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In situ detrimental impacts of *Prorocentrum donghaiense* blooms on zooplankton in the East China Sea

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ABSTRACT

Large-scale algal blooms of the dinoflagellate *Prorocentrum donghaiense* have occurred frequently in the East China Sea (ECS) in recent decades. However, its impacts on the zooplankton *in situ* are still under not well understood. During a spring *P. donghaiense* bloom (April–May 2013) along the northern coast of Fujian Province (120°–121°30″E, 26°30″–28°N), we found that the bloom decreased the abundance of copepods and had no significant effect on chaetognaths and small jellyfish. However, the abundance of small jellyfish increased over the course of the study. The zooplankton community changed from being copepod and small jellyfish- to small jellyfish-dominated during the bloom. In the bloom areas, the copepod *Calanus sinicus* showed higher mortality and lower egg production rates (EPR) than those in the non-bloom areas. The results suggested that *P. donghaiense* blooms had detrimental effects on the structure of zooplankton community and the recruitments of *C. sinicus*.

1. Introduction

In the recent years, large-scale harmful algal blooms (HABs) have occurred frequently in the coastal areas of China. Zooplankton species are responsible for the exchange of materials and energy in marine ecosystems. When exposed to harmful algae, zooplankton exhibit undesirable responses, including decreased survival and feeding rates, inhibition of growth and reproduction, changes in behavior, and abnormalities in larval development (Huntley et al., 1987; Hansen, 1989; Poulet et al., 1995; Yan et al., 2009; Ianora and Miralto, 2010).

The dinoflagellate *Prorocentrum donghaiense* is the dominant species in spring algal blooms in the East China Sea (ECS). *P. donghaiense* blooms have affected large areas (>1000–10,000 km^2) for long periods of time (>30 d) in the ECS almost every spring since the 1990s (Zhou et al., 2003; Lu et al., 2005). These blooms are massive, with high biomass of 10^7 cell L^{-1} and dominance of 90% in the phytoplankton community (Lu et al., 2005). The affected areas covered the largest estuary in China (Changjiang River estuary), the well-known upwelling systems (i.e. Zhoushan fishing ground), and systems strongly influenced by eutrophication. Such typical and complex environments made the large-scale bloom more representative and worth researching.

P. donghaiense is a non-toxic dinoflagellate and does not release known phytotoxins (Glibert et al., 2012). However, in laboratory studies, some zooplankton species exhibited significantly decreased viability or fecundity when fed with high concentrations of *P. donghaiense* (10^8 cells L^{-1}). For example, the brine shrimp *Artemia salina* and the cladoceran *Moina mongolica* showed a decline in survival rates (Chen et al., 2007a,b), and the rotifer *Brachionus plicatilis* displayed inhibited swimming activity, decreased egg production rates and population growth (Wang et al., 2003; Yan et al., 2009). Several laboratory studies demonstrated that exposure to *P. donghaiense* caused significant inhibition of egg production, and hatching success of the copepod *C. sinicus*, which might be related to the low contents of some essential fatty acid in *P. donghaiense* (Jing–Jing Song, not published). However, these views are based solely on laboratory experiments, and relatively little is known about *in situ* effects of *P. donghaiense* blooms on zooplankton. Although studies showed that *C. sinicus* exhibited low survival rates in bloom areas compared to non-bloom areas during the spring bloom in 2005, the bloom was co-dominated by the toxic dinoflagellate *Karenia mikimotoi* and the non-toxic *P. donghaiense*. Therefore, field evidence of the impact of *P. donghaiense* blooms on zooplanktons is still scarce.

In spring, coastal areas in the ECS are important spawning grounds for many commercially important fish species, including large head hairtail *Trichiurus japonicus*, small yellow croaker *Larimichthys polyactis*, and chub mackerel *Scomber japonicas* (Hu, 2004). Copepods, jellyfish, and chaetognaths are the main

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zooplankton groups in these coastal areas. Copepods are the main food source of larvae of many fish, such as anchovy and mackerel (Uye et al., 1999; Meng, 2000). In contrast, jellyfish adversely affect fishery resources through competition and predation (Lynam et al., 2006). Changes in zooplankton community structure can have both direct and indirect effects on fish recruitments and resources. Decomposition and respiration of massive algal blooms always lead to depletion of oxygen and production of ammonia and/or toxic sulfides (Landsberg, 2002; Branch et al., 2013), which are also harmful to aquatic fauna. Therefore, clarification of the influence of *P. donghaiense* bloom (i.e. the peak and decay phases) on zooplankton groups (copepods, jellyfish, and chaetognaths) is key to understand its possible effects on the fishery resources in the ECS.

C. sinicus is an ecologically important copepod species in the ECS (Chen, 1964) and the dominant species in bloom areas (i.e., ~68.09% of zooplankton abundance) (Xu et al., 2003). The survival and recruitment of this species is crucial for the structure of zooplankton community. Therefore, the impact of *in situ P. donghaiense* bloom on the recruitments of *C. sinicus* is still in need of concern.

In this study, several cruises were conducted during *P. donghaiense* blooms in the ECS in 2013. The objectives of this study were twofold: (1) to illustrate the effects of different phases of the *P. donghaiense* bloom on zooplankton community structure; (2) to measure the adverse effects of *P. donghaiense* blooms on the copepod *C. sinicus* and discuss the causes of the observed detrimental effects. The results will help us identify the potential threats to the marine ecosystem posed by large-scale *P. donghaiense* blooms.

2. Materials and methods

2.1. Study location and field sampling

During the spring *P. donghaiense* bloom outbreaks in the ECS, four cruises were carried out along the northern coast of Fujian Province from April to May in 2013 (Fig. 1). Large-scale and high biomass blooms were found in two sections, ZE and FA (120°–121°30′E, 26°30′–28°N), where sampling was conducted at 25 stations during the four cruises. The changes in abundances of zooplankton, and the mortality of the copepod *C. sinicus*, during the blooms were studied at these stations. Therein, egg production rates and essential fatty acid measurements of *C. sinicus* were studied in some representative stations. Zooplankton samples were collected by towing a net (mouth area, 0.5 m^2; mesh size, 500 μm; length, 2 m) vertically from the bottom to the sea surface. At each sampling station, at least two zooplankton samples were collected; one was preserved in a 5% formalin-seawater solution, and the other sample was kept alive for onboard experiments.

2.2. Abundance of key zooplankton groups

All samples preserved in 5% formalin-seawater solution were analyzed in the laboratory. Copepods, small jellyfish, and chaetognaths were picked out and counted under a stereomicroscope. The abundance (ind m^{-3}) of each zooplankton group was obtained using the zooplankton abundance per net divided by the volume of the filtered seawater (measured by multiplying the area of net mouth by the vertical distance through which the net was towed). All determinations were performed by strictly following the Specifications of Oceanographic Surveys (State Oceanic Administration, 1991).

2.3. Mortality of C. sinicus

At each sampling station, the living sample was used to analyze the proportion of dead *C. sinicus* in the total count. After capture, the numbers of total individuals and dead individuals in the sample were measured immediately on board the research vessel.

2.4. Egg production rates of C. sinicus

2.4.1. Egg production experiments

At some non-bloom stations (ZE5 on 25 April, FA 7 on 26 April, FA1 on13 May, ZE7 on 14 May, and ZE3 on 22 May) and representative peak bloom stations (FA5 on 26 April and 7 May, ZE4 on 14 May), one living sample was collected for on board egg production experiments. Plankton samples were diluted into a 20 L bucket filled with surface water and delivered immediately to the laboratory, where healthy adult females were picked out as soon as possible. Ninety fresh adult females were transferred into plastic bottles (with false bottoms of 220 μm mesh size to avoid cannibalism; 10 females per bottle). Next, 500 mL of *in situ* surface seawater was added to each bottle for incubation. Prior to the experiments, the surface seawater were filtered through a 100 μm mesh and then checked carefully under a stereomicroscope to avoid the unwanted introduction of nauplii and eggs. The adult females in the bottles were incubated for 1 d in an incubator set on a 12:12 h light: dark cycle. Temperature in the incubator was set at the surface seawater temperature (17–19 °C). After 24 h, eggs in each bottle were counted under a stereomicroscope. Egg production rate (EPR) was expressed as eggs $female^{-1}$ day^{-1}.

2.4.2. Shipboard incubation experiments

A shipboard incubation experiment was conducted to further clarify the harmful effects of *P. donghaiense* on EPR. We conducted additional vertical hauls with the same net in a non-bloom area (station: ZE7, date: 14 May). One hundred and eighty fresh adult females were transferred into plastic bottles (18 bottles, 10 individuals per 500 mL bottle). Two series of experiments were conducted at the same time. The surface seawater collected at station ZE7 (with no *P. donghaiense* cells) were added to nine of the bottles as controls. The water collected in the bloom area (station: ZE2, date: 14 May), which contained high density of *P. donghaiense* cells at 10^7 cell L^{-1}, were added to the other replicate bottles as experimental groups. The surface water were also filtered through a 100 μm mesh and checked to avoid the unwanted introduction of nauplii and eggs before the experiment. Other conditions for incubation were the same as those used for the egg incubation experiment. After 24 h, eggs in each bottle were counted and EPR in each treatment was measured.

2.5. Essential fatty acid measurements of C. sinicus

Additional vertical hauls were conducted and fresh, healthy *C. sinicus* females were collected from non-bloom stations (ZE5 on 25 April, FA 7 on 26 April, ZE7 on 14 May, FA1 on13 May, and ZE3 on 22 May) and stations with blooms in the peak phase (FA5 on 26 April and 7 May, ZE4 on 14 May). For each sample, 100 individuals were picked on the GF/F filter membrane and then preserved in liquid nitrogen. All samples were processed according to Folch et al. (1957) and Parrish (1999). Analysis of fatty acid composition was carried out using an Agilent 7890 AGC instrument.

2.6. Data analysis

Data were analyzed using the Excel 2003, Origin 8.5, Surfer 8.0, and SPSS 16.0 software packages. No transformation did not meet the assumptions for the analysis of variance, so nonparametric analogs were used for all the data. For the mortality, percentages were transformed to arcsine square root, and then analyzed by nonparametric analogs (Kruskal–Wallis test). For the others, the original data were directly used. Pair-wise comparisons were