Picoplankton Dynamics and Their Trophic Roles in the Microbial Food-Web Processes in the Southern East China Sea Upwelling Region During Summer

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ABSTRACT

We monitored the abundance and biomass of nanoflagellates and their potential prey, heterotrophic bacteria, and *Synechococcus* spp. five times during the summers in 2010 and 2011. We used size-fractionation to measure growth and grazing rates of heterotrophic bacteria and *Synechococcus* spp. Temporal changes in surface water chlorophyll *a* (Chl *a*) concentrations during our study were caused by an influx of nutrient-rich water upwelling into the surface water. The bacterial growth rates ranged from 0.01 to 0.08 h^{-1} and grazing rates from 0 to 0.06 h^{-1}. Bacterial growth rate had a positive relationship with Chl *a* concentration. Furthermore, growth and grazing rates of *Synechococcus* spp. ranged from 0.01 to 0.09 and 0 to 0.02 h^{-1}, respectively. During the study period about 68% of the bacterial production and 41% of the *Synechococcus* spp. production was grazed by nanoflagellates in the southern East China Sea upwelling region, thus, a large fraction of bacterial biomass is transferred to higher trophic levels via nanoflagellate grazing.

1. INTRODUCTION

Ubiquitous heterotrophic bacteria dominate the mineralization of dissolved organic matter (DOM) in the sea (Azam and Hodson 1977). It is generally agreed that bacteria in the open ocean derive their nutrition from DOM originally derived from phytoplankton. In the pelagic zones, primary production is generally thought to be sustained more by the recycling of growth-limiting nutrients than by the diffusion of nutrients through the pycnocline. In upwelling systems, autochthonously produced DOM is an important component of C and N budgets (Hill and Wheeler 2002; Wetz and Wheeler 2004), and bacterial abundance is closely related to the upwelling cycle and production of phytoplankton (Bak and Nieuwland 1993; Wetz and Wheeler 2004). For instance, in the upwelling area off Concepción, central Chile, bacterial production represents 18.9 - 28.6% of the primary production. Significant correlations have been

found between primary and bacterial production as well as between bacterial abundance and chlorophyll *a* (Chl *a*) (Cuevas et al. 2004).

Picophytoplankton contributes significantly to the plankton biomass and productivity in oligotrophic regions of the ocean (Gradinger et al. 1992; Iriarte and Purdie 1994; Agawin and Agustí 1997). In oceanic upwelling systems, larger algal species, especially diatoms, often dominate the phytoplankton community (Lassiter et al. 2006). However, phytoplankton of smaller size fractions have been observed to respond faster to the upwelling events than those of larger size fractions by Lips and Lips (2010). Likewise, *Synechococcus* spp. has been found to vary positively with Chl *a* concentrations and dominate the phytoplankton community's response in a coastal upwelling system (Garrison et al. 2000; Tsai et al. 2010b). However, the factors that regulate size structure of phytoplankton under different trophic conditions are still being debated (Agawin et al. 2000; Echevarría et al. 2009). For instance, in the Oregon coastal upwelling ecosystem, Sherr et al. (2005) found a negative relationship

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between integrated abundance of picophytoplankton cells and Chl *a* concentrations.

Nanoflagellates play an important role in microbial food webs, where they are a major cause of mortality for smaller primary producers (e.g., *Synechococcus* spp.) as well as heterotrophic bacteria (Sanders et al. 2000; Sherr and Sherr 2002; Tsai et al. 2005). Highly productive upwelling systems are commonly characterized as having short food chains composed of large-sized phytoplankton, zooplankton and pelagic fish (Ryther 1969). However, the microbial components of food webs are ubiquitous with important features of both oligotrophic and eutrophic systems, including seasonally variable upwelling areas (Cuevas et al. 2004; Worden et al. 2004; Vargas et al. 2007). Protists in upwelling regions can be an important component of the plankton community (Neuer and Cowles 1994; Vargas and González 2004). Cuevas and Morales (2006) observed that, in the northern Humboldt Current System upwelling area, for example, nearly 100% of the bacterial production and 17% of the cyanobacterial production is grazed by nanoflagellates in the water column.

Extending from the coast of mainland China to the offshore region northeast of Taiwan, the southern East China Sea (ECS) is a highly dynamic region because of the interaction of different water types, including China Coastal Water, Taiwan Current Warm Water and Upwelling Water (Gong et al. 1996). The upwelling events are particularly important in the pelagic ecosystem of the southern ECS, because the input of nutrients into the euphotic zone during summer makes it possible for an increase in microbial biological production when an oligotrophic situation is expected. In the southern ECS, hydrographic and nutrient conditions regulate the phytoplankton biomass and primary production (Liu et al. 1995). Previous studies have shown

that microbial populations are affected by environmental factors in the ECS (Jiao et al. 2005; Tsai et al. 2010a; Tsai et al. 2011). There are few ecological studies on the distributions of microbial populations in the upwelling region of southern ECS. In this investigation, we studied summertime dynamics and the trophic role of the nanoflagellate assemblage in the microbial food web of an upwelling region of the southern ECS. To do this, we monitored the cell abundance and biomass of nanoflagellates in this region for two years. In addition, using size-fractionation, we measured the growth and grazing rates of the picoplankton nanoflagellate prey (heterotrophic bacteria and *Synechococcus* spp.).

2. MATERIAL AND METHODS

2.1 Sampling

We collected samples from 13 stations in the southern ECS along an inshore-offshore transect on board the R/V Ocean Researcher II (Fig. 1). Samples were collected during five short cruises: two in August 2010 (2 - 5 August and 23 - 25 August 2010) and three in 2011 (8 - 10 June, 1 - 3 August, and 4 - 6 September). The hydrography along this transect is fairly well-described (Gong et al. 1996; Chiang et al. 1997) and it is known to be influenced by different water masses, including China Coastal Water, Taiwan Current Warm Water and Upwelling Water (Gong et al. 1996). Seawater was collected using a SeaBird CTD-General Oceanic Rosette assembly with 20 L Go-Flo bottles at different water depths. Temperature and salinity was measured in depth profiles with a SeaBird CTD.

For Ch1 *a* analysis 1 L water samples were first filtered (25 mm GF/F) and stored at -20°C in darkness. Samples were then extracted in cold 90% acetone in darkness for 24 h before analysis using a Turner Design Model 10-AU-005
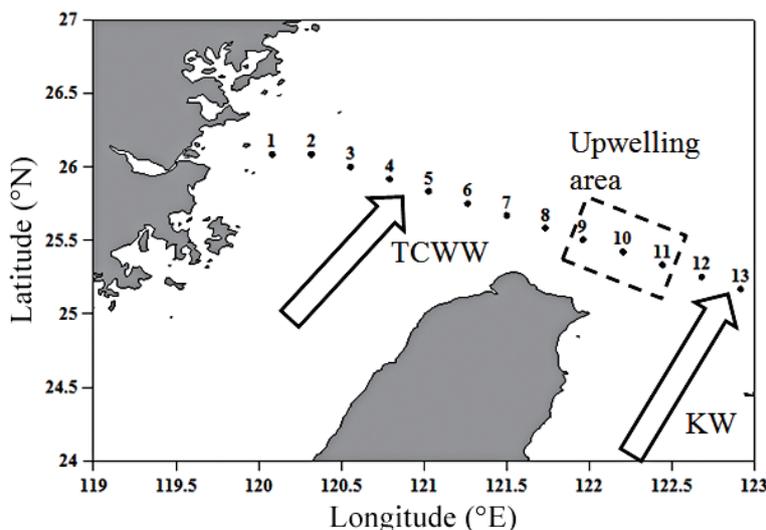

Fig. 1. Location of sampling site in the southern East China Sea. TCWW: Taiwan Current Warm Water, KW: Kuroshio Water. The region between stations 9 and 11 is the upwelling system.

fluorometer (Parsons et al. 1984). Water samples for nutrient analyses were placed in clean 100-mL polypropylene bottles and frozen immediately with liquid nitrogen. Nitrate was reduced to nitrite with a cadmium wire, being activated with copper sulfate solution, and was then analyzed with a self-designed flow injection analyzer (Gong et al. 1992). Primary productivity (PP) in surface waters was measured by ^{14}C assimilation method (Parsons et al. 1984). Water samples were pre-screened through 200 μm woven mesh (Spectrum) to remove large organisms and particles. Next, two light and one dark 250 mL clean polycarbonate bottles (Nalgene) were filled with filtrate and inoculated with 10 μCi NaH^{14}CO$_3$. Primary productivity samples were incubated on deck from dawn to dawn and cooled with running seawater. Duplicate light bottles from a given depth were covered with several layers of nylon stockings to simulate local light penetration.

2.2 Bacteria, *Synechococcus* Spp., Heterotrophic Nanoflagellates (HNF) and Pigmented Nanoflagellates (PNF)

To count bacteria, *Synechococcus* spp., HNF and PNF, we fixed 50 ml water samples with glutaraldehyde to a final concentration of 1% (Christaki et al. 1999; Sanders et al. 2000), with 1 - 2 or 20 mL filtered onto 0.2 or 0.8 μm black Nuclepore filters for picoplankton (bacteria and *Synechococcus* spp.) and nanoflagellates, respectively. Samples were stained with DAPI at a final concentration of 1 μg mL^{-1} (Porter and Feig 1980). PNF and HNF were counted based on the presence or absence of chlorophyll autofluorescence using a separate filter set optimized for chlorophyll or DAPI under a 1000X epifluorescence microscope (Nikon-Optiphot-2). Bacteria and HNF were identified by their blue fluorescence under UV illumination. *Synechococcus* spp. and PNF were identified by their orange and red autofluorescence under blue excitation light. To obtain reliable estimates of abundance, we counted 30 and 50 fields of view for bacteria and nanoflagellates, respectively.

2.3 Growth and Grazing Estimates

Using size-fractionation (Wright and Coffin 1984) we estimated heterotrophic bacteria and *Synechococcus* spp. growth and grazing rates near the upwelling area during the study period (Fig. 1). All water samples were collected from 5 m depth and used for the following size fractionations. Samples were treated twice to remove predators of different sizes. A 2-μm pore size polycarbonate filter was used to remove bacteria and *Synechococcus* spp. predators, and a 10-μm pore size polycarbonate filter was used to remove nanoflagellate predators. The size fractionation used for grazers (< 10 μm) was chosen based on previous studies at this site to eliminate ciliates but not nanoflagellates (Tsai et al. 2008). Each size fraction was then transferred

into polycarbonate bottles to a volume of 125 mL (run in triplicate). The bottles were incubated in a water bath at in situ temperature and under natural light for 8 h. At the beginning and end of each incubation period triplicate samples (30 mL) were taken to count pico- and nanoplankton as described above.

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

$$\mu = (\ln N_f - \ln N_i)/(T_f - T_i) \tag{1}$$

where N_i and N_f are cell concentrations (cell mL^{-1}) at the beginning (T_i) and end (T_f) of the incubation period in corresponding size fractions.

The grazing rates (g, h^{-1}) of bacteria and *Synechococcus* spp. were calculated based on the results obtained from the difference between < 2 and < 10 μm filtrate growth rates.

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

where $\mu_{2\mu m}$ and $\mu_{10\mu m}$ are the growth rates of bacteria and *Synechococcus* spp. in the < 2 and < 10 μm filtrates.

Microbial abundance was converted into carbon biomass (B, μg C L^{-1}) using the following equation:

$$B = N \times C \tag{3}$$

where N is cell density (cells mL^{-1}) and C is estimated cell carbon content (fg C cell^{-1}).

Cell carbon was estimated using 15 fg C cell^{-1} for bacteria (Lee and Fuhrman 1987), 250 fg C cell^{-1} for *Synechococcus* spp. (Kana and Glibert 1987) and 220 fg C μm^{-3} for nanoflagellates (Børsheim and Bratbak 1987). Linear dimensions (length and width) of at least 20 cells were measured for each sample, with the cell volume calculated as an elliptical sphere to provide the cell volume.

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

$$P = \mu \times B_i \tag{4}$$

where B_i is the in situ cell biomass (μg C L^{-1}) at the sampling time.

Furthermore, consumption rates (G, μg C L^{-1} h^{-1}) of bacteria and *Synechococcus* spp. were estimated using the following equation:

$$G = g \times B_i \tag{5}$$

3. RESULTS

3.1 Physical and Chemical Environment

Water temperature ranged from 15.4° to 29.9°C, with higher values in the shallower layers (depth < 20 m). Deeper temperature and salinity isolines indicated upwelling events occurred mainly between stations 9 and 11 (Fig. 2). Furthermore, surface nitrate concentrations generally decreased from the coastal station towards the offshore station (Figs. 3a, c, e, g, i). The upwelling events that occurred during all of the cruises brought high nitrate concentrations (> 10 μm) into the upper water column layers (Figs. 3a, c, e, g, i).

3.2 Chl *a*, Bacterial, *Synechococcus* Spp. and Nanoflagellate Abundance Variations in the Surface Water

Chl *a* concentrations in the surface water ranged from 0.1 to 3.2 mg m^{-3}(Figs. 3b, d, f, h, j). The concentrations and distributions of Chl *a* appeared typical of those found in the ECS, with relatively high concentrations generally found in the coastal waters (Figs. 3b, d, f, h), except for the September 2011 cruise (Fig. 3j). High levels of Chl *a* were usually observed at the outer fringe of the upwelling. The injections of nutrients by the upwelling water into the upper layers of stations 9 to 11 were followed by increases in Chl *a* (maximum > 2.5 mg m^{-3}) in September 2011 (Fig. 3j), corresponding to the upwelling event that reached the shallowest depth in the water column.

The pico- and nanoplankton distributions from all depths were compared using data collected at stations 8 to 12 (Figs. 4 and 5). Bacterial and *Synechococcus* spp. abundance ranged from 0.3 to 30 × 10^5 cells mL^{-1} and from 0.5 to 25 × 10^4 cells mL^{-1}, respectively, with higher values found in the shallow layers (depth 0 - 30 m) in the upwelling area (Fig. 4). Furthermore, PNF abundance for all depths peaked between 10 and 60 m (Figs. 5a, c, e, g, i), the highest value being > 40 × 10^2 cells mL^{-1}.
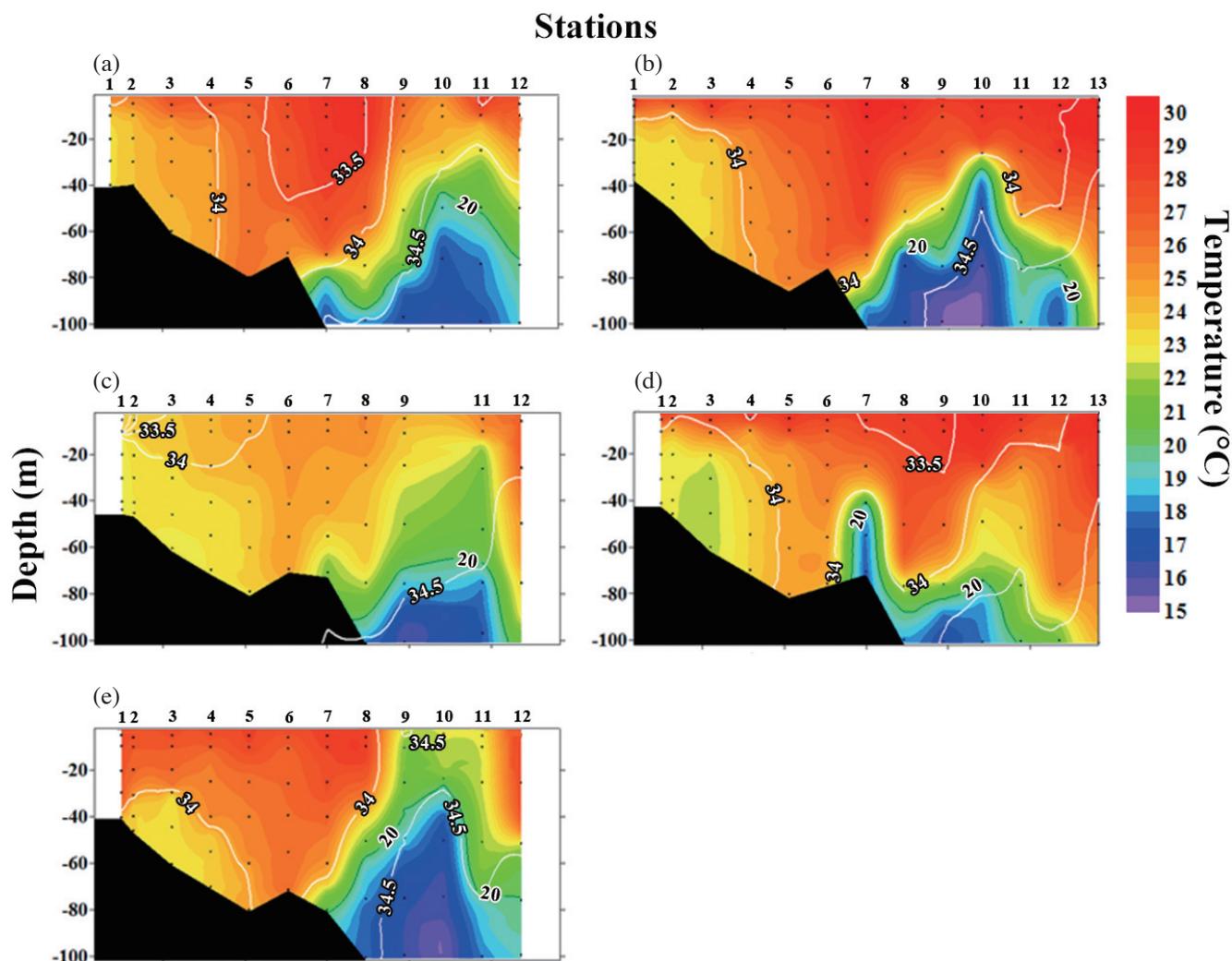
Stations

Fig. 2. Vertical distributions across the temperature and salinity (white line) during each of the cruises; (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.
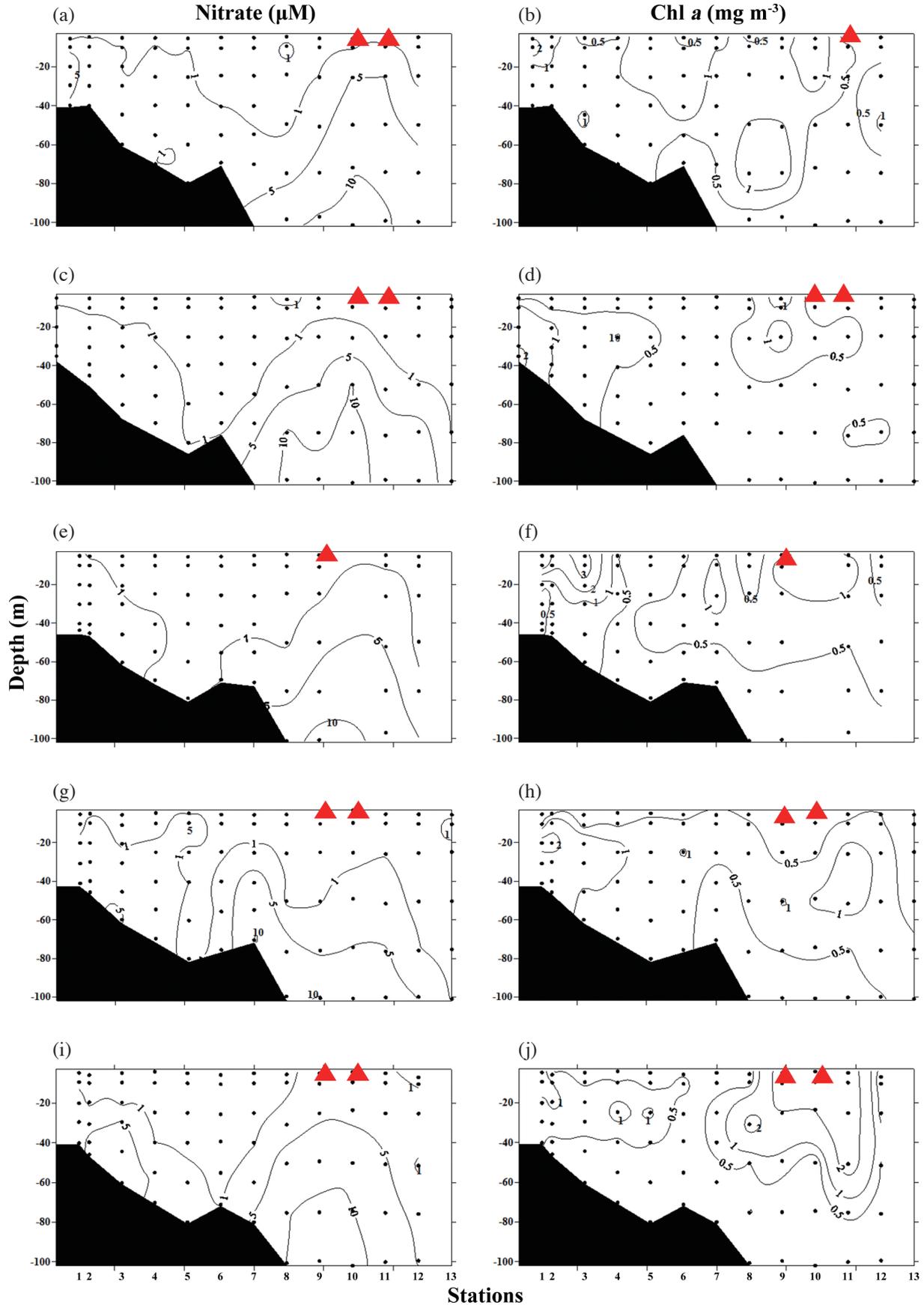
Fig. 3. Vertical variations across the transect in NO$_3$ concentrations (µm, panel a - e) and Chl a concentrations (mg m^{-3}, panel f - j); (a) and (b): 2 - 5 August 2010, (c) and (d): 23 - 25 August 2010, (e) and (f): 8 - 10 June 2011, (g) and (h): 1 - 3 August 2011, and (i) and (j): 4 - 6 September 2011.

bacteria
(10^5 cells mL^{-1})

Synechococcus spp.
(10^4 cells mL^{-1})

Fig. 4. Vertical variations across the transect in bacterial (10^5 cells mL^{-1}, panel a - e) and *Synechococcus* spp. abundance (10^4 cells mL^{-1}, panel f - j); (a) and (b): 2 - 5 August 2010, (c) and (d): 23 - 25 August 2010, (e) and (f): 8 - 10 June 2011, (g) and (h): 1 - 3 August 2011, and (i) and (j): 4 - 6 September 2011. The red symbol (▲) shows the sampling site for the incubation experiments.

Fig. 5. Vertical variations across the transect in pigmented nanoflagellate (PNF, 10² cells mL⁻¹, panel a - e) and heterotrophic nanoflagellate abundance (HNF, 10² cells mL⁻¹, panel f - j); (a) and (b): 2 - 5 August 2010, (c) and (d): 23 - 25 August 2010, (e) and (f): 8 - 10 June 2011, (g) and (h):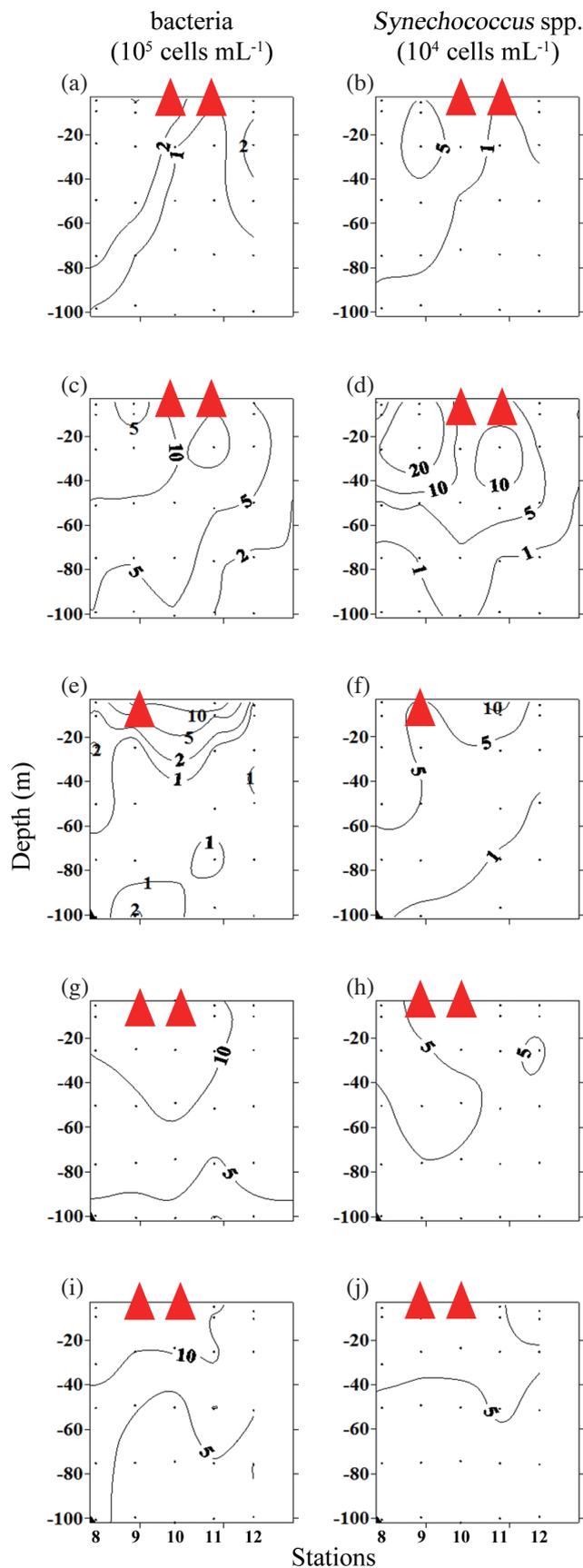 1 - 3 August 2011, and (i) and (j): 4 - 6 September 2011. The red symbol (▲) shows the sampling site for the incubation experiments.

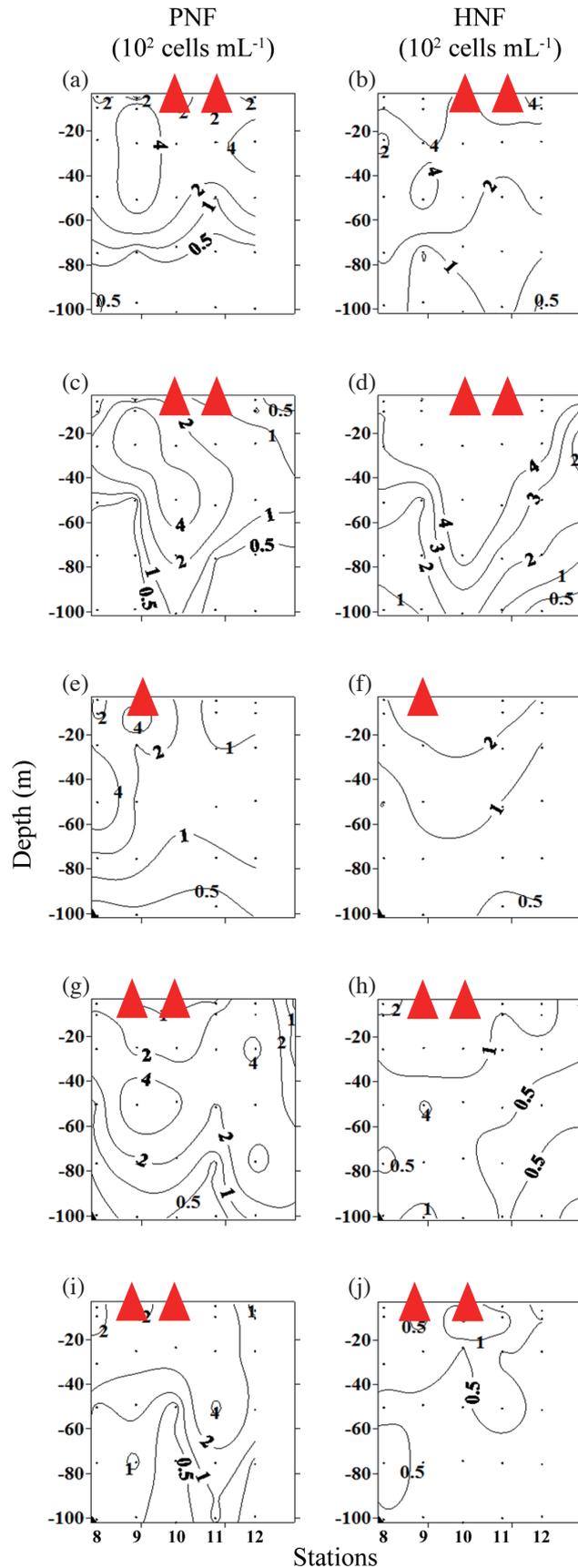Analyzing the surface water (≤ 10 m) data set for the upwelling area, we found weakly significant positive relationships between bacterial, *Synechococcus* spp. and picoplankton biomass (bacterial and *Synechococcus* spp. biomass) and HNF biomass (bacteria: $r = 0.49$, $p < 0.05$; *Synechococcus* spp.: $r = 0.57$, $p < 0.05$; picoplankton: $r = 0.6$, $p < 0.05$) (Fig. 6), which suggested that the distributions of HNF was influenced by food availability.

3.3 Pico- and Nanoflagellate Growth and Grazing Rates

Nine experiments were prepared with seawater samples showing contrasting *in situ* temperature, salinity, NO_3, Chl *a* and PP (Table 1). As can be seen in Table 2, a summary of pico- and nanoflagellate grazing and growth coefficient estimates based on data obtained by size-fractionation, the growth and grazing rates of bacteria ranged from 0.01 to 0.08 h^{-1} and from 0 to 0.06 h^{-1}, respectively. The results showed a positive and significant correlation between Chl *a* concentrations and bacterial growth rates (Fig. 7). Furthermore, the growth rates of *Synechococcus* spp. varied between 0.01 d^{-1} and 0.09 h^{-1} during the summer period (Table 2).

HNF and PNF growth rates ranged from 0 to 0.05 h^{-1} and from 0 to 0.06 h^{-1}, respectively (Table 1). Prey growth was always higher than the nanoflagellate grazing pressure (68% consumption of bacteria and 41% *Synechococcus* spp. production) (Fig. 8).

4. DISCUSSION

Temporal changes in the surface water Chl *a* concentration during our study occurred as a result of an influx of nutrient-rich upwelled water into the surface water. The results showed a positive and significant correlation between Chl *a* concentrations and bacterial growth rates. Previous studies suggest that a nutrient supply carried from deep waters into surface layers can enhance primary production (Bode et al. 1997; Lips and Lips 2010). Previous investigators of one upwelling area used the rate of primary production normalized by the Chl *a* (mg C mg Chl a^{-1} h^{-1}) concentration as an indicator of phytoplankton growth potential (specific-rate of photosynthesis) (Hanson et al. 1986). The specific photosynthesis rates in this study ranged from 1.3 to 5.7 mg C mg Chl a^{-1} h^{-1}, which are relatively low compared to other coastal upwelling
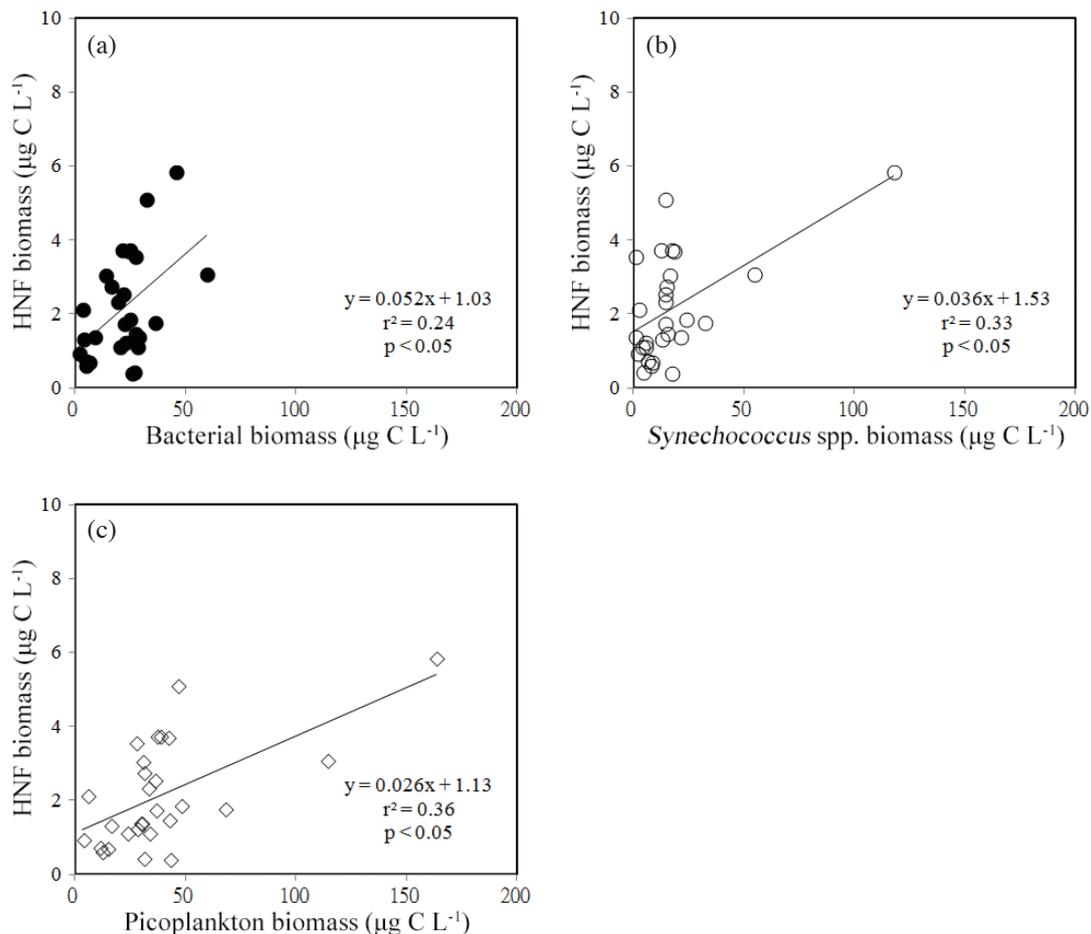

Fig. 6. Relationship between heterotrophic nanoflagellate (HNF) and bacterial biomass (a), *Synechococcus* spp. (b) and picoplankton biomass (bacteria + *Synechococcus* spp.) (c) for all surface water depths (≤ 10 m) in the upwelling region.

Table 1. Physicochemical and biological characteristics in the upwelling region used for the experiments. PP: Primary productivity, —: no data.

Sampling date	station (5m)	temperature (°C)	salinity (psu)	NO3 (µM)	Chl a (mg m^{-3})	PP (mg C m^{-3} d^{-1})
2 - 5 August 2010	St. 10	28.35	34.03	0.10	0.40	—
	St. 11	27.33	34.19	0.00	0.29	14.68
23 - 25 August 2010	St. 10	28.90	33.72	0.29	0.39	53.39
	St. 11	29.73	33.71	0.00	0.36	29.75
8 - 10 June 2011	St. 9	24.57	34.31	0.00	0.72	22.49
1 - 3 August 2011	St. 9	28.99	33.34	0.00	0.22	22.95
	St. 10	29.79	33.88	0.00	0.20	—
4 - 6 September 2011	St. 9	22.09	34.47	3.06	2.66	148.75
	St. 10	22.62	34.30	1.42	2.33	—

Table 2. Picoplankton and nanoflagellate growth and grazing rates for the size-fractionation experiments conducted in the upwelling region. sd: Standard deviation.

		bacteria		*Synechococcus* spp.		HNF	PNF
Sampling date	station (5m)	µ ± (sd) (h^{-1})	g ± (sd) (h^{-1})	µ ± (sd) (h^{-1})	g ± (sd) (h^{-1})	µ ± (sd) (h^{-1})	µ ± (sd) (h^{-1})
2 - 5 August 2010	St. 10	0.04 (0.02)	0.01 (0.01)	0.04 (0.02)	0.01 (0.01)	0.03 (0.04)	0.06 (0.03)
	St. 11	0.04 (0.03)	0.02 (0.02)	0.04 (0.03)	0.01 (0.01)	0.04 (0.03)	0.05 (0.04)
23 - 25 August 2010	St. 10	0.01 (0.01)	< 0.005 (0.005)	0.03 (0.03)	< 0.005 (< 0.005)	0.03 (0.03)	0.02 (0.02)
	St. 11	0.02 (0.02)	0.01 (0.02)	0.05 (0.02)	0.02 (0.02)	0.04 (0.05)	0.03 (0.04)
8 - 10 June 2011	St. 9	0.08 (0.04)	0.06 (0.03)	0.03 (0.02)	0.02 (0.03)	0.05 (0.03)	0.03 (0.03)
1 - 3 August 2011	St. 9	0.04 (0.04)	0.02 (0.03)	0.04 (0.03)	< 0.005 (0.01)	0.01 (0.02)	0.05 (0.04)
	St. 10	0.02 (0.02)	< 0.005 (0.02)	0.15 (0.08)	0.02 (0.02)	< 0.005 (0.01)	0.04 (0.05)
4 - 6 September 2011	St. 9	0.07 (0.03)	0.01 (0.04)	0.01 (0.01)	< 0.005 (0.01)	< 0.005 (< 0.005)	< 0.005 (0.01)
	St. 10	0.06 (0.03)	< 0.005 (0.01)	0.09 (0.06)	0.013 (0.02)	< 0.005 (0.01)	< 0.005 (0.01)

$y = 0.016x + 0.029$
$r^2 = 0.40$
$p < 0.05$
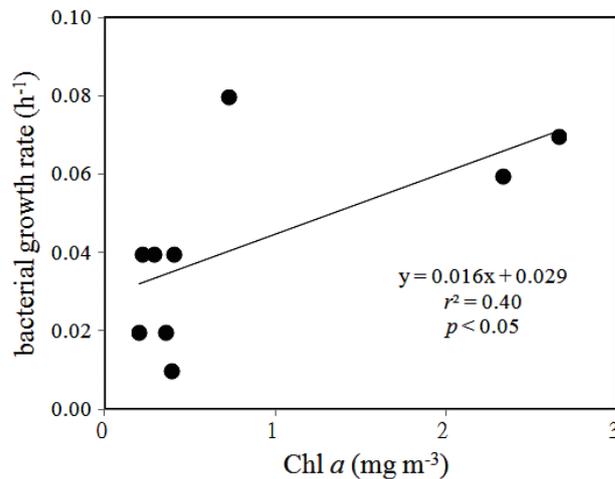
Fig. 7. Relationship between Chl a concentrations and bacterial growth rates during the study period.
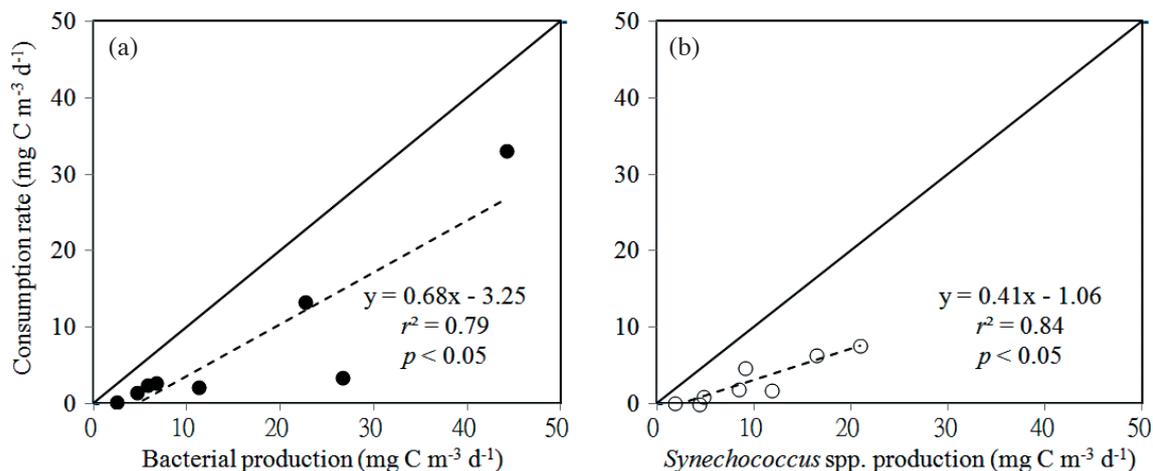
Fig. 8. Relationship between consumption rates and bacterial production (a) and *Synechococcus* spp. production (b). The solid line is 1:1 line.

studies (Malone and Neale 1981; Hanson et al. 1986). These low specific rates indicate that phytoplankton were growing slower and were in bad physiological condition in the surface water of the southern ECS. Harrison et al. (1981) showed that, in nutrient-stressed phytoplankton populations, once NO_3 was depleted, specific rates declined to < 2 mg C mg Chl a^{-1} h^{-1}. This result suggests that phytoplankton populations in the southern of ECS frequently shift between nutrient-limited and growth periods following an influx of upwelled waters. In addition, phytoplankton species composition and pigment composition within a species changes with irradiance levels (Falkowski 1981). However, in the current study light limitation seems unlikely since the Chl a and primary production values were measured in the surface waters during the summer. Phytoplankton growth is also controlled by factors other than light, such as nutrients and temperature. We expected a coupling of temperature and Chl a concentration, given the fact that phytoplankton growth increases with increasing temperature (Eppley 1972). However, we found a negative relationship between the temperature and Chl a concentrations (Fig. 9) ($r = -0.91$, $p < 0.05$). The results from our regression analyses suggest that the upwelling introduced low temperature waters and the nutrient supply associated with upwelling inputs to surface waters accounted for a significant fraction of variability in phytoplankton growth.

Primary production (DOC and POC) is a major source of readily available metabolites for oceanic bacteria (Azam et al. 1983). As has been found in the Humboldt Current System upwelling areas, an important proportion (up to 24%) of the organic matter produced by phytoplankton is channeled through bacteria (Troncoso et al. 2003), making the microbial loop an important pathway for the recycling of organic matter in the upwelling water column. We found a correlation between the bacterial growth rate and Chl a concentration (Fig. 7) but not bacterial growth rate and primary production (Table 1), which is not unusual for

oligotrophic environments, especially in this region. The relationship between bacterial activity and phytoplankton production may be blurred or shifted by other processes. For example, changes in bacterial community structure (Schultz and Ducklow 2000) may change how organic substrates are utilized. Bacterial utilization of DOC and POC from turbulence and the upward displacement of water as well as temporal lags between phytoplankton production and bacterial utilization will obscure such relationships.

In oceanic upwelling systems larger algal species, especially diatoms, often dominate the phytoplankton community (Lassiter et al. 2006). However, previous studies observed that the smaller phytoplankton size fractions showed a faster response to the upwelling events than the larger size fractions (Lips and Lips 2010; Selph et al. 2011). Likewise, other studies report that *Synechococcus* spp. varies positively with Chl a, which could dominate the phytoplankton community response in a coastal upwelling system (Garrison et al. 2000; Tsai et al. 2010b). Conventional thinking is represented by Li (2002), who found inverse relationships between picoplankton cell abundance and biomass relative to total Chl a values from a series of Atlantic Ocean studies, whereas small nanoplankton were relatively invariant across the orders of magnitude of Chl a concentration. More recently, it has been reported that there is a negative trend in picoplankton cell reduction under eutrophic conditions in three coastal systems south of the Iberian Peninsula (Echevarría et al. 2009). Ning et al. (2000) observed that the *Synechococcus* spp. biomass component became significant during periods of low phytoplankton biomass. Our observation of the upwelling area is also consistent with the notion that the *Synechococcus* spp. component of biomass becomes significant during periods of low phytoplankton biomass (Ning et al. 2000).

It is known that nanoflagellates influence the abundance of bacteria and picophytoplankton by grazing (James
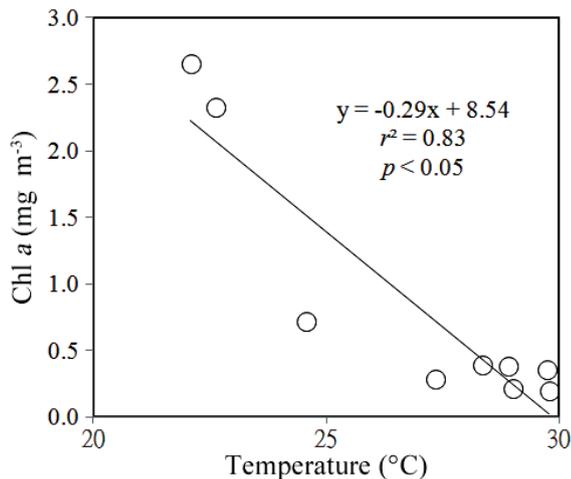
Fig. 9. Relationship between Chl *a* concentration and surface water temperature during the study period.

and Hall 1995; Tsai et al. 2008; Tsai et al. 2010b) and that grazing by nanoflagellates on bacteria and picophytoplankton serves as an essential link in the transfer of carbon to higher trophic levels. From our experiments, the significant, albeit weak, positive correlation of HNF biomasses with the picoplankton biomass (bacterial and *Synechococcus* spp. biomass) is an indication of the potential importance of food availability in the regulation of nanoflagellate abundance (Fig. 6). Furthermore, the overall variability in the relationship between picoplankton and nanoflagellates may reflect changes in the physical and chemical environment. Changing light, temperature and nutrient regimes will affect picoplankton production as well as changes in the rate of production when combined with increased nanoflagellate grazing. Furthermore, it is now well known that dinoflagellates, ciliates and metazooplankton are recognized predators on nanoflagellates, and impact nanoflagellate abundance (Suzuki et al. 1998; Wieltschnig et al. 1999; Berglund et al. 2005). In this upwelling region, a study found that ciliate community grazed about 43% of nanoflagellate production (Chen et al. 2012). Therefore, these ciliates may play an important role linking the nanoflagellate carbon source to higher trophic levels in the southern ECS.

Estimates of nanoflagellate grazing in the upwelling region we studied are also scarce. If we measured grazing rates of the total nanoflagellate assemblage on bacteria and *Synechococcus* spp., nanoflagellates would remove an average of 68% of the bacteria and 41% of the *Synechococcus* spp. production (Fig. 8). Similar results have also been found for the northern Humboldt Current System upwelling area, where there was higher grazing pressure on bacteria than cyanobacteria (Cuevas and Morales 2006). We found an imbalance between picoplankton production and nanoflagellate grazing in this study. Estimates of the impact of ciliate grazing suggest that they can control phytoplankton

production (Verity et al. 1993) and play a significant role in the fate of bacteria (Stelfox-Widdicombe et al. 2000).

In summary, in the upwelling area of the southern ECS system we found a positive and significant correlation between Chl *a* concentrations and bacterial growth rates. These findings also suggest that food availability exerts a great influence in the regulation of nanoflagellate abundance. The results of this study highlight the potentially important role that the nutrient supply associated with upwelling inputs to surface waters accounting for a significant fraction of variability in phytoplankton growth and in regulating the growth of picoplankton and nanoplankton in the southern ECS.

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