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Coupling of the spatial dynamic of picoplankton and nanoflagellate grazing pressure and carbon flow of the microbial food web in the subtropical pelagic continental shelf ecosystem

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Abstract

In order to investigate the mechanism of spatial dynamics of picoplankton community (bacteria and *Synechococcus* spp.) and estimate the carbon flux of the microbial food web in the oligotrophic Taiwan Warm Current Water of subtropical marine pelagic ecosystem, we conducted size-fractionation experiments in 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 bettween bacteria growth rate and temperature, indicating that the active growth of heterotrophic bacteria might be induced by nutrients lifted from deep layer by cold upwelling water. This finding suggests that the area we studied was a bottom-up control pelagic ecosystem. We suggest that the microbial food web of an oligotrophic ecosystem may be changed from top-down control to resource supply (bottom-up control) when a physical force brings nutrient into the oligotrophic ecosystem. Upwelling brings nutrient-rich water to euphotic zone and promotes bacteria growth, increasing the picoplankton biomass which increased the consumption rate of nanoflagellate. 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 abundances of bacteria ($10^5 - 10^6$ cells mL^{-1}) and *Synechococcus* spp. ($10^4 - 10^5$ cells mL^{-1}) within a narrow range, forming a predator-prey eddy. Meanwhile, 62 % of bacteria production and 55 % of *Synechococcus* spp. production are transported to higher trophic level (nanoflagellate), though the cascade effect might cause an underestimation of both percentages of transported carbon. Based on the increasing number of sizes we found in the size-fractionation experiments, we estimated that the predation values were underestimated by 28.3 % for bacteria and 34.6 % for *Synechococcus* spp. Taking these corrections into consideration, we conclude that

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picoplankton production is balanced by nonoflagellate grazing and the diet of nanoflagellate 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 level in a linear food chain via bacteria, nanoflagellates, and ciliates was formalized as the "microbial loop" (Azam et al., 1983). When picophytoplankton was added as a primary producer, this loop became 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. Our previous studies demonstrated that bacteria were mostly consumed by nanoflagellates of size < 6 µm, and *Synechococcus* was consumed mainly by pigmented nanoflagellates of 3–10 µm in subtropical western Pacific coastal ecosystem (Chan et al., 2009; Tsai et al., 2011).

Factors that regulate the standing stock of picoplankton include bottom-up control on the growth environment (temperature, nutrients, and substrate supply) (Almeida et al., 2001; Schultz Jr. et al., 2003; Ameryk et al., 2005) as well as top-down mortality pressure, especially 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 grazer (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 nature environment, however, suggests a typical bottom-up control relationship. In

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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 bacteria production and bacteria biomass (Ducklow, 1992), to the coupling between the abundance of bacteria and their main predator (heterotrophic nanoflagellate; Gasol 1994; Gasol et al., 2002), and to the relationship between bacteria growth rate and bacteria 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 top-down in most oligotrophic situations and 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). Tanaka et al. (1997) proposed a predator-prey eddy to illustrate the temporal variation in the numerical relationship between nanoflagellate and bacteria. On annual scales, the position and magnitude of the eddy differed between seasons due to changes in environmental conditions. It has been hypothesized that the formation of the predator-prey eddy is promoted by nanoflagellate grazing process (Tanaka and Taniguchi, 1999). However, we know little about how growth and mortality rates regulate the spatial dynamic of bacterial community and the predator-prey eddy does little to explain this relationship.

This study investigated the impact of the substrate supply and the grazing of nanoflagellate on picoplankton communities in an oligotrophic pelagic marine ecosystem (Taiwan Warm Current Water) of the subtropical western Pacific during the summer season (June to September). We investigate the existence of a predator-prey eddy of nanoflagellate-bacteria association in spatial scale and identify the mechanisms underlying the formation of predator-prey eddy.

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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). Seawater for microscopic counting of picoplankton (bacteria and *Synechococcus* spp.) and nanoflagellate was collected by 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 bottom using a Sea Bird CTD-General Oceanic Rosette.

2.2 Flow cytometric 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° for later analysis (Campbell and Vaulot, 1993). Abundances of picoplankton (heterotrophic bacteria and *Synechococcus* spp.) were determined by flow cytometry (Marie et al., 1997) using an 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 in a plot of fluorescence FL3 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 enumeration of nanoplankton, 50 mL water samples were fixed with glutaraldehyde to a final concentration of 1 % (Christaki et al., 2002; Sanders et al., 2000). Subsamples (20 mL each) for pigmented and non-pigmented cells were filtered onto a 0.8 µm

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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 epifluorescence microscope at 1000 × (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 fractionation method (Wright and Coffin, 1984) at seven stations, including two coastal stations (St. 1 and 2), two middle stations usually influenced by oligotrophic Taiwan Current Warm Water (St. 5 and 6), and three offshore stations often affected by Kuroshio Upwelling Water (St. 9 and 10 or 11) (Gong et al., 1996). At each station, surface seawater sample was collected. One subsample (500 mL each) was filtered through a 2 µm pore size polycarbonate membrane to remove predators of bacteria and *Synechococcus* spp.; another through a 10 µm pore size polycarbonate membrane to remove predators of nanoflagellates. Each size fraction was then transferred into polycarbonate bottles of 500 mL (run in triplicate). The subsamples were incubated in a water bath at in situ temperature and light intensity for 24 h. At the beginning and end of each incubation period, triplicate samples (30 mL) were taken to count the number of pico- and nanoplankton as described above.

Growth rates (μ, d^{-1}) of bacteria and *Synechococcus* spp. (μ, d^{-1}) were calculated on the basis of the results from the < 2 µm filtrates, and those of nanoflagellates were calculated from the < 10 µm filtrates according to the following equation:

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$$\mu = \frac{(\ln N_f - \ln N_i)}{(T_f - T_i)} \quad (1)$$

where N_i and N_f are cell numbers at the initial (T_i) and final (T_f) incubation time corresponding to size fractions.

Grazing rates of nanoflagellate on bacteria and *Synechococcus* spp. (g, d^{-1}) were obtained from the difference of growth rate between the < 2 µm filtrates and the < 10 µm filtrates based on the following equation:

$$g = \mu_{2}\mu m - \mu_{10}\mu m \quad (2)$$

Microbial abundance was converted into carbon biomass (B, µg C L^{-1}) according to the conversion coefficient of 20 fg C $cell^{-1}$ for bacteria (Lee and Fuhurman, 1987), 250 fg C $cell^{-1}$ for *Synechococcus* spp. (Li et al., 1983), and 220 fg C μm^{-3} for nanoflagellates (Børsheim and Bratbak, 1987). For cell volume of nanoflagellates, linear dimensions (length and width) of at least 20 cells were measured in each sample, and the cell volume was calculated as an elliptical sphere.

Production rates (P, µg C L^{-1} d^{-1}) of bacteria and *Synechococcus* spp. were estimated from the < 2 µm filtrates using the following equation:

$$P = \mu \times B_i \quad (3)$$

where B_i is the in situ cell biomass (µg C L^{-1}) at the sampling time i. Production rates (mg C L^{-1} d^{-1}) of nanoflagellates were similarly estimated in the < 10 µm filtrates.

Consumption rates of nanoflagellates (G, µg C L^{-1} d^{-1}) on picoplankton (bacteria or *Synechococcus* spp.) were calculated according to the following equation:

$$G = g \times B_i \quad (4)$$

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3 Results

Spatial changes in temperature and salinity observed during the summer period of 2010 and 2011 are shown in Fig. 2 and Table 1. There was no significant difference in distribution patterns of temperature and salinity through the water column between the five cruises. The distribution pattern of water mass in summer remained typical in southern ECS. Throughout the surface water column water temperature was high and salinity low ($>25^{\circ}$, <34 psu), and under the surface layer of offshore stations (St. 8–12) was Kuroshio subsurface upwelling water ($<22^{\circ}$, >34.5 psu).The upwelling water reached the surface layer or intruded into the coastal area (St. 1–3) along the shelf bottom. We conducted size-fractionation experiments with samples collected from seven stations to measure the growth and grazing rates of nanoplankton at surface waters characterised, according to $T-S$ diagram, as Taiwan Current Warm Water during the study period (Fig. 3; Gong et al., 1996).

Throughout the whole water column, abundnce of bacteria and *Synechococcus* spp. ranged between 7×10^{3}–3.5×10^{6} cells mL^{-1} and $<2.2\times10$–4.7×10^{5} cells mL^{-1}, respectively. Both picoplankton abundances were different, though not significant, between cruises or stations of the same cruise, showing high abundance in surface layer and no close relationship with water mass. Nanoflagellate abundance ranged between 1.7×10–1.5×10^{3} cells mL^{-1}, and varied less than picoplankton. In samples collected from surface water, bacteria and *Synechococcus* spp. abundance ranged from 1.33×10^{4} to 3.3×10^{6} cells mL^{-1} and from 1.0×10^{3} to 4.4×10^{5} cells mL^{-1}, respectively, showing a spatial and temporal variation of 2 orders of magnitude (Fig. 4; Table 1). Nanoflagellate ranged from 6.2×10 to 1.04×10^{3} cells mL^{-1}, and varied within 1 order of magnitude. It was found to be more abundant in Taiwan Current Warm Water than in Kuroshio Water (Fig. 4; Table 1).

Growth rate of bacteria and grazing rate of nanoflagellates on bacteria ranged from 0.22 to 1.99 d^{-1} and from -0.40 to 1.77 d^{-1}, respectively. Bacteria growth rate was negatively correlated with temperature (Fig. 5), suggesting that the active growth of

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heterotrophic bacteria might occur under the influence of cold upwelling water. The growth rate of *Synechococcus* spp. and grazing rate of nanoflagellate on *Synechococcus* spp. ranged from 0.21 to 4.84 d^{-1} and from −0.30 to 1.33 d^{-1}, respectively. The growth rate was not affected by upwelling water. Comparing picoplankton growth rates and nanoflagellate grazing rates, we found a positive relationship between bacteria growth and nanoflagellate grazing (Fig. 6). The high bacteria growth rate corresponded with the high nanoflagellate grazing rate ($p < 0.05$). This study found a weak coupling relationship between *Synechococcus* growth rate and nanoflagellate grazing rate ($p > 0.05$; Fig. 6). However, the spatial variations in picoplankton growth rate showed a negative relationship with picoplankton abundance (Fig. 7), and nanoflagellate grazing rate showed no significant correlation with picoplankton abundance. A negative relationship between net growth rate and abundance in picoplankton was found (Fig. 8).

The production rate of bacteria varied between 1.13 μg C L^{-1} d^{-1} and 58.77 μg C L^{-1} d^{-1} (mean 21.81 μg C L^{-1} d^{-1}) and consumption rates of nanoflagellates on bacteria ranged from −2.30 μg C L^{-1} d^{-1} to 46.33 μg C L^{-1} d^{-1} (mean 21.81 μg C L^{-1} d^{-1}). Both rates showed a close positive correlation with a slope of 0.61 ($p < 0.05$; Fig. 9). A similar positive relationship was also found between the production rate of *Synechococcus* spp. (1.85–32.02 μg C L^{-1} d^{-1}, mean 12.12 μg C L^{-1} d^{-1}) and consumption rates of nanoflagellates on *Synechococcus* spp. (−0.97–45.26 μg C L^{-1} d^{-1}, mean 5.22 μg C L^{-1} d^{-1}) with a slope of 0.55 (Fig. 9).

To characterize the interaction of trophic coupling between picoplankton and nanoflagellates and to estimate the growth rate and grazing rate of picoplankton in the presence of nanoflagellates of different sizes, successive size-fractionation experiments were undertaken in three cruises from June 2011 to September 2006. We truncated the food web by removing organisms in different body sizes (< 2 μm, < 5 μm, < 10 μm, and < 20 μm) (Lin et al., 2009). Our studies have found the trophic cascade effect (e.g., St. 5 of July 2011) and nanoflagellates of size 10–20 μm were the main grazers at some stations (e.g., St. 1 of July 2011) (Fig. 11).

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4 Discussion

Our study of the environment variables in the southern ECS suggest that the marine environment is a typical summer water column. A warm oligotrophic Taiwan Current Warm Water intruded into the southern ECS from the Taiwan Strait and affected the pelagical ecosystem throughout the surface water column in aread we studied. A persistent upwelling system, located at the offshore near Kuroshio, is known to be a major nutrient source. The uplifted depth of the upwelling in summer is controlled by the intensity of southwestern monsoon, which brings a strong Taiwan Strait Warm Current that suppresses the lifting of upwelling water to the surface water column (Gong et al., 1992; Shen et al., 2011).

4.1 Bottom-up versus top-down control of picoplankton

There have been some discussion regarding whether planktonic bacteria abundance is controlled by resource supply such as organic carbon or inorganic nutrient (bottom-up control) or by predation of bacterivores (top-down control) (Sander et al., 1992; Simek et al., 1995; Lee et al., 2001; Murrell, 2003; Bouvy et al., 2004). The relative importance of population regulation is an old topic with many arguments for and against both types of control mechanisms, with the degree of this importance varing by location and period of time (Goosen et al., 1997; Ferrier-Pagés and Gattuso, 1998). However, no theory about the regulation of bacterial stock and production in pelagic ecosystem has been universally accepted (Hariston et al., 1960; Thingstad, 2000). The positive correlations between bactera abundance and phytoplankton, dissolved organic carbon, or inorganic nutrient were used initially as evidence of bottom-up control, and, based on these correlations, conclusions were drawn regarding the prevailing control model in a given ecosystem (Billen et al., 1990; Gasol et al., 2002; Gasol and Duarte, 2000; Duarte and Agustí, 2005; Tsai et al., 2010). In the southern ECS, we found the spatial variation of heterotrophic bacteria abundance at the surface water during summer to be within a narrow range, between 5×10^4 and 3.3×10^6 cells mL^{-1}. This range is

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equivalent to the feeding threshold for nanoflagellate on bacteria (e.g., Fenchel et al., 1982; Andersen and Fenchel, 1985; Wikner and Hagström, 1991). Our study found high bacteria growth rates to occur under low temperatures, hence spatial variability in growth rate of heterotrophic bacteria was negatively influenced by temperature (Fig. 5). This relationship was unexpected because bacteria abundance and production in aquatic ecosystems have been shown to vary positively with temperature (Hoch and Kirchman, 1993; Shiah and Ducklow, 1997; Schultz Jr. et al., 2003) and the importance of temperature as a positive regulator of marine bacteria growth rate is well recognized (White et al., 1991; Tsai et al., 2005, 2008). The low temperature we found suggested that the waters we studied were affected by Kuroshio Upwelling Water. The active growth of heterotrophic bacteria might have been induced by nutrient brought upward by the cold upwelling water, indicating possible bottom-up control. However, we did not observe a significant relationship between the growth rate of *Synechoccous* spp. and temperature, suggesting that the cold upwelling water did not influence the *Synechoccous* spp. community. In addition, we found a negative correlation between growth rate and abundance in bacteria (Fig. 7). Based on the density-dependent logistic growth of bacteria, Wright and Coffin (1984) used an empirical model relating bacteria growth rate with bacteria abundance to estimate the ecological state of bacteria in a given system. When their abundance was close to the carrying capacity, bacteria were limited by resource availability (bottom-up control), a negative relationship between bacteria growth rate and abundance appeared. Generally, bacteria growth appears to be top-down control in most nutrient-poor environments and bottom-up control in eutrophic environments (Gasol et al., 2002). The results of our study are in accordance with this relationship and demonstrate that abundance of resource-dependent bacteria is common in oligotrophic Taiwan Current Warm Water, where influence of high-nutrient upwelling water is prevailing. In other words, the abundance of bacteria is generally regulated by predators in most oligotrophic environments, but may be ameliorated with a sustained resource supply, e.g., from the upwelling system. Our study illustrates that the microbial food web of an oligotrophic ecosystem may be changed

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from top-down control to resource supply (bottom-up control) with the presence of a physical force to bring nutrient into the oligotrophic ecosystem.

4.2 Spatial relation of predator-prey eddy in picoplankton and nanoflagellate

Tanaka et al. (1997) and Tanaka and Taniguchi (1999) proposed predator-prey eddy to describe the temporal variation in the numerical relationship between nanoflagellates and bacteria. In that model, tightly and stably coupled relationships between two components of abundance are confined within a narrow range on short temporal scales and continuously migrate over a certain region with season–bearing-environment factors. Based on the spatial data sets obtained within oligotrophic Taiwan Current Warm Water in summer, we plotted nanoflagellate abundance against bacteria abundance in a phase space (Fig. 4). Data sets of each cruise showed a circular orbit roughly similar to the graphic diagrams in Tanaka et al. (1997) and Tanaka and Taniguchi (1999). Location and magnitude of the orbits were clearly different between the five cruises, so these eddies were confined to a narrow range in spite of sporadic and drastic change of environmental variables. The observed circular orbits, however, sometimes showed wide protrusion where the bacteria abundance had a low density due to the cold upwelling water. Our results support the Tanaka et al. (1997) postulation that the predator-prey eddy of the nanoflagellate-bacteria system also exists in subtropical marine ecosystem and that the eddy can be shown on spatial basis, if it has similar environment condition.

No adequate explanation has been given regarding the mechanism underlying the predator-prey eddy in the nanoflagellate-bacteria system. Tanaka and Taniguchi (1999) suggested that the formation of the predator-prey eddy can be explained by both the intensive feeding by nanoflagellate on increasing bacteria and the inability of nanoflagellate to feed on bacteria if its concentration is lower than feeding threshold value. In the present study, the spatial dynamics of the bacteria and *Synechococcus* spp. communities were affected by both growth and grazing rates. The abundances of bacteria, *Synechococcus* spp., and nanoflagellates clearly varied within a narrow range. However, the spatial variations in picoplankton growth rate showed a negative relationship

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with picoplankton abundance (Fig. 7), and nanoflagellate grazing rate showed no significant correlation with picoplankton abundance. Growth rates of both picoplankton (bacteria and *Synechococcus* spp.) decreased with increasing picoplankton abundance, while picoplankton production rate continuously increased due to the increase of picoplankton biomass ($P = \mu \times B_i$), resulting in a gradual increase in picoplankton production. Moreover, while nanoflagellate grazing rate was not significantly correlated with prey, the consumption rate of nanoflagellates also was enhanced due to increased picoplankton biomass ($G = g \times B_i$). Therefore, both picoplankton production rate and consumption rate increase following the increase in picoplankton biomass. In fact, the predator-prey eddy of the nanoflagellate-picoplankton system is a reflection of the changing net growth rates (growth rate – grazing rate). We found a negative relationship between net growth rate and picoplankton abundance (Fig. 8). The highest net growth rates for both bacteria and *Synechococcus* spp. occurred when the respective abundance was lowest, and the net growth rate sharply decreased following the increases in abundances of the bacteria and *Synechococcus* spp. communities. Subsequently, the net growth rate became negative after abundance reached a density smaller than the threshold value. The abundances of bacteria and *Synechococcus* spp. consequently declined (Fig. 8).

Based on these findings, the observed spatial variations in abundance (predator-prey eddy) can be explained by a scenario in which both picoplankton growth and nanoflagellate grazing influence the dynamics of the bacteria and *Synechococcus* spp. communities. In addition, the growth rate of picoplankton is controlled by its abundance or upwelling water. Under low temperature or low picoplankton abundance, the active growth of heterotrophic bacteria might be induced by nutrient brought upward by the cold upwelling water. Upwelling brings nutrient-rich and low-abundance water to euphotic zone with high temperature, and promotes picoplankton growth, consequently increasing abundance. When abundances reach the threshold value of picoplankton abundance, the growth rate and grazing rate are in balance, and the net growth rate approaches zero. When prey abundances exceed the threshold value, the rate of grazing

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upon them increases and exceedes their growth rate, and their net growth rate becomes negative. Hence, the abundance of bacteria and *Synechococcus* spp. gradually decreases. This scenario has also been used to describe seasonal dynamic of picoplankton community by Tasi et al. (2008) and Kobari et al. (2010).

4.3 Carbon flow in microbial food web in the oligotrophic Taiwan Strait Warm Current Water

If bacteria production is not balanced by grazing, other factors that can cause bacterial loss, such as cell death, viruses, and sedimentation (Pace, 1988), may account for the imbalance. In our study, there was an clear imbalance between picoplankton growth and grazing, and the consumption of nanoflagellate accounted for the removal of about 62 % of bacteria production and 55 % of *Synechococcus* spp. production, respectively. We, therefore, suggest that nanoflagellates are major consumers of picoplankton (Fig. 9).

Nanoflagellates are known to potentially be able to regulate the production and abundance of picoplankton and are, therefore, thought to play a key role in the transfer of picoplanktonic carbon to higher trophic levels (Hahn and Hofle, 2001; Tsai et al., 2005). This study evaluated the dynamics of bacteria, *Synechococcus* spp., and nanoflagellates affecting the energy flow in the microbial food web in an oligotrophic subtropical pelagic marine ecosystem. For bacteria, the production and grazing carbon fluxes ranged from 1.13 to 58.77 and −2.30 to 46.33 $\mu g\,C\,L^{-1}\,d^{-1}$, respectively; likewise for *Synechococcus* spp., these ranges were 1.85 to 32.02 and −0.97 to 45.26 $\mu g\,C\,l^{-1}\,h^{-1}$, respectively. We also found that 64 % and 36 % of carbon consumed by nanoflagellates came from bacteria and *Synechococcus* spp., respectively. A significant part of bacteria and *Synechococcus* spp. carbon was channeled through the microbial food web, possibly making it an important link between primary production and higher trophic levels (Fig. 10).

Due to the trophic cascade effect (e.g., St. 5 of July 2011) and the main grazers of nanoflagellates of size 10–20 µm, the consumption rate of nanoflagellate could be

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underestimated (Fig. 11). We tried to correct the grazing rate of nanoflagellate by the maximum grazing rate in three treatments of successive size-fraction experiments. The corrected result showed that the consumption rate of nanoflagellate could be underestimated about 28.3 % for bacteria and 34.6 % for *Synechococcus* spp., respectively. The corrected result of our study clearly indicates that picoplankton production was balanced by nonoflagellate grazing and that there was a close coupling trophic relationship between picoplankton and nanoflagellate.

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Table 1. The surface temperature, salinity, NO_3, and Chl *a*, as well as the abundance and biomass of bacteria, *Synechococcus* spp. and total nanoflagellate.

		2–5 Aug 2010	23–25 Aug 2010	8–10 Jun 2011	1–3 Aug 2011	4–6 Sep 2011
T (°)		27.1 ± 1.4	28.7 ± 0.7	25.0 ± 0.9	28.3 ± 0.7	26.5 ± 2.4
S (psu)		33.9 ± 0.3	33.8 ± 0.1	33.7 ± 0.8	33.7 ± 0.2	33.9 ± 0.3
NO_3 (µM)		0.025 ± 0.05	0.27 ± 0.75	0.21 ± 0.52	0.07 ± 0.16	0.59 ± 0.94
Chl *a* (mg m^{-3})		0.89 ± 0.69	0.39 ± 0.42	1.57 ± 2.18	0.32 ± 0.13	0.96 ± 1.02
Bacteria	abun.	3.0 ± 1.4	10.5 ± 7.3	6.2 ± 6.1	13.9 ± 3.7	18.7 ± 9.2
	bio.	7.6 ± 3.8	22.3 ± 13.7	12.4 ± 12.2	27.9 ± 7.3	37.3 ± 18.3
Syn.	abun.	72.1 ± 117.4	59.9 ± 54.0	52.3 ± 34.5	25.1 ± 28.8	67.0 ± 85.0
	bio.	18.8 ± 29.0	16.2 ± 14.0	13.1 ± 8.6	6.3 ± 7.2	16.8 ± 21.3
TNF	abun.	674.2 ± 227.4	715.9 ± 169.7	569.3 ± 206.2	322.1 ± 114.2	300.9 ± 146.9
	bio.	3.2 ± 2.1	3.6 ± 1.3	6.7 ± 2.8	3.8 ± 2.2	3.8 ± 2.8

All data were taken from Sea Bird CTD-General Oceanic Rosette assembly with 20 L Go-Flo bottles from the surface water. T, temperature; S, salinity; *Syn.*, *Synechococcus*; TNF, total nanoflagellate; abun., abundance (10^5 cells mL^{-1} in bacteria and 10^3 cells mL^{-1} in *Synechococcus*); bio., biomass (µg C L^{-1}).

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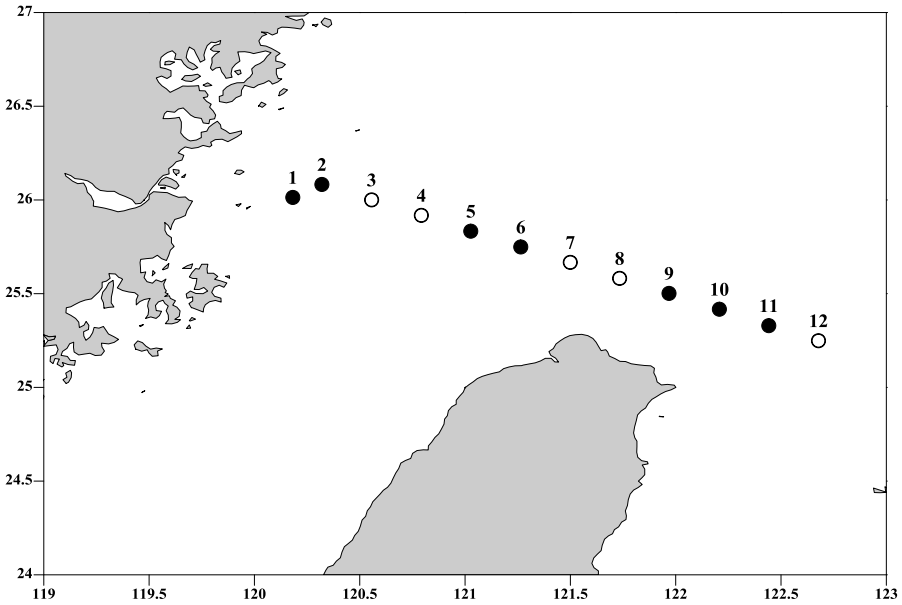

Fig. 1. Sampling stations 1–12 were located along a cross-shelf transect in the southern East China Sea on the five cruises of present study during the summer period of 2010 and 2011. Solid circles indicate the stations where culture experiments with fractionation method were performed.

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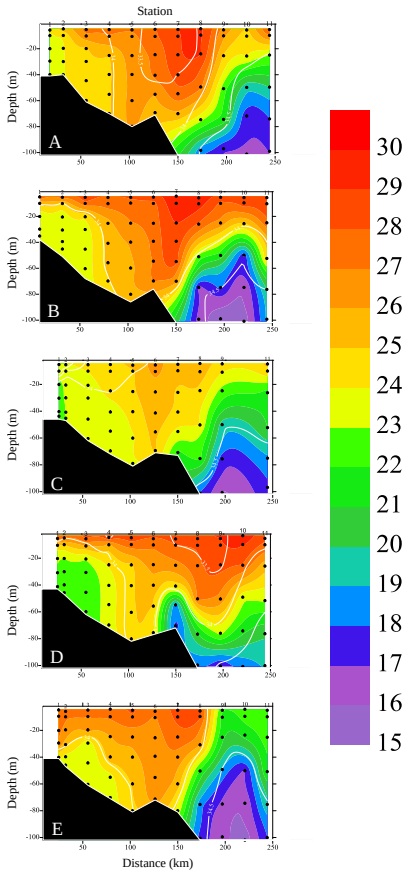

Fig. 2. Vertical profiles of temperature (°C) and salinity (psu, solid line) along a cross-shelf transect on **(A)** 2–5 August 2010, **(B)** 23–25 August 2010, **(C)** 8–10 June 2011, **(D)** 1–3 August 2011 and **(E)** 4–6 September 2011.

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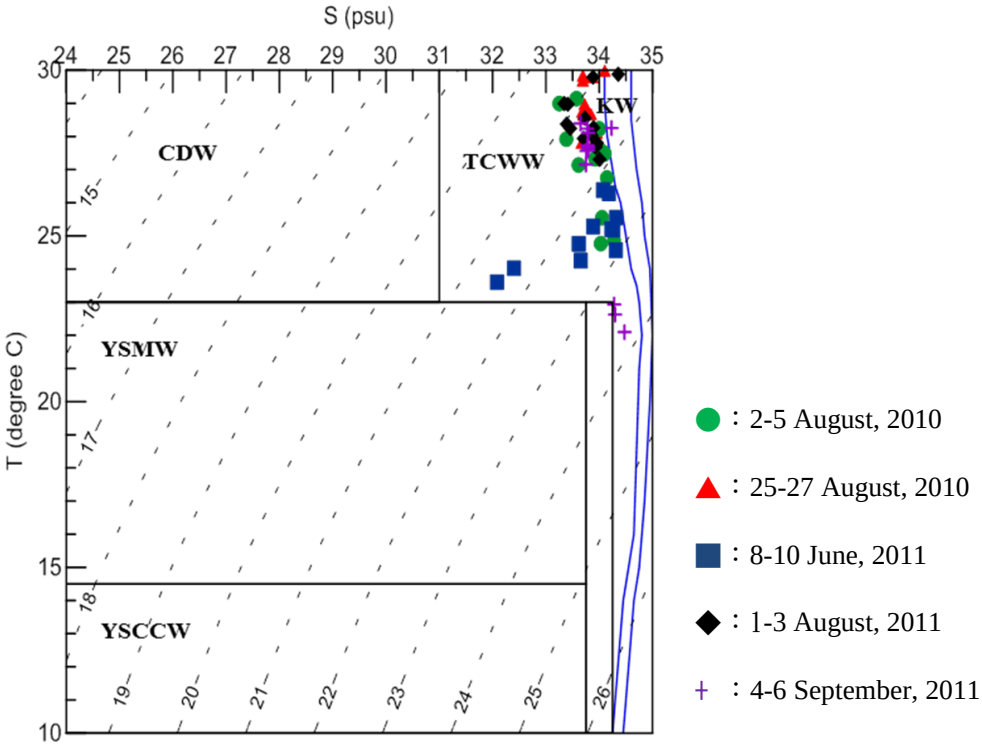

Fig. 3. The surface $T - S$ relationship of sea water in the five cruises of present study. CDW, Chiangjiang Diluted Water; KW, Kuroshio Water; TCWW, Taiwan Current Warm Water; YSCW, Yellow Sea Cold Water; YSMW, Yellow Sea Mixed Water.

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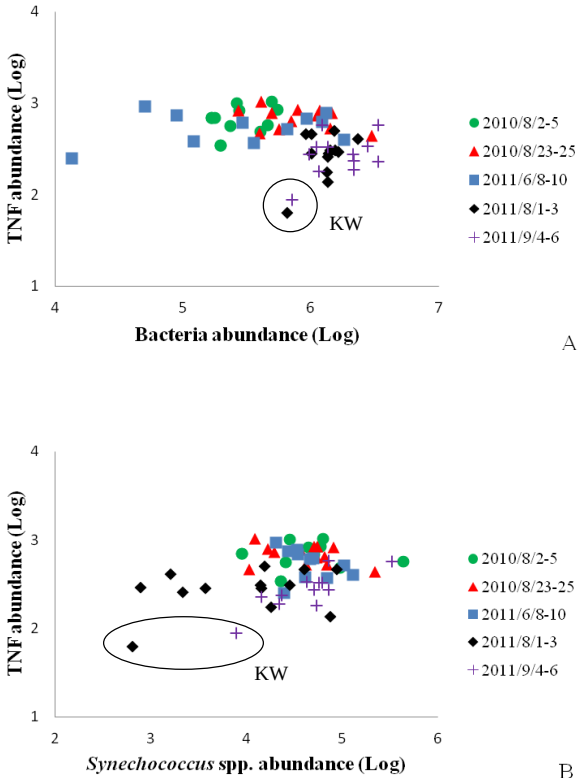

Fig. 4. The spatial relationship between the abundance of total nanoflagellate and picoplankton and abundance of **(A)** bacteria and **(B)** *Synechococcus* spp. for the surface of all stations in the five cruises of the present study. KW, Kurashio Water; TNF, total nanoflagellate.

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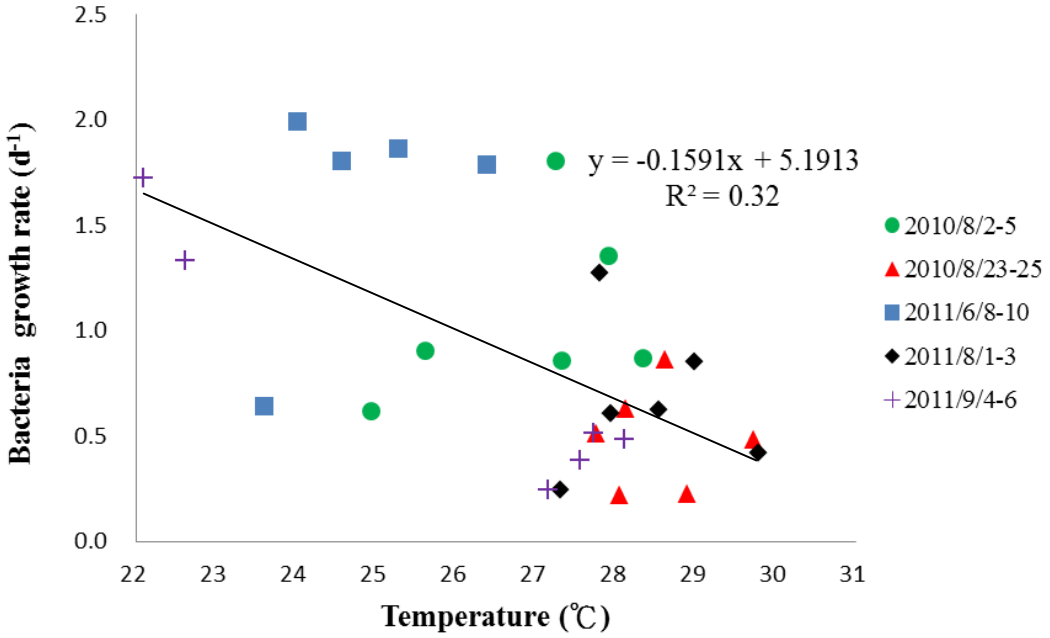

Fig. 5. The relationship between specific growth rate of bacteria and temperature of surface water at culture experiment stations.

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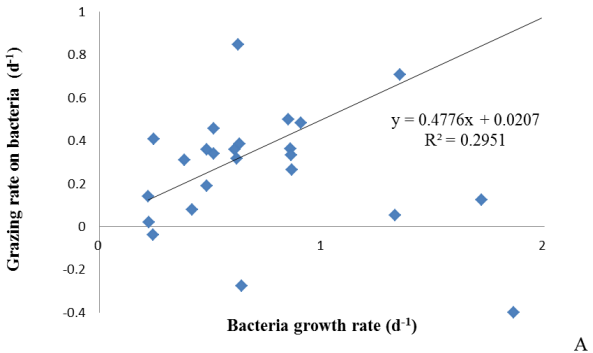

Fig. 6. The relationship between picoplanktonal specific growth rate and nanoflagellate grazing rate on picoplankton. **(A)** Bacteria and **(B)** *Synechococcus* spp.

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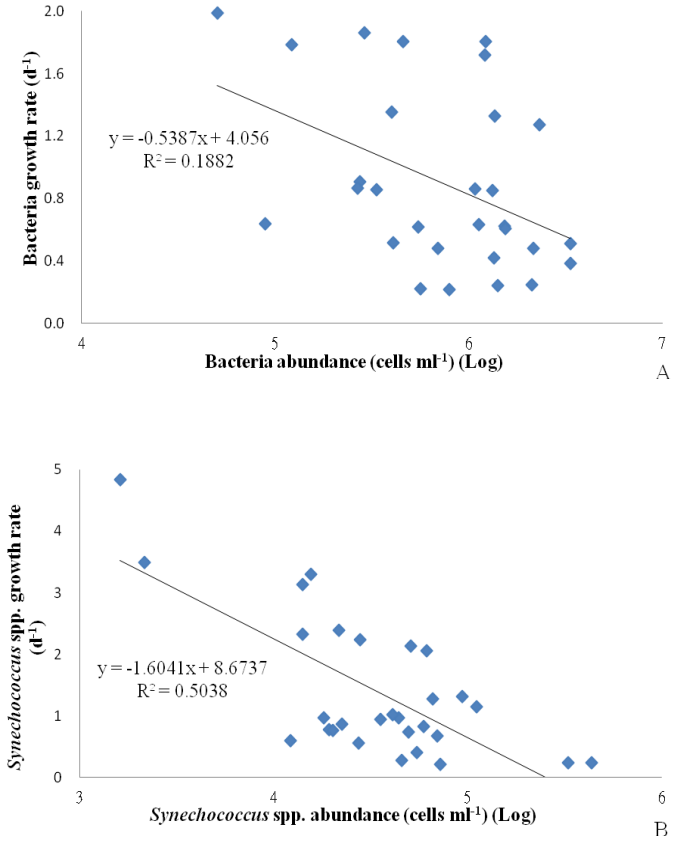

Fig. 7. The relationship between abundance and specific growth rate of picoplankton. **(A)** Bacteria; **(B)** *Synechococcus* spp.

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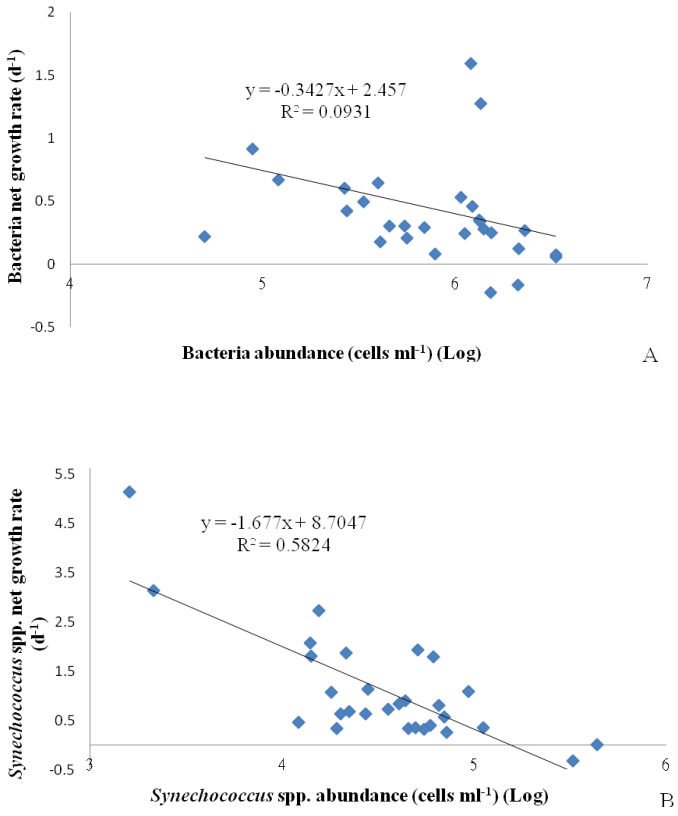

Fig. 8. The relationship between net growth rate and abundance of picoplankton. **(A)** Bacteria; **(B)** *Synechococcus* spp.

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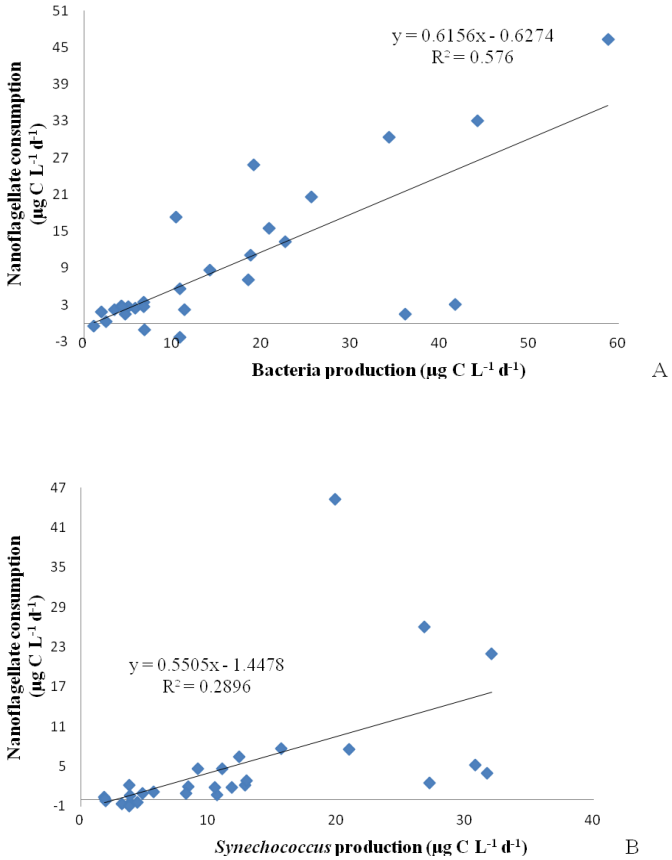

Fig. 9. The relationship between nanoflagellate consumption rate on picoplankton and picoplankton production rate. **(A)** Bacteria; **(B)** *Synechococcus* spp.

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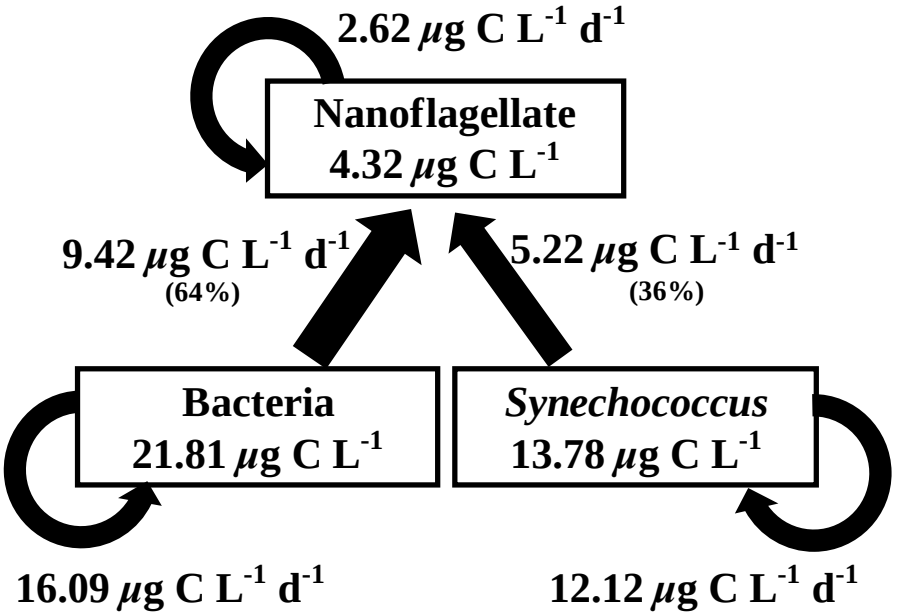

Fig. 10. Schematic carbon flow diagram depicting warm seasonal variation in energy transfer of picoplankton production to nanoflagellates in oligotrophic Taiwan Current Warm Water, the Subtropical Pelagic Continental Shelf Ecosystem. The numbers within individual picoplankton and nanoflagellates boxes refer to their biomass. The numbers next to looped arrow represent picoplankton production rate (μg C L^{-1} d^{-1}). Straight arrow pointing to nanoflagellate show their grazing rate (μg C L^{-1} d^{-1}).

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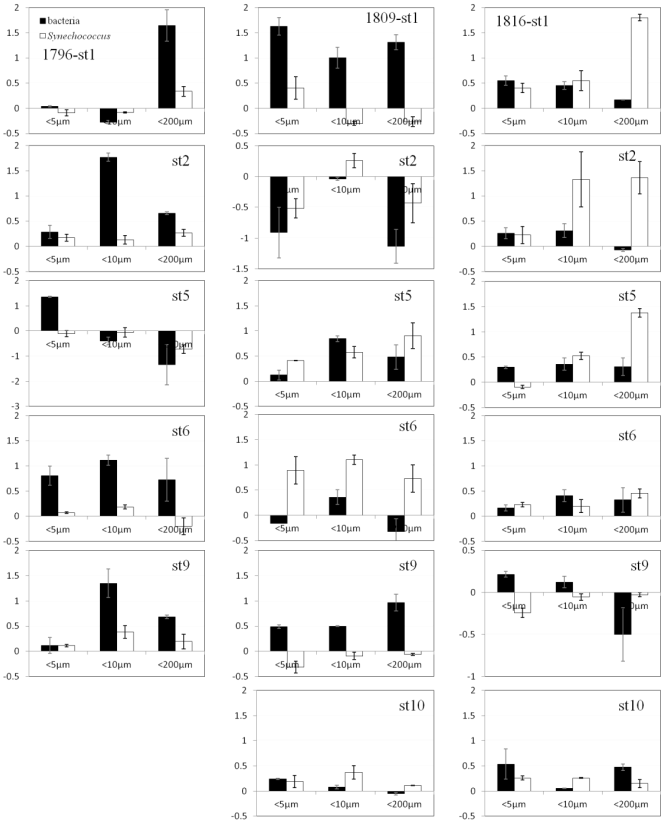

Fig. 11. Effect of removing different size classes of picoplankton. Treatments of seawater samples filtered through 5, 10 and 200 μm filters. The unit on y-axis is d^{-1}.

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