

Picoplankton dynamics during contrasting seasonal oceanographic conditions at a coastal upwelling station off Northern Baja California, México

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The ecological dynamics of picoplankton were investigated at a coastal upwelling system of northern Baja California during six cruises (September 2007–November 2008). Populations of *Prochlorococcus*, *Synechococcus*, PicoEukaryotes and heterotrophic bacteria were assessed by flow cytometry (FCM). On each sampling date, we used an abbreviated three-treatment dilution technique and ¹⁴C-uptake experiments to determine population (FCM) and community (TChl *a*) rates of growth, grazing and production from 24-h *in situ* incubations at three to four euphotic depths. Overall, picoplankton comprised an active and important component of the community, with biomass values (2.3–69.8 μg C L⁻¹) and production rates (0.8–68.4 μg C L⁻¹ day⁻¹) that varied positively with Chl *a* and community ¹⁴C-production. The exception was an intense algal bloom (>25 μg Chl *a* L⁻¹) during La Niña-intensified upwelling conditions in April 2008, during which biomass and production estimates of picophytoplankton were at their lowest levels, suggesting that the smallest primary producers were being replaced by larger cells. Thus, for most of the environmental circumstances encountered during our study, our results supported the recent “rising tide” hypothesis that improved growth (nutrient) conditions benefit all size classes, including picophytoplankton. Under extreme conditions of upwelling, however, the picophytoplankton declined abruptly, despite seemingly strong (average) growth rates. Future studies need to provide a better mechanistic understanding of the physical (advection), physiological (nutrient uptake and temperature) and ecological (food web) factors that result in this dramatic nonlinearity in picophytoplankton response to system forcing and richness.

KEYWORDS: picoplankton dynamics; dilution method; growth rate; grazing rate; coastal upwelling system

INTRODUCTION

The coastal region off western Baja California (WBC) is the southern limit of the California Current System (CCS). The hydrography of WBC is characterized by an alongshore near-surface equatorward flow carrying relatively cool and fresh water from the subarctic, a sub-surface poleward current flowing along the edge of the continental slope, and coastal upwelling episodes driven by northerly winds during most of the year. At the seasonal scale, subarctic waters dominate during the peak of the upwelling season in spring and summer, while tropical and subtropical influences are commonly observed during later summer and fall (Lynn and Simpson, 1987; Durazo *et al.*, in press).

Physical and chemical characteristics of the coastal waters from a monitoring observatory off Ensenada, México (ENSENADA station), display the seasonal and interannual characteristics derived from 11 years of recent oceanographic measurements in the northern region off WBC (Linacre *et al.*, in preparation), ranging from strong seasonal upwelling that produces dense algal blooms in the euphotic layer to periods of strong stratification and oligotrophy. Seasonal climatological means from ENSENADA station demonstrate that this coastal site is representative of a broader area (Linacre *et al.*, in preparation). Consequently, it is a convenient site for the study of plankton community and production responses to strongly variable conditions throughout this dynamic coastal ecosystem.

Classical and microbial pathways are a useful dichotomy for distinguishing among the alternate fates of primary production carbon in marine ecosystems (Calbet and Landry, 2004). In highly productive coastal upwelling areas, it has long been assumed that carbon production via chain-forming diatoms is either efficiently transferred to higher trophic levels through the classical food web (Ryther, 1969) or exported from the euphotic zone as fecal pellets, detritus or by sedimentation of “marine snow” aggregates (Turner, 2002). However, there is increasing evidence that the microbial components of food webs are an ubiquitous and important feature of not only oligotrophic but also eutrophic systems, including seasonally variable coastal upwelling areas (Cuevas *et al.*, 2004; Worden *et al.*, 2004; Vargas *et al.*, 2007). Most productive and seasonal systems involve multivorous food webs, where both classical and microbial trophic components play significant roles (Legendre and Rassoulzadegan, 1995). It has also been recently noted that the onset of favorable growth conditions for diatom-dominated blooms does not necessarily lead, as often assumed, to the successional replacement of the smaller cells that dominate during

less productive times (Barber and Hiscock, 2006). The picophytoplankton assemblage can respond positively in growth rates and biomass to improved growth conditions, although its biomass increase is often modest compared with larger phytoplankton, which escape control by protistan grazers via the “loophole” blooming mechanism (Irigoiien *et al.*, 2005; Barber and Hiscock, 2006).

Heterotrophic protists (here defined as nano- to micro-sized grazers $<200\ \mu\text{m}$) play an important role in pelagic food webs, where they are a major source of mortality for small and large primary producers, as well as heterotrophic bacteria (Sherr and Sherr, 2002). Globally, grazing impact by protists has been estimated to consume two-thirds of phytoplankton daily growth (production), with moderate variations among marine habitats and regions (Calbet and Landry, 2004). In coastal environments, protistan grazing accounts for $\sim 60\%$ of daily primary production on average, although this value appears to vary dynamically with seasonality and the state of the phytoplankton bloom and bust cycles (Neuer and Cowles, 1994; Böttjer and Morales, 2005; McManus *et al.*, 2007). Similar to protistan consumption of autotrophs, strong grazing pressure is exerted on heterotrophic bacteria mainly by nanoflagellates. Removal of $>100\%$ of daily bacterial production has been reported in the coastal upwelling areas of the Humboldt Current System, mostly during non-upwelling seasons (Cuevas *et al.*, 2004; Vargas *et al.*, 2007). Since bacterial growth is ultimately supported by autotrophic sources of DOC, even when the seasons of production and utilization are temporally separate, a significant fraction of annual primary production is channeled through bacteria to heterotrophic nanoflagellates, reflecting the importance of microbial food webs in carbon cycling in coastal upwelling systems (Cuevas *et al.*, 2004; Vargas *et al.*, 2007).

The aim of the present study was to assess the temporal dynamics of phytoplankton and bacteria in the coastal upwelling system off northern WBC, focusing on abundance, biomass, growth, grazing mortality and production of autotrophic and heterotrophic picoplankton populations under contrasting seasonal oceanographic conditions.

METHOD

In situ dilution experiments

Experimental studies of phytoplankton growth and protistan grazing were conducted at the ENSENADA station ($31^{\circ}40.105'N$, $116^{\circ}41.596'W$) at the northern

region off WBC (Fig. 1), as part of the FLUCAR (*Carbon Sources and Sinks in the Continental Margins of the Mexican Pacific Waters*) project. During the period from 24 September 2007 to 11 November 2008, six sets of experiments were incubated *in situ* for 24 h at a fixed coastal station, following the experimental approach of Landry *et al.* (Landry *et al.*, 2008). Routine station activities included CTD/rosette casts to 100 m depth with continuous measurements of pressure, temperature, conductivity, dissolved oxygen, chlorophyll fluorescence and PAR light, as well as seawater collection with 5-L Niskin bottles for flow cytometric (FCM) and nutrient analyses ($\text{NO}_3^- + \text{NO}_2^-$, $\text{Si}(\text{OH})_4$ and PO_4^{3-}) at 10 depths which were variable among cruises. Nutrient analyses were performed with a Skalar SAN^{plus} segmented-flow nutrient analyzer; $\text{NO}_3^- + \text{NO}_2^-$ determination was based on a modification of the Armstrong *et al.* (Armstrong *et al.*, 1967) procedure. Seawater for the experiments was also collected with 5-L Niskin bottles at variable depths among cruises within the euphotic zone (from 56 to 0.3% of average PAR light from the first meter, %I₀). Based on the *in situ* fluorescence profiles, the collection depths were at the deep chlorophyll maximum (DCM) and one level above and one level below the DCM. For each set of experiments, the treatments were prepared in clear polycarbonate bottles (2.0 L) with 100 (undiluted), 30 and 10% of whole seawater (diluted with 0.1- μm filtered seawater) at each depth. All dilution treatments were done by filtering directly from the Niskin bottles using a peristaltic pump, silicone tubing and an in-line Suporcap filter

capsule that had previously been acid washed (10% trace-metal grade HCl followed by distilled water and seawater rinses). Each bottle was sub-sampled for FCM analysis to confirm initial concentrations and volume dilutions. The bottles were tightly capped, then placed into net bags and secured with snap hooks to a weighted line hanging from surface floats and attached to a fixed buoy at the depths of initial sample collection. All experiments were started in the early morning and deployed prior to sunrise; the total elapsed time from Niskin sampling to array deployment was about 2.0 h.

Pigment and picoplankton analyses

Samples were taken for chlorophyll *a* (Chl *a*) and FCM analyses at the start and end of each experiment to determine initial abundances and dilution concentrations and to compute growth and grazing loss rates in the dilution incubations. Triplicate samples (250 ml) for Chl *a* analyses were filtered onto 25-mm Gelman GF/F filters, analyzed by the fluorometric non-acidification method (Welshmeyer, 1994). For this, we used a Turner Designs Trilogy fluorometer previously calibrated with pure Chl *a*.

For enumeration of picophytoplankton and heterotrophic bacteria, 2-mL samples were collected, preserved (0.5% paraformaldehyde, final concentration), flash frozen and stored in liquid nitrogen until analysis on the flow cytometer. Samples were thawed in batches, then stained with Hoechst 34 442 (1 $\mu\text{g mL}^{-1}$, final concentration) (Monger and Landry, 1993). For this analysis, a Beckman–Coulter Altra cytometer was used (operated by the SOEST Flow Cytometry Facility, www.soest.hawaii.edu/sfcf), with an Harvard Apparatus syringe pump for quantitative volume sampling and two argon ion lasers for UV (200 mW) and 488 nm (1 W) excitation. Scatter (side and forward) and fluorescence signals were collected using filters as appropriate, including those for Hoechst-bound DNA, phycoerythrin and chlorophyll. Fluorescence signals were normalized to 0.5 and 1.0 μm yellow–green (YG) polystyrene beads (Polysciences Inc., Warrington, PA, USA). Sample list-mode files were analyzed using FlowJo software (Treestar, Inc., www.flowjo.com). Cell abundances were converted to biomass using carbon per cell conversion factors, computed based on literature values used in coastal waters of the CCS. Estimates of carbon content were made using mean cell estimates of 20, 39, 82 and 1000 fg C cell⁻¹ as a starting point for heterotrophic bacteria (H-Bact), *Prochlorococcus* spp. (PRO), *Synechococcus* spp. (SYN) and PicoEukaryotes (P-Euk), respectively (Lee and Fuhrman, 1987; Worden *et al.*, 2004; Sherr *et al.*, 2005). Cell size variability around these values was

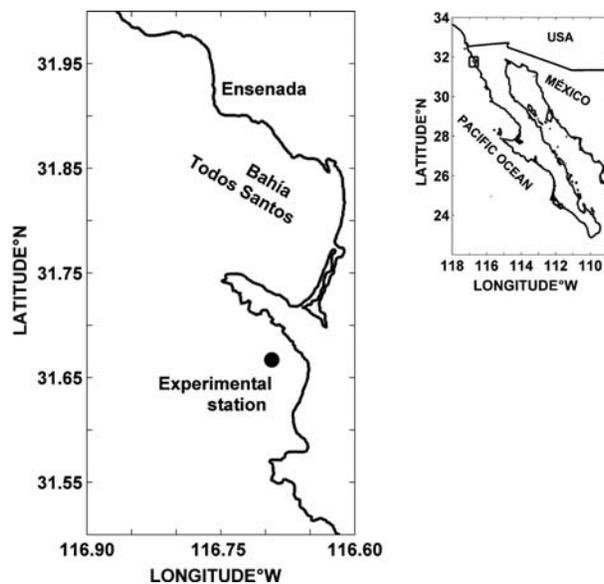


Fig. 1. Location of experimental station in the coastal waters off WBC, México.

then determined using bead-normalized Forward Angle Light Scattering (FALS) as a relative measure of biovolume (BV) and carbon content, assuming constant cell carbon density (C:BV) for each population category. Thus, using the scaling factor $FALS^{0.55}$ (Binder *et al.*, 1996; Landry *et al.*, 2003), the carbon content for each category was determined for each cruise and depth (sample = *i*) from the taxon-specific mean cell carbon from the literature and the FALS ratio = $(FALS_i / FALS_{mean})^{0.55}$.

Growth and grazing estimates

Instantaneous rates of phytoplankton growth (μ) and mortality loss (m) to protistan grazers were estimated from dilution incubations according to Landry and Hassett (Landry and Hassett, 1982), using an abbreviated three-treatment dilution protocol. Initial pigment concentrations and FCM population abundances (C_o) were determined for each dilution treatment from measured concentrations in the unfiltered seawater and the proportion of unfiltered (D_i) seawater in the treatment. Final concentrations (C_t) were measured in each bottle at the end of the 24-h incubations (t). Daily net rates of change (day^{-1}) were determined as $k_i = \ln(C_t / C_o) / t$. The typical linear relationship between k_i and D_i allowed estimation of m and μ daily rates from the slope and intercept of the trendline, respectively. Although the responses were linear in most of the experiments, estimates of k_i from the most diluted treatment (10%) were lower than the 30% treatment, on a few occasions in these analyses, typically toward the base of the euphotic zone where the net rates and the population concentrations were both low and difficult to measure, and perhaps a feeding threshold response occurred (Frost, 1972). In these cases, μ and m rates were estimated following the two-treatment dilution approach of Landry *et al.* (Landry *et al.*, 2008, 2009), using two equations: $m = (k_d - k) / (1 - 0.30)$ and $\mu = k + m$, where k_d and k are the daily net rates of change in the 30% and undiluted treatments, respectively. The lack of nutrient-added treatments in the experimental design may mean that the computed rates are underestimates. However, a systematic bias was not observed in comparing the results from three-bottle *in situ* experiments to several full dilution experiments with nutrient treatments (data not shown) that were done in the laboratory during the same period of this work.

Mortality rate estimates were assumed to be unaffected by changes in phytoplankton physiological condition during the experimental incubations. However, growth rate estimates could be affected by physiological adjustments. We attempted to correct for such effects

using FCM measurements of red fluorescence to account for cellular changes in Chl *a* from each group of photosynthetic picophytoplankton (PRO, SYN and P-Euk). For each category, FCM analyses provided initial (*i*) and final (*f*) estimates of bead-normalized Chl *a* red fluorescence per cell ($F1$), from which a “weighted mean normalized red fluorescence” ($WF1$) was used to compute daily instantaneous rates of change for the 24-h (t) incubations as $\ln(WF1_f / WF1_i) / t$. These computations were made for each initial and final whole seawater samples and applied to μ based on measured Chl *a* changes, as a correction factor for pigment photoadaptation. Thus, the corrected growth rate was calculated as, $\mu - [\ln(WF1_f / WF1_i) / t]$ (Landry *et al.*, 2003).

Experimental values of μ and m were combined with biomass estimates to compute rates of production and grazing for populations of autotrophic and heterotrophic picoplankton according to Calbet and Landry (Calbet and Landry, 2004). Production estimates (PP , $\mu\text{g C L}^{-1} \text{day}^{-1}$) were calculated as, $PP = \mu * (C_o [e^{(\mu - m)t} - 1] / (\mu - m)t)$, where C_o is expressed in carbon term ($\mu\text{g C L}^{-1}$). Similarly, the biomass consumption of protistan grazers (G , $\mu\text{g C L}^{-1} \text{day}^{-1}$) was computed as, $G = m * (C_o [e^{(\mu - m)t} - 1] / (\mu - m)t)$. Finally, the ratios of grazing to growth ($m:\mu$) were estimated as a measure of protistan grazing impact on the production of the phytoplankton community (based on Chl *a*) and the individual populations of picoplankton.

¹⁴C-Uptake primary production

As a basis of comparison to population production estimates from dilution experiments, we also measured primary productivity by the standard ¹⁴C-bicarbonate-uptake technique (Steemann Nielsen, 1952). Seawater samples collected on the same hydrocast as dilution experiments were screened through a 150- μm net to exclude macrozooplankton, and inoculated with $\sim 5 \mu\text{Ci NaH}^{14}\text{CO}_3$ in 250-mL polycarbonate bottles. Replicated light and dark bottles were placed into net bags on the array line together with dilution bottles and deployed at the initial collection depth for 24 h. After incubation, the labeled particulate matter was filtered onto 0.45- μm pore Millipore HA filters. To purge $\text{NaH}^{14}\text{CO}_3$ that was not fixed by photosynthesis, filters were placed in 20-mL scintillation vials with 0.5 mL of 10% HCl for 3 h. Scintillation cocktail (10 mL Ecolite) was added to each vial, and the radioactivity was determined with a Beckman LS-6500 scintillation counter. Primary production rates were calculated from these radioactivity counts according to Parsons *et al.* (Parsons *et al.*, 1984), subtracting the carbon uptake of the dark bottles.

RESULTS

Environmental conditions

Seasonal and interannual variability in the physical and chemical conditions at ENSENADA station are described in detail by Linacre *et al.* (Linacre *et al.*, in preparation). However, a brief description of the oceanographic conditions during the sampling for the dilution experiments is given in Table I and Fig. 2. The upwelling is a semi-permanent feature in northern coastal waters off WBC; however, it is stronger and more frequent during spring and summer seasons (Lynn and Simpson, 1987; Durazo *et al.*, in press). Figure 2A shows the daily Upwelling Indices at 30°N 119°W (solid line) and the monthly means (dashed line) for the sampling period at ENSENADA station. The index for each incubation day is highlighted in enlarged gray circles. Except for a few notable days in January 2008, the values over the sampling periods were neutral to positive in this region, ranging from 0 to 250 m³ s⁻¹ per 100 m of coastline. The upwelling indices for individual incubation days were variable, but were close to monthly means. The highest index value occurred during the April 2008 experiments (154 m³ s⁻¹ per

100 m of coastline), while the lowest values were found during November 2007 and January 2008 (15 m³ s⁻¹ per 100 m of coastline).

The vertical distributions of seawater density (σ_t , kg m⁻³) and *in situ* fluorescence ($\mu\text{g Chl L}^{-1}$) for each cruise are shown in Fig. 2B and C, respectively. The summer periods (September 2007 and August 2008) displayed a strong and marked pycnocline around 10 m, while the colder periods (November 2007–08, January 2008 and April 2008) showed a more homogeneous water column. The density values for all periods oscillated between 24.5 and 25.5 kg m⁻³ throughout 0–100 m of depth, except for April 2008, when anomalously high values (>25.7 kg m⁻³) associated the cold and salty upwelled water reached to the surface (Fig. 2B). In this particular case, the normal seasonal intensification of upwelling was enhanced by La Niña conditions observed during winter to spring 2008 in the northern region off WBC (McClatchie *et al.*, 2008; Durazo, 2009) and indicated by the Oceanic NINO Index reported by the NOAA Climate Prediction Center (<http://www.cpc.noaa.gov/>) for the period from December 2007 to January/February 2008, as well as by the Multivariate ENSO Index (Wolter and Timlin, 1998)

Table I: Initial environmental conditions for *in situ* dilution experiments conducted at ENSENADA station located in the northern region off WBC, México

Cruise	Depth (m)	Light (%I ₀)	Temp (°C)	Dissolved nutrients ^a			Total phyto TChl <i>a</i> ($\mu\text{g L}^{-1}$)	Picoautotrophs (cell L ⁻¹ × 10 ⁶)			Heterotrophic Bacteria (cell L ⁻¹ × 10 ⁸)
				NO ₃ ⁻ (μM)	PO ₄ ³⁻ (μM)	Si(OH) ₄ (μM)		PRO	SYN	P-Euk	
September 2007	2	56	15.4	0.3	0.2	2.3	4.7	15	187	53	30
September 2007	7	23	14.6	5.5	0.6	9.1	5.2	18	160	45	23
September 2007	10	15	13.2	7.2	0.7	6.1	2.4	21	49	15	12
September 2007	35	1	11.9	11.0	0.9	10.7	1.1	2	11	11	11
November 2007	10	13	15.2	4.2	0.9	6.5	1.3	18	71	21	11
November 2007	20	6	14.0	4.4	0.9	6.1	1.5	32	46	16	7
November 2007	30	2	12.8	6.0	1.0	6.0	1.1	46	35	9	6
January 2008	5	25	13.8	0.6	0.5	4.0	2.7	33	223	38	26
January 2008	20	2	13.2	7.7	1.6	7.7	4.5	12	140	43	18
January 2008	30	0.3	12.9	12.6	1.6	13.0	1.9	5	42	15	11
April 2008 ^b	5	22	11.6	–	–	–	25.1	0	3	7	11
April 2008 ^b	10	8	11.3	–	–	–	13.2	0	3	4	7
April 2008 ^b	15	3	10.5	–	–	–	9.4	0	4	5	8
August 2008	5	36	16.3	0.3	0.5	5.4	4.4	99	120	14	16
August 2008	10	17	14.4	8.7	0.7	7.7	2.6	36	80	11	10
August 2008	20	4	12.3	17.0	1.3	11.5	1.1	7	12	3	6
November 2008	5	47	16.0	0.1	0.7	5.1	2.9	88	105	8	16
November 2008	12	14	15.0	1.8	0.8	6.2	3.2	56	80	10	11
November 2008	30	1	13.9	6.6	1.1	8.0	1.0	11	6	2	5

Nutrients and light conditions were measured at midday CTD-cast. Temperature and initial concentrations of chlorophyll *a* and picoplankton abundance were measured in pre-dawn CTD cast during the collection of seawater for *in situ* dilution experiments. Groups of picoautotrophs distinguished by flow cytometry were *Prochlorococcus* spp. (PRO), *Synechococcus* spp. (SYN) and PicoEukaryotes (P-Euk). Because of high winds, station operations were abandoned in April 2008 before the hydrographic cast for nutrient sampling.

^aValues were acquired from samples analyzed by Dr Víctor Camacho-Ibar from IIO-UABC.

^bAn algal bloom condition was recorded during this season.

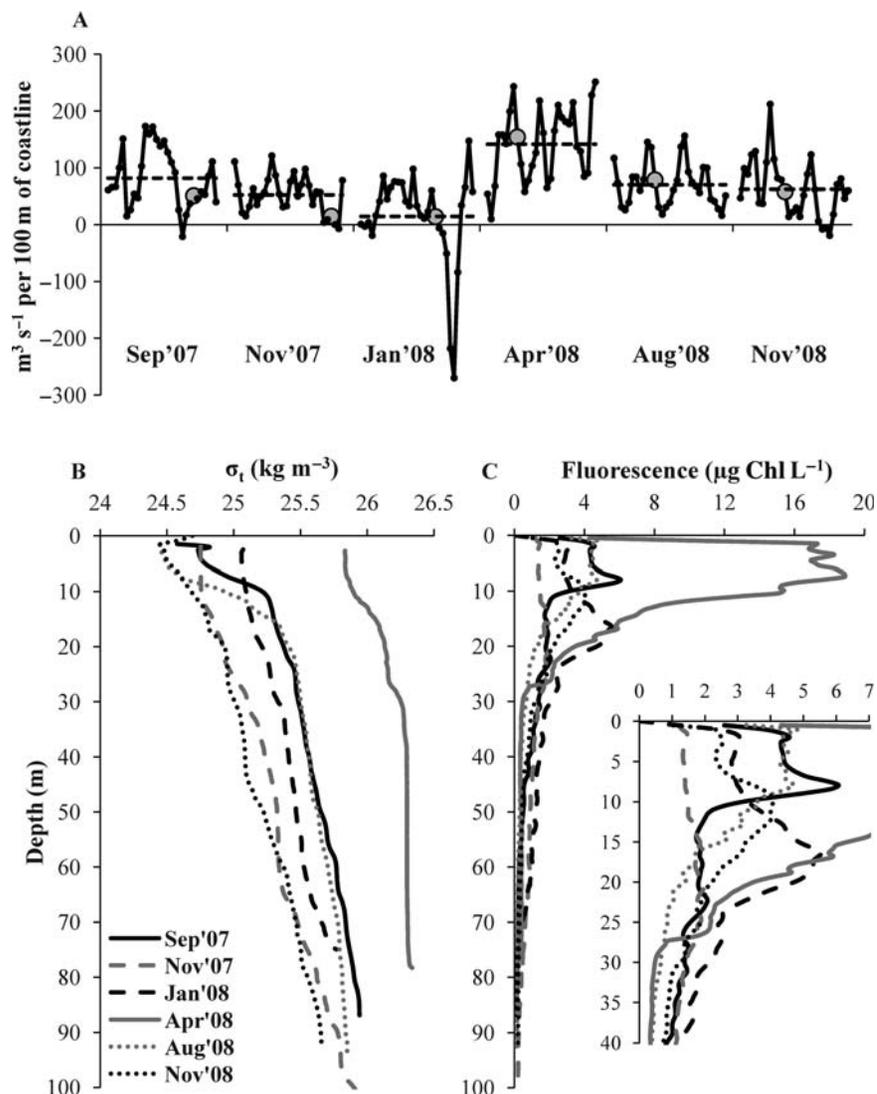


Fig. 2. (A) Bakun upwelling index ($\text{m}^3 \text{s}^{-1}$ per 100 m of coastline; solid line) and monthly mean ($\text{m}^3 \text{s}^{-1}$ per 100 m of coastline; dashed line) from NOAA/NMFS/PFEG for $30^\circ\text{N } 119^\circ\text{W}$, during the six periods when the dilution experiments were done at ENSENADA station in the northern region off WBC, México. The gray and enlarged circles indicate the index value for the day when incubation dilutions were done. (B) Profiles of water density (σ_t ; kg m^{-3}) and (C) *in situ* fluorescence ($\mu\text{g Chl L}^{-1}$), measured at the time of the seawater collection for the dilution experiments during each cruise.

(<http://www.cdc.noaa.gov/people/klaus.wolter/MEI/>). At ENSENADA station, this cold event in April 2008 was evident in anomalously high salinity (>33.9), low temperature ($<12^\circ\text{C}$) and consequently high density values ($>25.7 \text{ kg m}^{-3}$) throughout the water column (Table I, Fig. 2B), as well as euphotic zone waters depleted in dissolved oxygen ($\text{dO}_2 < 200 \mu\text{mol L}^{-1}$) but rich in dissolved inorganic carbon ($\text{DIC} > 2140 \mu\text{mol kg}^{-1}$) (Linacre *et al.*, in preparation).

In situ fluorescence profiles showed highest values in the first 30 m of the water column during all periods (Fig. 2C), where the Chl *a* concentrations were typically in the range of $1\text{--}5 \mu\text{g L}^{-1}$ (Table I) and the DCM was

in general above 15 m of depth, except for January 2008, when the DCM was notably deeper ($\sim 20 \text{ m}$; Fig. 2C). The lowest fluorescence values in the water-column occurred in November 2007, and they appeared close to Chl *a* concentrations measured at the depths sampled for the dilution experiment done in this season (Table I, Fig. 2C). Conversely, in April 2008, an algal bloom with surface Chl *a* concentrations of $>25 \mu\text{g L}^{-1}$ (Table I) and a dense layer of cells was evident in the upper 20 m ($\sim 20 \mu\text{g Chl L}^{-1}$; Fig. 2C). The depth of the euphotic zone, defined here by 0.1% surface light penetration ($\%I_0$ relative to the average measurement for the first meter), varied from 37 m in

January 2008 to 63 m in November 2007. Despite this variability, the euphotic zone was always deeper than the base of pycnocline, allowing upwelled water with high nutrients to penetrate into euphotic depths during all cruises (Table I).

Taxon-specific carbon content and biomass

Population variability of normalized FALS, a proxy of cell size, and the estimated carbon contents of picoplankton cells are shown in Fig. 3. P-Euk exhibited the most size variability associated with light level (depth) on a given cruise, which is understandable since this category is composed of different taxa, compared at least to the genus-level grouping of photosynthetic bacteria. We also use the “pico” size category non-rigorously for P-Euk since our FCM measurements likely include some cells that are larger than the formal 2- μm diameter cut-off. Perhaps due to compositional

changes, the patterns of variability were not consistent for P-Euk, which sometimes increased (September 2007, November 2007 and January 2008) and sometimes decreased in size (April 2008, August 2008, November 2008) with depth, as opposed to the typical slight increase in size with depth of the photosynthetic bacteria. Overall, H-Bact showed very little size differences among depths within cruises, but there was a general shift from larger to smaller sizes over the study period and especially between January 2008 and April 2008 (Fig. 3). Seasonally, larger SYN and P-Euk cells (but relatively small H-Bact) were found during April 2008, coincident with the algal bloom episode at ENSENADA station during that time (Table I). PRO was too scarce to enumerate in the samples collected during this major coastal bloom.

Estimates of picoplankton carbon biomass ranged from 2 to 60 $\mu\text{g C L}^{-1}$ for picoautotrophs (A-Pico combined PRO, SYN and P-Euk) and from 9 to

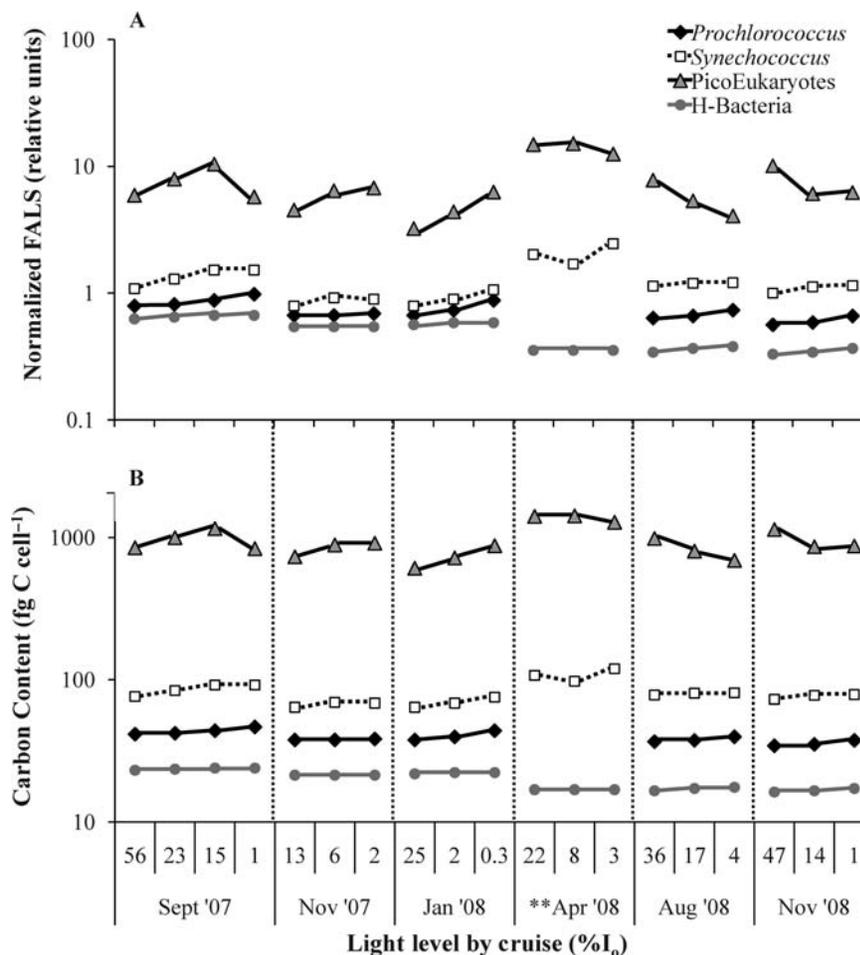


Fig. 3. Initial (A) bead-normalized forward angle light scattering (FALS) and (B) carbon content per cell (log scale) for autotrophic and heterotrophic populations, from 19 dilution experiments conducted in the euphotic zone from September 2007 to November 2008 at ENSENADA station in the northern region off WBC, México. The ** symbol denotes that a major algal bloom occurred during this cruise.

70 $\mu\text{g C L}^{-1}$ for H-Bact (Fig. 4). Biomass of picoautotrophs and heterotrophs were strongly correlated ($R^2 = 0.89$, $P < 0.0001$), with both showing highest values during September 2007 and secondary peaks in January 2008. The lowest values were registered during April 2008, when A-Pico was represented mainly by P-Euk (Fig. 4B). Depth distributions of picoplankton typically showed the highest abundance and biomass at near-surface light levels and the lowest at the deepest light levels (Fig. 4, Table I). Among populations of autotrophic picoplankton, SYN had the highest abundances on most sampling dates (Table I). However, SYN only contributed an overall average of 25% of the total A-Pico carbon biomass, compared with 70% for the larger P-Euk. PRO is both the smallest A-Pico cell and occurs in relatively low abundance at this coastal site relative to the open ocean. PRO therefore comprised a

minor portion of A-Pico biomass (5%) throughout the year, and was notably absent during the upwelling bloom in April 2008 (Table I, Fig. 4B).

Concentrations of total chlorophyll *a* (TChl *a*) in the euphotic zone, a proxy for relative biomass of the total phytoplankton community, were relatively high throughout the study period, varying from ~ 1 to 25 $\mu\text{g Chl } a \text{ L}^{-1}$. As previously noted, strong upwelling and La Niña conditions produced extraordinary bloom levels of TChl *a* in April 2008 (Table I). Biomass of P-Euk and photosynthetic bacteria, in particular, was at their lowest levels, in absolute and relative terms, during this bloom, which therefore mostly involved larger phytoplankton (Fig. 4B). In contrast, A-Pico components were at their highest values during the more modest secondary peak of TChl *a* in September 2007. The lowest TChl *a* values were observed throughout the euphotic

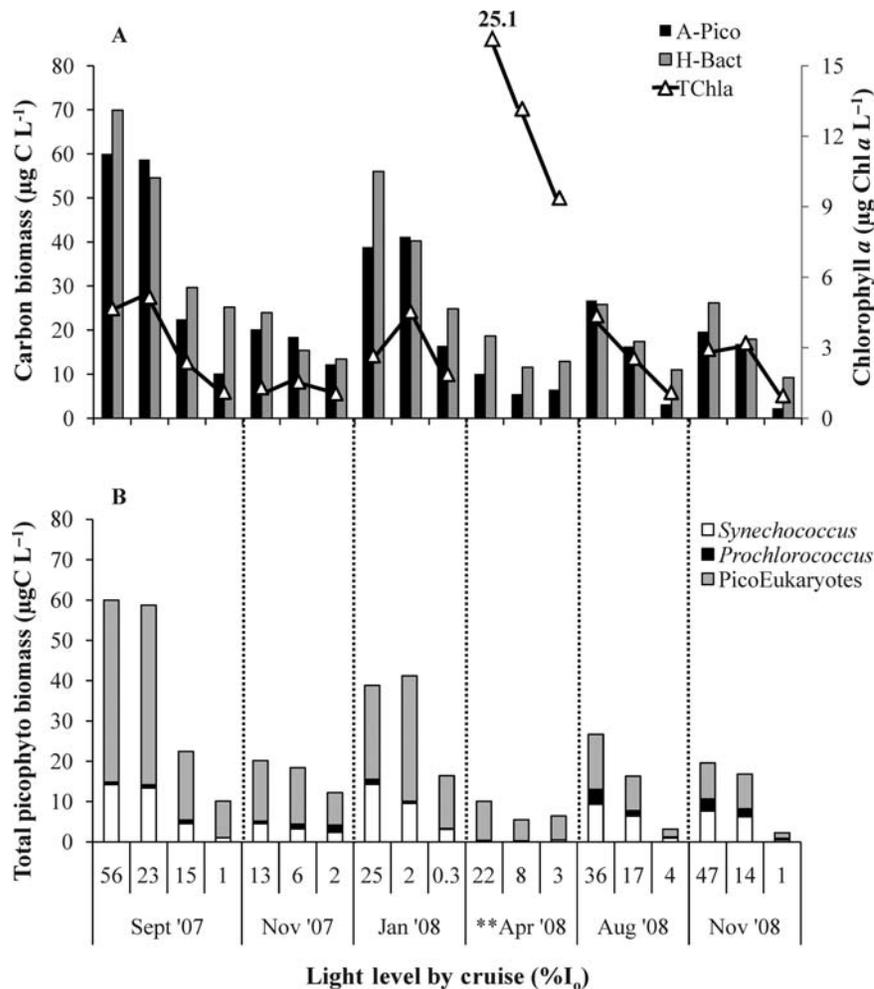


Fig. 4. (A) Initial biomasses of picoplankton (autotrophic and heterotrophic) populations (left *x*-axis) and initial total concentration of chlorophyll *a* (right *x*-axis), and (B) contribution of autotrophic populations (PicoEukaryotes, *Prochlorococcus* and *Synechococcus*) to total picophyto biomass, from 19 dilution experiments conducted in the euphotic zone from September 2007 to November 2008 at ENSENADA station in the northern region off WBC, México. The ** symbol denotes that a major algal bloom occurred during this cruise.

zone in November 2007 and at the deepest light levels in August 2008–November 2008 (Table I, Fig. 4A).

For most of the sampling times, a positive relationship between picoplankton populations and the phytoplankton community was found, except for April 2008, when the lowest biomass levels of picoplankton populations were measured (Fig. 5). Excluding the April 2008 data, A-Pico biomass ($R^2 = 0.73$, $P < 0.0001$) and H-Bact biomass ($R^2 = 0.52$, $P = 0.0017$) were both positively and significantly correlated with TChl *a* (Fig. 5A and B).

Experimental rate estimates

Community- and population-level estimates of growth (μ) and grazing mortality (m) rates were computed, respectively, from the measured net changes in TChl *a*

and cell abundances of picoplankton (A-Pico and H-Bact) (Fig. 6). TChl *a* estimates of μ ranged seasonally from 0.06 to 2.06 day^{-1} , and m varied from 0.05 to 1.09 day^{-1} (Fig. 6A). Picoplankton growth rates ranged from 0.14 to 2.02 day^{-1} and 0.19 to 1.71 day^{-1} for A-Pico and H-Bact, respectively. Protistan grazing rates on picoplankton varied between 0.21–1.37 and 0.09–1.53 day^{-1} for A-Pico and H-Bact, respectively (Fig. 6B and C).

Among all cruises and light levels, TChl *a*-based estimates of μ and m were highly correlated ($R^2 = 0.67$, $P < 0.0001$). Seasonally, the highest community rates ($>1 \text{ day}^{-1}$) were found during experiments conducted in September 2007 and April 2008, when TChl *a* was also high. However, growth rates were lower during the massive bloom in April 2008, compared with the

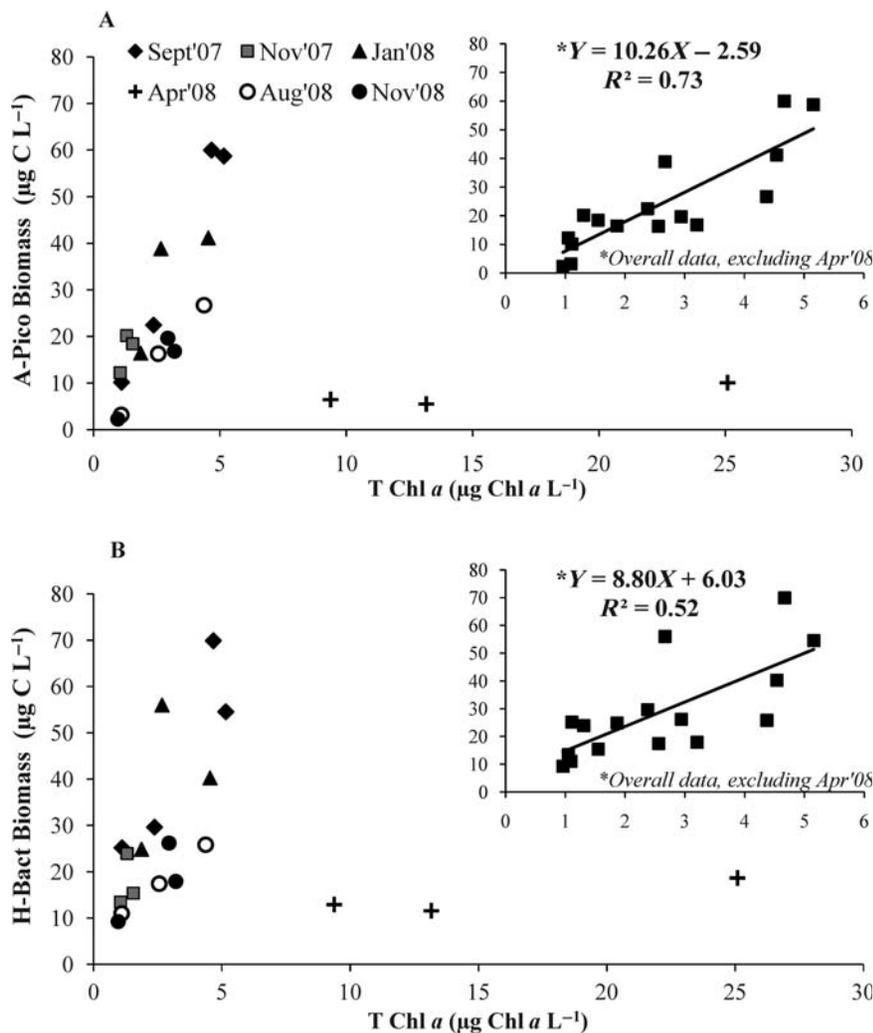


Fig. 5. Scatter plots between initial total concentration of chlorophyll *a* (TChl *a*) and initial biomasses of (A) autotrophic picoplankton (A-Pico) and of (B) heterotrophic picoplankton (H-Bact), from data of 19 dilution experiments conducted in the euphotic zone from September 2007 to November 2008 at ENSENADA station in the northern region off WBC, México. The top (A) and bottom (B) panels show significant linear regressions from overall data, excluding April 2008 experiments.

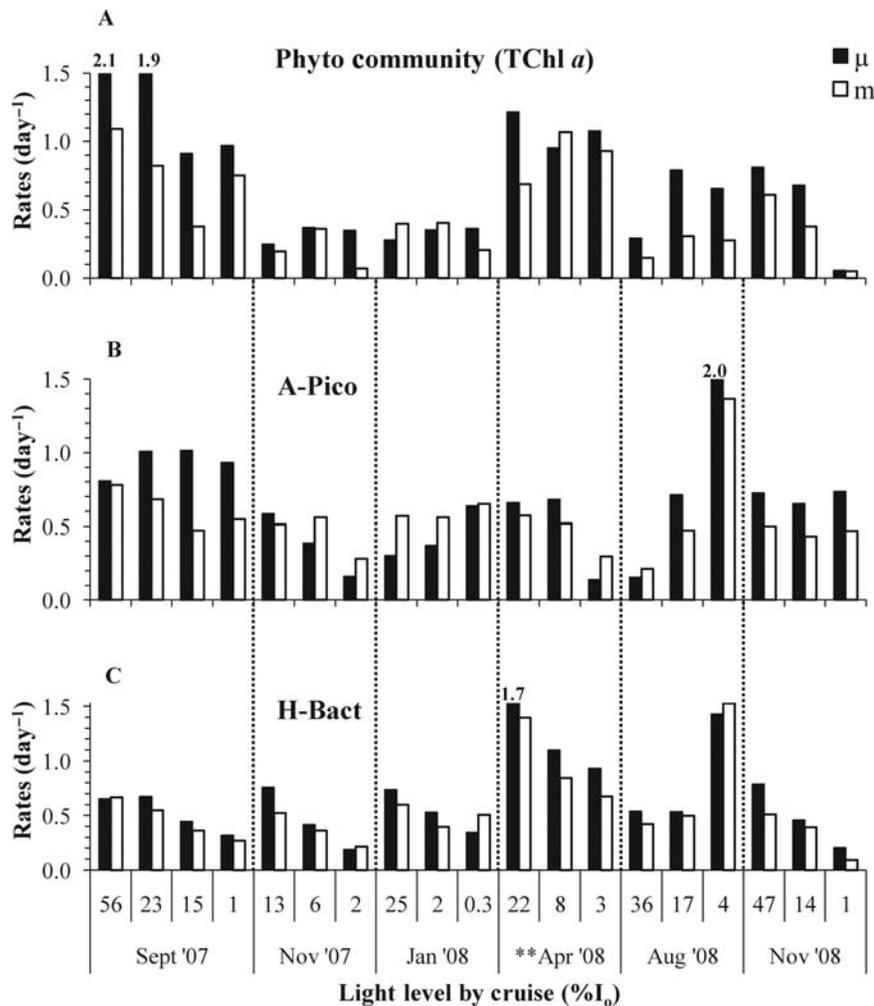


Fig. 6. Growth (μ) and protistan grazing (m) rates of (A) total phytoplankton community (TChl *a*), (B) picoautotrophic populations (A-Pico) and (C) heterotrophic bacteria (H-Bact), from 19 dilution experiments conducted in the euphotic zone from September 2007 to November 2008 at ENSENADA station in the northern region off WBC, México. The ** symbol denotes that a major algal bloom occurred during this cruise.

almost 2X higher estimates of September 2007. The lowest community rates were measured seasonally during autumn (November 2007) and winter (January 2008) experiments. Nonetheless, substantially higher community growth rate and TChl *a* were found at surface light levels in November 2008 compared with November 2007, despite 40X higher surface nitrate concentrations in November 2007 (Fig. 6A, Table I).

Cell-based μ and m rates of the picoautotrophs, calculated as the “weighted mean” from population-specific rate assessments of PRO, SYN and P-Euk, generally followed the trends for TChl *a* community rates, although the range was suppressed, especially in the higher values of the upper mixed-layer μ for A-Pico (0.7–1.0 day⁻¹) during experiments conducted in September 2007 and April 2008 (Fig. 6B). On half of the experimental dates (January 2008, August 2008 and

November 2008), A-Pico rates at the lowest light levels greatly exceeded the rates inferred from TChl *a* changes. For August 2008, in particular, exceptionally high cell growth and grazing rates for A-Pico populations were found at the pycnocline base, at 4% I_0 light level (Figs 6B and 2B, Table I). Grazing losses exceeded growth rates during autumn (November 2007) and winter (January 2008) experiments when growth rates in the upper light levels were lowest (Fig. 6B). Growth and grazing mortality rates for A-Pico were significantly correlated ($R^2 = 0.73$, $P < 0.0001$) throughout the year at different light levels.

Similar to TChl *a* data, the relationship between m and μ rates for H-Bact cells was significant and strong ($R^2 = 0.86$, $P < 0.0001$). H-Bact rate estimates, however, were typically low compared with A-Pico or TChl *a*-based rates, except during the April 2008

phytoplankton bloom (Fig. 6C). We generally found high rates for H-Bact in the upper mixed-layer and low rates in deeper incubations, except during August 2008, when coincident with A-Pico, high growth and grazing rates were observed at the 4% light level (Fig. 6C).

Grazing impact assessments

Grazing mortality (m) rates for TChl a and picoplankton populations often equaled or exceeded 50% of μ , indicating a substantial removal of autotrophic and heterotrophic planktonic cells by protists at this coastal station throughout the year (Fig. 7). The $m:\mu$ ratios for TChl a showed higher predatory pressure during autumn-winter seasons (November 2007–08 and January 2008) and at light levels $<10\%$ I_0 during the April 2008 bloom. Grazing impact on the

phytoplankton community was lower in late summer (September 2007 and August 2008) than the seasonal average. Overall, $m:\mu$ averaged (\pm SD) 0.66 ± 0.20 for TChl a ($n = 19$; these and other $m:\mu$ data were arc-tangent transformed, averaged, then tangent back-converted, as in Calbet and Landry, 2004). This result indicates that protistan grazers consumed about two-thirds of total phytoplankton cell production in this coastal area (Fig. 7A).

A-Pico cells were even more strongly impacted by nano/micrograzers than the phytoplankton community as a whole (Fig. 7B). The weighted average impact of protistan grazers on A-Pico cells was 0.94 ± 0.22 . The highest A-Pico $m:\mu$ ratios (>1.5) were mainly observed during autumn-winter experiments (November 2007–January 2008) and at the base of the euphotic zone, close to the bottom of the algal bloom, in April 2008

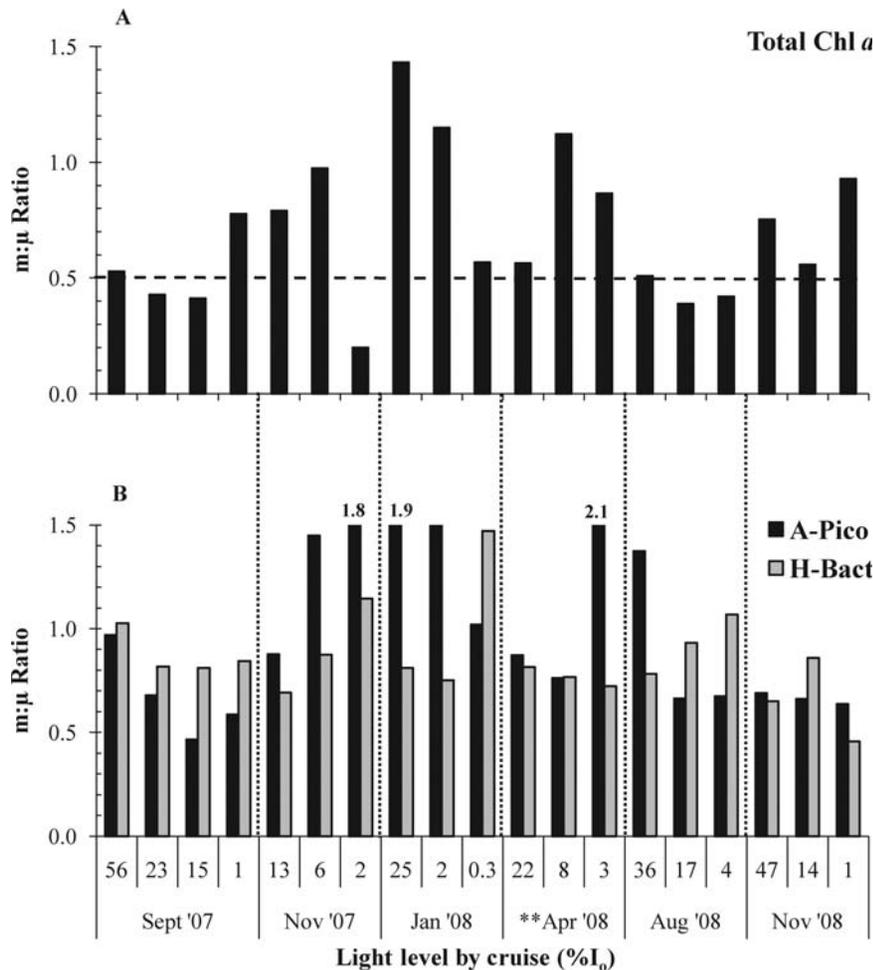


Fig. 7. Ratio of protistan grazing (m) to growth (μ) rates of (A) total phytoplankton community (TChl a) and (B) picoplankton (autotrophic and heterotrophic), from 19 dilution experiments conducted in the euphotic zone from September 2007 to November 2008 at ENSENADA station in the northern region off WBC, México. The ** symbol denotes that a major algal bloom occurred during this cruise. Dashed horizontal line on top panel indicates grazing equivalent to half of phytoplankton growth.

(Figs 7B and 2C). In order of population-specific percentage losses of A-Pico production to protistan consumers, PRO ($>100 \pm 25\%$) was highest, followed by P-Euk ($96 \pm 25\%$) and SYN ($93 \pm 23\%$). Similarly, the $m:\mu$ ratios for H-Bact oscillated around an average value of 0.84 ± 0.11 and was distributed more uniformly with time and depth (Fig. 7B).

¹⁴C-uptake primary production and biomass production-loss estimates

Throughout the euphotic zone, the measured rates of ¹⁴C-uptake primary production were variable among seasons and light levels, ranging from 0.24 to

$217 \mu\text{g C L}^{-1} \text{day}^{-1}$. Highest productivity rates were found during September 2007 and April 2008 within the mixed layer and above $\sim 10\% I_0$. The lowest values were registered during the November cruises (2007–08) and at the deepest light levels close to $1\% I_0$ (Fig. 8A). During the high production period of September 2007, A-Pico exhibited their highest rates of biomass production and grazing losses. That was not the case, however, for the high production period of April 2008, when A-Pico populations, represented basically by P-Euk, had their lowest production and grazing loss estimates (Fig. 8).

Among A-Pico populations, P-Euk contributed the most to production estimates throughout the study, with population-specific rates ranging from 1.13 to

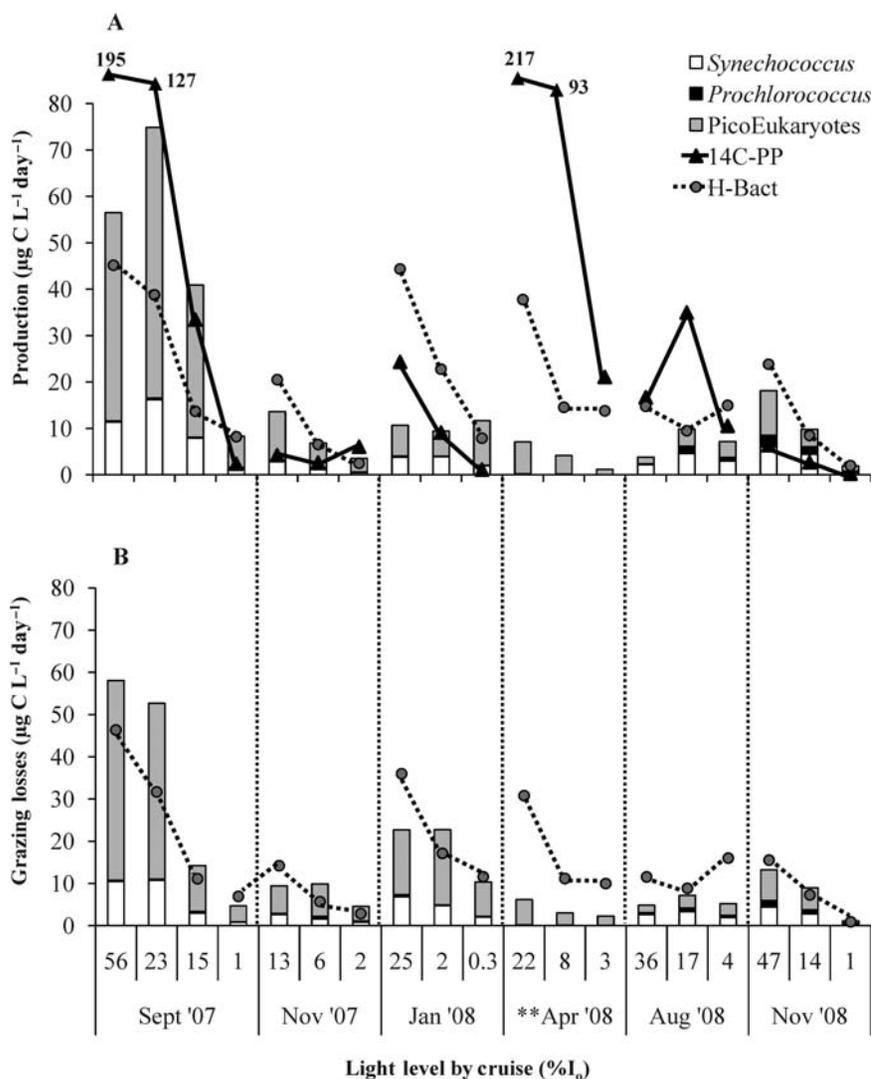


Fig. 8. Daily rates of (A) production and (B) grazing losses by protists of picoautotrophic populations (*PicoEukaryotes*, *Prochlorococcus* and *Synechococcus*), heterotrophic bacteria (H-Bact) and ¹⁴C-uptake primary production (only panel A), from 19 dilution experiments conducted in the euphotic zone from September 2007 to November 2008 at ENSENADA station in the northern region off WBC, México. The ** symbol denotes that a major algal bloom occurred during this cruise.

58.3 $\mu\text{g C L}^{-1} \text{ day}^{-1}$ (Fig. 8A). In comparison, production estimates varied between 0.02–16.2 $\mu\text{g C L}^{-1} \text{ day}^{-1}$ and 0.08–2.5 $\mu\text{g C L}^{-1} \text{ day}^{-1}$ for SYN and PRO, respectively. Higher production values of A-Pico were found at the surface light levels and lower towards the base of euphotic layer, as was the general tendency for ^{14}C -uptake rates. The notable exception was in January 2008, when the production estimates were relatively similar among all experiments and even slightly higher at the lowest light level. On several occasions (mainly November 2007–08 cruises), estimates of A-Pico cell biomass production exceeded contemporaneous estimates of community production by ^{14}C -uptake. The strongest discrepancy was at the lowest light level (0.3% I_0) in January 2008, when ^{14}C -uptake was 10X lower than estimated A-Pico production (Fig. 8A). Secondary production estimates for H-Bact varied between 2.0 and 45.2 $\mu\text{g C L}^{-1} \text{ day}^{-1}$ and displayed their higher values during September 2007, January 2008 and April 2008 at the highest light levels (Fig. 8A).

In order of A-Pico biomass losses to protistan grazing, P-Euk rates were highest (0.58–47.3 $\mu\text{g C L}^{-1} \text{ day}^{-1}$), followed by SYN (0.07–10.7 $\mu\text{g C L}^{-1} \text{ day}^{-1}$) and PRO (0.09–1.58 $\mu\text{g C L}^{-1} \text{ day}^{-1}$). Grazing losses of H-Bact ranged from 0.91 to 46.3 $\mu\text{g C L}^{-1} \text{ day}^{-1}$ (Fig. 8B). The highest consumption rates of picoplankton were found in September 2007 in the upper mixed-layer.

Overall, A-Pico production estimates and ^{14}C -primary production were slightly correlated with a low significance ($R^2 = 0.26$, $P = 0.0261$; Fig. 9A), but this positive relationship was much stronger when the April 2008 data were excluded ($R^2 = 0.74$, $P < 0.0001$; Fig. 9A, inset). The slope of the linear regression indicated that A-Pico production rate typically increased as 13% of the ^{14}C -uptake rate. However, this production rate increased as 29% of the community production when the April 2008 bloom data were excluded from the regression analysis (Fig. 9A). H-Bact production was also significantly correlated with ^{14}C -primary production ($R^2 = 0.49$, $P = 0.0008$; Fig. 9B) for all experiments at the ENSENADA station, and increased only slightly when the April 2008 data were excluded ($R^2 = 0.51$, $P = 0.0020$; Fig. 9B, inset). The slopes of both relationships (including and excluding April 2008 data) indicated that H-Bact production rate increased as 14–19% of the ^{14}C -uptake rate (Fig. 9B).

Picoautotroph populations and nutrients in the euphotic layer

Over all cruises and euphotic sampling depths, the log-scale abundance of A-Pico was negatively correlated with nitrate + nitrite concentration ($R^2 = 0.45$, $P < 0.0001$, Fig. 10). Lower abundances of A-Pico when

nutrients are high could reflect recently upwelled water in the euphotic layer; conversely, high abundance of picophytoplankton is also naturally associated with stratified, nutrient-poor waters.

DISCUSSION

The present data were not collected at sufficient temporal resolution to define the seasonal dynamics of phytoplankton in the northern region off WBC. They do, however, provide six contrasted temporal “snapshots” that capture the system at differing states of productivity. The most productive conditions encountered during our study (September 2007 and April 2008) were associated with high nutrient delivery into the euphotic zone from episodic upwelling. Such events occur stronger and more frequently during spring and summer months in the coastal eastern boundary ecosystem off northern Baja California (Lynn and Simpson, 1987; Durazo *et al.*, in press). Particularly during September 2007 and April 2008, upwelling indices showed strong increases during or a few days before the dilution incubations were conducted (Fig. 2A). Although September 2007 and April 2008 were marked by comparable high levels of ^{14}C production in the upper 10 m of the water column, September 2007 results are more coherent with other times sampled, while the situation in April 2008 stands out as extraordinary in terms of TChl *a* accumulation and community structure. The normal seasonality of phytoplankton was intensified during this period by La Niña conditions (McClatchie *et al.*, 2008; Durazo, 2009), which have the effect of bringing the pycnocline and nutricline closer to the ocean surface and more easily entrained into the upper euphotic zone by upwelling favorable winds. The very cold sea surface temperature during our sampling in April 2008 was indicative of a massive influx of nutrients from deeper waters, which had subsequently been incorporated into phytoplankton. Although the circumstances leading up to the bloom were not observed, increased offshore transport of surface water is implied by the strong upwelling-favorable winds in the week prior to our sampling in April 2008 (Fig. 2A). Physically, this could have had the effect of moving the community that had established itself in upper euphotic zone at this site further offshore. Thus, advective transport may have contributed to changes observed in community composition, in addition to *in situ* biological responses to the altered hydrography (Li, 2002). Turbulence and the upward displacement of water associated with upwelling would also help increase the residence time of large primary producers in the euphotic layer against their tendency to sink (Rodríguez *et al.*, 2001).

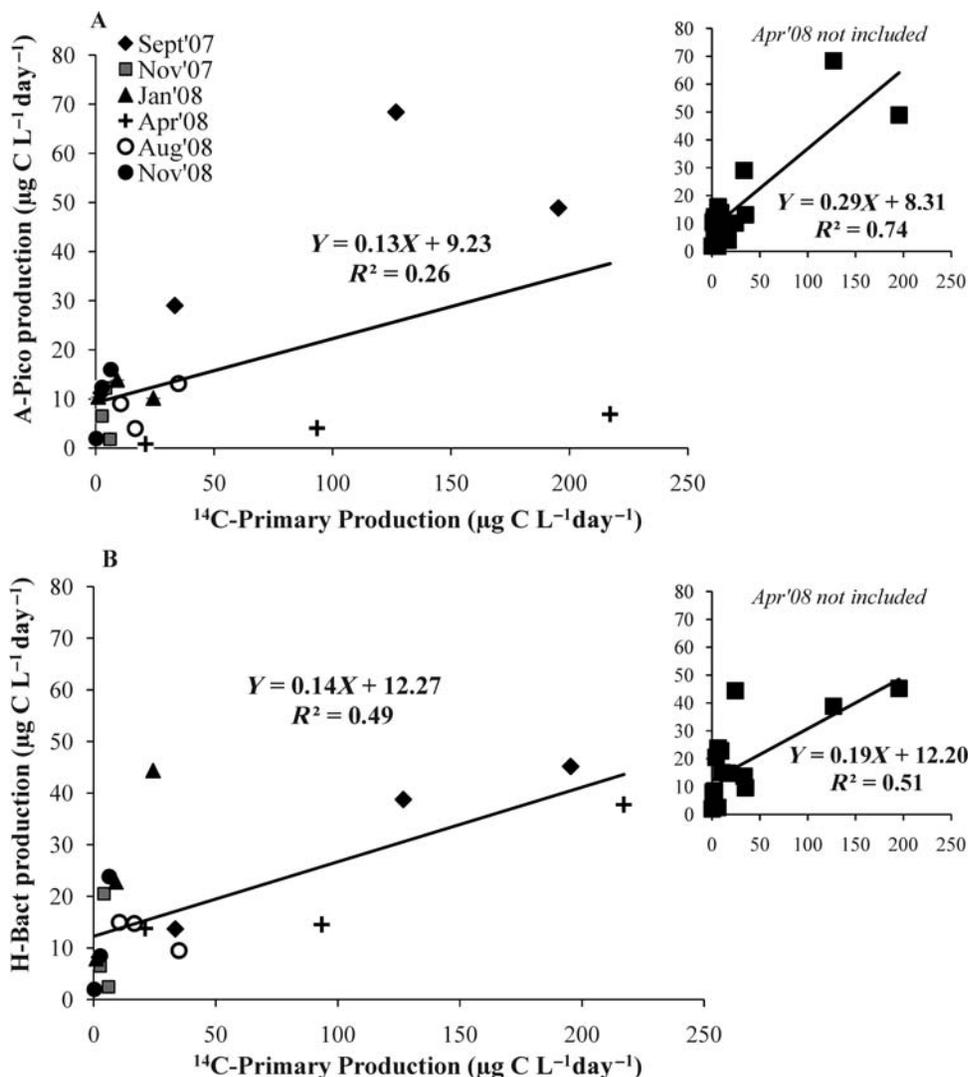


Fig. 9. Scatter plots between ^{14}C -uptake primary production and production estimates of (A) autotrophic picoplankton (A-Pico) and (B) heterotrophic picoplankton (H-Bact), from data of 19 dilution experiments conducted in the euphotic zone from September 2007 to November 2008 at ENSENADA station in the northern region off WBC, México. Top (A) and bottom (B) panels show significant linear regressions from overall data. Top (A) and bottom (B) inset panels show the significant linear regressions excluding the April 2008 experiments.

The phytoplankton community structure at ENSENADA station may, therefore, be shaped in part by the direct effects of physical processes (advection and turbulence), as well as their indirect effects on nutrient availability and biological interactions. From spring to late summer in the coastal waters off Oregon, an upwelling front physically separates high abundances of coccoid cyanobacteria and small eukaryotic phototrophs seaward of the front from low abundances on the coastal side (Sherr *et al.*, 2005). We do not know if a similar type of hydrographic barrier may have been set up off of ENSENADA station in April 2008, but it is a possibility.

The factors that regulate size structure of phytoplankton under different trophic conditions are still under

debate (Agawin *et al.*, 2000; Sherr *et al.*, 2005; Echevarría *et al.*, 2009). Conventional thinking is represented by Li (Li, 2002), who found inverse relationships between picoplankton cell number and biomass relative to total chlorophyll values from a series of cruises crossing biogeographical provinces in the North Atlantic. On a smaller scale, Sherr *et al.* (Sherr *et al.*, 2005) also obtained a negative relationship between integrated abundance of P-Euk cells and Chl *a* for samples collected from a cross-shelf transect of stations in the Oregon coastal upwelling ecosystem. More recently, a negative trend of reduced picoplankton cells under eutrophic conditions has also been reported from three coastal systems south of the Iberian Peninsula

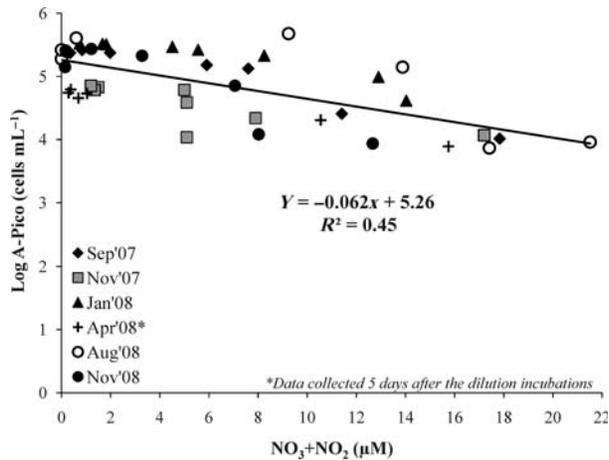


Fig. 10. Scatter plot between log-scale abundance of A-Pico populations from discrete depths and nitrate + nitrite concentrations for individual water samples collected within euphotic layer in six periods during September 2007–November 2008 at ENSENADA station in the northern region off WBC, México. All the samples were collected in the same day of incubation dilutions, excepting April 2008, when the data were sampled 5 days after dilution incubations.

(Echevarría *et al.*, 2009). In contrast, during seasonal studies in the Arabian Sea, the highest biomass levels of P-Euk occurred coincident with the highest biomass of diatoms and total phytoplankton at coastal upwelling stations during the SW Monsoon (Garrison *et al.*, 2000), though that was not the case for photosynthetic bacteria, especially *Prochlorococcus*. Recent work in the equatorial Pacific upwelling system, however, has shown that *Prochlorococcus* varies positively with TChl *a* and can dominate the phytoplankton community response to open-ocean upwelling (Selph *et al.*, in preparation). In as far as Barber and Hiscock (Barber and Hiscock, 2006) have developed their rising tide hypothesis largely based on observations of phytoplankton response to iron fertilization experiments in the equatorial Pacific, there may be cause to think that picophytoplankton responses to perturbations in tropical/subtropical systems may differ from those in more temperate and highly seasonal environments. The warmer systems, for example, tend to maintain a strong interactive network of producers and consumers throughout the year and may promote more tightly coupled trophic responses at all levels that help regulate the explosive growth potential of bloom taxa.

At our study location, which has strong tropical/subtropical influences, increases in TChl *a* were generally paralleled by increases in picoplankton biomass (Fig. 5A, inset). Thus, over a productive range, but excluding extreme environmental conditions, our results are consistent with the rising tide hypothesis (Barber and Hiscock, 2006). Although picophytoplankton

carbon grew with total phytoplankton, the rate of A-Pico increase was slower. If we assume a carbon conversion factor of 30 $\mu\text{g C}$ per $\mu\text{g TChl } a$ (Eppley, 1968) to compute total phytoplankton carbon for comparison with A-Pico biomass, the resulting regression gives a slope of 0.34 (data not shown). This less than 1:1 relationship indicates in our case, as well as in previous observations (Agawin *et al.*, 2000; Li, 2002; Sherr *et al.*, 2005; Echevarría *et al.*, 2009), that the relative contribution of picoautotrophs to total phytoplankton biomass decreases significantly under more productive conditions. The positive relationship between A-Pico production estimates and ^{14}C -primary production at our coastal station, also had a <1.0 positive slope (Fig. 9A, inset), even when the most productive period of April 2008 was considered (Fig. 9A). Thus, in the majority of our temporal “snapshots”, favorable environmental conditions tended to lift all phytoplankton together, although small cells increased slower than large cells.

Mechanistically, smaller cells are better adapted to low-nutrient conditions due to larger surface to BV ratios (Chisholm, 1992), while large cells may have physiological advantages, such as better internal nutrient storage or more effective nutrient uptake, under high nutrient conditions (Echevarría *et al.*, 2009). Our finding of an overall negative relationship between the log-abundance of A-Pico cells and euphotic zone nitrate + nitrite concentrations (Fig. 10) suggests that small cells were not able to grow well at the expense of high nutrient concentrations in recently upwelled water, which can, however, support a large biomass accumulation of large-sized diatoms, and oppositely, the highest abundances of A-Pico cells were able to grow efficiently at nitrate + nitrite concentrations $<2 \mu\text{M}$ (Fig. 10). Similar results were found in other ecosystems such as the coastal waters off Oregon (Sherr *et al.*, 2005), and even, in contrasting trophic environments (Agawin *et al.*, 2000; Echevarría *et al.*, 2009). However, nutrient availability *per se* could be not the direct cause of A-Pico diminished abundance or relative performance with increasing trophic state; temperature for example has a strong impact on *Prochlorococcus*, which disappeared when colder waters were present during April 2008 (Table I), and generally lack the functional capability to utilize nitrate (Moore *et al.*, 2002; Martiny *et al.*, 2009).

April 2008 was the most productive sampling period at our coastal station, generating a strong bloom of phytoplankton and providing an interesting counterpoint to the rising tide hypothesis (Barber and Hiscock, 2006). During this period, we found evidence of reasonably good growth conditions for picophytoplankton; instantaneous growth rates were about equal to seasonally averaged levels (Fig. 6B), and a significant correlation

was found between A-Pico production estimates and contemporaneous estimates of community production by ^{14}C -uptake (Fig. 9A). However, the low biomass of A-Pico (Fig. 4A), mainly represented by P-Euk, and the strong mortality impact of protistan grazers (the highest $m:\mu$ ratio of all cruises was found at the base of pycnocline; Fig. 7B) point to the possibility that, while large phytoplankton cells were still growing and accumulating in absence of high losses by consumers, the small cells were already top-down controlled (A-Pico production and grazing losses showed similar and low estimates; Fig. 8). Thus, the greatly reduced production of A-Pico during the April 2008 bloom (Fig. 8A) was determined principally by the decline in biomass, which could be related at some point to inadequate growth to accumulate, or even maintain, picophytoplankton standing stock during the bloom. As noted previously, however, advective losses of surface waters away from the upwelling station may also have contributed to this dramatic decline, greatly exacerbating the appearance of a rapid replacement of small by large cells. In effect, the virtual collapse of A-Pico during April 2008 bloom appears to be consistent with the conventional view of a successional replacement of small phytoplankters by the larger responders to bloom-favorable growth conditions, but the mechanisms in our system (biological versus physical) are not clear. At the very least, the rising tide hypothesis needs to be carefully examined in tropical/subtropical systems for circumstances that are more typical of highly seasonal temperate environments, where growth-favorable nutrient conditions are strongly associated with colder waters and uncoupled food webs.

Another possible explanation for the low abundances (biomass) of picoautotrophs during April 2008 is the elevated concentration of trace metals (particularly cadmium) in the upwelled waters. As noted off Oregon, for example, cadmium may be differentially toxic to small versus large phytoplankton, especially to coccoid cyanobacteria, for which only ~ 0.1 nM can greatly affect growth rate (Sherr *et al.*, 2005). During 2008 at a coastal site off Punta Banda, Ensenada, cadmium concentrations averaged 0.27 nM (± 0.03) and increased (>0.3 nM) in surface waters from winter to spring (F. Delgadillo, Ensenada, BC, personal communication). From these results, it is possible that during the initial and peak phase of the April 2008 bloom, a high input of trace metals to surface layers favored the predominance of larger phytoplankton to the detriment of the smaller cells. High abundances of large diatoms and autotrophic dinoflagellates were clearly evident during April 2008 via epifluorescence microscopy. Consequently, the outcome is consistent with what we might expect for a classic spring bloom, where large

primary producers with high growth rate potential, like chain-forming diatoms, can effectively decouple from grazing losses (the “loophole” mechanism; Irigoien *et al.*, 2005) and dominate the phytoplankton community response.

Except for the April 2008 bloom, picoplankton comprised an important component of the community under all other conditions sampled at the ENSENADA coastal station. A-Pico carbon biomass almost always exceeded $20 \mu\text{g C L}^{-1}$ in the upper euphotic zone, and was typically comparable to the biomass of H-Bact (Fig. 4). Small eukaryotes dominated A-Pico biomass, especially at elevated concentrations of TChl *a*. Although small-sized phytoplankton are typically thought to be characteristic of oligotrophic open-ocean systems, high abundance of autotrophic bacteria and P-Euk have also been observed in other upwelling ecosystems of the CCS (Worden *et al.*, 2004; Sherr *et al.*, 2005). However, this fraction was higher in our uppermost experimental incubations of <10 – 12 m depth and relatively high light, where A-Pico typically accounted for half or more of the corresponding estimates from ^{14}C -PP incubated under the same *in situ* conditions and 24-h time interval (Fig. 8A). Especially in near-surface incubations during November 2007 and November 2008, A-Pico production estimates exceeded ^{14}C by double (Fig. 8A). Some of this difference reflects the fact that while our estimates measure the total production of new A-Pico cells over the incubation period, which may be closer to a gross production rate, a good deal of the ^{14}C uptake during the incubation can be lost to cycling before the net total particulate label remaining is measured. It is also possible, however, that our mean carbon content assumed as a starting point for estimations of biomass and production of P-Euk for cells size on average ~ 2.1 - μm diameter ($1000 \text{ fg C cell}^{-1}$, from Sherr *et al.*, 2005) might be too high for this system. If that is the case, then the biomass and production estimates would also be systematically high. Because of the diversity in types of cells that comprise the P-Euk category and the vagaries of size inferences from FCM, independent assessment of mean P-Euk carbon content is therefore very important for future analyses of production size structure in plankton communities.

Carbon content alone cannot account, however, for the relative production rates many times higher than the contemporaneous ^{14}C results at the light levels closer or lower than $1\% I_0$, as were showed in September 2007, January 2008 and November 2008 (Fig. 8A). The inverse pattern with depth of the growth rates (Fig. 6B) and the production estimates (Fig. 8A) for January 2008 suggest, for example, that an error

(switch) was made in incubating the bottles at the correct depth of collection or in sub-sampling the bottles for FCM analyses. Either of these possibilities would have left unmistakable signs in the FCM results, which were carefully examined to diagnose the problem. The key signal is the bead-normalized red fluorescence per cell, which rigorously defines the depth order of the samples collected (deeper cells have substantially higher chlorophyll content) and would show marked changes if low-light adapted cells had been inadvertently incubated at a near-surface light (the January conditions were bright sunlight). We found no evidence of a sub-sampling or incubation problem in the raw cytograms, the cell counts or the Chl *a*-content inferences for these experiments. The relatively high growth rates measured at low light, not only in this particular experiment, but also in September 2007, August 2008 ($\mu = 2 \text{ day}^{-1}$) and November 2008 (Fig. 6B), beg the question of whether A-Pico populations may be able to use alternate carbon sources for growth (i.e. mixotrophy of dissolved organics or particulate prey) under low light or benefit in some other way from the physical-chemical conditions at the base of the euphotic zone.

During the study period, more than half of phytoplankton community production was typically lost daily to protistan grazers in the coastal waters off WBC (Fig. 7A). On average, nano/micrograzer consumption was equivalent to 66% of the phytoplankton community growth, suggesting a close coupling between daily production and consumption of phytoplankton in this region, as has been previously reported for other coastal systems (Strom *et al.*, 2001; McManus *et al.*, 2007). Also, this assessment based on TChl *a* agrees with global estimates of percent primary production grazed by microzooplankton in temperate (61–69%) and coastal systems (57–60%) (Calbet and Landry, 2004). The seasonal variability of the grazing impact measured in this study is similar to mixed-layer results from other upwelling regions with contrasting trophic conditions along the year (Neuer and Cowles, 1994; Böttjer and Morales, 2005; McManus *et al.*, 2007). We also found heavy predatory pressure on picoplankton populations for most of the year (Fig. 7B). Overall, the consumption of A-Pico averaged 94% of their daily production. Grazing vulnerability of A-Pico also varied slightly among populations (PRO > 100%, P-Euk = 96% and SYN = 93%) and was likely associated with their relative biomass and presence during the year (PRO < SYN and P-Euk). This pattern has been reported previously in coastal waters of the Southern California Bight, where carbon consumed:produced ratios ranged seasonally from 23 to >100% per day among A-Pico populations, with

highest ratios for PRO relative to P-Euk and SYN populations, as well as total absence of PRO cells during spring months (Worden *et al.*, 2004), as was described in our work. Similarly, high percentages of A-Pico production grazed per day have been reported for the western South China Sea, with highest mean values for PRO ($83 \pm 57\%$) and lowest for SYN ($61 \pm 25\%$), although population growth rates $< 0.2 \text{ day}^{-1}$ were excluded from these computations (Chen *et al.*, 2009). Furthermore, strong grazing impact accounted for 84% of the daily production of H-Bact on average, indicating that the top-down processes play an important role in regulating bacterial biomass at the ENSENADA station. In fact, the predatory pressure was similar to other coastal upwelling regions, as was reported for the Humboldt Current system, where >100% of H-Bact biomass was daily removed mainly by heterotrophic nanoflagellates, mostly during the non-upwelling seasons (Cuevas *et al.*, 2004; Vargas *et al.*, 2007). Based on measured rates and stocks, our estimates of bacterial production (BP) were significantly correlated with primary production (Fig. 9B), but BP values were many times higher than ^{14}C -uptake rates during the less productive autumn-winter periods of the sampling (Fig. 8A), when protistan grazing on A-Pico populations was strongest (m: μ values >1.5) (Fig. 7B). This may reflect a more tightly coupled dependency of BP on DOC production from grazing processes during such times, or the utilization of previously fixed, excess DOC that is stored and returned to the system by the upwelling circulation.

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 temporal snapshots of plankton dynamics described here for the coastal upwelling system of WBC show an active and generally significant component associated with the growth, production and grazing turnover of picophytoplankton populations. For most of the year, the high production contributions of A-Pico and H-Bact compared with ^{14}C -uptake measurements and the strong grazing impacts on A-Pico and H-Bact populations indicated that a significant fraction of production was channeled through the microbial food web. The situation observed in April 2008 was exceptional, however, in terms of TChl *a* accumulation and community structure associated with intensified spring upwelling under La Niña conditions. While picophytoplankton as a group varied positively with the rest of the community in response to less extreme variations in growth conditions, the “rising tide” hypothesis therefore appears to apply under most conditions in our coastal upwelling area. However,

under extreme conditions, as in April 2008, the dynamics of the picoplankton could be modulated by physical forcing (low standing stocks due to increased offshore advection of small cells in surface waters) and/or by a metabolic response (no evidence of A-Pico biomass accumulation in cold and rich-nutrient upwelled water) that lead to a replacement of small cells by the larger phytoplankters in this coastal upwelling station. Given the significance of microbial trophic pathways in coastal upwelling systems, further studies are needed to understand at a mechanistic level what gives rise to these unexpected dynamics.

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