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Maternal consumption of non-toxic *Microcystis* by *Daphnia magna* induces tolerance to toxic *Microcystis* in offspring

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SUMMARY

1. Freshwater cyanobacterial blooms are a worldwide environmental issue. These blooms often comprise both non-toxic and toxic species and strains. Populations of some zooplankton, including *Daphnia*, have been shown to adapt locally to toxic *Microcystis* through maternal effects. However, *Microcystis* populations vary spatially and temporally in the absolute and relative abundances of non-toxic and toxic genotypes.

2. We examined variation in induction of tolerance to toxic cyanobacteria in offspring from two *Daphnia magna* clones (JS and AH) fed diets containing *Scenedesmus* by itself or in combination with either a non-toxic or a toxic clone of *Microcystis aeruginosa*. The diets containing *Microcystis* included relatively more cyanobacteria within each week of the 3 week experiment (week 1–10%, week 2–20%, week 3–40%). *Daphnia* neonates were collected from these three treatments at the end of the third week and fed a diet containing *Scenedesmus* and toxic *Microcystis* for 3 weeks before a suite of life-history, physiological and biochemical measurements were made on the surviving animals.

3. Neonates from mothers fed toxic and non-toxic *Microcystis* showed enhanced growth and reproduction compared to neonates produced from mothers fed only *Scenedesmus*. Our results showed that *Daphnia* neonates could be induced to tolerate toxic cyanobacteria when their mothers were fed diets containing non-toxic or toxic strains of cyanobacteria. Furthermore, elevated RNA–DNA ratios, superoxide dismutase activity and catalase activity in neonates fed diets containing non-toxic or toxic *Microcystis* clones suggested that the mechanisms behind these changes involved processes associated with metabolism and antioxidation.

4. Our study shows that non-toxic cyanobacteria can induce tolerance to toxic cyanobacteria and further elucidates the general importance of maternal effects on tolerance of cyanobacteria in herbivorous zooplankton.

Keywords: adaptive fitness, cyanobacteria, genotype, maternal effects, zooplankton

Introduction

Cyanobacteria regularly form blooms in recreational lakes, drinking water reservoirs and protected wetland areas due to eutrophication (Paerl & Paul, 2012; Chislock *et al*., 2013). The frequency and intensity of these blooms are projected to increase under a changing climate (Paerl & Huisman, 2008; Kosten *et al*., 2012). A subset of clones of the well-studied, bloom-forming taxa, *Microcystis*, carries a 55 kb microcystin synthetase (*mcy*) gene cluster required for the production of the potent hepatotoxin microcystin, whereas non-toxin producing genotypes generally lack or contain an incomplete copy of this gene cluster and thus lack the ability to produce the toxin (Kaebernick & Neilan, 2001). Both microcystin-producing and non-microcystin-producing strains can be isolated from the same water source (Wilson *et al*., 2005), and reports show that the composition of toxic and

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non-toxic populations within *Microcystis* species change over time depending on environmental factors including zooplankton grazing (Kardinaal *et al*., 2007; Rinta-Kanto *et al*., 2009; Van De Waal *et al*., 2011; Haney & Lampert, 2013).

During cyanobacterial blooms, the transfer of energy from phytoplankton to zooplankton has been shown to be inefficient because cyanobacteria may produce toxic secondary metabolites such as microcystins (Yang *et al*., 2012), grow in forms that mechanically interfere with zooplankton filtering apparatus (DeMott, Gulati & Van Donk, 2001; Wilson, Sarnelle & Tillmanns, 2006) or are deficient in essential nutritional components such as sterols and fatty acids (Von Elert & Wolffrom, 2001; Martin-Creuzburg, Von Elert & Hoffmann, 2008). Short-term exposure to toxic cyanobacteria has been shown to improve the fitness of *Daphnia magna* upon further exposure to toxic prey during development (Gustafsson & Hansson, 2004). Enhanced tolerance in *D. magna* after previous exposure to toxic cyanobacteria was shown to be an inducible response developed over the lifetime of the individual, and further, this trait could be transferred to offspring via maternal effects (Gustafsson, Rengefors & Hansson, 2005). However, such adaptations in *Daphnia* may be rather clone-specific (Jiang *et al*., 2013). Recent evidence for the resistance to toxic cyanobacteria based on differential gene expression in *Daphnia* suggested that the increased expression of ATP-binding cassette transporters and arginine kinase constitutes an adaptive mechanism enabling *Daphnia* resistance to toxic cyanobacteria (Schwarzenberger *et al*., 2014; Lyu *et al*., 2015). Overall, the existence of tolerant zooplankton clones in populations is well supported (Hairston *et al*., 1999), but understanding of the mechanisms influencing the range of tolerance in zooplankton is still limited.

Maternal effects occur whenever environmental cues experienced by mothers result in a modification of offspring phenotype. Although maternal effects were originally deemed nuisance sources of variation in quantitative genetic studies (Falconer, Mackay & Frankham, 1996), they are now recognized as an important mechanism for rapid, multigenerational responses to environmental change, empowering offspring with optimal life-history strategies, mate choice or improved behaviour to avoid predation (reviewed by Marshall & Uller, 2007). Consequently, maternal effects are suggested to strongly affect population dynamics (Rossiter, 1996), community interactions (Agrawal, 2001) and the rate and direction of evolutionary change (Day & Bonduriansky, 2011). Zooplankton offspring fitness is often influenced by maternal conditions (LaMontagne & McCauley, 2001; Garbutt *et al*., 2013; Garbutt & Little, 2014; Gribble *et al*., 2014; Walsh *et al*., 2015). For example, Garbutt & Little (2014) showed that *Daphnia* mothers maintained at an elevated temperature or fed a restricted diet produced offspring that were more resistant to pathogen infection.

In freshwater systems with abundant cyanobacteria, the dominant taxa may shift from non-toxic to toxic strains (Davis *et al*., 2009; Li *et al*., 2014) during the seasonal succession of phytoplankton communities. Hence, zooplankton offspring might be expected to experience more toxic environments than their mothers during the summer growing season. Prior work has shown that exposure to a toxic strain enhances fitness in *Daphnia* offspring (Gustafsson *et al*., 2005). Here, we extend this prior work by testing the maternal influence of both toxic and non-toxic strains of *Microcystis* to obtain a better understanding of the underlying mechanisms driving phenotypic responses to low-quality algal diets. We hypothesized that offspring produced from *Daphnia* fed a diet containing non-toxic *Microcystis* would also enhance offspring performance relative to conspecifics fed a high-quality diet without cyanobacteria. Since *Microcystis* strains that produce microcystin cause oxidative damage (Ortiz-Rodríguez & Wiegand, 2010), we further examined whether a correlation exists between activation of maternal effects of inducible defences against *Microcystis* and offspring antioxidant ability.

Methods

Daphnia *and algae culture*

We conducted experiments using two genotypes of the cyclically parthenogenetic freshwater cladoceran, *Daphnia magna*. These clones (JS and AH) originated from Lake Taihu (China) and Lake Chaohu (China), respectively. The two lakes are 500 km apart, have similar physical and chemical parameters (Chen *et al*., 2003; Deng *et al*., 2008) and annually experience heavy cyanobacterial blooms (Deng *et al*., 2008; Zhang *et al*., 2010). Consequently, both *Daphnia* clones were expected to be more adapted to toxic cyanobacteria than clones from less productive lakes with few cyanobacteria (Sarnelle & Wilson, 2005). The two *Daphnia* clones were maintained in a state of clonal replication under controlled conditions: temperature 25 °C, 14 h : 10 h light : dark cycle in M4 medium (Elendt & Bias, 1990). Zooplankton medium was gently aerated with filtered air for 24 h before use and totally renewed twice

weekly. Animals were maintained with daily additions of *Scenedesmus obliquus* (FACHB-416; 1.5 mg C L^{-1}).

The high-quality alga *Scenedesmus obliquus*, the toxic *Microcystis aeruginosa* strain (PCC7806) and the non-toxic *M. aeruginosa* strain (FACHB-469) were obtained from the Freshwater Algae Culture Collection of the Institute of Hydrobiology at the Chinese Academy of Sciences. Both *M. aeruginosa* strains, which grow as single cells (5–6 μm diameter spheres) or paired cells, were chosen for this study to avoid any mechanical interference issues from large colonies that might influence *Daphnia* fitness. We also conducted a pilot experiment and confirmed via high-performance liquid chromatography (Gan *et al.*, 2010) that the toxic *M. aeruginosa* (PCC7806) produces at least two types of microcystin (MC-LR and MC-RR) with a total content of 3.6 pg per cell. All three algal strains were maintained in 1-L flasks containing 400 mL of BG-11 medium (Stanier *et al.*, 1971), grown semi-continuously at 25 °C under fluorescent light at 40 μmol photons m^{-2} s^{-1} supplied in a 14 h : 10 h light : dark cycle and were then harvested for feeding to *Daphnia*.

Exposure protocol

Life-history tables were constructed for F_0 (mother) and F_1 (offspring) generations of *D. magna* to study the effect of *M. aeruginosa* on *Daphnia* fitness (Fig. 1). The F_0 generation was maintained (total food concentration for each diet = 1.5 mg C L^{-1}) for 21 d and divided into three groups: (i) 100% *S. obliquus* (hereafter called the S group), (ii) an increasing amount of non-toxic *M. aeruginosa* (N group) or (iii) an increasing amount of toxic *M. aeruginosa* (T group) with five replicates of each group. Each replicate in 200 mL containers contained five individuals. During the first week of exposure, N group and T group *Daphnia* were fed 10% (by carbon content) *M. aeruginosa* with increasing relative abundances of *M. aeruginosa* in the second (20%) and third (40%) weeks. For the three groups, single offspring (<24 h old) from parthenogenetic females were transferred to individual 50-mL beakers under identical experimental conditions to those used during culturing.

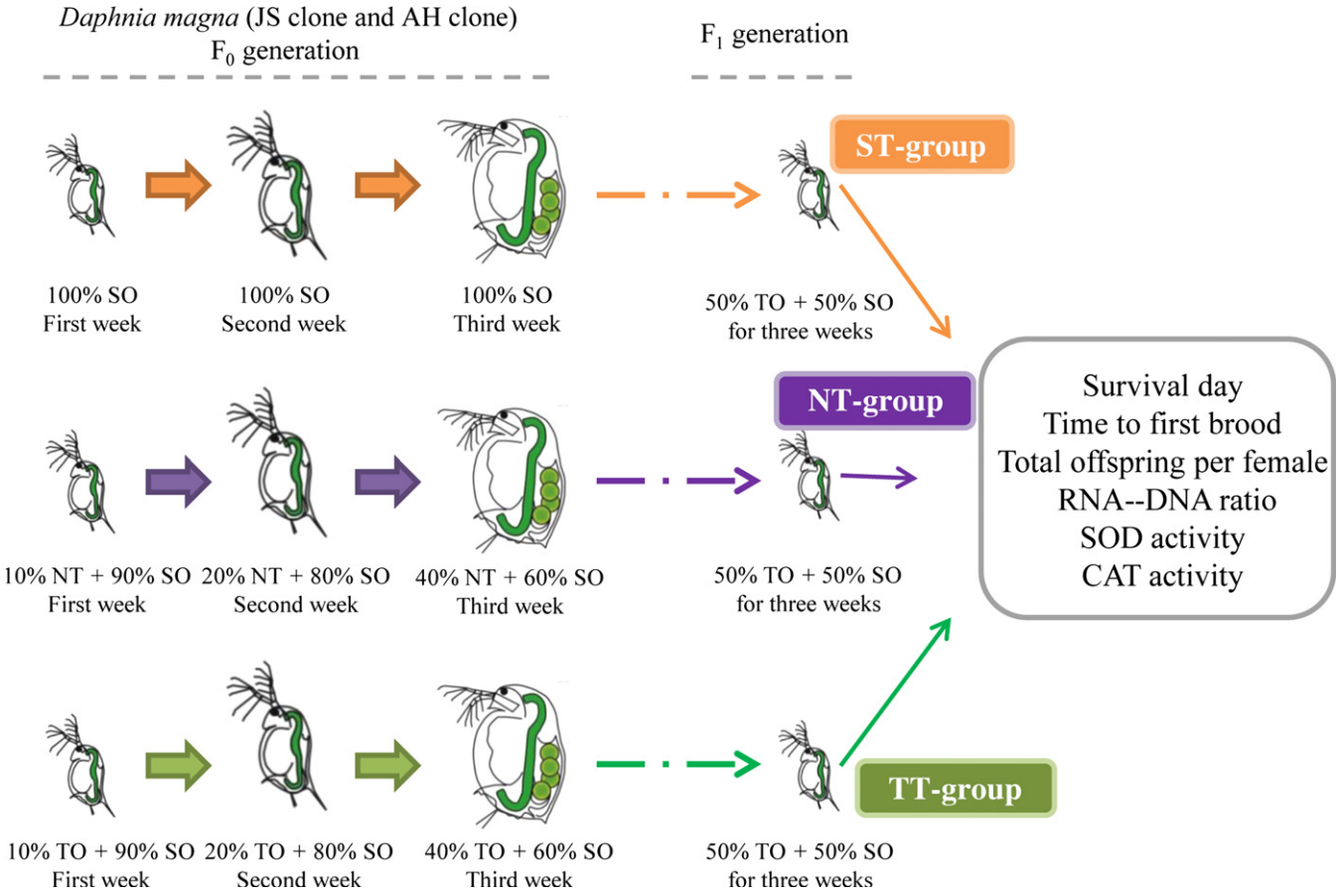

Fig. 1 Schematic diagram of the experiment. Diet abbreviations: SO (*Scenedesmus obliquus*); NT (Non-toxic *Microcystis aeruginosa*); TO (Toxic *Microcystis aeruginosa*).

The F_1 generation was obtained from the last brood of the F_0 generation during the 21-d exposure. Newly hatched offspring (<24 h old) from each of the three treatment groups were transferred to a mixture of 50% toxic *M. aeruginosa* + 50% *S. obliquus* treatments (total food concentration = 1.5 mg C L^{-1}) in the F_1 generation with 10 (JS clone) and 30 (AH clone) replicates of each group (labelled as ST group, NT group and TT group, respectively) for a 21-d observation. All 10 replicate offspring (JS clone) or 10 of the 30 replicate offspring (AH clone) were used for estimating life-history traits. The remaining 20 replicates of the AH clone were used for determining enzyme activity and RNA–DNA ratio.

In this study, individual fitness of F_1 offspring was considered as survival and reproduction. Individual survival was monitored daily. Dead individuals were confirmed using microscopy and were removed. Offspring production was measured daily; once counted offspring were removed. Time to first brood (i.e. maturation) and the total number of offspring per female were recorded.

Determination of enzyme activity and RNA–DNA ratio

To determine whether improved offspring fitness is correlated with elevated antioxidant activities and metabolic capacities, we observed changes in catalase (CAT), superoxide dismutase (SOD) and the RNA–DNA ratio for the different maternal food treatments in the offspring generation. On the 21st day of F_1 generation exposure, living females in each group of AH clone were collected and washed three times with physiological saline (PS; 0.68% NaCl). Next, 200 μL of PS was added prior to sonication (40% amplification, 5 s on/5 s off, 5 times) on ice for the purpose of breaking the cells and the body of the *Daphnia*. The lysis solutions were centrifuged at 600 ***g*** (Thermo Fresco 21, Langenselbold, Gremany) for 10 min at 4 °C to eliminate cellular debris and cartilage fragments. The supernatant was removed and used for antioxidant parameters assays. Protein (mg mg^{-1}), CAT (U mg^{-1} protein) and SOD (U mg^{-1} protein) were estimated by the Diagnostic Reagent Kits (Jiancheng Bioengineering Institute, Nanjing, China). Enzyme activities were related to total protein per extract, determined according to Bradford (1976) with Bradford protein dye reagent (Generay, China) measured at 595 nm. RNA–DNA ratio was measured by monitoring fluorescence intensity in a NanoDrop 2000 UV-Vis Spectrophotometer (Thermo Scientific, Willmington, DE, U.S.A.) (Gorokhova & Kyle, 2002; Goto *et al.*, 2012). Briefly, five living females were disrupted in a 1.5-mL tube containing dBiozol (Bioflux, Hangzhou, China) for DNA extraction and five living females were extracted for RNA (Trizol, Takara, Japan), respectively.

Statistical analysis

All biochemical data were expressed as mean ± 1 standard error (SE). Significant differences in life-history variables were evaluated by two-way analysis of variance (ANOVA) followed by the Duncan multiple range test ($\alpha = 0.05$). Also, effects of maternal food types on enzyme activity and RNA–DNA ratio in the AH clone were assessed by one-way ANOVA followed by Duncan's multiple range test (a = 0.05). All tests were run with the SigmaPlot 11.0 software package.

Results

During the 21-d exposure, all individuals in the ST group survived and no significant differences were detected for survivorship among the three groups, regardless of *Daphnia* genotype (Fig. 2a; Table 1). Of the three life-history variables examined in the offspring (F_1 generation), maternal food experience significantly affected growth and reproduction in the offspring (Fig. 2b and c). Specifically, NT group and TT group of the two clones reached maturity (the age at the first brood) 30% faster than did those in ST group, respectively (Table 1). Consequently, both the NT group and TT group of each clone had a higher number of total offspring during the exposure to toxic cyanobacteria (Table 1). There was also a significant effect of *Daphnia* genotype on the fecundity in the NT group and TT group (Table 1), reflecting the 30% higher fecundity in the AH clone than the JS clone.

For the AH clone, changes in RNA–DNA ratio were significantly elevated (2 to 4-fold) in both the NT group and TT group in comparison with the ST group (Fig. 3a; one-way ANOVA, $F_{2,\ 8} = 48.269$, $P < 0.001$). Similar responses for the NT group and TT group were observed for SOD and CAT relative activity in comparison with the ST group (Fig. 3b and c; one-way ANOVA, $F_{2,\ 8} = 65.620$, $P < 0.001$ for SOD; $F_{2,\ 8} = 6.012$, $P = 0.037$ for CAT).

Discussion

In line with previous studies (Gustafsson *et al.*, 2005; Jiang *et al.*, 2013), our results clearly showed that offspring produced from both of the two *D. magna* clones whose mothers previously experienced diets containing toxic *M. aeruginosa* had higher reproduction and shorter time to first brood in the presence of toxic *M. aeruginosa*

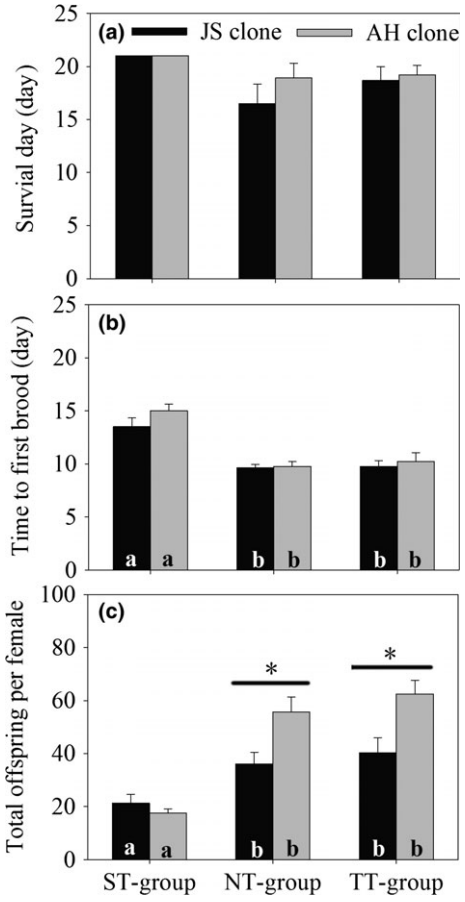

Fig. 2 Life-history variables of the F_1 (offspring) generation from two clones of *Daphnia magna* (JS and AH) fed a mixture of 50% *Scenedesmus obliquus* and 50% *Microcystis aeruginosa* (1.5 mg C L^{-1}). Error bars indicate 1 standard error (SE). Significant differences ($P < 0.05$) among the three diet groups in each clone are indicated by different letters. Asterisks (*) denote significant differences ($P < 0.05$) between clones under certain diet groups.

than offspring from mothers fed a high-quality diet of *Scenedesmus* (Fig. 2). Interestingly, offspring produced by mothers pre-exposed to non-toxic *M. aeruginosa* had improved fitness when fed a diet containing toxic *Microcystis* relative to offspring produced from mothers that were fed only high-quality *Scenedesmus*. Thus, the transgenerational effect leading to adaptive phenotypic plasticity in offspring was evident in offspring whose mothers had experienced either toxic or non-toxic *Microcystis* strains. In addition, the two *Daphnia* clones used in this study were isolated from two productive lakes 500 km apart showing that the observed patterns may be generalizable to other eutrophic lakes that suffer from cyanobacterial blooms. However, we encourage future research to conduct similar types of experiments with more clones across diverse zooplankton taxa to confirm the generality of our findings.

Table 1 Summary of two-way ANOVA analysis of life-history variables in offspring generation (F_1)

Life-history variable	Effect	d.f.	*F*	*P*-value
Survival day	Clone genotype	1	0.950	0.334
	Maternal food	2	1.998	0.146
	Clone genotype × Maternal food	2	0.0508	0.950
Time to first brood	Clone genotype	1	1.706	0.198
	Maternal food	2	30.918	<0.001
	Clone genotype × Maternal food	2	0.550	0.558
Total offspring per female	Clone genotype	1	12.198	0.001
	Maternal food	2	31.555	<0.001
	Clone genotype × Maternal food	2	5.535	0.007

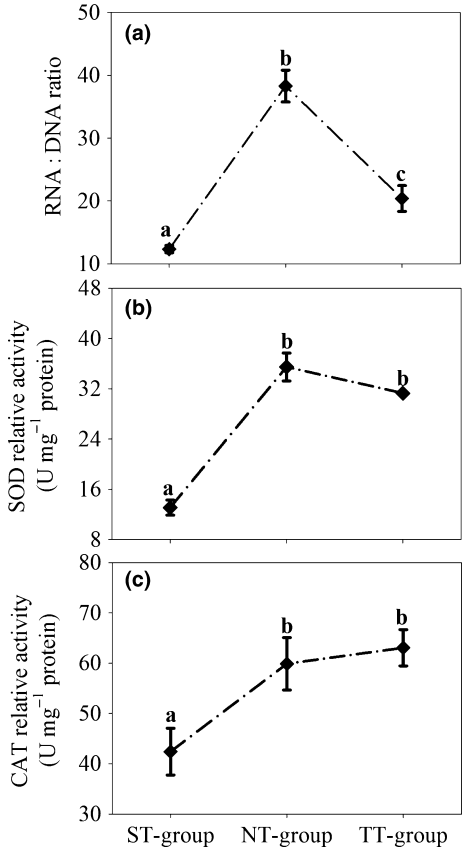

Fig. 3 Changes in a) RNA–DNA ratio, b) SOD and c) CAT activities of the F_1 (offspring) generation from *Daphnia magna* AH clone fed a mixture of 50% *Scenedesmus obliquus* and 50% *Microcystis aeruginosa* (1.5 mg C L^{-1}). Error bars indicate 1 SE. Significant differences ($P < 0.05$) among the three diet groups are indicated by different letters.

Negative effects of toxic *Microcystis* on *Daphnia* could be assigned to toxic substances, such as microcystins (DeMott, Zhang & Carmichael, 1991), or poor food

quality, such as the absence of sterols and certain essential PUFAs (Martin-Creuzburg *et al*., 2008). The frequently observed mechanical obstruction of *Daphnia* filtering apparatus (DeMott *et al*., 2001) did not apply in our study because we used *Microcystis* strains that consisted primarily of single cells. Therefore, the observed fitness increase in offspring derived from mothers exposed to *Microcystis* can be best attributed to the toxic substances or poor food quality. Previous studies of zooplankton resistance to toxic cyanobacteria have showed the harmful effects of microcystins while generally ignoring the potential effects of nutritional inadequacy of cyanobacteria (Gustafsson *et al*., 2005; Jiang *et al*., 2013). Since non-toxic *Microcystis* induced tolerance of *Daphnia* offspring to toxic *Microcystis*, our results suggest that the low nutritional value of cyanobacteria for *Daphnia* was the main factor promoting offspring tolerance to toxic *Microcystis*. Gustafsson *et al*. (2005) showed that *Daphnia* mothers exposed to toxic *Microcystis* (NIVA-CYA 228/1) produced offspring that had higher fitness when fed toxic diets, whereas non-toxic *Microcystis* failed to induce offspring tolerance. One explanation for this difference from our results could be the lower proportion of non-toxic *Microcystis* in the maternal daily diet (5% *Microcystis* and 95% *Scenedesmus*) than in our study (increasing *Microcystis* portion from 10% to 40%). We are not aware of published evidence that maternal exposure to pure microcystins or to microcystin crude extracts helps *Daphnia* offspring develop tolerance to the toxin. Furthermore, Dao, Do-Hong & Wiegand (2010) found that offspring of microcystin-treated mothers suffered from mortalities and became more vulnerable than those of mothers fed a high-quality diet. Hence, our results can help to disentangle which adverse factors in *Microcystis* trigger *Daphnia* offspring tolerance to toxic *Microcystis*.

In essence, our finding of increased fitness of *Daphnia* offspring following transfer of tolerance against *Microcystis* was consistent with a previous study (Jiang *et al*., 2013) on *D. carinata*. However, the explanatory mechanisms differ between the two species. For example, the increased fitness in *D. carinata* offspring mainly resulted from improved survival and reproduction but did not depend on fast growth (Jiang *et al*., 2013). In contrast, the maternal effect in *D. magna* was associated with enhanced reproduction and faster development to maturity (i.e. time to first brood) but not with improved survivorship. Regardless of the precise mechanisms and induction factors in *Microcystis*, the fitness increases observed in *D. carinata* and *D. magna* clearly suggest the general importance of maternal effects in adaptations of zooplankton to toxic cyanobacteria. Furthermore, given the importance of *Daphnia* in freshwater food webs (Stollewerk, 2010; Miner *et al*., 2012), the presence of maternally based inducible defences would sustain herbivore production and food availability for higher trophic levels in the event of a cyanobacterial bloom.

We showed that a poor maternal diet significantly increased offspring defence against toxic cyanobacteria and, therefore, poor quality resources may be a cue to the risk of future challenge by toxic cyanobacterial blooms. This pattern may reflect that maternal effects in daphnids could minimize the lag between exposure to cyanobacteria and inducible defences, consequently improving offspring fitness (Marshall & Uller, 2007). However, several studies have also demonstrated that maternal effects could reduce offspring tolerance and enhance the lag between environmental stresses and inducible defences (Scheirs, De Bruyn & Verhagen, 2000; Papchenkova, Golovanova & Ushakova, 2009). For example, *Daphnia* offspring had higher sensitivity to glyphosate herbicide if their mother had been exposed to that herbicide, reflected in inhibited fecundity, lower quality of progeny and smaller body size (Papchenkova *et al*., 2009). Recent studies have reported that, under simulated conditions of ecological stress, senescing animals can mount a glucocorticoid stress response (Cook *et al*., 2011) and reproduction can be impaired, for example as incomplete egg release (McConnachie *et al*., 2012). These seemingly contradictory roles of maternal effects show the complexity associated with exploring the influence of maternal effects on species interactions, especially in the context of global change (Hoffmann & Sgrò, 2011).

In our study, maternal effects promoted *Daphnia* offspring tolerance to cyanobacteria via faster growth and elevated reproduction at the individual level. Recent studies have revealed that improved invertebrate offspring tolerance to toxicants and pathogen infection involved enhanced antioxidative and immune activity mediated by maternal effects (Kaneko *et al*., 2011; Boots & Roberts, 2012). In response to toxic cyanobacteria, resistant *Daphnia* specifically upregulated some gene transcripts functional in cellular transportation (Schwarzenberger *et al*., 2014), energy production (Lyu *et al*., 2015) and antioxidation (Ortiz-Rodríguez, Dao & Wiegand, 2012). Microcystins were suggested to disrupt the mitochondrial electron transport chain (ETC.), thus favouring ROS generation that culminates in oxidative damage (Ding, Shen & Ong, 2002). Accordingly, the increase of SOD and CAT enzymatic activity in the NT group and TT group maintained cellular homeostasis by

avoiding overloading ROS. Meanwhile, elevated RNA–DNA ratio in the NT group and TT group revealed that a stronger metabolic process, which was also supported by enhanced energy production (Lyu *et al*., 2015), probably was necessary to buffer microcystin-induced damage, such as a stronger excretion of microcystins from the cells by permeases (Schwarzenberger *et al*., 2014). Previous research has highlighted the possibility of maternal transfer of immunity in invertebrates (Kurtz & Franz, 2003; Little *et al*., 2003), although the mechanisms underlying these effects are poorly understood. Thus, future studies should test whether or not enhanced antioxidant enzyme is transferred through parthenogenetic reproduction. Also, we cannot rule out the possibility that the enzyme activity was increased in eggs of mothers in the NT group and TT group. Further experiments using genome editing, such as the new CRISPR–Cas system (Hwang *et al*., 2013; Nakanishi *et al*., 2014), may confirm the relationship between the increased activities of antioxidants and maternal transfer.

Blooms of *Microcystis* are genetically diverse (Ye *et al*., 2009) and usually comprise both non-toxic and toxic genotypes (Kardinaal *et al*., 2007; Rinta-Kanto *et al*., 2009; Van De Waal *et al*., 2011; Haney & Lampert, 2013). Several studies have showed that non-toxic genotypes dominate earlier in the growing season than toxic genotypes (Davis *et al*., 2009; Ye *et al*., 2009; Li *et al*., 2011). An alternative explanation for *Microcystis* subpopulation shifting from non-toxic to toxic clones could be resource competition between the strains. For example, Yoshida *et al*. (2007) found that nitrate concentration might be a significant factor promoting the microcystin-producing subpopulation within the *Microcystis* population in a Japanese lake. Also, non-toxic *Microcystis* have been shown to dominate the *Microcystis* community under elevated CO_2 and lower light levels (Kardinaal *et al*., 2007; Van De Waal *et al*., 2011) and therefore could rapidly proliferate in the early stage of blooms. Consequently, induction of tolerance to toxic cyanobacteria in offspring produced by *Daphnia* mothers fed non-toxic *Microcystis*, as shown in the present study, could be an important mechanism for survival of *Daphnia* populations during a developing toxic bloom. Alternatively, *Microcystis* could sense *Daphnia* grazing and increase *mcy* gene expression, and consequently, microcystin production could be used as a defence mechanism against grazing (Pineda-Mendoza, Zúñiga & Martínez-Jerónimo, 2014). We further speculate that a shift in the *Microcystis* subpopulation from non-toxic to toxic strains could be due to re-activation of microcystin synthesis genes in non-toxic strains stimulated by *Daphnia* grazing, as Rantala *et al*. (2004) suggested that non-toxic strains of cyanobacteria may retain the genes necessary for their synthesis. Hence, in the context of the arms-race hypothesis, tolerance to toxic *Microcystis* in *Daphnia* offspring that is induced by maternal non-toxic *Microcystis* exposure appears to be an unexplored, yet potentially important, factor that could be mediating zooplankton–cyanobacteria interactions.

In conclusion, our study showed that diets containing non-toxic or toxic *Microcystis* may trigger tolerance to toxic *Microcystis* in *Daphnia* offspring. In addition, physiological responses revealed that the increased tolerance induced by maternal exposure to toxic *Microcystis* was related to elevated metabolic processes and antioxidative activity in offspring. Our work highlights the importance of including maternal effects in the understanding of *Daphnia*–cyanobacteria co-evolution, which may have wide-reaching implications for deciphering complex interactions involving phenotypic plasticity in defended prey and adapted consumers.

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