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The effect of grazing and viral lysis on the diel variations of *Synechococcus* spp. abundance in the East China Sea

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ABSTRACT

Diel variations in the nanoflagellate grazing and viral-mediated mortality of *Synechococcus* spp. were simultaneously estimated using a dilution and size-fractionation approach in the inner (I-1 and I-2) and outer regions (O-1, O2 and O-3) of the Changjiang River plume in the East China Sea during summer 2014. *Synechococcus* spp. abundance generally tended to increase during the dark period, followed by a plateau until midnight at all sampling stations. Overall, gross growth rate of *Synechococcus* spp. ranged from 0.069 h^{-1} to 0.122 h^{-1} during the growth phase. Microzooplankton, nanoflagellate grazing, and viral lysis had no effect on the *Synechococcus* spp. abundance during this phase. Moreover, nanoflagellate grazing was the largest cause of *Synechococcus* spp. mortality during the loss phase at nighttime. Compared to the predators, viruses exerted only a minor impact on mortality at St. I-1, where we detected some of the effect that this community had on *Synechococcus* spp.. Little is known about the impact of nanoflagellates and viruses on the short-term dynamics of *Synechococcus* spp. in the East China Sea. Therefore, this study's characterization of the relative importance of nanoflagellates and viruses may help provide a better understanding of trophic structures and the energy flow within the microbial loop.

1. Introduction

The fate of primary production has important implications for food web structure and biogeochemical cycling. In oceanic communities, picophytoplankton often dominate phytoplankton biomass and productivity (Agawin et al., 2000; Veldhuis et al., 2005) and thus play a key role in marine carbon and nutrient cycles (Campbell et al., 1994). In natural environments, phytoplankton are influenced by a variety of environmental factors, including nutrient availability, temperature, mixing and irradiance level. Among these, the daily alternation of light and darkness is undoubtedly a very important external stimulus. Recently, most oceanographic time series have used the month, the week or, in the most extreme case, the day as their minimum sampling period. However, shorter timescales, in particular the daily scale, are arguably more relevant to understanding the physiology and ecology of these microbial communities.

Diel variations in picophytoplankton cell abundance, growth and division have been well documented (Vaulot and Marie, 1999; Christaki et al., 2002; Jacquet et al., 2002; Tsai et al., 2005, 2009; Lefort and Gasol, 2014). Cell division in *Synechococcus* spp., for example, has been found to obey a clock-controlled circadian regulation (Jacquet et al., 1998; Johnson and Golden, 1999). *Synechococcus* spp. have been found to have distinct diel changes in abundance, with higher division rates at dusk (Vaulot and Marie, 1999; Christaki et al., 2002; Tsai et al., 2009) and maximum abundances at night (Christaki et al., 2002; Tsai et al., 2009). Studies of small-scale variability in picophytoplankton abundance have found that temporary imbalances between growth and loss rates throughout a day generate important daily variation in aquatic environments. It is well-known that grazing and viral lysis are the two main factors responsible for *Synechococcus* spp. mortality in aquatic environments (Suttle and Chan, 1994; Dolan and Šimek, 1999; Suttle, 2000; Christaki et al., 2002; Tsai et al., 2009, 2012a). However, studies of diel variations in *Synechococcus* spp. abundance have found that loss processes do not occur at a uniform rate during the day and suggest that grazing activity of nanoflagellate could vary with *Synechococcus* spp. cell cycle (Christaki et al., 2002; Tsai et al., 2009). Viral infection also varies over the course of a day (Weinbauer et al., 1995; Suttle, 2000).

The East China Sea (ECS) is located on the western edge of the Northwest Pacific Ocean, and is characterized by dynamic

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interactions among several water systems including the nutrient enriched freshwater input from the Changjiang River (Gong et al., 1996, 2003). The other three water masses influencing the ECS are the Yellow Sea Coastal Water (YSCW) from north to south along the northwest coast of the sea, the Kuroshio Water (KW) coming from the western equatorial Pacific in the east and Taiwan Current Warm Water (TCWW) from the south. Fresh water discharge before 2003 peaked between June and August (summer), during which time the mixing of fresh water and seawater formed Changjiang Diluted Water (CDW) (Gong et al., 1996). The CDW, which has an average salinity of <31, is generally influenced by the southwest monsoon, although this zone has undergone significant change since a reservoir was created by the building of Three-Gorges Dam in June 2003 (Gong et al., 2006). Typically, before the dam, in the subtropical shelf water of the East China Sea, picophytoplankton account for 29–86% of the chlorophyll *a* and 19–72% of the primary productivity (Chen, 2000; Chiang et al., 2002). Although the distribution and abundance of *Synechococcus* spp. in the East China Sea has been well documented (Chiang et al., 2002; Jiao et al., 2005; Pan et al., 2007), gaps remain in our understanding of the mechanisms that control the growth and loss of *Synechococcus* spp. and the multiple interactions that occur among these picophytoplanton, viruses, and nanoflagellates in the ECS (Tsai et al., 2012a).

Therefore, the goal of the present study was to simultaneously compare the roles of viral lysis and nanoflagellate bacterivory in the diel variability of *Synechococcus* spp. in the East China Sea during summer. Knowledge of the relative contribution of these loss factors for picophytoplankton mortality is critical for an optimal understanding of the flow of energy and nutrients in the marine environments.

2. Materials and methods

2.1. Sampling

Research was conducted aboard the R/V Ocean Research V along the cross-shelf transects in the ECS from 15 to 30 July, 2014 (Fig. 1). To characterize growth and removal rates that might account for diel variations of *Synechococcus* spp. in the inner and outer regions of Changjiang River plume, we performed a series of incubation experiments at five sites; two in the inner region (I-1 and I-2) and three in the outer region (O-1, O-2 and O-3) of the Changjiang River plume (Fig. 1). During this cruise, seawater samples were collected at depths of 2 m using a SeaBird CTD-General Oceanic Rosette assembly with 20 L Go-Flo bottles. Nutrients of seawater samples were measured as previously described by Gong et al. (1995). Water samples were filtered (25 mm GF/F) for Chl *a* analysis and measured after extraction with an in vitro fluorometer (Turner Design 10-AU-005) (Parsons et al., 1984).

2.2. Incubation experiments

After collection, water samples were immediately filtered through 200 μm mesh to remove mesozooplankton. Subsamples (1000 mL) were filtered through 47 mm Nuclepore filters (type PC), which had a pore size of 10 μm to remove ciliates. The size fractionation used for grazers (<10 μm) was chosen following that used by a previous study in the ECS, where it was shown that the majority of nanoflagellates ranged < 10 μm in size during the summer periods (Tsai et al., 2010). Other subsamples (2000 mL) were filtered through 2 μm pore size Nuclepore filters under low pressure (<50 mm Hg) to remove nanoflagellates (Fig. 2). We assumed that the filtrates of the 2 μm filters contained picoplankton and viruses (Tsai et al., 2005, 2008), those of the 10 μm filters contained nanoflagellates, picoplankton, and viruses (Tsai et al., 2011), and those of the 200 μm mesh plankton net contained ciliates, nanoflagellates, picoplankton, and viruses. An additional dilution experiment was designed to examine the impact of viruses on *Synechococcus* spp. abundance. Water was filtered in a series through 2 μm and 0.2 μm pore-size, 47 mm diameter polycarbonate filters (AMD Manufacturing), with the first filter removing nanoflagellate grazers and the second concentrating picoplankton (Wilhelm et al., 2002). A transfer pipette was used to keep the *Synechococcus* spp. in suspension above the 0.2 μm filter. Viruses were removed using a Prep Scale-TFF Cartridge (Millipore) with a 30 kDa molecular weight cut-off (virus-free water). Subsequently, dilution was performed by adding 20 mL of *Synechococcus* spp. concentrate to 230 mL of virus-free water. All treatments were set up in triplicate in clear 500 mL polycarbonate bottles and incubated under natural light in thermo-controlled incubators for 24 h. Subsamples were then taken from each bottle at 2 h intervals after the experiments were set up. Net growth rates (b) of *Synechococcus*

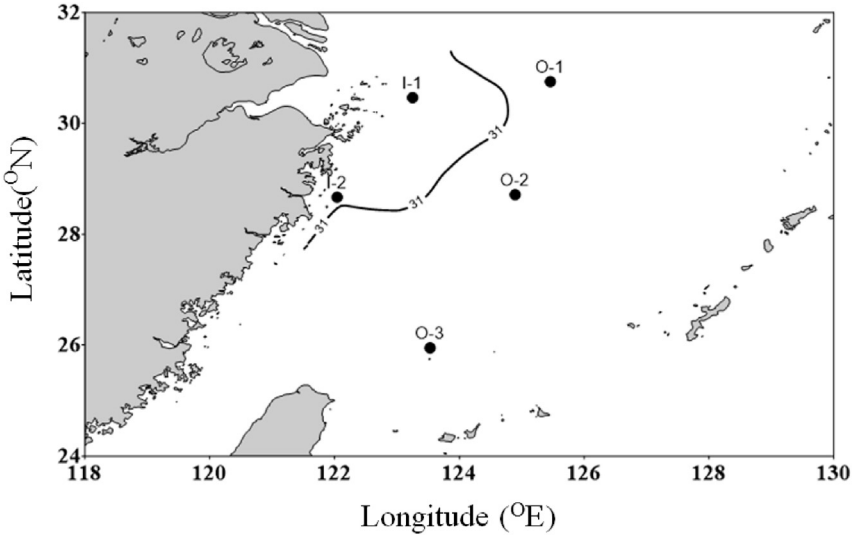

Fig. 1. Map of sampling stations (I-1, I-2, O-1, O-2 and O-3) and the solid line is contour of surface salinity of 31 (isohaline). CDW: Changjiang Diluted Water, TCWW: Taiwan Current Warm Water.