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Vertical distribution of pigmented and non-pigmented nanoflagellates in the East China Sea

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ABSTRACT

Nanoflagellates can be separated into two groups according to their trophic mode, i.e. pigmented nanoflagellates (PNF) and heterotrophic nanoflagellates (HNF). However, a newly identified group, mixotrophic nanoflagellates (MNF), are pigmented and show the ability of prey on bacteria. To examine the vertical variations in PNF and HNF abundances, as well as their relationships and the nutritional strategies that they might use, two summer cruises were undertaken in the East China Sea in July 2011 (OR1 966) and July 2012 (OR1 1004). The results show that both HNF and PNF abundances decline with increasing water depth. Vertical variations of abundances are believed to be influenced by prey and light, for HNF and PNF respectively. Over a large part of the sampling area, the ratio of PNF to HNF abundances is about 1:1 in the disphotic and euphotic zones, but exceeds 1.5 in the nutrient-depleted environment along the margin of the continental shelf. The correlation between PNF abundance and bacteria/*Synechococcus* abundance is positive where PNF/HNF >1.5. However, there is no significant correlation between PNF/HNF abundance when PNF/HNF >1.5 and light/nutrients, indicating that vertical distributions are influenced mainly by prey (bacteria and *Synechococcus*) in the nutrient-depleted environment. This study assumes that PNF consists mostly of MNF. In the euphotic zone they receive energy from photosynthesis, which is stimulated by the available nutrients from grazing. Their abundance is thus higher than that of HNF. However, in the disphotic zone, both PNF and HNF satisfy their nutrient demands by grazing, and PNF/HNF is close to 1. In other words, mixotrophy might be the main trophic mode for PNF in the nutrient-depleted, oligotrophic environment. Meanwhile, in deeper water (300 m), the much lower prey density means that MNF cannot satisfy the basic energy demands of metabolism and photosynthesis, and thus HNF abundance exceeds that of PNF.

1. Introduction

As primary consumers of bacteria and picophytoplankton, both pigmented and non-pigmented nanoflagellates play an important role in the trophic fluxes in the microbial food web (Hahn and Höfle, 2001; Hartmann et al., 2012; Sanders et al., 2000; Tsai et al., 2007). They, in turn, are potentially a major food source for ciliates and metazoans (Cleven, 1996; Jürgens et al., 1996; Sanders and Wickham, 1993). Non-pigmented nanoflagellates (HNF) have been identified as a major cause of bacterial mortality in many aquatic ecosystems (Christaki et al., 2001; McManus and Fuhrmann, 1986; Sherr and Sherr, 1994, 2001; Šolić and Krstulović, 1994). However, a growing number of studies indicate that bacterivory by pigmented nanoflagellates (PNF) is another major cause of bacterial mortality (Bennett et al., 1990; Hall et al., 1993; Havskum and Riemann, 1996; Sanders et al., 1989).

In recent years, mixotrophic nanoflagellates (MNF), which combine both modes of nutrition, photosynthesis and bacterivory, within a single cell, have been recognized as bacterivory, and their importance to the carbon cycle in the microbial food web has been noted (Ward and Follows, 2016; Caron, 2000; Sanders and Porter, 1988; Stickney et al., 2000). Initially, they were most commonly found in freshwater ecosystems (Bennett et al., 1990; Berninger et al., 1992; Bird and Kalff, 1986). However, later studies in pelagic marine communities have shown that MNF abundances can exceed 50% of the total abundance of nanophytoplankton, and that bacterivory contribution can be higher than that of HNF (Arenovski et al., 1995; Hall et al., 1993; Havskum and Hansen, 1997; Li et al., 1996; Tsai et al., 2007). Mitra et al. (2014) show the importance of an explicit mixotrophic community through simulations in the pelagic environment, and suggest an alternative new paradigm with a bulk of mixotrophic nanoflagellate community.

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MNF have been shown to possess several possible advantages for survival and growth, including the ability to acquire organic carbon (energy) under poor phototrophic conditions (Bird and Kalff, 1986; Caron et al., 1993; Maranger et al., 1998; McKenrie et al., 1995), and to obtain macronutrients (nitrogen, phosphorus) (Arenovski et al., 1995; Legrand et al., 1998; Nygaard and Tobiesen, 1993) and micronutrients (vitamins, specific lipids, iron) under nutrient-limited environments for photosynthetic growth (Maranger et al., 1998). Mixotrophs rely more on phagotrophy as a means to alleviate nutrient stress, as indicated by a strong inverse relationship between the proportion of community FLB uptake and ambient nutrient concentration (Stukel et al., 2011). MNF thus exhibit a range of nutritional strategies, from absolute autotrophy to absolute heterotrophy (Jones, 1994), in which mixotrophy is used to gain a competitive advantage over purely phototrophic and heterotrophic species.

Although many laboratory experiments have been carried out to examine the nutrition modes of MNF (Jones et al., 1995, 1993; Sanders et al., 1990), the understanding of the ecological role of natural populations of MNF is still poor. Similarly, little is known about the relationship between HNF and PNF abundances in the water column. The objective of this study is to examine the vertical distribution of HNF and PNF abundances by means of microscopical examination.

2. Materials and methods

2.1. Sampling

Samples were collected during two cruises of the R/V Ocean Research I in the East China Sea (ECS) in the summers of 2011 and 2012, from 35 and 28 stations respectively (Fig. 1), and also at greater depths (> 300 m) from four stations in the oligotrophic subtropical Western Pacific Ocean (WPO) near eastern Taiwan in 2013 (Fig. 1).

To count picoplankton (bacteria and *Synechococcus* spp.) and nanoplankton, seawater was collected using a Sea Bird CTD-General Oceanic Rosette assembly with 20 l Go-Flo bottles at six levels within the upper 100 m water column in ECS, and at four levels within the upper 300 m water column in WPO. Temperature and salinity profiles were taken from the surface to near the bottom using the same assembly. Nitrate and phosphate were measured according to Gong et al. (1995). Water samples (500 ml) were filtered (25 mm GF/F) for Chl *a* analysis which was then measured after extraction with an in vitro fluorometer (Turner Design 10-AU-005) (Parsons et al., 1984).

2.2. Epifluorescence microscopic analysis

The samples (50 ml) were fixed immediately by adding glutaraldehyde to a final concentration of 1% (Christaki et al., 2002; Sanders et al., 2000). To count bacteria and *Synechococcus*, subsamples of 4 ml seawater were filtered onto 0.2 μm black Nuclepore filters, while for nanoplankton, subsamples of 20 ml seawater were filtered onto 0.8 μm black Nuclepore filters. They were then placed under low pressure (< 100 mmHg) with a 0.45 μm pore size Millipore filter used as a backing-pad to obtain an even distribution of cells. The cells left on the filter membranes were stained with 4′6-diamidino-2-phenylindole (DAPI) at a final concentration of 1 μg ml^{-1} (Porter and Feig, 1980) and counted under an epifluorescence microscope at 1000 × magnification (Nikon Optiphot-2). Bacteria and HNF were identified by their blue fluorescence under UV illumination, while *Synechococcus* and pigmented nanoflagellates were identified by their orange and red fluorescence under blue excitation light. To obtain reliable estimates of abundance in each sample, at least 800 bacteria, 400 *Synechococcus* and 100 nanoflagellates were counted, respectively.

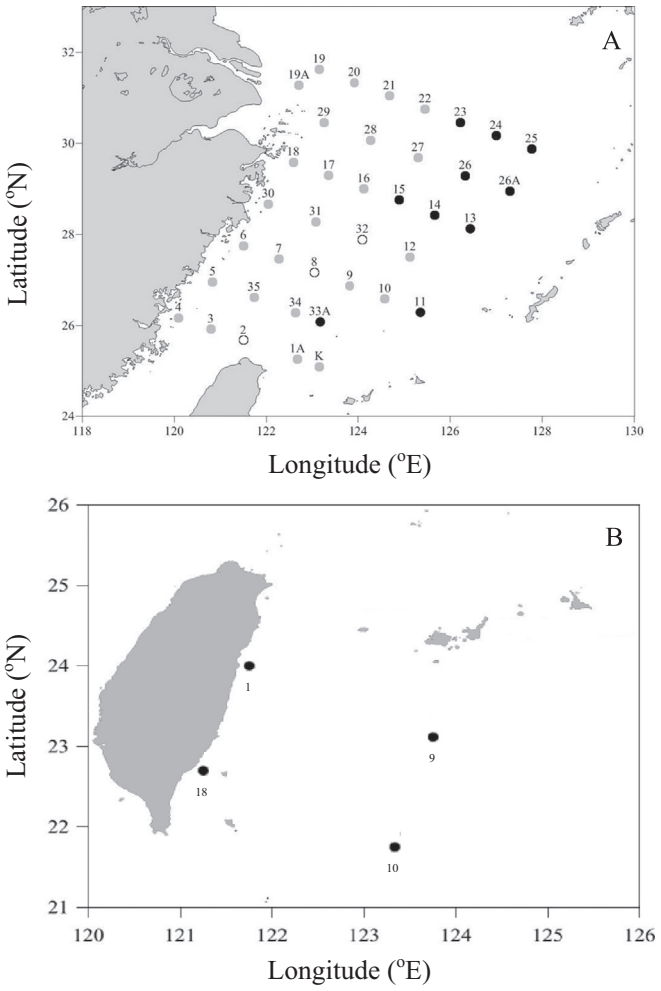

Fig. 1. Spatial pattern of sampling sites during cruises conducted in July 2011 (black and gray circles) and July 2012 (white and gray circles) in A, and in January 2013 (gray circles) in B.

2.3. Calculation of euphotic zone depth

The euphotic zone depth is the point at which only 1% of the surface photosynthetic available radiation (PAR) remains (Kirk, 1994). This is not only a quality index of an ecosystem but also an important property for primary production in the upper water column (Behrenfeld and Falkowski, 1997; Platt and Sathyendranath, 1988). The PAR penetrating to a depth z below the surface of the water column is assumed by Cole (1975) to be:

$$I_z = I_0 e^{(-Kd)z} \quad (1)$$

where I_z = solar radiation intensity at depth z, I_0 = net solar radiation penetrating the surface of the water column, and Kd = bulk extinction coefficient.

In the present study, the values of I_0, I_z and the euphotic zone depth (z) at each station were estimated using a photometer attached to the Rosette during daylight, and the values of Kd were calculated by formula (1). Night time values of the euphotic zone depth at other stations were estimated by correlation between the euphotic zone depth and water column depth at nearby stations:

$$Z = 0.5766 \times D + 6.5018 \quad (2)$$