Interaction between simulated dense *Scenedesmus dimorphus* (Chlorophyta) bloom and freshwater meta-zooplankton community

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ABSTRACT

Algal bloom has been a subject of much research, especially the occurrence of blue-green algae (cyanobacteria) blooms and their effects on aquatic ecosystems. However, the interaction between green algae blooms and zooplankton community was rarely investigated. In the present study, the effects exerted by *Scenedesmus dimorphus* (green alga) bloom on the community structure of zooplankton and the top-down control of the bloom process mediated by the zooplankton were evaluated using a series of laboratory cultures. The results showed that a dense *S. dimorphus* bloom could change the zooplankton community structure by decreasing its diversity indices, leading to the enrichment of a particular zooplankton species, *Brachionus calyciflorus*. In the presence of mixed species of zooplankton, the density of *S. dimorphus* in the culture was decreased as determined by a change in total chlorophyll *a* (Chl *a*) concentration, which was about 200 μg L⁻¹ lower than that of the zooplankton-free culture. Furthermore, the number of species belonging to Cladocera, Copepoda and Rotifera all decreased, with all the cladocerans disappeared in the co-culture within 2 weeks of culturing, while the density of rotifers increased from 818 (±243) ind L⁻¹ at the time of inoculation to 40733 (±2173) ind L⁻¹ on the 14th day post-inoculation. Grazing of *S. dimorphus* by the rotifer *B. calyciflorus* neutralized its growth, and the gradual increase in *B. calyciflorus* density eventually led to the collapse of the bloom. Furthermore, grazing by *B. calyciflorus* also led to a decrease in the maximal photochemical efficiency (*Fv/Fm*) of photosystem II (PSII). The combined changes occurring in the zooplankton community structure during the process of *S. dimorphus* bloom and the negative effects of grazing on algal growth, morphology and photosynthetic activities confirmed the key role of zooplankton in the control of algal bloom. The results of the study therefore indicated that dense algal blooms caused by non-toxic algae could still remain a threat to aquatic ecosystems.

**Key words**: Algal bloom; *Scenedesmus dimorphus*; zooplankton; Rotifera; Copepoda; Cladocera; morphology; photochemical efficiency.

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INTRODUCTION

Aquatic food webs depend on a balance between bottom-up availability of nutrients and top-down control via grazing (Shurin et al., 2012). Phytoplankton blooms usually occur as a result of net biomass production in response to favorable growth conditions and the removal of suppressors (Donaghay et al., 1997; Kang et al., 2015). In China, algal blooms are caused by advanced eutrophication of freshwater lakes, which occur as a result of dramatic increase in nutrient loading and the weak bearing capacity of shallow lakes to nutrient loading (Havens et al., 2001; Paerl and Huisman, 2008; Qin et al., 2010; Xu et al., 2010; Paerl et al., 2011). As a result of reduced light transmission in water, algal bloom can enhance water turbidity, which threatens the survival of benthic algae and aquatic macrophytes (Scheffer et al., 1997, 2011; Jeppesen et al., 2007). Furthermore, oxygen depletion caused by algal respiration during night time can also lead to the loss of other aquatic organisms (Paerl and Fulton, 2006; Zhang et al., 2011).

Zooplankton constitute an important link in the food web. They feed on the bacteria and algae and become a source of food for aquatic insects and small fish (Turner, 2004, 2014). The community of zooplankton within any natural pond can be very diverse, usually comprising hundreds of protozoa, several rotifers and 10 to 30 microcrustacean species (Norlin et al., 2006; Merrix-Jones et al., 2013). The composition of species within a habitat is influenced by local climate, diurnal cycles, water quality, trophic state, pH, direct predation, food composition (Guisande et al., 2003; Ban et al., 2008; Verschoor et al., 2009; Guo et al., 2011). Moreover, microalgal species and their abundance are directly controlled by zooplankton,
resulting in complex mutual interactions (Steiner, 2003; Verschoor et al., 2007). Therefore, algal bloom has been suggested to affect the zooplankton community and energy flow in aquatic ecosystems (Gressel et al., 2013; Katsanevakis et al., 2014).

Several studies have reported the decline in abundance of larger-sized zooplankton and increase in abundance of smaller-sized zooplankton following the occurrence of a cyanobacterial bloom, which supplied the toxic food (Hansson et al., 2007; Ke et al., 2008; Deng et al., 2010; Sun et al., 2012). This indicates that the tolerance of zooplankton to algal blooms might be species-dependent, thus the interspecific differences among zooplankton species should also be considered when investigating the interaction between filter feeders and phytoplankton (Tillmanns et al., 2008). However, very few studies have been undertaken to investigate the changes in zooplankton community structure during the process of an algal bloom (Xie et al., 1998; Michaloudi et al., 2009; Gorokhova et al., 2014; Walsh and O’Neil, 2014). This has seriously impeded effort to reveal the relationship between algal bloom and the underlying changes in the community structure of zooplankton.

To our knowledge, no study on the effect of green algae blooms on zooplankton community has been reported. *Scenedesmus dimorphus*, one of the most common species of freshwater green algae (Trainor, 1992), is commonly observed as colonies in the field, but it often fails to form colonies in long-term axenic cultures (Lürling and Beekman, 1999; Lürling, 2003). Field studies have suggested that *Scenedesmus* spp. can quickly respond to environmental changes, which explains why *Scenedesmus* may dominate in turbid and continuous mixed shallow water bodies (Oliver and Ganf, 2000). Furthermore, it grows rapidly and is commonly the dominant species in eutrophic water, making it a likely candidate to give rise to a bloom (Garg and Gard, 2002). The ability of infochemicals released by zooplankton to induce changes in the biochemical composition and morphology of *Scenedesmus* has been proven (Lürling et al., 1997; Lürling, 2003). In addition, the species-dependent tolerance of zooplankton to algal blooms has been suggested to be an evolutionary mechanism in a natural system that has a long history of algal bloom (Gilbert, 1990; Gustafsson and Hansson, 2004). Zooplankton species that co-exist with dense algal populations may be more able to digest these algae than the species that are mainly found in algal bloom-free water (Sarnelle and Wilson, 2005; Paes et al., 2016). On these bases, we predicted that a dense *Scenedesmus* bloom could still change the zooplankton community structure by altering the food composition and quality, which would then influence the filter feeding efficiency of zooplankton and can be regarded as a challenge, although *Scenedesmus* is considered as a non-toxic species. Based on the above hypothesis, the effect of *S. dimorphus* bloom on the community structure of zooplankton over time, and the downward regulation by meta-zooplankton on the growth as well as on the photosynthetic activity of *S. dimorphus* were studied under a series of laboratory experiments.

**METHODS**

**Organism and culture media**

*Scenedesmus dimorphus* was isolated from the Wenruitang River (China) in the spring of 2015. Its identification was performed by classical morphological methods. The Wenruitang River is a typical eutrophic urban river, with shallow water and the phytoplankton community in the water is dominated by green algae and diatoms throughout the year (Sun et al., 2018). A single eight-celled colony of *S. dimorphus* approximately 16 µm long and 3 µm wide was isolated and propagated for all subsequent cultures. *Scenedesmus dimorphus* was cultured in sterile BG11 medium (Allen and Stanier, 1968) at 25°C with light irradiance of 100 µmol photons m⁻² s⁻¹ and under a 12:12 h light-dark regime. The culture was routinely shaken (2-3 times per day) and the cells in the exponential growth phase were harvested and used in the following experiments.

**Zooplankton sampling and experiment preparation**

Zooplankton species were collected from the main course of the Wenruitang River that flows through the campus of Wenzhou University in the middle of April 2016. In spring, the chlorophyll a (Chl a) concentration in the Wenruitang River is around 40 µg L⁻¹ and the water temperature is about 25°C (Sun et al., 2018). Green algae account for a dominant proportion of the algae in spring and summer (Sun et al., 2018). The total zooplankton species were collected by vertically hauling a net (diameter 20 cm, length 60 cm, and mesh diameter 76 µm) from the bottom to the surface of the river. All samples were immediately removed from the net and temporarily cultured in distilled water inside a 5-L Erlenmeyer flask. A 100-mL sample of the suspension was removed and preserved in formalin followed by staining with Bengal’s red for microscopic identification. All the zooplankton members were identified down to the genus or species level, and the average abundance of each species was calculated from three random samples. The zooplankton community structure obtained was used as a control that denoted the zooplankton community before encountering the dense *S. dimorphus* bloom. The remaining zooplankton in the culture were used for subsequent experiments within 2 h.
S. dimorphus cultured in presence of mixed zooplankton

The harvested S. dimorphus cells were diluted with fresh BG11 medium to yield a Chl a concentration of 47.19 ± 4.92 µg L⁻¹ and cultured in six transparent plastic tanks, each containing 20 L of culture. The cultures were aerated with ambient air at a flow rate of 500 mL min⁻¹ and irradiated with 100 µmol photons m⁻² s⁻¹ PAR at 25°C. Three of the six tanks were kept as controls while the other three tanks were each inoculated with 500 mL of well-mixed zooplankton culture. The 500-mL well-mixed zooplankton culture was first filtered through a nylon net (mesh diameter 76 µm) and the zooplankton retained on the net were then added to the S. dimorphus cultures on the 5th day when the Chl a concentration was about 277.21 ± 20.97 µg L⁻¹. Both sets of cultures were incubated continuously for two weeks. Samples (2-L) of the culture were removed from each tank on the 4th, 8th, 12th and 14th days and preserved in formalin for zooplankton identification. At the same time, 2 mL of the culture was also removed and directly used to determine the concentration of Chl a. Triplicate samples were determined for each treatment, and the data were reported as the mean value and standard deviation for each treatment. The abundance of total zooplankton used in these experiments was slightly higher than the abundance found in the Wenruitang River (Xiao et al., 2012). However, it still had the same order of magnitude, and should therefore reflect the actual zooplankton abundance in the river.

Determination of Chl a concentration in S. dimorphus culture

In the present study, the Chl a concentration in the S. dimorphus culture was determined using a Phyto-PAM Phytoplankton Analyzer (Heinz Walz GmbH, Effeltrich, Germany). In the Phyto-PAM, μsec measuring light pulses were generated by an array of light-emitting diodes (LED) featuring 4 different colors: blue (470 nm), green (520 nm), light red (645 nm) and dark red (665 nm). The different color-measuring light pulses were applied alternately at a high frequency, such that quasi-simultaneous information on Chl fluorescence excited at the four different wavelengths was obtained. This feature is very useful for distinguishing algae with different types of light harvesting pigment antenna (Schreiber, 1994; Ma et al., 2015). Therefore, changes in Chl a concentration in the S. dimorphus culture were detected as groups of green algae.

Determination of the biodiversity index of zooplankton community in S. dimorphus culture

To measure the species diversity, we used richness (e.g., the number of species), evenness (the relative abundance distribution of those species) and proportional diversity (a combination of richness and evenness) as defined by the following three indexes: (1) Shannon \( H=-\sum(n_i/N)\ln(n_i/N) \) (Shannon and Weaver, 1949), (2) Pielou \( J=H/H_{max} \) (Pielou, 1966), (3) Simpson index \( Si=\sum(n_i/N)^2 \) (Simpson, 1949), where \( H \) and \( S \) represent the species diversity index and number of species respectively, \( n_i \) and \( N \) are the number of individuals of the \( i \)th species and the total number of individuals, respectively.

Effect of B. calyciflorus on S. dimorphus culture

To evaluate the downward regulation of meta- zooplankton in the process of S. dimorphus bloom as well as the photosynthetic activity and cellular phenotype of the bloom-forming species, different amounts of the micro-zooplankton B. calyciflorus Pallas (Rotifera) were added to the S. dimorphus cultures followed by incubation in transparent plastic tanks (each containing 10 L culture). Brachionus calyciflorus from the initial culture sampled from the Wenruitang River was propagated by incubation with the green alga Chlorella vulgaris at a density of about 10⁵ cells L⁻¹. The cultures were aerated with ambient air at a flow rate of 500 mL min⁻¹ and irradiated with 100 µmol photons m⁻² s⁻¹ PAR at 25°C. To obtain cultures with increasing abundances of rotifer, different (200, 400, 800, 1,200 and 2,000 mL) volumes of a well-mixed culture of high B. calyciflorus were filtered through a nylon net (mesh diameter 76 µm) and the retained B. calyciflorus cells were inoculated into a 10-L S. dimorphus culture. For ease of referencing, inoculums corresponded to 200, 400, 800, 1,200 and 2,000 mL of B. calyciflorus culture were referred to as 1Z, 2Z, 4Z, 6Z and 10Z, respectively, where 1Z was defined as a B. calyciflorus density of 3344 ind. L⁻¹. Triplicates were conducted at the same time, and the data were reported as the mean value and standard deviation from each treatment.

Photosynthetic activities and morphological examination of S. dimorphus

To evaluate the impacts of zooplankton grazing on the biological response of S. dimorphus, the algal cells co-cultured with B. calyciflorus were sampled at the end of the 1st, 2nd and 3rd day after B. calyciflorus inoculation. The PSII quantum yield of the cells was subsequently determined using a Phyto-PAM (Heinz Walz GmbH, Effeltrich, Germany). To obtain the PSII quantum yield, the minimum fluorescence (F₀) was initially determined by illuminating the samples with low intensity light (600 Hz, 665 nm, 0.3 µmol photons m⁻² s⁻¹) after the samples had been kept in the dark for 10 min. Subsequently, the maximal fluorescence (Fm) was determined with a 0.8-s pulse of saturating red light of 5,000 µmol photons m⁻² s⁻¹.
The variable fluorescence \( (F_v) \) was defined as \( F_v = F_m - F_0 \), and the optimal quantum yield was set as \( F_v/F_m \).

Meanwhile, the morphological changes of \( S. \ dimorphus \) were examined with a microscope (Zeiss Axioplan 2; Carl Zeiss, Germany). Digital images were recorded weekly with a Zeiss Axicam HRC color camera (Carl Zeiss, Jena, Germany), and analyzed with a Vision Analysis system (Axio Vision 3.0).

**Statistical analysis**

Data from the different measurements were reported as means and standard deviations. One-way ANOVA and Post-hoc tests (Tukey) were used to establish differences among treatments, with a significant level set at 5% \( (P=0.05) \). Statistical analysis was performed using the SPSS V.16.0 for windows (SPSS inc., Chicago, IL, USA) and all charts were generated using Origin 8.0 (Origin Lab, Northampton, MA, USA).

**RESULTS**

**Changes in zooplankton community structure during the \( S. \ dimorphus \) culture**

Twenty zooplankton species were identified in the mixed zooplankton sample before it was added to the \( S. \ dimorphus \) culture. These included 5 Cladocera species, 3 Copepoda species and 12 Rotifera species (Tab. 1). The dominant species were Mesocyclops leuckarti, Asplanchna brightwellii, B. calyciflorus, Keratella valga and Trichocerca cylindrica. However, only three species (\( M. \ leuckarti, \ B. \ calyciflorus \) and \( B. \ urceolaris \)) survived to the end of the experiment (Tab. 1). The abundances of cladocerans, copepods and rotifers were 180 (±59), 437 (±82) and 818 (±243) ind. L\(^{-1}\), respectively, at the time of inoculation. All the species belonging to the cladocerans disappeared from the \( S. \ dimorphus \) culture at the end of the experiment, but the abundance of rotifer increased to 40733 (±2173) ind. L\(^{-1}\) on the 14\(^{th}\) day (Fig. 1a). The total zooplankton abundance in the culture at the time of inoculation consisted of 12.75%, 31.05% and 56.20% of cladocerans, copepods and rotifers species, respectively. The proportion of cladocerans and copepods decreased with prolonged culturing time, but that of rotifers increased rapidly, reaching 99.68% of the total zooplankton abundance by the end of the experiment (Fig. 1b).

The species diversity index (\( H \)), evenness index (\( J \)) and Simpson index (\( S_i \)) all changed with prolonged culturing time. Increased culturing time and algal cell density resulted in decreased \( H \) and \( J \) values, which were 3.67 and 0.82, respectively, at the time of zooplankton inoculation, but decreased to 0.08 and 0.02 on the 14\(^{th}\) day.

**Tab. 1. Changes in abundance of zooplankton species during the emergence and disappearance of \( S. \ dimorphus \) bloom simulated in the laboratory.**

| Zooplankton species                  | Class       | Bloom emergence abundance (ind. L\(^{-1}\)) | Bloom disappearance abundance (ind. L\(^{-1}\)) |
|--------------------------------------|-------------|--------------------------------------------|-----------------------------------------------|
| Diaphanosoma leuchtenbergianum       | Cladocera   | 57.14                                      | —                                             |
| Bosmina fatalis                      | Cladocera   | 28.57                                      | —                                             |
| Moina macrrocopa                     | Cladocera   | 85.71                                      | —                                             |
| Bosmina coregoni                     | Cladocera   | 57.14                                      | —                                             |
| Mesocyclops leuckarti                | Copepoda    | 142.86                                     | 133.33                                        |
| Schmackeria inopinus                 | Copepoda    | 57.14                                      | —                                             |
| Copepoda larvae                      | Copepoda    | 114.29                                     | —                                             |
| Asplanchna brightwellii              | Rotifera    | 85.72                                      | —                                             |
| Trichocerca similis                  | Rotifera    | 28.57                                      | —                                             |
| Brachionus calyciflorus              | Rotifera    | 142.85                                     | 40 133.33                                     |
| Brachionus quadridentatus            | Rotifera    | 57.14                                      | —                                             |
| Brachionus urceolaris                | Rotifera    | 85.71                                      | 600.00                                        |
| Brachionus diversicornis             | Rotifera    | 28.57                                      | —                                             |
| Filinia longisela                    | Rotifera    | 28.57                                      | —                                             |
| Keratella valga                      | Rotifera    | 142.85                                     | —                                             |
| Brachionus angularis                 | Rotifera    | 28.57                                      | —                                             |
| Trichocerca tenutor                  | Rotifera    | 57.14                                      | —                                             |
| Trichocerca cylindrica               | Rotifera    | 114.28                                     | —                                             |
| Polyaertha trigla                    | Rotifera    | 28.57                                      | —                                             |
post inoculation. At the same time, the value of Si increased from 0.11 to 0.98 (Tab. 2).

Effects of zooplankton-mediated downward regulation on the dynamics of \textit{S. dimorphus} bloom

The mean Chl \textit{a} concentration of the \textit{S. dimorphus} culture increased from 47.19 (±4.92) µg L\(^{-1}\) at the time of inoculation to 1742.88 (±119.89) µg L\(^{-1}\) on the 13\(^{th}\) day and then sharply decreased to 64.29 (±6.42) µg L\(^{-1}\) on the 18\(^{th}\) day post-zooplankton inoculation (Fig. 2). The addition of zooplankton did not entirely suppress the continuous development of the algal bloom. However, their presence led to a significant (P<0.05) decrease in the concentration of \textit{S. dimorphus} cells (represented by reduction in Chl \textit{a} concentration) and resulted in a faster collapse of the simulated algal bloom. The differences in Chl \textit{a} concentration over time between the control \textit{S. dimorphus} culture and \textit{S. dimorphus} culture containing the mixed zooplankton ranged from 25.67 to 574.87 µg L\(^{-1}\) (average of 253.40 µg L\(^{-1}\)), with the latter having less Chl \textit{a} (Fig. 2).

Effect of \textit{B. calyciflorus} abundance on \textit{S. dimorphus} growth and photosynthetic activity

The Chl \textit{a} concentration of the \textit{S. dimorphus} culture increased with prolonged incubation time (Fig. 3a). However, the growth of \textit{S. dimorphus} was completely neutralized within 2 days when it was inoculated with 1Z of \textit{B. calyciflorus} (Fig. 3b). In the case of the \textit{S. dimorphus} culture inoculated with 1Z \textit{B. calyciflorus}, the Chl \textit{a} concentrations were 236.74 (±15.63), 237.47 (±6.67), 238.09 (±11.15) and 220.34 (±13.83) µg L\(^{-1}\) on days 0, 1, 2 and 3 post-inoculation, respectively. However, when the \textit{B. calyciflorus} inoculum was increased to 2Z, 4Z, 6Z and 10Z, the Chl \textit{a} concentration decreased to 50.25 (±5.77), 35.11 (±5.77), 31.43 (±7.20) and 26.22 (±4.53) µg L\(^{-1}\), respectively, on the 3\(^{rd}\) day post-inoculation (Fig. 3b).

The Chl \textit{a} concentration of the \textit{S. dimorphus} culture inoculated with 1Z \textit{B. calyciflorus} only decreased by 6.9\%
in 3 days (Fig. 3b). Therefore, we assumed that the consumption of *S. dimorphus* by *B. calyciflorus* in this case was almost equal to the amount of increase in phytoplankton production. The average specific growth rates (μ) of the *S. dimorphus* culture were 0.25, 0.31 and 0.34 d⁻¹ on the 1st, 2nd and 3rd days, respectively, following *B. calyciflorus* inoculation (Fig. 3A). Thus, if the Chl a concentrations of the culture at the beginning of each day were kept equal to that at the time of inoculation, the corresponding average increase in Chl a concentration per day should be 65.47, 87.53 and 96.91 µg L⁻¹ on day 1, day 2 and day 3, respectively, in the absence of *B. calyciflorus* grazing. This suggested that a biomass corresponding to a Chl a content of 6.20 to 28.98 ng was consumed by one individual *B. calyciflorus* per day.

The optimal quantum yield (F_v/F_m) of the *S. dimorphus* culture remained stable (0.67±0.01) during the culturing period. However, in the presence of *B. calyciflorus*, it decreased with increasing zooplankton abundance and prolonged culturing time when the size of the *B. calyciflorus* inoculum was increased (Fig. 3c). The yield of *S. dimorphus* from cultures inoculated with 1Z, 2Z, 4Z, 6Z and 10Z *B. calyciflorus* decreased to 0.63 ±0.01, 0.56 ±0.02, 0.52 ±0.01, 0.38 ±0.02 and 0.34 ±0.02, respectively, on the 3rd day post-inoculation. Furthermore, there were significant (P<0.05) differences among the cultures, except between the culture inoculated with 6Z and that inoculated with 10Z (P=0.07). Within a 3-day period, the abundance of *B. calyciflorus* increased by 215%, 195% and 36% in the cultures inoculated with 1Z, 2Z and 4Z, respectively. However, for the cultures inoculated with 6Z and 10Z, the abundance of *B.

![Fig. 3. Changes in Chl a of *S. dimorphus* culture in the absence of rotifer *B. calyciflorus* (a), Chl a (b) and optimal quantum yield (F_v/F_m) of *S. dimorphus* (c) in the presence of different abundance of rotifer *B. calyciflorus* as well as abundance of *B. calyciflorus* (d) in the 3-day cultures with the same Chl a concentration at the time of inoculation. Data are the means±standard errors obtained from triplicate samples.](image-url)
Interactions between green algal bloom and zooplankton

Calyciflorus decreased by 10% and 33%, respectively (Fig. 3d).

Effects of B. calyciflorus graze on the morphology of S. dimorphus

In addition to the inhibitory effect on the Fv/Fm of S. dimorphus, grazing by B. calyciflorus also destroyed its single-celled and colony morphologies. Compared to the colonies of S. dimorphus cultured in the absence of B. calyciflorus (Fig. 4a), the colonies of S. dimorphus from the culture inoculated with 100B. calyciflorus lost their regular conformation, and the cells were damaged and lysed (Fig. 4b). In fact, the damage to algal cells occurred regardless of the abundance of B. calyciflorus but was most obvious in the culture containing the highest B. calyciflorus abundance (10Z). Furthermore, the damaged cells readily formed flocs and sank to the bottom.

DISCUSSION

Biodiversity is known to affect the stability and productivity of communities and the trophic interactions among these communities in a natural ecosystem (McArt et al., 2012; Tilman et al., 2014). Our experiments showed that the bloom caused by S. dimorphus could decrease the diversity indices of the zooplankton community, while increasing the abundance of one particular species of zooplankton, B. calyciflorus (Tabs. 1 and 2), suggesting that the dense bloom caused by an nontoxic alga could decrease the community stability and lead to the rapid population expansion of the species that could adapt to the bloom more quickly. It should be pointed out that the changes in zooplankton community structure due to algal bloom exposure were assessed by comparing the zooplankton community cultured in the presence of the Scenedesmus bloom to the community initially present in the water sample collected from the river, which was considered as the control. A better control should perhaps consist of the same water sample subjected to the same laboratory culturing conditions and time as the mixed culture, but without the addition of S. dimorphus, since it is well known that some zooplankton species are more difficult to culture than others under laboratory conditions, and some zooplankton species may die because they cannot adapt to the lab culturing conditions. This can also lead to the loss of zooplankton abundance. However, if such a control was included, it would have been necessary to feed the zooplankton with some microalgae species because the population of zooplankton would decline sharply when there is a shortage of food as shown in Fig. 3d. Adding microalgae to the zooplankton culture as food would change the culture condition, making it unsuitable as a control for our purpose. Therefore, the control treatment we used was a realistic and practical choice, though it could not entirely discriminate the impact caused only by the algal bloom from the impact caused by laboratory conditions.

The downward regulation of algal bloom by zooplankton plays an important role in constraining the abundance of phytoplankton in an aquatic ecosystem (Calbet and Landry, 2004; Smayda, 2008), and a lack of control exerted by predators could ultimately lead to a phytoplankton bloom (Irigoien et al., 2005; Modigh and Franzè, 2009). The presence of zooplankton significantly decreased the concentration of S. dimorphus cells, thereby

**Fig. 4.** Morphology of S. dimorphus colonies in the absence (a) and presence (b) of B. calyciflorus in a 3-day culture.
accelerating the collapse of the bloom (Fig. 2). Furthermore, the bloom caused by \textit{S. dimorphus} was completely constrained by the rotifer \textit{B. calyciflorus} when it was present in sufficiently high abundance (Fig. 3b). \textit{Brachionus calyciflorus} was not able to fully suppress the algal bloom (Fig. 2), despite reaching a density (up to 40,000 ind. L$^{-1}$) comparable to those used in Fig. 3b, and this might be related to the timing of the inoculation of \textit{B. calyciflorus} (during the initial vs. exponential algal growth phase) besides the much higher concentration of \textit{S. dimorphus} before the inoculation of \textit{B. calyciflorus} (Fig. 2). Rotifers are small zooplankton, usually less than 200 µm in size, and they are often the most abundant organisms in highly eutrophic freshwater bodies, having a density between 1000 and 500,000 ind. L$^{-1}$ (Roche, 1995; Sarma et al., 2003). Rotifers usually feed on small organisms with a diameter of 20 µm or less (Pourriot, 1977; Riemann and Ahrlich, 2008). \textit{Brachionus calyciflorus} feeds on organisms having a wide range of cell sizes but has a clear preference for the large algae such as \textit{Cyclotella} sp. and \textit{Scenedesmus opoliensis}, which have particle size between 10 and 33 µm (Pagano, 2008). In natural water, \textit{S. dimorphus} usually exists as colonies of the four- and eight-celled stages (Hessen and Van Donk, 1993) as shown in Fig. 4a, making it an ideal food for \textit{B. calyciflorus}.

In general, cyanobacteria can cause the rapid decrease of large crustaceans because they can interfere with the filter feeding behavior of these animals (Chow-Fraser, 1986; DeMott, 1999). The toxins produced by the cyanobacteria may also be lethal to the crustaceans (DeMott, 1999; Carmichael, 2001; Ke et al., 2008). In addition, the diversity of phytoplankton is thought to be one of the main factors determining the seasonal succession of crustacean zooplankton (Abrantes et al., 2006). Other noticeable features of our experiment were a reduced number of Cladocera, Copepoda and Rotifer species and the complete disappearance of cladocers at the end of the \textit{S. dimorphus} bloom. However, the abundance of rotifers increased sharply at the end of the \textit{S. dimorphus} bloom, reaching almost 50 times the amount present at inoculation (Fig. 1a). The disappearance of most copepods and all cladocerans allowed the rotifers to become the absolute dominant thriving species in the presence of \textit{S. dimorphus} bloom (Fig. 1b). In addition to the decreased cladocerans and copepods abundance and biodiversity index (Fig. 1a, Tab. 2), the increased growth of \textit{B. calyciflorus} (Fig. 1 a,b) suggested that \textit{S. dimorphus} bloom might have detrimental effects on the zooplankton community and the response of zooplankton to the bloom appeared to be species-dependent. Therefore, the zooplankton species that could not adapt to the experimental conditions had probably died out in a short time, reducing the biodiversity indices of the zooplankton population (Tab. 2).

Micro-zooplankton grazing can account for more than half of the phytoplankton production in the coastal and estuarine systems (Calbet and Landry, 2004) and their preferential predation plays a key role in altering the composition of the phytoplankton communities (Strom, 2008). In this study, the growth of \textit{S. dimorphus} was completely neutralized by \textit{B. calyciflorus} grazing and even collapsed when the inoculum of \textit{B. calyciflorus} reached a certain concentration (Fig. 3b). In addition, the abundance of \textit{B. calyciflorus} also increased in the mixed culture (Fig. 3c). Although there are many mechanisms that may result in the loss of phytoplankton, zooplankton grazing and trophic interactions among the zooplankton are widely considered to be the major biological factors influencing the top-down control throughout the development of an algal bloom (Turner, 2004; Irigoien et al., 2005; Smayda, 2008). The balance between phytoplankton growth and total grazing pressure is regarded as the factor that determines the magnitude and duration of the bloom (Turner, 2004, 2014; Smayda, 2008). However, this balance does vary spatially and temporally as specific growth and grazing rates may vary with nutrient condition as well as grazer community composition and abundance. The decline in phytoplankton population inflicted by zooplankton grazing had the potential to suppress bloom development when the grazers were present in high abundance. This could both regulate the bloom magnitude and contribute to the rapid population decline, and the eventual collapse of the bloom (Fig. 2).

Decreases in zooplankton diversity and community composition (Fig. 1, Tab. 2) may have numerous negative consequences for the ecosystem, including a reduction in the net energy transfer to higher trophic levels (Sunda et al., 2006, 2012) and in the efficiency of energy fluxes through the food webs. The flow of water in the Wenruitang River is very slow and the water is often in a stagnant state. The annual concentration of total nitrogen (TN) ranges from 5.33 (±0.81) to 9.40 (±1.25) mg L$^{-1}$ and that of total phosphorus (TP) ranges from 0.32 (±0.18) to 0.95 (±0.25) mg L$^{-1}$, both of which are considered as hypereutrophic level (Sun et al., 2018). \textit{Scenedesmus dimorphus} is also the dominant species in other eutrophic freshwater rivers besides the Wenruitang River, and it can be used as an indicator of water quality (Trainor, 1992; Jafari and Gunale, 2006; Sun et al., 2018). Under suitable conditions, the occurrence of dense \textit{S. dimorphus} blooms in hypereutrophic rivers can be expected. Therefore, the changes in zooplankton population observed in this study should be applicable to natural environments, although the bloom was stimulated in a capacity-limited container.

Grazing pressure has been associated with changes in productivity, colony size and biomass settleability (Xie et al., 1998; Michaloudi et al., 2009; Gorokhova et al., 2014;
Grazing of *S. dimorphus* by *B. calyciflorus* was found to decrease the yield of *S. dimorphus* (Fig. 3c) and destroy its regular colony morphology (Fig. 4 a,b). Therefore, it is reasonable to assume that decreases in algal cell density, photosynthetic activity and morphology were caused by the strong grazing pressure exerted by the zooplankton. The results also indicated that the occurrence of *S. dimorphus* bloom might have detrimental effects on the structure of zooplankton community, but could be favorable to some rotifer species such as *B. calyciflorus* due to the provision of adequate food supply throughout the bloom.

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