Understanding interactive inducible defenses of *Daphnia* and its phytoplankton prey

Siddiq Akbar[a], Jingjing Du[b], Hong Lin[a], Xiangshi Kong[a], Shucun Sun[a], Xingjun Tian[a,*]

[a] *School of Life Sciences, Nanjing University, Nanjing 210093, China*
[b] *Henan Collaborative Innovation Center of Environmental Pollution Control and Ecological Restoration, School of Material and Chemical Engineering, Zhengzhou University of Light Industry, Zhengzhou 450002, China*

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ABSTRACT

Cyanobacterial and zooplankton inducible defenses are important but understudied process that regulate the trophic interactions of freshwater ecosystem. *Daphnia* due to its large size is considered an important zooplankton with the high potential to control cyanobacterial blooms. It has been shown that *Daphnia* through maternal induction transfer tolerance to their next generation against *Microcystis* toxicity. Maternal induction has been investigated in different *Daphnia* species without considering phenotypic plasticity of prey. Laboratory experiments were performed to explore cyanobacteria-*Daphnia* inducible defenses in order to better understand their interactions. Two *Daphnia* species were fed either with *Microcystis aeruginosa* PCC7806 (Ma) or *Microcystis flos-aquae* (Mf) mixed with *Chlorella vulgaris* (Cv) (exposed *Daphnia*), and or pure Cv (unexposed *Daphnia*). Exposed prey cultures were produced by prior exposure to *Daphnia* infochemicals. Neonates produced by exposed and unexposed *Daphnia* were fed with mixed diet (*Microcystis* + Cv) of either exposed and or unexposed prey. Growth parameters and toxin production of exposed prey cultures were significantly different than that of control. Exposed *Daphnia* fecundity and survival was higher as compared to unexposed *Daphnia*. Growth and reproduction was reduced in exposed *Daphnia* when fed with exposed prey as compared to those fed with unexposed prey. This study provides information on the interactive inducible defenses between cyanobacteria and its grazer under laboratory conditions and may increase our understanding of cyanobacteria and *Daphnia* interactions in the freshwater ecosystem.

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1. Introduction

Phytoplankton and zooplankton are important components of freshwater ecosystem (Elser and Goldman, 1991; Menden-Deuer and Rowlett, 2014), and their interactions are critical for regulating productivity and efficient transfer of carbon and energy to higher trophic levels (Garcia-Comas et al., 2016; Paul, 2008; Kang et al., 2015). Recently, some phytoplankton, such as cyanobacteria, due to eutrophication and warming overgrow in freshwater ecosystem and form blooms (Gobler et al., 2016; Paerl and Otten, 2013). These blooms forming species due to their toxicity and poor nutritional value, not only deter grazing, but also reduce zooplankton reproductive success and survival of their future generations (Hülsmann and Voigt, 2002; Jang et al., 2003; Martin-Creuzburg et al., 2008). Understanding of such interactions is important for controlling these bloom forming species and for balanced freshwater ecosystem (Urrutia-Cordero et al., 2016; Turner and Tester, 1997).

Cyanobacterial dominance is linked to their potential of various morphological, biochemical and behavioral defense mechanisms against grazing of zooplankton (Jang et al., 2007; van Gremberghe et al., 2009). Cyanobacteria through inducible defenses produce different phenotypes which resist zooplankton grazing (Lemaire et al., 2012; Oberhaus et al., 2007). It has been shown for several phytoplankton species that herbivorous zooplankton can induce defenses by releasing infochemicals (Lürling and Van Donk, 1996; Van Donk, 2007). Phytoplankton respond to these infochemicals by changing morphology (such as cell and colony size as well as length) (Bergkvist et al., 2008; Hansson et al., 2007a), physiology and increased toxin production (Jang et al., 2003; Selander et al., 2012).

On one hand cyanobacteria negatively affect zooplankton growth and reproduction (Wilson et al., 2006a). Alternatively,

* Corresponding author.
E-mail addresses: Siddiq_sa28@gmail.com, DG1230065@smail.nju.edu.cn
(S. Akbar), Dujj@zuli.edu.cn (J. Du), tianxj@nju.edu.cn (X. Tian).

other studies have provided evidence that some zooplankton are also capable to produce physiological and behavioral traits to survive in the presence of certain toxic cells (Semyalo et al., 2009; Urrutia-Cordero et al., 2016). Recent observations suggest that zooplankton populations may adapt to tolerate toxic cyanobacteria (Sarnelle and Wilson, 2005), and that this trait can be transferred from mother to offspring (Gustafsson et al., 2005), indicating that it has a genetic basis. Many investigators observed that previous exposure to Microcystis enhanced the resistance of Daphnia to toxic M. aeruginosa through maternal induction (Jiang et al., 2013b; Sarnelle et al., 2010). Among the interactions of cladocerans and cyanobacterial bloom, microcystin has been extensively studied. Several studies showed pronounced negative effects of micro-cystins on Daphnia fitness parameters (Lurling, 2003; Rohrlack et al., 2005). Cellular toxin content in response to specific zooplankton grazers was increased in the freshwater cyanophy-cean Microcystis (Jang et al., 2003) and the marine dinoflagellates A. tamarense. These toxins resist phytoplankton grazing while harmful for zooplanktons (Senft-Batoh et al., 2015b; Wohlrab et al., 2010) In addition to microcystin toxicity, some other studies have been shown that non- microcystin producing strains and other unknown substances released from M. aeruginosa are also detrimental to cladoceran (Carmichael, 1992; Semyalo et al., 2010).

Predator-prey inducible defenses are important for growth and survival of organisms (Agrawal, 1998; Van Donk et al., 2011). Different cyanobacteria and zooplankton respond to these defenses by producing different phenotypes, changes in life history, morphology and biochemical parameters (Sarnelle et al., 2010; Verschoor et al., 2004). (Bolnick et al., 2011; Chipps et al., 2004; Lundgren et al., 2016; Pfennig et al., 2010). Inducible defenses allow phytoplankton to resist the risk of being grazed under the threat of predation and save extra energy to maintain the defense system in the absence of predators (Hansson et al., 2007b; Zhu et al., 2016). Predicting the responses of prey and predators due to inducible defenses is integral to understanding the ecosystem disruptive effects of algal blooms (Sunda and Hardison, 2007). Researchers have separately investigated effects of cyano-bacteria on zooplankton (Tillmann et al., 2007), and or zooplank-ton resistance to cyanobacteria through maternal induction and co-evolutionary adaptability (Gustafsson et al., 2005; Jiang et al., 2013a; Sarnelle, 2007). Remarkably, despite its importance for resource management and regardless of the very many laboratory and field observations, our understanding of the interactive effects of cyanobacteria and zooplankton remains inadequate and contradictory (Twombly et al., 1998; Wilson et al., 2006a). Cyanobacteria and zooplankton interactions is an evolutionary arms race, in which the zooplankton adapts for higher tolerance, while cyanobacteria develop grazing defenses (Ger et al., 2016). Despite this two-way interaction between the groups, research has mainly focussed on one way relationship (Gustafsson and Hansson, 2004; Hairston et al., 2001; Jang et al., 2003; Jiang et al., 2013b). The negative effects of cyanobacteria on Daphnia growth, reproduction and survival have been documented in which Daphnia are fed various species and strains of cyanobacteria and their performance (survival and/or fecundity) is compared with standard diet such as nutritious chlorophytes (Lampert, 1981; Lurling, 2003; Wilson et al., 2006a). Previous laboratory experiments of Daphnia maternal induction detected stronger effects and enhanced fecundity and survival against Microcystis toxicity (Gustafsson and Hansson, 2004; Jiang et al., 2013b; Sarnelle and Wilson, 2005), however these experiments are often performed with phytoplank-ton from grazer free laboratory cultures. In most laboratory studies the potential effects of prey exposure on induced defenses have been ignored. Therefore, we investigate the inducible response of both prey and predator in order to better predict natural interactions through laboratory studies. We hypothesized that

without considering prey inducible defenses, maternal induction in Daphnia may be overestimated.

2. Materials and methods

2.1. Cultures

The microcystin (MC) producing cyanobacterium Microcystis aeruginosa PCC7806 FACHB-915 (Ma), Microcystis flos-aquae FACHB-905 (Mf) and the green alga Chlorella vulgaris FACHB-1227 (Cv) were provided by the Freshwater Algae Culture Collection of the Institute of Hydrobiology (FACHB), Chinese Academy of Sciences, Wuhan, China. They were mass cultured separately in BG-11 medium at $24 \pm 1\,^\circ\text{C}$ under a light intensity of approximately $50\,\mu\text{mol}\,\text{m}^{-2}\,\text{s}^{-1}$ with a 12:12-h light: dark cycle (Akbar et al., 2017). The log phase microalgae were harvested by centrifugation for 10–12 min at 3300 rpm.

The cladoceran Daphnia carinata (DC) was originally isolated from Huaihe River Basin, while Daphnia magna (DM) was isolated from Taihu Lake, and grown in our laboratory. The stock cultures of DM and DC were raised in COMBO media, and fed with Cv $(1 \times 10^5\,\text{cells}\,\text{mL}^{-1})$ every other day. The cultures were kept in a constant temperature chamber at $22 \pm 1.2\,^\circ\text{C}$ at $50\,\mu\text{mol}\,\text{m}^{-2}\,\text{s}^{-1}$ with a 12:12-h light: dark cycle. These animals were maintained under these conditions for at least one month and served as mothers for the animals used in the experiments. All neonates for a given experiment were taken from a single brood and within 12–15 h after birth.

2.2. Exposure experiments

2.2.1. Daphnia infochemicals
10 DM and DC were taken separately in 500 mL COMBO media. They were fed with a mixture of (Ma + Cv) and or (Mf + Cv) at 1:3 with total C of $1\,\text{mg}\,\text{L}^{-1}$. The COMBO + mixed phytoplankton culture without Daphnia was also filtered and used as a control. After 24 h the suspension of each culture was filtered ($0.2\text{-}\mu\text{m}$ pore size, Whatman) in sterile environment to remove microalgal cells, bacteria, and other particulates. These filtered suspensions were labeled as DM infochemicals, DC infochemicals and control, respectively. After collection of Daphnia infochemicals, Daphnia were individually kept in 100 mL beaker with 60 mL COMBO media.

2.2.2. Prey exposure
To determine Microcystis and Chlorella vulgaris response to Daphnia infochemicals, Ma, Mf and Cv were separately cultured in flasks with 120 mL BG-11 medium. Filterates of COMBO + phytoplankton cultures without Daphnia were inoculated in to separate cultures to use as control. Each treatment and control culture was grown in triplicate. 10 mL of filtered suspension (DM infochemicals, DC infochemicals or control) were added to fresh prey cultures. The parameters of these cultures including chlorophyll-a (Chl-a), microcystin-LR and cells density were measured on Day 0, 2, 4 and 6. New cultures of Ma, Mf and Cv were inoculated with $10^5\,\text{cells}\,\text{mL}^{-1}$ from infochemicals exposed and control cultures. These exposed and control cultures were harvested in log phase by centrifugation for 10–12 min at 3300 rpm. All the flasks and the culture medium were sterilized at $121\,^\circ\text{C}$ for 20 min, and all the operations were carried out in the axenic condition.

Chl-a concentration of subsamples was measured using the ethanol extraction method as prescribed by the national standard method (Eaton et al., 1998), and the absorbance was measured on spectrophotometer (756MC, Shanghai Jinghua Science and Tech-nology Instrument Co., Ltd., China) at 680 nm (Jingjing et al., 2015).