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Phosphorus availability, phytoplankton community dynamics, and taxon-specific phosphorus status in the Gulf of Aqaba, Red Sea

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Abstract

The relationships among phytoplankton taxon-specific phosphorus-status, phytoplankton community composition, and nutrient levels were assessed over three seasons in the Gulf of Aqaba, Red Sea. During summer and fall, stratified surface waters were depleted of nutrients, and picophytoplankton populations comprised the majority of cells (80% and 88%, respectively). In winter, surface nutrient concentrations were higher and larger phytoplankton were more abundant (63%). Cell-specific alkaline phosphatase activity (APA) derived from enzyme-labeled fluorescence was consistently low (<5%) in the picophytoplankton population throughout the year, whereas larger cells expressed increased APA (up to 68% labeling in some taxa) during the summer and fall but less in the winter. A nutrient addition bioassay during the fall showed that after addition of orthophosphate along with a nitrogen source, APA in larger cells was reduced by half relative to the control, whereas the APA of picophytoplankton groups remained low (<1%) across all treatments. These results indicate that the most abundant phytoplankton in the gulf are not limited by orthophosphate, and only some subpopulations (particularly of larger cells) exhibit orthophosphate limitation throughout the year, and more so in the summer and fall. Our results indicate that orthophosphate availability influences phytoplankton ecology, correlating with shifts in phytoplankton community structure and the nutrient status of individual cells. The role of dissolved organic phosphorus as an important phosphorus source for marine phytoplankton in oligotrophic settings and the need for evaluating nutrient limitation at the taxa and/or single cell level (rather than inferring it from nutrient concentrations and ratios or bulk enzyme activity measurements) are highlighted.

In oligotrophic oceans, phytoplankton growth and community dynamics are strongly influenced by nutrient

availability, and which nutrient ultimately limits production depends on the relative abundance of these nutrients with respect to the phytoplankton nutritional requirements (Redfield et al. 1963). Nutrient limitation of primary production in the ocean has traditionally been attributed to nitrogen (N) and, more recently, iron (Fe) availability; however, phosphorus (P) has been suggested as the ultimate limiting nutrient over geologic timescales (Redfield 1958; Follmi 1996; Tyrrell 1999). The major source of P to the ocean is the weathering of minerals on land that are subsequently introduced into the ocean by fluvial and aeolian sources (Filippelli and Delaney 1996; Benitez-Nelson 2000). Because there is no P input process analogous to N fixation, marine productivity over geological timescales is considered to be a function of the supply rate of P from continental weathering and the rate at which P is recycled in the ocean (Scanlan and Wilson 1999). Accordingly, these factors and their effect on P input to the ocean are believed to be responsible for long-term changes in

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marine ecology, phytoplankton productivity, carbon dioxide fixation, and carbon cycling through the biological pump (Follmi 1996; Toggweiler 1999; Tyrrell 1999).

In recent years, it has been recognized that P limitation in the ocean may be more prevalent than previously estimated, and that the efficiency of P uptake among individual groups of phytoplankton may in fact control the phytoplankton species composition observed in a given community. In the Sargasso Sea, orthophosphate (Pi) is suspected to play an important role in limiting production (Wu et al. 2000; Ammerman et al. 2003). Research in the Pacific Ocean gyres indicates that biological Pi uptake rates far surpass the combined input from atmospheric and deepwater sources, suggesting that P is efficiently recycled within oligotrophic euphotic zones (Björkman and Karl 1994). Further, it has been suggested that a transition from N limitation to P limitation has taken place over the last two decades in the North Pacific Subtropical Gyre, and that this favors the growth of prokaryotic picophytoplankton, such as *Prochlorococcus* and *Synechococcus*, which have a large surface area-to-volume ratio and take up nutrients more efficiently than larger phytoplankton (Karl et al. 2001). It is generally accepted that Pi is the limiting nutrient in parts of the Mediterranean sea on the basis of findings of high N/P ratios (Krom et al. 1991), short turnover times for Pi (Thingstad and Rassoulzadegan 1995), and high alkaline phosphatase activity (Thingstad and Mantoura 2005). However, a recent study revealed that whereas the bacterial and copepod populations were P limited, the phytoplankton populations in the Mediterranean Sea were limited by both N and P (Thingstad et al. 2005).

The nitrogen to phosphorus molar ratio (N:P) of dissolved inorganic nitrogen to soluble reactive phosphorus (SRP) in surface seawater, when compared with the Redfield ratio of 16:1 (originally described by Redfield et al. in 1963), traditionally forms the basis to evaluate which nutrient is limiting marine primary productivity (Redfield et al. 1963; Tyrrell 1999). This approach, however, may be a poor measure of nutrient limitation for several reasons: (1) analytically determined dissolved inorganic nitrogen and SRP may deviate from "true" nutrient concentrations and do not necessarily reflect the bioavailable fractions (Baldwin 1998; Benitez-Nelson 2000), (2) nutrient requirements and uptake rates change with taxonomic affiliation and phytoplankton nutrient status and could differ from the Redfield ratio (Falkowski 2000; Geider and La Roche 2002; Arrigo 2005), and (3) efficient nutrient uptake and recycling may satisfy the nutrition demands of the phytoplankton community despite low absolute nutrient concentrations. Accordingly, interpreting nutrient concentrations and their ratios in the water column as indicators of phytoplankton growth limitation must be done with caution (Cañellas et al. 2000; Hudson et al. 2000); physiological (Scanlan et al. 1997) and molecular (Lindell et al. 2005) measures of nutrition status are better indicators of nutrient limitation of the growth of a particular phytoplankton taxon.

The activity of the enzyme alkaline phosphatase (AlkP) is induced by low Pi concentrations in many phytoplankton species (Dyrman and Palenik 1999; Lomas et al. 2004).

Therefore, alkaline phosphatase activity (APA) is indicative of the P status (specifically Pi limitation) of the phytoplankton groups in which it is measured. This enzyme hydrolyzes dissolved organic P to Pi, which can then be taken up by the phytoplankton (Chrost 1991; Karl and Yanagi 1997). APA can be measured in environmental samples either through bulk (community) or cell-specific assays. Bulk APA has been used as a physiological indicator of Pi stress in marine (Li et al. 1998; Stihl et al. 2001) and other aquatic systems (Jansson et al. 1998; Rose and Axler 1997). However, bulk APA does not provide information about the specific groups of organisms expressing the enzyme activity. Moreover, it may include heterotrophic bacterial activity, which may overestimate the autotrophic component (Nicholson et al. 2006). To obtain information regarding the P status of specific groups of marine phytoplankton, a cell-specific APA enzyme label fluorescence (ELF) assay, allowing rapid qualitative assessment of the in situ physiological condition of phytoplankton with respect to Pi limitation, has been developed and used (Gonzalez-Gil et al. 1998; Dyrman and Palenik 1999).

In this study, we examined seasonal changes in the cell-specific APA of phytoplankton populations collected at several locations and water depths in the northern Gulf of Aqaba. The Gulf of Aqaba, Red Sea, is an oligotrophic region with a predictable seasonal cycle in macronutrient concentrations and phytoplankton community structure that is similar to other large oligotrophic areas of the world's oceans, such as the open ocean gyres. The gulf's accessibility from land therefore provides a convenient opportunity to conduct investigations on the interplay between phytoplankton nutrient status, community dynamics, and water chemistry in an oligotrophic marine system. Changes in the dominance of phytoplankton groups occur as the deep mixing conditions in the winter relax and stratification intensifies, beginning in the early spring and continuing through the summer. Specifically, a bloom of eukaryotic nanophytoplankton characteristic of the winter-summer transition is replaced by a summer community dominated by picophytoplankton (cells $<2 \mu\text{m}$), of which *Prochlorococcus* and *Synechococcus* are the most numerous (Lindell and Post 1995). Previous research (Lindell and Post 1995; Labiosa et al. 2003), however, could only speculate about the driving forces behind this seasonal succession. Although Pi limitation among the bulk community has been suggested on the basis of bulk APA (Li et al. 1998), the authors were not able to comment on the limitation status of individual plankton taxa. The goal of the present study was to investigate how the Pi status varies among various phytoplankton groups in the gulf during three major seasons (winter, summer, and fall), and to determine how the nutrient dynamics of this system may influence natural phytoplankton assemblages from the individual to community level.

Materials and methods

Sampling—Water samples were collected from the northern Gulf of Aqaba (Fig. 1) during three field

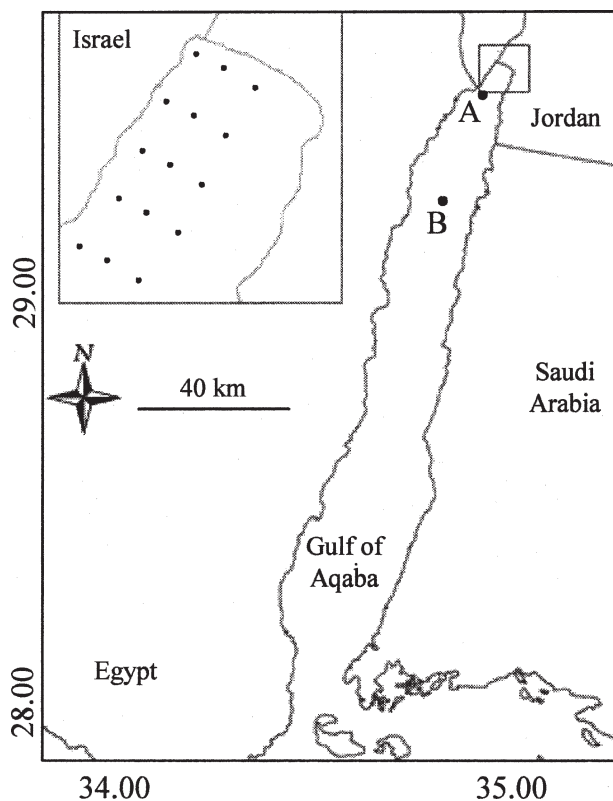


Fig. 1. Map of the Red Sea and surrounding lands and location of Sta. A and B. Inset shows the northern tip of the Gulf of Aqaba and the area over which surface samples were collected.

excursions in the summer (August 2003), winter (March 2004), and fall (November 2004) seasons. Depth profiles were taken at Sta. A ($29^{\circ}28'N$, $34^{\circ}55'E$) and B ($29^{\circ}22'N$, $34^{\circ}53'E$) in Israeli and Jordanian waters, respectively, by using a sampling CTD-Rosette (SeaBird) equipped with 12-liter Niskin bottles. Water was transferred to large (8–20 liter) low-density, flexible polyethylene cubitainers that had been previously rinsed with sample water. Sta. A and B are located away from shore in open water. Both stations are located in deep (>650 m) regions of the northern gulf away from coastline areas, thereby allowing for deep profiles to be taken and for the entire euphotic zone to be sampled. In addition, surface samples were collected from ~ 1 m depth at 15 sites located at various distances from shore and proximity to recreational and industrial areas along the coast. These samples were typically taken 1–2 d before or after the depth profile samples were taken and thus represent different conditions both spatially and temporally. Surface temperature and salinity for the latter samples were measured with a digital probe (YSI). Water samples were prefiltered over a $100\text{-}\mu\text{m}$ nylon mesh to remove zooplankton grazers and collected into cubitainers as described above. The cubitainers were shaded during transport and were delivered to the laboratory at the Inter University Institute (IUI) for Marine Science in Eilat, Israel, within 2 h of collection. Samples from all sites and depths were analyzed for dissolved nutrient concentrations, and samples from within the euphotic zone were also

analyzed for chlorophyll *a* (Chl *a*) and cell-specific APA. Typical euphotic depths were ~ 60 m in the winter and 100 m in the summer and fall.

Nutrient analysis—Nitrate, nitrite, and SRP samples were analyzed by the colorimetric methods described by Hansen and Koroleff (1999) and modified for a flow injection autoanalyzer (FIA, Lachat Instruments Model QuickChem 8000). SRP was preconcentrated by a factor of ~ 20 when the magnesium coprecipitation (MAGIC) method was used (Karl and Tien 1992), followed by measurement that used FIA. Total oxidized nitrogen and nitrite were measured by FIA, and nitrate concentrations were calculated as the difference of total oxidized nitrogen and nitrite. The FIA was fully automated, and peak areas were calibrated by using standards prepared in low-nutrient filtered seawater (summer surface water from the gulf) over a range of $0\text{--}10\ \mu\text{mol L}^{-1}$. By use of standard additions, the recovery of inorganic P in this procedure was determined to be 100%, and the blank was always below detection limits. The precision of our methods is $0.05\ \mu\text{mol L}^{-1}$ for NO_2 and NO_3 , and $0.02\ \mu\text{mol L}^{-1}$ for SRP. The detection limit was $0.02\ \mu\text{mol L}^{-1}$ for SRP and $0.02\ \mu\text{mol L}^{-1}$ for nitrate and nitrite. At SRP levels near the limit of detection, dissolved arsenate has been shown to contribute to the analytically determined SRP in some systems (Johnson 1971; Karl and Tien 1992); however, a test for arsenate showed that it did not contribute significantly to the analytically determined SRP in our samples (T. Rivlin pers. comm.). Ammonium levels were assessed with the fluorescence method by use of ortho-phthaldehyde as described by Holmes et al. (1999) with a precision of $0.02\ \mu\text{mol L}^{-1}$ and a detection limit of $0.01\ \mu\text{mol L}^{-1}$. We note that Pi is the biologically relevant form of P and low Pi levels stimulate APA; however, SRP is the form measured analytically. SRP concentrations tend to closely reflect the amount of Pi in the water although they are not necessarily identical (Benitez-Nelson 2000). Therefore, to distinguish between these components in this study, we use SRP when actual measurements are reported, whereas Pi is used when discussing APA and the physiological responses of cells.

Chl *a* measurement—Chl *a* measurements were made fluorometrically. Duplicate water samples (250 mL) were filtered through Whatman GFF filters. Surface samples were filtered upon arrival at the IUI laboratories, whereas samples from the deep casts were immediately filtered aboard the research vessel. Filters were placed in 10-mL vials and extracted with 90% acetone saturated with MgCO_3 (10 mL per filter) for 24 h in the dark at 4°C . The extract was analyzed fluorometrically before and after acidification with 3.7% HCl on a Turner fluorometer.

ELF assay—Cell-specific APA was measured by using the procedure for ELF-97 labeling as described in Dyhrman and Palenik (1999). In brief, ~ 5 liters of seawater was filtered through Supor filters (Pall) ($0.8\ \mu\text{m}$ filters in August 2003 and $0.2\ \mu\text{m}$ filters in March and November 2004) under a maximum pressure of 15 kPa, either in the laboratory at IUI (for surface-water samples) or aboard

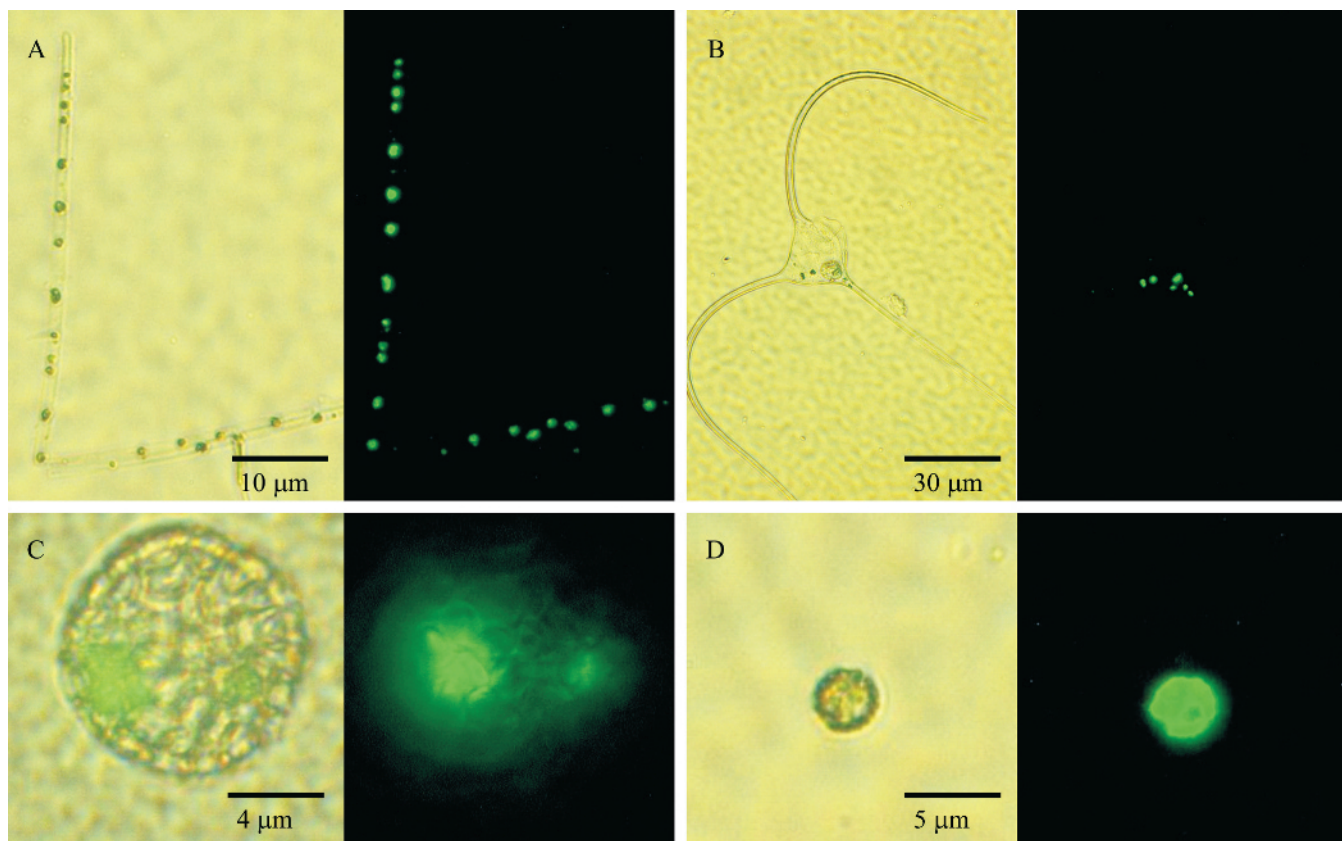


Fig. 2. Micrographs of ELF-97-labeled phytoplankton from the euphotic zone in the Gulf of Aqaba (A) *Trichodesmium* sp., (B) nanoplankton (*Ceratum* sp.), (C) coccolithophore, and (D) *Cyanothece* sp. For each pair, the left panel is a view under visible light and the right under ultraviolet (UV) illumination. ELF-97-labeled areas appear as bright areas under UV illumination.

ship (for the depth profiles). Cells were gently eluted from the filter with 1 mL of 70% ethanol, transferred into sterile microcentrifuge tubes, and allowed to incubate in the dark at 4°C for 30 min. The cells were then pelleted by centrifugation at 3,000 rpm for 5 min, and the supernatant was discarded. The pellet was resuspended and incubated in 95 μ L sterile seawater and 5 μ L ELF-97 reagent (Molecular Probes) for 45 min in the dark, followed by centrifugation and removal of the supernatant. Cells were washed three times by alternating suspension and centrifugation in 100 μ L sterile seawater, followed by a final suspension in 35 μ L sterile seawater. Samples were stored in the dark at 4°C until microscopic analyses were done.

Microscopy—Samples were mounted on microscope slides with a small drop of mounting medium plus 5–8 μ L of the seawater–cell suspension, and were viewed under a Nikon epifluorescent microscope with a DAPI filter set at 400 \times magnification. Digital photographs of the slides were captured for visible light and ultraviolet irradiated conditions. Slides were visually scanned, and each cell was tallied as either positive or negative for ELF labeling on the basis of the presence or absence of the fluorescent green ELF-97 precipitate. For the purposes of this analysis, cells other than picophytoplankton (i.e., cells >2 μ m in diameter) were binned into four groups on the basis of their taxonomy. The groups included two

diazotrophic genera, *Trichodesmium* and *Cyanothece*, of the division Cyanophyta (cyanobacteria), members of the division Prymnesiophyta (coccolithophores), and a fourth group composed of all other cells >2 μ m, referred to as the nanoplankton group (cells between 2 and 20 μ m). This last group included all other families that are less abundant in the gulf, such as Chlorophyta (green algae), Bacillariophyta (diatoms), and Dinophyta (dinoflagellates). Although coccolithophores, *Trichodesmium*, and *Cyanothece* are classified on the basis of size as nanoplankton, they were grouped independently in this study; therefore, our references to “nanoplankton” refer only to other groups of phytoplankton >2 μ m that were not coccolithophores, *Trichodesmium*, or *Cyanothece*. The term “nonpicophytoplankton” is used when referring to the coccolithophore, *Cyanothece*, *Trichodesmium*, and nanoplankton groups collectively. The majority of phytoplankton cells in our samples were <20 μ m in diameter. Figure 2 shows representative micrographs of ELF-97-labeled cells for the phytoplankton groups discussed herein.

The relative number of cells counted from each group provides a semiquantitative estimate of phytoplankton group abundance for each sample, and because of the large volume filtered, it offers the benefit of including groups of organisms that are relatively rare. The cell abundance data should be considered qualitative because the actual cell number per volume seawater was not determined on the

same sample aliquots, and it is possible that some cells were lost during the ELF sample preparation procedure. It is also possible that because of their small size, the picophytoplankton cell numbers were underestimated by this method (particularly in summer, when filters with a larger pore size were used). However, because the same procedure was applied the general differences in distribution of various taxa between seasons, it should be internally consistent across all samples compared herein. The average standard error for triplicate counts of 100 cells using the ELF-97 labeling technique was determined to be 3% by Dyhrman and Palenik (1999), and a similar error is expected in the present study, where >100 cells in each group were counted for each sample whenever possible (and in the case of picophytoplankton, the number of counts was often much higher). Counts from all 15 surface samples were combined into one analysis for each season (likely reducing the error to <3% for these measurements), whereas depth profile measurements each reflect one sample per depth.

Nutrient addition bioassays—Nutrient addition bioassays were conducted to ascertain phytoplankton APA responses to the individual and combined effects of fertilization with inorganic nutrients and aerosol dust. Surface water was collected at 1 m depth from sampling Sta. A in November 2004. Eight liters of surface water per sample was prefiltered over 100 μm nylon mesh to remove zooplankton grazers and collected into large (10 liter) sample-rinsed, low-density, translucent polyethylene cubitainers. Water was shaded during transport and arrived at the laboratory (IUI) within 2 h of collection.

Samples were treated with various combinations of inorganic nutrients and aerosol dust. Samples treated with inorganic nitrogen received sodium nitrate (NaNO_3 , final concentration 20 $\mu\text{mol L}^{-1}$) and ammonium chloride (NH_4Cl , final concentration 20 $\mu\text{mol L}^{-1}$) together; samples treated with inorganic phosphorus received sodium phosphate monobasic (NaH_2PO_4 , final concentration 1 $\mu\text{mol L}^{-1}$); and samples treated with inorganic iron received iron(II) sulfate (FeSO_4 , final concentration 0.016 $\mu\text{mol L}^{-1}$). Samples receiving dust treatments were fertilized with ~ 6 mg aerosol dust collected previously on site at IUI, and dust additions were selected from a series of days with a common air mass back trajectory. After nutrient additions, the samples were incubated for 4 d within a large flow-through outdoor tank through which water from the gulf was circulated. A mesh tarp was used to attenuate the sunlight intensity reaching the samples. After 4 d of incubation, 5 liters of each sample was assayed for APA by the ELF-97 assay as described above.

Results

Density, Chl *a*, and nutrient concentrations of samples from depth transects and surface stations (Figs. 3 and 4) reveal a well-defined seasonal pattern that is driven by alternating periods of stratification and mixing in the Gulf of Aqaba. During the winter season, the upper 300 m of the water column was well mixed, and at the end of winter

(March), surface nutrient concentrations were consistently higher than during the summer stratified season (August) (Fig. 4). November represents the beginning of fall, a time when short-term mixing episodes take place (adding nutrients to the euphotic zone), followed by reestablishment of stratified conditions during warm days and rapid depletion of nutrients in the surface layer.

The nutrient depth profiles (Fig. 3) reveal nitrate concentrations that were 20 times higher in the winter (1 $\mu\text{mol L}^{-1}$) than in the summer and fall (<0.05 $\mu\text{mol L}^{-1}$) in the top 120 m of the water column. SRP concentrations remained low and close to the detection limit throughout the upper 120 m in the summer, increasing gradually with depth <120 m, whereas winter concentrations were low but measurable (0.04–0.08 $\mu\text{mol L}^{-1}$). Mixing during the winter was also evident from the uniform distribution of Chl *a* with depth. The Chl *a* concentration was ~ 0.35 $\mu\text{g L}^{-1}$ throughout the euphotic zone in the winter, whereas in the summer and fall, Chl *a* was lower at the surface (0.1 and 0.2 $\mu\text{g L}^{-1}$) and reached maximal concentrations (0.3 and 0.4 $\mu\text{g L}^{-1}$) deeper within the euphotic zone (deep Chl *a* maxima).

The averaged nutrient concentrations of the 15 surface-water samples (Fig. 4) indicate that in winter, SRP, nitrate, and ammonium concentrations were 0.04, 0.37, and 0.06 $\mu\text{mol L}^{-1}$, respectively, whereas in the summer and fall, these levels were near the limit of detection. It should be noted that nutrient concentrations for the combined surface samples (e.g., the average of 15 distinct stations sampled at 1 m depth) differ somewhat from the surface nutrient levels reported in the depth profiles for Sta. A and B. This is due to spatial and temporal variability, as discussed in Materials and Methods. Specifically, in winter, the samples were collected during the transitional period at the end of winter–beginning of spring, and the surface-water samples were taken 1–2 d after the depth profiles. During this time, the water column began to stratify, leading to relaxation of light limitation and subsequent increases in phytoplankton growth, which drew down nutrient levels within the surface water.

The ratios of nitrate plus nitrite to SRP (Fig. 5) ranged from 14 to 22 throughout the water column during the winter and were in that range below the euphotic zone during the summer and fall. However, during the summer and fall, the ratios in the euphotic zone decreased dramatically, reaching values as low as 3 in the surface and increasing with depth. The ratios in the euphotic zone in the summer and fall, however, are not reliable because the SRP concentrations were at or below the detection limit of the analysis.

Microscopic enumeration of cells from the ELF-97 assay provided a semiquantitative measure of the relative abundance of dominant taxa comprising the phytoplankton communities during the winter, summer, and fall. The relative abundance of each phytoplankton group was estimated as the fraction of cells in a specified group divided by the total number of phytoplankton cells counted, and is reported as a percentage. These relative abundances therefore include all cells within a given group regardless of ELF-97 labeling. Analysis of the combined

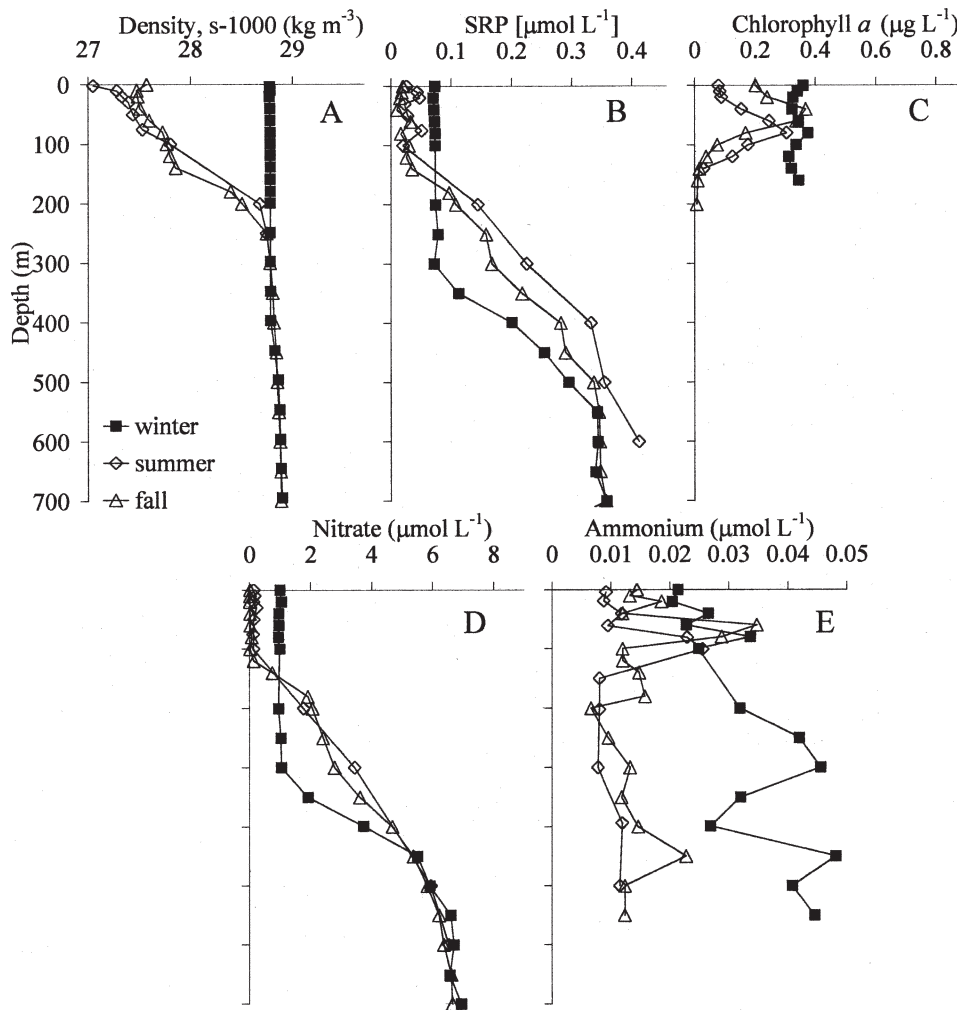


Fig. 3. Typical depth profiles of (A) density, (B) SRP concentration, (C) Chl *a* concentration, (D) nitrate concentration, and (E) ammonium concentration for winter, summer, and fall. Profiles for Sta. A and B were similar; for simplicity, only the Sta. A profile is shown.

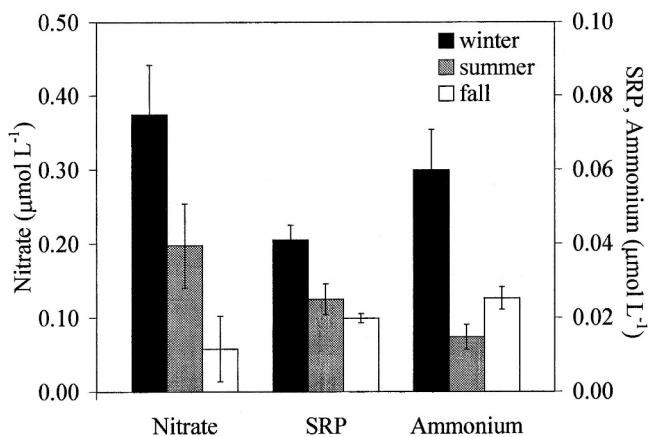


Fig. 4. Surface nutrient concentrations in winter ($n = 12$), summer ($n = 28$) and fall ($n = 15$) for nitrate, SRP, and ammonium. Error bars represent standard error of the mean.

surface-water samples indicated that picophytoplankton (cells $< 2 \mu\text{m}$) represented a significant portion of the phytoplankton community throughout the year, particularly in the summer and fall, when nutrient concentrations were very low (Fig. 6). This group represented about 37% of cells counted in the winter, whereas they represented 80–88% of cells in summer and fall, increasing in relative abundance as stratification progressed and nutrients became scarce. In the summer and fall, when the water column was stratified, the relative abundance of the picophytoplankton remained high throughout the euphotic zone ($\sim 100 \text{ m}$); all other groups showed a subsurface maximum in the abundance (at $\sim 10 \text{ m}$) during the summer (Fig. 7A).

Microscopic enumeration of the nonpicophytoplankton taxa in the combined surface samples revealed seasonal shifts in their relative abundances. *Trichodesmium* was present in low numbers ($\sim 2\%$ of cells) during the winter season; however, *Trichodesmium* filaments were even scarcer throughout the summer and fall, representing a negligible fraction of the phytoplankton community. By

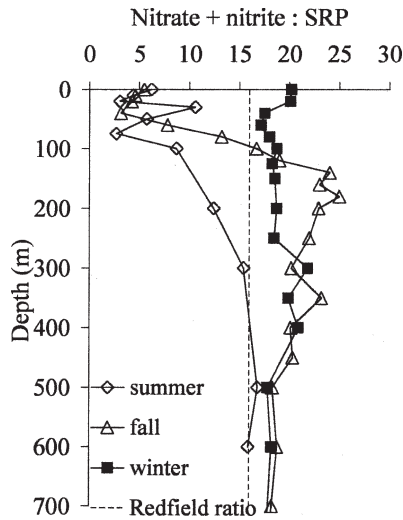


Fig. 5. Depth profiles of the ratio between nitrate plus nitrite and SRP during winter, summer, and fall. Dashed line indicates the canonical Redfield ratio value of 16. Please note that the summer and fall ratios in samples shallower than 150 m are unreliable because they are calculated from concentrations of N + N and SRP below detection limits.

contrast, *Cyanothece* (a smaller N-fixing phytoplankter) represented a major portion (42%) of the phytoplankton community in the winter and was present (albeit in lower abundance) throughout the summer (7%) and fall (2%), following the observed decrease in surface nutrient levels (see Figs. 4 and 6). The coccolithophore population underwent a similar decline in relative abundance as the seasons progressed from winter through summer and into fall; however, the decline was not as gradual as observed in the *Cyanothece* group. Rather, the coccolithophores represented a constant proportion (~7% of cells) through winter and summer, but declined to <1% of the phytoplankton cells by the fall. The nanoplankton group, which

comprised several different taxa of phytoplankton, was less abundant in the summer than in the winter, representing 6% and 9% of phytoplankton cells in the summer and fall, respectively, compared with 12% in the winter.

ELF-97 labeling was used to measure cell-specific APA within phytoplankton communities throughout the year, and cells were binned into groups as described above (Figs. 2 and 7). The overall ELF-97 labeling efficiency for all phytoplankton groups combined (e.g., number of cells labeled from total cells counted) did not change much over the year, ranging from 11% in winter to 16% in the summer, and was only 6% in the fall, despite considerable changes in SRP concentrations. The ELF-97 labeling of the picophytoplankton was consistently low across all seasons (1.5–5% of cells). A small increase from 1.5% in winter to 5% in summer was observed as SRP concentration decreased; however, the percentage labeling decreased from 5% in summer to 3.5% in early fall despite sustained low SRP concentrations, although these values are similar given the error estimate for this method. It is possible that the low ELF labeling observed in the picophytoplankton population in general (all seasons) is not a result of low APA but rather stems from lack of labeling of this group when the ELF protocol is used (Rengefors et al. 2003). However, the few instances in which picophytoplankton were labeled in our field samples suggest that that this is not the case, as also shown by Lomas et al. (2004), where a stronger cell-penetrating procedure was applied. In addition, when performed on pure cultures of *Prochlorococcus* sp. strain MED4 and *Synechococcus* sp. strain WH8102 grown in the laboratory, the procedure also showed that these cells could indeed exhibit ELF labeling (Fig. 8). Thus, the lack of substantial ELF-97 labeling among picophytoplankton indicates that these populations are generally not Pi limited and are able to satisfy their P requirements in the very low nutrient concentrations seen in the gulf waters in the summer and fall months (see Stihl et al. 2001; Fuller et al. 2005).

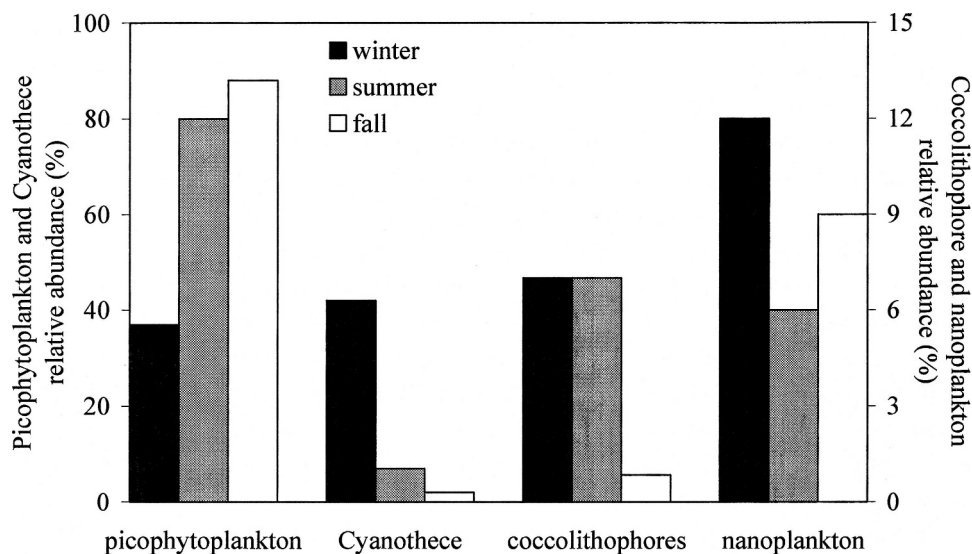


Fig. 6. Relative abundances of phytoplankton groups in surface water for the winter, summer, and fall. Note the different scales for the various species.

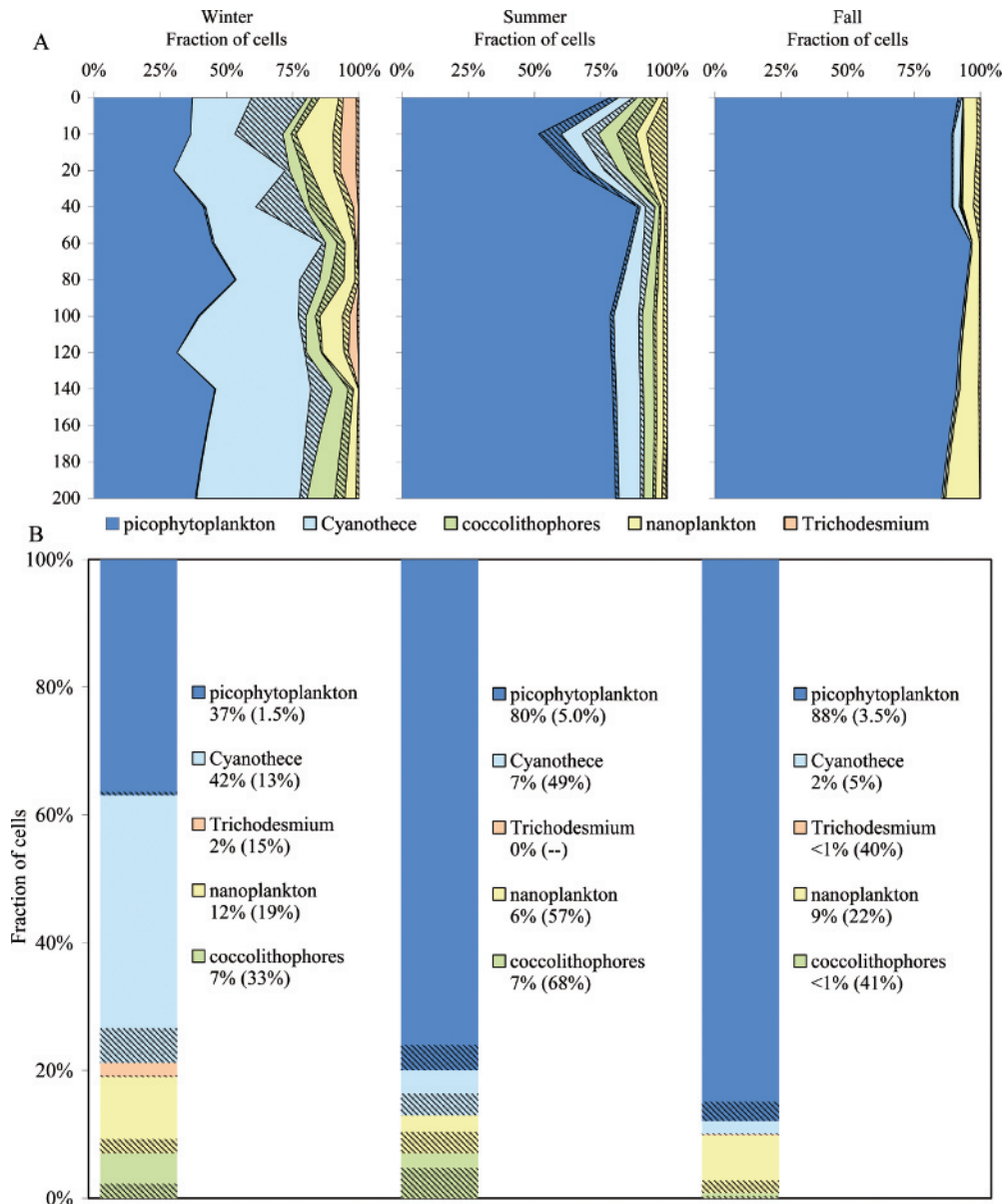


Fig. 7. (A) Depth profiles of the relative abundances of phytoplankton groups throughout the euphotic zone for (from left to right) the winter, summer, and fall. Hatched areas represent the percentage ELF-labeled cells from each group (e.g., percentage picophytoplankton labeled from total picophytoplankton counted). (B) Community alkaline phosphatase activity showing relative abundance (solid colors) and ELF-97-labeled fractions (hatched colors) for each phytoplankton group in the combined surface samples for (from left to right) the winter, summer, and fall. The percent values are the percentage of each group of phytoplankton from the total counted population. Hatched areas represent the percentage of ELF-labeled cells from each group (i.e., percentage of picophytoplankton labeled from total picophytoplankton counted).

ELF-97 labeling throughout the year was higher among the nonpicophytoplankton, with a threefold increase from winter (when average labeling was 17%) to summer (58% labeling), followed by a decrease in fall, when 21% of cells expressed ELF-97 labeling. The *Cyanothecae* cells, which decreased in abundance from winter to summer and fall, were characterized by a more than threefold increase in ELF-97 labeling between winter (13% labeled) and summer (49% labeled), whereas fall showed the lowest level of

labeling (5%) (Fig. 7B). Similar trends were observed in the coccolithophore and nanoplankton groups. ELF-97 labeling within the coccolithophore group underwent a twofold increase between winter and summer (increasing from 33% labeling to 68% labeling), but decreased in the fall (43% labeling). Likewise, ELF-97 labeling in nanoplankton showed a threefold increase between winter (19% labeling) and summer (57% labeling), followed by a decrease in fall (23% labeling). When present (during the winter), 15% of

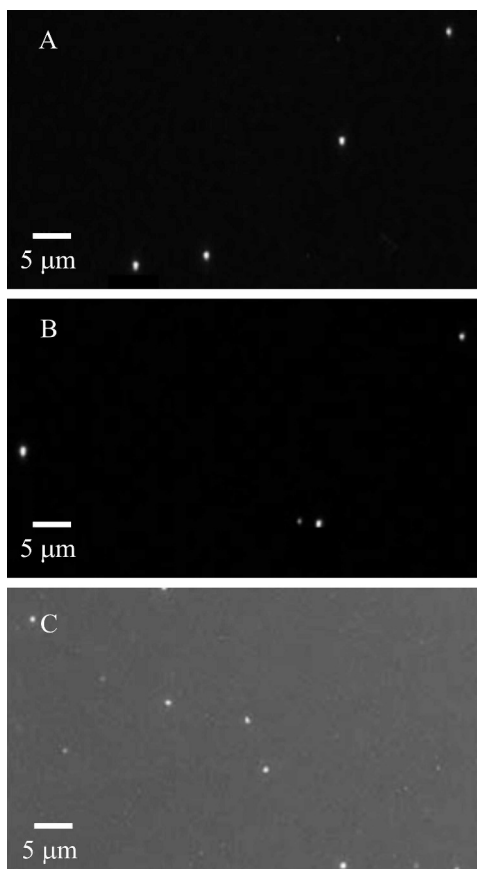


Fig. 8. Micrographs of ELF-97-labeled picophytoplankton (A) *Prochlorococcus* sp. strain MED4 grown in culture (B) *Synechococcus* sp. strain WH8102 grown in culture, (C) picophytoplankton from the euphotic zone in the Gulf of Aqaba. Pictures taken under UV illumination; bright spots indicate ELF-97-labeled cells.

Trichodesmium cells exhibited ELF-97 labeling (see Fig. 7B). *Trichodesmium* was not sufficiently abundant to accurately enumerate ELF-97 labeling during the summer; however, a general increasing trend in labeling was observed throughout the summer in the cells that were counted.

Analysis of samples from the nutrient addition bioassay demonstrated that samples receiving dust alone or together with N and/or P, as well as samples receiving inorganic N and P together showed two- or threefold increases in chlorophyll *a* (Chl *a*) relative to the control (no nutrient addition) sample (data not shown; Mackey et al. 2006). Of these samples, nonpicophytoplankton represented ~10% of the population. At the beginning of incubation, ELF-97 labeling indicated that 32% of the nonpicophytoplankton cells were labeled. The picophytoplankton group (~90% of cells) showed very low (<1%) ELF-97 labeling at the beginning of the experiments and remained low across all treatments. The ELF-97 labeling of nonpicophytoplankton in the above addition treatments decreased by half when P was added (e.g., samples receiving dust and P with or without other nutrients or with out dust but with N and P together) (Fig. 9). By contrast, treatments receiving dust

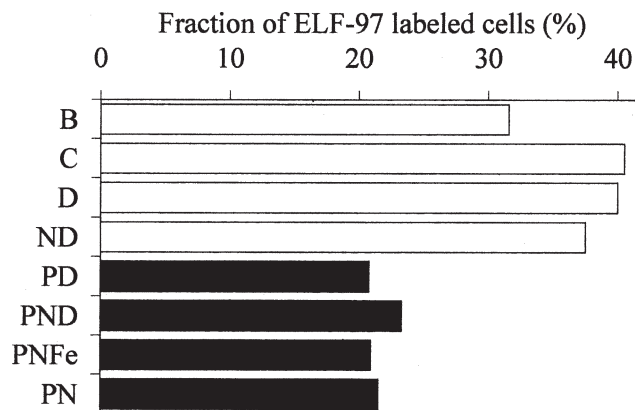


Fig. 9. Percent ELF-97 labeling of nonpicophytoplankton cells (>2 µm) in samples that showed Chl *a* increases greater than the control in enrichment experiments. Values are expressed in percentages as the fraction of ELF-97-labeled cells out of the total number of cells counted. Shaded bars represent samples receiving phosphate; B, baseline; C, control (no nutrient addition); D, dust; N, nitrate + ammonium; P, phosphate; Fe, iron.

alone or in combination with N (but not phosphate) showed labeling similar to the control (~40%) despite having increased levels of Chl *a*. Samples receiving single nutrient amendments of N or P alone or with Fe had Chl *a* levels similar to the control and had no clear change in APA.

Discussion

Density and nutrient measurements in this study (Figs. 3 and 4) are consistent with previous reports for the Gulf of Aqaba (Lindell and Post 1995; Badran et al. 2005; Fuller et al. 2005) and show the seasonal patterns typical of this and other oligotrophic oceans (Venrick 1993). The phytoplankton community dynamics in the gulf appear to be heavily influenced by the seasonal changes in physical and chemical characteristics of the water column, as found in other studies (Lindell and Post 1995; Li et al. 1998; Labiosa et al. 2003). Seasonal shifts in the relative abundances of various phytoplankton groups correspond to pronounced seasonal changes in the trophic state of the gulf, switching from mesotrophic conditions in the winter to oligotrophic conditions in the summer and fall (Lindell and Post 1995).

The increase in the relative abundance of picophytoplankton as stratification progresses and nutrient concentrations in the surface decrease indicates that there is an advantage for small cells in the low nutrient waters of the gulf. This may be due to the greater cellular surface area to volume ratios, higher nutrient uptake efficiencies (Bertilsson et al. 2003; Heldal et al. 2003), and/or lower nutrient requirements of these organisms (Van Mooy et al. 2006). Further, flow cytometry and cell count data show that the relative abundances of the phytoplankton groups comprising the picophytoplankton population (i.e., *Prochlorococcus*, *Synechococcus*, and picoeukaryotes) also varied seasonally as nutrient levels changed (R. Labiosa unpubl. data). By contrast, the relative abundances of all nonpicophytoplankton groups decrease from winter to summer, and with the exception of the group composed of

mixed nanoplankton cells, continue to decrease from summer to fall as oligotrophic conditions progress.

In general, the picophytoplankton showed low levels of ELF-97 labeling throughout the year (Fig. 7A,B). Depth profiles show very little labeling for picophytoplankton throughout the water column in the winter, whereas in the summer and fall, labeling was particularly low at greater depths where nutrients are recycled (potentially yielding higher Pi availability) (Fig. 3). Further, although the ELF-97 labeling of picophytoplankton in the combined surface samples was consistently low across all seasons, winter labeling (1.5%) was lower than summer (5.0%) and fall (3.5%) labeling (although the error associated with these measurements should be kept in mind when comparing the fall and winter values). These trends are consistent with decreased APA with higher Pi concentrations, but they also indicate that picophytoplankton in the gulf were, for the most part, not Pi limited during all seasons.

In contrast to the picophytoplankton, ELF-97 labeling trends in all other phytoplankton groups reveal seasonal variability, indicative of various degrees of Pi limitation. Cells other than picophytoplankton are therefore responsible for the majority of the autotroph APA in our samples, despite representing only a fraction (and sometimes a very small fraction) of the entire phytoplankton community. Overall, ELF-97 labeling among the nonpicophytoplankton was lowest in winter, highest in summer (two- to threefold greater than winter), and moderately low in fall (approximately twofold less than summer). The trend of increased labeling between winter and summer is expected, as APA would increase with decreasing SRP concentrations in each of these groups. The observed decreases in APA between summer and fall despite SRP concentrations below the detection limit during both sampling periods is less intuitive and could be related to a transient increase in Pi within the euphotic zone preceding our sampling in the fall. In the fall, air temperatures begin to decrease, and when they are below the surface-water temperature, transient mixing (brought about by the initial degradation of stratified layers) occurs. This can be followed by restratification of the upper water column if warm days reoccur. Field observations and modeling indicate that this process begins in the gulf around the month of November (R. Labiosa unpubl. data). We suspect that such a transient mixing and restratification event may have introduced Pi from depth before our sampling and led to a corresponding decrease in APA in our fall samples. The low nutrient concentration we measured resulted from rapid uptake following restratification. Lower levels of APA within this time frame (compared with summer) is feasible given that there is a lag between AlkP expression and Pi concentration due to, on the one hand, the time required for enzyme synthesis and depletion of internal P stores and, on the other hand, the time required for enzymes to be broken down when they are no longer needed by the cell (Dyhrman and Palenik 1999; Lomas et al. 2004). If Pi were transiently available from a brief mixing event before our sampling date, as might be expected for this season, the resulting decrease in APA could have been sustained at the time of our sampling in spite of the low ambient Pi concentrations because of the lag time to

synthesize new AlkP and may have led to the reduced labeling (relative to summer) of the coccolithophore, *Cyanothece*, and nanoplankton populations in surface waters in the fall (Fig. 7B). Interestingly, *Cyanothece* labeling in the fall is even lower than in the winter. Although a presampling mixing event could explain lower APA activity in the fall than that expected on the basis of SRP concentrations, the activity is not likely to be lower than the winter values. It may be possible that a different strain of *Cyanothece* with different constitutive levels of APA is present in winter than in fall; however, our data cannot resolve this, and more work should be done to address this possibility.

When considering the bulk ELF labeling of the entire phytoplankton community (expressed as percentage of all cells labeled from total cells counted), our study indicates that the observed seasonal changes are relatively small throughout the year (6–16%). Similarly, in both surface samples and along depth profiles the total fraction of ELF-97-labeled cells does not seem to decrease in a consistent manner as SRP concentrations increase. At first glance, this would seem to imply little or no effect of the seasonal SRP concentration changes (from $<0.01 \mu\text{mol L}^{-1}$ in summer to a maximum of $0.08 \mu\text{mol L}^{-1}$ in winter) on the Pi status of the phytoplankton. However, when shifts in phytoplankton relative abundances are taken into account along with labeling, the effects of Pi deficiency are better resolved. Our data show that during seasons when Pi and other nutrient levels are low, the phytoplankton population is dominated by picophytoplankton that are generally not Pi limited, whereas higher levels of Pi and other nutrients support a greater diversity of phytoplankton taxa (some of which may still be limited by Pi despite the detectable SRP concentrations). Therefore, although variation in Pi availability is not the only factor controlling seasonal phytoplankton succession, it does affect the nutrient status of some phytoplankton groups and parallels changes in community composition.

It has been suggested that in warm ($>25^\circ\text{C}$), highly stratified, calm water where nitrogen sources are impoverished and Fe is available, the diazotroph *Trichodesmium* may be abundant (Carpenter and Romans 1991; Mulholland et al. 2002). It is interesting that *Trichodesmium* in the Gulf of Aqaba was not abundant in the summer months during the time of our sampling despite these favorable conditions. We suggest that *Trichodesmium* populations were limited by Pi availability, and because of the low Pi concentrations in the euphotic zone, they could not flourish during the summer months, despite their ability to use dissolved organic P, concentrations of which were 10-fold higher than SRP (Mulholland et al. 2002; Dyrman et al. 2006). In contrast, during the winter, when Pi was more abundant, *Trichodesmium* cells were observed, and a substantial portion of them exhibited APA, indicative of their higher P requirements and ability to use organic P to satisfy the P nutrition needs. This confirms work of Stihl et al. (2001), which determined the bulk APA of *Trichodesmium* colonies (when these were found) in the gulf and suggested that they were Pi stressed.

Interestingly, the diazotroph *Cyanothece* is more abundant than *Trichodesmium* in all seasons, comprising $\sim 42\%$

of cells in the winter. As with *Trichodesmium*, there is a measurable decline in *Cyanothece* abundance as stratification progresses and Pi becomes scarce. However, compared with *Trichodesmium*, *Cyanothece* is present in higher numbers throughout the summer, indicating that it may be better adapted than *Trichodesmium* to survival under low Pi conditions. Because both *Trichodesmium* and *Cyanothece* are able to utilize dissolved organic P, the ability to synthesize AlkP is probably not the source of *Cyanothece*'s competitive advantage. Rather, *Cyanothece* likely dominates because of its smaller size, which may enable it to grow more efficiently than *Trichodesmium* when acquiring Pi from the limited pool. The dominance of *Cyanothece* over *Trichodesmium* under extremely low Pi conditions underscores the fact that a growth-limiting nutrient, in this case Pi, likely influences competition among the phytoplankton here. Although the majority of phytoplankton (i.e., the picophytoplankton) in the gulf are not Pi limited, some subgroups within the population exhibit Pi deficiency, and the ability of these phytoplankton groups to use organic P sources may enable them to survive extreme oligotrophic conditions. Further, these data demonstrate that although increased APA can be used as an indicator of Pi limitation, it does not necessarily confer a competitive advantage to cells; cell size may in fact provide a more important competitive edge in oligotrophic waters.

These results highlight the variability in Pi status of different taxa within the same community. Traditional Pi limitation assays that measure the bulk APA of the community are therefore somewhat limited because they cannot attribute enzyme activity to specific individuals and groups. Fortunately, ELF-97 labeling allows APA to be assessed in a way that is taxonomically meaningful and ecologically relevant. When we compare each group across the seasons, we generally observe lower ELF-97 labeling during the winter, indicating less APA when Pi (and other nutrients) is more abundant. Thus, despite an apparently small seasonal change in APA for the population as a whole, change in APA within each nonpicophytoplankton group is evident. These conclusions are also supported by the nutrient addition bioassay, which demonstrated that the numerous picophytoplankton were not limited by Pi alone, and only some of the larger cells (representing a small fraction of the overall phytoplankton community) were Pi limited; this limitation was at least partially relieved with nutrient additions.

In addition to highlighting the importance of interpreting community dynamics and nutrient status together, our results also underscore two potential difficulties associated with inferring nutrient limitation from nutrient concentration and ratio data, particularly in oligotrophic waters where the nutrient concentrations are close to the limits of detection. First, dissolved nutrient ratios neglect organic nutrient sources and can fail to account for variable nutrient demands (i.e., the range in N:P ratio demands among species) and preferences for nutrient source among different taxonomic groups. Specifically, these ratios may not be a good measure of nutrient status in waters where *Prochlorococcus* dominates because recycled sources of N

(e.g., ammonium, urea, dissolved organic N), which are not considered in the N:P ratio calculation, are the primary N sources for these cells (Dufresne et al. 2003). The second difficulty is that as nutrient levels approach their respective detection limits, the ratios of these values become more prone to error. To illustrate this point, our data show that in the winter, surface N:P ratios range from 15 to 20 (in the range of the Redfield ratio; see Fig. 5), and nonpicophytoplankton cells are abundant. By contrast, in the summer, when picophytoplankton are dominant, surface ratios fall to 3, a ratio that would typically be interpreted as indicative of N limitation. However, the large error associated with this ratio and its inability to account for some biologically relevant nutrient sources for picophytoplankton suggest that the ratio may not be a good indicator of nutrient limitation during this oligotrophic season. Indeed, trends in seasonal succession and ELF-97 labeling indicate that Pi may limit some phytoplankton groups in the summer despite the low calculated N:P ratios. Therefore, although N:P ratios can still provide information about nutrient levels in many marine systems and may have validity at the community level, they should not be applied indiscriminately or exclusively when assessing nutrient limitation. Rather, the approach is strengthened by including measures that account for taxonomic variability.

The relation between Pi concentrations and the degree of response of individual phytoplankton taxa to Pi availability is reflected in the APA status of the cells and, to an extent, in the relative abundances of phytoplankton groups. Our data show that although Pi availability may limit some phytoplankton taxa in this system, the majority of cells (i.e., the picophytoplankton) are not Pi limited throughout the year. Indeed, Pi is likely not the sole nutrient affecting phytoplankton distribution patterns; SRP and oxidized nitrogen concentrations in the water column are highly coupled (i.e., both have higher concentrations in the winter and lower concentrations in the summer), and co-control of phytoplankton production and abundance patterns is very likely. Similar investigation of the physiological responses of various phytoplankton groups to N and light limitation should be carried out.

Our data have shown that although coexisting groups of phytoplankton may encounter identical environmental conditions, physiological differences among them, such as variability in cellular nutrient quotas and acquisition mechanisms, ultimately cause each group to have a different nutrient status. The importance of collecting accurate nutrient status information over a broad range of ecological scales becomes apparent as more complex and robust biogeochemical models require such information. In many regions, changes in phytoplankton community structure resulting from changing nutrient availability and species-specific adaptations to variable nutrient levels may directly affect the amount of export production from these systems because cell size, density, and aggregation potential affect sinking rate (Karl et al. 1997; Arrigo et al. 1999; Boyd and Newton 1999). Accordingly, because phytoplankton are major contributors to global primary production (Behrenfeld and Falkowski 1997; Partensky et al. 1999), understanding nutrient controls and stress responses

of natural phytoplankton populations is necessary for understanding the interplay between nutrient cycling, phytoplankton community composition, primary production, and the global carbon cycle at present and in the future.

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