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The influence of nanoflagellates on the spatial variety of picoplankton and the carbon flow of the microbial food web in the oligotrophic subtropical pelagic continental shelf ecosystem

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ABSTRACT

To investigate the mechanism of the spatial dynamics of picoplankton community (bacteria and Synechococcus spp.) and to estimate the carbon flux of the microbial food web in the oligotrophic Taiwan Warm Current Water of the subtropical marine pelagic ecosystem, we conducted size-fractionation experiments during five cruises by the R/V Ocean Research II during the summers of 2010 and 2011 in the southern East China Sea. We carried out culture experiments using surface water, which according to a temperature–salinity (T–S) diagram, is characterized as oligotrophic Taiwan Current Warm Water. We found a negative correlation between bacteria growth rate and temperature, and another negative correlation between nitrate and temperature indicating that the active growth of heterotrophic bacteria might be induced by nutrients lifted from a deep layer by cold upwelling water. This finding suggests that the area we studied was a bottom-up control pelagic ecosystem. Upwelling brings nutrient-rich water to the euphotic zone and promotes bacterial growth, resulting in increased picoplankton biomass, which increases the consumption rate of nanoflagellates. The net growth rate (growth rate–grazing rate) becomes negative when the densities of bacteria and Synechococcus spp. are lower than the threshold values. The interaction between growth and grazing will limit the abundance of bacteria (10^5–10^6 cells ml^{-1}) and Synechococcus spp. (10^4–10^5 cells ml^{-1}) within a narrow range. Meanwhile, 61% of bacteria production and 54% of Synechococcus spp. production are transported to a higher trophic level (nanoflagellate), though the cascade effect might cause an underestimation of both percentages of transported carbon. Based on the successive size-fractionation experiments, we estimated that the predation values were underestimated and that the diet of nanoflagellates is composed of 64% bacteria and 36% Synechococcus spp.

1. Introduction

Bacteria are very important energy and carbon sources in the marine pelagic ecosystem (Pomeroy, 1974; Azam et al., 1983). The transfer of bacterial organic carbon to higher trophic levels in a food chain via bacteria, nanoflagellates, and ciliates was formalized as the "microbial loop" (Azam et al., 1983). When picophytoplankton were described as a primary producer, this loop came to be referred to as a complex "microbial food web" (Sherr and Sherr, 1994). Picoplankton, including heterotrophic bacteria and picophytoplankton (*Prochlorococcus*, *Synechococcus* and picoeukaryotes), are generally thought to be consumed mainly by nanoflagellates in a marine pelagic ecosystem. Zubkov and Tarran (2008) indicated that small algae (<5 μm) were responsible for substantial part of bacterivory in the oligotrophic marine environment. Our previous studies demonstrated that bacteria were mostly consumed by nanoflagellates <6 μm in size, and *Synechococcus* was consumed mainly by pigmented nanoflagellates of 3–10 μm in the subtropical western Pacific coastal ecosystem (Chan et al., 2009; Tsai et al., 2011). In contrast, the information about grazing on picoeukaryotes is limited compared to that available for *Prochlorococcus*, *Synechococcus*, and heterotrophic bacteria (Hirose et al., 2008).

Factors that regulate the standing stock of picoplankton include bottom-up control on the growth environment (temperature,

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nutrients, and substrate supply) (Almeida et al., 2001; Schultz et al., 2003; Ameryk et al., 2005) as well as top-down mortality pressure, especially due to grazing and viral lysis (Wilhelm et al., 2002; Taira et al., 2009). With regard to marine systems, there is an ongoing debate on whether the standing stock and production of picoplankton are mainly controlled by bottom-up or top-down mechanisms. The close coupling between picoplankton and bacterivores in experiments were used initially as evidence of top-down control by protistan grazers (Ducklow, 1983; Tanaka et al., 1997; Calbet et al., 2001; Hirose et al., 2008). The positive correlation between resource supply (phytoplankton, nutrient, dissolved organic carbon) (Gasol and Duarte, 2000; Duarte and Agustí, 2005) and picoplankton standing stock in the natural environment, however, suggests a typical bottom-up control relationship. In addition, many empirical models have drawn conclusions on the relative importance of top-down and bottom-up controls by referring to the slope of the regression between bacterial production and bacterial biomass (Ducklow, 1992), to the coupling between the abundance of bacteria and their main predator (heterotrophic nanoflagellates; Gasol, 1994; Gasol et al., 2002), and to the relationship between the bacterial growth rate and bacterial abundance (Wright and Coffin, 1984; Zubkov et al., 2000; Jochem, 2003). Studies applying several of these methods to the marine pelagic ecosystem have concluded that bacteria are commonly regulated from the top-down in most oligotrophic situations and are regulated bottom-up in eutrophic environments (Gasol et al., 2002).

Generally, bacteria have high growth rates in both marine and freshwater environments, yet their growth is often balanced by the effect of predation, e.g., nanoflagellate grazing (Sanders et al., 1992; Zubkov et al., 2000; Vaqué et al., 2002; Tsai et al., 2005, 2008). Therefore, bacterial abundance is less spatially and temporally variable and remarkably constant (Cole and Caraco, 1993; Tsai et al., 2005). Nanoflagellate abundance, on the other hand, has marked seasonal fluctuation (Tanaka et al., 1997; Tanaka and Taniguchi, 1999; Granda and Álvarez, 2008), but we know little about how growth and mortality rates regulate the spatial dynamic of bacterial community. This study investigated the impact of the population growth and nanoflagellate grazing on picoplankton communities, and identified the mechanism of spatial variation in picoplankton and nanoflagellate abundances in an oligotrophic pelagic marine ecosystem (Taiwan Warm Current Water) of the subtropical Western Pacific during the summer season (June–September).

2. Materials and methods

2.1. Sampling

Samples were collected during five cruises of the R/V Ocean Research II in the summers of 2010 and 2011 at 12 stations crossing the continental shelf in the southern East China Sea (ECS) (Fig. 1). To count picoplankton (bacteria and *Synechococcus* spp.) and nanoflagellates, sea water was collected using a Sea Bird CTD-General Oceanic Rosette assembly with 20 l Go-Flo bottles at six water depths (5, 10, 25, 50, 75 and 100 m). Temperature and salinity profiles were taken from the surface to near the bottom using a Sea Bird CTD-General Oceanic Rosette.

Water samples collected for the determination of nitrate concentration were placed in 100 ml polypropylene bottles and stored in liquid nitrogen. Samples used to determine the chlorophyll *a* concentration were obtained by filtering 2 l of sea water through a GF/F filter. The filter was stored in liquid nitrogen and then transferred to a freezer. Nitrate concentrations were analyzed with a self-designed flow injection analyzer according to Gong et al. (1992). Chlorophyll *a* on the filter was measured using a Turner Designs Fluorometer (Model 10-Au-005) (Strickland and Parsons, 1972).

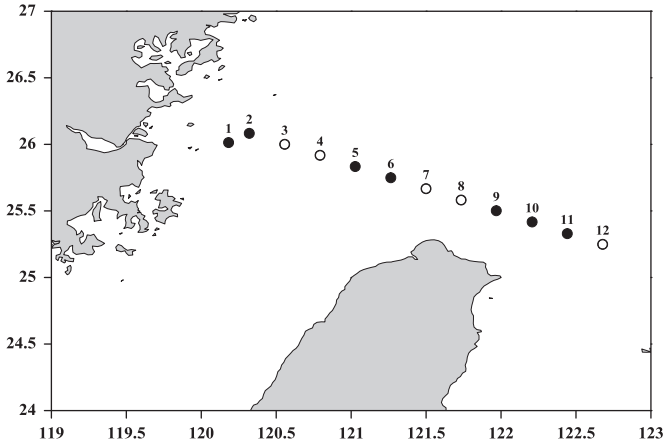

Fig. 1. Sampling stations 1–12 were located along a cross-shelf transect in the southern East China Sea on the five cruises of present study during the summer period of 2010 and 2011. Solid circles indicate the stations where culture experiments with fractionation method were performed.

2.2. Flow cytometry analysis of picoplankton

Each 2-ml subsample used in the flow cytometry analyses was fixed with 40 μl paraformaldehyde (0.2% final concentration), quickly frozen in liquid nitrogen, and stored in a freezer at −75 °C for later analysis (Campbell and Vaulot, 1993). The abundances of picoplankton (heterotrophic bacteria and *Synechococcus* spp.) were determined by flow cytometry (Marie et al., 1997) using a FACSAria flow cytometer (Becton Dickinson). Samples were run on the low rate setting for 2 min. *Synechococcus* spp. specimens were distinguished according to their positions in plots of orange fluorescence (FL2) and red fluorescence (FL3). Bacteria were identified by using SYBR Green I (Molecular Probes) as a nucleic acid stain (Marie et al., 1997) for a plot of FL3 fluorescence versus green fluorescence (FL1). Internal calibration beads (1-μm yellow-green fluorescence beads) were added to each sample as an internal reference.

2.3. Epifluorescence microscopic analysis of nanoplankton

For nanoplankton enumeration, 50 ml water samples were fixed with glutaraldehyde to a final concentration of 1% (Sanders et al., 2000; Christaki et al., 2002). Subsamples (20 ml each) for pigmented and non-pigmented cells were filtered onto a 0.8-μm black nuclepore filter 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). Non-pigmented nanoflagellates were identified by their blue fluorescence under UV illumination, and pigmented nanoflagellates were identified by their orange and red autofluorescence under blue excitation light. To obtain reliable estimates of abundance, at least 100 nanoflagellates were counted per sample.

2.4. Growth and grazing rates

The growth and grazing rates were estimated using a fractionation method (Wright and Coffin, 1984) at six stations, including