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Blooming cyanobacteria alter water flea reproduction via exudates of estrogen analogues

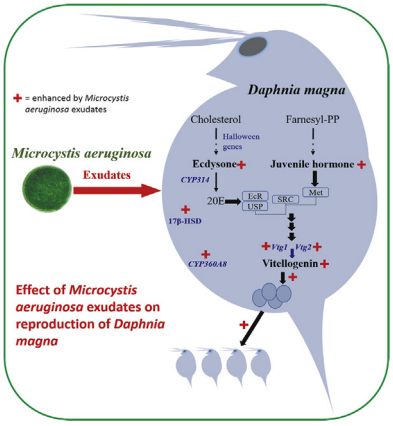
Runbing Xu [a,1], Yao Jiang [a,d,1], Hugh J. MacIsaac [a,b], Liqiang Chen [c], Jingjing Li [a], Jun Xu [a], Tao Wang [a], Yuanyan Zi [a], Xuexiu Chang [a,b,*]

[a] School of Ecology and Environmental Science, Yunnan University, Kunming 650091, PR China
[b] Great Lakes Institute for Environmental Research, University of Windsor, Windsor, ON N9B 3P4, Canada
[c] Institute of International Rivers and Eco-security, Yunnan Key Laboratory of International Rivers and Trans-boundary Eco-security, Yunnan University, Kunming 650091, PR China
[d] Xi'an Institute for Innovative Earth Environment Research, Xi'an 710061, PR China

HIGHLIGHTS

- Whether/how blooming cyanobacteria induce reproductive disorder of aquatic animals remains unclear.
- Effect of *Microcystis* exudates on *Daphnia* reproduction were addressed.
- Physiological, biochemical and molecular characteristics of *Daphnia* were analyzed via both chronic and acute exposures.
- *Microcystis* stimulated 17β-HSD, ecdysone, juvenile hormone, and vitellogenin biosynthesis in *Daphnia*.
- This paper provides insights into novel risks of cyanobacterial blooms via estrogenic effects based upon in vivo trials.

GRAPHICAL ABSTRACT



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ABSTRACT

Cyanobacteria blooms are increasing globally, with further increases predicted in association with climate change. Recently, some cyanobacteria species have been identified as a source of estrogenic effects in aquatic animals. To explore possible estrogenic effects of *Microcystis aeruginosa* (an often-dominant cyanobacteria species) on zooplankton, we examined effects of cyanobacteria exudates (MaE, 2×10^4 and 4×10^5 cells/ml) on reproduction in *Daphnia magna*. We analyzed physiological, biochemical and molecular characteristics of exposed *Daphnia* via both chronic and acute exposures. MaE at both low and high cell density enhanced egg number (15.4% and 23.3%, respectively) and reproduction (37.7% and 52.4%, respectively) in *D. magna* similar to 10 μg/L estradiol exposure. In addition, both MaE of low and high cell densities increased population growth rate (15.8% and 19.6%, respectively) and reproductive potential (60% and 83%, respectively) of *D. magna*. These exudates promoted *D. magna* reproduction by stimulating 17β-hydroxysteroid-dehydrogenase (17β-HSD) activity and production of ecdysone and juvenile hormone, and by enhancing vitellogenin biosynthesis via up-regulating expression of *Vtg1* and *Vtg2*. However, increased expression (6.6 times higher than controls) of a detoxification gene *(CYP360A8)* indicated that MaE might also induce toxicity in *D. magna*. Reproductive interference of zooplankton

* Corresponding author at: School of Ecology and Environmental Science, Yunnan University, Kunming 650091, PR China.
E-mail addresses: hughm@uwindsor.ca (H.J. MacIsaac), chenlq@ynu.edu.cn (L. Chen), changxx@ynu.edu.cn (X. Chang).
[1] Runbing Xu and Yao Jiang contributed equally to this work.

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Endocrine disorder
Daphnia magna

by blooming cyanobacteria might negatively affect foodwebs because MaE-induced zooplankton population increase would enhance grazing and reduce abundance of edible algae, thereby adding to the list of known disruptive properties of cyanobacterial blooms.

1. Introduction

Cyanobacteria blooms are increasing in frequency and severity on a worldwide basis, with further increases predicted in association with climate change (Paerl and Paul, 2012; Visser et al., 2016). Severe cyanobacterial blooms have been observed in all of the Great Lakes in North America (Birbeck et al., 2019; Bullerjahn et al., 2016; Newell et al., 2019; Wynne and Stumpf, 2015), Lake Victoria in Africa (Simiyu et al., 2018), the Baltic Sea in Northern Europe (Suikkanen et al., 2007), Lake Biwa in Japan (Nalewajko and Murphy, 2001), lakes Taihu and Dianchi in China (Shan et al., 2019), and many other lakes globally (Cirés and Ballot, 2016; Moorhouse et al., 2018). These hazards increase individually or in combination with other environmental stressors (Loick-Wilde et al., 2019; Richardson et al., 2018, 2019). Review of publications reveals a clear increase in publications related to cyanobacteria blooms in general, and *Microcystis* – a common bloom-forming genus – in particular (see in Fig. 1). Dense cyanobacterial blooms in lakes and reservoirs can produce diverse bioactive compounds that when released are toxic to green algae, macrophytes, zooplankton and fishes (Chia et al., 2018; Song et al., 2017; Whitmore et al., 2018; Zi et al., 2018). The production of toxins by bloom-forming cyanobacteria can lead to drinking water crises, such as the one experienced by the city of Toledo, Ohio in August 2014, when the city shut off its drinking water supply for >2 days owing to a cyanobacteria bloom in Lake Erie (Steffen et al., 2017).

Cyanotoxins such as microcystins, anatoxins and cylindrospermopsin, are the most widely studied cyanobacterial metabolites (Corbel et al., 2014). Recently, however, estrogenic effects were reported for cyanobacteria species (Prochazkova et al., 2018; Smutná et al., 2014; Sychrová et al., 2012), beyond that more commonly linked to organic pollutants (Bertram et al., 2018; Wang et al., 2016a; Yu et al., 2019). For instance, estrogenic activity of cyanobacterial hepatotoxins microcystin-LR and nodularin-R was demonstrated in vitro in a stably-transfected cell line with an estrogen-regulated luciferase gene (Oziol and Bouaïcha, 2010). Gong et al. (2014) observed a correlation between estrogenicity of sea water and phytoplankton concentration and reported estrogenic activity of two isolated phytoplankton species using human cell-based bioassays. Štěpánková et al. (2011) demonstrated using in vitro reporter gene trans-activation assays that both single- and mixed-species of cyanobacteria collected from the environment contained compounds with the potential for interaction with signaling pathways of estrogen receptors. Finally, Sychrová et al. (2012) assessed estrogenic activity in extracts and exudates of cyanobacteria by use of in vitro trans-activation assays. However, the nature and consequences of exposure to estrogenic compounds released by cyanobacteria to co-occurring animals remains unknown.

Estrogens are involved in various processes linked to reproduction (metabolic, morphological and behavioral changes) and development via estrogen receptor (ER) signaling (Korach et al., 2019; Miah et al., 2019). Disruption of estrogen signaling leads to impaired gonadal development, feminization, alteration of the sex ratio and inhibition of metamorphosis (Orton and Tyler, 2015; Söffker and Tyler, 2012). Other disorders in which neurohormonal regulation plays a role have also been associated with disruption by estrogenic compounds, including altered immunity, metabolism, and tissue homeostasis among others (Casals-Casas and Desvergne, 2011; Khan and Ansar Ahmed, 2016). Phytoestrogens have lower estrogenic potency than animal hormones, but they can reach relatively high concentrations in rivers (Hoerger et al., 2009; Jarošová et al., 2015). Therefore, their contributions to estrogenic effects should not be overlooked, especially considering the discovery of phytoestrogens in blooming cyanobacteria (Goiris et al., 2014; Klejdus et al., 2010; Procházková et al., 2017). While previous studies have demonstrated that cyanobacteria toxins could induce reproductive endocrine disorders in animals (Liu et al., 2018) or have estrogenic potency (Hou et al., 2018), and may accumulate organic pollutants from water to act as sink and source of endocrine disruptors (Jia et al., 2019), few studies have addressed the presence and role of other non-toxin, estrogenic compounds produced by blooming cyanobacteria (Procházková et al., 2017).

Zooplankton play important roles in aquatic ecosystems and their response to environmental changes can cascade to other elements of the food web (Li et al., 2019; Moody and Wilkinson, 2019). *Daphnia*

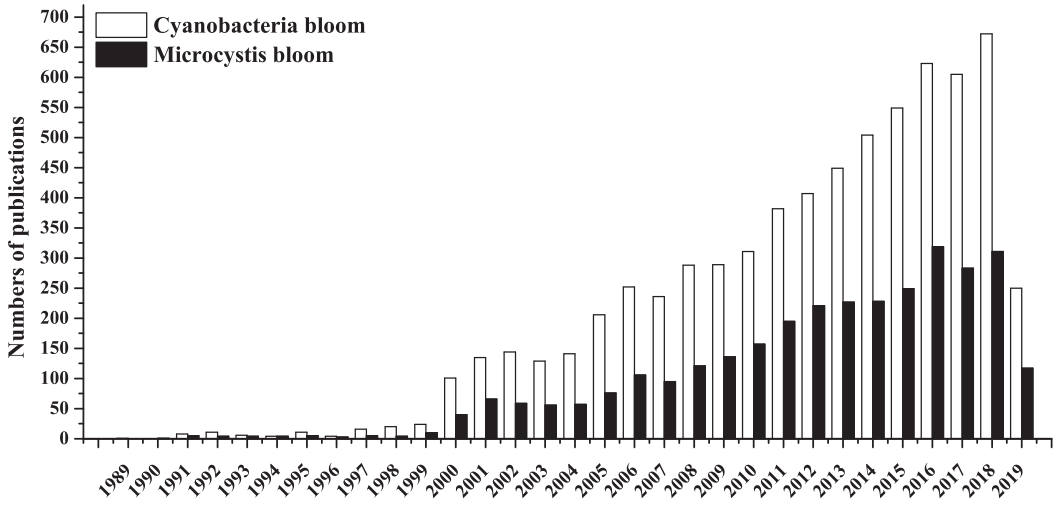

Fig. 1. Publications on cyanobacteria/*Microcystis* blooms in the past 30 years. Database search (Web of Science, Thomson Reuters) performed on 20 May 2019. Y axis: total number of publications by boolean search topic: cyanobacteria* AND bloom or *Microcystis* AND bloom.

magna is the most common freshwater invertebrate model species used in ecotoxicology studies because of its relatively high sensitivity to toxicants, rapid reproduction, and short lifetime (OECD, 2004). Several studies have examined effects of microcystins on *D. magna* in both the laboratory (Hulot et al., 2012; Yang et al., 2012) and nature (Smutná et al., 2014). However, comprehensive studies of estrogenic effects of cyanobacteria on reproductive, physiological, biochemical and molecular systems of planktonic invertebrate species have not been conducted.

The objective of this study was to reveal the reproductive response of *D. magna* to *Microcystis aeruginosa* via alteration of its physiological, biochemical and molecular characteristics. Specifically, we sought to assess changes in population characteristics (e.g., growth rate and reproduction), vitellogenin biosynthesis, production of hormones involved in vitellogenin regulation, and expression of four genes (*CYP314*, *CYP360A8*, *Vtg1* and *Vtg2*) in *D. magna* exposed to exudates of *Microcystis aeruginosa* - the most dominant species associated with cyanobacteria blooms globally.

2. Material and methods

2.1. Algae cultivation and M. aeruginosa exudates (MaE) production

Cultures of *M. aeruginosa* (FACHB-905) and *Chlorella vulgaris* (FACHB-32) were obtained from the Freshwater Algae Culture Collection of the Institution of Hydrobiology (FACHB-Collection) at the Chinese Academy of Sciences. *M. aeruginosa* and *C. vulgaris* were axenically cultured with COMBO medium. Cultures of algae were grown semi-continuously (daily additions of fresh nutrient solution) in a climate-controlled room at 25 ± 1 °C in a 12:12 h light–dark cycle with light of 80 mmol quanta m^{-2} s^{-1} measured as photosynthetic photon flux density (PPFD) by a Quantum Meter (Spectrum Technology, Inc., USA), and manually shaken twice daily.

M. aeruginosa cells of two groups were counted manually by hemocytometer with an optical microscope (Olympus, BX51, Japan) to obtain initial inoculation densities of 1×10^4 cells/ml and 2×10^5 cells/ml, respectively. *M. aeruginosa* culture of the low density group was harvested after 5 or 6 days' growth to achieve a density of approximately 2×10^4 cells/ml while the high density group was harvested at 4×10^5cells/ml after around 3 days. *M. aeruginosa* cell densities were set within the range of those observed in Lake Dianchi, in which the highest cell density was 4.21×10^4 cells/ml in winter and 1.1×10^6 cells/ml in summer (Ma et al., 2015; Wu et al., 2016). The two solutions of *M. aeruginosa* cells (2.0×10^4 cells/ml and 4×10^5 cells/ml) were centrifuged for 10 min at 11,000 rpm, 4 °C. Supernatant was filtered through a glass-fiber filter (0.22 μm) (MiLiMo separation technology limited company, Shanghai) to obtain the *M. aeruginosa* exudates (MaE) solution. MaE of two groups was freshly prepared in advance and kept at 4 °C a few hours just before use.

2.2. Daphnia magna cultivation

D. magna was originally obtained from Guangdong Laboratory Animals Monitoring Institute (Guangzhou, China) and maintained under a constant 14-h light/10-h dark cycle at 25 °C in Yunnan University (Kunming, China). Animals were cultured in glass beakers containing COMBO medium with 20 ml of solution per daphnia individual and daily fed 5×10^5 cells mL^{-1} of *C. vulgaris*, with renewal of the medium twice a week. Healthy and reproductive *D. magna* were selected to produce neonates (<24 h) for experiments, about 6 h before the exposure experiments. We chose third or fourth brood neonates (<24 h) because they were produced exclusively by parthenogenesis.

Chronic and acute toxicity tests were performed by exposing *D. magna* to COMBO medium with: 1) a negative control without any treatment (CK); 2) positive control with the addition of 10 μg/L of estradiol (E2), which is higher than most reported environmental concentrations (e.g. concentrations of 1 to 80 ng/L in municipal WWTP effluents of USA (Wright-Walters and Volz, 2009) and of 40.0 to 117 ng/L for Taihu Lake, China) (Wang et al., 2015); 3 and 4) *M. aeruginosa* exudates (MaE) of low (2×10^4 cells/mL) or high (4×10^5 cells/mL) cell densities.

2.3. Chronic exposure test

In the chronic exposure test, *D. magna* was exposed to the above solutions for 21 days. Exposure was carried out in a 100 ml glass beaker containing 50 mL of the solution of each treatment, with one neonate was placed in each beaker, with twelve replicates. Two replicate animals died in the positive control group, and two died in each of MaE low and high densities, while none died in the negative control group thus during the 21-d exposure experiment, thus only live individuals (ten replicates for each treatment) were used in statistical analyses. *D. magna* was fed daily (5×10^5 cells mL^{-1} of *C. vulgaris*) and the test solution was refreshed daily. The survival, growth, development and reproduction of *D. magna* was monitored daily and the following parameters recorded: number of molts per individual, days to first brood, days to first egg production, number of eggs in first brood, total number of molts per individual, total egg production per individual, and mortality rate of produced offspring. At the end of the experiment, we measured body length (from the top of its head to the base of its tail spine) of each female under the microscope with a micrometer. We divided total number of offspring by mortality to obtain the death rate of produced offspring, and calculated the intrinsic rate of growth (r) and net reproduction rate (R_o) using the Euler-Lotka eq. (1–2): (De Coen and Janssen, 2003; Lotka, 1913)

$$\sum l_x m_x e^{-rx} = 1 \tag{1}$$

$$R_0 = \sum l_x m_x \tag{2}$$

where l_x is the proportion of individuals surviving to age × (days), and m_x is age-specific fecundity (number of neonates produced per surviving female between age x and x + 1).

2.4. Acute exposure test

In the acute exposure test, exposure was carried out in 1000 ml glass beakers for 24, 48 and 96 h, respectively. Four hundred individuals of *D. magna* were used in ten beakers (40 individuals per beaker containing 800 ml of the solution) for each replicate, with four replicates for each treatment in the 24 and 48-h experiments (400 individuals × 4 replicates) and eight replicates for each treatment in the 96-h experiment (400 individuals × 8 replicates). *D. magna* was fed daily (5×10^5 cells mL^{-1} of *C. vulgaris* for per individual) and the test solution was refreshed daily. At the end of each experiment by 24 or 48 h, all individuals of each replicate ($40 \times 10 = 400$) were combined into one sample after harvest, and four replicates of each treatment were collected to analyze the level of vitellogenin, ecdysone and juvenile hormone, as well as the activities of 17β-hydroxysteroid-dehydrogenase (17β-HSD) involved in hormone biosynthesis in *D. magna* by using commercial ELISA kits (Jiangsu Kete, China) with a microtiter plate reader (Lab Systems Multiskan® MS, Finland). For the 96-h experiment, four replicates of each treatment were collected for ELISA test while the rest of the eight replicates were used for total RNA extraction.

2.5. Total RNA isolation and quantitative real-time PCR

In each treatment, neonates were transferred into a 2 mL centrifuge tube, rinsed with distilled water and placed and kept on ice. Total RNA was isolated from neonates with RNAiso plus reagent (Takara, Japan), following the instructions of the manufacturer. After isolation, the concentrations and purity of the total RNA in the samples were measured using a SpectraMax® QuickDrop™ (Molecular Devices, USA). We then

diluted RNA samples with RNase free H_2O to maintain consistent concentrations. Reverse transcription was performed using a PrimeScriptTM RT reagent Kit (Takara, Japan) according to the manufacturer's protocol, then cDNA was obtained for *D. magna* in each treatment.

Quantitative real-time polymerase chain reaction (RT-PCR) was used to analyze gene expression of *D. magna* exposed to *M. aeruginosa* exudates. qPCR primers were designed according to known sequences listed in Table S1, where the β-actin gene was used as an internal standard, and the other four genes used to analyze the response of expression in *D. magna* after exposure to MaE. Primers of all genes were designed according to previous studies (Liu et al., 2017). The availability of these primers and the cDNA obtained was checked by a series of conventional PCR experiments before qPCR experiments.

qPCR experiments were carried out by using TB Green™ Premix Ex Taq™ II(Tli RNaseH Plus) (Takara, Japan) with an ABI QuantStudio™ 7 Flex analyzer (ABI, USA) to amplify these genes. Cycling conditions were as follows: 95 °C for 30 s, 40 cycles of 95 °C for 5 s, 60 °C for 30 s. At the end of each test, a melting curve analysis was conducted (plate read every 0.5 °C from 55 to 95 °C) to determine the formation of specific products. Each 25 μL volume of reaction mixture contained 12.5 μL SYBR Premix Mix Taq (2×), 1 μL for each primer (10 μM), 2 μL cDNA, and was completed to a final volume of 25 μL with ultrapure ddH_2O. Gel electrophoresis and melting curve analyses were performed to confirm correct amplicon size and the absence of nonspecific bands. Gene expression results were calculated by the $2^{-\triangle\triangle CT}$ method (Livak and Schmittgen, 2001). Each reaction was conducted in triplicate to ensure reproducibility of results.

2.6. Statistical analysis

Statistical analysis was performed with SPSS Statistics 19.0 software after normality and homoscedasticity of data were checked. One-way analysis of variance (ANOVA) and LSD post-hoc tests were used to evaluate the significance of differences between the exposure groups and the control. Repeated-measures ANOVA and subsequent post-hoc tests were used to evaluate the significance of differences among 24 h, 48 h and 96 h, subsequently between the exposure groups and the control. The relationships between every two tested parameters of *D. magna* were determined by Pearson's correlation analysis. Experimental data was reported as mean ± standard error, and the 95% confidence limits were calculated using the trimmed Spearman-Karber method.

3. Results

3.1. Growth and reproduction in Daphnia magna exposed to MaE

The day to first brood of the positive control group (E2) treated with 10 μg/L of estradiol was earlier than the negative control (CK, Fig. 2A); also size of the first brood was significantly larger than that of the negative control ($p < 0.05$). On average, one egg emerged on the 4th day in the former group, followed by 11.6 new eggs the next day, which differed significantly from the negative control (only 7.8 eggs on the 5th day as the first brood). However, the negative control and two MaE treatment groups did not produce eggs until day 5. MaE increased the number of eggs, with the MaE of low cell density exerting a stronger stimulus than the higher one (Fig. 2A). However, there was no significant difference between MaE treatments and the negative control ($p > 0.05$). Compared with the CK group, the total number of eggs produced by *D. magna* during the 21 days was significantly increased in both MaE treatments of low and high cell densities as well as in the E2 treatment (27%, 15% and 23%, respectively) (Fig. 2B and Table S2, $p < 0.05$). However, there were no significant differences in total egg production among the MaE treatments and the E2 treatment ($p > 0.05$). Also, both MaE of high and low cell densities as well as E2 stimulated total offspring number, though only the former MaE treatment differed significantly from the negative control (elevated by 52%) (Fig. 2C and Table S2, $p < 0.05$). The MaE of high cell density as well as E2 also resulted in increased body length of adults, though only the latter treatment differed significantly from the negative control (Fig. 2D, $p < 0.05$). Adults of both MaE treatments did not differ significantly in body length from CK ($p > 0.05$).

The intrinsic growth rate of *D. magna* significantly increased in both MaE treatments relative to negative controls (by 15.8% and 19.6%, respectively) (Fig. 2 and Table S2, $p < 0.05$). Also, both MaE treatments induced a greater increase in net reproduction rate of *D. magna* than E2 treatment, increasing by 60% and 83% in the low and high MaE treatments, respectively (Fig. 2 and Table S2, $p < 0.05$). Significant positive correlations between net reproduction rate and total neonates, and between net reproduction rate and intrinsic growth rate, were observed (Fig. 3).

3.2. Physiological parameters of Daphnia magna after acute exposure in MaE

There were no significant differences in vitellogenin concentration in vivo among treatments after 24 h, although the MaE treatments and E2 group were slightly higher than the negative control (Fig. 4A, $p > 0.05$). Compared with the CK treatment, the concentration of *D. magna* vitellogenin after 48 h or 96 h was significantly higher in both MaE treatments as well as E2 groups versus the negative control (Fig. 4A, $p < 0.05$); the low cell density MaE treatment demonstrated greater elevation of vitellogenin than the higher one, and 141% and 67% more than the CK group after 48 h and 96 h, respectively (Table S2). However, there was no significant difference in vitellogenin concentrations among the two MaE treatments and the E2 group either after 48 h or 96 h ($p > 0.05$). Although the level of vitellogenin was unstable during the total 96-h exposure experiment in the negative control group, it was always elevated in both MaE and E2 treatments at each investigated time. We also observed a positive correlation between vitellogenin concentration after 24 h and 48 h (Fig. 3).

Both MaE treatments as well as E2 exhibited elevated concentrations of ecdysone in *D. magna* at each tested time, with significant differences from the negative control after 24 h or 96 h (Fig. 4B, $p < 0.05$). The low cell density MaE treatment had significantly elevated ecdysone level than the higher cell density treatment and 54.5% more than the CK group after 96 h (Table S2), while the direct addition of estradiol after 96 h caused an in vivo increase of 54.4% in ecdysone ($p < 0.05$). Except for the MaE treatment of low cell density, the concentration of ecdysone in the rest three treatments did not exhibit significant differences across time periods. We did observe positive correlations between the concentration of ecdysone and vitellogenin after 24 h, and between the concentration of ecdysone after 24 h and total neonates after 21 days (Fig. 3).

The concentration of juvenile hormone was significantly elevated in both MaE treatments as well as E2 at each tested time, with differences occurring from the negative control after 48 h and 96 h (Fig. 4C, $p < 0.05$). Juvenile hormone of the low cell density MaE treatment achieved the highest observed concentration of all treatments after 96 h, achieving a level 52% higher than the CK group though it was not significantly different from the positive control. This hormone was also stimulated significantly over time in the low cell density MaE treatment and in the E2 treatment ($p < 0.05$). We observed synchronous and significant positive correlations between juvenile hormone concentration and ecdysone level after 24 h and 96 h, and between juvenile hormone and vitellogenin after 24 h (Fig. 3).

Compared with the CK group, the activity of 17β-HSD increased significantly in all treatments after 48 h and 96 h (Fig. 4B, $p < 0.05$), though there were no differences among the MaE treatments and the E2 treatment for any time period ($p > 0.05$). Moreover, this enzyme did not vary significantly across time periods in each treatment. The activity of 17β-

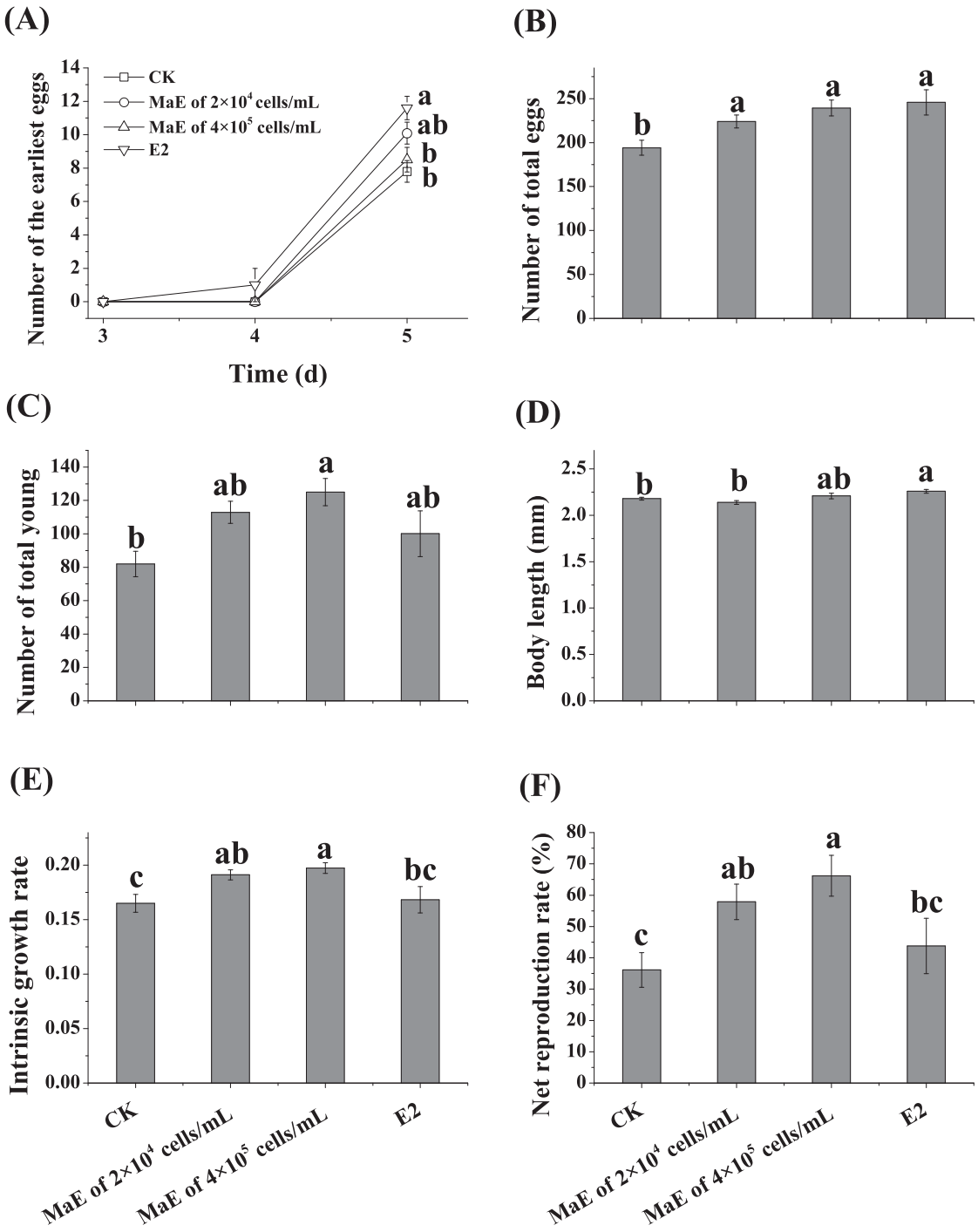

Fig. 2. Performance of growth and reproduction of *Daphnia magna* during a 21-days MaE exposure (CK: negative control group; low MaE: treatment with exudates from 2×10^4 cells/mL of *M. aeruginosa*; high MaE: treatment with exudates from 4×10^5 cells/mL of *M. aeruginosa*; E2: positive control group treated with 10 μg/L of estradiol). (A) Number of the earliest eggs produced by per adult at the 4th or 5th day; (B) Number of total eggs produced by per adult after 21 days; (C) Number of total young produced by per adult after 21 days; (D) Body length of adults after 21 days; (E) Intrinsic growth rate during 21 days; (F) Net reproduction rate during 21 days (mean ± SE, $n = 10$). Different letters on the bars indicate a significant difference between the treatments using one-way ANOVA (LSD, $p < 0.05$).

HSD was positively correlated to levels of vitellogenin, ecdysone and juvenile hormone in vivo (Fig. 3).

3.3. Gene expression of Daphnia magna exposed to MaE

We observed no significant difference in relative mRNA expression of *CYP314* in *D. magna* after 96 h among the negative and positive controls, as well as the two MaE treatments (Fig. 5A, $p > 0.05$). *CYP360A8* expression was similar among the negative control and E2 groups and low cell density MaE treatment after 96 h, however this gene was significantly and dramatically (6×) up-regulated in the high cell density MaE treatment (Fig. 5B, $p < 0.05$). Expression of *Vtg1* after 96 h increased in both the MaE treatments and in the E2 treatment, though only the low MaE treatment was significantly different from the CK control (Fig. 5C, $p < 0.05$). All treatments experienced significant up-regulation of *Vtg2* expression after 96 h relative to the negative control (Fig. 5D, $p < 0.05$); expression was highest for the high cell density MaE and E2 treatments. We observed a significant positive correlation

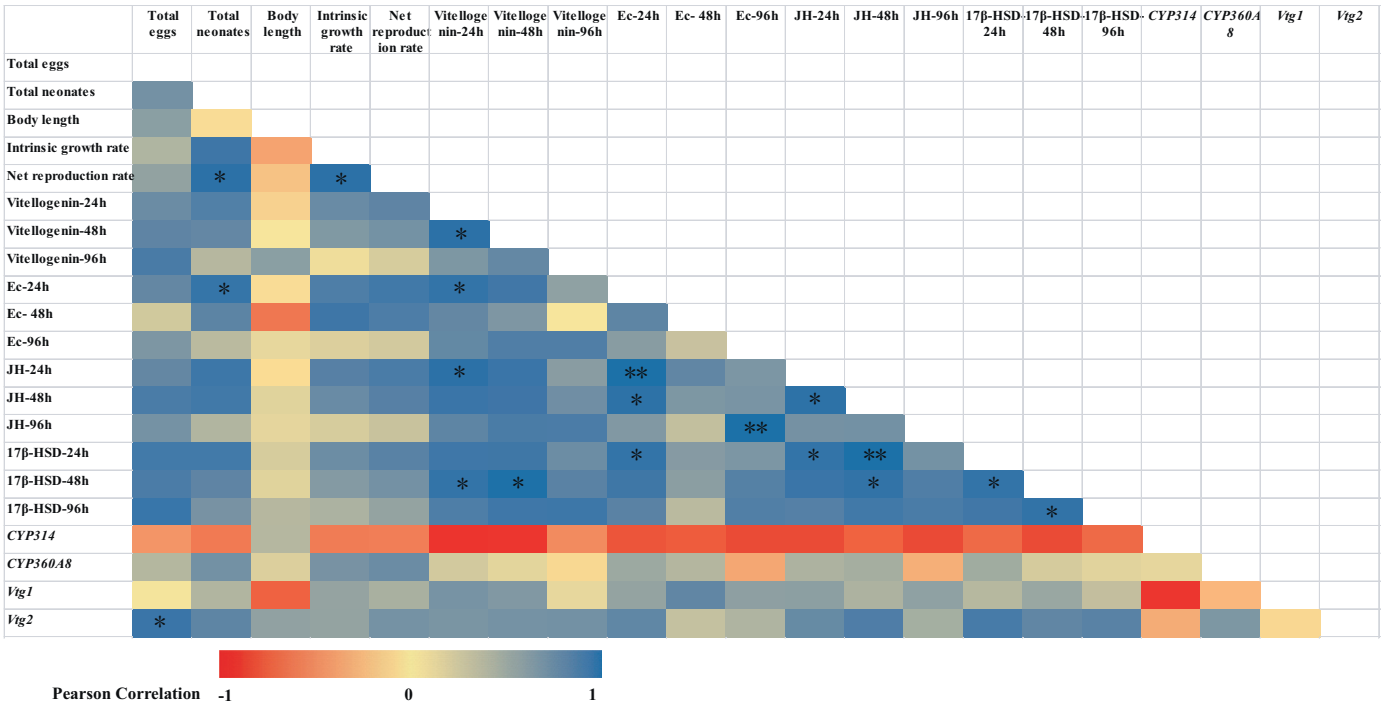

Fig. 3. Heatmap plotted by Pearson correlations between every two tested parameters of *D. magna*. Asterisk indicates a significant correlation between the two parameters (*, $p < 0.05$; **, $p < 0.01$).

between *Vtg2* expression and total egg production after 21 days in the chronic exposure experiment (Fig. 3).

4. Discussion

4.1. Estrogenic effect of M. aeruginosa exudates on D. magna

Endocrine disruptive potential was recently associated with algal blooms, which could contribute to elevated levels of phytoestrogens in aquatic environments (Procházková et al., 2017; Prochazkova et al., 2018). However, estrogenic activity of cyanobacteria in vivo, as well as the action mode of cyanobacteria estrogen (or estrogen analogues), remains unclear. In this study, we demonstrated pronounced estrogenic effects of *M. aeruginosa* exudates on the reproductive, physiological, and molecular characteristics of *D. magna* in the laboratory. These exudates performed very similar to the direct addition of 10 μg/L estradiol on most of the tested demographic parameters (Figs. 2, 4 and 5). Although MaE did not affect body length of *D. magna*, both low and high concentration of MaE experienced increased total egg production.

Vitellogenin (Vtg) is the precursor of vitellin, which provides an energy supply for embryo development in oviparous animals. In parthenogenetically-reproducing *Daphnia,* after vitellogenesis Vtg is transferred via the haemolymph into the oocytes where it is further processed to yolk protein. Production of Vtg is controlled by estrogen hormones, e. g., estradiol in vertebrates, or ecdysteroids and juvenile hormone in crustacea, thus both Vtg production and the expression of Vtg-coding genes (*Vtg1* and *Vtg2*) are often used as biomarkers for estrogenic compound exposure (Jones et al., 2000; Matozzo et al., 2008) or toxicant assessments with cladocerans (Soetaert et al., 2006). Induction of Vtg was reported after exposure to a mixture of cyanobacteria cell cultures in zebrafish and medaka. (Marie et al., 2012; Rogers et al., 2011). We observed increased Vtg levels in *D. magna* when animals were exposed to *M. aeruginosa* exudates (Fig. 4A), suggesting that bioactive chemicals with estrogenic effects might be present with effects similar to that of direct-supplied estradiol. Also, significantly increased expression of *Vtg1* in *D. magna* when exposed to the low concentration of MaE, as well as slightly up-regulated *Vtg2* at both two concentrations of MaE (Fig. 5C and D), suggest that the exudate might act as a source of estrogen.

Induction of Vtg may be estrogen-independent in arthropods (Hannas et al., 2011). Ecdysteroids (e. g., ecdysone) and juvenile hormone may play roles in vitellogenesis of daphnids owing to their definite function in reproduction of insects (Miyakawa et al., 2014; Miyakawa et al., 2018; Qu et al., 2018), and the identification of genes *Vtg1* and *Vtg2* in *D. magna* that contain both juvenile hormone- and ecdysteroid-responsive elements in the promoter and showed expected responses to juvenile hormone agonists and ecdysteroid (Tokishita et al., 2006). The ecdysone receptor (EcR) activates ecdysone production by forming a heterodimer with ultraspiracle (USP) in insects and daphnids (Hopkins, 2009; Kato et al., 2007). EcR and USP have been cloned in *D. magna* (Kato et al., 2007). In this study, we observed significant positive correlations between production of Vtg and levels of the two hormones. 17β-HSD, which is responsible for catalyzation of the last steps of steroid synthesis and its primary metabolism, is involved in ecdysteroid biosynthesis (Janer and Porte, 2007). Thus, the enhancement of 17β-HSD activity by MaE in our study is consistent with the increase in ecdysone production (Fig. 4B and D).

CYP314 in our study is one member of the subfamily CYP19 in the mitochondrial CYPs, a family of the cytochrome P450 superfamily (CYPs), and is involved in conversion of ecdysone to its active form (Rewitz and Gilbert, 2008; Rewitz et al., 2007; Baldwin et al., 2009). *CYP314* gene has already been treated as a biomarker to identify the effects of pollutants on *D. magna* molting and reproduction (Le et al., 2010; Liu et al., 2017; Wang et al., 2016b). Therefore, we sought to determine whether the impact of MaE on *D. magna* reproduction might be indirectly reflected in the change of *CYP314* gene expression. We observed that *CYP314* gene expression was not affected by exposure to either MaE or E2 exposure over 96 h (Fig. 5A). Given that significant correlations exist among most of the tested parameters of *D. magna* affected by MaE (Fig. 3), mechanisms involved in reproductive promotion of *D. magna* by MaE in this study could be elaborated. MaE enhanced the activity of 17β-HSD. Subsequently, these enzymes promoted biosynthesis of ecdysone. Meanwhile, production of juvenile hormone was also enhanced. In turn, increased ecdysone and juvenile hormone had

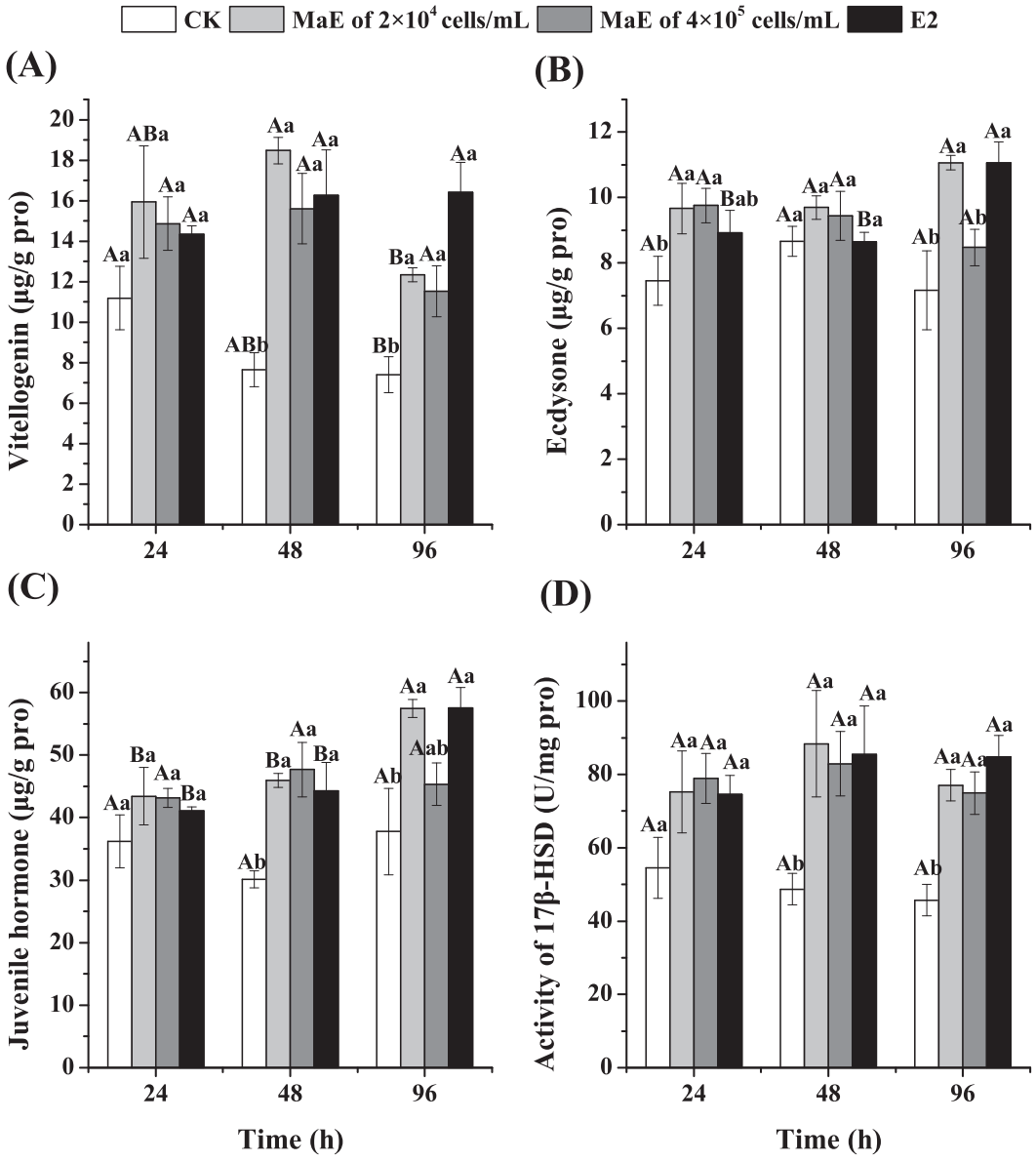

Fig. 4. Performance of physiological parameters of *Daphnia magna* after a 96-h MaE exposure (CK: negative control group; low MaE: treatment with exudates from 2×10^4 cells/mL of *M. aeruginosa*; high MaE: treatment with exudates from 4×10^5 cells/mL of *M. aeruginosa*; E2: positive control group treated with 10 μg/L of estradiol). (A) Concentration of vitellogenin; (B) Concentration of ecdysone; (C) Concentration of juvenile hormone; (D) Activity of 17β-hydroxysteroid-dehydrogenase (17β-HSD) (mean ± SE, $n = 4$). Different small letters indicate significant differences between different solutions applied while capital letters indicate significant differences between values of different time using repeated-measures ANOVA and subsequent post hoc tests (LSD, $p < 0.05$).

greater efficiency to bind to their receptors which collectively induced the subsequent steps of vitellogenesis. At the same time, the expression of genes *Vtg1* and *Vtg2* were also enhanced by MaE exposure, resulting in higher Vtg production. The massive Vtg increase could, in turn, provide added nutritional support for developing eggs. Consequently, affected adult *D. magna* should produce more offspring.

Like previous studies, we were not able to identify the specific compounds in cyanobacteria exudates responsible for observed estrogenic effects. Smutná et al. (2014) indicated that unknown metabolites other than microcystins were likely responsible for toxic effects on *D. magna* reproduction. However, cyanobacteria and algae contain phytoestrogens such as estrogenic flavonoids (Goiris et al., 2014; Klejdus et al., 2010). These compounds can act as both estrogens and antiestrogens, with the contradictory effects related to phytoestrogens being weak estrogen receptor agonists. At low concentrations of the endogenous hormone, weak agonists can elicit estrogenic effects while they block the binding sites of the receptors (D'Alessandro et al., 2005). Therefore, it may be risky if animals are exposed to exogenous hormones originating from cyanobacteria blooms in nature. More research is needed to identify the chemicals responsible.

4.2. Ecological consequence of estrogenic effects caused by M. aeruginosa

Pelagic crustaceans, represented here by daphnids, play important roles in aquatic ecosystems. Changes in their population abundance can have cascading effects in foodwebs (Tessier and Woodruff, 2002). *Daphnia* are adversely affected by cyanobacterial blooms, mainly due to the toxicity of microcystins (Hietala et al., 1995; Hulot et al., 2012; Reinikainen and Walls, 1999). However, we observed that cyanobacteria exudates could have the opposite effect by increasing reproduction in *D. magna*. If this finding applies under natural conditions, enhanced reproductive output by *Daphnia* could impose a stronger top-

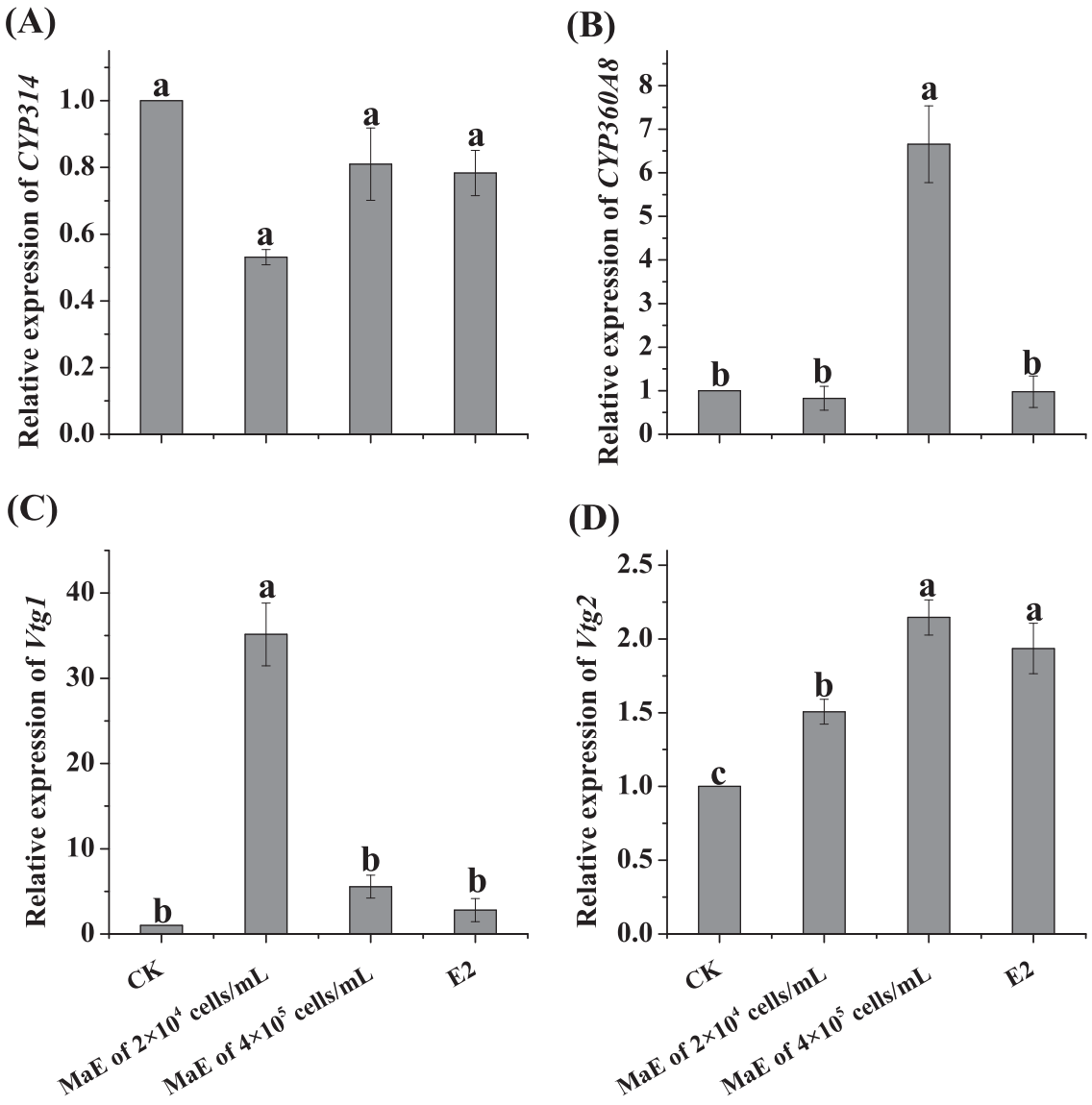

Fig. 5. The relative mRNA expression (mean ± SE, n = 4) of *Daphnia magna* genes related to reproduction ((A) *CYP314*, (B) *CYP360A8*, (C) *Vtg1*, and (D) *Vtg2*) after a 96-h MaE exposure (CK: negative control group; low MaE: treatment with exudates from 2×10^4 cells/mL of *M. aeruginosa*; high MaE: treatment with exudates from 4×10^5 cells/mL of *M. aeruginosa*; E2: positive control group treated with 10 μg/L of estradiol). Different letters on the bars indicate a significant difference between the treatments using one-way ANOVA (LSD, $p < 0.05$).

down effect on phytoplankton. The effect would be most pronounced on edible species such as green algae and diatoms, and less or not at all on less edible cyanobacteria (Ger et al., 2018; Solis et al., 2018). For example, after a cyanobacterial bloom, edible *Cryptomonas* spp., *Dolichospermum* spp., and Bacillariophyceae were differently consumed by different crustaceans (Solis et al., 2018). Cyanobacteria would potentially benefit by enhancing abundance of their competitor's predator (i.e. enemy of my enemy). Nevertheless, it is difficult to conclude that promotion of *D. magna* reproduction by cyanobacteria is beneficial given widespread problems associated with cyanobacteria blooms (Dao et al., 2018; Pawlik-Skowrońska et al., 2019).

On the other hand, our study provides some clues as to unexpected effects of cyanobacteria. *CYP360A8* is also a member of *Daphnia* CYP3 clan family CYP360, which are the closest phylogenetic relatives of the CYP6 and CYP9 subfamily members of insects involved in endobiotic and xenobiotic metabolism and detoxification (Baldwin et al., 2009). Its homologue gene in vertebrates, *CYP3A*, is involved in oxidative metabolism of various drugs in vivo (Yamano et al., 1990). Also, Liu et al. (2017) found that *CYP360A8* in *D. magna* play an important role in catalyzing detoxification of the pollutant diclofenac. Thus, increased expression of *CYP360A8* by the high concentration of MaE in our study (Fig. 5B) suggests that MaE might act as a source of inhibitor, inducing some adverse effect on *D. magna*. In our previous study, MaE induced embryonic malformations and heart failure in a fish species (Zi et al., 2018). MaE exposure over a longer time scale poses greater risk to the target species. For example, estrogenic inhibition to *D. magna* caused by some pharmaceuticals was stronger in chronic than in acute exposures (Le et al., 2011; Liu et al., 2017). Furthermore, longer exposure to MaE may affect this population in other ways, including growth, development or even reproduction of the next generation. For example, offspring body size, which was not measured in this study, could be affected by intact *Microcystis* cells after longer exposure in both laboratory and field conditions (Lampert, 1993; De Senerpont Domis et al., 2013). Filtration rate of *Daphnia* would in turn be affected since it is size-dependent (Davis et al., 2012). Dao et al. (2018) observed strong direct, accumulated and carried-over impacts of cyanobacterial toxins on life history traits on F1 and F2 generations of *D. lumholtzi*, including reductions in survival and reproduction, though there were only weak negative effects on the first generation (F0). Moreover, the situation may be more complex in the field where *D. magna* would be exposed to

complex cyanobacterial communities and a variety of bioactive chemicals, toxins and effects (Smutná et al., 2014). Dao et al. (2018) also concluded that chronic exposure to long-lasting blooms, even at low density, reduced survival of *D. lumholtzi* in tropical lakes and reservoirs, with ecological consequences. In addition, a recent report indicated that natural cyanobacteria can accumulate endocrine hormones from polluted water which could be transferred via the food chain (Jia et al., 2019). Similarly, estrogenic chemicals released by cyanobacteria could directly threaten species at higher trophic levels or by indirect accumulation through the food chain (Jonas et al., 2015; Ziková et al., 2013).

It is generally accepted that cyanobacteria have strong negative effects on survivorship, growth and reproduction of *Daphnia* due to their toxins, "bad taste", poor nutritional value for lacking essential fatty acids and lipids, and morphological features that hinder ingestion (Lürling, 2003). Toxicity of microcystins is generally considered as the major cause of reduced growth and increased mortality in *Daphnia* (Gustafsson and Hansson, 2004; Liu et al., 2011). As well, *Daphnia* and other aquatic invertebrates have been reported to accumulate cyanobacterial toxins and are speculated to then transfer them to higher trophic levels (Bownik, 2016). However, these studies demonstrated negative effects of cyanobacteria on *Daphnia* mostly by experiments with intact cyanobacteria cells, which may trigger other problems such as altered ingestion and filtration rates. In addition, toxin production of cyanobacteria cells could be increased by exposure to zooplankton (Jang et al., 2003, 2008). On the other hand, cyanobacteria toxins are generally thought to be released into the aquatic environment mainly during cell lysis. Thus, reproductive disruption of *D. magna* caused by exponential-growth-phase-*Microcystis* exudates can occur even in the absence of microcystins (Zi et al., 2018; Zheng et al., 2013). Lürling (2003) also found that adverse effects on *Daphnia* growth and survival were not necessarily related to presence and concentration of microcystins but rather other substances.

Cyanobacteria blooms are an unfortunate by-product of modern civilization and have adverse impacts on other aquatic species (Cirés and Ballot, 2016). Some of these hazards are well known (Mello et al., 2018). However, estrogenic effects associated cyanobacterial blooms that we have demonstrated here are less well-appreciated. Our study demonstrated that blooming cyanobacteria could release bioactive compounds that induce reproductive endocrine disorder in a common and important aquatic species. It provides insights into potential risks of cyanobacterial blooms via estrogenic effects based upon in vivo trials. However, the role of cyanobacteria in reproductive disorders under natural conditions warrants further attention. In particular, studies on the chemical nature, modes of action, and consequences of the unknown compounds associated with estrogenic activity are needed. In addition, physiological factors such as cyanobacteria strain differences, species tolerance and age/generation of target animals, population density, and physical factors must be considered when investigating effects of cyanobacterial blooms.

5. Conclusion

In the present study, the effects of two levels of *M. aeruginosa* exudates (MaE, 2×10^4 cells/ml and 4×10^5 cells/ml) on the reproductive, physiological, and molecular characteristics of *D. magna* were investigated. Totally, *M. aeruginosa* exudates had significant promotion in *D. magna* reproduction including increased egg and neonate production, intrinsic growth rate, and net reproduction rate. And *M. aeruginosa* exudates promoted *D. magna* reproduction by stimulating the activity of 17β-HSD and biosynthesis of ecdysone and juvenile hormone, also by enhancing its vitellogenin biosynthesis with up-regulating the expression of *Vtg1* and *Vtg2*. However, the increased expression of *CYP360A8*, a gene responsible for detoxification, suggested that MaE might induce some toxicity in *D. magna*. Also, strong estrogenic effects on *D. magna* induced by cyanobacteria might enhance grazing and reduce abundance of edible algae, and negatively affect other filter feeders.

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References

Baldwin, W.S., Marko, P.B., Nelson, D.R., 2009. The cytochrome P450 (CYP) gene superfamily in *Daphnia pulex*. BMC Genomics 10, 169.
Bertram, M.G., Saaristo, M., Martin, J.M., Ecker, T.E., Michelangeli, M., Johnstone, C.P., Wong, B.B.M., 2018. Field-realistic exposure to the androgenic endocrine disruptor 17β-trenbolone alters ecologically important behaviours in female fish across multiple contexts. Environ. Pollut. 243, 900–911.
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, 13.
Bownik, A., 2016. Harmful algae: effects of cyanobacterial cyclic peptides on aquatic invertebrates-a short review. Toxicon 124, 26–35.
Bullerjahn, G.S., McKay, R.M., Davis, T.W., Baker, D.B., Boyer, G.L., D'Anglada, L.V., Doucette, G.J., Ho, J.C., Irwin, E.G., Kling, C.L., Kudela, R.M., Kurmayer, R., Michalak, A.M., Ortiz, J.D., Otten, T.G., Paerl, H.W., Qin, B., Sohngen, B.L., Stumpf, R.P., Visser, P.M., Wilhelm, S.W., 2016. Global solutions to regional problems: collecting global expertise to address the problem of harmful cyanobacterial blooms. A Lake Erie case study. Harmful Algae 54, 223–238.
Casals-Casas, C., Desvergne, B., 2011. Endocrine disruptors: from endocrine to metabolic disruption. Annu. Rev. Physiol. 73, 135–162.
Chia, M.A., Jankowiak, J.G., Kramer, B.J., Goleski, J.A., Huang, I.S., Zimba, P.V., do Carmo Bittencourt-Oliveira, M., Gobler, C.J., 2018. Succession and toxicity of *Microcystis* and *Anabaena* (*Dolichospermum*) blooms are controlled by nutrient-dependent allelopathic interactions. Harmful Algae 74, 67–77.
Cirés, S., Ballot, A., 2016. A review of the phylogeny, ecology and toxin production of bloom-forming *Aphanizomenon* spp. and related species within the Nostocales (cyanobacteria). Harmful Algae 54, 21–43.
Corbel, S., Mougin, C., Bouaïcha, N., 2014. Cyanobacterial toxins: modes of actions, fate in aquatic and soil ecosystems, phytotoxicity and bioaccumulation in agricultural crops. Chemosphere 96, 1–15.
D'Alessandro, T.L., Boersma-Maland, B.J., Greg Peterson, T., Sfakianos, J., Prasain, K., Patel, R.P., Darley-Usmar, V.M., Botting, N.P., Barnes, S., 2005. Metabolism of phytoestrogen conjugates. Methods in Enzymology. Academic Press, pp. 316–342.
Dao, T.-S., Vo, T.-M.-C., Wiegand, C., Bui, B.-T., Dinh, K.V., 2018. Transgenerational effects of cyanobacterial toxins on a tropical micro-crustacean *Daphnia lumholtzi* across three generations. Environ. Pollut. 243, 791–799.
Davis, T.W., Koch, F., Marcoval, M.A., Wilhelm, S.W., Gobler, C.J., 2012. Mesozooplankton and microzooplankton grazing during cyanobacterial blooms in the western basin of Lake Erie. Harmful Algae 15, 26–35.
De Coen, W.M., Janssen, C.R., 2003. The missing biomarker link: relationships between effects on the cellular energy allocation biomarker of toxicant-stressed *Daphnia magna* and corresponding population characteristics. Environ. Toxicol. Chem. 22, 1632–1641.
De Senerpont Domis, L.N., Bartosiewicz, M., Davis, C., Cerbin, S., 2013. The effect of small doses of toxic cyanobacterial food on the temperature response of *Daphnia galeata*: is bigger better? Freshw. Biol. 58, 560–572.
Ger, K.A., Naus-Wiezer, S., De Meester, L., Lürling, M., 2018. Zooplankton grazing selectivity regulates herbivory and dominance of toxic phytoplankton over multiple prey generations. Limnol. Oceanogr. 64, 1214–1227.
Goiris, K., Muylaert, K., Voorspoels, S., Noten, B., De Paepe, D., E Baart, G.J., De Cooman, L., 2014. Detection of flavonoids in microalgae from different evolutionary lineages. J. Phycol. 50, 483–492.
Gong, Y., Wang, X., Indran, I.R., Zhang, S.-J., Lv, Z., Li, J., Holmes, M., Tang, Y.Z., Yong, E.L., 2014. Phytoplankton blooms: an overlooked marine source of natural endocrine disrupting chemicals. Ecotoxicol. Environ. Saf. 107, 126–132.
Gustafsson, S., Hansson, L.-A., 2004. Development of tolerance against toxic cyanobacteria in *Daphnia*. Aquat. Ecol. 38, 37–44.
Hannas, B.R., Wang, Y.H., Thomson, S., Kwon, G., Li, H., LeBlanc, G.A., 2011. Regulation and dysregulation of vitellogenin mRNA accumulation in daphnids (*Daphnia magna*). Aquat. Toxicol. 101, 351–357.
Hietala, J., Reinikainen, M., Walls, M., 1995. Variation in life history responses of *Daphnia* to toxic *Microcystis aeruginosa*. J. Plankton Res. 17, 2307–2318.
Hoerger, C.C., Wettstein, F.E., Hungerbühler, K., Bucheli, T.D., 2009. Occurrence and origin of estrogenic Isoflavones in Swiss River waters. Environ. Sci. Technol. 43, 6151–6157.

Hopkins, P.M., 2009. Crustacean ecdysteroids and their receptors. In: Smagghe, G. (Ed.), Ecdysone: Structures and Functions. Springer Netherlands, Dordrecht, pp. 73–97.
Hou, J., Su, Y., Lin, W., Guo, H., Li, L., Anderson, D.M., Li, D., Tang, R., Chi, W., Zhang, X., 2018. Estrogenic potency of MC-LR is induced via stimulating steroidogenesis: *In vitro* and *in vivo* evidence. Environ. Pollut. 240, 615–622.
Hulot, F.D., Carmignac, D., Legendre, S., Yéprémian, C., Bernard, C., 2012. Effects of microcystin-producing and microcystin-free strains of *Planktothrix agardhii* on long-term population dynamics of *Daphnia magna*. Annales de Limnologie – Int. J. Limnol. 48, 337–347.
Janer, G., Porte, C., 2007. Sex steroids and potential mechanisms of non-genomic endocrine disruption in invertebrates. Ecotoxicology 16, 145–160.
Jang, M.H., Ha, K., Joo, G.J., Takamura, N., 2003. Toxin production of cyanobacteria is increased by exposure to zooplankton. Freshw. Biol. 48, 1540–1550.
Jang, M.H., Ha, K., Takamura, N., 2008. Microcystin production by *Microcystis aeruginosa* exposed to different stages of herbivorous zooplankton. Toxicon 51, 882–889.
Jarošová, B., Javůrek, J., Adamovský, O., Hilscherová, K., 2015. Phytoestrogens and mycoestrogens in surface waters — their sources, occurrence, and potential contribution to estrogenic activity. Environ. Int. 81, 26–44.
Jia, Y., Chen, Q., Crawford, S.E., Song, L., Chen, W., Hammers-Wirtz, M., Strauss, T., Seiler, T.-B., Schäffer, A., Hollert, H., 2019. Cyanobacterial blooms act as sink and source of endocrine disruptors in the third largest freshwater lake in China. Environ. Pollut. 245, 408–418.
Jonas, A., Scholz, S., Fetter, E., Sychrova, E., Novakova, K., Ortmann, J., Benisek, M., Adamovsky, O., Giesy, J.P., Hilscherova, K., 2015. Endocrine, teratogenic and neurotoxic effects of cyanobacteria detected by cellular *in vitro* and zebrafish embryos assays. Chemosphere 120, 321–327.
Jones, P.D., De Coen, W.M., Tremblay, L., Giesy, J.P., 2000. Vitellogenin as a biomarker for environmental estrogens. Water Sci. Technol. 42, 1–14.
Kato, Y., Kobayashi, K., Oda, S., Tatarazako, N., Watanabe, H., Iguchi, T., 2007. Cloning and characterization of the ecdysone receptor and ultraspiracle protein from the water flea *Daphnia magna*. J. Endocrinol. 193, 183.
Khan, D., Ansar Ahmed, S., 2016. The immune system is a natural target for estrogen action: opposing effects of estrogen in two prototypical autoimmune diseases. Front. Immunol. 6.
Klejdus, B., Lojková, L., Plaza, M., Šnóblová, M., Štěrbová, D., 2010. Hyphenated technique for the extraction and determination of isoflavones in algae: ultrasound-assisted supercritical fluid extraction followed by fast chromatography with tandem mass spectrometry. J. Chromatogr. A 1217, 7956–7965.
Korach, K.S., Hewitt, S.C., Hamilton, K.J., Li, Y., Ramsey, J.T., Garcia, M., Mathura, E., Arao, Y., 2019. Physiological and pathological roles of estrogen receptor. In: Zhang, X. (Ed.), Estrogen Receptor and Breast Cancer: Celebrating the 60th Anniversary of the Discovery of ER. Springer International Publishing, Cham, pp. 15–47.
Lampert, W., 1993. Phenotypic plasticity of the size at 1st reproduction in *Daphnia*: the importance of maternal size. Ecology 74, 1455–1466.
Le, T.-H., Lim, E.-S., Lee, S.K., Choi, Y.-W., Kim, Y.-H., Min, J., 2010. Effects of glyphosate and methidathion on the expression of the Dhb, Vtg, Arnt, CYP4 and CYP314 in *Daphnia magna*. Chemosphere 79, 67–71.
Le, T.-H., Lim, E.-S., Lee, S.-K., Park, J.-S., Kim, Y.-H., Min, J., 2011. Toxicity evaluation of verapamil and tramadol based on toxicity assay and expression patterns of Dhb, Vtg, Arnt, CYP4, and CYP314 in *Daphnia magna*. Environ. Toxicol. 26, 515–523.
Li, C., Feng, W., Chen, H., Li, X., Song, F., Guo, W., Giesy, J.P., Sun, F., 2019. Temporal variation in zooplankton and phytoplankton community species composition and the affecting factors in Lake Taihu—a large freshwater lake in China. Environ. Pollut. 245, 1050–1057.
Liu, L., Li, K., Chen, T., Dai, X., Jiang, M., Diana, J.S., 2011. Effects of *Microcystis aeruginosa* on life history of water flea *Daphnia magna*. Chin. J. Oceanol. Limnol. 29, 892–897.
Liu, Y., Wang, L., Pan, B., Wang, C., Bao, S., Nie, X., 2017. Toxic effects of diclofenac on life history parameters and the expression of detoxification-related genes in *Daphnia magna*. Aquat. Toxicol. 183, 104–113.
Liu, G., Ke, M., Fan, X., Zhang, M., Zhu, Y., Lu, T., Sun, L., Qian, H., 2018. Reproductive and endocrine-disrupting toxicity of *Microcystis aeruginosa* in female zebrafish. Chemosphere 192, 289–296.
Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25, 402–408.
Loick-Wilde, N., Fernández-Urruzola, I., Eglite, E., Liskow, I., Nausch, M., Schulz-Bull, D., Wodarg, D., Wasmund, N., Mohrholz, V., 2019. Stratification, nitrogen fixation, and cyanobacterial bloom stage regulate the planktonic food web structure. Glob. Chang. Biol. 25, 794–810.
Lotka, A.J., 1913. A natural population norm. I. J. Wash. Acad. Sci. 3, 241–248.
Lürling, M., 2003. *Daphnia* growth on microcystin-producing and microcystin-free *Microcystis aeruginosa* in different mixtures with the green alga *Scenedesmus obliquus*. Limnol. Oceanogr. 48, 2214–2220.
Ma, X., Wang, Y., Feng, S., Wang, S., 2015. Vertical migration patterns of different phytoplankton species during a summer bloom in Dianchi Lake, China. Environ. Earth Sci. 74, 3805–3814.
Marie, B., Huet, H., Marie, A., Djediat, C., Puiseux-Dao, S., Catherine, A., Trinchet, I., Edery, M., 2012. Effects of a toxic cyanobacterial bloom (*Planktothrix agardhii*) on fish: insights from histopathological and quantitative proteomic assessments following the oral exposure of medaka fish (*Oryzias latipes*). Aquat. Toxicol. 114-115, 39–48.
Matozzo, V., Gagné, F., Marin, M.G., Ricciardi, F., Blaise, C., 2008. Vitellogenin as a biomarker of exposure to estrogenic compounds in aquatic invertebrates: a review. Environ. Inter. 34, 531–545.
Mello, F.D., Braidy, N., Marçal, H., Guillemin, G., Nabavi, S.M., Neilan, B.A., 2018. Mechanisms and effects posed by neurotoxic products of cyanobacteria/microbial eukaryotes/dinoflagellates in algae blooms: a review. Neurotox. Res. 33, 153–167.
Miah, S., Bagu, E., Goel, R., Ogunbolude, Y., Dai, C., Ward, A., Vizeacoumar, F.S., Davies, G., Vizeacoumar, F.J., Anderson, D., Lukong, K.E., 2019. Estrogen receptor signaling regulates the expression of the breast tumor kinase in breast cancer cells. BMC Cancer 19, 78.
Miyakawa, H., Toyota, K., Sumiya, E., Iguchi, T., 2014. Comparison of JH signaling in insects and crustaceans. Current Opinion in Insect Sci 1, 81–87.
Miyakawa, H., Sato, T., Song, Y., Tollefsen, K.E., Iguchi, T., 2018. Ecdysteroid and juvenile hormone biosynthesis, receptors and their signaling in the freshwater microcrustacean *Daphnia*. J. Steroid Biochem. Mol. Biol. 184, 62–68.
Moody, E.K., Wilkinson, G.M., 2019. Functional shifts in lake zooplankton communities with hypereutrophication. Freshw. Biol. 64, 608–616.
Moorhouse, H.L., McGowan, S., Taranu, Z.E., Gregory-Eaves, I., Leavitt, P.R., Jones, M.D., Barker, P., Brayshaw, S.A., 2018. Regional versus local drivers of water quality in the Windermere catchment, Lake District, United Kingdom: the dominant influence of wastewater pollution over the past 200 years. Glob. Chang. Biol. 24, 4009–4022.
Nalewajko, C., Murphy, T.P., 2001. Effects of temperature, and availability of nitrogen and phosphorus on the abundance of *Anabaena* and *Microcystis* in Lake Biwa, Japan: an experimental approach. Limnology 2, 45–48.
Newell, S.E., Davis, T.W., Johengen, T.H., Gossiaux, D., Burtner, A., Palladino, D., McCarthy, M.J., 2019. Reduced forms of nitrogen are a driver of non-nitrogen-fixing harmful cyanobacterial blooms and toxicity in Lake Erie. Harmful Algae 81, 86–93.
OECD, 2004. Test No. 202: Daphnia Sp. Acute Immobilisation Test.
Orton, F., Tyler, C.R., 2015. Do hormone-modulating chemicals impact on reproduction and development of wild amphibians? Biol. Rev. 90, 1100–1117.
Oziol, L., Bouaïcha, N., 2010. First evidence of estrogenic potential of the cyanobacterial heptotoxins the nodularin-R and the microcystin-LR in cultured mammalian cells. J. Hazard. Mater. 174, 610–615.
Paerl, H.W., Paul, V.J., 2012. Climate change: links to global expansion of harmful cyanobacteria. Water Res. 46, 1349–1363.
Pawlik-Skowrońska, B., Toporowska, M., Mazur-Marzec, H., 2019. Effects of secondary metabolites produced by different cyanobacterial populations on the freshwater zooplankters *Brachionus calyciflorus* and *Daphnia pulex*. Environ. Sci. Pollut. Res. 26, 11793. https://doi.org/10.1007/s11356-019-04543-1.
Procházková, T., Sychrová, E., Javůrková, B., Večerková, J., Kohoutek, J., Lepšová-Skácelová, O., Bláha, L., Hilscherová, K., 2017. Phytoestrogens and sterols in waters with cyanobacterial blooms - analytical methods and estrogenic potencies. Chemosphere 170, 104–112.
Prochazkova, T., Sychrova, E., Vecerkova, J., Javurkova, B., Otoupalikova, A., Pernica, M., Simek, Z., Smutna, M., Lepsova-Skacelova, O., Hilscherova, K., 2018. Estrogenic activity and contributing compounds in stagnant water bodies with massive occurrence of phytoplankton. Water Res. 136, 12–21.
Qu, Z., Bendena, W.G., Tobe, S.S., Hui, J.H.L., 2018. Juvenile hormone and sesquiterpenoids in arthropods: biosynthesis, signaling, and role of microRNA. J. Steroid Biochem. Mol. Biol. 184, 69–76.
Reinikainen, M.H. Jaana, Walls, Mari, 1999. Reproductive allocation in *Daphnia* exposed to toxic cyanobacteria. J. Plankton Res. 21, 1553–1564.
Rewitz, K.F., Gilbert, L.I., 2008. *Daphnia* Halloween genes that encode cytochrome P450s mediating the synthesis of the arthropod molting hormone: evolutionary implications. BMC Evol. Biol. 8, 60.
Rewitz, K.F., O'Connor, M.B., Gilbert, L.I., 2007. Molecular evolution of the insect Halloween family of cytochrome P450s: phylogeny, gene organization and functional conservation. Insect Biochem. Mol. Biol. 37, 741–753.
Richardson, J., Miller, C., Maberly, S.C., Taylor, P., Globevnik, L., Hunter, P., Jeppesen, E., Mischke, U., Moe, S.J., Pasztaleniec, A., Søndergaard, M., Carvalho, L., 2018. Effects of multiple stressors on cyanobacteria abundance vary with lake type. Glob. Chang. Biol. 24, 5044–5055.
Richardson, J., Feuchtmayr, H., Miller, C., Hunter, P., Maberly, S.C., Carvalho, L., 2019. The response of cyanobacteria and phytoplankton abundance to warming, extreme rainfall events and nutrient enrichment. Glob. Chang. Biol. https://doi.org/10.1111/gcb.14701.
Rogers, E.D., Henry, T.B., Twiner, M.J., Gouffon, J.S., McPherson, J.T., Boyer, G.L., Sayler, G.S., Wilhelm, S.W., 2011. Global gene expression profiling in larval zebrafish exposed to microcystin-LR and *Microcystis* reveals endocrine disrupting effects of cyanobacteria. Environ. Sci. Technol. 45, 1962–1969.
Shan, K., Song, L., Chen, W., Li, L., Liu, L., Wu, Y., Jia, Y., Zhou, Q., Peng, L., 2019. Analysis of environmental drivers influencing interspecific variations and associations among bloom-forming cyanobacteria in large, shallow eutrophic lakes. Harmful Algae 84, 84–94.
Simiyu, B.M., Oduor, S.O., Rohrlack, T., Sitoki, L., Kurmayer, R., 2018. Microcystin content in phytoplankton and in small fish from eutrophic Nyanza gulf, Lake Victoria, Kenya. Toxins 10, 275.
Smutná, M., Babica, P., Jarque, S., Hilscherová, K., Maršálek, B., Haeba, M., Bláha, L., 2014. Acute, chronic and reproductive toxicity of complex cyanobacterial blooms in *Daphnia magna* and the role of microcystins. Toxicon 79, 11–18.
Soetaert, A., Moens, L.N., Van der Ven, K., Van Leemput, K., Naudts, B., Blust, R., De Coen, W.M., 2006. Molecular impact of propiconazole on *Daphnia magna* using a reproduction-related cDNA array. Comp. Biochem. Physiol., Part C: Toxicol. Pharmacol. 142, 66–76.
Söffker, M., Tyler, C.R., 2012. Endocrine disrupting chemicals and sexual behaviors in fish – a critical review on effects and possible consequences. Crit. Rev. Toxicol. 42, 653–668.
Solis, M., Pawlik-Skowrońska, B., Adamczuk, M., Kalinowska, R., 2018. Dynamics of small-sized Cladocera and their algal diet in lake with toxic cyanobacterial water blooms. Annales de Limnologie-Int. J. Limnol. 54, 6.
Song, H., Lavoie, M., Fan, X., Tan, H., Liu, G., Xu, P., Fu, Z., Paerl, H.W., Qian, H., 2017. Allelopathic interactions of linoleic acid and nitric oxide increase the competitive ability of *Microcystis aeruginosa*. ISME J 11, 1865.
Steffen, M.M., Davis, T.W., McKay, R.M.L., Bullerjahn, G.S., Krausfeldt, L.E., Stough, J.M.A., Neitzey, M.L., Gilbert, N.E., Boyer, G.L., Johengen, T.H., Gossiaux, D.C., Burtner, A.M.,

Palladino, D., Rowe, M.D., Dick, G.J., Meyer, K.A., Levy, S., Boone, B.E., Stumpf, R.P., Wynne, T.T., Zimba, P.V., Gutierrez, D., Wilhelm, S.W., 2017. Ecophysiological examination of the Lake Erie *Microcystis* bloom in 2014: linkages between biology and the water supply shutdown of Toledo, OH. Environ. Sci. Technol. 51, 6745–6755.

Štěpánková, T., Ambrožová, L., Bláha, L., Giesy, J.P., Hilscherová, K., 2011. *In vitro* modulation of intracellular receptor signaling and cytotoxicity induced by extracts of cyanobacteria, complex water blooms and their fractions. Aquat. Toxicol. 105, 497–507.

Suikkanen, S., Laamanen, M., Huttunen, M., 2007. Long-term changes in summer phytoplankton communities of the open northern Baltic Sea. Estuar. Coast. Shelf Sci. 71, 580–592.

Sychrová, E., Štěpánková, T., Nováková, K., Bláha, L., Giesy, J.P., Hilscherová, K., 2012. Estrogenic activity in extracts and exudates of cyanobacteria and green algae. Environ. Int. 39, 134–140.

Tessier, A.J., Woodruff, P., 2002. Cryptic trophic cascade along a gradient of lake size. Ecology 83, 1263–1270.

Tokishita, S.-i., Kato, Y., Kobayashi, T., Nakamura, S., Ohta, T., Yamagata, H., 2006. Organization and repression by juvenile hormone of a vitellogenin gene cluster in the crustacean, *Daphnia magna*. Bioche. Biophys. Res. Commun. 345, 362–370.

Visser, P.M., Verspagen, J.M.H., Sandrini, G., Stal, L.J., Matthijs, H.C.P., Davis, T.W., Paerl, H.W., Huisman, J., 2016. How rising CO_2 and global warming may stimulate harmful cyanobacterial blooms. Harmful Algae 54, 145–159.

Wang, Y., Wang, Q., Hu, L., Lu, G., Li, Y., 2015. Occurrence of estrogens in water, sediment and biota and their ecological risk in northern Taihu Lake in China. Environ. Geochem. Health 37, 147–156.

Wang, C., Zhang, S., Zhou, Y., Huang, C., Mu, D., Giesy, J.P., Hu, J., 2016a. Equol induces gonadal intersex in Japanese medaka (*Oryzias latipes*) at environmentally relevant concentrations: comparison with 17β-estradiol. Environ. Sci. Technol. 50, 7852–7860.

Wang, L., Peng, Y., Nie, X., Pan, B., Ku, P., Bao, S., 2016b. Gene response of CYP360A, CYP314, and GST and whole-organism changes in *Daphnia magna* exposed to ibuprofen. Comp. Biochem. Physiol., Part C: Toxicol. Pharmacol. 179, 49–56.

Whitmore, T.J., Riedinger-Whitmore, M.A., Lauterman, F.M., Curtis, J.H., 2018. Cyanobacterial influence on diatom community lifeform dynamics in shallow subtropical lakes of Florida USA. J. Paleolimnol. 60, 223–246.

Wright-Walters, M., Volz, C., 2009. Municipal Wastewater Concentrations of Pharmaceutical and Xeno-Estrogens: Wildlife and Human Health Implications. Springer New York, New York, NY, pp. 103–113.

Wu, Y., Li, L., Zheng, L., Dai, G., Ma, H., Shan, K., Wu, H., Zhou, Q., Song, L., 2016. Patterns of succession between bloom-forming cyanobacteria *Aphanizomenon flos-aquae* and *Microcystis* and related environmental factors in large, shallow Dianchi Lake, China. Hydrobiologia 765, 1–13.

Wynne, T.T., Stumpf, R.P., 2015. Spatial and temporal patterns in the seasonal distribution of toxic cyanobacteria in western Lake Erie from 2002–2014. Toxins 7, 1649–1663.

Yamano, S., Tatsuno, J., Gonzalez, F.J., 1990. The CYP2A3 gene product catalyzes coumarin 7-hydroxylation in human liver microsomes. Biochemistry 29, 1322–1329.

Yang, Z., Lü, K., Chen, Y., Montagnes, D.J.S., 2012. The interactive effects of ammonia and microcystin on life-history traits of the cladoceran *Daphnia magna*: synergistic or antagonistic? PLoS One 7, e32285.

Yu, W., Du, B., Yang, L., Zhang, Z., Yang, C., Yuan, S., Zhang, M., 2019. Occurrence, sorption, and transformation of free and conjugated natural steroid estrogens in the environment. Environ. Sci. Pollut. Res. 26, 9443. https://doi.org/10.1007/s11356-019-04402-z.

Zheng, G., Xu, R., Chang, X., Hilt, S., Wu, C., 2013. Cyanobacteria can allelopathically inhibit submerged macrophytes: effects of *Microcystis aeruginosa* extracts and exudates on *Potamogeton malaianus*. Aquat. Bot. 109, 1–7.

Zi, J., Pan, X., MacIsaac, H.J., Yang, J., Xu, R., Chen, S., Chang, X., 2018. Cyanobacteria blooms induce embryonic heart failure in an endangered fish species. Aquat. Toxicol. 194, 78–85.

Ziková, A., Lorenz, C., Lutz, I., Pflugmacher, S., Kloas, W., 2013. Physiological responses of *Xenopus laevis* tadpoles exposed to cyanobacterial biomass containing microcystin-LR. Aquat. Toxicol. 128-129, 25–33.