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Ecotoxicological investigation of cyanobacterial crude extracts to *Daphnia magna* under subchronic test conditions

Tan-Duc NGUYEN[1,2,*], Xuan-Quang NGO[2], Thanh-Luu PHAM[2], Thanh-Son DAO[3]

[1]Department of Science and Technology, Graduate School of Engineering, Nagasaki University, Nagasaki City, Japan
[2]Department of Environmental Management and Technology, Institute of Tropical Biology, Vietnam Academy of Science and Technology, Ho Chi Minh City, Vietnam
[3]Department of Environmental Management, Faculty of Environment and Natural Resources, Ho Chi Minh City University of Technology (HCMUT), Ho Chi Minh City, Vietnam

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Abstract: Cyanobacterial blooms often consist of mixtures of microcystin-producing and microcystin-free species, and both can cause unpredictable effects on aquatic organisms. In this work, the subchronic effects of the cyanobacterial crude extracts (CCEs) from microcystin-producing and microcystin-free cyanobacteria with different microcystin concentrations (1, 10, and 50 μgL^{-1})on *Daphnia magna* were investigated. The life-history trait responses of *D. magna* to CCEs were determined based on survival, reproduction, and somatic growth. In addition, the physiological response, represented by the feeding rate of *D. magna* on green algae (*Scenedesmus* sp.), after exposure to both types of crude extracts was estimated. Our results showed that both microcystin-containing (MCCE) and microcystin-free (NCCE) crude extracts insignificantly reduced survival but strongly enhanced reproduction and somatic growth of organisms. However, degradation of eggs and neonates of the gravid females exposed to CCEs was observed. In addition, the feeding rate of *D. magna* exposed to MCCE increased significantly, whereas no change in the feeding rate was observed for NCCE-exposed *D. magna*. In general, new and interesting aspects of the toxicity of MCCE and NCCE were revealed by this study, which contributes to the understanding of the toxicity of *Pseudanabaena* sp. extract on *D. magna* under bioassays.

Keywords: Cyanobacteria, ecotoxicology, feeding rate, life-history traits, somatic growth

1. Introduction

Lakes and reservoirs are one of the major potable water resources for humans and other living beings and are habitats for a wide variety of species (Cooke et al., 2005). Unfortunately, more than 40% of lakes and reservoirs in the world are in eutrophic condition, which is favorable for cyanobacterial bloom events (Chorus and Bartram, 1999). Cyanobacteria are harmful to aquatic organisms in water bodies due to their ability to produce a variety of toxic secondary metabolites, particularly during their mass development (Sivonen, 1996). Therefore, cyanobacterial blooms are major concerns for both human and ecological health (Bláha et al., 2009). In general, cyanobacteria can induce numerous negative effects on zooplankton including inhibition of feeding rate and reductions in survival rate, reproduction, and somatic growth (Ferrao-Filho et al., 2000; Dao et al., 2010). However, zooplanktonic species can respond differently to toxins or other bioactive compounds produced by cyanobacteria (Ferrao-Filho et al., 2000).

Among the toxic secondary metabolites produced by cyanobacteria, microcystins (MCs) are most commonly investigated (Guzmán-Guillén et al., 2017), particularly as they relate to zooplankton (e.g., *Daphnia* spp.), due to their toxicity and ubiquitous distribution (Díez-Quijada et al., 2019). Therefore, the adverse effects of the microcystin-producing strain of cyanobacteria on zooplankton have been extensively investigated (Liang et al., 2017). Although the microcystin-free strain does not contain known microcystin toxins, its negative effects on *Daphnia magna* have been reported (e.g., Lurling, 2003; Hulot et al., 2012). A number of previous studies used living cells (Ferrao-Filho et al., 2000; Lurling, 2003; Dionisio Pires et al., 2005; da Costa et al., 2013; Pham et al., 2015a), purified MCs (Chen et al., 2005; Ortiz-Rodríguez et al., 2010; Ortiz-Rodríguez et al., 2012; Hulot et al., 2012), or cyanobacterial crude extracts (CCEs) (Pietsch et al., 2001; Dao et al., 2010; Hulot et al., 2012; Dao et al., 2013a; Pham et al., 2016; Pawlik-Skowrońska et al., 2019; Toporowska et al., 2020) in toxicity tests. In fact, water from cyanobacterial blooms

* Correspondence: bb52218481@ms.nagasaki-u.ac.jp

contains MCs as well as a mixture of multiple substances which can cause unpredictable effects on zooplankton (e.g., *Daphnia* spp.) (Okumura et al., 2007). Therefore, the use of CCEs as the toxicant to evaluate the harmful effects of water bodies on aquatic organisms during cyanobacterial bloom events is highly recommended (Pietsch et al., 2001). Our current knowledge about the response of *D. magna* (e.g., life-history characteristics and feeding rate) to the microcystin-free crude extract (NCCE) of *Pseudanabaena* sp. is limited.

In Vietnam, toxicities of the microcystin-containing crude extract (MCCE) of *Microcystis* spp. sampled from Dau Tieng Reservoir, which is the largest irrigation reservoir in Vietnam and largest potable water resource in Southern Vietnam (Pham et al., 2015b), has not been evaluated completely. Although a few notable studies (Dao et al., 2013b; Dao et al., 2014; Pham and Ngo, 2017; Pham et al., 2017; Dao et al., 2018) have indicated the adverse effects of cyanobacterial blooms (including the *Microcystis* spp. living cells and MCCE) collected from Dau Tieng Reservoir on several model organisms, the life-history traits and feeding rate of *D. magna* in early development, a highly important stage that can influence the lifespan of organisms (e.g., reproduction) (Van Leeuwen et al., 1985), have not been reported. As a continuation of previous work, the aim of this study was to investigate the effects of subchronic MCCE and NCCE toxicity on the life-history traits and feeding rate of *D. magna* in its early stage of development.

2. Materials and methods

2.1. Test organisms

Samples of *D. magna* obtained from Microbiotests Inc. (Belgium) were used for the toxicity test. These organisms were raised in ISO medium (Dao et al., 2010) and fed a mixture of viable green algae *Chlorella* sp. and *Scenedesmus* sp., which were cultivated in COMBO medium (Kilham et al., 1998) with continuous aeration. Both *D. magna* and green algae were maintained at a temperature of 25 ± 1 °C and a 12/12 h light/dark cycle.

2.2. Cyanobacterial crude extract preparation

Biomasses extracted from *Microcystis* spp. and *Pseudanabaena* sp. blooms were used as cyanobacterial materials for the toxicity tests. The scum of cyanobacteria produced during blooms events, mainly *Microcystis* spp., was collected from Dau Tieng Reservoir located around 85 km northwest of Ho Chi Minh City, Vietnam. The cyanobacterial sample was dried under sunlight and kept at −20 °C prior to extraction. The microcystin-free cyanobacterial strain (*Pseudanabaena* sp.) isolated from the Dau Tieng Reservoir (Pham et al., 2015b) was cultured in Z8 medium and harvested onto GF/C glass fiber filters (Whatman, Kent, England). The filters containing *Pseudanabaena* sp. were dried at 45 °C and kept at −20 °C until the experiment. The CCEs were prepared as previously reported by Pietsch et al. (2001): two gram dry weight (DW) of the bloom material or *Pseudanabaena* sp. isolate were put into distilled water, frozen at −70 °C, and then thawed at room temperature. After the materials were thawed completely, they were sonicated for 3 min. This freeze-thaw-sonicate cycle was repeated five times, and then the samples were centrifuged at 2000 × g for 10 min to remove cell debris. The supernatants were collected and kept at −20 °C until the toxicity experiments. For analysis of MC content, a concentration of 8 g L^{-1} of CCEs (w/v) was prepared. Subsamples of the CCE supernatant were used for MC analysis as previously reported (Pham et al., 2015b): one hundred mL of the supernatants were centrifuged at 6000 × g at 4 °C for 15 min. The supernatants were collected, dried completely, and redissolved in 500 µL of 100% MeOH. The samples were analyzed by a high-performance liquid chromatography (HPLC) system with an ultraviolet-visible photodiode array detector (Shimadzu 10A series, Kyoto, Japan), and MC-RR, MC-LR, and MC-YR (Wako, Osaka, Japan) were used as standards. The HPLC analysis showed that CCE from natural cyanobacterial blooms contained three MC congeners, MC-RR, MC-LR, and MC-YR, at a total concentration of 670 µgg^{-1} DW(Pham et al., 2015b), whereas MCs were not detected in the *Pseudanabaena* sp. extract.

2.3. Ecotoxicologicalexperiments

Experiment on life-history traits of *D. magna*

The ecotoxicological test on life-history traits of *D. magna* was conducted according to Dao et al. (2010). The experiments consisted of control and CCE exposures. For the control, *D. magna* was raised in ISO medium without the addition of cyanobacterial extract. For CCE exposures, MCCE was added into the medium to reach the desired concentrations (1, 10, and 50 µg MCL^{-1}), i.e. those MC concentrations that are often found in the natural environment during cyanobacterial blooms (Chorus and Bartram, 1999). Then, NCCE was used at the same concentrations (i.e. the injected volume) as MCCE, and they are hereafter referred to as M1, M10, and M50 (MCCE) and N1, N10, and N50 (NCCE), respectively. For each exposure, fifteen neonates (<24h old) were randomly collected and individually transferred into 50 mL beakers containing 30 mL of ISO medium. The organisms were fed with *Scenedesmus* sp. at a concentration of 1 mg carbon source L^{-1} (approximately 140,000 cells mL^{-1}) during the test. Test medium and food were renewed simultaneously every two days. In addition, the pH and dissolved oxygen in each culture beaker were measured for fresh and spent medium. The measured pH and dissolved oxygen (mg L^{-1}) in culture medium were within a range of 7.1 to 7.6

and 7.4 to 7.7, respectively; the test lasted for 14 days. During experiments, the life-history trait responses were evaluated based on survival rate, reproduction, and somatic growth. The survival rate of the initial maternal *D. magna* was recorded daily. The reproduction endpoints included the number of neonates per female, brood size, time to maturation, time to first reproduced brood, number of broods per female, and the intrinsic rate of natural increase. Note that the produced neonates were counted daily and discarded. The degradation of eggs and neonates represented by dead eggs, dead neonates, and neonate malformation, if any, was recorded when the dead eggs, dead neonates, and neonate malformations were detectable. The intrinsic rate of natural increase (r) was calculated according to the jackknife procedure (Meyer et al., 1986):

$$\sum_{x=1}^{\max age} e^{-rx} l_x m_x = 1$$

where, l_x is the proportion of individuals surviving to age x, m_x is the number of neonates produced per surviving females at age x, and x is days. Calculations of the intrinsic rate of natural increase over 14 days are indistinguishable from those for an entire lifespan; due to the great importance of early-stage reproduction (Van Leeuwen et al., 1985), calculations of intrinsic rate of natural increase based on 14-day experiments were considered acceptable. The somatic growth of surviving individuals of each exposure was determined when the test was completed. Specifically, the body length of each surviving individual was measured from the apex of the helmet to the base of the tail spine under an inverted microscope (Villarroel et al., 2003).

Experiment on the feeding rate of *D. magna*

The feeding rate experiment was conducted according to the description of Ferrando et al. (1993), with minor modifications. Briefly, three concentrations (the same used in the life-history trait experiment) of CCEs (MCCE and NCCE) were used for the feeding rate study, and each concentration consisted of five replicates. Another exposure in which *D. magna* was incubated in medium without CCEs was implemented for control. One additional replicate without *D. magna* was used for calculating the correction factor. The experiment was conducted in 50 mL glass beakers containing 50 mL of ISO medium and 10 of *D. magna* (<24 h old) without green algae as food. The beakers were kept at 25 ± 1 °C under dark and static conditions. After 24 h, *Scenedesmus*sp. was added to each beaker to reach the desired concentration of 10^5 cells mL^{-1}, and the experiment lasted for 5 h . At the end of the experiment, *D. magna* was removed, and the density of *Scenedesmus* sp. in the beakers was estimated by hemocytometer counting chamber under an inverted microscope. For the calculations of filtration rate (μL $individual^{-1}$ h^{-1}) (FR) and ingestion rate (cells $individual^{-1}$ h^{-1}) (IR), the equations of Gauld (1951) were used:

$$FR = \frac{V(\ln C_0 - \ln C_t)}{nt} - A$$

$$A = \frac{\ln C'_0 - \ln C'_t}{t}$$

$$IR = FR\sqrt{C_0 C_t}$$

where, C_0 and C_t are initial and final algae densities, respectively (cells μL^{-1}), t is time (period of the experiment in hours), and n is the number of *D. magna* in volume V (μL). A correction factor (A) is a change in the initial (C'_0) and final (C'_t) algae densities after time t (in one additional replicate). The expression $\sqrt{C_0 C_t}$ represents the geometric mean of algae density during time t.

2.4. Data mining and analysis

All data were presented as mean ± standard deviation except the survival rate. Type I error level was set at P≤.05, which is considered the statistically significant level to reject the null hypothesis. The statistical analysis was carried out using R software (version 3.4.3, Lucent Technologies, Inc., Murray Hill, New Jersey, USA) combined with R Studio (version 1.2.5033, Lucent Technologies, Inc.). One-way analysis of variance (ANOVA) was applied to detect the significant difference of reproduction and somaticg rowth, as well as the feeding rate of *D. magna*, between control and CCE exposures (CCE concentrations were assigned as the factor variables), followed by many-to-one comparison Dunnett's test using the multcomp package. The assumptions of the ANOVA test were confirmed prior to the test. Levene's test (in multcomp package) and Shapiro–Wilk's were applied to check the homogeneity of variances and normality of residuals, respectively. If the assumptions were not satisfied, the nonparametric Kruskal–Wallis rank–sum test was applied, followed by the nonparametric Wilcoxon rank–sum test for multiple comparisons. The highest correlation between the *D. magna* responses (reproduction endpoints, somatic growth, and feeding rates) and CCE concentrations, if any, was evaluated by running simple linear (or nonlinear) models for each of the CCEs with the CCE concentrations assigned as the numeric variables.

3. Results

3.1. Effects of cyanobacterial crude extracts on life-history traits of *D. magna*

Effects on survival rates

No mortality occurred in the control, M1, N10, N50, and M50 exposures over 14 days of the test, whereas exposures to M10 and N1 decreased the survival rates of *D. magna* slightly at 6.7% and 13.3% of total initial females, respectively.

Effects on reproduction

The number of neonates per female in MCCE-and NCCE-exposed *D. magna* at all exposures was significantly higher than the control (Table 1). The time to maturation of *D. magna* exposed to both CCEs at all exposures (except M1 exposure) was significantly earlier than the control (Table 1). Subsequently, *D. magna* belonging to all MCCE and NCCE exposures (except M1 exposure) reached their first reproduced brood days sooner relative to the control *D. magna* (Table 1). The number of broods per female in N10, N50, M10, and M50 exposures was significantly greater than the control (Table 1). Under exposures to M1, M10, M50, and N50, the first brood size was significantly larger than the control, whereas all MCCE and NCCE exposures significantly increased the second brood size compared to the control (Figures 1A, 1B). The intrinsic rate of natural increase of *D. magna* at all MCCE and NCCE exposures was significantly higher than the control (Table 1). Dead eggs, dead neonates, and neonate malformations of gravid females were observed in the M50 and N50 exposures (represented by Figures 2A–2D as control represented by Figures 2A–2D as control), whereas only dead neonates were observed in M1, M10, N1, and N10 exposures (Figure 2B). Among reproduction endpoints, somatic growth, and feeding rate of *D. magna*, the number of neonates per female analyzed had the highest correlation with the tested concentrations of both CCEs (Figure 3).The regression equations were calculated by the nonlinear model (or quadratic equation) (Figure 3).

Effects on somatic growth

Daphnia magna body length in all MCCE and NCCE exposures was significantly longer than the control (Figure 4).

3.2. Effects of cyanobacterial crude extracts on the feeding rate of *D. magna*

The filtration and ingestion rates of *D. magna* in all MCCE exposures were significantly higher than those in the control, whereas no change in *D. magna* feeding rate in all NCCE exposures was observed relative to the control (Figures 5A, 5B).

4. Discussion

Daphnia magna mortality rates under the effects of MCCE and NCCE were insignificant according to OECD (2012), where the regulated the survival rate was ≥80%. As expected, NCCE did not cause strong adverse effects on survival rates because iit was MC-free crude extracts" with "it did not contain the known survival-toxic MC (Ortiz-Rodríguez et al., 2012). As evidenced by Hulot et al. (2012), the survival rate of *D. magna* exposed to NCCE did not significantly decrease over a long-term test. In terms of MCCE effects on survival rates, Dao et al. (2010) reported that MCCE at a low concentration of 5 μg MC L^{-1} insignificantly decreased *D. magna* survival rates over the 60 days of the test. Moreover, Smutná et al. (2014) pointed out that MCCE at a medium concentration of 9.2 μg MC L^{-1} did not significantly reduce the *D. magna is italicized* over 21 days of testing. At a high concentration (50 μg MC L^{-1}) the harmful effects of MCCE on *D. magna* were likely to come about over time. Thus the adverse effects of MC and/or harmfully bioactive compounds on *D. magna* over 14 days were not enough to induce mortality. As proof, Dao et al. (2010) revealed that MCCE at a high concentration (50 μg MCL^{-1}) caused high mortality for *D. magna* after the 21st day. In addition, the dissolved MC content in aqueous extracts was likely to decline as a consequence of the process of adsorption on particulate materials, photo degradation, and biodegradation (Schmidt et al., 2014).Therefore, we suggest that future research should measure the MC concentration in spent medium (the second-day medium before renewed) to confirm remaining MC concentrations. Moreover, the toxic effects of MCCE on survival rates were perhaps compensated by multiple substances (considered nutrients); hence, MC in a mixture did not cause negative

Table 1. The reproduction of *Daphnia magna* exposed to cyanobacterial crude extracts over the 14-day test.

Exp.	The number of neonates per female (ind.)	Time to maturation (d.)	Time to first reproduced brood (d.)	The number of broods per female (br.)	r (day $^{-1}$)
CT	8.9 ± 2.9	9.1 ± 1.4	11.4 ± 1.2	1.8 ± 0.4	0.15 ± 0.03
M1	16.8 ± 4.2a	7.9 ± 1.6	10.6 ± 1.3	2.3 ± 0.6	0.20 ± 0.02c
M10	42.2 ± 8.9c	5.5 ± 0.8c	7.7 ± 0.8c	3.6 ± 0.6c	0.26 ± 0.02c
M50	74.3 ± 13.0c	5.1 ± 0.8c	7.1 ± 0.8c	4.3 ± 0.6c	0.31 ± 0.01c
N1	19.3 ± 5.8b	7.3 ± 0.5b	9.4 ± 0.5c	2.5 ± 0.5	0.20 ± 0.02c
N10	45.3 ± 5.7c	5.3 ± 0.6c	7.3 ± 0.5c	3.7 ± 0.5c	0.27 ± 0.01c
N50	60 ± 20.0c	5.1 ± 1.3c	7.1 ± 1.3c	3.6 ± 0.8c	0.29 ± 0.04c

*Small letters indicate significant differences by the ANOVA test, followed by the many-to-one comparison Dunnett's test ($^{a}P \leq .05$, $^{b}P \leq .01$, $^{c}P \leq .001$). Exp.: exposure, ind.: individual, d.: day, br.: brood, r: the intrinsic rate of natural increase.

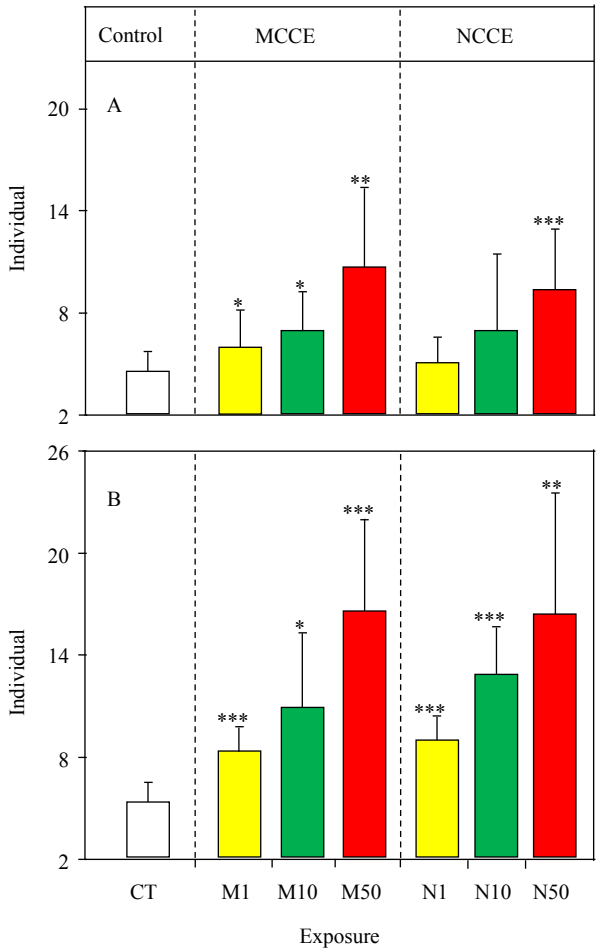

Figure 1. The effects of cyanobacterial crude extracts on the brood size. A: the first brood, B: the second brood. Asterisks indicate significant differences by the Kruskal-Wallis test, followed by the Wilcoxon rank-sum test for multiple comparison (*P < .05, **P < .01, ***P < .001).

effects on the survival of *D. magna* compared to purified MC (Ortiz-Rodríguez et al., 2012). A meta-analysis (Smutná et al., 2014) indicated that the toxic effects of MC-containing cyanobacterial bloom materials on survival rates in *D. magna* decreased significantly depending on the complexity of tested materials with a toxic rank of several-substances-containing extract > total aqueous extract > biomass. Our MCCE consisted of a mixture of MC-RR, -LR, and -YR, in which MC-RR was the dominant congener (Pham et al., 2015b). Thus, MC-RR is likely to induce low toxicities compared to MC-YR or MC-LR by a toxic rank of MC-YR > MC-LR > MC-RR (Puerto et al., 2009). In addition, Campos et al. (2014) offered proof of a multixenobiotic resistance mechanism of *D. magna* which could recognize a wide variety of toxicants (e.g., MC and/or harmfully bioactive compounds in MCCE) and keep them in cells at low levels (Epel et al., 2008).

Our results of reproduction-stimulated effects, in which *D. magna* was exposed to MCCE at concentrations of 5 and 50 μgMC L^{-1}, were in good agreement with Dao et al. (2010). However, the higher levels of stimulation of reproduction in our study compared to the previous study (Dao et al., 2010) were probably due to the lower total MC content in MCCE. Specifically, the MCCE of Dao et al. (2010) contained 6.92 mg L^{-1} of total MCs, whereas our MCCE contained 5.36 mg L^{-1} of total MCs derived from 670 μgMC g^{-1} DW of cyanobacterial bloom materials (see Section 2.2). Therefore, higher beneficial compound concentrations, which are considered nutrients, were injected into the culture medium provided with the specific concentrations (e.g., 50 μgMC L^{-1}). The bioactive compounds in CCEs included vital components, such as lipopeptides (40%), amino acids (5.6%), fatty acids (4.2%), macrolides (4.2%), and amides (9%) (Lau et al., 2015), which likely improved *D. magna* fitness and, probably, reproduction (Hulot et al., 2012). The possible effects of CCEs were definitely dependent on species-specific cyanobacteria (Hulot et al., 2012). Our study yielded interesting results showing that the reproduction-stimulated effects of two CCEs from two kinds of cyanobacteria species on *D. magna* were close to each other, implying that the two CCEs had similar properties or that some of the compounds that stimulate dreproduction were contained in both CCEs (Hulot et al., 2012). Our results were in line with Dao et al. (2010) who observed the death of eggs and neonates and neonate malformations of *D. magna* females exposed to 5 and 50 μgMC L^{-1} of MCCE or purified MC. Interestingly, in our study, harmed offspring of MCCE-and NCCE-exposed *D. magna* were observed; this implies not only that MC is responsible for the harmed offspring but that other, unknown harmful compounds should be considered. In fact, Bednarska and Slusarczyk (2013) also proved that the microcystin-free filamentous cyanobacteria could cause maternal abortion of *Daphnia pulicaria*. It is likely that the offspring of MCCE- and NCCE-exposed *D. magna* suffered from toxic pressures and therefore needed to pay the energy cost for resisting the toxins, even from the embryonic development stage (Dao et al., 2010). In addition, the degradation of offspring was derived directly from maternal biotransformation of MC and/or harmfully bioactive compounds to eggs (Wiegand, 2009). To the best of our knowledge, modelizations of stimulated responses of life-history traits and physiological endpoints versus CCE concentrations have not yet been implemented. A few meta-analyses (Lurling, 2003; Herrera et al., 2015) indicated that the linear and logistic models were usually applied for describing correlations of adverse responses versus CCE concentrations or cyanobacterial cells. In this study, we suggested the quadratic equation, which

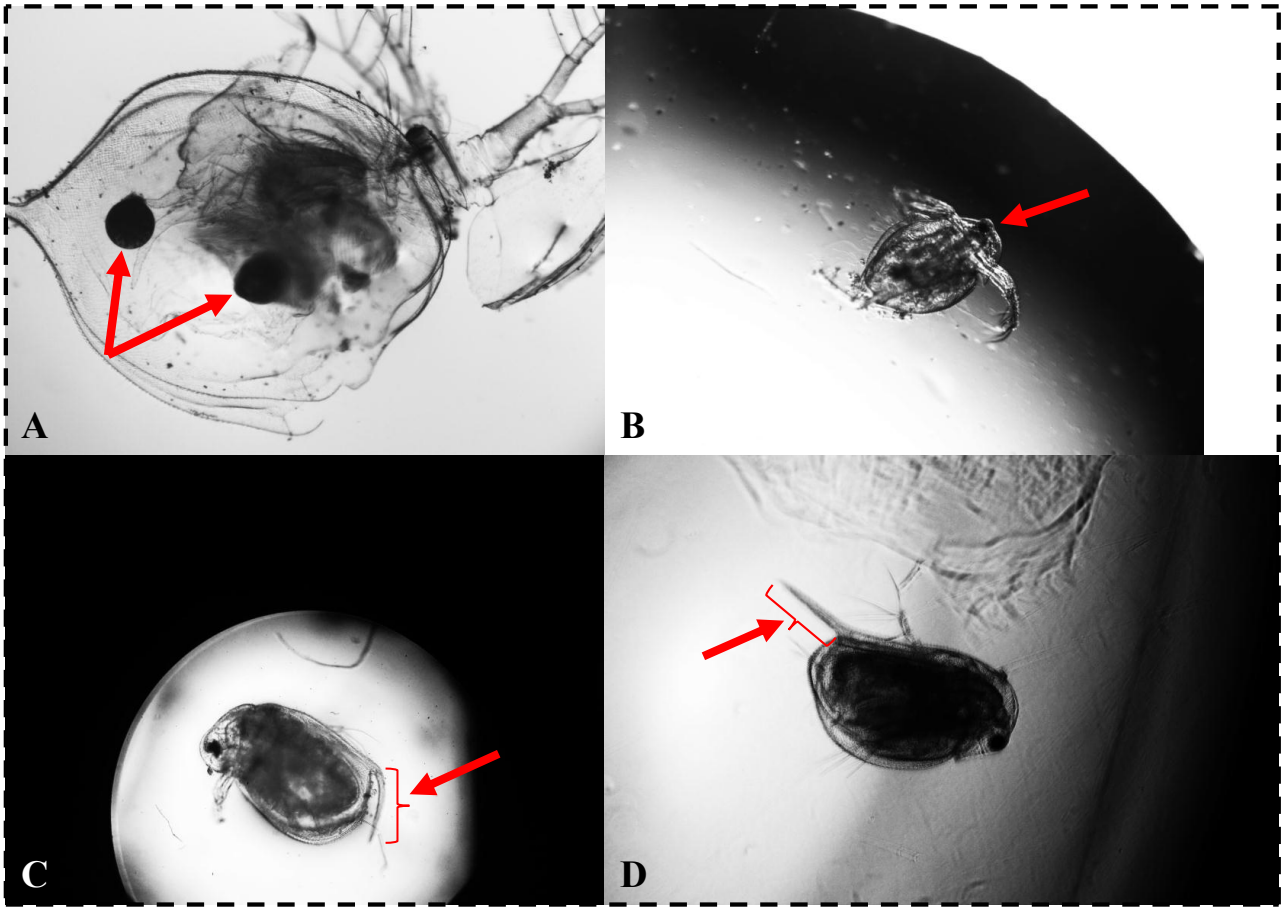

Figure 2. The degradation of eggs and neonates (red arrows) of the *Daphnia magna* gravid females exposed to cyanobacterial crude extracts. A: dead eggs, B: dead neonate, C: malformation of the tail, and D: normal tail of control *Daphnia magna*.

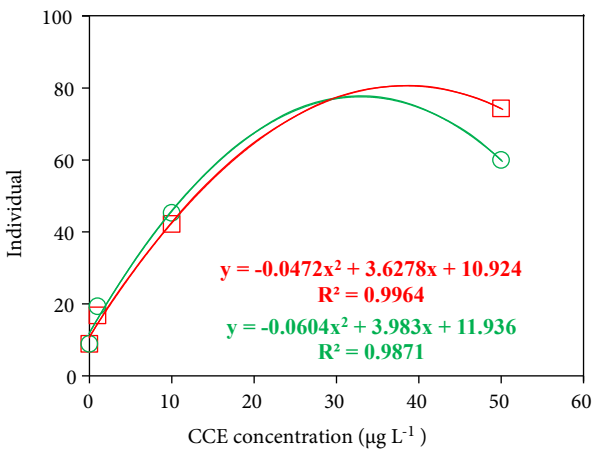

Figure 3. Regression equations and correlation coefficients (R^2) describing the highest correlations between the number of neonates per female (as mean) and CCE concentrations. The red color indicates the microcystin-containing crude extract and the green color indicates the microcystin-free crude extract.

is able to demonstrate the correlation between *D. magna* responses and CCE concentrations. Specifically, CCEs at concentrations assumed to be undera tolerance threshold did support *D. magna* reproduction (e.g., number of neonates per female) as the nutrient supplement, whereas when CCE concentrations increased beyond this tolerance threshold, harmful expressions were observed (Herrera et al., 2015). We recommend further research with higher CCE concentrations to test the aforementioned hypothesis. Interestingly, among many endpoints belonging to the life-history traits and physiological responses evaluated, the number of neonates per female endpoint exhibited the highest correlation to both MCCE and NCCE exposures. It is reasonable as Cui et al. (2016) and Sancho et al. (2016) observed that the number of neonates per female was a very important endpoint for ecotoxicological evaluation because it was highly reflective of the toxic levels of tested substances on model organisms.

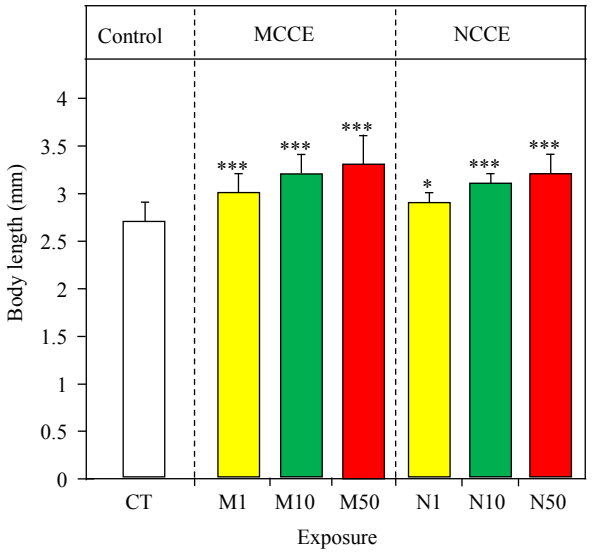

Figure 4. The effects of cyanobacterial crude extracts on somatic growth of *Daphnia magna*. Asterisks indicate significant differences by the Kruskal-Wallis test, followed by the Wilcoxon rank-sum test for multiple comparison (*P < .05, **P < .01, ***P < .001).

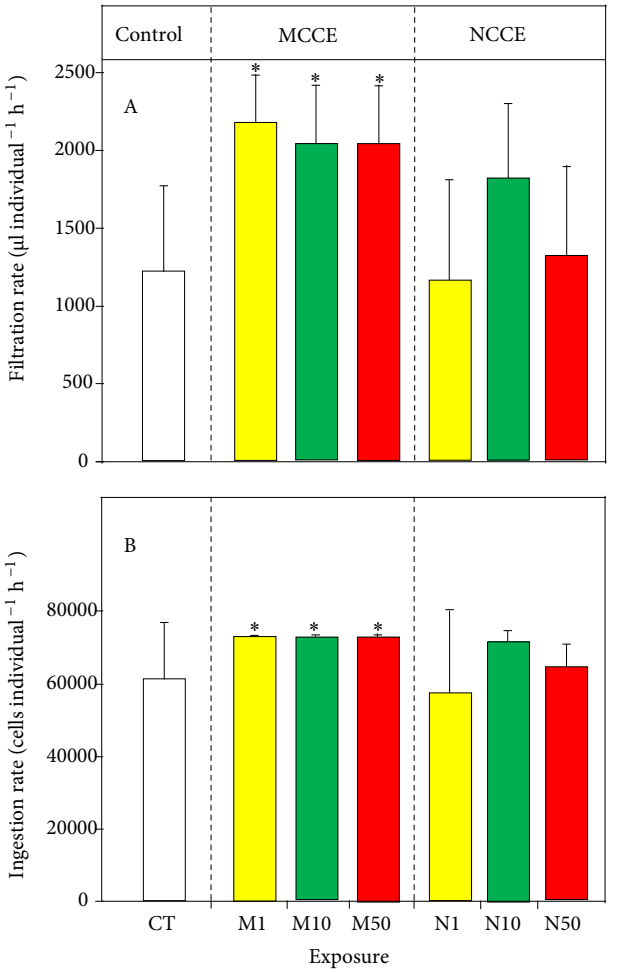

Figure 5. Filtration (A) and ingestion (B) rates of *Daphnia magna* after exposing to cyanobacterial crude extracts. Asterisks indicate significant differences by the Kruskal-Wallis test, followed by the Wilcoxon rank-sum test for multiple comparison (*P ≤ .05).

The enhanced somatic growth of *D. magna* exposed to MCCE in our study was in line with Dao et al. (2010), in which *D. magna* was exposed to MCCE at concentrations of 5 and 50 µgMC L^{-1}. However, purified MC at concentrations of 5 and 50 µg L^{-1} also increased *D. magna* somatic growth (Dao et al., 2010). Therefore, our results confirmed that MC is likely to enhance *D. magna* somatic growth, and beyond that it is reasonable to consider other compounds that may be responsible for the increase in somatic growth in CCE-exposed *D. magna*. Another highly possible scenario is additional nutrients stimulating somatic growth, and this has been proven by Herrera et al. (2015). Specifically, the body length of *Moinamicrura* and *Daphnia similis* increased significantly relative to the control when exposed to MCCE at concentrations of 4.5–21.7 µgMC L^{-1} (Herrera et al., 2015).

In our results the stimulated feeding rate only occurred in MCCE-exposed *D. magna* not in NCCE-exposed *D. magna*. Interestingly, according to Ghadouani et al. (2004), exposure to purified MC at a concentration of 50 µg L^{-1} did not change the feeding behavior of *Daphnia pulicaria*. In addition, exposure to MCCE at a concentration of 134.5 µgMC L^{-1} strongly inhibited *Daphnia similis* feeding behavior (Herrera et al., 2014). Thus, there must be some unknown substances at the relevant concentrations contained in the MCCE investigated in the current study. We suggest the further determination of chemical composition of the two CCEs in order to facilitate clear interpretation of the differences in the feeding rate effects of both CCEs.

In summary, the *D. magna*survival rate was insignificantly reduced by both CCEs. In addition, the fertility of *D. magna* exposed to both CCEs was drastically improved, even at the lowest concentration. Although the reproduction-stimulated effects on *D. magna* were expressed clearly at the evaluated endpoints, offspring-toxic effects were also recorded in all MCCE and NCCE exposures. Additionally, somatic growth in *D. magna* increased under exposures to MCCE and NCCE at all tested concentrations. However, the feeding rate was only enhanced by MCCE exposures, which indicated dissimilar properties between CCEs that remain unknown. Our results are new and ecotoxicologically

indicate feeding rate as well as life-history trait responses of *D. magna* exposed to MCCE and NCCE at an early stage of development. The toxicities of *Pseudanabaena* sp. extract on *D. magna* obtained in our study are considered the first report for further investigations. Ecotoxicological tests with reservoir water collected directly from locations of cyanobacterial blooms using *D. magna* and/or a variety of species will provide material for future investigations.

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