

Assessing the dynamics and ecology of marine picophytoplankton: The importance of the eukaryotic component

Alexandra Z. Worden¹

Marine Biology Research Division, Scripps Institution of Oceanography, University of California, San Diego, 9500 Gilman Drive, La Jolla, California 92093-0202

Jessica K. Nolan²

Integrative Oceanography Division, Scripps Institution of Oceanography, University of California, San Diego, 9500 Gilman Drive, La Jolla, California 92093

B. Palenik

Marine Biology Research Division, Scripps Institution of Oceanography, University of California, San Diego, 9500 Gilman Drive, La Jolla, California 92093-0202

Abstract

We assess population dynamics of picophytoplankton groups ($\leq 2 \mu\text{m}$ diameter; *Prochlorococcus*, *Synechococcus*, and picoeukaryote) at a Pacific Ocean coastal site in the Southern California Bight. Weekly sampling (August 2000 to January 2002), dilution experiments, and flow cytometric analysis were combined with an instrument-specific calibration for cell size determination, allowing biovolume and carbon biomass estimation. *Synechococcus* was almost always numerically dominant, accounting for $60 \pm 12\%$ of the total picoplankton cells over time. It had moderately high growth rates ($0.52\text{--}0.86 \text{ d}^{-1}$) and was subject to low grazing mortality (-0.14 to -0.39 d^{-1}). *Prochlorococcus* growth and mortality rates were roughly balanced ($0.33 \pm 0.14 \text{ d}^{-1}$ and $-0.36 \pm 0.06 \text{ d}^{-1}$, respectively). Picoeukaryotes had the highest growth rates ($0.71\text{--}1.29 \text{ d}^{-1}$) and were responsible for, on average, 76% of net carbon production (NCP), amounting in up to $32.05 \pm 1.31 \mu\text{g C L}^{-1} \text{ d}^{-1}$ produced and $28.31 \pm 2.61 \mu\text{g C L}^{-1} \text{ d}^{-1}$ consumed. In order to better define the eukaryotic component of these populations, an isolate was characterized via small subunit rRNA gene sequencing, transmission electron microscopy, and growth experiments and was identified as the prasinophyte *Ostreococcus*, not previously known to the Pacific Ocean. Our results show that although picoeukaryotes do not stand out as particularly important players in this system on the basis of cell abundance, they dominate in terms of picophytoplankton biomass and trophic transfer potential of carbon in this size class.

The ecology of picophytoplankton ($\leq 2 \mu\text{m}$ diameter) has been a major area of oceanographic research since the discovery of the abundant marine cyanobacteria, *Synechococcus* (Waterbury et al. 1979) and *Prochlorococcus* (Chisholm et al. 1988). Photosynthetic picoeukaryotes comprise a third ubiquitous picophytoplankton group that is numerically less abundant. Members of this “group” can be difficult to dis-

tinguish on a morphological basis, and only recently have molecular techniques been applied to better define these populations (e.g., Guillou et al. 1999). In part because of their lower abundance, as well as their complexity and diversity as a group, picoeukaryotes have been less well studied than the marine cyanobacteria. However the work of Li (1994) indicates that photosynthetic picoeukaryotes could indeed be responsible for a large portion of the primary production in the picoplankton size fraction. Using simplified carbon conversion factors, other studies have shown that picoeukaryotes can be responsible for a greater portion of carbon biomass than either *Prochlorococcus* or *Synechococcus* (e.g., Partensky et al. 1996; Blanchot et al. 2001). Given the importance in marine biogeochemical cycling attributed to picophytoplankton, it is essential that factors critical to ecosystem function such as carbon biomass, growth, and mortality rates, as well as physiology of different types of picophytoplankton, be elucidated in greater detail.

Recently, studies have addressed variations in cellular abundance, fluorescence properties, and carbon biomass of natural phytoplankton populations of *Prochlorococcus*, *Synechococcus*, and eukaryotic phytoplankton (DuRand et al. 2001; Shalapyonok et al. 2001). By calibrating flow cytometric scatter properties with cell diameter data, DuRand et al. (2001) estimated the absolute and relative carbon contri-

¹ Corresponding author (azworden@alum.mit.edu).

² Present address: York College of Pennsylvania, Department of Biological Sciences, Annex 2, York, Pennsylvania 17405.

Acknowledgments

We are indebted to R. Chastain and D. Landry for help in attaining samples, in addition to R. Gausling for help with experiments. We thank B. Binder for use of dilution experiment equipment, R. Goericke for both equipment and comments on the manuscript, and S. W. Chisholm for comments on the manuscript. We appreciate time and expertise shared by the S.D.V.A. Hospital Electron Microscopy Core Facility. D. Vaultot and F. Le Gall kindly provided *Ostreococcus tauri*. Weekly pier water collection was supported by a grant from the U.S. EPA (R827107-01-0) to B.P. A.Z.W. was supported by an NSF microbial biology fellowship, as were sequencing costs and electron microscopy costs (NSF award 0074392). We are also grateful to two anonymous reviewers whose suggestions improved the manuscript.

bution of the different groups at the Bermuda Atlantic Time Series (BATS) site, as did Shalapyonok et al. (2001) in the Arabian Sea. The combination of such studies with molecular work on ecotypic differentiation and related physiological capabilities aids comprehension of population distributions and biomass (for review, see Partensky et al. 1999; Scanlan and West 2002). However, factors other than growth potential, such as grazing mortality, undoubtedly play a significant role in population regulation. Such controls are essential to carbon cycling models that aim to incorporate phytoplankton standing stocks and production rates, as well as the fate of that fixed carbon.

Estimates of specific growth and grazing mortality rates of *Prochlorococcus* and *Synechococcus* from widely disparate oceanographic regimes (e.g., Landry et al. 1995a; Reckermann and Veldhuis 1997; Rivkin et al. 1999; Kuipers and Witte 2000; Sherry and Wood 2001; Worden and Binder 2003) show that these rates are frequently different for the two groups, suggesting they are subject to different grazing pressure terms, as has been shown in laboratory experiments (Christaki et al. 1999; Guillou et al. 2001). Few rate measurements are available for photosynthetic picoeukaryotes. In the Sargasso Sea, small eukaryotes were found responsible for a large portion of picoplanktonic primary production despite their low abundance (Li 1994). In larger size fractions, Landry et al. (1995a) addressed nanoeukaryote growth rates in the equatorial Pacific and found that this group grew faster than the photosynthetic prokaryotes.

We are interested in the dynamics of picophytoplankton and especially in better defining the role and composition of eukaryotes in this size fraction. Therefore, we have undertaken time series sampling at a Pacific Ocean coastal monitoring site and used size-calibrated flow cytometry in combination with culture-based approaches and dilution experiments to explore these dynamics. Evidence is presented that photosynthetic picoeukaryotes, including the prasinophyte *Ostreococcus*, contribute significantly to biomass, carbon production, and trophic transport of carbon and, by these measures, are the dominant picophytoplankton at the coastal site studied.

Materials and methods

Study site location and weekly sampling—Time series sampling and experimental studies were conducted at the Scripps pier (32°53'N, 117°15'W), a Pacific Ocean coastal site bound by the California Current. Water samples were collected from surface water at the end of the 330-m pier on a weekly basis in the morning hours. Samples were fixed in 0.25% glutaraldehyde (final concentration) and frozen at -80°C until later flow cytometric analysis. Midday seawater temperature data was attained from recordings taken by the Climate Research Division, Scripps Institution of Oceanography (meteora.ucsd.edu/weather/observations/sio_pier).

Culturing and isolate characterization—A picoeukaryote was isolated from a pier sample by nitrate enrichment of a 1- μ m filtrate, followed by plating in 0.3% agar solidified F/4 (Guillard 1975) and colony isolation. After isolation, the picoeukaryote strain (hereafter termed CCE9901) was main-

tained in F/4 media at 20°C and rendered axenic using antibiotics for later laboratory studies. Axenic cultures were then grown in F/4 amended with 10 nmol L⁻¹ selenium (SeF/4), which improved growth reliability. *Ostreococcus tauri* was obtained from D. Vaultot and F. Le Gall and also rendered axenic. Culture growth was monitored by in vivo fluorescence and cells were diluted into fresh media prior to the onset of stationary phase to ensure constant exponential growth, as described previously (Binder and Chisholm 1990). For comparison of nitrogen utilization, the two organisms were first acclimated to constant light of 40 μ mol quanta m⁻² s⁻¹ (fluorescent cool-white light) and then maintained for at least 10 generations prior to experiments. Cells were then inoculated into SeF/4 media, in which the standard nitrogen source had been replaced by test source (200 μ mol L⁻¹ urea + 80 nmol L⁻¹ Ni, 100 μ mol L⁻¹ NH₃, 500 μ mol L⁻¹ NaNO₃), as well as no nitrogen SeF/4 and Ni-SeF/4 media as controls. All growth experiments were replicated.

DNA of both CCE9901 and *O. tauri* was isolated using the DNeasy extraction kit (Qiagen) according to the manufacturer's recommendations. PCR amplification of the small subunit (SSU) rRNA gene was accomplished using the primers 5'-ACCTGGTTGATCCTGCCAG-3' (500 nmol L⁻¹ final concentration) and reverse 5'-TGATCCTTYGCAGGTT-CAC-3' (500 nmol L⁻¹ final concentration) in conjunction with 2.5 U HotStar *Taq* polymerase (Qiagen), 200 μ mol L⁻¹ each dNTP, and 1.5 mmol L⁻¹ MgCl₂ in a 50- μ l reaction volume. After a 15-min hot start period (95°C), 32 amplification cycles were performed, consisting of 1 min denaturation (94°C), 1 min annealing (55°C), 2 min elongation (72°C), followed by a 10-min final extension (72°C). PCR products were cloned with the TOPO-TA[®] cloning kit (Invitrogen) and plasmids were isolated with the Quiprep[®] kit (Qiagen) according to manufacturers' protocols. Subsequently, plasmids were sequenced using ABI Prism BigDye terminators on an ABI377XL (Applied Biosystems). 18S rRNA gene sequences for CCE9901 and *O. tauri* have been deposited in GenBank under the accession numbers AY329636 and AY329635, respectively. The *O. tauri* SSU rRNA gene sequence has previously been reported under the accession number Y15814.

For transmission electron microscopy (TEM), 10 ml of cultures of CCE9901 were concentrated in an ultracentrifuge at 5,473 \times g for 10 min at 20°C. The supernatant was removed, and the pellet was resuspended in 1 ml F/4 medium. The cells were then spun at 7,000 \times g in a microcentrifuge for 10 min, and the supernatant was aspirated. The remaining cells were fixed overnight at 4°C in modified Karnovsky's fixative (2% paraformaldehyde, 1% glutaraldehyde in 0.2 μ m filtered seawater, 0.2 mol L⁻¹ Na cacodylate buffer, pH 7.4) followed by 1% OsO₄ in 0.1 mol L⁻¹ Na cacodylate buffer, pH 7.4, and subsequently dehydrated with a graded series of ethanol solutions (50%, 70%, 80%, and 95%, and 100% twice) followed by propylene oxide and infiltration with epoxy resin (Scipoxy 812, Energy Beam Sciences). After polymerization at 65°C overnight, thin sections were cut and stained with uranyl acetate and lead citrate. Sections were examined at an accelerating voltage of 60 kV with a Zeiss EM10B electron microscope.

Four photosynthetic picoplankton isolates, *Prochlorococ-*

Table 1. Dilution-based estimates of growth (μ) and grazing mortality (g) rate for the respective picophytoplankton groups and mean cellular carbon at the experiment onset. Values for net carbon produced (NCP) and carbon consumed (CC) are followed by the standard deviation (in parentheses, calculated from experiment replicates). CC/NCP represents the fraction of NCP consumed.

Experiment date	Abundance ($\times 10^4$ ml $^{-1}$)	μ (d $^{-1}$)	g (d $^{-1}$)	Mean cellular carbon (fg C cell $^{-1}$)	NCP (μ g L $^{-1}$ d $^{-1}$)	CC (μ g L $^{-1}$ d $^{-1}$)	CC/NCP
<i>Picoeukaryotes</i>							
29 Dec 00	1.6	0.75	0.27	354.40	5.65(0.50)	2.34(0.68)	0.41
19 May 01	3.0	1.24	0.43	162.01	13.14(3.07)	6.03(1.85)	0.46
30 Jul 01	2.2	1.29	1.09	488.00	32.05(1.31)	28.31(2.61)	0.88
19 Oct 01	2.6	1.22	0.42	307.70	17.32(1.80)	8.24(0.95)	0.48
8 Dec 01	2.5	0.75	0.34	312.86	8.82(0.63)	5.07(0.08)	0.58
18 Jan 02	1.1	0.71	0.17	341.26	5.45(0.09)	1.67(0.36)	0.31
<i>Synechococcus</i>							
29 Dec 00	3.3	0.52	0.27	77.68	1.71(0.14)	0.90(0.28)	0.53
19 May 01	8.2	0.86	0.31	65.14	7.23(0.21)	3.24(0.02)	0.45
30 Jul 01	10	0.69	0.39	58.50	6.08(0.07)	4.58(0.49)	0.75
19 Oct 01	3.8	0.77	0.15	73.89	2.64(0.20)	0.62(0.01)	0.23
8 Dec 01	6.5	0.56	0.22	69.92	3.45(0.18)	1.50(0.52)	0.43
18 Jan 02	4.2	0.58	0.14	71.21	2.85(0.10)	0.74(0.12)	0.26
<i>Prochlorococcus</i>							
29 Dec 00	1.1	0.30	0.36	41.56	0.16(0.00)	0.19(0.03)	1.15
19 May 01	3.2	0.48	0.33	38.0	0.73(0.10)	0.54(0.04)	0.74
30 Jul 01	3.1	0.09*	0.35	39.5	0.12(0.00)	0.50(0.01)	4.22
19 Oct 01	2.5	0.46	0.48	42.7	0.55(0.08)	0.46(0.10)	0.83
8 Dec 01	0.8	0.27	0.34	41.88	0.09(0.00)	0.10(0.04)	1.04
18 Jan 02	1.6	0.35	0.30	38.2	0.32(0.03)	0.27(0.07)	0.87

* Growth rate was estimated from the 0.1 dilution bottle because of relationship nonlinearity (see Methods).

cus marinus strain MED4, *Synechococcus* sp. strain WH8102, CCE9901, and *Pelagomonas calceolata* strain CCMP2006, were grown in standard media (Pro99, SN, F/4, and K media, respectively) at 20–22°C and constant light (28 μ mol quanta m $^{-2}$ s $^{-1}$, CCE9901, or 25 μ mol quanta m $^{-2}$ s $^{-1}$, all other strains) for flow cytometric size calibration and were fixed while in the exponential phase of growth. Samples were preserved as above with an initial 30-min freeze in liquid nitrogen and subsequent storage at -80°C .

Dilution experiments—Dilution experiments were based on the methods of Worden and Binder (2003), a modification of the approach of Landry et al. (see 1995b). Experiments were conducted on six dates between December 2000 and January 2002 (Table 1). Briefly, in each experiment, a nutrient-amended series (880 nmol L $^{-1}$ NO $_3$ and 72 nmol L $^{-1}$ PO $_4$, final concentrations) was employed of five different dilutions corresponding to fractions of unfiltered seawater equal to 1, 0.7, 0.5, 0.3, and 0.1 and a series of nonnutrient-amended bottles corresponding to unfiltered seawater fractions equal to 1, 0.5, and 0.1. Seawater was collected from the top 1 m of the sea surface (~ 0.5 m) in the dark. Seawater for filtration (to be used for dilution) was collected first; gravity filtered through a 0.2- μ m sterile acid-rinsed Gelman Supor filter capsule, and distributed to acid-cleaned 1-liter polycarbonate bottles. Subsequently, seawater for the unfiltered fraction was collected, sieved through 110- μ m Nitex mesh, and distributed to the bottles, in duplicate. Nutrient supplements were added just prior to the initial sampling. Bottles were placed randomly in a Plexiglas on-deck (on-

pier) water bath cooled with continuously flowing seawater to maintain near-ambient temperature conditions. On-deck water bath light levels were adjusted with neutral density screening to reduce the photosynthetically available radiation intensity to that at 0.5 m water depth. Because these incubations were conducted outside, the day–night cycle was identical to that of ambient conditions. Bottles were placed in the water bath at dawn, and incubations were terminated after 24 h. Initial (T_0) and final (T_{24}) time point samples were taken from every bottle immediately before placement in the water bath and at the end of the 24-h period, respectively. Samples were preserved in 0.25% glutaraldehyde (final concentration), frozen in liquid nitrogen for 30 min, and subsequently stored at -80°C . All preparations and sampling were performed under low light conditions in order to avoid light shock.

Flow cytometry—Samples were analyzed on a Becton Dickinson FACSort flow cytometer equipped with a 488-nm laser (15 mW output). Forward angle light scatter (FALS), right angle light scatter (RALS), orange fluorescence from phycoerythrin (PE, 564–606 nm), and red fluorescence from chlorophyll (CHL, >650 nm) were measured after 488-nm laser excitation. Just before analysis, 0.974- μ m-diameter fluorescent latex beads (Duke) were added to samples for later signal normalization. Samples were delivered at 12 μ l min $^{-1}$ for 4 min prior to data collection, and subsequently, data were acquired from a volume of 48 μ l of the sample. Data acquisition was triggered on RALS, and count rates generally ranged from 350 to 900 events s $^{-1}$. All samples

were fixed and frozen prior to flow cytometric analysis. Samples were run for dilution experiments and weekly samples taken from August 2000 to January 2002, except for the following dates: 1 March 2001, 14 March 2001, 7 November 2001, 14 November 2001, and 21 November 2001.

Cell size—Size distributions were determined with a Coulter Counter Multisizer II equipped with a 15- μm orifice for *Prochlorococcus*, *Synechococcus*, and CCE9901 and with a 20- μm orifice for *P. calceolata*. The apertures were calibrated with 0.97- and 2.08- μm beads. Filtered seawater (0.2 μm) was used as a blank and to dilute samples to maintain the coincidence below 3%. At least 1.5×10^4 cells were counted per sample. A lognormal curve was fit to all size distributions, and data from the curve fit were used for subsequent analysis.

Data analysis—Flow cytometry data were analyzed using WinList (Verity Software House). *Prochlorococcus* and *Synechococcus* were identified and enumerated on the basis of light scatter and fluorescence signals as described previously (Olson et al. 1990). In samples where *Prochlorococcus* appeared to be absent or unambiguous determination was difficult (counts <100, close to the baseline), a value of zero was entered for cell counts. Picoeukaryotes were defined empirically as those cells that fell within a predefined analysis window in a RALS versus CHL two-parameter histogram. The analysis window was defined on the basis of a histogram generated from a picoeukaryote culture with a mean population diameter of 2.0 μm (determined by Coulter counter). Furthermore, the analysis window included all cells passing through a 2- μm filter (with no vacuum applied) prior to fixation, which were subsequently preserved and run on the flow cytometer. *Synechococcus* cells were removed from the defined window by gating based on PE fluorescence because these populations frequently overlap in both CHL fluorescence and light scatter properties. Throughout the study the analysis window allowed collection of picoeukaryote cell counts without truncation of natural populations. Mean fluorescence and RALS values were linearized and normalized to the linearized bead mean. Data from 25 July 2001 was discarded because of extremely high noise that made unambiguous definition of populations impossible.

Biovolume measurements and volume-to-carbon conversion factors were generated by empirically derived calibrations. Here, biovolume was determined by first converting RALS data to cell diameter, and then cell diameter was converted to biovolume assuming spherical shape. A linear regression was fitted to laboratory calibrations between multiple determinations of mean RALS and mean Coulter counter cell diameter measurements of picoplankton cultures (MED4, WH8102, CCE9901, CCMP2006) preserved while in the exponential phase of growth. Cultures used for the calibration ranged from a minimum Coulter cell diameter of $0.70 \pm 0.00 \mu\text{m}$ (MED4) to a maximum of $2.02 \pm 0.02 \mu\text{m}$ (CCMP2006), with intermediate sizes of $1.09 \pm 0.01 \mu\text{m}$ (WH8102) and $1.19 \pm 0.01 \mu\text{m}$ (CCE9901). The empirically derived linear regression ($r^2 = 0.99$) yielded the relationship $d (\mu\text{m}) = [4.1566 \times (L_{\text{RALS}})] + 0.5918$, where d is the mean diameter and L_{RALS} is the bead-normalized, linearized mean

RALS. This equation fit the data better than the power relationships commonly used for FALS on the basis of Mie light scattering theory (see, e.g., Jacquet et al. 2001).

Three approaches were used for carbon conversion. First, average cell biovolume was converted to carbon with the value 237 fg C μm^{-3} , an average of CHN measurements for the groups of interest: *Synechococcus* (230 fg C μm^{-3}) and *Prochlorococcus* (240 fg C μm^{-3}) from Nolan (unpubl. data) and *Micromonas pusilla* (238 fg C μm^{-3}) from DuRand et al. (2002). This average value was also consistent with our own CHN analysis of *Ostreococcus* sp. strain CCE9901 (single determinations of two cultures showed 233 and 247 fg C μm^{-3}). Second, carbon content was calculated with the equation $\log \text{carbon (pg cell}^{-1}) = 0.94 \times \log \text{volume } (\mu\text{m}^3) - 0.6$ (Eppley et al. 1970). Third, carbon content was calculated as $\text{C (pg)} = 0.433 \times (\text{Biovolume})^{0.863}$ (Verity et al. 1992). Carbon biomass was then determined for each group by multiplying carbon per cell by cell concentration.

Correlation analysis was performed between all parameters both within and between populations; reported values reflect the Pearson correlation coefficient. The fraction change between T_{24} and T_0 (T_{24}/T_0) was also analyzed across dilutions for CHL and RALS for all three populations.

Net growth rate was calculated for the different groups in each bottle on the basis of the T_0 and T_{24} cell counts from the dilution experiments. Dilution data were first tested with an ANOVA according to the methods of Worden and Binder (2003) to determine the suitability of regression analysis. In one experiment (30 July 2001), the assumption of linearity on which this method is based was violated for *Prochlorococcus*. Therefore, we determined the growth and grazing mortality rate using end-point analysis as discussed elsewhere (see Worden and Binder 2003 and references therein). For all other data growth and grazing mortality rates were taken as the Y -intercept and slope, respectively, from the linear regression of the observed net growth rates versus dilution (e.g., Landry et al. 1995b).

Results

Environmental abundance and population characteristics—Photosynthetic picoeukaryotes and *Synechococcus* were always present at this monitoring site, whereas *Prochlorococcus* populations were sometimes absent (Fig. 1). *Synechococcus* concentrations reached $2.6 \times 10^5 \text{ ml}^{-1}$, *Prochlorococcus* reached $7.2 \times 10^4 \text{ ml}^{-1}$, and the picoeukaryote maximum was $5.9 \times 10^4 \text{ ml}^{-1}$. *Synechococcus* dominated numerically on all but five dates: on three dates *Prochlorococcus* dominated, and on two dates picoeukaryotes dominated (Fig. 1). *Prochlorococcus* was low in abundance or absent during the winter and spring months from late January to mid-May 2001. Overall *Synechococcus* corresponded to $60 \pm 12\%$ of the total picoplankton cells, whereas picoeukaryotes comprised $24 \pm 9\%$ and *Prochlorococcus* $16 \pm 13\%$. Seawater temperatures ranged from 13 to 24°C ($17 \pm 3^\circ\text{C}$). Population abundance did not appear to be sensitive to temperature in a coherent fashion, although maximum concentrations occurred below 18°C. Picoeukaryote and *Synechococcus* cell concentrations were well correlated

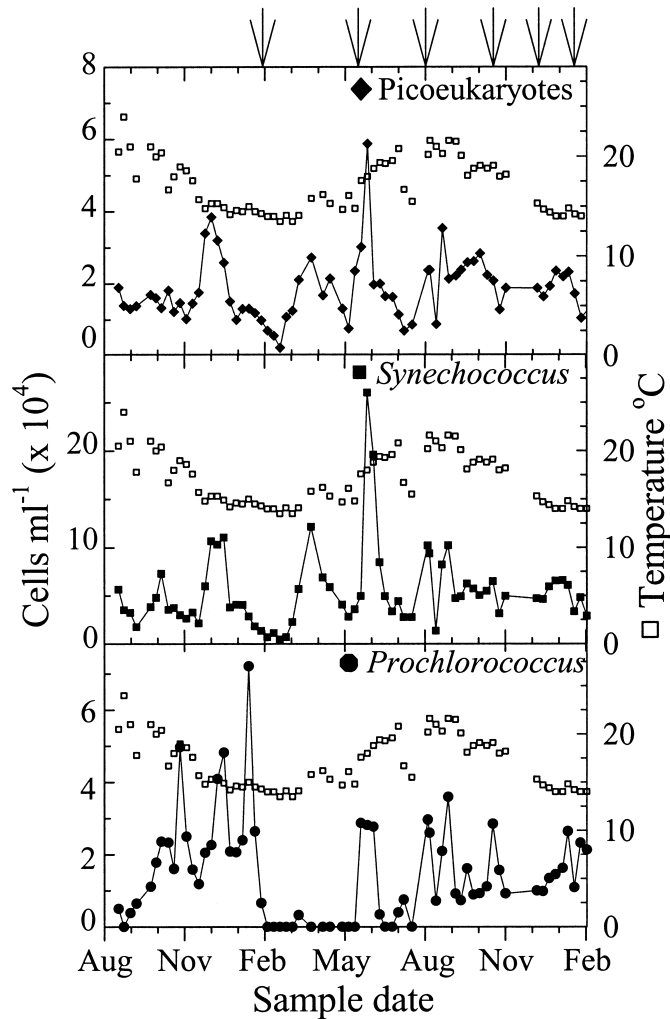


Fig. 1. Picoeukaryote, *Synechococcus*, and *Prochlorococcus* cell concentrations and temperatures from August 2000 through January 2002 at the Pacific Ocean coastal site. Ticks represent approximately the first of the month. Arrows indicate dates on which dilution experiments were performed. Note the different *Synechococcus* y-axis scale.

with one another ($r = 0.76$, $P < 0.0001$). *Prochlorococcus* concentrations were weakly correlated with the latter two populations ($r = 0.27$ and 0.31 , respectively, $P < 0.02$).

CHL fluorescence per cell varied dramatically but non-systematically for all groups, whereas changes in RALS (and cell diameter) were greatest for the eukaryotes (Fig. 2). Picoeukaryotes had the highest CHL fluorescence per cell and the largest mean cell size and *Prochlorococcus* the lowest and smallest. RALS was not related to cell abundances, whereas cellular CHL fluorescence showed a weak negative correlation in both picoeukaryotes ($r = -0.288$, $P < 0.02$) and *Synechococcus* ($r = -0.316$, $P < 0.02$). In picoeukaryotes, CHL fluorescence per cell and mean population cell size were positively and significantly related ($r = 0.53$, $P < 0.0001$). This was not the case for either *Prochlorococcus* or *Synechococcus*. *Synechococcus* cellular CHL fluorescence and temperature were negatively correlated ($r = -0.606$, $P < 0.0001$).

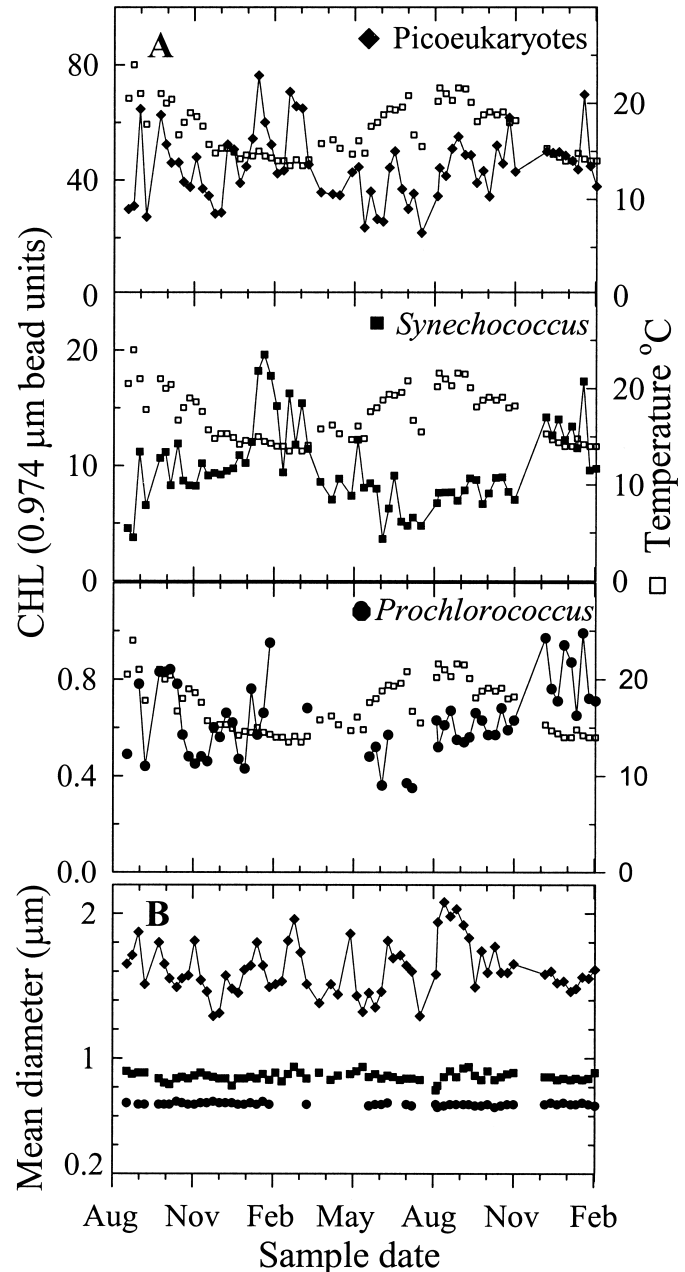


Fig. 2. (A) Changes in picoeukaryote, *Synechococcus*, and *Prochlorococcus* CHL fluorescence per cell over the time series, normalized to linearized bead means. Note the different y-axis scales for each group. The bottom panel (B) shows the respective mean diameters for each group.

Carbon content estimates for picoeukaryotes were calculated with biovolumes determined through size-calibrated RALS data, similar to the approach of DuRand et al. (2001), with the use of our conversion factor ($237 \text{ fg C } \mu\text{m}^{-3}$) rather than theirs ($325 \text{ fg C } \mu\text{m}^{-3}$; see Discussion) and with the equations of Eppley et al. (1970) and Verity et al. (1992). Eppley's approach yielded values (average $533 \text{ fg C cell}^{-1}$, $\text{SD} = 175$) close to ours (average $530 \text{ fg C cell}^{-1}$, $\text{SD} = 185$), whereas Verity's approach yielded higher values (average $863 \text{ fg C cell}^{-1}$, $\text{SD} = 258$). For further interpretation

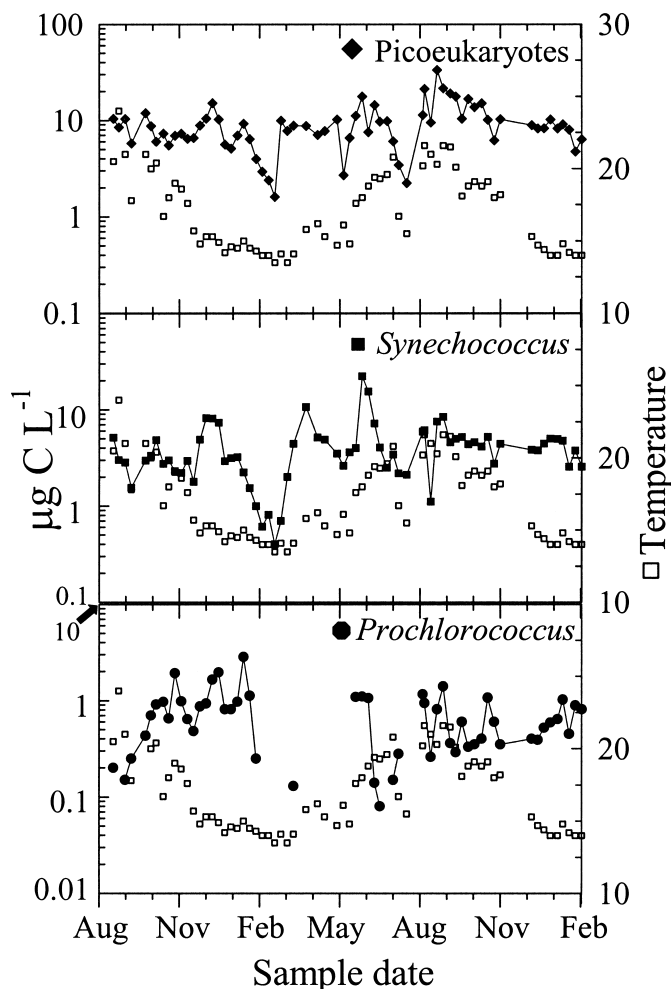


Fig. 3. Changes in standing stock carbon biomass calculated for each of the three groups on the basis of population biovolume and abundance combined with a picoplankton carbon conversion factor. Note the different left y-axis log scales. In all plots, the temperature scale (right y-axis) ranges from 10°C to 30°C.

of the data set, values based on the DuRand et al. approach are employed (see Discussion). *Synechococcus* carbon content averaged 82 fg C cell⁻¹ (SD = 8), and *Prochlorococcus* averaged 39 fg C cell⁻¹ (SD = 1). Picoeukaryotes contained considerably more carbon per cell as well as more variation from date to date (average 530 fg C cell⁻¹, SD = 185).

Fluctuations in carbon biomass over time (Fig. 3) were highly related to changes in cell concentration (*Prochlorococcus*, $r = 0.998$; *Synechococcus*, $r = 0.990$; picoeukaryotes, $r = 0.644$; for all, $P < 0.0001$). Picoeukaryotic biomass was also correlated with size ($r = 0.507$, $P < 0.0001$) and temperature ($r = 0.499$, $P < 0.0001$). The picoeukaryotic component contained the largest fraction of carbon biomass in the picoplankton size class (Fig. 3), with values ranging from 1.60 to 33.28 $\mu\text{g C L}^{-1}$ (average 9.40 $\mu\text{g C L}^{-1}$, SD = 5.20). *Prochlorococcus* carbon biomass ranged from 0.0 to 2.82 $\mu\text{g C L}^{-1}$ (average 0.58 $\mu\text{g C L}^{-1}$, SD = 0.55) and *Synechococcus* from 0.40 to 22.20 (average 4.32 $\mu\text{g C L}^{-1}$, SD = 3.33). Averaged over the entire sampling period, this corresponded to $66.44 \pm 10.86\%$ of carbon bio-

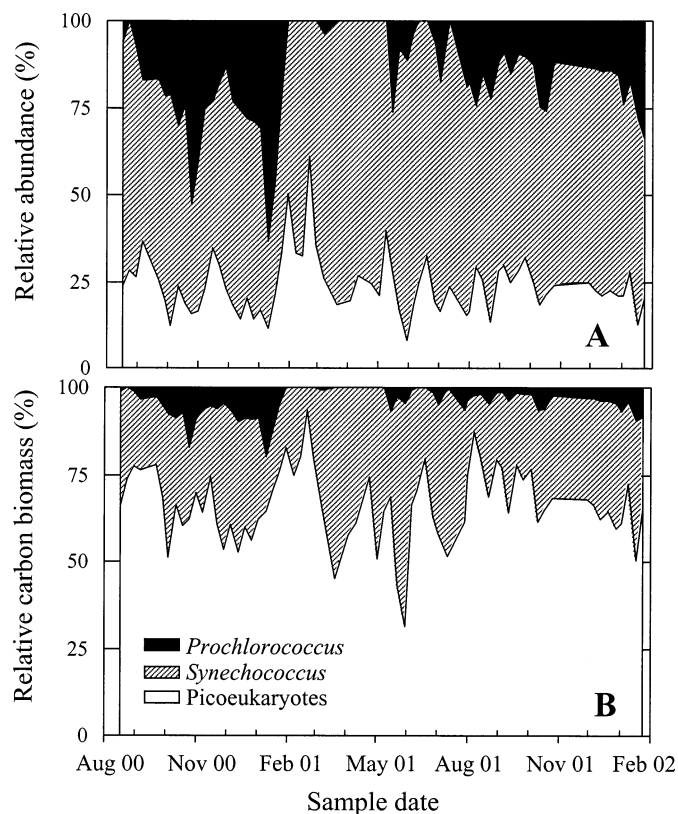


Fig. 4. (A) The relative contribution to picophytoplankton abundance (%) and (B) the relative contribution to picophytoplankton standing stock carbon biomass (%) for each group from August 2000 through January 2002.

mass being picoeukaryotic, $29.36 \pm 10.44\%$ from *Synechococcus*, and $4.20 \pm 4.07\%$ from *Prochlorococcus* (Fig. 4).

Characterization of a study site isolate—To better define the picoeukaryotic component of these populations, we isolated and characterized a picoeukaryotic organism from these waters. On the basis of SSU rRNA gene sequencing, the isolate (CCE9901) is a prasinophyte bearing highest identity (99%) to *O. tauri* (Courties et al. 1994; Courties et al. 1998). TEM showed this small ovoid organism to have no cell wall, just a thin membrane surrounding the cell (Fig. 5). Cell diameter of CCE9901 ranged from 0.7 to 1.2 μm in culture, although no experimental effort was made to define the upper and lower limits of cell size for this organism; indeed, later laboratory experiments have shown it can be 1.5 μm in diameter. The isolate was capable of growth on NH_4 , NO_3 , and urea as sole nitrogen sources at rates higher than *O. tauri* when grown under the same light conditions (Table 2).

Dilution experiments—Field growth and grazing mortality rates were substantially different among the three groups (Table 1). The highest growth rates estimated on each of the experiment dates were those of the picoeukaryotes, with an observed maximum of 1.29 d^{-1} (average $0.99 \pm 0.28 \text{ d}^{-1}$). *Synechococcus* growth rates varied within a fairly small

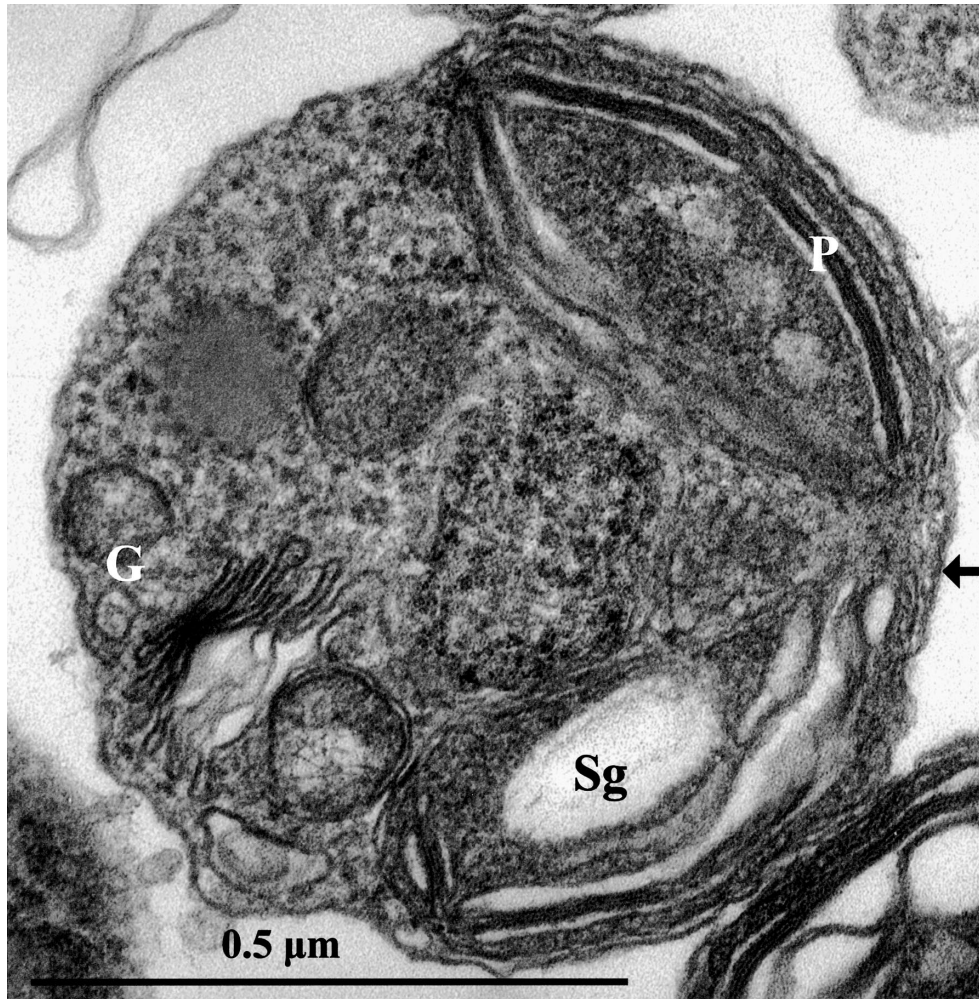


Fig. 5. TEM of *Ostreococcus* CCE9901, a picoeukaryotic prasinophyte present at the study site not previously known to the Pacific Ocean. P, chloroplast; Sg, starch grain within the chloroplast; G, Golgi body. The arrow indicates the cell membrane; notice the absence of a cell wall.

range ($0.66 \pm 0.13 \text{ d}^{-1}$). *Prochlorococcus* consistently had the lowest growth rates measured (average $0.33 \pm 0.14 \text{ d}^{-1}$).

Grazing mortality rates were not directly related to growth rates. Picoeukaryote grazing mortality rates ranged widely, from -0.17 to -1.09 d^{-1} . Although picoeukaryote grazing mortality rates were higher on the three dates with the highest growth rates (1.24, 1.29, and 1.22 d^{-1}), the fraction of growth consumed was not necessarily higher on these dates (Table 1). *Synechococcus* was under the lowest grazing pres-

ures observed on all but one date, when its mortality rate was comparable to that of *Prochlorococcus*. Furthermore, *Synechococcus* abundance and T_0 cell size were negatively correlated ($r = -0.982$, $P < 0.0001$) across the dilution experiments. In two of the three experiments with particularly low grazing mortality, dilution (release from grazing) resulted in a statistically significant (as determined by *t*-test) 10% reduction in mean cell size in the most dilute bottles versus either T_0 cells or undiluted T_{24} cells. *Prochlorococcus* grazing mortality rates were frequently equal to or higher than its growth rate and remarkably constant (-0.36 ± 0.06) regardless of cell concentration or growth rate. In general, variable physiological responses as evaluated by changes in mean fluorescence per cell and not related to incubation light level (see Worden and Binder 2003) were observed in some nutrient-amended treatments but did not appear to affect growth or grazing mortality rates or dynamics relative to nonamended treatments.

Temperature was significantly correlated with growth and grazing mortality rates of picoeukaryotes only. Picoeukaryote growth rates were systematically higher in higher tem-

Table 2. Growth rates of the Pacific Ocean *Ostreococcus* CCE9901 isolate versus *O. tauri* in nitrogen source experiments performed in duplicate. Shown are the average and standard deviation of three successive transfers in the respective nitrogen source.

Nitrogen source	CCE9901 (d^{-1})	<i>O. tauri</i> (d^{-1})
NO_3	0.89 ± 0.04	0.77 ± 0.05
NH_4	0.77 ± 0.01	0.62 ± 0.04
H_2NCONH_2	1.14 ± 0.03	0.78 ± 0.06

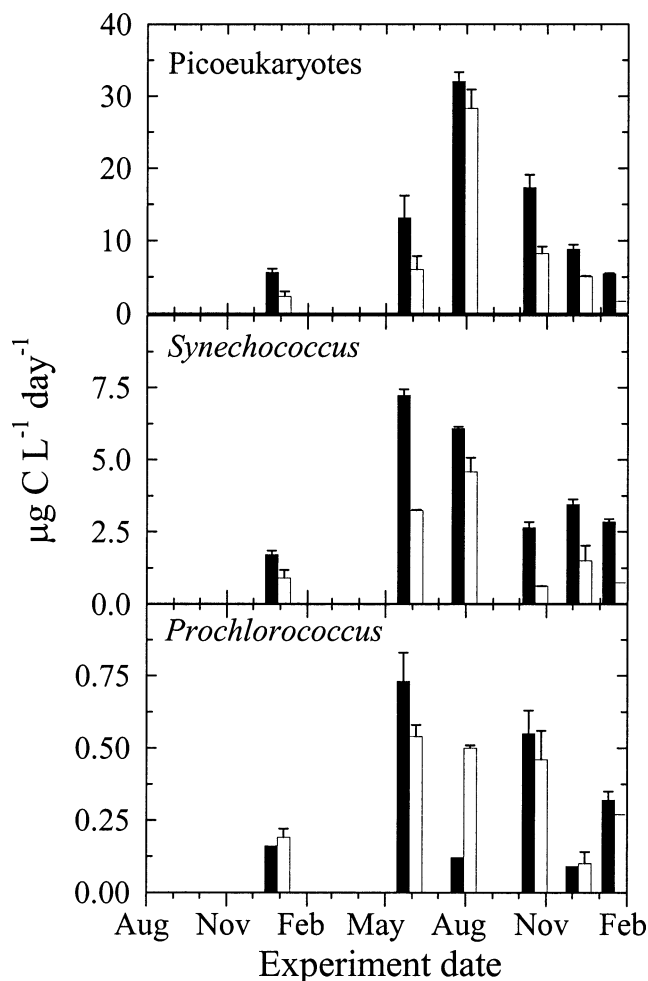


Fig. 6. Net carbon production (dark bars) and carbon consumption (light bars) per day for each picophytoplankton group. Error bars represent the standard deviation for NCP estimates and CC estimates calculated individually for experiment replicates. Note the different y-axis scales.

perature waters ($r = 0.906$, $P < 0.02$), as were grazing mortality rates ($r = -0.917$, $P < 0.02$; greater mortality in higher temperature waters).

Combining carbon biomass and rate measurements—To assess the amount of daily production consumed, we combined carbon biomass data with that of the dilution experiments, yielding six individual “snapshots” of trophic transfer in this system. Net carbon produced per day (NCP) was based on the carbon biomass of the total number of cells produced (as calculated from the T_0 concentration and growth rate) minus that of the initial T_0 cell concentration. Carbon consumed per day (CC) was based on the difference between the carbon biomass of the total number of cells and the carbon biomass of the cells in the T_{24} undiluted bottles. Picoeukaryotes accounted for a large portion of NCP (76%) and CC (79%) in this size fraction (Fig. 6). The ratio of CC/NCP was highest for *Prochlorococcus* on all experiment dates relative to that of picoeukaryotes and *Synechococcus*.

At a more detailed level, passage of carbon through the

picophytoplankton can be seen by incorporating this rate-based dynamic component into the data. For example, although picoeukaryotes abundance was moderate on 19 May 2001 relative to immediately subsequent dates when a bloom appeared to occur, the growth rate (1.24 d^{-1}) was quite high. The grazing mortality rate (0.43 d^{-1}) was comparatively low on this date, and presumably, this relaxed grazing pressure allowed the accumulation of cells represented by the bloom. On 30 July 2001, picoeukaryote abundance was lower than on 19 May 2001 (with a dramatic rise and decline of cell numbers between these dates), growth rate was slightly higher, but grazing mortality was much higher (1.09 d^{-1}). These trends viewed in terms of picoeukaryote standing stock carbon show lower biomass on 19 May 2001 ($4.58 \pm 0.09 \mu\text{g C L}^{-1}$) than on 30 July 2001 ($12.17 \pm 0.50 \mu\text{g C L}^{-1}$) because of the significantly larger mean cell size of the picoeukaryote population on the latter date. The May NCP ($13.14 \pm 3.07 \mu\text{g C L}^{-1} \text{ d}^{-1}$) was greater than the standing stock, with slightly $<50\%$ of this production consumed, reflected in the large carbon biomass increase on subsequent dates. In July, the picoeukaryote NCP ($32.05 \pm 1.31 \mu\text{g C L}^{-1} \text{ d}^{-1}$) was again considerably higher than the standing stock biomass, but the vast majority of this carbon was “unseen” because $\sim 88\%$ of it was consumed immediately.

Discussion

We have focused on the picoplanktonic fraction of the phytoplankton, defined as those cells $\leq 2 \mu\text{m}$, because of their ubiquity, abundance, and persistence, all of which contribute to their potential importance to primary production and carbon cycling. To explore the dynamics of these organisms, we undertook time series sampling at a Pacific Ocean coastal site and used an approach similar to that taken by DuRand et al. (2001) in combination with dilution experiments and culture-based techniques.

The monitoring site employed is a dynamic coastal environment that has been studied for many years (e.g., Eppley 1986). Measurements from 1983 to 1997 show chlorophyll *a* (Chl *a*) to range over four orders of magnitude (0.02 to over $80 \mu\text{g L}^{-1}$), with a long-term mean of $1.58 \mu\text{g L}^{-1}$, whereas nutrient concentrations are generally below detection levels (McGowan pers. comm.). A wide variety of phytoplankton contribute to primary production at this site (see Eppley 1986).

Basic population dynamics—*Synechococcus* and picoeukaryotes were present on all sample dates. *Prochlorococcus* was frequently absent during the spring (Fig. 1), although the role of instrument sensitivity in this absence cannot be ruled out. *Prochlorococcus* populations had low red fluorescence, as has been found in stratified surface waters of the Sargasso Sea (Olson et al. 1990). Low-power lasers (e.g., 15 mW) such as that used in this study are known to be insufficient for discrimination of some *Prochlorococcus* field populations. Other factors, such as advection or heavy grazing, might account for the absence of noticeable *Prochlorococcus* populations during the spring months (Fig. 1). Absence is supported by HPLC pigment analysis showing divinyl Chl *a*, indicative of *Prochlorococcus* consistently

missing during February, April, and early May from 1998 through 2000 (Goericke unpubl. data).

Light scatter, cell size, biovolume, and carbon biomass—Because of the relationship between light scatter and size characteristics (see Morel and Bricaud 1986), flow cytometer-specific calibrations have been used to generate cell biovolume measurements. Olson and coworkers showed FALS to be strongly correlated with Coulter counter size determinations (see Olson et al. 1989; DuRand et al. 2001), whereas Jacquet et al. (2001) defined the relationship between FALS, RALS, and confocal microscopy determinations of cell size. Because biovolume conversion factors are sensitive to cell-size estimates, we derived the calibration for this factor empirically with the use of cultures within the same picophytoplankton groups and size ranges as the field populations under observation. RALS exhibited a very tight relationship with Multisizer cell size measurements best described by a linear regression ($r^2 = 0.99$). In addition to cell size, both refractive index and cell shape can influence the scattering properties of a cell, and these factors might have influenced the linearity of the observed relationship between cell size and RALS. The average cell sizes for *Prochlorococcus* and *Synechococcus* were identical to those reported by DuRand et al. (2001) at BATS (0.68 μm and 0.87 μm , respectively) and slightly lower than those attained by Shalapyonok et al. (2001) in the Arabian Sea. The calculated biovolumes were used to estimate carbon content on the basis of a carbon conversion factor generated with triplicate diel CHN measurements of *Prochlorococcus* and *Synechococcus* (Nolan, unpubl. data) as well as *M. pusilla* (DuRand et al. 2002), a phytoplankter also found at this site. Published *Nannochloris* values (see DuRand et al. 2002) were not used in the carbon conversion factor, although close (252 fg C μm^{-3}), because the mean diameter of these cells (2.71 μm) is significantly larger than those under study. CHN analysis of two CCE9901 cultures (single determinations, growth stage not monitored) also yielded values similar to the average used in this analysis (233 and 247 fg C μm^{-3}). Thus, the carbon conversion used here (237 fg C μm^{-3}) may provide a fairly robust representative value for picophytoplankton. The equation of Eppley et al. (1970) developed for eukaryotic algae yielded very close carbon estimates for the picoeukaryotes, whereas that of Verity et al. (1992) yielded values on average 39% higher than ours. Our estimates showed picoeukaryote cellular carbon content to be 6.5–14 times that of the picocyanobacteria. As discussed elsewhere, there is considerable uncertainty over carbon conversion factors (e.g., DuRand et al. 2001; Shalapyonok et al. 2001); however, the use of cell size in initial biovolume calculations, as well as CHN analysis of relevant organisms, should serve to strengthen these data.

It should be noted that glutaraldehyde can cause cell shrinkage. For instance, Verity et al. (1992) found a 7% reduction in *Synechococcus bacillaris* biovolume after fixation in 0.5% glutaraldehyde (final concentration), and Booth (1987) found a 14% reduction in *M. pusilla* biovolume using 2% fixation (final concentration). Should the fixation procedures used in our study result in cell shrinkage, then picophytoplankton contribution to total carbon would be un-

derestimated. Data are not currently available to assess which species are more or less affected by cell shrinkage or whether glutaraldehyde concentration is a factor (0.25% final concentration was used in this study); however, on the basis of the above studies, shrinkage could result in an underestimation of picoeukaryote carbon relative to *Synechococcus* carbon.

Like DuRand et al. (2001), we found that fluctuations in picophytoplanktonic carbon over time were often determined by changes in cell abundance. Because of the stability in mean cell size of *Prochlorococcus* and *Synechococcus* populations, close to 100% of the variance in total carbon was explained by changes in cell abundance, whereas for picoeukaryotes variation in total carbon was a function of both changes in cell abundance and size. These shifts in mean population size are likely to reflect changes in species composition of the picoeukaryotic fraction. Because all samples were taken in the morning hours, it is unlikely that they are heavily influenced by diel variations in cell size or volume, as observed previously (e.g., Binder et al. 1996; DuRand et al. 2002). The contribution of picoeukaryotes to the average total phytoplanktonic carbon can be calculated on the basis of the long-term mean Chl *a* (1.58 $\mu\text{g Chl a L}^{-1}$) and published C:chlorophyll values for this site. Eppley (1968) found average ratios of 90 for nutrient-depleted waters and 30 for nutrient-rich waters, the latter of which agrees well with an average of ratios (C:chlorophyll 26.23) in Geider (1987) for nutrient-replete cultures grown at temperatures corresponding to those at our site. The Eppley ratios yield average total phytoplankton biomass of 48 $\mu\text{g C L}^{-1}$ and 142 $\mu\text{g C L}^{-1}$ for nutrient-replete and deplete conditions, respectively. The average biomass of picoeukaryotes during our study (9.40 $\mu\text{g C L}^{-1}$) comprises 7% to 20% of the total phytoplankton carbon as calculated above. Depending on the respective production rates of larger phytoplankton versus that of these relatively fast-growing picoeukaryotes, the overall contribution of picoeukaryotes to primary production could be much greater than indicated by standing stock estimates.

Defining the picoplankton community and interactions—To address the competitive abilities of these picoeukaryotes with respect to their prokaryotic counterparts, picoeukaryotes relevant to the system under study must be identified. As a first step, we isolated and characterized a picoeukaryote (CCE9901) from this site initially identified as a prasinophyte on the basis of high-performance liquid chromatography (HPLC)-determined pigment characteristics (Goericke unpubl. data). SSU rRNA gene sequence confirmed this classification and showed highest relatedness to *O. tauri*, the smallest free-living eukaryote known in the world (Courties et al. 1994). CCE9901 provides the first *Ostreococcus* discovered and isolated from the Pacific Ocean. Seasonal 18S rRNA environmental clone libraries confirm the frequent presence of *Ostreococcus* at the study location (Worden and Palenik unpubl. data). Higher growth rates of CCE9901 relative to *O. tauri* on a variety of nitrogen sources indicate this isolate is a physiologically distinct strain (Table 2). Comparison of chromosomal banding on the basis of pulse field gel electrophoresis and other phylogenetic markers sup-

port this finding (Worden and Palenik unpubl. data). This is not surprising considering the different environments from which the strains were isolated, *O. tauri* being from an enclosed, shallow oyster production lagoon (Thau lagoon) and CCE9901 from open coastal waters, often having relatively low nutrient concentrations. The presence of *Ostreococcus* at this Pacific Ocean coastal site, as well as environmentally derived *Ostreococcus* sequences in the Mediterranean (Diez et al. 2001), Antarctic (Diez et al. 2001), and North Atlantic (Vaulot et al. 2002), suggests its role in marine ecosystems is likely more important than previously recognized.

Similarities in *Synechococcus*, *Ostreococcus*, and *M. pusilla* (Cochlan and Harrison 1991) nitrogen utilization abilities could provide insight into the strong positive correlations between *Synechococcus* and picoeukaryote field populations in terms of abundance, growth rate, and CHL fluorescence per cell. Like *Ostreococcus* CCE9901, isolates of *Synechococcus* from this site are capable of using nitrate and ammonia (Palenik unpubl. data), and most *Synechococcus* strains are capable of using urea (Waterbury et al. 1986; Collier et al. 1999). Increases in growth rate and cellular chlorophyll concentration are both well-established responses to a reduction in nutrient stress (e.g., Caperon and Meyer 1972; Sosik and Mitchell 1991), suggesting that the positive relationship between these two picoplankton groups might be founded in similar response to relief from nutrient stress. Clearly, simply considering nitrogen uptake differences is an oversimplification of the complexity of factors affecting these populations; however, it is a step in exploring the underlying reasons for the observed dynamics.

In contrast to the strong positive relationships between picoeukaryote and *Synechococcus* populations, *Prochlorococcus* CHL and abundance were weakly correlated with that of eukaryotes and *Synechococcus*. In addition, *Prochlorococcus* growth rates were not correlated with those of either of the other groups. Interestingly, although no *Prochlorococcus* strain to date can utilize nitrate (see Moore et al. 2002), this organism is reported to use organic nitrogen in the natural environment, and transporter systems for uptake of such sources have been found by genome analysis (Zubkov et al. 2003). Thus, one can hypothesize that although some picoeukaryotic organisms and *Synechococcus* thrive under similar conditions, competing directly for macronutrients such as NO_3 , *Prochlorococcus* relies either on its superior surface area to volume ratio to more efficiently access NH_4 or its ability to take up organic nitrogen compounds at high rates. We found that *Prochlorococcus* numbers were higher during and after the summer when dynamics of this system are thought to be dominated in part by recycled nutrients.

Several other studies of *Prochlorococcus*, *Synechococcus*, and picoeukaryote abundance and biomass support the above hypothesis. The tendency for *Synechococcus* and small eukaryotes to covary and *Synechococcus* to have opposing patterns of abundance with *Prochlorococcus* has been noted in the Arabian Sea (Campbell et al. 1998; Shalapyonok et al. 2001) and the Sargasso Sea (e.g., DuRand et al. 2001). By evaluating different regions of the monsoonal Arabian Sea, Shalapyonok et al. (2001) observed that small eukaryotes were highly successful in terms of abundance and biomass

in coastal upwelling regions and in regions with moderately elevated nitrate levels. In contrast, *Prochlorococcus* dominated in oligotrophic regions in the Arabian Sea. Similar results were found for the low-nutrient, permanently stratified waters (no measurable nitrate) at Station ALOHA in the North Pacific Ocean where picoeukaryotes are generally minor relative to *Prochlorococcus* in terms of both abundance and biomass (Campbell et al. 1994; Campbell et al. 1997).

Differential grazing mortality—Many explanations are possible for the differential grazing mortality observed. A simple explanation is that distinct predator populations specifically or preferentially consume each group. For instance, laboratory experiments with two ciliate grazers showed higher removal rates of *Synechococcus* compared to *Prochlorococcus* and improved growth on *Synechococcus* for one of the ciliate predators (Christaki et al. 1999). In contrast, heterotrophic flagellates underwent significant growth on *Prochlorococcus* but not *Synechococcus* (Guillou et al. 2001). *Synechococcus* has also been found to be selectively egested by some nanoflagellates, whereas other bacteria are digested (Boenigk et al. 2001). *Synechococcus* isolates from Lake Constance were shown to be subject to prey selection, which appears to be based on isolate cell surface characteristics with less-grazed *Synechococcus* having a highly glycosylated, paracrystalline layer (Postius and Ernst 1999). Our study of *Ostreococcus* CCE9901 shows that these cells have no cell wall, only a thin membrane, which we hypothesize could enhance its value (or desirability) as a prey item (Fig. 5).

In the grazing experiments, there was a significant negative relationship between *Synechococcus* cell size and abundance. Furthermore, in two of the three experiments yielding the lowest mortality rates, mean cell size decreased in diluted T_{24} bottles (low grazer concentration) versus undiluted T_{24} bottles (higher grazer concentration). These results indicate that smaller cells were more heavily grazed than larger cells. Grazer size selectivity in field populations has been suggested previously (Dolan and Simek 1999; Worden and Binder 2003). Potentially, several ecotypes are represented within one flow cytometric population, and within populations, ecotype-specific grazing might occur (see Postius and Ernst 1999), or physiological constraints might render different cell sizes. It is unlikely that the observed cell size differences are related to differences in the proportion of larger replicating cells versus smaller divided cells given the sampling time (early morning). Field studies of *Synechococcus* have found that division generally occurs after the onset of morning and before late evening (Sherry and Wood 2001; Binder and Durand 2002, Worden and Binder 2003). Similar results have been demonstrated in laboratory experiments with *Synechococcus* (Jacquet et al. 2001).

Decrease of Prochlorococcus numbers in coastal environments—The decline of *Prochlorococcus* abundance in coastal zones has been hypothesized to be a result of high rates of grazing mortality relative to growth rates, whereas the sufficiently higher growth rates of *Synechococcus* allow it to maintain a sizable population despite grazing pressure (Chisholm pers. comm.). Our results support the hypothesis that

Prochlorococcus numbers are low in these environments because of heavy grazing mortality and suggest that *Synechococcus* may be numerically dominant not only because of high growth rates but also because grazing pressure on this group is lower than on *Prochlorococcus* and picoeukaryotes. The mortality rates we observed here resulted in a greater proportion of *Prochlorococcus* daily growth consumed than seen in some oligotrophic systems (e.g., Worden and Binder 2003). Thus, *Prochlorococcus* might be present close to coastal zones under heavy grazing pressure relative to its growth rate, so essentially it is grazed to extinction unlike the more abundant or faster growing picophytoplankton populations. In environments such as the study site employed here, where upwelling occurs periodically, *Prochlorococcus* might routinely be reintroduced from oligotrophic offshore populations as well.

Implications for trophic transfer of carbon—In all of the experiments conducted, grazing pressure was significantly different for each of the groups, as were growth rates. In several, a significant portion of picoeukaryote and *Synechococcus* NCP was not consumed (on experiment day). This apparent imbalance between phytoplankton growth or carbon production and subsequent consumption has been observed elsewhere (e.g., Worden and Binder 2003), although balance may occur at different temporal and spatial scales than studied here or previously. Understanding the fate of this carbon, whether it undergoes processes such as large grazing events, viral lysis, or advection, is a necessary step toward understanding carbon fluxes in marine systems.

Conclusions—At this study site, picoeukaryotes were the greatest in terms of standing stock carbon, as well as NCP and CC, although not numerically dominant (see Fig. 4). This occurred because of the multifactorial effect of greater biovolume, higher growth rates, and high grazing mortality rates. In some cases, more than 2.5 times the visible picoeukaryote standing stock was produced and consumed in a fashion that was effectively invisible without growth and grazing mortality rate measurements. A member of this picoeukaryotic community is *Ostreococcus*, a potentially important prasinophyte not reported previously in the Pacific Ocean. In contrast, *Synechococcus* was numerically dominant but had relatively low NCP and CC. Clearly, the large differences in NCP and CC between populations are of central importance to understanding trophic transfer in these systems. Furthermore, given the extreme differences in carbon transfer efficiency reported for heterotrophic flagellate consumption of *Prochlorococcus* (23%) versus *Synechococcus* (0.9%) (Guillou et al. 2001), it is critical that greater attention be focused on quantifying carbon transfer efficiency to fully comprehend relative ecosystem roles.

References

- BINDER, B. J., AND S. W. CHISHOLM. 1990. Relationship between DNA cycle and growth rate in *Synechococcus* sp. strain PCC 6301. *J. Bacteriol.* **172**: 2313–2319.
- , AND M. D. DURAND. 2002. Diel cycles in surface waters of the equatorial Pacific. *Deep-Sea Res. II* **49**: 2601–2617.
- , S. W. CHISHOLM, R. J. OLSON, S. L. FRANKEL, AND A. Z. WORDEN. 1996. Dynamics of picophytoplankton, ultraphytoplankton and bacteria in the central equatorial Pacific. *Deep-Sea Res. II* **43**: 907–931.
- BLANCHOT, J., J. M. ANDRE, C. NAVARETTE, J. NEVEUX, AND M. H. RADENAC. 2001. Picophytoplankton in the equatorial Pacific: Vertical distributions in the warm pool and in the high nutrient low chlorophyll conditions. *Deep-Sea Res. I* **48**: 297–314.
- BOENIGK, J., C. MATZ, K. JURGENS, AND H. ARNDT. 2001. The influence of preculture conditions and food quality on the ingestions and digestion process of three species of heterotrophic nanoflagellates. *Microb. Ecol.* **42**: 168–176.
- BOOTH, B. C. 1987. The use of autofluorescence for analyzing oceanic phytoplankton communities. *Bot. Mar.* **30**: 101–108.
- CAMPBELL, L., H. A. NOLLA, AND D. VAULOT. 1994. The importance of *Prochlorococcus* to community structure in the central North Pacific Ocean. *Limnol. Oceanogr.* **39**: 954–961.
- , H. B. LIU, H. A. NOLLA, AND D. VAULOT. 1997. Annual variability of phytoplankton and bacteria in the subtropical North Pacific Ocean at Station ALOHA during the 1991–1994 ENSO event. *Deep-Sea Res. I* **44**: 167–192.
- , AND OTHERS. 1998. Response of microbial community structure to environmental forcing in the Arabian Sea. *Deep-Sea Res. II* **45**: 2301–2325.
- CAPERON, J., AND J. MEYER. 1972. Limited growth of marine phytoplankton, Part 1: Changes in population characteristics with steady-state growth rate. *Deep-Sea Res.* **19**: 601–618.
- CHISHOLM, S. W., R. J. OLSON, E. R. ZETTLER, R. GOERICKE, J. B. WATERBURY, AND N. A. WELSCHEMEYER. 1988. A novel free-living prochlorophyte abundant in the oceanic euphotic zone. *Nature* **334**: 340–343.
- CHRISTAKI, U., S. JACQUET, J. R. DOLAN, D. VAULOT, AND F. RAS-SOULZADEGAN. 1999. Growth and grazing on *Prochlorococcus* and *Synechococcus* by two marine ciliates. *Limnol. Oceanogr.* **44**: 52–61.
- COCHLAN, W. P., AND P. J. HARRISON. 1991. Uptake of nitrate, ammonium, and urea by nitrogen-starved cultures of *Micromonas pusilla* (prasinophyceae): Transient responses. *J. Phycol.* **27**: 673–679.
- COLLIER, J. L., B. BRAHAMSHA, AND B. PALENIK. 1999. The marine cyanobacterium *Synechococcus* sp. 7805 requires urease (urea aminohydrolase, E.C. 3.5.1.5.) to utilize urea as a nitrogen source: Molecular-genetic and biochemical analysis of the enzyme. *Microbiology* **145**: 447–459.
- COURTIES, C., AND OTHERS. 1994. Smallest eukaryotic organism. *Nature* **370**: 255.
- , R. PERASSO, M.-J. CHRETIENNOT-DINET, M. GOUY, L. GUILLOU, AND M. TROUSSELLIER. 1998. Phylogenetic analysis and genome size of *Ostreococcus tauri* (Chlorophyta, Prasinophyceae). *J. Phycol.* **34**: 844–849.
- DIEZ, B., C. PEDROS-ALIO, AND R. MASSANA. 2001. Genetic diversity of eukaryotic picoplankton in different oceanic regions by small-subunit rRNA gene cloning and sequencing. *Appl. Environ. Microbiol.* **67**: 2932–2941.
- DOLAN, J. R., AND K. SIMEK. 1999. Diel periodicity in *Synechococcus* populations and grazing by heterotrophic nanoflagellates: Analysis of food vacuole contents. *Limnol. Oceanogr.* **44**: 1565–1570.
- DURAND, M. D., R. J. OLSON, AND S. W. CHISHOLM. 2001. Phytoplankton population dynamics at the Bermuda Atlantic time-series station in the Sargasso Sea. *Deep-Sea Res. II* **48**: 1983–2003.
- , R. E. GREEN, H. M. SOSIK, AND R. J. OLSON. 2002. Diel variations in optical properties of *Micromonas pusilla* (Prasinophyceae). *J. Phycol.* **38**: 1132–1142.

- EPPLEY, R. W. 1968. An incubation method for estimating the carbon content of phytoplankton in natural samples. *Limnol. Oceanogr.* **13**: 574–582.
- . 1986. Plankton dynamics of the Southern California Bight: Lecture notes on coastal and estuarine studies. Springer-Verlag.
- , F. M. H. REID, AND J. D. H. STRICKLAND. 1970. Estimates of phytoplankton crop size, growth rate and primary production off La Jolla, CA in the period April through September 1967, p. 33–42. *In* J. D. H. Strickland [ed.], *Bulletin of the Scripps Institution of Oceanography*. University of California Press.
- GEIDER, R. J. 1987. Light and temperature dependence of the carbon to chlorophyll *a* ratio in microalgae and cyanobacteria. *New Phytol.* **106**: 1–34.
- GUILLARD, R. R. L. 1975. Culture of phytoplankton for feeding marine invertebrates, p. 29–60. *In* W.C.S.a.M.H. Chanley [ed.], *Culture of marine invertebrate animals*. Plenum.
- GUILLOU, L., M.-J. CHRETIENNOT-DINET, L. K. MEDLIN, H. CLAUSTRE, S. LOISEAUX-DE GOER, AND D. VAULOT. 1999. *Bolidomonas*: A new genus with two species belonging to a new algal class, the Bolidophyceae (Heterokonta). *J. Phycol.* **35**: 368–381.
- GUILLOU, L., S. JACQUET, M.-J. CHRETIENNOT-DINET, AND D. VAULOT. 2001. Grazing impact of two small heterotrophic flagellates on *Prochlorococcus* and *Synechococcus*. *Aquat. Microb. Ecol.* **26**: 201–207.
- JACQUET, S., F. PARTENSKY, J. F. LENNON, AND D. VAULOT. 2001. Diel patterns of growth and division in marine picoplankton in culture. *J. Phycol.* **37**: 357–369.
- KUIPERS, B. R., AND H.J. WITTE. 2000. Prochlorophytes as secondary prey for heterotrophic nanoflagellates in the deep chlorophyll maximum layer of the (sub)tropical North Atlantic. *Mar. Ecol. Prog. Ser.* **204**: 53–63.
- LANDRY, M. R., J. CONSTANTINOU, AND J. KIRSSTEIN. 1995a. Microzooplankton grazing in the equatorial Pacific during February and August 1992. *Deep-Sea Res.* **42**: 657–671.
- , J. KIRSSTEIN, AND J. CONSTANTOU. 1995b. A refined dilution technique for measuring the community grazing impact of microzooplankton, with experimental tests in the central equatorial Pacific. *Mar. Ecol. Prog. Ser.* **120**: 53–63.
- LI, W. K. W. 1994. Primary production of prochlorophytes, cyanobacteria, and eukaryotic ultraphytoplankton: Measurements from flow cytometric sorting. *Limnol. Oceanogr.* **39**: 169–175.
- MOORE, L. R., A. POST, G. ROCAP, AND S. W. CHISHOLM. 2002. Utilization of different nitrogen sources by the marine cyanobacteria, *Prochlorococcus*, and *Synechococcus*. *Limnol. Oceanogr.* **47**: 989–996.
- MOREL, A., AND A. BRICAUD. 1986. Inherent optical properties of algal cells including picoplankton theoretical and experimental results. *Can. Bull. Fish. Aquat. Sci.* **214**: 521–560.
- OLSON, R. J., E. R. ZETTLER, AND O. K. ANDERSON. 1989. Discrimination of eukaryotic phytoplankton cell types from light scatter and autofluorescence properties measured by flow cytometry. *Cytometry* **10**: 636–643.
- , S. W. CHISHOLM, E. R. ZETTLER, AND E. V. ARMBRUST. 1990. Spatial and temporal distributions of prochlorophyte picoplankton in the North Atlantic Ocean. *Deep-Sea Res.* **37**: 1033–1051.
- PARTENSKY, F., J. BLANCHOT, F. LANTOINE, J. NEVEUX, AND D. MARIE. 1996. Vertical structure of picophytoplankton at different trophic sites in the northeastern Atlantic Ocean. *Deep-Sea Res.* **43**: 1191–1213.
- PARTENSKY, F., J. BLANCHOT, AND D. VAULOT. 1999. Differential distribution and ecology of *Prochlorococcus* and *Synechococcus* in oceanic waters: A review. *In* L. Charpy and A. W. D. Larkum [eds.], *Marine cyanobacteria*. Bulletin de l'Institut Oceanographique de Monaco No. sp. **19**: 457–475.
- POSTIUS, C., AND A. ERNST. 1999. Mechanisms of dominance: Coexistence of picocyanobacterial genotypes in a freshwater ecosystem. *Arch. Microbiol.* **172**: 69–75.
- RECKERMANN, M., AND M. J. W. VELDHUIS. 1997. Trophic interactions between picophytoplankton and micro- and nanozooplankton in the western Arabian Sea during the NE monsoon 1993. *Aquat. Microb. Ecol.* **12**: 263–273.
- RIVKIN, R., J. PUTLAND, M. ANDERSON, AND D. DEIBEL. 1999. Microzooplankton bacterivory and herbivory in the NE subarctic Pacific. *Deep-Sea Res. II* **46**: 2579–2618.
- SCANLAN, D. J., AND N. J. WEST. 2002. Molecular ecology of the marine cyanobacterial genera *Prochlorococcus* and *Synechococcus*. *FEMS Microb. Ecol.* **40**: 1–12.
- SHALAPYONOK, A., R. J. OLSON, AND L. S. SHALAPYONOK. 2001. Arabian Sea phytoplankton during southwest and northeast monsoons 1995: Composition, size structure and biomass from individual cell properties measured by flow cytometry. *Deep-Sea Res. II* **48**: 1231–1261.
- SHERRY, N. D., AND A. M. WOOD. 2001. Phycoerythrin-containing picocyanobacteria in the Arabian Sea in February 1995: Diel patterns, spatial variability, and growth rates. *Deep-Sea Res. II* **48**: 1263–1283.
- SOSIK, H. M., AND G. C. MITCHELL. 1991. Absorption fluorescence and quantum yield for growth in nitrogen-limited *Dunaliella tertiolecta*. *Limnol. Oceanogr.* **36**: 910–921.
- VAULOT, D., K. ROMARI, AND F. NOT. 2002. Are autotrophs less diverse than heterotrophs in marine picoplankton. *Trends in Microbiology* **10**: 266–267.
- VERITY, P. G., C. Y. ROBERTSON, C. R. TRONZO, M. G. ANDREWS, J. R. NELSON, AND M. E. SIERACKI. 1992. Relationships between cell volume and the carbon to nitrogen content of marine photosynthetic nanoplankton. *Limnol. Oceanogr.* **37**: 1434–1446.
- WATERBURY, J. B., S. W. WATSON, R. R. L. GUILLARD, AND L. E. BRAND. 1979. Wide-spread occurrence of a unicellular cyanobacterium *Synechococcus*. *Nature* **277**: 293–294.
- , ———, F. W. VALOIS, AND D. G. FRANKS. 1986. Biological and ecological characterization of the marine unicellular cyanobacteria *Synechococcus*. *Can. Bull. Fish. Aquat. Sci.* **214**: 71–120.
- WORDEN, A. Z., AND B. J. BINDER. 2003. Application of dilution experiments for measuring growth and mortality rates among *Prochlorococcus* and *Synechococcus* populations in oligotrophic environments. *Aquat. Microb. Ecol.* **30**: 159–174.
- ZUBKOV, M. V., B. M. FUCHS, G. A. TARRAN, P. H. BURKILL, AND R. AMANN. 2003. High rate of uptake of organic nitrogen compounds by *Prochlorococcus* cyanobacteria as a key to their dominance in oligotrophic oceanic waters. *Appl. Environ. Microbiol.* **69**: 1299–1304.

Received: 14 April 2003

Accepted: 15 August 2003

Amended: 21 August 2003