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Impact of several harmful algal bloom (HAB) causing species, on life history characteristics of rotifer *Brachionus plicatilis* Müller*

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Abstract In recent years, harmful algal blooms (HABs) have occurred frequently along the coast of China, and have been exhibiting succession from diatom- to dinoflagellate-dominated blooms. To examine the effects of different diatom and dinoflagellate HABs, the life history parameters of rotifers (*Brachionus plicatilis* Müller) were measured after exposure to different concentrations of HAB species. The HAB species examined included a diatom (*Skeletonema costatum*) and four dinoflagellates (*Prorocentrum donghaiense*, *Alexandrium catenella*, *Prorocentrum lima* and *Karlodinium veneficum*). Compared with the control treatment (CT), the diatom *S. costatum* showed no adverse impacts on rotifers. Exposure to dinoflagellates at densities equivalent to those measured in the field resulted in a reduction in all the life history parameters measured. This included a reduction in: lifetime egg production (CT: 20.34 eggs/ind.) reduced to 10.11, 3.22, 4.17, 7.16 eggs/ind., life span (CT: 394.53 h) reduced to 261.11, 162.90, 203.67, 196 h, net reproductive rate (CT: 19.51/ind.) reduced to 3.01, 1.26, 3.53, 5.96/ind., finite rate of increase (CT: 1.47/d) reduced to 1.16, 1.03, 1.33, 1.38/d, and intrinsic rate of population increase (CT: 0.39/d) reduced to 0.15, 0.03, 0.28, 0.32/d, for the dinoflagellates *P. donghaiense*, *A. catenella*, *P. lima* and *K. veneficum*, respectively. The results showed that the diatom *S. costatum* had no detrimental consequences on the reproduction and growth of *B. plicatilis*, however, the four dinoflagellates tested did show adverse effects. This suggests that dinoflagellate HABs may suppress microzooplankton, resulting in an increase in algal numbers.

Keyword: harmful algal bloom (HAB); dinoflagellates; *Brachionus plicatilis*; reproduction; population dynamics

1 INTRODUCTION

In recent years, large-scale harmful algal blooms (HABs) have occurred frequently along the coast of China. These HABs have affected large areas (>1 000–10 000 km^2) for long periods of time (>20 d), in the areas adjacent to the Changjiang River estuary in the East China Sea almost every spring since the 1990s (Zhou et al., 2003; Lu et al., 2005). Marine HABs have been gradually exhibiting succession from diatom- to dinoflagellate-dominated blooms in these areas. During the 1980s and 1990s, the dominant species was the diatom *Skeletonema costatum* sensu lato, which reached a density of 10^5 cells/mL, accounted for 95%–99% of the total biomass of phytoplankton (Zhou et al., 2008), and supported a high production fishery in the adjacent coastal waters. By the early 2000s, the dominant HAB species were dinoflagellates, including *Prorocentrum donghaiense*

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Lu, *Karenia mikimotoi* (Miyake et Kominami ex Oda) G. Hansen et Ø. Moestrup, and *Alexandrium catenella* (Whedon et Kofoid) Balech (Zhou, 2010). Zhou et al. (2003) reported that maximum densities of the dinoflagellates *P. donghaiense* and *A. catenella* reached 10^5 and 10^2 cells/mL, respectively. Other toxic species such as *Prorocentrum lima* (Ehrenberg) Dodge, and *Karlodinium veneficum* (D. Ballantine) J. Larsen have also been found (Wang et al., 2011; Zhou et al., 2011). Therefore, it is important to assess and compare the toxicities of different kinds of HABs.

Marine HAB species have been reported to adversely affect components of the marine food web, such as zooplankton, shellfish, fish, marine mammals and benthic crabs (Landsberg, 2002). Zooplankton species, which are responsible for the exchange of materials and energy in marine ecosystems, show undesirable responses when exposed to harmful algae. Reduced survival and feeding rates, inhibition of growth and reproduction, changes in behavior, and abnormalities in embryonic and larval development have been reported (Huntley et al., 1987; Hansen, 1989; Poulet et al., 1995; Yan et al., 2009; Ianora and Miralto, 2010).

The rotifer *Brachionus plicatilis* Müller, which is a polyphagous microplanktonic filter feeder characterized by rapid growth, a high reproductive rate and short generation time, has been used extensively in aquaculture and ecotoxicology (Snell et al., 1983; Snell and Janssen, 1995; Kostopoulou et al., 2012). Previous studies demonstrate that the rotifer *B. plicatilis* displays inhibited swimming activity, low ingestion rates and reduced individual survival when exposed to many dinoflagellate species including: *K. mikimotoi*, most species of *Alexandrium*, *Heterocapsa circularisquama*, *Heterosigma akashiwo*, *P. donghaiense* and *Prorocentrum micans* (Kim et al., 2000; Wang et al., 2005; Xie et al., 2008; Yan et al., 2009; Zou et al., 2010; Zhang and Geng, 2012). Nevertheless, relatively little is known about the effects of diatom and dinoflagellate blooms on rotifers.

A life table can be used to derive variables such as the duration of development, average lifespan (ML), gross and net reproductive rates (R_0), generation time (T) and population growth rates (Krebs, 1985). These life history variables may be sensitive to heavy metals, pesticides and other environmental contaminants (Marcial and Hagiwara, 2007; Zha et al., 2007; Zhao et al., 2008; Huang et al., 2012). In this study, the effects of different harmful diatom and dinoflagellate blooms on the life history characteristics of rotifers were examined.

We evaluated the effects of five different HAB species, including a diatom strain (*S. costatum*) and four dinoflagellate strains (*P. donghaiense* (non-toxin producer), *A. catenella* (paralytic shellfish poison (PSP) producer), *P. lima* (diarrhetic shellfish poison (DSP) producer), and *K. veneficum* (karlotoxin producer)) on the life history parameters of the rotifer, *B. plicatilis*. The objectives of this study were twofold: 1) to test and compare the toxicities of different diatom and dinoflagellate HABs on the life history characteristics of the rotifer *B. plicatilis* and 2) to discuss potential causes of adverse effects of these HAB species on *B. plicatilis*. The results will help to identify potential threats to the marine ecosystem posed by diatom and dinoflagellate HABs.

2 MATERIAL AND METHOD

2.1 Strains and culture conditions

The diatom *Skeletonema costatum* was isolated from Jiaozhou Bay in the Yellow Sea of China. The dinoflagellates *P. donghaiense*, *A. catenella* and *K. veneficum* were isolated from the East China Sea and provided by two National Basic Research Priority Programs of China (CEOHAB-I, II). *Prorocentrum lima* (strain CCMP1966) was obtained from the Provasoli-Guillard National Center for Marine Algae and Microbiota (NCMA, formerly the CCMP) (East Boothbay, ME, USA). *Chlorella* sp. served as the control algae and was added to the mixed diet used in every treatment. *Chlorella* sp. was provided by the Algal Culture Center of the Institute of Oceanology, Chinese Academy of Sciences (IOCAS). All algae, except for *S. costatum*, were cultured in modified f/2 medium in flasks without added silicate. All algae were cultured at 20±1°C with irradiance at 56 μE/(m^2·s) and a 12 h light:12 h dark photoperiod. The rotifer *B. plicatilis* was supplied by IOCAS and cultured under the same conditions as the algae throughout the year.

The natural seawater used in this study was pumped from Taipingjiao (a clean site with no known pollution history) at Qingdao and sand filtered prior to use in the laboratory. Prior to the experiments, the seawater was filtered through a 0.45 μm pore size cellulose nitrate membrane, and treated by boiling and air saturation. Salinity was adjusted to 31±1 using distilled water as determined using an ATAGO hand-held refractometer. The pH of seawater was measured using a HI991000 pH instrument.

Table 1 List of algal species used in the experiment

Algal species	Algal cell size (μm)	Carbon Content (pg C/cell)	Density (×10³ cells/mL)			
Chlorella sp.	3.55±0.31	5.75±1.20	2000.0			
S. costatum	Length: 29.65±1.84; Width: 5.05±0.57	43.71±0.37	10.0	50.0	100.0	150.0
P. donghaiense	Length: 17.58±1.21; Width: 12.07±0.59	226.50±6.94	10.0	50.0	100.0	150.0
A. catenella	Length: 24.58±3.06; Width: 26.18±2.58	2341.85±34.52	0.1	0.2	0.4	0.6
P. lima	Length: 46.91±0.78; Width: 32.94±1.40	1863.0±91.59	0.5	1.0	5.0	10.0
K. veneficum	Length: 12.56±0.29; Width: 9.36±0.63	140.0 (Li et al., 2000)	1.0	5.0	10.0	50.0

Chlorella sp. (density of 2×10^6 cells/mL) was added to each diatom and dinoflagellate treatments. Data represent means±SE.

2.2 Experimental design

2.2.1 Method

The density gradients for each algal species in the experiment were based on the bloom density measured in the field (*S. costatum* and *P. donghaiense* reached 10^5 cells/mL (Zhou et al., 2003, 2008); *K. veneficum* reached more than 10^3 cells/mL (Wang et al., 2011); and *A. catenella* and *P. lima* reached more than 10^2 cells/mL (Wang et al., 1998; Zhou et al., 2003)). Algal cells of the five HAB species were harvested at the exponential phase. A 1-mL subsample was taken and algal cells were counted under the microscope after fixation in Lugol's solution. Based on this cell count, the algae were diluted to the densities shown in Table 1. *Chlorella* sp. (density of 2.0×10^6 cells/mL) was added into each diatom and dinoflagellate treatment to produce a mixed diet. We also measured the carbon content of the used algae in lab, except for *K. veneficum* (Table 1). The densities of HAB species used in the experiment were based on the densities of algal blooms recorded in the field, therefore, the carbon levels of each species differed (*P. donghaiense* > *P. lima* > *K. veneficum* > *S. costatum* > *A. catenella*).

Brachionus plicatilis neonates were hatched from gravid females for use in the experiment. Hatching took place in 96-well tissue culture plates, with one gravid rotifer in each well. Each well contained 100 μL of fresh seawater and no food. Healthy and active neonates (<2 h old) were collected and placed in 24-well tissue culture plates, with one neonate per well. Each well contained 1.0 mL of test solution, which consisted of a mixed diet (one of the HAB species and *Chlorella* sp. at a density of 2.0×10^6 cells/mL). The control solution contained only *Chlorella* sp. (2.0×10^6 cells/mL). Every treatment consisted of three replicates, with thirty neonates in total. The hatching and culture of neonates was conducted at 22±1°C with irradiance at 56 μE/(m^2·s) and a 12 h light:12 h dark photoperiod.

The rotifers in the 24-well tissue culture plates were checked every 4–6 h, and the following values were recorded: time at which the first egg and neonate were produced, time at which the last egg was produced, the number of eggs and neonates produced, and the number of original test individuals that remained alive. Neonates were removed every 8 h. The original rotifers that were still alive were transferred into a freshly prepared test solution every 24 h. The life table experiments were performed in darkness at 22±1°C, salinity 31±1, and pH 8.0±0.2, until each individual of every cohort died. Salinity was checked every 24 h and kept at 31±1 by adding appropriate amounts of diluted water.

2.2.2 Calculation of life history parameters

Using the data recorded in this experiment, the duration of the principal developmental periods (embryonic development (ED), juvenile period (JP), reproductive period (RP), and post-reproductive period (PP)), mean life span (ML, ML=the sum of JP, RP and PP), and lifetime egg production (NE) of the rotifers were calculated. Age-specific survivorship (l_x, l_x=proportion of survivorship per day) and fecundity (m_x, m_x=proportion of offspring produced per female per day) were determined for each replicate using conventional life table techniques (Poole, 1974). Net reproductive rate (R_0, $R_0=\Sigma l_x\ m_x$), generation time (T, $T=\Sigma l_x\ m_x\ x/R_0$), intrinsic rate of population increase (R_m, $R_m=\ln R_0/T$), and finite rate of increase (λ, λ=anti $\log_e R_m$) were calculated (Birch, 1948; Krebs, 1985; Sarma et al., 2001).

2.3 Analysis

Data were analyzed using Excel 2003, Origin 8.5 and SPSS 16.0 software packages. As the concentrations of algae used in the experiment differed among species, the data could not be analyzed

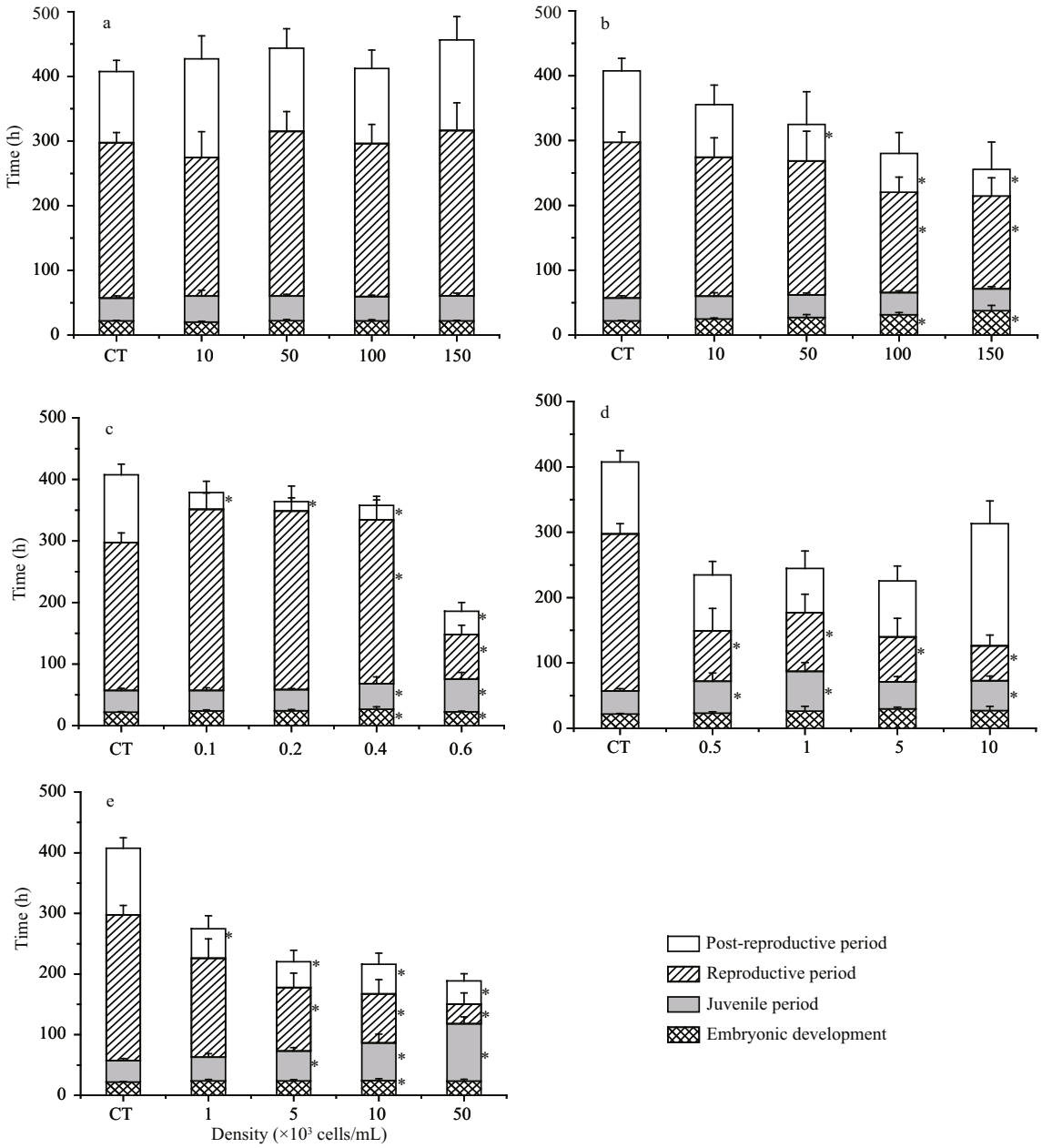

Fig.1 Durations of embryonic development (ED), juvenile period (JP), reproductive period (RP), and post-reproductive period (PP) of *Brachionus plicatilis* exposed to different algal densities

CT: *Chlorella* sp. at 1 000×10³ cells/mL; a. *Skeletonema costatum*; b. *Prorocentrum donghaiense*; c. *Alexandrium catenella*; d. *Prorocentrum lima*; e.*Karlodinium veneficum*). Data points represent means±SE; *: significance (P<0.05) compared to the control.

using a two-way analysis of variance (ANOVA) with algal species and algal density as factors. Therefore, one-way ANOVA was applied for statistical evaluations. Prior to statistical analysis, all data were tested for normality and homogeneity (SPSS 16.0). Mean differences were considered to be significant at the 0.05 level. If the overall ANOVA results were significant, a Fisher's least significance difference (LSD) post-hoc test was performed to test among experimental combinations.

3 RESULT

3.1 Duration of developmental periods, mean life span, and lifetime egg production

The duration of the principal developmental periods (embryonic development (ED), juvenile period (JP), reproductive period (RP), and post-reproductive period (PP)) of *B. plicatilis* exposed to different algal densities are shown in Fig.1. Most algal species including *S. costatum*, *A. catenella*,

P. lima, and *K. veneficum* had no marked impact on the duration of ED. However, exposure to *P. donghaiense* (>1×10^5 cells/mL) significantly prolonged the duration of ED ($P<0.05$), which increased with increasing algal cell density.

Rotifers did not exhibit a significantly prolonged JP ($P>0.05$) when exposed to *S. costatum* and *P. donghaiense* at densities ranging from 10^4 to 1.5×10^5 cells/mL. However when exposed to *A. catenella* (>400 cells/mL), *P. lima* (>500 cells/mL), and *K. veneficum* (>5×10^3 cells/mL), the duration of the juvenile period was significantly prolonged ($P<0.05$).

Skeletonema costatum had no significant impact on the duration of the reproductive period (RP) of rotifers ($P>0.05$). Compared with the control group, an increased RP was observed in rotifers exposed to *A. catenella* at low densities (100, 200 and 400 cells/mL). Exposure to *P. donghaiense* (>1×10^5 cells/mL), *A. catenella* (only at a density of 600 cells/mL), *P. lima*, and *K. veneficum* significantly shortened the RP of rotifers ($P<0.05$). When cultured with *P. donghaiense* and *K. veneficum*, the reproductive period of rotifers decreased as algal density increased. For every dinoflagellate species tested, harmful effects on rotifers occurred at cell densities equivalent to those found in the field. The duration of the reproductive period (*Chlorella* sp. (control): 240.08 h) sharply declined to 72.20 h when rotifers were exposed to *A. catenella* at a density of 10^2 cells/mL and to 77.12 h when exposed to *P. lima* at the same concentration. Reproductive period also declined to 104.42 h when rotifers were exposed to a density of 10^3 cells/mL of *K. veneficum*, and to 154.78 h at a density of 10^5 cells/mL of *P. donghaiense*.

Skeletonema costatum had no significant impact on the duration of the post-reproductive period (PP) of rotifers ($P>0.05$). An increased PP was observed in rotifers exposed to *P. lima* at densities of 10^4 cells/mL. The duration of the post-reproductive period in rotifers showed a marked decrease when cultured with *P. donghaiense* (>1×10^5 cells/mL), *A. catenella*, *P. lima* (except at a the density of 10^4 cells/mL) and *K. veneficum* ($P<0.05$).

The mean life span (ML) of rotifers was calculated based on the values for JP, RP and PP (Fig.2). The diatom *Skeletonema costatum* had no significant impact on the mean life span of rotifers ($P>0.05$). Exposure to *P. donghaiense*, *A. catenella* (except at a density of 100 cells/mL), *P. lima* and *K. veneficum* significantly shortened the ML of rotifers. The mean life span of rotifers exposed to *P. donghaiense* and *K. veneficum* declined as algal densities increased ($P<0.05$). However, the decline in the mean life span of rotifers exposed to *A. catenella* and *P. lima*, was not dependent on algal concentration. The duration of mean life span (*Chlorella* sp. (control): 394.53 h) significantly declined to 162.90 h in rotifers exposed to a density of 10^2 cells/mL of *A. catenella*, and to 203.67 h for rotifers exposed to a density of 10^2 cells/mL of *P. lima*. Mean life span declined to 196.00 h at a density of 10^3 cells/mL of *K. veneficum* and to 261.11 h at a density of 10^5 cells/mL of *P. donghaiense*.

The number of eggs produced over a lifetime (NE) for *B. plicatilis* exposed to different algal densities is shown in Fig.3. There was no significant impact of the diatom *Skeletonema costatum* on the NE of rotifers ($P>0.05$). A marked decrease in NE was observed in rotifers exposed to the dinoflagellates *P. donghaiense*, *A. catenella*, *P. lima* and *K. veneficum* ($P<0.05$). The NE of rotifers exposed to *A. catenella*, *P. lima* and *K. veneficum* declined as algal densities increased. When cultured with *P. donghaiense*, the decline in NE of rotifers was not dependent on the density of algae.

3.2 Effects of HAB-causing species on the population growth of *B. plicatilis*

Based on the age-specific survival and fertility of the rotifers exposed to several algal species that cause HABs, we calculated the net reproductive rate (R_0), generation time (T), intrinsic rate of population increase (R_m), and finite rate of increase (λ) for rotifers (Fig.4). The diatom *Skeletonema costatum* had no significant impact on R_0 ($P>0.05$). When exposed to the dinoflagellate treatments, rotifers showed significantly shortened R_0 values. When rotifers were cultured with *P. donghaiense*, *A. catenella* or *K. veneficum*, R_0 decreased as algal densities increased.

Rotifers did not exhibit a significantly shortened T ($P>0.05$) when exposed to *S. costatum*, *P. donghaiense* (except at a density of 1.5×10^5 cells/mL) and *A. catenella* (except at a density of 600 cells/mL). Exposure to *P. lima* and *K. veneficum* significantly shortened the T of rotifers ($P<0.05$).

There were no adverse effects of *S. costatum* on the R_m of rotifers at any of the concentrations tested ($P>0.05$). The intrinsic rate of population increase (R_m) significantly decreased in rotifers exposed to the dinoflagellate treatments (except for *P. lima* at densities of 0.5 and 1.0×10^3 cells/mL and *K. veneficum* at a density of 1.0×10^3 cells/mL). The intrinsic rate of population increase (R_m) gradually decreased with

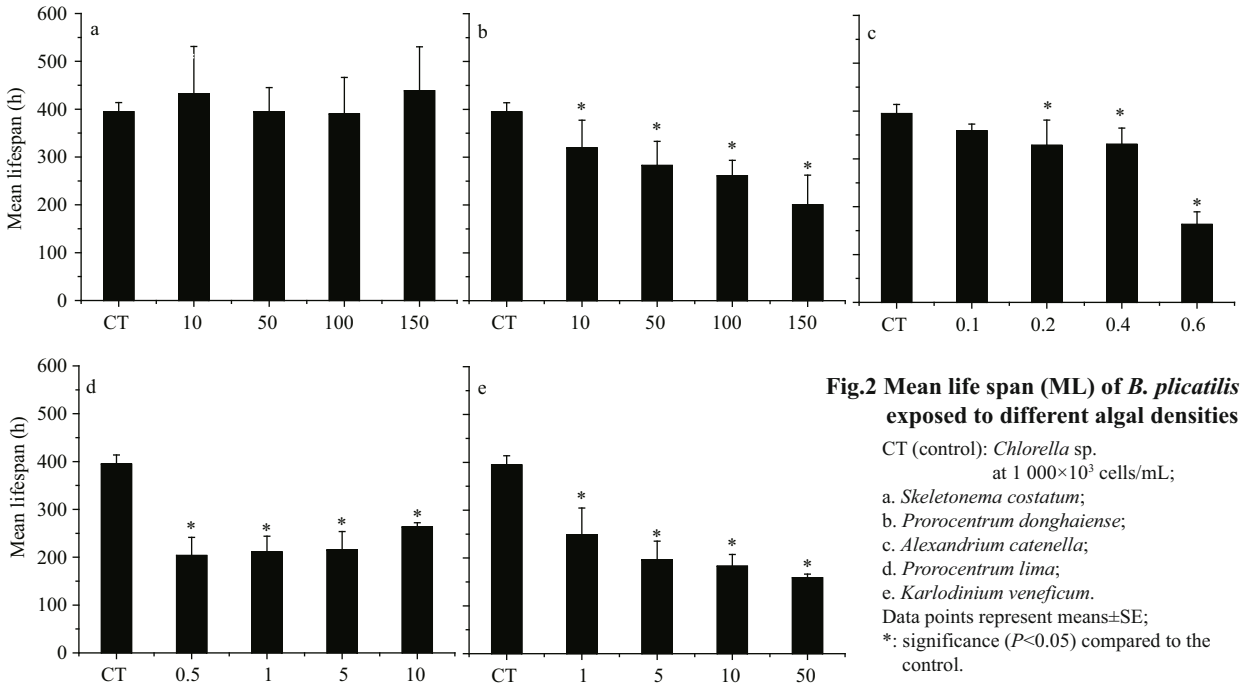

Fig.2 Mean life span (ML) of *B. plicatilis* exposed to different algal densities

CT (control): *Chlorella* sp. at 1 000×10^3 cells/mL;
a. *Skeletonema costatum*;
b. *Prorocentrum donghaiense*;
c. *Alexandrium catenella*;
d. *Prorocentrum lima*;
e. *Karlodinium veneficum*.
Data points represent means±SE;
*: significance (P<0.05) compared to the control.

exposure to increasing concentrations of *P. donghaiense* and *A. catenella*.

Skeletonema costatum had no adverse effects on the λ of rotifers. The dinoflagellate treatments (except for *P. lima* and *K. veneficum* at 1.0×10^3 cells/mL) significantly decreased the λ of rotifers as algal densities increased.

4 DISCUSSION

4.1 The impacts of different HAB species on rotifers

The densities of HAB species used in the experiment were based on the densities of algal blooms recorded in the field, therefore, the carbon levels of each species differed (*P. donghaiense* > *P. lima* > *K. veneficum* > *S. costatum* > *A. catenella*). Rotifers did not exhibit any adverse responses to the diatom *S. costatum*, even at the highest carbon content used in the experiment (6.45 μg/mL, equal to 10^4 to 1.5×10^5 cells/mL). Similarly, *S. costatum* did not have any adverse effects on the copepod *Calanus sinicus*, during its feeding and reproduction (Jing-Jing Song, unpublished). However, the effects of diatoms on zooplankton may be species-specific. Several researchers have reported adverse effects of the diatoms (e.g. *Thalassiosira rotula*) on copepods (e.g. *Temora stylifera*, *Calanus helgolandicus* and *Pseudocalanus newmani*), including reduced hatching success, abnormal development and high larval mortality rates (Poulet et al., 1995; Carotenuto et al., 2002; Ianora et al., 2004; Halsband-Lenk et al., 2005; Ianora and Miralto, 2010). It has been widely reported that toxic compounds (including short chain polyunsaturated aldehydes, some oxylipins) and nutritional quality may be the major cause of adverse effects of diatoms on zooplankton (Jónasdóttir, 1994; Miralto et al. 1999; Ianora et al., 2003, 2004; Fontana et al., 2007; Jónasdóttir et al., 2009; Amin, 2011; Barreiro et al., 2011).

Rotifers in the experiment showed a decline in all of the life history parameters measured (ML, NE, R_0, R_m and λ) when exposed to the dinoflagellates *A. catenella*, *P. lima*, *K. veneficum* and *P. donghaiense*, at carbon levels of <6.45 μg/mL. These dinoflagellates also have detrimental effects on copepods and some early life stages of bivalves, and may cause decreased egg production rates and hatching success, reduced ingestion rates, lower nauplii production and increased deformities (Frangópulos et al., 2000; da Costa et al., 2005; Dam and Colin, 2005; Glibert et al., 2007; Stoecker et al., 2008). Exposure to the dinoflagellates *A. catenella*, *P. lima* and *K. veneficum* resulted in a reduced duration of rotifer development (JP, RP and PP), even at a very low carbon content (i.e. *A. catenella* at 0.92 μg/mL carbon, equal to 400 cells/mL, *P. lima* at 0.93 μg/mL carbon, equal to 500 cells/mL and *K. veneficum* at 0.7 μg/mL carbon, equal to 5 000 cells/mL). As the carbon content of the dinoflagellate cells causing these effects were low, it was not possible to distinguish if any of these strains had more impact on the rotifers, compared with others. A reduction in the

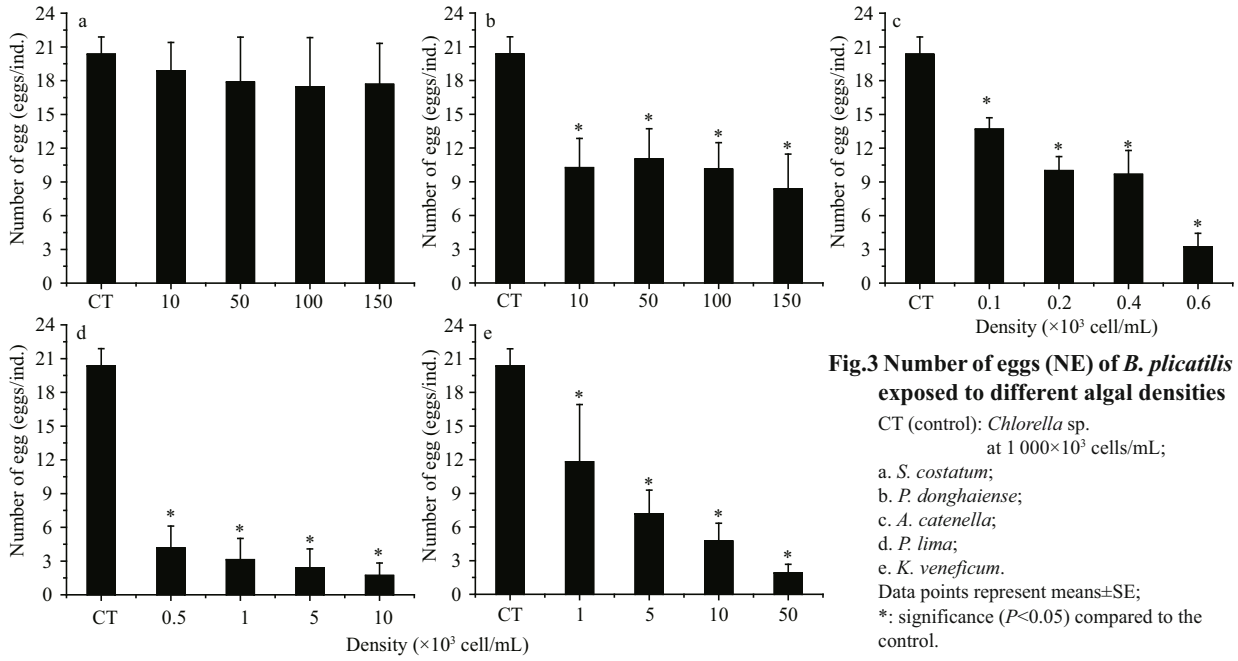

Fig.3 Number of eggs (NE) of *B. plicatilis* exposed to different algal densities

CT (control): *Chlorella* sp. at 1 000×10³ cells/mL;
a. *S. costatum*;
b. *P. donghaiense*;
c. *A. catenella*;
d. *P. lima*;
e. *K. veneficum*.
Data points represent means±SE;
*: significance (P<0.05) compared to the control.

duration of development only occurred in rotifers exposed to a carbon content >22.65 μg/mL (or 10^5 cells/mL) of *P. donghaiense*, suggesting that *P. donghaiense* had the weakest effects on *B. plicatilis* among the dinoflagellates tested. Similarly, other *Prorocentrum* species, especially *P. minimum*, also showed weaker effects on the oyster *Crassostrea ariakenisis*, compared with *K. veneficum* (Glibert et al., 2007).

4.2 The possible causes of adverse effects of dinoflagellates on rotifers

Previous studies have indicated that the adverse effects of several HAB species on zooplankton may be associated with algal toxicity (Ives, 1987; Hansen, 1989; Poulet et al., 1995; da Costa et al., 2005; Wu et al., 2006), inadequate algal body size and poor nutritional quality (Dam and Colin, 2005; Zheng et al., 2011) or mechanical damage (Wang et al., 2006).

Rotifers prefer to consume algal cells with a diameter less than 10 μm, and seldom consume algae with a diameter of >22–30 μm (Turner et al., 1998). For *B. plicatilis*, the optimal prey diameter was approximately 8 μm, and the upper limit for retention was a diameter 20–25 μm (Hansen et al., 1997). The dinoflagellates *A. catenella* and *P. lima* are not in the optimal size range for rotifer feeding. Some algal species have poor nutritional quality. The dinoflagellate *P. donghaiense* has low amounts of phenylalanine, histidine and lysine (Chen et al., 2007), and may be a poor food source for rotifers. Researchers in our lab found that *B. plicatilis* seldom ingest these dinoflagellate strains (Wang, 2004; unpublished data). New-born rotifers almost died of starvation within one week when fed a diet that consisted of only a single dinoflagellate species (unpublished data). The algae *Chlorella* sp. was added to every algal treatment at the same density as that used in the control. This mixed diet ruled out the possibility that large cell diameter or insufficient nutrients in the dinoflagellate HAB species led to low population growth of *B. plicatilis*. This suggests that algal toxicity or mechanical damage may be the potential causes of harmful effects on the rotifers.

4.2.1 Algal toxicity of *Karlodinium veneficum* and *Alexandrium catenella*

The strain of *K. veneficum* used in our experiment, produces a hemolytic toxin (Qing-Chun Zhang, unpublished). A similar strain also isolated from the East China Sea has shown hemolytic activity, which is associated with karlotoxins (Zhou et al., 2011). The toxin has been associated with massive fish kills and has delayed or restricted larval development of marine invertebrates (Deeds et al., 2002; Kempton et al., 2002; Brownlee et al., 2008; Zhou et al., 2011). In this research, the life stage characteristics of rotifers were strongly reduced after exposure to the dinoflagellates. *Karlodinium veneficum* can produce toxic substances that are released when cells are disturbed or damaged (Deeds et al., 2002). Therefore, direct contact between rotifers and these algal cells may facilitate the release

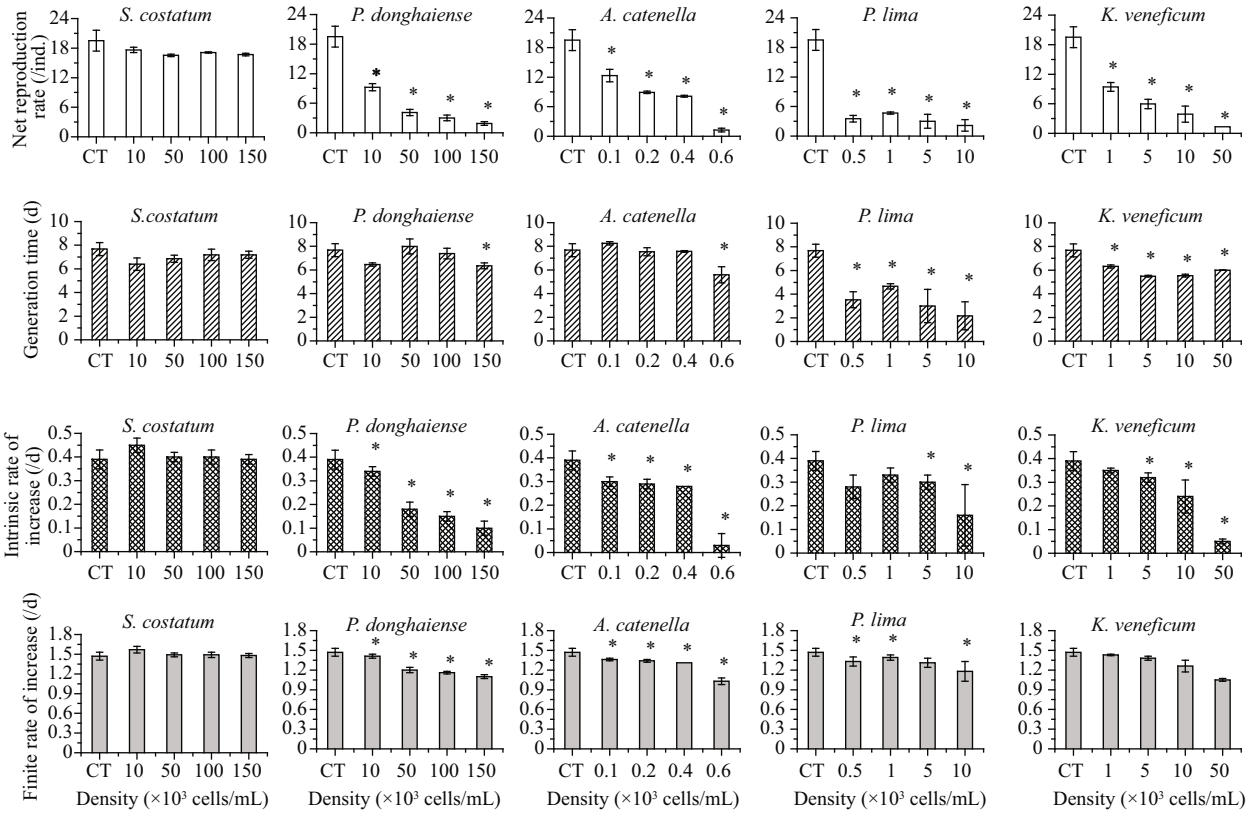

Fig.4 Parameters of population growth for the rotifer *B. plicatilis* at several algal densities

Data points represent means±SE, *: significance (P<0.05) compared to the control (CT).

of toxin. It is possible that sublethal effects of the toxin inhibited some facets of rotifer reproductive behavior.

The strain of *A. catenella* used in the experiment produces paralytic shellfish poison (PSP) (Mons et al., 1998). The known PSP toxins are mainly stored inside the cells (Ma et al., 2009). By measuring changes in chlorophyll a content in the gut of rotifers, it was shown that rotifers seldom ingest this strain of *A. catenella* (Wang, 2004). Therefore, given that the PSP was not ingested by rotifers, the adverse effects observed may not be caused by the PSP toxin. Similarly, Wang et al. (2005) reported that the effects of *Alexandrium* sp. on rotifers were not related to PSP toxins. In addition to producing PSP toxin, *Alexandrium* sp. produces unknown non-PSP toxicants, such as polysaccharide-based compounds (Yamasaki et al., 2008) and amphipathic compounds (Ma et al., 2009, 2011), which are harmful to marine organisms through hemolytic or other lytic activities (Simonsen et al., 1995; Bagøien et al., 1996; Lush et al., 2001; Gribble et al., 2005). Seven strains of *Alexandrium* sp. studied in our lab, including *A. catenella*, have been shown to produce hemolytic compounds other than PSP toxins (Tan et al., 2008). Although *Chlorella* sp. (carbon content of 11.4 μg/mL) was added to each treatment in this study, rotifers exposed to *A. catenella* had a prolonged juvenile period (JP). Based on the close relationship between the JP and food quality (King, 1967; Pilarska, 1977), we hypothesized that unknown toxic substances produced by *A. catenella* might affect the ingestion of *Chlorella* sp. by *B. plicatilis*. Chen et al. (2007) also demonstrated that substances produced by *Alexandrium* sp. may inhibit the ingestion of other algal cells by the brine shrimp, *Artemia salina*. Further research on the ingestion of mixed diets by rotifers needs to be carried out to test this hypothesis. The unknown toxic substances may also have other effects on rotifers, such as a hemolytic or cytolytic response.

4.2.2 Mechanical damage caused by *Prorocentrum lima* and *Prorocentrum donghaiense*

The cells of *P. lima* appeared to be clustered and held together by mucous-like substances that seemed viscous and gummy (Fig.5a). Light microscopy revealed that many *P. lima* cells adhered to the surface of rotifers (Fig.5b), and inhibited them from normal swimming and movement. This phenomenon was

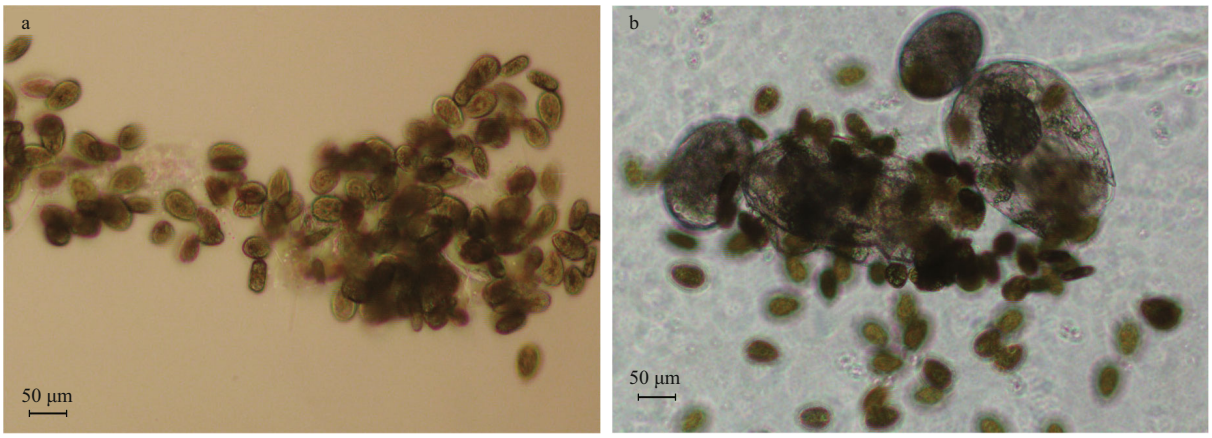

Fig.5 Adhesion of *Prorocentrum lima* cells to the surface of rotifer *Brachionus plicatilis*

a. many *P. lima* cells in clustered arrangements; b. rotifers adhered by *P. lima* through mucous-like substances after exposure to *P. lima* at 1.0×10^4 cells/mL.

also documented for the dinoflagellates *K. mikimotoi* and *H. akashiwo*, which exerted mechanical damage on zooplankton or juvenile sea bass by producing mucous-like substances (Wang et al., 2006; Ajuzie, 2008; Zou et al., 2010). Interestingly, *B. plicatilis* tended to escape from the adhesion, expending energy to disentangle themselves from the mucus. The prolonged JP and decreased fecundity observed in the experiment may be associated with a reduced amount of energy available. This finding is supported by studies which showed that the life history characteristics of rotifers is effected by a low food intake or low levels of energy (King et al., 1967; Halbach et al., 1974; Pilarska et al., 1977; Schmid-Araya et al., 1991; Galindo et al., 1993; Xi et al., 2001).

Most planktonic rotifers (e.g. the genus *Brachionus*, *Keratella* and *Anuraeopsis*), carry eggs attached to the posterior end of the body (Gilbert, 1983), although some rotifer taxa (e.g. *Epiphanes brachionus*, *Notholca*, Trichocercidae) do not (Ruttner-Kolisko, 1974; Pontin, 1978). When rotifers were exposed to high densities of *P. donghaiense* in our experiment, most eggs of *B. plicatilis* were easily detached. We suspect that this may be related to the presence of mucus. Wang et al. (2003) suggested that rotifers showed inhibited swimming activity due to mucus present on the cell surface of *P. donghaiense*. It has been reported that loose eggs have a high hatching success and could constitute up to 10% of the total production of rotifers (Sarma, 1987). However, the dropped eggs in the *P. donghaiense* treatment had little chance to develop into neonates, which may result in low population reproduction rates. Determining if this is related to the mucus produced by *P. donghaiense* requires further investigation.

4.3 The potential threats of dinoflagellate HABs to the marine ecosystem in China

In recent years, HABs in China have been gradually exhibiting succession from diatom-dominant to dinoflagellate-dominant, and harmful dinoflagellates have become the dominant species in HABs (Zhou and Yu, 2007). In our study the diatom *S. costatum* did not affect any of the life history parameters measured in the rotifer *B. plicatilis*, whereas the dinoflagellates *P. donghaiense*, *A. catenella*, *P. lima* and *K. veneficum* all had detrimental effects. Although rotifers are not a key species along the coast of China, they are representative of other micro-zooplankton of the same size and with similar characteristics. Micro-zooplankton, as the main food source for other organisms, play an important role in material transfer and energy flow in the marine ecosystem. If reproduction in these organisms is threatened, fisheries productivity and ecosystem balance also become endangered. Thus, the continued prevalence of dinoflagellate HABs could pose risks to the structure and function of the marine ecosystem. Additionally, zooplankton plays a key role in the occurrence of HABs and in the promotion or inhibition of HAB development. In this study, the life history characteristics of the rotifer *B. plicatilis* were significantly suppressed when exposed to dinoflagellates, which may promote dinoflagellate bloom formation.

5 CONCLUSION

The diatom *S. costatum* had no adverse impacts on

the life history parameters of the rotifer *B. plicatilis*. The dinoflagellates *P. donghaiense*, *A. catenella*, *P. lima* and *K. veneficum* all had adverse effects on the reproduction and growth of *B. plicatilis*. The continued prevalence of dinoflagellate-dominated HABs along the coast of China, could pose risks to the structure and function of the marine ecosystem.

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