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Temperature-dependent elimination efficiency on *Phaeocystis globosa* by different initial population sizes of rotifer *Brachionus plicatilis*

Yunfei Sun, Yuanyuan Wang, Jin Lei, Chenchen Qian, Xuexia Zhu, Siddiq Akbar, Yuan Huang, Zhou Yang*

Jiangsu Key Laboratory for Biodiversity and Biotechnology, School of Biological Sciences, Nanjing Normal University, 1 Wenyuan Road, Nanjing 210023, China

HIGHLIGHTS

- The rotifer population increased rapidly when feeding on harmful *Phaeocystis*.
- The optimal temperature for rotifer to eliminate *Phaeocystis* is 28 °C.
- With increased rotifer density and temperature, *Phaeocystis* were eliminated earlier.
- Effect of rotifer density on clearing *Phaeocystis* decreased with rising temperature.
- Increased temperature improves consumer's ability to control autotrophic organism.

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ABSTRACT

Due to sea water eutrophication and global warming, the harmful *Phaeocystis* blooms outbreak frequently in coastal waters, which cause a serious threat to marine ecosystem. The application of rotifer to control the harmful alga is a promising way. To investigate the influence of initial rotifer density and temperature on the ability of rotifer *Brachionus plicatilis* to eliminate *Phaeocystis globosa* population, we cultured *P. globosa* with different initial rotifer densities (1, 3, 5 inds mL^{-1}) at 19, 22, 25, 28, and 31 °C for 9–16 d. Results showed that the population of rotifer feeding on *Phaeocystis* increased rapidly and higher temperatures favored the growth of *P. globosa* and *B. plicatilis*. With increased initial rotifer density and temperature, both the clearance rate of rotifer and the reduction rate of *P. globosa* increased, and thus *P. globosa* were eliminated earlier. Both temperature and initial rotifer density had significant effects on clearance rate of rotifer and the time to *Phaeocystis* extinction, and there was a significant interaction between the two factors on the two parameters, i.e., the effect of initial rotifer density on eliminating *Phaeocystis* decreased with increasing temperature. The rotifer in 5 inds mL^{-1} at 28 °C eliminated *P. globosa* in 4 d, whereas the rotifer in 1 ind mL^{-1} at 19 °C spent about 16 d on eliminating *P. globosa*. In conclusion, higher temperature and bigger initial rotifer density promote rotifer to eliminate the harmful *P. globosa*, and the optimal temperature for rotifer to clear *P. globosa* is 28 °C.

1. Introduction

In recent years, anthropogenic activities have caused the consequent marine eutrophication and global warming, which lead to harmful algal blooms (Anderson et al., 2002; Tas, 2011; Zhang et al., 2016; Dai et al., 2016). The widespread genus *Phaeocystis* is an important bloom-forming harmful alga as well as a recurrent event in many marine ecosystems (Baudoux and Brussaard, 2005; Lamy et al., 2009). *Phaeocystis globosa* has two major morph types: single cells and mucilaginous colonies (Rousseau et al., 2007). The high biomass blooms, production of haemolytic toxins, and formation of foam cause severe damage to marine coastal systems, fisheries, and tourism (Tan et al., 2016; Baudoux and Brussaard, 2005). It was reported that some of toxic compounds have significant lethality on the *Gadus morhua* larva, the brine shrimp *Artemia salina*, and the juvenile *Epinephelus akaara* fish (Stabell et al., 1999; Long et al., 2015). Such negative consequences caused by *Phaeocystis* blooms attract extensive attention

* Corresponding author.
E-mail address: yangzhou@njnu.edu.cn (Z. Yang).

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worldwide, so it is necessary to find some effective methods to control the harmful alga.

Up to now, quite a lot of studies have focused on finding the effective methods to control the harmful algal blooms, including physical (Pierce et al., 2004; Sengco et al., 2001), chemical (Sun et al., 2004), and biological methods (Zheng et al., 2013; Santini et al., 2013). Although chemical and physical methods have timely effect on controlling harmful algal blooms, they also damage aquatic environment once again and waste large amounts of resources. Therefore, biological control of harmful algae is thought to be a better choice for environment management, which includes microbial degradation and zooplankton ingestion. It was reported that some microzooplankton and bacteria can well control the single-celled harmful algae, such as *Microcystis* (Zhang et al., 2017) and *P. globosa* (Baudoux et al., 2006; Gumbo et al., 2008). A few studies also found that copepod (*Acartia tonsa*) can effectively consume *P. globosa* (Weisse et al., 1994; Verity, 2000; Tang et al., 2001), whereas rotifer can ingest small colonies and single cells of *P. globosa* (Nejstgaard et al., 2007). In our previous study, we also indicated that *Brachionus plicatilis* can survive well when feeding on *P. globosa* (Sun et al., 2017).

The above-mentioned exploration related to biomanipulation is considered as an environment-friendly method to control *Phaeocystis*. In marine, rapid reproduction rate, relative small size, and slow swimming behavior make rotifer suitable live prey for newly-hatched fish larvae (Lubzens et al., 1989, 2001). Therefore, when rotifer feeding on *P. globosa*, it can transfer energy from primary producers (the harmful alga) to higher trophic levels (such as fish larvae). As an important component of zooplankton community, rotifer accounts for a large proportion of zooplankton in marine and can transfer a large amount of matter and energy into higher trophic levels (Tinh et al., 2006). Moreover, rotifer also can survive by adjusting their life history strategies when the food condition is unfavorable (Yoshinaga et al., 2003; Kirk, 1997). Consequently, the application of rotifer to control *Phaeocystis* is a promising way.

Climate warming, one of the most important drivers of ecosystem change (Alsterberg et al., 2013), not only promotes growth of primary producers, but also boosts growth and grazing of consumers (Doney, 2006; O'Connor, 2009; George et al., 2015; Basen et al., 2017). Based on the possibility of application of rotifer to control *Phaeocystis*, it is necessary to investigate the effect of rising temperature on rotifer eliminating *P. globosa* and to find an optimal temperature to control the harmful alga. We hypothesized that both high temperature and large initial rotifer population can enhance rotifer to eliminate *P. globosa*. To test the hypotheses, we respectively exposed *P. globosa* in different densities of rotifers (1, 3, 5 inds mL^{-1}) at 19, 22, 25, 28, and 31 °C until all the algae were eliminated. The population dynamics of rotifer and *Phaeocystis*, clearance rate of rotifer, time to *Phaeocystis* extinction, and reduction ratio of *Phaeocystis* were measured. Our results demonstrated that high initial rotifer density accelerates rotifer to clear *P. globosa* and the elimination efficiency is temperature-dependent.

2. Materials and methods

2.1. Plankton and culturing

P. globosa was cultivated in autoclaved synthetic seawater (salinity 33‰, pH = 8.3) with f/2 medium at 25 °C under fluorescent light at 50 μmol photons $m^{-2}s^{-1}$ with a 14 h: 10 h light: dark cycle. The resting eggs of *B. plicatilis* were hatched and cultured in beakers with the autoclaved synthetic seawater (salinity 33‰, DO > 6 mg mL^{-1}) using *Chlorella* as food at the same conditions as described above.

2.2. Experimental design

To acclimate the experimental temperatures, both the rotifer and algae were exposed to a series of temperatures (19, 22, 25, 28, 31 °C) for 4 d prior to the experiment, respectively. *B. plicatilis* were fed on *P. globosa* (~1.0×10^6 cells mL^{-1}) under similar conditions as described above. In this experiment, we set five temperatures; each temperature included the control group and three different initial rotifer densities (1, 3, 5 inds mL^{-1}). The control group contained *P. globosa* only. The initial abundance of *P. globosa* was approximately 1.0×10^6 cells mL^{-1} in all treatments, which was based on the range of abundance in natural ecosystems (Li et al., 2015). All experiments were conducted in 150 mL Erlenmeyer flasks with 100 mL sterilized f/2 medium, and incubated at above-mentioned five temperatures under fluorescent light at 50 μmol photons $m^{-2}s^{-1}$ with a 14 h:10 h light: dark cycle. All the treatments were performed in triplicate. All the flasks were shaken every 12 h by hand to avoid the sedimentation of algal cells.

Samples (2 mL) were collected every day and then separated to enumerate. Algae from treatment and control were fixed with Lugol's solution (2%), and their abundance were measured using a hemocytometer under a light microscope. The rotifer abundances were counted using a 6-welled culture plates under a stereoscopic microscope. These data were used to calculate growth rate, clearance rate, and reduction ratio and also to fit the population

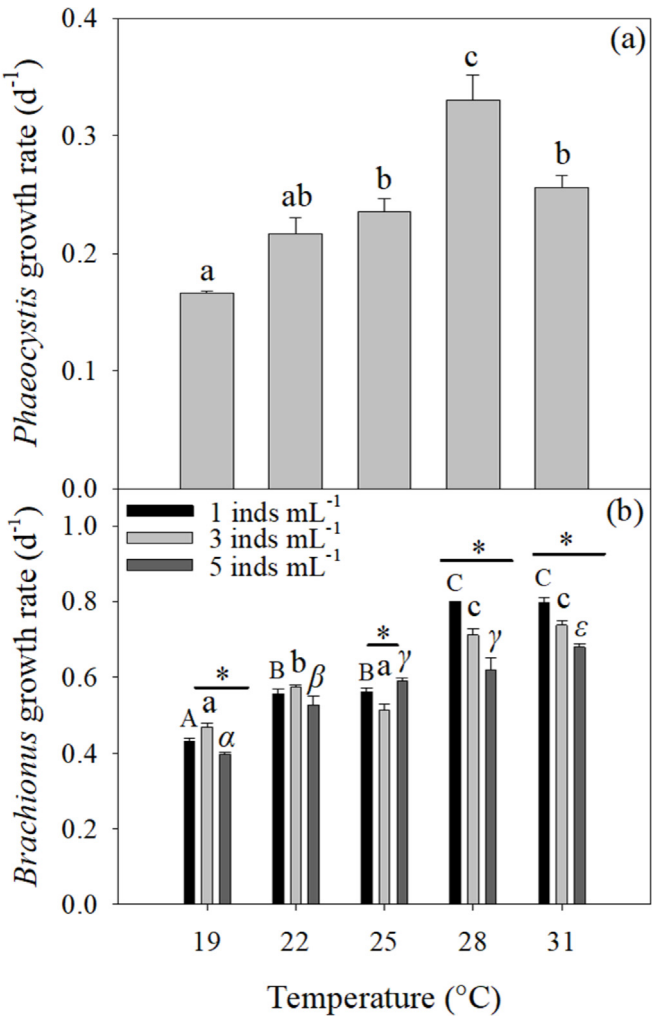

Fig. 1. Growth rate of *P. globosa* (a) and *B. plicatilis* (b). Significant differences are indicated by different letters in the growth rates ($P < 0.05$).