Cladoceran offspring tolerance to toxic *Microcystis* is promoted by maternal warming[☆]

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A B S T R A C T

Elevated temperatures and nutrients can favor phytoplankton dominance by cyanobacteria, which can be toxic to zooplankton. There is growing awareness that maternal effects not only are common but can also significantly impact ecological interactions. Although climate change is broadly studied, relatively little is known regarding its influence on maternal effects in zooplankton. Given that lakes are sentinels for climate change and that elevated temperatures and nutrient pollution can favor phytoplankton dominance by toxic cyanobacteria, this study focused on elucidating the effects of maternal exposure to elevated temperatures on the tolerance of zooplankton offspring to toxic cyanobacteria in the diet. Three different maternal thermal environments were used to examine population fitness in the offspring of two cladoceran species that vary in size, including the larger *Daphnia similoides* and the smaller *Moina macrocopa*, directly challenged by toxic *Microcystis*. *Daphnia* and *Moina* mothers exposed to elevated temperatures produced offspring that were more resistant to *Microcystis*. Such findings may result from life-history optimization of mothers in different temperature environments. Interestingly, offspring from *Moina* fed with toxic *Microcystis* performed better than *Daphnia* offspring, which could partially explain the dominance of small cladocerans typically observed during cyanobacterial blooms. The present study emphasizes the importance of maternal effects on zooplankton resistance to cyanobacteria mediated through environmental warming and further highlights the complexities associated with the abiotic factors that influence zooplankton-cyanobacteria interactions.

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1. Introduction

For more than two centuries, freshwater systems around the world have been negatively impacted by nutrient inputs from agricultural, industrial, and urban development (O'Neil et al., 2012). One of the negative consequences of eutrophication is the proliferation of harmful cyanobacterial blooms, which represents one of the greatest threats to the quality, usage, ecological integrity, and sustainability of our water resources worldwide (Paerl et al., 2001). When abundant, cyanobacteria can negatively affect herbivorous zooplankton as a result of poor food quality or toxic secondary metabolites (Ger et al., 2014). In addition, cyanobacterial blooms

have been shown to shift zooplankton communities towards dominance from larger (e.g. *Daphnia* with adult size >2.0 mm) to smaller genera (e.g., *Bosmina* and *Moina* with adult size < 1.0 mm) (Sun et al., 2012). Such foodweb changes can interfere with the transfer of energy across trophic levels (Burns, 1969; Persson et al., 1996). However, the generality of the negative correlation between cyanobacterial abundance and cladoceran fitness has been debated based on laboratory and field-based studies for a long time (Hairston et al., 1999; Gustafsson et al., 2005; Sarnelle and Wilson, 2005; Wilson et al., 2006). For example, the phenotype of an individual is influenced by transgenerational experiences as well as its environment. Consequently, maternal effects may provide insights regarding adaptations in cladocerans for tolerating dietary toxic cyanobacteria (Gustafsson et al., 2005; Jiang et al., 2013).

Maternal effects occur when the environment a mother experiences influences the phenotype of her offspring over and above the direct effect of transmitted genes (Marshall and Uller, 2007).

Ecologists now recognize that maternal effects contribute to offspring phenotypic change over the lifetime of an individual (Lamontagne and Mccauley, 2001; Mitchell and Read, 2005; Marshall and Uller, 2007), as they are widespread in nature (Mousseau and Fox, 1998). Although maternal effects were originally deemed nuisance sources of variation in quantitative genetic studies, they are now seen as an important mechanism for rapid multi-generational responses to environmental change providing offspring with optimal life history strategies, mate choice, or improved behavior to avoid predation (Marshall and Uller, 2007). As keystone species in freshwater ecosystems, cladocerans have been the subject of much work on phenotypic plasticity and maternal effects, partly because they offer ideal examples of these phenomena, but also due to their parthenogenetic life-history. Such asexual reproduction facilitates experiments that simultaneously separate effects of environment and genotype. Past studies have demonstrated that maternal effects in cladocerans can moderate the detrimental effects of environmental stresses when maternal experiences adaptively reshape offspring fitness (Gustafsson et al., 2005; Mitchell and Read, 2005; Garbutt et al., 2013). For example, genetically identical *Daphnia* mothers grown at low food levels produced small clutches of larger eggs, and their offspring, albeit low in numbers, were able to survive long periods of starvation (Gliwicz and Guisande, 1992). Moreover, maternal exposure to predator cues significantly modifies the phenotypes of offspring to avoid subsequent predation risk (Tollrian, 1995; Agrawal et al., 1999). Thus, understanding the role of maternal effects can have far-reaching consequences for population dynamics (Rossiter, 1996), community interactions, and the rate and direction of evolutionary change (Agrawal et al., 1999; Day and Bonduriansky, 2011).

Few doubt that global warming will lead to intensified cyanobacterial dominance of aquatic ecosystems (Paerl and Paul, 2012), particularly in shallow lakes (Kosten et al., 2012) which are especially susceptible to warmer air temperatures and nutrient inputs (Adrian et al., 1999; Gerten and Adrian, 2000). Past studies have shown that temperature-mediated, within-generational survival and reproduction in cladocerans affected by cyanobacteria was dependent on the species, and even genotype, of cyanobacteria and cladoceran (Hietala et al., 1997; Claska and Gilbert, 1998; Hochmuth and De Schamphelaere, 2014). However, offspring tolerance to cyanobacteria may also be influenced by temperature in the maternal generation, arising either due to a general stress response or as an adaptation to a stressed environment where temperature fluctuations reliably correlate with cyanobacterial dominance (Liu et al., 2011).

The purpose of the present study was to improve our understanding regarding the role of thermal maternal effects on offspring fitness (reflected by the intrinsic rate of increase) in cladocerans fed toxic *Microcystis* (one of most studied cyanobacterial genera) through the interaction of the maternal environment $(E_m) \times$ offspring environment (E_o). Previous studies have shown that thermal fluctuation and food deficiency in mothers can influence offspring resistance to pathogens (Mitchell and Read, 2005; Garbutt et al., 2013). Hence, we tested whether the thermal maternal environment reshaped offspring sensitivity in two cladoceran species that varied in size to toxic *Microcystis*. Field studies have revealed that small-sized cladocerans tend to dominate zooplankton communities during cyanobacterial blooms (Sun et al., 2012). To further explore one factor that may mediate this pattern, we used two different body-sized cladocerans (i.e., large-sized *Daphnia similoides* (mean adult size 2.7 mm) and small-sized *Moina macrocopa* (mean adult size 1.0 mm)) to understand the consequences of maternal effects on taxa that tend to vary in dominance during phytoplankton seasonal succession. To answer these questions, individuals from the above two cladoceran species were raised at three temperatures (15, 20, and 30 °C) and the performance (life span, reproduction, and population growth) of their offspring was studied when fed with diets containing different ratios of toxic *Microcystis*.

2. Materials and methods

2.1. Collection and culture of test organisms

Scenedesmus obliquus (green alga; high quality food for cladocerans; FACHB416) and toxic *Microcystis aeruginosa* (cyanobacterium; poor quality food for cladocerans, PCC7806) were obtained from the Institute of Hydrobiology, Chinese Academy of Sciences. Both algal cultures were grown in sterilized liquid BG-11 medium in 1-L Erlenmeyer flasks at 25 °C under fluorescent light at 40 μE m^{-2} s^{-1} with a light-dark period of 14: 10 h. A pilot experiment confirmed that this strain of *M. aeruginosa* grew as single or paired cells and produced at least two types of microcystin (MC-LR and MC-RR) with a total content of 3.6 pg per cell via HPLC method (Gan et al., 2010).

Two different-sized species of cladocerans, including one large-sized *Daphnia similoides* clone and one small-sized *Moina macrocopa* clone, used in the experiments were originally isolated from Lake Taihu, China, and cultured in the laboratory at 20 °C in 1-L jars containing 800 mL M4 medium (Elendt and Bias, 1990). Cladocera stock cultures were fed daily with *S. obliquus* (1.5 mg C L^{-1}). Carbon levels per cell were determined biweekly using a Dohrmann Carbon Analyzer (Lyu et al., 2014). Both phytoplankton species (*Scenedesmus*: 7.5 μm × 5 μm, *Microcystis*: 3.5 μm) were within the size range of particles that are efficiently consumed by *Daphnia* and *Moina* (DeMott, 1989). Neither *Microcystis* nor *Scenedesmus* produced colonies or gelatinous sheaths of any substantial size. Culture media was refreshed twice each week. All cladoceran mothers (10 individuals per 500 mL) were acclimated for a minimum of three generations to minimize transgenerational effects before being used in the following experiments.

2.2. Experimental design

Cladoceran mothers were exposed to three ecologically relevant temperatures: 15 (low), 20 (medium), and 30 °C (high). These temperatures relate to late spring and early summer temperatures (15 °C and 20 °C) in temperate environments or summer temperatures in subtropical and tropical environments (30 °C) (Hansen et al., 2006). Five neonates (<12-h-old; F_0) from the third brood of the third acclimated generation of each replicate were assigned into the 500-mL flask containing 200 mL M4 medium and exposed to the three temperature treatments (five replicates per treatment; See Fig. 1). Experimental medium was renewed every two days and individuals were fed daily a high quality diet of *S. obliquus* (1.5 mg C L^{-1}). Life-history data (i.e., age at the first and third broods, initial body-length of offspring at the third brood, and number of newly-released offspring each day over the first to third brood) were recorded to quantify direct (within generation) effects of temperature variation.

The filial 1 generation (F_1) was obtained from the third brood of F_0 animals (i.e., mothers). Newly released offspring (<12-h-old) from the three temperature treatments were transferred to three food treatments in the F_1 generation, resulting in nine treatments with five individuals per replicate (five replicates per treatment; See Fig. 1). The three different food treatments comprised either 100% *S. obliquus*, 50% *M. aeruginosa* + 50% *S. obliquus*, or 100% *M. aeruginosa* (Sarnelle et al., 2010). The three different food treatments received an equal food concentration of 1.5 mg C L^{-1} per

day. We chose to account only for the indirect effects of maternal temperature on the offspring resistance to toxic *Microcystis*, therefore offspring were cultured at a single, optimum temperature (20 °C) for 14 days. Offspring were fed daily and media was renewed every two days. Temperature shifts from 15 or 30 °C of F_0 to 20 °C for F_1 offspring did not cause negative effects on this F_1 generation (Fig. 2E and F showed that no significant difference in intrinsic rate of population across maternal temperatures under the control diet (100% *S. obliquus*)). To estimate newly-released F_1 generation provisioning state, we measured protein (mg g^{-1}) in newly-released offspring at the third brood, using the Diagnostic Reagent Kits following manufacturer instructions (Jian Cheng Bioengineering Institute, Nanjing, China). We also measured the initial body length of the newly-released F_1 generation.

(a) Formulas for demography parameters in F_1

The demography parameters in F_1 were calculated using the formulae:

Average life span : $L = \sum l_x$

Net reproductive rate : $R_0 = \sum l_x m_x$

Generation time : $T = \dfrac{\sum l_x m_x x}{R_0}$

where x is the age in days, l_x is the age-specific probability of survival, and m_x is the age-specific fecundity. Intrinsic rate of population increase (r) was estimated iteratively using the Euler-Lotka equation:

$$1 = \int e^{-rx} l_x m_x dx$$

The intrinsic rate of population increase can be treated as cladoceran fitness under the conditions of no or low predation activities (Lampert and Trubetskova, 1996). In the case of no reproduction ($b = 0$), the intrinsic rate of population increase (r) was determined from mortality rates (d) because $r = b - d$. Mortality

rates were estimated from Type II survivorship curves after $\ln(n+1)$ transformation by linear regression, assuming that death of cladoceran was entirely determined by their interaction with the environment (Hutchinson, 1978; Lürling, 2003).

(b) Cladoceran 24-h grazing experiment

A grazing experiment was designed to test the possible inhibitory effects of toxic *Microcystis* on the feeding rate of F_1 generation from mothers experiencing 15, 20, and 30 °C, following the method previously described in Lürling (2003). Briefly, the grazing experiment was run in 6-well culture plates, and each well was filled with 3 mL of food suspensions containing 50% *M. aeruginosa* + 50% *S. obliquus*. The food suspensions were made up in four replicates at equal food levels of 1.5 mg C L^{-1}. Newly-released F_1 generation of *D. similoides* and *M. macrocopa* from mothers experiencing 15, 20, and 30 °C were transferred individually into four wells per species per maternal temperature. The well plates were incubated for 24 h at 20 °C in the dark. The clearance rates were calculated from the decrease in algal density (AD) over time, according to the equation:

$$CR = \frac{[\ln(AD_0) - \ln(AD_t)]}{t} \times 3$$

in which CR is the clearance rate (mL $ind.^{-1}$ h^{-1}), AD_0 is the algal density at the start of the experiment, AD_t = the AD at the end of the experiment after incubation period t (hour; here t = 24 h), and 3 is the amount of medium (mL) per individual *D. similoides* or *M. macrocopa* at 20 °C in the dark.

2.3. Data analysis

The difference in life history parameters for F_0 generations, biochemical parameter (i.e., protein) of newly-released F_1 generations were compared by one-way ANOVA followed by Duncan's multiple range test ($\alpha = 0.05$). Two-way ANOVA followed by Holm-Sidak test was employed to detect significant ($\alpha = 0.05$) differences in life history parameters and clearance rate for F_1 generation. In addition, the intrinsic rates of population increase were compared between *Moina* and *Daphnia* fed with 50% *M. aeruginosa* + 50%
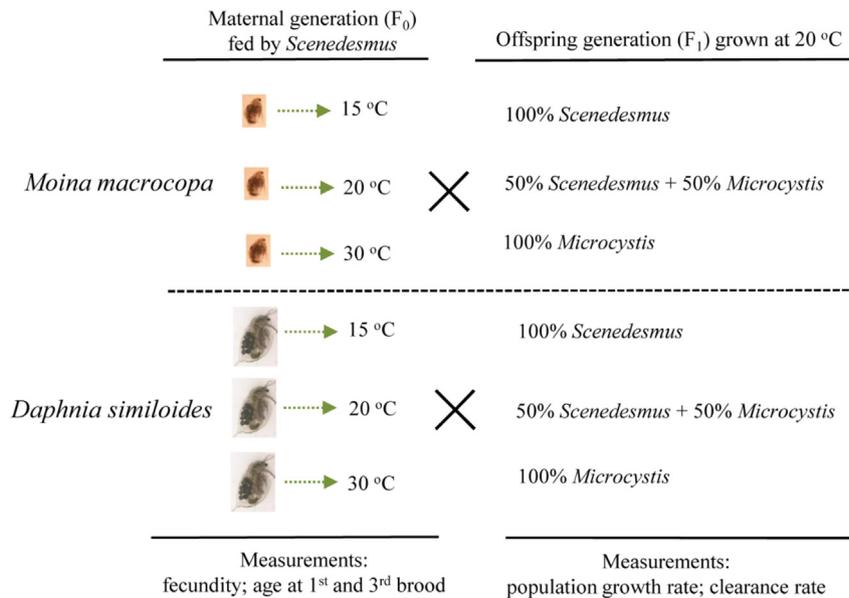

Fig. 1. Scheme illustrating the experimental design of the current study.
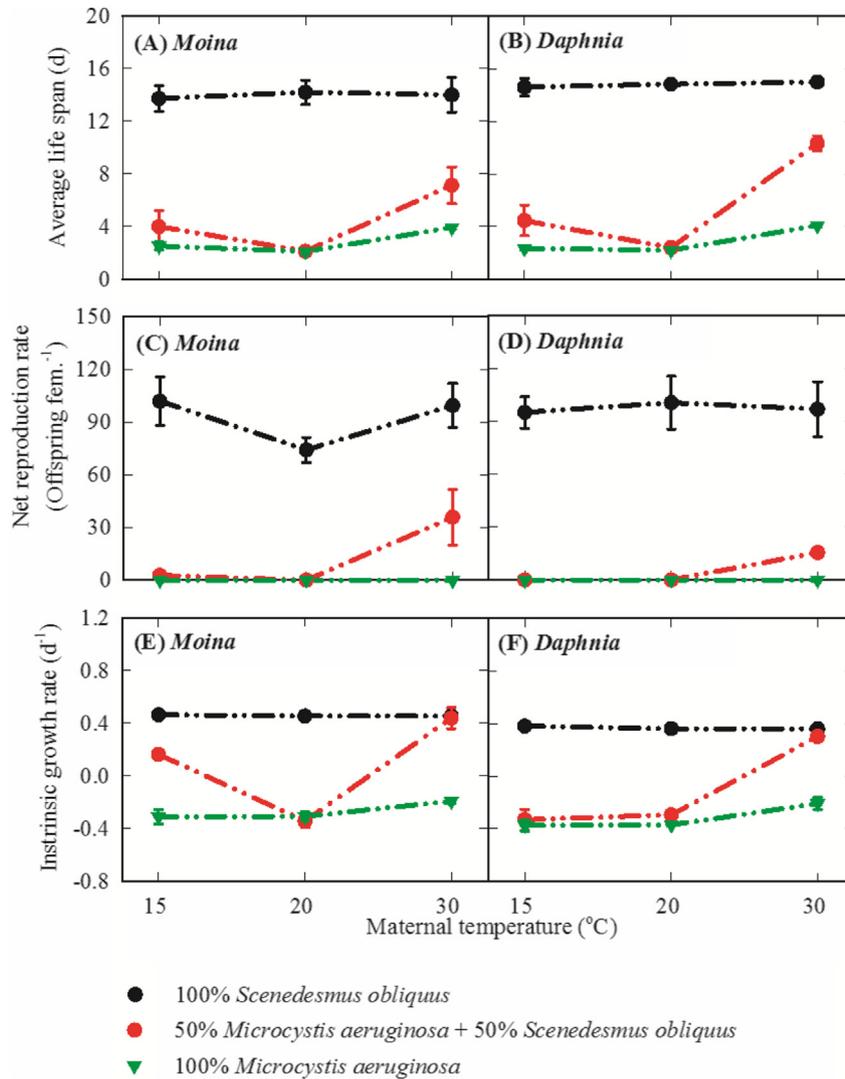
Fig. 2. Maternal effect of temperature (15, 20, and 30 °C) on life span (A and B), net reproductive rate (C and D) and intrinsic rate of population increase (E and F) of *Moina* and *Daphnia* offspring generation (F_1) when fed with different food treatments comprised either 100% *Scenedesmus* (black), 50% *Microcystis* + 50% *Scenedesmus* (red) or 100% *Microcystis* (green). Significant difference ($p < 0.05$) among treatment levels was detected by two-way ANOVA (maternal temperatures and offspring food types) followed by Holm-Sidak test. Error bars indicate 1 SD. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

S. obliquus at 15 and 30 °C maternal temperature, using an independent *t*-test after determining that the data were normal and variances did not differ. All statistical analyses were performed with Sigmaplot (Version 11.0 for Windows).

3. Results

3.1. Thermal effects on the maternal (F_0) generation

Temperature significantly affected the growth and reproduction in cladoceran mothers (Figs. S1 and S2; Table S1, Supporting Information (SI)). Specifically, *Moina* raised at 30 °C reached maturity (the age at the first brood) twice as fast ($p < 0.001$; Tables S1 and SI) as did conspecifics raised at 15 or 20 °C (Figs. S2 and SI). Similarly, *Daphnia* raised at 30 °C reached maturity faster than those grown under the other two temperatures ($p < 0.001$; Tables S1 and SI). Similar to the age at the first brood, both *Moina* and *Daphnia* raised at 30 °C achieved their third brood by more than 3 days earlier ($p < 0.001$; Tables S1 and SI) than did cladocerans raised at lower temperatures (Figs. S2 and SI). Consequently, both *Moina* and

Daphnia grown at higher temperatures had higher net reproductive rates ($p < 0.001$; Fig. S2; Tables S1 and SI).

3.2. Maternal temperature alters offspring resistance to toxic *Microcystis*

Before conducting the *Microcystis* exposure experiment, we determined changes in the initial body length and protein content of the newly-released (<12 h) F_1 generation (Fig. 3). The two parameters decreased with increasing maternal temperatures ($p < 0.05$; Table 1). Furthermore, initial body length of *Daphnia* was significantly higher than those of *Moina* at the same maternal temperature (Fig. 3A; Table 1). During the 14-day exposure, both maternal temperature and diet significantly affected offspring (F_1) survival (Fig. 2A and B; Table 1). When fed with toxic *M. aeruginosa*, offspring from higher maternal temperature had longer life spans than those from lower maternal temperature, regardless of species (Fig. 2A and B), but did not differ when offspring were fed with the high quality diet containing only *S. obliquus*. Moreover, 14-d exposure to *M. aeruginosa* negatively affected the life span in

both *Moina* and *Daphnia* offspring, regardless of maternal temperature (Fig. 2A and B; Table 1).

Both maternal temperature and diet tended to affect offspring (F_1) fecundity, as estimated by net reproductive rate (Fig. 2C and D; Table 1), but these relationships varied. As expected, both *Moina* and *Daphnia* offspring exposed to *M. aeruginosa* produced fewer offspring than did those exposed to the high quality diet of 100% *S. obliquus*. In particular, all offspring fed with 100% *M. aeruginosa* died before they reached maturity (Fig. 2C and D). However, a number of interactive effects revealed that maternal temperature at 30 °C had a clear positive impact on offspring reproduction only when offspring were exposed to a mix of *M. aeruginosa* and *S. obliquus* (there was no effect of maternal temperature on reproduction in offspring exposed to 100% *M. aeruginosa*) (Fig. 2C and D; Table 1). In addition, offspring fed with 100% *S. obliquus* had similar reproduction rates across all temperatures in *Daphnia*, whereas *Moina* offspring fed with 100% *S. obliquus* had lower neonate production in the 20 °C maternal temperature than did those raised in 15 and 30 °C (Fig. 2C and D; Table 1).

The fitness in offspring fed with 100% *M. aeruginosa* decreased and was much lower than that of cladocerans fed with 100% *S. obliquus* across all temperatures for both *Daphnia* and *Moina* (Fig. 2E and F; Table 1). However, higher maternal temperatures improved offspring fitness in the presence of *M. aeruginosa*. Offspring from 30 °C maternal temperature mothers fed with a mix of *M. aeruginosa* and *S. obliquus* had increased fitness that was not significantly different compared to offspring fed with 100% *S. obliquus*. These findings contrasted with fitness estimates of offspring produced from 20 °C maternal temperature. In addition, *Moina* offspring, but not *Daphnia* offspring, from 15 °C maternal temperature mothers exhibited increased fitness. When maternal temperatures were 15 and 30 °C, *Moina* had higher fitness than *Daphnia* when comparing offspring fitness between the two species fed with mixed diets (Fig. 4; *t*-test, $t_4 = 2.952$, $p = 0.042$ for 30 °C; *t*-test, $t_4 = 10.557$, $p < 0.001$ for 15 °C).

Clearance rates (CR) of *Daphnia* and *Moina* produced by mothers grown at 30 °C on mixtures with 50% toxic *Microcystis* were significantly lower than mothers maintained at 20 or 15 °C, while *Daphnia* and *Moina* offspring produced from mothers maintained at 20 °C were marginally different compared to offspring produced from mothers maintained at 15 °C (Fig. 5; Table 1). Also, CR in *Moina* was significantly lower than observed for *Daphnia*, regardless of maternal temperature (Table 1).

4. Discussion

To identify how an important environmental signal, such as elevated temperature, influences the response of zooplankton to harmful cyanobacterial bloom risks through maternal investments, we investigated the fitness of offspring produced from mothers maintained under three different temperatures fed diets containing toxic *Microcystis*. Mothers maintained at a higher temperature (i.e., 30 °C) released offspring that were more resistant to *Microcystis* (Fig. 2) than offspring from mothers raised at cooler temperatures. These findings are in accordance with previous studies that mothers from higher temperature improved the offspring fitness against external stressors, such as pathogen infection or global warming (Salinas and Munch, 2012; Garbutt et al., 2013; Shama et al., 2014; Zizzari and Ellers, 2014). In addition, offspring (F_1 generation) were cultured at a single and optimum temperature (20 °C), since the present study chose to account only for the indirect effects of maternal temperature on the offspring resistance to toxic *Microcystis*. It is of interest to examine the temperature effect across a range of maternal and offspring generations. We encourage that future experiments expand upon the groundwork laid presently to test in detail how any maternal effects might be mediated by offspring environment.

Temperature is one of the most important environmental variables that affect population dynamics, especially for freshwater plankton, because their metabolic rates are controlled directly by ambient temperature (Eppley, 1972; Savage et al., 2004). For cyanobacteria and zooplankton, elevated temperatures may promote cyanobacterial dominance and persistence (Imai et al., 2009), not only because growth rates of prokaryotes are optimized at relatively high temperatures; often in excess of 25 °C (Butterwick et al., 2005; Paerl and Otten, 2013), but also because cyanobacteria tend to be promoted when lakes are stratified allowing the formation of surface scums (Rigosi et al., 2014). On the other hand, increasing temperatures could exacerbate the inhibitory effect of toxic cyanobacteria on cladoceran population growth rates (Claska and Gilbert, 1998). In contrast, a recent study illustrated that a decrease in the harmful effects of cyanobacteria on reproduction was possible in *D. pulex* with increasing temperature when fed with diets containing *Microcystis*, *Nodularia*, or *Aphanizomenon* (Hochmuth and De Schamphelaere, 2014). Regardless of these conflicting findings, it is clear that water temperature can play an essential role in shaping cladoceran phenotypic responses to cyanobacteria. In the present study, we observed that high-temperature-derived offspring showed improved tolerance to toxic cyanobacteria, suggesting that temperature impacted offspring traits not just directly, but also indirectly. Given that such hidden effects triggered by maternal environments are often overlooked regarding cyanobacteria-zooplankton interactions, we contend that indirect maternal effects may strongly influence ecological interactions.

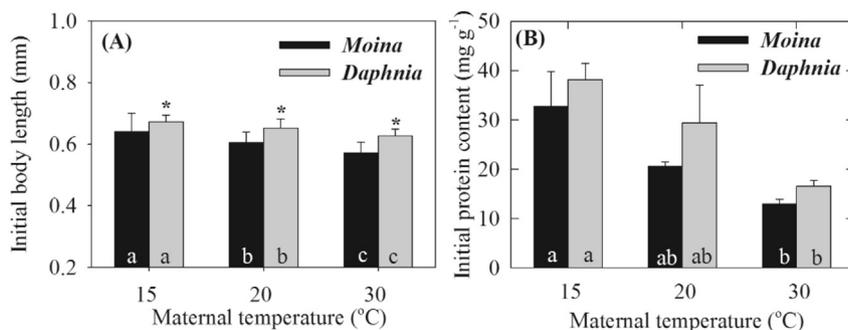The enhanced resistance of high-temperature-derived offspring

Fig. 3. Initial body length (A) and protein content (B) of offspring generation (F_1) at day 0 under different maternal temperatures (15, 20, and 30 °C). Error bars indicate 1 SD. Significant difference ($p < 0.05$) among treatment levels was indicated by different letters within a species.

Table 1
Results from two-way ANOVA for the factors maternal temperature and food type for each variable in offspring generation (F_1). d.f. = degrees of freedom.

Response	Species	Effect	d.f.	F	p-value
Average life span	Daphnia	Maternal temperature	2, 18	103.113	<0.001
		Food type	2, 18	1296.743	<0.001
		Maternal temperature × Food type	4, 18	48.763	<0.001
	Moina	Maternal temperature	2, 18	14.954	<0.001
		Food type	2, 18	414.695	<0.001
		Maternal temperature × Food type	4, 18	6.611	0.002
Net reproduction rate	Daphnia	Maternal temperature	2, 18	1.267	0.306
		Food type	2, 18	438.616	<0.001
		Maternal temperature × Food type	4, 18	1.484	0.248
	Moina	Maternal temperature	2, 18	12.796	<0.001
		Food type	2, 18	305.031	<0.001
		Maternal temperature × Food type	4, 18	6.640	0.002
Intrinsic growth rate	Daphnia	Maternal temperature	2, 18	145.454	<0.001
		Food type	2, 18	819.901	<0.001
		Maternal temperature × Food type	4, 18	77.344	<0.001
	Moina	Maternal temperature	2, 18	123.693	<0.001
		Food type	2, 18	730.593	<0.001
		Maternal temperature × Food type	4, 18	86.992	<0.001
Clearance rate	Both	Maternal temperature	2, 18	34.341	<0.001
		Species	1, 18	119.592	<0.001
		Maternal temperature × Species	2, 18	1.272	0.304
Initial body length	Both	Maternal temperature	2	32.768	<0.001
		Species	1	57.624	<0.001
		Maternal temperature × Species	2	1.140	0.322
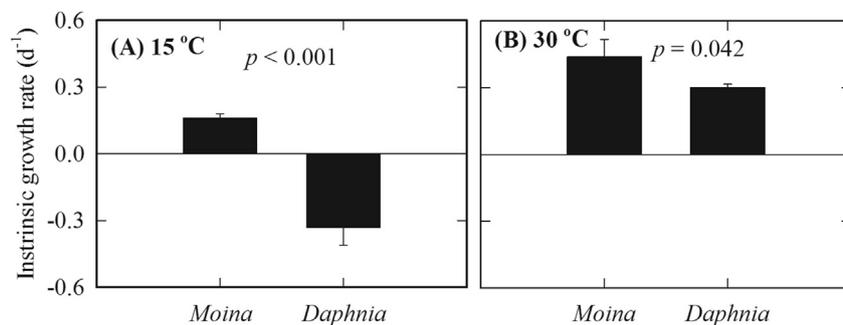
Fig. 4. Maternal effect of temperature (15 and 30 °C) on intrinsic rate of population increase of offspring generation (F_1) of Moina and Daphnia, respectively. These offspring were only fed with 50% Microcystis + 50% Scenedesmus. Error bars indicate 1 SD. Significant difference ($p < 0.05$) between treatment levels was detected by t-test.
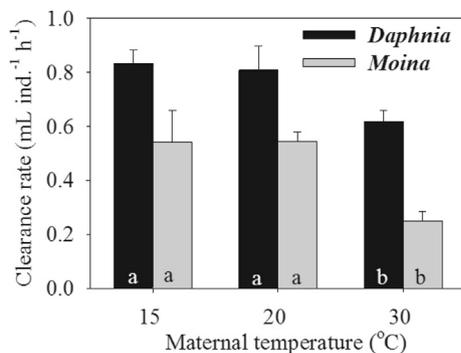
Fig. 5. Maternal effect of temperature (15, 20, and 30 °C) on clearance rate on the mixture of 50% Microcystis + 50% Scenedesmus. Significant difference ($p < 0.05$) among treatment levels was detected by two-way ANOVA (maternal temperatures and species) followed by Holm-Sidak test. Error bars indicate 1 SD.

may be partly caused by a general phenomenon that cladoceran optimize their reproductive allocation strategy. In the present study, we found that both Daphnia and Moina raised at warmer temperatures had larger broods (Figs. S2 and SI), indicating their offspring (F_1) had smaller body-length than those from mothers

exposed to 20 °C. Indeed, both Daphnia and Moina grown at 30 °C produced smaller neonates (Fig. 3), which is consistent with other ectotherms (but see Stillwell et al. (2008) and Blanckenhorn (2000)). Life-history theory holds that smaller offspring may generally confer a competitive disadvantage in stressful environments due to insufficient availability of energy reserves (Gliwicz and Guisande, 1992). However, since smaller offspring (i.e., high-temperature-derived offspring) did not exhibit lower fitness (i.e., intrinsic growth rate) in the absence of Microcystis (20 °C vs. 30 °C in pure Scenedesmus; Fig. 2), it seemed unlikely that inadequate provisioning to offspring was responsible for inhibited Microcystis resistance. We further found that even the nutritional state (i.e., proteins) in offspring from mothers exposed to 30 °C was lower than those from 20 °C (Fig. 3). Therefore, it is reasonable that some other size-dependent factor was implicated in the ability of the smaller offspring to resist Microcystis. As generalist grazers, cladocerans are relatively nonselective feeders that tend to ingest food particles in the same ratio as they are encountered (Kirk and Gilbert, 1992). When cyanobacteria become abundant, cladoceran ingestion rates generally decline or are inhibited altogether, preventing energy intake and reducing fitness (Ghadouani et al., 2004; Lyu et al., 2016). Indeed, the cladoceran grazing experiment revealed that smaller Daphnia and Moina offspring from high

maternal temperature (i.e., 30 °C) had lower clearance rate on toxic *Microcystis* than those from 15 to 20 °C maternal temperatures (Fig. 5). It is an important optimization in cladoceran reproductive strategy: high temperature causes mothers to release more but smaller offspring to help their offspring digest less toxic algae (*r*-strategy). Under this scenario, cladoceran populations may persist given that individuals of smaller species may have the opportunity to continue grazing on edible phytoplankton by finding areas free from inhibitory cyanobacteria. Previous studies have demonstrated that smaller body size may be an advantage in generalist cladoceran species by preventing significant ingestion of large cyanobacterial colonies or filaments and by resulting in coexistence with cyanobacteria (Hansson et al., 2007; Davis and Gobler, 2011; Haney and Lampert, 2013). Hence, our results showed for the first time that maternal effects that favor resistance to *Microcystis* were negatively correlated to zooplankton body size.

To our surprise, low-temperature-derived *Moina* offspring (15 °C) were more resistant to *Microcystis* compared to the medium temperature-derived offspring (20 °C) (Fig. 2E). Given that *Moina* tended to produce smaller broods when maintained at 15 °C (Fig. S2), we contended that these mothers released larger offspring with presumably better provisions (Gliwicz and Guisande, 1992; Burgess and Marshall, 2011). Indeed, we found that larger offspring (i.e., low-temperature-derived offspring) had higher reproductive output in the absence of *Microcystis* (15 °C vs. 20 °C in *Scenedesmus*; Fig. 2). An increase in cladoceran neonate size is often observed when food supply is scarce (Boersma, 1997), such as when phytoplankton densities wane during the winter (Rellstab and Spaak, 2007). Thus, elevated provisioning of offspring may have contributed to enhanced resistance in *Moina* to imminent *Microcystis* blooms. Our results show that *Moina* may exhibit different adaptive strategies depending on maternal effects in response to *Microcystis*.

In cladocerans, *Moina* (mean adult size 1.0 mm) and *Daphnia* (mean adult size 2.7 mm) are typical examples of small- and large-sized cladocerans, respectively (Guo and Xie, 2006). During the summer, cyanobacterial blooms may alter competitive outcomes between small- and large-sized cladocerans by facilitating the dominance of small cladocerans (Hanazato, 1991; Demott et al., 2001; Sun et al., 2012). Gliwicz (1990) described that feeding inhibition by the high abundance of colonial cyanobacteria favored small-sized cladoceran in eutrophic lakes. These findings were supported by our previous research that showed a small-sized *Daphnia* had higher survivorship than a large-sized *Daphnia* exposed to hypoxic events, which regularly occur during and following cyanobacterial blooms (Lyu et al., 2015). In the present study, elevated temperature maternal effects resulted in better fitness (~30%) against *Microcystis* in *Moina* than in *Daphnia* (Fig. 4B), despite that initial body length in *Daphnia* was only 10% larger than *Moina* (Fig. 3A). Despite the past study that show that small-sized zooplankton dominate during cyanobacterial blooms (Sun et al., 2012), the present results suggested that elevated maternal temperatures may indirectly induce changes in offspring tolerance that could partially contribute to reshaping zooplankton communities toward dominance by small-sized cladocerans. Guo and Xie (2006) found that *Moina* may develop stronger tolerance against toxic *Microcystis* than large-sized *Daphnia* when their mothers were pre-exposed to *Microcystis*, emphasizing that maternal effects leading to the dominance of small-sized cladocerans occurred under environmental homogeneity (i.e., mother (E_m) and offspring (E_o) experienced the similar environment, *Microcystis* exposure; $E_m \approx E_o$). Our results showed for the first time that this pattern can also extend to environmental heterogeneity (mothers and neonates were grown under different conditions; $E_m \neq E_o$). In addition, Sommer et al. (1986) and Deng et al. (2008) showed that seasonal

succession of crustacean zooplankton favored larger herbivorous species (e.g., *D. pulex* and *D. carinata*) during the spring season due to lower temperatures and reduced planktivory and smaller herbivores (e.g., *M. micrura*, *Ceriodaphnia cornuta* and *Bosmina coregoni*) during the warm summer season and elevated planktivory. Our results showed that *Moina* offspring had better fitness in response to *Microcystis* than *Daphnia* offspring produced from mothers who experienced a relatively low temperature, indicating that small herbivores may have a stronger capacity of resistance to *Microcystis* during the spring recruitment stage, laying the foundation for subsequent zooplankton community shifted towards small-sized cladocerans during the summer when cyanobacteria typically dominate. Given that both low and high temperatures promoted small-sized cladocerans (e.g. *Moina* genus), temperature-induced maternal effects could therefore have a considerable selective advantage, absent of a genetic change, to force zooplankton community shifts, particularly in the context of global warming.

As Mousseau and Fox (1998) proposed, maternal effects provide a mechanism for adaptive transgenerational phenotypic plasticity. In the strictest form of their hypothesis, mothers can prepare offspring to grow better in expected future environment based on current conditions and that this programming was particularly strong when their offspring faced environmental variation similar to the mother, termed as $E_m \approx E_o$. Obviously, a crucial prerequisite for maternal effects was that offspring environmental conditions were predictable from maternal phenotype or environmental conditions (Marshall and Uller, 2007). Our results show that, at least in cladocerans, a simple mismatch between the maternal and offspring environment ($E_m \neq E_o$) was sufficient to produce dramatic phenotypic changes in offspring, and hence could modify species diversity and evolutionary dynamics. As global temperature instability becomes more intense (Meehl and Tebaldi, 2004), such temperature-induced maternal effects could play a hidden but essential role in adaptations to novel environments (Bossdorf et al., 2008). Clearly, the presence of complex maternal effects will exacerbate the challenge of making accurate predictions about the ecological and interspecific co-evolutionary impacts of environmental change.

In conclusion, we have estimated the potential for the maternal thermal environment to modify offspring resistance to *Microcystis* in two different sized cladoceran species. Mothers exposed to elevated temperatures produced offspring that were more resistant to *Microcystis*. Maternal effects are often considered something to ignore or control for, particularly in experiments aiming at elucidating genetic effects. Given that epigenetic mechanisms (e.g., DNA methylation, histone modification) reported in host-parasite systems (Gómez-Díaz et al., 2012) and the rapidly expanding available genomic resources for cladocerans (Schwarzenberger et al., 2014; Druga et al., 2016), future research should consider how maternal effects in zooplankton link phenotype with molecular responses. A key message from this study is that maternal effects can be extremely large, namely that offspring resistance was strongly influenced by variation in the maternal environment to a far great extent than the current environment of the offspring. Such findings may have wide-reaching implications for forecasting the outcome of cyanobacteria-zooplankton interactions.

Founding of Scientific Research for the Introduction of Talents in Jiangsu University of Science and Technology (1732931609).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2017.04.095.

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