ORIGINAL ARTICLE

Seasonal variation in the response to a toxin-producing cyanobacteria in *Daphnia*

Alexander Hegg[1] | Reinder Radersma[1,2] | Tobias Uller[1]

[1]Department of Biology, Lund University, Lund, Sweden

[2]Centrum Wiskunde & Informatica, Amsterdam, The Netherlands

Correspondence
Tobias Uller, Department of Biology, Lund University, Sölvegatan 37, 223 62 Lund, Sweden.
Email: tobias.uller@biol.lu.se

Abstract

1. Many populations of water fleas (*Daphnia*) are exposed to algal blooms dominated by microcystin-producing cyanobacteria. However, the severity of these effects on *Daphnia* fitness remain poorly understood in natural populations.
2. We investigated seasonal changes in body size, reproduction and survival of *D. longispina* individuals from five eutrophic lakes in southern Sweden. We tested whether individuals collected before, during or following algal blooms differed in their reproduction and survival when experimentally exposed to microcystin-producing cyanobacteria.
3. The concentration of microcystin in the lakes was significantly higher during summer and autumn compared to spring, but there were substantial differences between lakes. The reproductive output of individuals declined consistently over the season, and this decline was stronger for *Daphnia* collected during periods of, or from lakes with, high microcystin concentration. There was little evidence that individuals adapted to the toxin over the season.
4. The strong seasonal changes in body size, reproduction and survival in these *D. longispina* appear to be caused partly by variation in the abundance of toxin-producing cyanobacteria. Populations were unable to adapt sufficiently quickly during summer and autumn to recover from the negative effects of microcystin. We therefore suggest that seasonal increases in tolerance to microcystin-producing cyanobacteria have limited effects on the eco-evolutionary dynamics between *Daphnia* and phytoplankton.

KEYWORDS
adaptation, cyanobacteria, microcystin, seasonality, tolerance

1 | INTRODUCTION

Organisms commonly encounter environments that negatively impact their fitness, and many environmental stressors recur on a seasonal basis. Representative examples are temperate eutrophic lakes and ponds that experience seasonal algal blooms dominated by toxic cyanobacteria (O'Neil et al., 2012; Paerl & Huisman, 2008). Zooplankton that feed on phytoplankton, such as the non-selective grazer *Daphnia*, come into direct contact with cyanobacteria during algal blooms, which typically occur in mid- to late summer (Hansson et al., 2007; Sivonen et al., 1990; Sommer et al., 1986).

Cyanobacteria are known to affect *Daphnia* negatively in several different ways, including through their low nutritional

value, mechanical interference with the filtering apparatus and through the production of toxins (e.g., Ger et al., 2016; Porter & Mcdonough, 1984; Rohrlack et al., 2001; von Elert et al., 2003, 2012). One of the best-known groups of cyanotoxins are the microcystins, which consist of c. 100 variants (Carmichael, 1994; Dawson, 1998; de Figueiredo et al., 2004; Meriluoto et al., 2017). In *Daphnia*, both purified and cell-bound microcystins have been shown to be acutely toxic and decrease survival (e.g., DeMott et al., 1991; Rohrlack et al., 2001), growth rates (e.g., Lürling, 2003) and reproductive output (e.g., Gustafsson et al., 2005). Despite the documented negative effects of the toxins, several zooplankton, including *Daphnia*, show a remarkable ability to co-exist in environments dominated by bloom-forming algae, and some studies even suggest that *Daphnia* not only can cope with, but also effectively graze down and reduce toxic cyanobacteria, even at high toxin concentrations (e.g., Chislock et al., 2013; Sarnelle, 2007).

Daphnia populations that live in lakes with regular cyanobacteria blooms can evolve local adaptation to their toxins, including microcystin (Hairston et al., 2001; Sarnelle & Wilson, 2005). However, this does not fully explain seasonal changes in susceptibility to microcystin-producing cyanobacteria (Hansson, Gustafsson, et al., 2007). Instead, *Daphnia* populations may only develop tolerance to microcystin during periods of high concentration, and lose that tolerance during times of the year when exposure is low (Schaffner et al., 2019). Such adaptive seasonal change could involve selective elimination of unfit individuals, lineages or genotypes, or via some form of non-genetic inheritance. A recent study by Schaffner et al. (2019) showed that clones collected during an algal bloom had higher juvenile growth rates when fed phytoplankton typical to a bloom event compared to clones collected before the bloom, suggesting adaptive change within months. Other studies suggest that *Daphnia* can respond adaptively to microcystin-producing cyanobacteria via phenotypic plasticity, and that such responses can accumulate across at least two generations (Gustafsson & Hansson, 2004; Gustafsson et al., 2005, Ortiz-Rodriguez et al., 2012; but see Radersma et al., 2018).

Studies like these typically are designed to test for differences between genotypes, not individuals, which means that often only a limited number of clones are isolated and kept for several generations before being tested. However, genetic differences and plastic responses in *Daphnia* reared for long periods in the laboratory may not reflect well the absolute and relative performance of individuals before, during and after exposure to toxic cyanobacteria in a natural environment. In fact, there are very limited data on how *Daphnia* that come directly from natural populations actually respond to cyanotoxins, and it is unknown if these responses change across the season and following a bloom in a manner expected under adaptive change (but see Schwarzenberger et al., 2013).

The aim of this study was to fill this gap in knowledge. We sampled *Daphnia* from five lakes in spring, summer and autumn, and quantified the body-size distribution of the different populations. To test whether or not *Daphnia* from these lakes and across the season differed in their tolerance to microcystin, we quantified

reproduction and survival in a laboratory experiment in which we exposed individuals to cyanobacteria that either produce or do not produce the cyanotoxin microcystin. By measuring the concentration of microcystin in the five lakes, we also were able to assess if recent exposure to microcystin were associated with changes in body size, reproduction and survival.

Based on previous laboratory experiments (e.g., Gustafsson et al., 2005; Hansson, Gustafsson, et al., 2007; Radersma et al., 2018), we hypothesised that exposure to toxic cyanobacteria in the lakes would generally reduce body size, viability and reproduction. Thus, viability and reproductive fitness should be highest in spring (i.e., before blooms) and decline as the concentration of microcystin in the lakes increases. We also predicted that, if populations successfully adapt to toxic cyanobacteria (through genetic change or transgenerational plasticity), it would change the relative fitness of individuals on a toxic versus non-toxic diet. Specifically, the negative effects of the toxin on reproduction and survival relative to the controls should be smaller following the bloom (even if both reproduction and survival may be worse for individuals collected after the bloom than those collected before the bloom).

2 | METHODS

This study included five lakes in southern Sweden: Bysjön, Häljasjön, Västra Ringsjön (hereafter: Ringsjön), Sövdesjön and Vombsjön. The lakes are all eutrophic to hypereutrophic (Total-P > 35 μg/L) and relatively shallow (mean depth 2.7–5.9 m) but vary across a wide range in size (area 0.2–14.4 km^2). Several toxin-producing cyanobacteria species (e.g., *Microcystis* spp.) have been found previously in many of these lakes (http://miljodata.slu.se/mvm/ [27–05–2020]; for more detailed information of each lake, see Table S1).

2.1 | Lake sampling

All five lakes were sampled on five occasions at intervals of 5 weeks from 22 May to 13 October 2017. Sampling was conducted by boat at the same location each time. Water was collected each sampling event, and *Daphnia* every other sampling event. Pooled water samples were created by collecting water using a 2-L sampler at three different depths (1, 2 and 3 m) and mixed in a bucket. Two such pooled samples (biological replicates) were taken at every sampling event, and from each replicate one aliquot of 10 ml and one of 1 L were used for analysis of microcystin and chlorophyll, respectively. All water samples were transported under cool conditions (8–10°C) and samples for microcystin analyses were frozen upon arrival at the laboratory. Samples for chlorophyll were first filtered through a GF/C filter and then frozen.

Daphnia were sampled on three occasions: 22–25 May (hereafter: spring), 31 July–4 August (hereafter: summer) and 9–13 October (hereafter: autumn). They were collected from the entire water column (0–3 m) using a plankton net (25 cm diameter; 335 μm mesh size) and stored in two 1-L bottles. In one of the lakes (Vombsjön), we

were able to find *Daphnia* only during spring and autumn, and thus this lake has missing data for summer. In addition, autumn data from Bysjön are based on a sampling event 2 weeks after that of the other lakes (on 27 October) since the original sampling failed to capture *Daphnia*. This sampling event also lacks data on body-size distribution. *Daphnia* sampled from the lakes were used in the laboratory experiment described below.

Because species identification in *Daphnia* requires careful inspection, it could not be verified with full certainty for all individuals at the time of lake sampling. Following the laboratory experiment, we therefore examined a sample of approximately five *Daphnia* from each lake, sampling event and experimental treatment to verify that individuals in the experiment classified as *D. longispina*. Although this is perhaps best considered a species complex, we refer to all individuals as belonging to the same species since considerable morphological variation and phenotypic plasticity makes it difficult to distinguish between lineages visually (Dlouhá et al., 2010).

2.2 | Culturing of algae

All *Daphnia* in this study were fed the green algae *Scenedesmus obliquus* (NIVA CHL-6) (Culture collection of Algae, Norwegian Institute for Water research). Rather than testing for response to cyanobacteria, we contrasted the response to microcystin-producing cyanobacteria and cyanobacteria that do not produce microcystin. Microcystin is one of the most potent and important cyanotoxins, and is associated with toxic algal blooms in these lakes (e.g., Hansson, Gustafsson, et al., 2007). Adaptive transgenerational plasticity and genetic variation in tolerance to microcystin have been studied extensively in the laboratory (Gustafsson & Hansson, 2004; Gustafsson et al., 2005; Lürling, 2003). We therefore used two strains of the cyanobacterium *Microcystis aeruginosa* in the laboratory exposure experiment: NIVA CYA-143 and NIVA CYA-228/1. One strain (CYA-143) does not produce any microcystin, and the effects on fitness under our laboratory conditions are small or absent (at least in *Daphnia magna*; Radersma et al., 2018). The other strain (CYA-228/1) produces different variants of microcystins, among them microcystin-LR (Lürling, 2003), and is known to reduce fitness in *Daphnia* (e.g., Gustafsson et al., 2005; Radersma et al., 2018). Since our main purpose was to test the effects of the toxin microcystin, we refer to the strains as toxic (CYA-228/1) and non-toxic (CYA-143), even though both strains produce other compounds that may be toxic to *Daphnia* (e.g., Lürling, 2003). Both strains have similarly sized cells and did not show any indication of colony formation during the experiments. Algal cultures were grown in 50-ml sterile cell culture flasks using Z8 medium (20°C; illumination: 30–40 μmol m^{-2} s^{-1}) during a 12 hr:12 hr, light:dark photoperiod. To control for variation in toxin production, we kept several replicate cultures which were maintained in the exponential growth phase, and only fresh cyanobacteria cultures that had just reached the stationary phase were fed to *Daphnia*.

2.3 | Acclimation and isolation of Daphnia

Collected *Daphnia* were allowed to adjust to laboratory temperature for approximately 1 hr before further handling. Culture conditions and food regime followed previous work (Radersma et al., 2018). In brief, all *Daphnia* in this study were kept at 18°C, with a 14 hr:10 hr, light:dark photoperiod. One hundred individuals from each lake, judged to be sufficiently large to be reproductively mature, were individually transferred to separate 100-ml jars filled with oxygenated tap water and 120,000 cells/ml of frozen green algae (CHL-6). At the same time, another c. 100 individuals were picked out and preserved in 95% ethanol. A subsample of these individuals then were used to quantify the body size distribution, measured as the distance from the middle of the eye to the base of the spine of 20 individual *Daphnia* from each population and sampling event.

After 2 days of acclimation, 60,000 cells/ml of frozen green algae (CHL-6; a standard food in *Daphnia* research) were added to the jars, and the number of surviving individuals was recorded (initial survival; see below). After another 2 days (4 days after isolation) the water was changed and 120,000 cells/ml was added. Jars with reproducing individuals were marked and any jars with dead *Daphnia* were discarded. The acclimation time reduced mortality following transfer to laboratory conditions and ensured that only reproducing individuals in good condition entered the microcystin exposure experiment (see below). After a total of 7 days of acclimation, 40 actively reproducing *Daphnia* (individuals that had reproduced or carried eggs) were selected randomly from each lake and distributed equally over the two treatments (toxic and non-toxic; see below).

2.4 | Experimental exposure to microcystin-producing cyanobacteria

At the start of the laboratory experiment, individual *Daphnia* were transferred to 100-ml jars filled with artificial lake water (ADaM) (Klüttgen et al., 1994) and fed 120,000 cells/ml frozen green algae (CHL-6), and 70,000 cells/ml of either live toxic (CYA-228/1) or live non-toxic (CYA-143) *M. aeruginosa*. We tracked reproductive output (number of offspring produced) and survival of each individual for a total of 24 days. Every other day, offspring produced were discarded and each individual was transferred to a new jar containing fresh medium and algae (CHL-6 + CYA-143 or CYA-228/1). This allowed us to maintain toxicity and food levels approximately constant throughout the duration of the experiment.

2.5 | Microcystin and chlorophyll-a analysis

Toxicity levels were analysed for both lake water (two samples per sampling event) and medium used in the laboratory experiment described above (the latter to make sure that experimental exposure remained the same across the season). We analysed the

total microcystin (free and cell-bound) using an enzyme-linked immunosorbent assay (ELISA) kit (Eurofins Abraxis, USA), according to the manufacturer's specifications. The chlorophyll samples (two samples per sampling event) were extracted from the GF/C filters soaked in 95% ethanol overnight and then chlorophyll-a levels were determined spectrophotometrically (Jespersen & Christoffersen, 1987).

These analyses confirmed that the microcystin concentration in the laboratory experiment (using the toxic strain CYA-228/1) ranged from 2 to 4 μg/L and did not vary over the course of the study. Thus, all *Daphnia* were exposed to similar toxin levels during the experiments, which was comparable to concentrations in Vombsjön and Sövdesjön during summer and autumn (Figure 1a; see Results).

2.6 | Data analysis

All data were analysed using R v.3.6.2 (R Core Team, 2019). To determine whether or not the body-size distribution and initial survival of the different *Daphnia* populations changed over the season, we fitted a linear and generalised linear model, respectively, with season and lake as fixed effects.
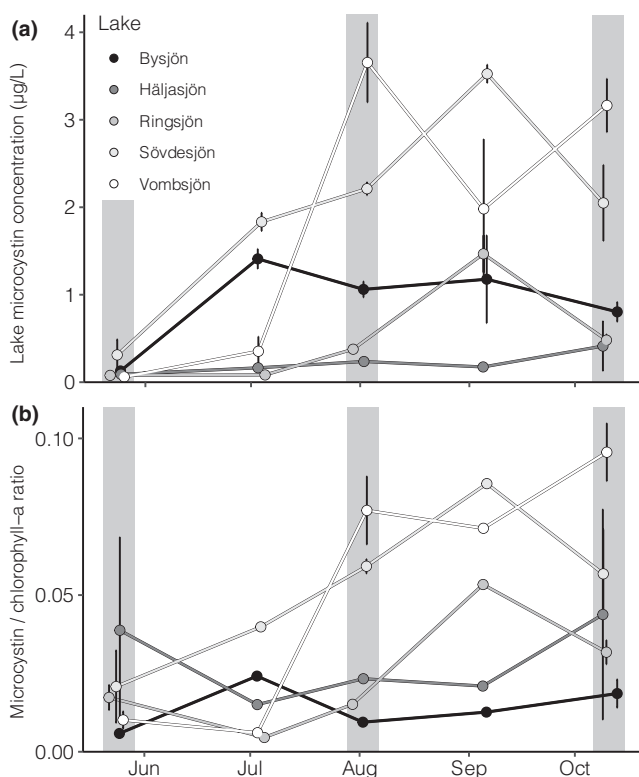

FIGURE 1 (a) Microcystin concentration (μg/L) and (b) microcystin / chlorophyll-a ratio (μg/L) at each sampling event, from 22 May to 13 October 2017, in the five investigated lakes. *Daphnia* were sampled in late May (Spring), late July/early August (Summer) and October (Autumn). The grey vertical lines further highlight the sampling events during which *Daphnia* were sampled. Data are based on two replicates per sampling event and error bars are ±SE

Reproductive output during each microcystin exposure experiment was measured as the total number of offspring produced between Day (D)2 and D24 (i.e., 22 days in total). Discarding the first 2 days effectively excluded offspring that were developing as eggs before the experimental treatment (under our laboratory conditions, *D. longispina* have a brood interval of approximately 2–3 days). Because the total number of offspring included a high number of zeros, we used zero-inflated generalised linear mixed models (GLMMs) ('glmmTMB' package in R; Brooks et al., 2017). Zero-inflated models fitted the data to two processes: one binary process that determines whether the value is zero or comes from the second process, in which the value is drawn from a specific distribution, typically a Poisson or negative binomial distribution. Here, these amount to whether or not an individual reproduced and, if it did, how many offspring it had. A first data exploration demonstrated that a negative binomial (both linear and quadratic parameterisation) distribution was superior to a Poisson distribution, and all model comparisons in the Results therefore rely on the former. The full model for the lake samples included treatment, season and treatment × season as fixed effects, and lake as a random effect for both zero-inflated (reproducing/not reproducing) and conditional (number of offspring if reproducing) models. We fitted simpler models by successively dropping terms and used corrected Aikaike Information Criteria (AICc) to retain the model with the best overall fit to the data. When two models demonstrated similar fit, we opted for the more parsimonious of the two and/or the one with less overdispersion.

Survival during the experiment was measured as the time until death during the 24 days of the laboratory experiment. Mixed effects cox models ('survival' and 'coxme' packages in R; Therneau, 2020) were used to fit models of survival. We included the same fixed and random effects as in the two models of reproductive output described above.

In order to investigate whether or not the seasonal variation in *Daphnia* fitness traits could be explained by recent exposure to microcystin, we estimated the recent levels of exposure by calculating for each lake the average concentrations of microcystin of the current and the previous (5 weeks before) sampling event. Since microcystin enters *Daphnia* through the algal cells that they consume, we also calculated the average microcystin per unit chlorophyll-a (hereafter: m:c ratio), which is an alternative, and potentially superior measurement of the actual toxin concentration experienced by individuals (Hansson, Gustafsson, et al., 2007). Since microcystin and chlorophyll-a were measured per sampling event, these analyses used the averaged body size, survival and number of offspring of *Daphnia* per sampling event and lake. Because season, microcystin and m:c ratio are highly correlated, we fitted models with one predictor at the time and used AICc to evaluate the best fit. The average reproductive output was log transformed before analyses. Reproductive output was analysed using linear models and survival using a GLM with binomial distribution and a logit link function. Throughout, all statistical tests for ANOVA refer to type III (for models with interactions) or type

II (without interactions). Figures were plotted using the package R/ ggplot2 (Wickham, 2016).

3 | RESULTS

The microcystin concentration of the lake water showed temporal variation both within and among the lakes. In general, microcystin was nearly undetectable in late spring but increased to moderate or high levels (2–4 μg/L) in summer and autumn (Figure 1a). Häljasjön deviated from this pattern by having a consistently low microcystin concentration throughout the season. Chlorophyll-a concentrations increased over the season in all lakes, and Bysjön showed consistently higher chlorophyll levels compared to the other lakes, which in turn resulted in a very low m:c ratio despite moderate levels of microcystin (Figure 1b).

3.1 | Body size and survival of individuals collected from the lakes

Overall, *Daphnia* body size differed between seasons ($F_{2,247} = 72.6$, $p < 0.001$) and lakes ($F_{4,247} = 14.2$, $p < 0.001$). Individuals were largest in spring (mean = 1.18 mm, $SD = 0.17$, $N = 100$), smallest in summer (mean = 0.92 mm, $SD = 0.14$, $N = 79$) and intermediate in autumn (mean = 1.01 mm, $SD = 0.21$, $N = 76$;

Figure 2a). Survival of *Daphnia* during the first 2 days of acclimation also differed between seasons ($\chi^2_2 = 141.1$, $p < 0.001$) and lakes ($\chi^2_4 = 18.8$, $p = 0.001$), and followed the same pattern as body size, being highest in spring, lowest in summer, and intermediate in autumn (Figure 2b).

3.2 | Responses to experimental exposure to microcystin-producing cyanobacteria

Fewer *Daphnia* reproduced in the laboratory experiment as the season progressed (spring: 89.4%; summer: 84.5%; autumn: 76.2%), and those that did reproduce produced fewer offspring (Table 1; Figure 3). There also was a strong negative effect of the microcystin treatment on reproductive output, but no significant effect on the likelihood to reproduce (Table 1; see Table S2 for a ranking of the top five models). *Daphnia* collected in autumn appeared to sustain reproduction better when exposed to microcystin than *Daphnia* collected in spring, but overall there was only a just not significant interaction between treatment and season (Figure 3; Table S2; treatment ×season: $\chi^2_1 = 5.6$, $p = 0.061$; see Table S3 for the full output of the model including the interaction between treatment and season). Survival of *Daphnia* sampled from the lakes was lower in the toxic compared to the non-toxic treatment ($\chi^2_1 = 22.1$, $p = 0.001$; Figure 4), but there was neither a seasonal effect, nor an interaction between season and microcystin exposure ($p > 0.05$).
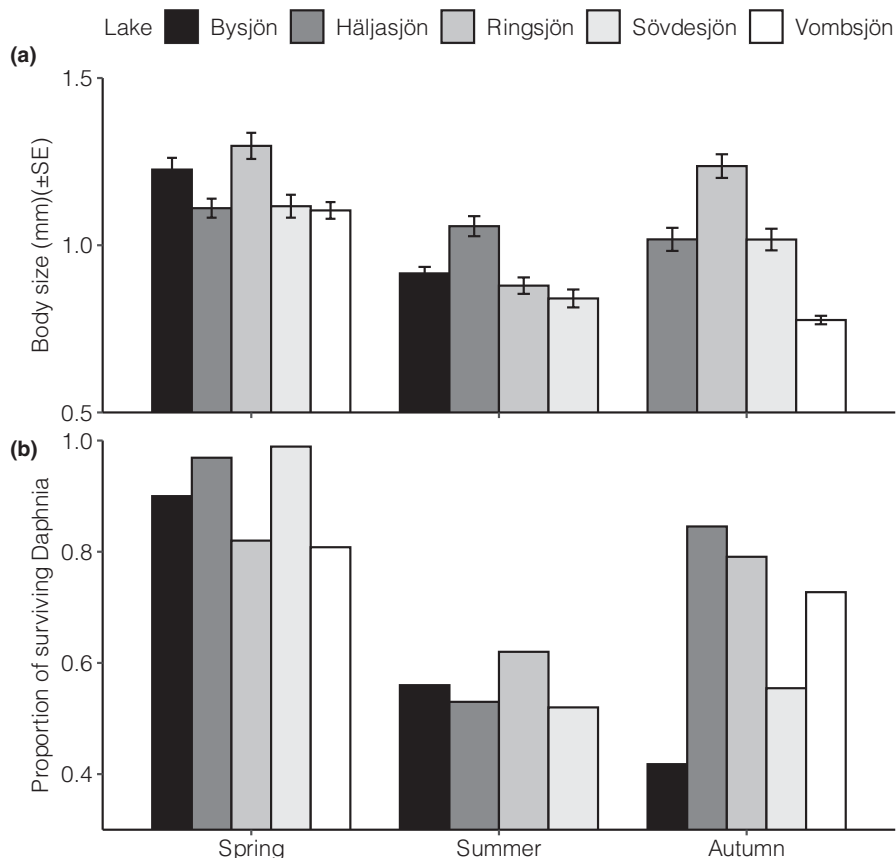

FIGURE 2 (a) Body size (mm) of *Daphnia* sampled from each of the lakes in spring, summer and autumn. Error bars are ±*SE*. See text for sample size. (b) Initial survival (measured as the proportion of c. 100 isolated individuals that survived 2 days of acclimation) for each population in spring, summer and autumn

Model part	Predictor	Estimate	SE	Z	p
Conditional	Intercept	3.307	0.089	37.270	**0.001**
	Treatment	−1.218	0.084	−14.580	**0.001**
	Season (Summer)	−0.574	0.110	−5.240	**0.001**
	Season (Autumn)	−0.824	0.101	−8.160	**0.001**
Zero-inflated	Intercept	−4.908	1.894	−2.591	**0.010**
	Season (Summer)	0.793	0.678	1.169	0.242
	Season (Autumn)	1.928	0.591	3.263	**0.001**

TABLE 1 Output of the zero-inflated negative binomial mixed model on reproduction in the microcystin exposure experiment, including both conditional (number of offspring if reproducing) and zero-inflated (reproducing/not reproducing) model parts of *Daphnia* sampled from the lakes

Note: The conditional part includes the main effects of toxic treatment (Treatment) and season (Season summer and Season autumn), and the zero-inflated part includes the main effects of season (Season summer and Season autumn). Intercept represents non-toxic treatment and spring. Total sample size was $n = 503$ (5 lakes ×3 seasons ×2 treatments ×20 individuals). See Table S2 for model comparisons, and Table S3 for a model that includes the interaction term in the conditional part of the model.

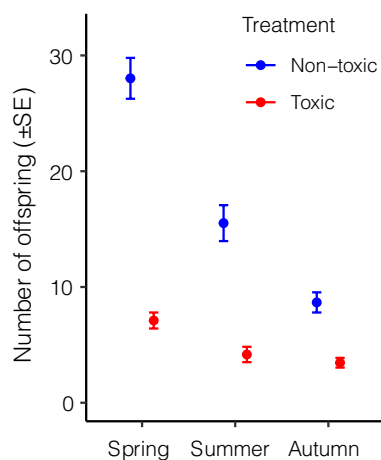Bold values indicates the significant *p*-values.

FIGURE 3 Average total number of offspring produced after 22 days of exposure in both the toxic (red) and non-toxic (blue) treatments, and comparisons of *Daphnia* sampled from the lakes in spring, summer and autumn. Error bars are $\pm SE$

3.3 | Relationship between fitness variables and lake microcystin

Daphnia sampled from the lakes following a period of high exposure to microcystin were significantly smaller ($F_{1, 11} = 6.8, p < 0.05$), but this statistical model (i.e., including microcystin concentration) did not perform substantially better than the models with season or the m:c ratio ($\Delta AICc = 1.4$ and 3.0, respectively; Table S4). The initial survival of *Daphnia* during the acclimation phase, before they entered the laboratory experiment, was strongly affected by season ($\chi_2^2 = 140.4, p < 0.001$), which performed significantly better than models with either microcystin or the m:c ratio ($\Delta AICc > 80$; Table S4).

The model that best explained average reproductive output included season: as demonstrated above, *Daphnia* produced fewer offspring as the season progressed (Figure 3). However, the model with m:c ratio alone received a similar level of support ($\Delta AICc = 2.5$;

Figure 5; Table S4), also indicating that reproduction was lower in *Daphnia* collected following a high exposure to microcystin through feeding ($F_{1, 25} = 12.8, p < 0.01$).

The corresponding results for survival during the course of the experiment revealed that the m:c ratio model performed the best (Table S4), with lower survival for *Daphnia* collected when the ratio between microcystin and chlorophyll was high ($\chi_1^2 = 3.8, p = 0.05$).

4 | DISCUSSION

Lakes are known to undergo changes in biotic and abiotic conditions from spring to autumn, and this can have a negative effect on population density of zooplankton (Sommer et al., 1986, 2012). However, how this is reflected at the level of individual growth, survival and reproduction remains poorly understood. In the present study of five eutrophic lakes, water fleas (*D. longispina*) collected from the water column showed a dramatic reduction in reproductive output over the season, a decrease in fitness that coincided with exposure to the cyanotoxin microcystin. At the same time, there was little evidence that individuals collected during or following exposure to high concentrations of microcystin had increased tolerance to the toxin. We therefore suggest that seasonal adaptation or acclimation to cyanotoxins is unlikely to have a substantial impact on the dynamics of the *Daphnia* populations in these lakes.

There was a steep decline in reproductive output in individuals collected in spring compared to those collected in autumn, both in the likelihood of reproduction and in the number of offspring produced. In addition to this seasonal change in reproduction, we also found that *Daphnia* sampled from the lakes in summer and autumn were smaller, and had lower viability than individuals sampled in spring. These results demonstrate that the absolute fitness of *Daphnia* (under laboratory conditions) strongly decreases over the season. The seasonal decline in body size and changes in life history could be part of an adaptive life history, or be the result of an inability to adapt to the changing environment. These questions warrant further study, and it would be particularly useful to understand if responses

FIGURE 4 Proportion of surviving *Daphnia* over 24 days of exposure in both the toxic (red) and non-toxic (blue) treatments. Proportions are based on the total number of individual from all lakes together and plotted for each season separately
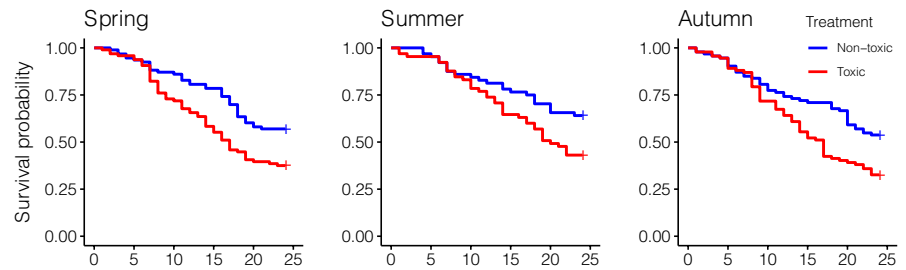
vary between species. Changes in ecosystems also can affect size-dependent predation by fish, which could be partly responsible for a seasonal shift in the body-size distribution (Brooks & Dodson, 1965). Furthermore, many biotic and abiotic variables are likely to exercise direct negative effects on individual growth, reproduction and survival. These include high temperature (e.g., Yampolsky et al., 2014), UV-radiation exposure (e.g., Hansson et al., 2007) or poor food quantity or quality (e.g., Vanni & Lampert, 1992). For example, cyanobacteria have low nutritional value and produce a suite of toxic compounds that compromise growth and fitness.

The seasonal changes in *Daphnia* body size, reproduction and survival were quite consistent between the five lakes (i.e., populations), suggesting that the causes of this pattern are general too. However, there also were some differences in the strength of this seasonal pattern, which may reflect a direct impact of local variation in biotic and abiotic conditions over the season. Here, we were particularly concerned with microcystin, a cyanotoxin known to have negative effects on fitness (DeMott et al., 1991; Lürling, 2003; Rohrlack et al., 2001; Sarnelle et al., 2010), but also a stressor to which *Daphnia* may be able to acclimatise or adapt over the course of the season (Gustafsson et al., 2005; Hansson, Gustafsson, et al., 2007). Although all lakes experience increases in microcystin in summer and early autumn, the intensity of the blooms varied between lakes herein. Our statistical analyses support the idea that the variation in *Daphnia* performance could, in part, have been caused by direct negative effects of exposure to microcystin. The ratio between microcystin and chlorophyll proved to be a particularly good fit to the reproductive output and viability data, a result consistent with the observation that *Daphnia* are exposed to cyanotoxins primarily through their non-selective feeding (e.g., Kirk & Gilbert, 1992). These negative fitness effects also mirror the direct negative effect of microcystin observed in the laboratory (DeMott et al., 1991; Gustafsson et al., 2005; Lürling, 2003; Radersma et al., 2018 for a meta-analysis), including the effects that we observed in our own experiments. We therefore suggest that the microcystin levels in these lakes are sufficiently high to contribute to the observed seasonal shift in body size, viability and reproduction.

Since exposure to microcystin-producing cyanobacteria reduces fitness, selection during blooms should remove individuals, lineages and genotypes with particularly low tolerance. Indeed, there is some evidence that clones isolated from lakes with a history of cyanobacteria blooms are more tolerant to cyanobacteria than clones from lakes with no history of blooms (Sarnelle & Wilson, 2005). Since selection on tolerance to microcystin and other toxins is likely to vary
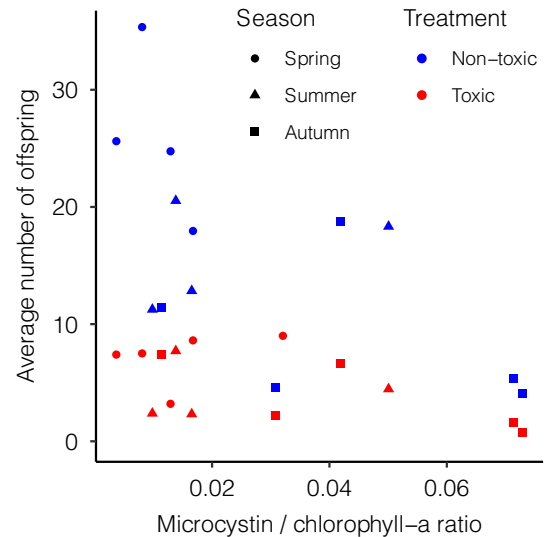

FIGURE 5 Relationship between the average lake microcystin / chlorophyll-a ratio of the past 5 weeks since sampling and the average total number of offspring in the microcystin exposure experiment of *Daphnia* in both toxic (red) and non-toxic (blue) treatments, collected in spring (circles), summer (triangles) and autumn (squares)

on both an annual and a seasonal basis, populations may harbour sufficient standing genetic variation to allow an adaptive genetic response from spring to autumn (Schaffner et al., 2019). Adaptive responses to seasonally occurring stressors also can occur via cumulative transgenerational effects, which have been suggested to be relevant for how *Daphnia* adapt (or acclimatise) to microcystin (e.g., Gustafsson & Hansson, 2004). We therefore hypothesised that *Daphnia* sampled after high exposure to microcystin, particularly in autumn, should show a relative increase in fitness when exposed to the toxin in our laboratory experiment. This relative increase in fitness on a toxic diet should be evident as a non-parallel relationship between fitness and season for the two experimental treatments, in addition to the consistent seasonal decline in absolute fitness. Exposure to microcystin in the laboratory did not appear to influence the likelihood that an individual reproduced, but it had a dramatic effect on the number of offspring produced over 3 weeks. However, individuals sampled in summer and autumn (i.e., during or following blooms) reproduced only marginally better on a diet including microcystin-producing cyanobacteria compared to individuals sampled in spring, before the microcystin concentration in the lakes increased.

It is difficult to know how to interpret the biological relevance of this marginal effect. However, given the overall low reproductive output in individuals collected in autumn, and that the negative effect of microcystin exposure on survival was consistent from spring to autumn, the overall effect on individual fitness is at best small. The results do not rule out some degree of adaptive plasticity or genetic change, however, because adaptive responses could be masked by negative counter-changes in the environment that exaggerate the negative effects of microcystin in summer and autumn. Further studies of reaction norms of genotypes collected over the season and kept under standardised conditions for several generations would be needed to test if this is the case (for general discussion on how to disentangle adaptive and non-adaptive responses that occur jointly, see Engqvist & Reinhold, 2016; Nettle & Bateson, 2015; Radersma et al., 2018). Nevertheless, our results do not support a restoration of fitness to the extent suggested by previous laboratory experiments (Gustafsson & Hansson, 2004; Gustafsson et al., 2005; Ortiz-Rodriguez et al., 2012; Schwarzenberger & Von Elert, 2013).

Why did we not see a more marked increase in tolerance to microcystin in this study? One possibility is that adaptive responses to microcystin in nature are specific to particular variants of microcystin. Another possibility is limited genetic variation. Schaffner et al. (2019) suggested that fluctuating selection within and between years results in high levels of genetic variation, and that this can explain the rapidly improved growth in response to cyanobacteria they observed. However, other studies have failed to detect tolerance differences between *D. magna* clones collected in spring (before a bloom peak) versus autumn (Kuster et al., 2013). Schwarzenberger et al. (2013) tested, in one of the lakes included in the present study (Lake Bysjön), whether clonal succession of *D. magna* was associated with seasonal changes in protease inhibitors (von Elert et al., 2012), but found no evidence of a change in tolerance. The authors suggested that this may the consequence of a lack of genetic variation and that the *Daphnia* population was already well-adapted to the toxins (see also Schwarzenberger et al., 2017, 2020). Unfortunately, the extent of relevant standing genetic variation, and if it hinders further adaptive change, is poorly known. Furthermore, few *Daphnia* populations have been studied in detail with respect to population genetic change across the season (but see, e.g., Steiner & Nowicki, 2019). Such studies could reveal whether or not there is opportunity, and evidence, for selective sweeps during environmental stress. More generally, the interactions between within-seasonal variation in selection and long-term evolution remain poorly understood. For *Daphnia* and cyanobacteria, one obvious missing link is the cost of tolerance during the years or parts of the season when cyanobacteria are present at very low levels. In fact, *Daphnia* populations that struggle to persist in a lake environment that is dominated by toxic cyanobacteria may reflect an inability to adapt, making the seasonal nature of algal blooms an interesting case of conditions that can allow persistence of maladaptation despite strong recurrent selection (Brady et al., 2019).

In summary, it appears that *D. longispina* populations are unable to adapt sufficiently quickly during summer and autumn to recover from the negative effects of toxic cyanobacteria. We therefore suggest that seasonal increase in the tolerance to toxin-producing cyanobacteria will have limited effects on the seasonal eco-evolutionary dynamics between *Daphnia* and phytoplankton in these lakes.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR'S CONTRIBUTIONS

A.H., R.R. and T.U. designed the study; A.H. performed the experiments; A.H., R.R. and T.U. analysed the data; and A.H. wrote the first draft of the manuscript. All authors participated in discussions and editing of the manuscript.

DATA AVAILABILITY STATEMENT

Data are available from the Zenodo Digital Repository, https://doi.org/10.5281/zenodo.6279105.

ORCID

Alexander Hegg https://orcid.org/0000-0003-4794-8352
Reinder Radersma https://orcid.org/0000-0001-8186-6348
Tobias Uller https://orcid.org/0000-0003-1293-5842

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