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Changes in size and trophic structure of the nanoflagellate assemblage in response to a spring phytoplankton bloom in the central Yellow Sea

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ABSTRACT

On a multidisciplinary investigation cruise from March 25 to April 14, 2009, the dynamics of the nanoflagellate (NF) assemblage in response to a spring phytoplankton bloom was observed in the central Yellow Sea (~33.5–37°N, ~120.5–124.5°E). The water mass of the bloom was followed with a drifting buoy-guided Lagrangian tracing approach. The results showed that the abundance, biomass, cell size composition and trophic structure of NFs dramatically changed with the succession of the bloom. Compared with that in the Pre-bloom phase, the cell abundance and biomass of NFs increased more than two-fold in the Bloom phase and the Post-bloom phase in the water column, respectively, especially in the deep chlorophyll maximum (DCM) layer. NF cell abundance was composed mainly of 2–5 μm pigmented photosynthetic nanoflagellates (PNFs), whose rapid growth in response to the bloom outbreak characterized the NF dynamics in the bloom phase. Although larger (5–20 μm) flagellates constituted less than 20% of NF cell abundance in most samples, they accounted for a rather larger percentage in NF biomass, especially in the bloom decline phase, when the response of the NF assemblage to the spring bloom was characterized by a dramatic increase in NF biomass, mainly contributed by 10–20 μm PNFs and non-pigmented heterotrophic nanoflagellates (HNFs). Compared to PNFs, HNFs showed a rather mild response during the bloom, especially in the 2–5 μm fraction, which decreased during the bloom, despite a significant increase of both HNFs and PNFs in the early stage of the bloom. However, when PNFs were declining in the Post-bloom phase, HNFs, especially the 5–10 μm fraction, had in contrast shown an ascending trend. This is the first systematic report on short-term dynamics of different nano-sized flagellate components, and our results suggested an inherent mechanism associated with cell size as well as trophic strategies for the dynamics of the NF assemblage in response to the spring phytoplankton bloom, within which mixotrophy in PNFs might play a substantially important role.

1. Introduction

The annual spring phytoplankton bloom is a crucial natural event in the dynamics of carbon flux during the year, with high new production, biomass accumulation and often high sedimentation rate, and it accounts for a significant percentage of the annual primary production in temperate coastal oceans (Parsons et al., 1984; Falkowski and Woodhead, 1992; Falkowski et al., 2003). It is believed that, during a spring phytoplankton bloom, high primary production would lead to a mass release of DOM from phytoplankton to the blooming water, and consequently result in both a dramatic increase of bacterial production and a remarkable succession in the pelagic microbial community (Larsen et al., 2004; Nagata, 2008). Several investigations of spring blooms have already confirmed a tight coupling between the blooming phytoplankton and the components of the microbial loop (Weisse et al., 1990; Hyun and Kim, 2003; Larsen et al., 2004; Aberle et al., 2007). However, most studies have focused on the interaction of bacteria, the predominant DOM consumer, and large (> 20 μm) ciliates and/or heterotrophic dinoflagellates, the active phytoplankton grazers, with the blooming phytoplankton. Works on the dynamic of nanoflagellates (NFs) during a spring phytoplankton bloom were much fewer, with merely a few reports on the dynamic of their biomass or abundance (Weisse et al., 1990; Tsuda et al., 1994; Kobari et al., 2010). We still have almost no knowledge about the dynamics of different nano-sized flagellate components of different trophic mode and cell size during the spring bloom, which are much more meaningful for the comprehensive understanding of NFs response to and their role in the bloom than merely the dynamic of their biomass or abundance.

For nanoflagellates, one of the major components in microbial loop, their major functions in pelagic nutrient cycling and organic carbon utilization and transfer were already uncovered some decades ago (Azam et al., 1983; Pomeroy, 1974). Based on the trophic

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strategies undertaken, NFs can technically be divided into two functional groups, non-pigmented heterotrophic flagellates (HNFs) and pigmented photosynthetic flagellates (PNFs) (Tsai et al., 2005). PNFs, including autotrophs and mixotrophs, are frequently found to dominate phytoplankton assemblages by cell abundance or biomass and usually account for a considerable proportion of the primary production (Booth et al., 1982; Booth and Smith Jr., 1997). HNFs are recognized as the most important consumers of bacterioplankton and active nutrient remineralizers in the pelagic ocean (Boenigk and Arndt, 2002; Laybourn-Parry and Parry, 2000; Jürgens and Massana, 2008), hence they might provide a valuable source of nutrients for phytoplankton growth or maintenance, especially in times of exogenous nutrient depletion such as in the late phase of a spring phytoplankton bloom (Laybourn-Parry and Parry, 2000). Therefore, both PNFs and HNFs may play an important role in the formation, maintenance and decline of phytoplankton blooms in the sea (Porter, 1988; Tiselius and Kuylenstierna, 1996). However, most of our knowledge about it has been obtained from laboratory studies (Christaki and Van Wambeke, 1995) and *in situ* bloom inducing experiments *via* nutrient enrichment (Hall and Safi, 2001; Thingstad et al., 2007; Christaki et al., 2008), while some were derived from studies in fresh water systems (Weisse et al., 1990; Carrias et al., 2001). The natural dynamics of the NF assemblage in response to the spring phytoplankton bloom in the coastal ocean have seldom been documented in field blooming conditions.

In this work, we focus on the changes in size and trophic structure of the NF assemblage. By adopting the drifting buoy-guided Lagrangian tracing approach in the central Yellow Sea in early spring, 2009, we expected to reveal the real dynamics of the NFs assemblage in the bloom water mass. This study may offer valuable evidence unveiling the community-level response of NFs, with emphasis on their trophic mode and cell size, to the spring phytoplankton bloom in the sea.

2. Methods

A multidisciplinary investigative cruise, aboard *R.V.* Beidou, was carried out in the Yellow Sea from March 24 to April 14, 2009. The observations were divided into two stages, a Pre-bloom stage and a Lagrangian bloom tracing stage. The scope of survey area (33.5–37°N, 120.5–124.5°E) and observing stations are shown in Fig. 1A. In the first stage (~March 24–April 2), the whole area was cruised in advance to find out where the bloom was most likely to start, based on chlorophyll fluorescence detected by an onboard probe. During this stage of the cruise, Stn.Z11 was found to have a high bloom probability, and thus was chosen as one of the candidate stations for time series observations in the second stage. On 4th April, when initiation of a diatom bloom was detected at the deep chlorophyll maximum (DCM) layer (about 15 m) at Stn.Z11 (see Chl *a* dynamics in Fig. 2), the bloom tracing observations were then started along with the drifting trajectory of the blooming water mass indicated by a floating buoy (Fig. 1B). Samples were taken immediately (0 h) and at 12 h, 30 h and 99 h thereafter by 1 L Niskin bottles on a "Sea Bird" CTD rosette at three selected depths, representing the surface layer, the DCM layer and the 30 m layer, respectively.

Samples (50–200 mL) for Chl *a* analyses were filtered onto 25-mm GF/F filters and extracted using acetone (90% final concentration) and determined fluorometrically (Parsons et al., 1984).

Samples for the enumeration of NF cells were pre-filtered through a nylon mesh of 20 μm pore size by gravity, and then fixed with cold glutaraldehyde (final concentration 0.5% (v/v)). Subsamples (20 mL) were filtered onto 0.2 μm pore size black polycarbonate membrane filters with glass filter holders (Millipore) at low vacuum pressure (<100 mm Hg). When 1 mL of the sample remained in the funnel, the vacuum pump was turned off and the sample was stained with DAPI (final concentration 10 μg mL^{-1}) for 5 min, then the pump was turned on again, to let the residual liquid completely pass through the membrane filter. The filter was then mounted on a microscope slide, a few drops of paraffin put in the center of the filter and a cover slip placed on the top. After the above procedures, the sample slide was immediately stored in the dark at −20 °C.

NFs cells were directly counted by epifluorescent microscopy (Leica DM 4500B) at 1000× magnification. PNFs were distinguished from HNFs by the presence of red-autofluorescence in the former with a blue excitation filter set (Tsai et al., 2005). At least 40 fields of view were examined. The abundance of flagellates was calculated from the average of cell counts made on duplicate samples.

The length (*L*) and width (*W*) of an NF cell were measured on photomicrographs using the Leica DM 4500 self-carried software. At least 60 cells (PNF plus HNF) were measured per sample. Cell volumes of NF individuals was estimated by assuming their nearest geometrical figures (Sun and Liu, 2003). The mean cell volumes were converted to carbon biomass using a conversion factor of 0.22 pg C μm^{-3} (Børsheim and Bratbak, 1987). For studying the size structure of NFs assemblage, flagellates were grouped into three size categories, 2–5 μm, 5–10 μm and 10–20 μm, according to their cell length.

3. Results

3.1. Dynamics of NFs abundance and biomass

Before the bloom outbreak (March 27), the cell abundance of NF in Stn.Z11was relatively low (<1600 cells mL^{-1}) in all three

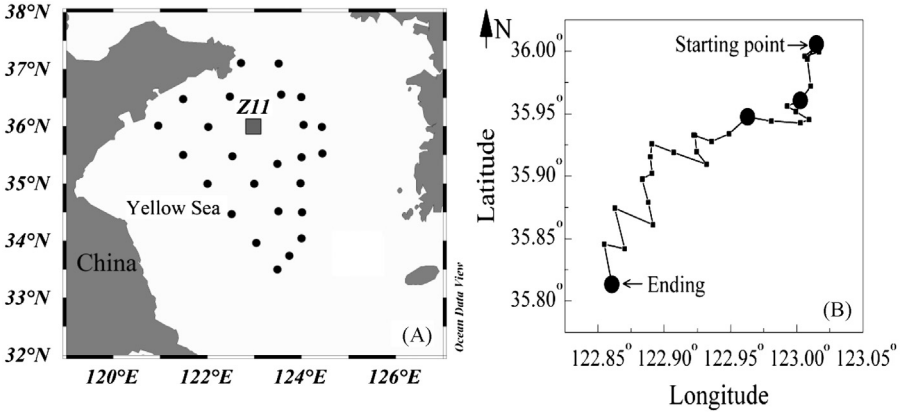

Fig. 1. Location of the spring bloom observation in the central Yellow Sea, ~March 24–April 14, 2009. (A) Survey area and bloom observation stations; (B) Bloom tracing route. The big solid points in B indicate the sampling site during the bloom tracing route.