coupled to liquid chromatography–quadrupole time-of-flight high resolution mass spectrometry. Anal. Bioanal. Chem. 31, 4959–4969. https://doi.org/10.1007/s00216-017-0437-0.
Palikova, M., Kovářů, F., Navratil, S., Kubala, L., Pešák, S., Vajcova, V., 1998. The effect of pure microcystin LR and biomass of blue-green algae on selected immunological indices of carp (Cyprinus carpio L.) and silver carp (Hypophthalmichthys molitrix Val.). Acta Vet. 67 (4), 265–272.
Pearson, L., Mihali, T., Moffitt, M., Kellmann, R., Neilan, B., 2010. On the chemistry, toxicology, and genetics of the cyanobacterial toxins, microcystin, nodularin, saxitoxin and cylindrospermopsin. Mar. Drugs 8, 1650–1680. https://doi.org/10.3390/md8051650.
Peng, L., Liu, Y., Chen, W., Liu, L., Kent, M., Song, L., 2010. Health risks associated with consumption of microcystin-contaminated fish and shellfish in three Chinese lakes: significance for freshwater aquacultures. Ecotoxicol. Environ. Saf. 73 (7), 1804–1811. https://doi.org/10.1016/j.ecoenv.2010.07.043.
Phillips, M.J., Roberts, R.J., Stewart, J.A., Codd, G.A., 1985. The toxicity of the cyanobacterium Microcystis aeruginosa to rainbow trout, Salmo gairdneri Richardson. J. Fish. Dis. 8 (4), 339–344. https://doi.org/10.1111/j.1365-2761.1985.tb00953.x.
Poste, A., Hecky, R., Guildford, S., Poste, A., 2011. Evaluating microcystin exposure risk through fish consumption. Environ. Sci. Technol. 45 (13), 5806–5811. https://doi.org/10.1021/es200285c.
Reid, A.J., Carlson, A.K., Creed, I.F., Eliason, E.J., Gell, P.A., Johnson, P.T., Kidd, K.A., MacCormack, T.J., Olden, J.D., Ormerod, S.J., Smol, J.P., Taylor, W.W., Tockner, K., Vermaire, J.C., Dudgeon, D., Cooke, S.J., 2019. Emerging threats and persistent conservation challenges for freshwater biodiversity. Biol. Rev. 94 (3), 849–873. https://doi.org/10.1111/brv.12480.
Rudman, S.M., Greenblum, S.I., Rajpurohit, S., Betancourt, N.J., Hanna, J., Tilk, S., Yokoyama, T., Petrov, D.A., Schmidt, P., 2021. Direct observation of adaptive tracking on ecological timescales in Drosophila. bioRxiv. https://doi.org/10.1101/2021.04.27.441526.
Rudman, S.M., Rodriguez-Cabal, M.A., Stier, A., Sato, T., Heavyside, J., El-Sabaawi, R. W., Crutsinger, G.M., 2015. Adaptive genetic variation mediates bottom-up and top-down control in an aquatic ecosystem. Proc. Biol. Sci. 282 (1812), 20151234. https://doi.org/10.1098/rspb.2015.1234.
Schmidt, J.R., Wilhelm, S.W., Boyer, G.L., 2014. The fate of microcystins in the environment and challenges for monitoring. Toxins 6 (12), 3354–3387. https://doi.org/10.3390/toxins6123354.
Simmons, D.B., Bols, N.C., Duncker, B.P., McMaster, M., Miller, J., Sherry, J.P., 2012. Proteomic profiles of white sucker (*Catostomus commersonii*) sampled from within the Thunder Bay area of concern reveal up-regulation of proteins associated with tumor formation and exposure to environmental estrogens. Environ. Sci. Technol. 46, 1886–1894. https://doi.org/10.1021/es204131r.
Shahmohamadloo, R.S., Almirall, X.O., Holeton, C., Chong-Kit, R., Poirier, D.G., Bhavsar, S.P., Sibley, P.K., 2019. An efficient and affordable laboratory method to produce and sustain high concentrations of microcystins by *Microcystis aeruginosa*. Methods (Duluth) (6), 2521–2535. https://doi.org/10.1016/j.mex.2019.10.024.
Shahmohamadloo, R., Poirier, D., Ortiz Almirall, X., Bhavsar, S., Sibley, P., 2020a. Assessing the toxicity of cell-bound microcystins on freshwater pelagic and benthic invertebrates. Ecotoxicol. Environ. Saf. 188, 109945. https://doi.org/10.1016/j.ecoenv.2019.109945.
Shahmohamadloo, R.S., Simmons, D.B., Sibley, P.K., 2020b. Shotgun proteomics analysis reveals sub-lethal effects in *Daphnia magna* exposed to cell-bound microcystins produced by *Microcystis aeruginosa*. Comp. Biochem. Physiol. Genom. Proteonomics 33, 100656. https://doi.org/10.1016/j.cbd.2020.100656.
Shahmohamadloo, R.S., Ortiz Almirall, X., Simmons, D.B.D., Lumsden, J.S., Bhavsar, S. P., Watson-Leung, T., Vander Eyken, A., Hankins, G., Hubbs, K., Konopelko, P., Sanarcki, M., Strong, D., Sibley, P.K., 2021. Cyanotoxins within and outside of *Microcystis aeruginosa* cause adverse effects in rainbow trout (*Oncorhynchus mykiss*). Environ. Sci. Technol. https://doi.org/10.1021/acs.est.1c01501.
Shimizu, K., Sano, T., Kubota, R., Kobayashi, N., Tahara, M., Obama, T., Sugimoto, N., Nishimura, T., Ikarashi, Y., 2014. Effects of the amino acid constituents of microcystin variants on cytotoxicity to primary cultured rat hepatocytes. Toxins 6 (1), 168–179. https://doi.org/10.3390/toxins601016.
Smith, R.B., Bass, B., Sawyer, D., Depew, D., Watson, S.B., 2019. Estimating the economic costs of algal blooms in the Canadian Lake Erie Basin. Harmful Algae 87, 101624. https://doi.org/10.1016/j.hal.2019.101624.
Smith, J., Haney, J., 2006. Foodweb transfer, accumulation, and depuration of microcystins, a cyanobacterial toxin, in pumpkinseed sunfish (*Lepomis gibbosus*). Toxicon 48 (5), 580–589. https://doi.org/10.1016/j.toxicon.2006.07.009.
Soares, R., Magalhães, V., Azevedo, S., 2004. Accumulation and depuration of microcystins (cyanobacteria hepatotoxins) in *Tilapia rendalli* (Cichlidae) under laboratory conditions. Aquat. Toxicol. 70 (1), 1–10. https://doi.org/10.1016/j.aquatox.2004.06.013.
Sotton, B., Paris, A., Le Manach, S., Blond, A., Lacroix, G., Millot, A., Duval, C., Huet, H., Qiao, Q., Labrut, S., Chiappetta, G., Vinh, J., Catherine, A., Marie, B., 2017. Metabolic changes in Medaka fish induced by cyanobacterial exposures in mesocosms: an integrative approach combining proteomic and metabolomic analyses. Sci. Rep. 7 (1), 1–13. https://doi.org/10.1038/s41598-017-04423-z.
Sun, Y., Zheng, Q., Sun, Y.T., Huang, P., Guo, Z.L., Xu, L.H., 2014. Microcystin-LR induces protein phosphatase 2A alteration in a human liver cell line. Environ. Toxicol. 29 (11), 1236–1244. https://doi.org/10.1002/tox.21854.
Tencalla, F.G., Dietrich, D.R., 1997. Biochemical characterization of microcystin toxicity in rainbow trout (*Oncorhynchus mykiss*). Toxicon 35 (4), 583–595. https://doi.org/10.1016/S0041-0101(96)00153-5.
Tencalla, F.G., Dietrich, D.R., Schlatter, C., 1994. Toxicity of *Microcystis aeruginosa* peptide toxin to yearling rainbow trout (*Oncorhynchus mykiss*). Aquat. Toxicol. 30, 215–224. https://doi.org/10.1016/0166-445X(94)90059-0.
Welker, M., Steinberg, C., Jones, G.J., 2001. Release and persistence of microcystins in natural waters. Cyanotoxins: Occ. Causes, Conseq. 83–101.
Welten, R.D., Meneely, J.P., Elliott, C.T., 2020. A Comparative review of the effect of microcystin-LR on the proteome. Expos. Health 12 (2), 111–129. https://doi.org/10.1007/s12403-019-00303-1.
Wendelaar Bonga, S.E., 1997. The stress response in fish. Physiol. Rev. 77 (3), 591–625. https://doi.org/10.1152/physrev.1997.77.3.591.
Wilhelm, S.W., Bullerjahn, G.S., McKay, R.M.L., 2020. The complicated and confusing ecology of Microcystis blooms. mBio 11 (3). https://doi.org/10.1128/mBio.00529-20.
Wood, S.A., Briggs, L.R., Sprosen, J., Ruck, J.G., Wear, R.G., Holland, P.T., Bloxham, M., 2006. Changes in concentrations of microcystins in rainbow trout, freshwater mussels, and cyanobacteria in Lakes Rotoiti and Rotoehu. Environ. Toxicol.: Int. J. 21 (3), 205–222. https://doi.org/10.1002/tox.20174.
Wood, J.D., Franklin, R.B., Garman, G., McIninch, S., Porter, A.J., Bukaveckas, P.A., 2014. Exposure to the cyanotoxin microcystin arising from interspecific differences in feeding habits among fish and shellfish in the James River Estuary, Virginia. Environ. Sci. Technol. 48 (9), 5194–5202. https://doi.org/10.1021/es403491k.
World Health Organization, 2020. Cyanobacterial Toxins: Microcystins (No. WHO/HEP/ECH/WSH/2020.6). World Health Organization.
Zhang, D., Xie, P., Liu, Y., Qiu, T., 2009. Transfer, distribution and bioaccumulation of microcystins in the aquatic food web in Lake Taihu, China, with potential risks to human health. Sci. Total Environ. 407 (7), 2191–2199. https://doi.org/10.1016/j.scitotenv.2008.12.039.