DYNAMICS OF PHOTOSYNTHETIC PLANKTON IN THE OLIGOTROPHIC NORTH PACIFIC SUBTROPICAL GYRE

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ABSTRACT

The North Pacific Subtropical Gyre (NPSG) is the largest marine ecosystem on Earth. However, the diversity and population dynamics of photosynthetic plankton in this ecosystem are not well understood, owing in part to undersampling of the habitat. In this dissertation, I utilized time-series observations at Station ALOHA (22°45’ N, 158° W), the field site for the Hawaii Ocean Time-series (HOT) program, to examine temporal dynamics in size-dependent photophysiology, population structure of eukaryotic phytoplankton, and contributions of several eukaryotic phytoplankton taxa to particle flux. Photosynthesis-irradiance (P-E) relationships for two size-fractions (> 2 µm and 0.2-2 µm) of phytoplankton were used to evaluate variability in photophysiology over a 5 year period (2004-2009) in the NPSG. Our results indicate although larger phytoplankton appear to constitute a relatively small fraction of phytoplankton biomass and production, the photophysiological responses of these organisms demonstrate high variability. Despite persistently oligotrophic conditions, phytoplankton in this larger size class appear to undergo temporally dynamic variations in growth. Population dynamics of three major groups of eukaryotic phytoplankton in the larger size class were further investigated over a 2 year period (2007-2009). Quantitative polymerase chain reaction (QPCR) assays were developed based on form 1D rbcL genes for diatoms, prymnesiophytes and pelagophytes. Diatom rbcL genes were typically the most abundant among these groups, with elevated abundances often occurring in the upper euphotic zone (0-45 m) during the summer. Abundances of prymnesiophyte and pelagophyte rbcL genes often increased in the lower euphotic zone (75-125 m) during fall and winter months. Analyses of upper ocean (150 m) sediment trap samples revealed that export of prymnesiophytes and pelagophytes from
the euphotic zone tended to be greatest in the spring and fall. In contrast, diatom $rbcL$
gene flux was often greatest in the summer when particulate carbon export was maximal.
To gain insight into the role of diatoms in biogeochemical dynamics in this ecosystem, I
examined temporal variability in upper ocean diatom population structure and their
contributions to particle export at Station ALOHA. PCR amplification, cloning and
sequencing of diatom $rbcL$ genes provided insight into the phylogenetic structure of
diatom populations in this ecosystem. QPCR amplification of $rbcL$ genes from five major
diatom genera (Chaetoceros, Pseudonitzschia, Nitzschia, Rhizosolenia and Hemiaulus)
revealed that diatom population structure has high temporal variability. Diatoms
belonging to the genera Pseudonitzschia/Nitzschia were typically the most abundant of
the $rbcL$ phylotypes examined, while Hemiaulus and Rhizosolenia $rbcL$ gene abundances
increased episodically in the upper euphotic zone in summer. Analyses of the upper
ocean (150 m) sediment trap samples suggested that Hemiaulus group contributed the
most to diatom $rbcL$ gene fluxes. Sediment trap collections in the deep sea (4000 m)
indicated a narrowly focused peak in the export of the Hemiaulus, Chaetoceros and
Rhizosolenia groups in the mid-summer months. Our study suggests that temporally
dynamic growth of the larger size phytoplankton, especially diatoms, contribute
significantly to the new production in the upper ocean and carbon flux into the deep
ocean.
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Chapter 1

General introduction: Phytoplankton dynamics in the North Pacific Subtropical Gyre

Unicellular photosynthetic plankton comprise the base of the ocean’s food web and are major components of the marine carbon cycle, contributing approximately 50% of global primary production (Falkowski 1994, Field et al. 1998). Oligotrophic ocean gyres occupy approximately 60% of the ocean surface area, making them important habitats for marine biodiversity and productivity. The North Pacific Subtropical Gyre (NPSG) is the largest of these open ocean habitats (Sverdrup et al. 1946, Karl 1999), and plays a major role in the global carbon cycle. Thus, understanding temporal and spatial variability in phytoplankton growth, biomass, and production in the NPSG, and how variations in environmental conditions affect phytoplankton dynamics has important ecological and biogeochemical implications.

The surface waters of the NPSG are optically very clear due to persistently low standing stocks of living organisms attributable to low nutrient concentrations. Penetration of light through the upper ocean permits net photosynthetic production to occur deep to depths > 125 m (Letelier et al. 1996, Karl 1999). The euphotic zone of the NPSG has been described as a two-layer system (Dugdale 1967, Eppley et al. 1973, Knauer et al. 1984, Small et al. 1987), where the upper layer is characterized by high light flux, extremely low nutrient concentrations and high rates of primary production. The lower region of the euphotic zone is light-limited, relatively nutrient-enriched, and maintains a persistent deep chlorophyll maximum layer (DCML, Fig. 1).
Thermal stratification of the upper ocean results in the majority of primary production in the well-lit layer of euphotic zone being supported by regenerated nutrients (Winn et al. 1995, Karl 1999, Dore et al. 2002). In the nutrient-replete lower layer, primary production appears to be predominately regulated by energy, namely solar irradiance. Time-series observations of light flux and primary production at Station ALOHA (22 ° 45’N, 158 ° 00’W), the field site for the Hawaii Ocean Time-series (HOT) program, reveal different temporal patterns of primary production and light flux between the upper euphotic zone (0-45 m) and lower euphotic zone (75-125 m). Sea-surface light flux and integrated primary production in the upper euphotic zone reach their annual maxima during the summer (June-August) and decrease in fall. In contrast, downwelling photosynthetically active radiation (PAR) and integrated primary production in the lower euphotic zone peak in the spring (April-May) and decrease through summer and fall (Fig. 2). The reduction of PAR at depth in summer suggests that the accumulation of plankton biomass in the upper euphotic zone increases light attenuation and decreases the flux of light into the lower layer of water. Such results suggest that elevated rates of productivity during the summer stem from increases in photosynthetic biomass throughout the spring and summer.

Physical climate variability and plankton community structure

Studies in the NPSG indicate that phytoplankton biomass and primary production can be spatially variable across basin, meso- and submeso- scales (e.g., Benitez-Nelson et al. 2007, Fong et al. 2008, Polovina et al. 2008, Wilson et al. 2008, Calil and Richards 2010), while time series observations at Station ALOHA indicate plankton community
structure varies across decadal, interannual, intra-annual time scales (Letelier et al. 1993, Karl et al. 1995, Karl 1999, Karl et al. 2001, 2002, Corno et al. 2007, Bidigare et al. 2009). In 2001, Karl et al. (2001) synthesized measurements of pigments and primary production in the NPSG over a 30-year period (1968-1998), reporting that Chl $a$ concentrations and rates of primary production had increased more than 2-fold. This increase in Chl $a$ and productivity was accompanied by an increase of Chl $b$ and decrease in dissolved phosphorus. The increase in Chl $b$ concentrations suggested that cyanobacteria, namely Prochlorococcus, had become more abundant in this region. The authors hypothesized that climate-forced successional changes in photosynthetic plankton, “a domain shift”, resulted in the observed changes in upper ocean biogeochemistry (Karl et al. 2001). The shift in plankton population structure appeared to be linked to climate variability, including largely the El Niño/Southern Oscillation (ENSO) and the Pacific Decadal Oscillation (PDO) (Karl et al. 1995, Karl 1999, Karl et al. 2001, 2002, Corno et al. 2007, Bidigare et al. 2009). During a positive phase of the PDO (1965-1977), Venrick et al. (1987) observed increased winter wind strength and decreased sea surface temperatures (SST), factors that contributed to a shift in carrying capacity of the NPSG ecosystem. Such shifts in primary producer population structure could affect the food web structure, nutrient dynamics and export production (Karl et al. 2001, 2002).

During the study period of the HOT program (1988-present), analyses of climate indices suggests three periods where the ENSO and PDO demonstrated similar dynamics: 1991-1993, 1997-2005 and 2008-2009 (Fig. 3) (Walter and Timlin 1998). Both the ENSO and PDO indices were relatively strongly positive (warm) during 1991-
1993 and 1997-1998, while the ENSO and PDO anomalies were relatively weak and in phase during 1999-2005 and 2008-2009 periods. Furthermore, there were 3 short transition periods: 1989-1990, 1994-1996 and 2006-2007, when the PDO and ENSO anomalies were out of phase and shifting between negative and positive phases (Fig 3). Near-surface temperature anomalies displayed a decrease from 1989 to 1990, an increase during 1994-1996 period and a decrease from 2007 to 2008, indicating that the large-scale climate forcing may affect local physical conditions during these transition periods.

Previous studies on climate forcing in the NPSG have been mainly focused on the strong impact of the ENSO/PDO warm event from 1997-1998 on the NPSG ecosystem (Lukas 2001, Corno et al. 2007, Bidigare et al. 2009). Corno et al. (2007) suggested that the interactions between ENSO and PDO may change upper ocean stability and influence the phytoplankton community structure, primary production and particle export. Bidigare et al. (2009) further assessed the biological responses to climate forcing in the NPSG, finding that plankton structure was sensitive to basin-scale destratification of the upper ocean and concomitant enhancement in nitrate flux accompanying changes in convective mixing. When ENSO and PDO were not in phase during ENSO warm events, the upper ocean was generally more stratified (Karl et al. 1995). In contrast, upper ocean stability appears to have decreased and become more susceptible to wind mixing during periods where ENSO and PDO were in phase and strong (Corno et al. 2007, Bidigare et al. 2009). Such contrary physical dynamics occurring during transition periods in the phasing of ENSO and PDO indices likely also play important roles in phytoplankton community structure.
Research on phytoplankton dynamics at Station ALOHA has emphasized the important role of picoplankton as key components of biomass and productivity in this ecosystem (Campbell and Vaulot 1993, Campbell et al. 1994, Karl et al. 2001, Church et al. 2006, Malmstrom et al. 2010). Photosynthetic prokaryotes, specifically Prochlorococcus, are dominant components of upper ocean plankton biomass in the NPSG (Campbell and Vaulot 1993, Letelier et al. 1993, Anderson et al. 1996).

Throughout the euphotic zone, picophytoplankton (0.2-2 µm in size) dominate the total phytoplankton biomass (Chl a) and primary production, while the larger phytoplankton (> 2 µm size) comprise a relative small portion of the total phytoplankton standing stock (Fig. 4).

Over the course of nearly 20 years (1968-1985), microscopic evaluations of phytoplankton community structure (taxa larger than 5 µm) at Station Climax (28°N, 155 °W) in the northern NPSG were examined by Venrick (1982, 1988, 1990, 1992, 1993, 1999). These studies identified two vertically separated phytoplankton assemblages in this environment (Venrick 1982). One group of photosynthetic plankton appeared largely relegated to the high-light, low-nutrient regions of the upper ocean, while the other assemblage typically associated with the deep chlorophyll maximum layer, where nutrient concentrations were elevated and seasonally variable (Venrick 1982, Letelier et al. 2004). More than 230 phytoplankton species were identified and listed as part of these studies, including 101 diatom species and 47 species belonging to the prymnesiophytes (Venrick 1982). In the summers of 1994 and 1996, Venrick (1997, 1999) compared phytoplankton species collected at Station ALOHA to those previously
observed at Station Climax, finding that similar species inhabited both of these locations, including similar shallow and deep plankton flora.

In addition to microscopic determinations of phytoplankton assemblages, time-series analyses of diagnostic algal pigments measured at Station ALOHA have also been utilized to examine time-variability in phytoplankton assemblages. Such measurements indicate that prymnesiophytes and pelagophytes comprise dominant components of eukaryotic phytoplankton biomass at Station ALOHA (Letelier et al. 1993, Anderson et al. 1996). The resulting time series of pigment concentration anomalies suggests variations in phytoplankton assemblages may be sensitive to basin-scale climate forcing (Fig. 5). For example, between 2005 and 2009, when ENSO/PDO indices were out of phase, upper euphotic zone (0-45 m) 19'-hexanoyloxyfucoxanthin (19-hex, a biomarker for prymnesiophytes) decreased (ANOVA, p < 0.05), while both 19’-hexanoyloxyfucoxanthin and 19’-butanoyloxyfucoxanthin (19-but, a biomarker for pelagophytes) in the lower euphotic zone decreased (75-125 m). In contrast, fucoxanthin (an accessory pigment produced by diatoms) concentrations in either the upper or lower euphotic zone did not appear as sensitive to variations in ENSO/PDO.

Letelier et al. (2004) explored the relationship between PAR and Chl $a$ concentrations in the lower euphotic zone of Station ALOHA and concluded that seasonal variability in Chl $a$ was driven by the annual light cycle at the base of the euphotic zone. Use of a pigment-based algorithm (Letelier et al. 1993, Chapter 3) to examine the contribution of the major eukaryotic phytoplankton groups in the lower euphotic zone (1989-2009) revealed that on average, prymnesiophytes, pelagophytes and
diatoms contributed ~26%, ~10% and ~2% to total Chl $a$ (Fig. 6). The percentage of Chl $a$ contributed by prymnesiophytes was generally lower between 2005 and 2009 than at other periods in the time series record (Fig. 6). The time series of derived contributions of pelagophytes to total Chl $a$ in the lower euphotic zone indicates that the biomass of these phytoplankton decreased toward the latter years of this time series record (2005-2009).

**Eukaryotic phytoplankton dynamics**

To date there are relatively few studies specifically focused on assessing temporal and spatial variability in eukaryotic phytoplankton assemblages in the NPSG. Cortés et al. (2001) investigated coccolithophore (prymnesiophytes) ecology at Station ALOHA over a 3-year study period, identifying upwards of 125 distinct species. This study concluded that abundances of coccolithophores typically peaked in the spring (March) and in the fall (September/October). Scharek et al. (1999a) investigated the abundance of diatoms during 11 cruises (1994-1995) to Station ALOHA, finding that two lightly silicified diatom species (*Hemiaulus hauckii* and *Mastogloia woodiana*) demonstrated large increases in abundance in July 1994 within the well stratified mixed layer; these groups were identified as key contributors to material export from the upper ocean to the deep sea.

Previous studies suggest that diatoms generally constitute a low fraction of phytoplankton biomass in the NPSG (Letelier et al. 1993, Anderson et al. 1996). Application of the previously described pigment algorithm (Letelier et al. 1993, Chapter 3) to assign contribution of major groups of eukaryotic phytoplankton to total Chl $a$ in the lower euphotic zone (75-125 m) suggests that the contributions of diatoms generally
increases during the winter, frequently peaking in April (Fig. 7), when downwelling PAR is near its annual maximum in the lower euphotic zone (Fig. 1). In contrast, such light-driven dynamics were not observed in the annual distribution of Chl $a$ contributed by prymnesiophytes and pelagophytes in the lower euphotic zone (Fig. 7).

Information on the ecological roles and potential importance of diatoms to oligotrophic ocean ecosystems remains limited. Globally diatoms are estimated to contribute up to 40% of the 45 to 50 billion metric tons of organic carbon produced each year in the sea (Falkowski et al. 2004). Moreover, export of diatom-derived organic matter is a major component of the ocean’s biological pump (Falkowski et al. 1998), whereby photosynthetically derived organic carbon is transported to the ocean’s interior by either sedimentation of particulate material or mixing of dissolved organic carbon (Carlson et al. 1994). In many regions of the world’s oceans, export of diatom biomass comprises the largest fraction of the annual particulate carbon export to the deep sea (Dugdale et al. 1995, Kemp et al. 2000), potentially linking diatom growth to geological variations in global climate via alteration in ocean-atmospheric fluxes of carbon dioxide (Brzezinski et al. 2002).

Various lines of evidence suggest that diatoms play central roles in the exchange of material between the upper ocean and the deep sea in oligotrophic ecosystems such as NPSG. Goldman (1993) formalized a hypothesis to explain how diatoms living in the nutrient-depleted open ocean could comprise important components of the carbon cycle in these systems. He suggested that the episodic injection of new nutrients into the lower portion of the euphotic zone could lead to rapid diatom cell growth with subsequent
export of this biologically produced material to the deep sea. Consistent with this hypothesis, time-series measurements of particulate carbon and particulate silica export at Station ALOHA and at the Bermuda Atlantic Time-series Study (BATS; 31° 40’N, 64° 10’W) in the Sargasso Sea demonstrate coupling in carbon and silica fluxes with export of both elements often occurring episodically (Deuser et al. 1995, Dore et al. 2008). Intriguingly, in the NPSG, export of both elements is generally greatest in the summer when the ocean is thermally stratified and vertical nutrient delivery to the upper ocean is restricted (Fig. 8). Scharek et al. (1999b) reported significant seasonal variability in diatom export (varying more than three orders of magnitude) to the deep sea at Station ALOHA. Such results suggest diatoms play important roles in driving seasonal and event-scale variability in organic matter export, coupling numerous bioelemental cycles (C, N, P, Si, Fe) between the epipelagic and deep ocean.

**Bloom observations and N₂ fixation**

The spring bloom is a prominent component of various polar and temperate latitude ecosystems. This recurring feature is triggered by thermal stabilization of the upper ocean following deep winter mixing; the resulting enrichment in nutrients and increased solar radiation promote the growth of photosynthetic plankton (Sverdrup 1953, Siegel et al. 2002). The NPSG does not display a recurring spring bloom, in part because winter mixing generally fails to penetrate into the nutricline, thereby restricting input of nutrient-enriched waters to the well lit upper ocean. Although enigmatic, phytoplankton blooms appear to be regular features of the low nutrient waters in the NPSG (Venrick 1974, Marumo and Asaoka 1974, Mague et al. 1974, Gundersen et al. 1976, Mague et al. 1976, Venrick et al. 2002).

Major blooming forming genera of diatoms (Hemiaulus, Rhizosolenia and Mastogloia) found at Station ALOHA have been observed in close association or in apparent symbiotic relationships with various genera of nitrogen-fixing cyanobacteria, including Richelia, Calothrix and Trichodesmium (Villareal and Carpenter 1989, Villareal 1991, 1994, Carpenter et al. 1999, Gómez et al. 2005, Foster et al. 2007, Wilson et al. 2008, Dore et al. 2008, Fong et al. 2008). Although Mastogloia woodiana has not been shown to harbor diazotrophic symbionts, it was suggested that Mastogloia woodiana may be able to obtain fixed nitrogen from other bloom cyanobacteria (Dore et al. 2008). Many lines of evidence from the HOT program measurements suggest that
Dinitrogen (N₂) fixation is a significant source of new nitrogen in the NPSG (Karl et al. 1997, Dore et al. 2002), which may contribute up to half of the nitrogen supporting the annual export production at Station ALOHA (Karl et al. 2008). Rates of N₂ fixation at Station ALOHA are typically maximal between June and August, consistent with the period of time when diatom biomass is elevated (Dore et al. 2002, Church et al. 2009). Thus increased rates of N₂ fixation could fuel subsequent diatom blooms in the upper euphotic zone of NPSG.

Objectives of the dissertation

Numerous lines of evidence indicate that eukaryotic phytoplankton undergo strong interannual and seasonal oscillations in productivity and biomass in the oligotrophic oceans. Our understanding of the roles that different groups of phytoplankton, especially diatoms, play in carbon and nutrient cycling in the NPSG is limited in part by our knowledge of temporal dynamics associated with phytoplankton population structure. The broad goal of my dissertation is to investigate population dynamics and photosynthetic characteristics of eukaryotic phytoplankton assemblages in the NPSG. The study includes several key elements: 1) evaluation of size-dependent differences in primary production and photosynthesis; 2) examination of population dynamics and vertical export of several prominent assemblages of eukaryotic phytoplankton; 3) elucidation of temporal variations in diatom population structure and its potential impact on upper ocean particle flux and productivity. I address these objectives through the following chapters.
Chapter 2 investigates the difference between two size classes of phytoplankton, 0.2-2 µm and > 2 µm, in primary production and photophysiological characteristics. Temporal and vertical variability in the photosynthetic characteristics was measured based on photosynthesis-irradiance (P-E) relationships. My results indicate that although larger phytoplankton appear to constitute a relatively small fraction of phytoplankton biomass and production in this ecosystem, the photophysiological responses of these organisms demonstrates high variability. Such results suggest that despite persistently oligotrophic habitat conditions, phytoplankton in this larger size class undergo temporally dynamic variations in growth.

Chapter 3 explores the temporal and vertical variability of eukaryotic phytoplankton population structure using both pigment-based and molecular analyses. The time-varying contributions of selected phytoplankton taxa to particle export in this ecosystem were also examined based on vertical fluxes of rbcL gene abundances. Quantitative polymerase chain reaction (QPCR) assays were developed based on form 1D rbcL genes for diatoms, prymnesiophytes and pelagophytes. Diatom rbcL genes were typically the most abundant among these groups, with elevated abundances often occurring in the upper euphotic zone during the summer. Abundances of prymnesiophyte and pelagophyte rbcL genes often increased in the lower euphotic zone during fall and winter months. Analyses of the upper ocean (150 m) sediment trap samples suggested that sinking fluxes associated with prymnesiophytes and pelagophytes tended to be greatest in the spring and fall. In contrast, diatom rbcL gene flux was often greatest in the summer, when particulate carbon export was maximal.
Chapter 4 focuses on diatom dynamics in the upper ocean and vertical export in the NPSG. Diatom population structure was examined using rbcL gene clone libraries. QPCR amplification of rbcL genes from five major diatom genera (Chaetoceros, Pseudonitzschia, Nitzschia, Rhizosolenia and Hemiaulus) revealed that diatom population structure was highly variable in time and space (vertically). Hemiaulus and Rhizosolenia rbcL gene abundances increased episodically in the upper euphotic zone in summer. The Hemiaulus phylotype was the greatest contributor to diatom rbcL gene fluxes in the upper ocean (150 m). Sediment trap collections in the deep sea (4000 m) indicated a narrowly focused peak in the export of the Hemiaulus, Chaetoceros and Rhizosolenia in the mid-summer months. My study suggests that episodic increase of abundances of specific diatom phylotypes in summer contribute significantly to the vertical fluxes into the deep ocean.

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Figure 1. Mean concentrations of nitrate + nitrite (N+N, yellow line) and Chl $a$ (green line), and rates of primary production (PP, red line) in the top 175 m at Station ALOHA from 1988 to 2009. Shaded area represents the lower euphotic zone (75-125 m) and deeper region.
Figure 2. (A) Box plots depicting monthly 11 year climatology (1998-2009) of surface (open boxes) and downwelling PAR at 100 m (gray); (B) Box plots depicting monthly 21 year climatology (1988-2009) of top 45 m (open boxes) and 75-125 m (gray) integrated primary production. Box boundaries define the 25th and 75th percentiles, lines inside the boxes represent median values, and the box whisker caps represent the 10th and 90th percentiles of the full time series.
Figure 3. (A) ENSO MEI and PDO index anomalies (z-scores) from 1989 to 2009. (B) Temporal variations of seawater temperature anomaly (z-scores) at 5 m from 1989 to 2009 at Station ALOHA. Blue line represents three-point running average.
Figure 4. Vertical distribution of size-fractionated Chl a (A) and primary production (B) between 2004 and 2007 at Station ALOHA. Depicted are time-averaged concentrations and rates for each discrete depth. Circles represent 0.2-2 µm size fraction; squares and triangles represent 2-10 µm and > 10 µm size fractions, respectively.
Figure 5. Temporal variations of 19’-hexanoyloxyfucoxanthin (19-hex), 19’-butanoyloxyfucoxanthin (19-but) and fucoxanthin anomalies (z-scores) in the upper (0-45 m) and lower (75-125 m) euphotic zone from 1989 to 2009; line represents three-point running average.
Figure 6. Temporal variations of percentage Chl $a$ contributed by prymnesiophytes (Chl $a_{pry}$ %, A), pelagophytes (Chl $a_{pel}$ %, B) and diatoms (Chl $a_{dia}$ %, C), in the lower euphotic zone (75-125 m) between 1989 and 2009 as estimated using a pigmented-based algorithm (Letelier et al. 1993). Blue line represents three-point running average. Solid black line represents the mean percentage.
Figure 7. Box plots depicting monthly 21 year climatology (1989-2009) of Chl \( a \) contributed by prymnesiophytes (A); pelagophytes (B) and diatoms (C) in the lower euphotic zone (75-125 m). Box boundaries define the 25th and 75th percentiles, lines inside the boxes represent median values, and the box whisker caps represent the 10th and 90th percentiles of the full time series.
**Figure 8.** Temporal variations in particulate silica (PSi) and particulate carbon (PC) fluxes at 150 m at Station ALOHA.
Chapter 2

Size-dependent photosynthetic variability in the North Pacific Subtropical Gyre

ABSTRACT

Photosynthesis-irradiance (P-E) relationships for two size-fractions (> 2 µm and 0.2-2 µm) of phytoplankton were used to evaluate variability in photophysiology over a 5 year period (2004-2009) in the North Pacific Subtropical Gyre (NPSG). Picophytoplankton (0.2-2 µm) were dominant contributors to euphotic zone chlorophyll (Chl) a concentrations (averaging 91% ± 2% of the 0-125 m depth integrated inventories) and accounted for a major fraction (averaging 74% ± 7%) of the depth integrated 14C-based primary production. In the well-lit upper ocean (0-45 m) Chl a normalized maximum rates of photosynthesis, P_{Chl max}, were significantly greater among the larger phytoplankton size class than in the smaller size fraction (one-way analysis of variance (ANOVA), p < 0.01). In contrast, in the dimly-lit region (125 m) of the euphotic zone there were no significant size-dependent differences in P_{Chl max} (one-way ANOVA, p > 0.05). Neither the initial slope of the P-E relationship, α, nor the light intensity required to saturate photosynthesis, E_k, varied significantly between the two size fractions. Although larger phytoplankton appear to constitute a relatively small fraction of phytoplankton biomass and production in this ecosystem, the photophysiological responses of organisms in this larger size class demonstrated considerable variability, suggesting despite persistently oligotrophic habitat conditions phytoplankton of this larger size class undergo temporally dynamic variations in growth.
INTRODUCTION

Organic matter production by photosynthetic plankton is a major control on the cycling of elements in the sea. Marine primary productivity accounts for nearly half of net global carbon fixation, and a major fraction of this productivity occurs in the low nutrient waters of the subtropical ocean gyres (Longhurst et al. 1995, Karl et al. 1996, Falkowski et al. 1998, Field et al. 1998, Behrenfeld et al. 2005). As a result, understanding the processes controlling the physiologies of photosynthetic plankton in oligotrophic gyres is critical to our understanding of the global carbon cycle.

The spatial and temporal patterns of primary production and the photophysiology of ocean phytoplankton have been intensively investigated (e.g., Platt et al. 1982, Sakshaug and Holm-Hansen 1986, Cullen et al. 1992, MacIntyre et al. 2002, Strzepek and Harrison 2004, Falkowski and Raven 2007). Such studies reveal that variations in phytoplankton physiology are reflected in characteristic patterns in the photosynthetic response to irradiance (P-E) (Jassby and Platt 1976, Falkowski and Owens 1980, Platt et al. 1980). P-E relationships demonstrate several well-defined characteristics, typically described using hyperbolic or negative exponential models (Jassby and Platt 1976, Platt et al. 1980). Various photophysiological parameters can be derived from such relationships, including the maximum rate of carbon fixation (termed $P_{\text{max}}$), the initial slope (termed $\alpha$) of the P-E response, which provides a measure of light harvesting efficiency by photosynthesis; the susceptibility of photosynthesis to inhibition at elevated light flux (termed $\beta$) and the irradiance required to saturate photosynthesis (termed $E_k$). When used in P-E relationships, rates of photosynthesis are frequently normalized to
concentrations of chlorophyll $a$ (Chl $a$), where the resulting normalized maximum photosynthetic rate is termed $P_{\text{Chl} \max}^\text{Chl}$.

When combined with information on temperature-dependent phytoplankton growth (Eppley 1972), P-E derived parameters can be incorporated into bio-optical models and used to estimate areal rates of primary production from remotely sensed determinations of phytoplankton pigments, temperature and light (Bidigare et al. 1992, Platt and Sathyendranath 1993, Behrenfeld and Falkowski 1997). Moreover, analyses of the P-E derived behavior provides information on the photophysiological flexibility of phytoplankton in specific environments, and can yield insight into controls underlying time and space variability in primary production (e.g., Harrison and Platt 1980, Cullen 1990, Ondrusek et al. 2001, Goebel and Kremer 2007, McAndrews et al. 2008, Isada et al. 2009). Most in situ studies aimed at characterizing rates of primary production and photosynthetic physiology are based on analyses of bulk phytoplankton properties, and thus do not resolve physiological variability that underpins measured rates of productivity. In this context, resolving how distinct phytoplankton size factions contribute to inorganic carbon fixation and the photophysiological behaviors of these size fractions may be useful for understanding processes controlling production in the marine environment.

The North Pacific Subtropical Gyre (NPSG) is the largest anticyclonic circulation feature on Earth (Sverdrup 1946) and forms the primary study region for the Hawaii Ocean Time-series (HOT) program. More than 22 years of observations at Station ALOHA ($22^\circ 45^\prime$N, $158^\circ 00^\prime$W), the field site for the HOT program, indicate moderate but consistent seasonal variations in environmental forcing, including properties that
influence phytoplankton growth. For example, surface photosynthetically active radiation (PAR) varies approximately two-fold seasonally, upper ocean temperatures fluctuate between 23° - 27°C, and weak to moderate wintertime mixing combined with shoaling of the nutricline entrains nutrients in the euphotic zone (Karl et al. 2001, Letelier et al. 2004, Church et al. 2009). Several studies have examined seasonal to interannual scale changes in phytoplankton biomass and productivity in the NPSG and concluded that variability in phytoplankton community structure forms an important control on net production and carbon export in this ecosystem (Letelier et al. 1993, 1996; Winn et al. 1995, Karl et al. 1996, 2001, 2002; Dore et al. 2008). Moreover, a bio-optical model based on measured P-E relationships at Station ALOHA concluded that the model’s ability to predict euphotic zone productivity could be improved through better understanding of how shifts in the phytoplankton community influence the measured photophysiological responses (Ondrusek et al. 2001). More recently, fast-repetition-rate fluorometry (FRRF) has been used to characterize photosynthetic processes at Station ALOHA (Corno et al. 2008), and results from this study suggested that the dynamics underlying time-space variability in plankton photophysiology were sensitive to changes in plankton community structure (Corno et al. 2008).

These past studies on the photosynthetic characteristics of the phytoplankton community in the NPSG, using different methods, suggest that changes in phytoplankton community structure play an important role in driving variability in ecosystem production. Picophytoplankton (0.2-2 µm), including cyanobacteria such as *Prochlorococcus* and *Synechococcus*, are dominant components of upper ocean plankton biomass in the NPSG.
(Campbell and Vaulot 1993, Letelier et al. 1993, Anderson et al. 1996); however, the contributions of larger phytoplankton, including filamentous and colonial cyanobacteria and protists, appear to contribute to regularly occurring bloom events observed in this region (White et al. 2007, Dore et al. 2008, Fong et al. 2008, Church et al. 2009). These previous studies emphasize that changes in plankton composition in the NPSG are often accompanied by shifts in phytoplankton size structure. The present study sought to examine the temporal and vertical variation in photophysiological parameters (e.g., $P^{\text{Chl}}_{\text{max}}$, $E_k$ and $\alpha$) of size-fractionated phytoplankton at Station ALOHA. Our goal was to assess time and depth variability underlying the photophysiology and productivity of different size-separated groups of phytoplankton in the NPSG.

MATERIALS AND METHODS

**Chl a and in situ measurements of $^{14}$C-bicarbonate assimilation**

Seawater samples for determinations of size partitioning of Chl $a$ concentrations and rates of $^{14}$C-bicarbonate assimilation were collected from 6 discrete depths in the upper ocean (5, 25, 45, 75, 100 and 125 m) at Station ALOHA during near-monthly HOT program cruises between October 2004 and December 2009. Pre-dawn samples were collected using a vertically profiling conductivity-temperature-density (CTD) rosette sampler equipped with twenty four 10 L polyvinyl chloride bottles.

In the initial 3 years of this study (October 2004 - October 2007), seawater for determinations of Chl $a$ concentrations was subsampled into 1 L amber polyethylene bottles; the entire volume was sequentially filtered by positive pressure through in-line 25 mm diameter 10 $\mu$m and 2 $\mu$m pore size polycarbonate filters. The 2 $\mu$m filtrate was
collected and 150 ml of this filtrate was vacuum filtered onto 25 mm diameter 0.2 µm pore size polycarbonate filters. Filters were placed in 7 ml of 100% HPLC grade acetone, extracted in the dark at -20 °C for 7 days (Letelier et al. 1996), and concentrations of Chl a in the acetone extracts were determined fluorometrically using a Turner Designs Model 10-AU fluorometer. In the second part of this study (October 2007 - December 2009), coinciding with the period when P-E experiments were conducted, Chl a concentrations were determined from plankton concentrated onto 25 mm diameter 2 µm and 0.2 µm pore size polycarbonate filters. For these measurements, 150 ml of seawater was vacuum filtered onto both 2 µm and 0.2 µm pore size polycarbonate filters, and concentrations of Chl a were determined fluorometrically. The Chl a concentrations in the 0.2-2 µm size-fraction were calculated by subtracting the > 2 µm Chl a from the > 0.2 µm Chl a. We have compared these two procedures for size-fractionating Chl a samples and found that the latter approach resulted in concentrations of Chl a in the > 2 µm size-fraction ~15% greater than separation of Chl a based on the sequential fractionation approach (data not shown).

Rates of 14C-bicarbonate assimilation were determined from seawater samples collected from the same pre-dawn CTD casts described above. Water was subsampled under subdued light from the CTD rosette bottle into 3 replicate 500 ml polycarbonate bottles and the bottles were transferred to a shipboard laboratory van where they were inoculated with approximately 0.0125 mCi of NaH14CO3. Samples were incubated over the full daylight period on a surface tethered, in situ array (Letelier et al. 1996). At the end of the incubation period, 250 µl aliquots from each sample were placed in a 20 ml
glass scintillation vial containing 500 μl phenylethylamine for subsequent determination of the 14C activity in each sample. During the initial period of this study (October 2004 - October 2007), triplicate 500 ml bottles were vacuum filtered through sequential 25 mm diameter 10 μm pore size polycarbonate filters, followed by 25 mm diameter 2.0 μm pore size polycarbonate filters. The < 2 μm filtrates were retained and 250 ml of this filtrate was then filtered onto 25 mm diameter 0.2 μm pore size filters. All filters were stored frozen in 20 ml borosilicate scintillation vials until processed. In the shorebased laboratory, 1 ml of 1M HCl was added to each filter to remove adsorbed [14C] bicarbonate. After 24 h, 10 ml of Ultima Gold scintillation cocktail was added to the scintillation vials and the 14C-activity was determined using a TRI-CARB 4640 liquid scintillation counter.

**Size fractionated P-E experiments**

P-E experiments were conducted on 14 HOT cruises to Station ALOHA between October 2007 and December 2009. Water samples for P-E experiments were collected from four depths (25, 45, 75 and 125 m) in the euphotic zone. Water was subsampled from the CTD rosette bottles into light-shielded, 1 L polycarbonate bottles. Approximately 0.1 mCi of [14C] bicarbonate was added to 500 ml subsamples of seawater, and 24 separate aliquots (15 ml) of the radiolabeled seawater was transferred to 20 ml borosilicate vials. These vials were incubated at in situ temperatures for 2 h at irradiances varying between 8 and 2049 μmol quanta m⁻² s⁻¹ in a photosynthetron apparatus (Lewis and Smith 1983). The light intensity of each sampling well was measured using a scalar irradiance meter (Biospherical QSL2101).
To terminate the incubations, samples were sequentially filtered onto 25 mm diameter 2 \( \mu \)m and 0.2 \( \mu \)m pore sized polycarbonate filters using a 12 place vacuum filtration system (Millipore). The 2 \( \mu \)m filtrates were collected into 15 ml conical centrifuge tubes and filtered onto 0.2 \( \mu \)m polycarbonate filters. Filters were stored frozen in 20 ml borosilicate scintillation vials and processed as described above. Carbon fixation rate in each sample was calculated as: Carbon fixation = \((^{14}\text{C}_{\text{filter}}/\text{Vol}_{\text{filtered}})/^{14}\text{C}_{\text{total}}\) * ([DIC]) * 12.011 * 1.06, where \(^{14}\text{C}_{\text{filter}}\) represents the \(^{14}\text{C}\) activity on the acidified filters; \(\text{Vol}_{\text{filtered}}\) represents the volume filtered; \(^{14}\text{C}_{\text{total}}\) is the total \(^{14}\text{C}\) activity in the 250 \( \mu \)l aliquot of the radiolabeled seawater; [DIC] is the concentration of dissolved inorganic carbon measured by the HOT program (2004-2009) at the sampled depths; 12.011 is the molar mass of carbon; and 1.06 represents a \(^{14}\text{C}/^{12}\text{C}\) isotopic fractionation factor (Steeman-Nielsen 1952).

**Upper ocean habitat characteristics**

Incident solar irradiance (400-700 nm) was measured on each HOT cruise using a LI-COR LI-1000 cosine collector and data logger. In addition, daily vertical profiles of downwelling photosynthetically active radiation (PAR) were conducted at approximately noon on each HOT cruise using a Profiling Reflectance Radiometer (PRR; Wetlabs). High-sensitivity measurements of nitrate + nitrite (N+N) and soluble reactive phosphorus (SRP) were determined as described by Dore and Karl (1996) and Karl and Tien (1992), respectively.

**Data Analysis**
The measured rates of $^{14}$C-bicarbonate assimilation were used to construct P-E relationships, and the photosynthetic parameters were computed using the negative exponential formulation described in Platt et al. (1980):

$$P = P_s[1 - \exp(-\alpha E/P_s)] \exp(-\beta E/P_s)$$

where $P$ is the photosynthetic carbon fixation rate, $P_s$ is the maximum rate of photosynthesis without photoinhibition, $\alpha$ is the initial slope, $E$ is the light flux, and $\beta$ is the rate of photoinhibition. The maximum rates of $^{14}$C-bicarbonate assimilation normalized to concentrations of Chl $a$, $P_{\text{Chl max}}$ were calculated as:

$$P_{\text{Chl max}} = P_{\text{Chl s}} \left(\frac{\alpha}{\alpha + \beta}\right) \left(\frac{\beta}{\alpha + \beta}\right) \frac{\beta}{\alpha}$$

where $P_{\text{Chl s}}$ is the maximum rate of Chl $a$ normalized photosynthesis in the absence of photoinhibition (mg C (mg Chl)$^{-1}$ h$^{-1}$), $\alpha$ describes the initial slope of the P-E relationship ((mg C (mg Chl)$^{-1}$ h$^{-1}$)(µmol quanta m$^{-2}$ s$^{-1}$)$^{-1}$) and $\beta$ describes the magnitude of photoinhibition of the photosynthetic rate ((mg C (mg Chl)$^{-1}$ h$^{-1}$)(µmol quanta m$^{-2}$ s$^{-1}$)$^{-1}$).

Uncertainties associated with the P-E curve fitting routine were determined and propagated through the subsequent derivations of the P-E parameters. In addition, the P-E curve fitting routine was used to estimate $P_{\text{opt}}$, a term used to describe the optimal rate of photosynthesis from the measured vertical profiles of in situ primary productivity. Similarly, $P_{\text{Chl opt}}$ was also determined as the optimal rate of photosynthesis normalized to Chl $a$.

Finally, $E_k$, the irradiance required to saturate carbon fixation, an index of photoadaptation, was computed as:

$$E_k = P_{\text{Chl max}} / \alpha$$
Statistical comparisons of photosynthetic parameters between size-fractions and among different depths were evaluated using one-way analysis of variance (ANOVA). Relationships between the derived photosynthetic parameters and various environmental properties (temperature, light, nutrients) were examined based on least-squares linear regression analyses.

RESULTS

Variability in mixing and light

The depth of the upper ocean mixed layer (based on the 0.125 unit change in potential density from the surface ocean, Levitus 1982) during this study varied between 16 and 124 m, with the upper ocean most stratified during warm summer months (mixed layer depths between May and October averaged 46 ± 15 m) and deepening in the winter (averaging 77 ± 26 m between November and April). Incident irradiance varied from 12 to 56 mol quanta m\(^{-2}\) d\(^{-1}\) during the study period, with solar flux at the sea-surface elevated in the summer and decreasing in the winter. In the well-lit upper ocean (0-45 m), downwelling irradiance varied ~ 5 fold at each of the discrete depths sampled (5, 25 and 45 m) over the course of this study. Temporal variability in downwelling irradiance increased with depth, with light fluxes varying as much as 11-fold (0.04-0.44 mol quanta m\(^{-2}\) d\(^{-1}\)) in the dimly-lit region of the lower euphotic zone (125 m) (Table 1). Downwelling irradiance was generally ~2-fold higher in summer months compared to the winter at each of the discrete depths (Table 1). The median light levels at the depths where primary production and Chl \(a\) concentrations were measured (5, 25, 45, 75, 100, 125 m) were 79%, 30%, 12%, 3.2%, 1.0%
and 0.3%, relative to the surface PAR, respectively. N+N concentrations throughout the upper 75 m did not demonstrate clear differences between the summer and winter (Table 1); however, throughout the dimly lit region of the euphotic zone, N+N concentrations increased (on average by 4- to 5-fold) in the winter months compared to the summer.

**Size-fractionated Chl a and in situ $^{14}$C-bicarbonate assimilation**

Throughout this study, Chl a concentrations in 0.2-2 µm size fraction consistently exceeded concentrations measured in the >2 µm size class (calculated as the sum of 2-10 µm and >10 µm Chl a). In the well-lit upper ocean (where light flux varied 1.7-44.0 mol quanta m$^{-2}$ d$^{-1}$), 0.2-2 µm Chl a concentrations varied between 0.02 and 0.21 µg l$^{-1}$ (with maximum concentrations in July 2005 and minimum concentrations in December 2005, respectively). Chl a concentrations in the upper ocean associated with the larger phytoplankton size-fraction (>2 µm) ranged from 0.01 to 0.03 µg l$^{-1}$ (September 2005 and January 2006, respectively) (Fig. 1A). On average, the Chl a concentrations in the lower euphotic zone, where light flux varied 0.04-2.8 mol quanta m$^{-2}$ d$^{-1}$ were ~2-fold greater than concentrations measured in the upper ocean (0-45 m; Fig. 1A).

Concentrations of Chl a in >2 µm size class were less variable with depth than concentrations measured in the 0.2-2 µm size class. On average, concentrations in the >2 µm size classes increased ~1.2-fold into the lower euphotic zone relative to concentrations measured in the well-lit upper ocean (Fig. 1A), while concentrations of Chl a in the 0.2-2 µm plankton size classes increased by ~2 fold in the lower euphotic zone relative to the well-lit upper ocean (Fig. 1A). The resulting depth-integrated (0-125 m) Chl a concentrations in 0.2-2 µm size fraction were 7- to 18-times greater than Chl a
inventories measured in the > 2 µm size fraction (Fig. 2A), with the 0.2-2 µm size fraction accounting for 91% (± 2%) of the total depth integrated (0-125 m) Chl a.

Vertical profiles of $^{14}$C-bicarbonate assimilation by > 2 µm and 0.2-2 µm plankton size classes demonstrated dominance of carbon fixation by the smaller phytoplankton size classes at all depths examined (Fig. 1B). Normalization of the $^{14}$C-bicarbonate assimilation to size-fractionated concentrations of Chl a ($P_{Chl}^{Chl}$) revealed that $P_{Chl}$ in the > 2 µm size class always exceeded $P_{Chl}$ derived from the 0.2-2 µm size class (Fig. 1C).

Depth-integrated (0-125 m) rates of $^{14}$C-bicarbonate assimilation in 0.2-2 µm size fraction ranged between 172-348 mg C m$^{-2}$ d$^{-1}$, and were 1.1- to 5.5-fold greater than in the > 2 µm size fraction (Fig. 2B), with the 0.2-2 µm accounting for 74% (± 7%) of the average $^{14}$C-bicarbonate assimilation in the euphotic zone. In contrast, rates of $P_{Chl}$ (depth-integrated primary production normalized by depth-integrated Chl a) in the > 2 µm size class were 1.7- to 9-fold greater than $P_{Chl}$ derived from the 0.2-2 µm size class (Fig. 2C).

Rates of $^{14}$C-bicarbonate assimilation by both phytoplankton size classes were consistently greatest in the well-lit upper ocean. Measurements of downwelling irradiance and the size fractionated rates of in situ $^{14}$C-bicarbonate assimilation were fitted to the Platt et al. (1980) model describing photosynthesis as a function of irradiance (Fig 1B). The resulting relationships suggested that variation in the downwelling light field explained 52- 57% and 55-67% of the variability associated with primary production and $P_{Chl}$ for the 0.2-2 µm and > 2 µm size classes, respectively. Moreover, these analyses indicated that on average $P_{opt}$ and $\alpha$ in the 0.2-2 µm size fraction were
greater than that observed in the > 2 µm size fraction (Fig. 1B). After normalization to concentrations of Chl \(a\), the \textit{in situ} \(P^{\text{Chl}}_{\text{opt}}\) and \(\alpha\) were 3- to 4-fold greater in the > 2 µm size fraction than in the 0.2-2 µm size fraction (Fig. 1C). In contrast, the derived values of \(E_k\) from the \textit{in situ} production rates were similar for the 0.2-2 µm and > 2 µm size-fractions (3.4 ± 0.7 and 3.8 ± 0.5 mol quanta m\(^{-2}\) d\(^{-1}\), respectively).

**Temporal and vertical variability of P-E derived parameters**

A total of 88 individual P-E curves were conducted and analyzed as part of this study. The Platt et al. (1980) model described 35-96% of the variance in 71 of these relationships (Fig. 3; \(r^2 = 0.35-0.96, p < 0.01\)). Results from the remaining 17 experiments did not demonstrate statistically significant relationships when fitted to the Platt et al. (1980) model (\(p > 0.01\)), and thus were excluded from further analyses. In general, the P-E relationships demonstrated several consistent patterns: 1) rates of \(^{14}\text{C-}\)bicarbonate assimilation were dominated by the 0.2-2 µm plankton size fraction at all depths sampled; 2) the initial slopes (\(\alpha\)) derived from the P-E relationships of both size fractions were greater in those samples collected from the dimly-lit region of the lower euphotic zone compared to samples collected in the well-lit upper ocean; 3) the irradiance required to saturate photosynthesis (\(E_k\)) was significantly lower in those samples collected from the dimly-lit region of the lower euphotic zone for both size fractions; and 4) both phytoplankton size fractions demonstrated photoinhibition in the dimly-lit region of the lower euphotic zone where downwelling irradiance decreased to between 0.04-0.44 mol quanta m\(^{-2}\) d\(^{-1}\), while none of the samples collected from the well-lit upper ocean demonstrated significant photoinhibition (\(\beta \approx 0\); Fig. 3, Table 2).
There were several notable differences in the P-E derived photosynthetic responses among the different phytoplankton size classes, some of which appeared depth-dependent (Fig. 4). The Chl $a$-normalized rates of maximum photosynthesis ($P_{\text{Chl \ max}}$) from the 0.2-2 $\mu$m phytoplankton assemblages did not show clear vertical structure; however, $P_{\text{Chl \ max}}$ derived from the > 2 $\mu$m size fractions were significantly lower in the dimly-lit region of the euphotic zone than in the upper 75 m (one-way ANOVA, $p < 0.01$). Although temporally variable, on average in the well-lit upper ocean (0-45 m) $P_{\text{Chl \ max}}$ of the > 2 $\mu$m size class was significantly greater than $P_{\text{Chl \ max}}$ derived for the 0.2-2 $\mu$m size class (one-way ANOVA, $p < 0.01$). Moreover, $P_{\text{Chl \ max}}$ of the > 2 $\mu$m phytoplankton size class varied as much as 8-fold through time (1.7-14.2 mg C (mg Chl)$^{-1}$ h$^{-1}$) in the well-lit upper ocean. In comparison, downwelling irradiance in the upper ocean at the depths where Chl $a$ and primary production were measured (5, 25 and 45 m) varied ~ 2 fold during the study period. In the lower regions of the euphotic zone, variability in $P_{\text{Chl \ max}}$ in the > 2 $\mu$m phytoplankton appeared similar to variations in downwelling irradiance (Fig. 4A).

In addition to these differences in $P_{\text{Chl \ max}}$, on average, $\alpha$ of the 0.2-2 $\mu$m size class varied significantly with depth with largest values observed in the dimly-lit region of the lower euphotic zone (Table 2, one-way ANOVA, $p < 0.001$). In contrast, although not statistically different (one-way ANOVA, $p > 0.05$) $\alpha$ in the > 2 $\mu$m size class was often greater at deeper depths in the euphotic zone. Temporal and vertical variability in derived values of $E_k$ resulted in no significant differences between the two phytoplankton size classes at any of the depths sampled (one-way ANOVA, $p > 0.05$).
Values of $E_k$ from both size fractions were significantly lower in the dimly-lit region of the lower euphotic zone (125 m), where downwelling irradiance decreased to 0.04-0.44 mol quanta m$^{-2}$ d$^{-1}$, compared to $E_k$ values derived from the well-lit upper ocean (one-way ANOVA, $p < 0.01$). In the well-lit upper ocean (0-45 m), $E_k$ values ranged between 37 and 286 µmol quanta m$^{-2}$ s$^{-1}$ for the 0.2-2 µm size class, and 37 and 319 µmol quanta m$^{-2}$ s$^{-1}$ for the > 2 µm size. With two exceptions, values of $E_k$ in both size fractions from the well-lit upper ocean were lower than the measured downwelling irradiance at the depths where samples were collected. The two exceptions occurred from samples collected at 45 m depth in December 2008 and December 2009; on both of these sampling occasions the mixed layer depth exceeded 75 m. In contrast, throughout the lower euphotic zone (75-125 m), $E_k$ ranged between 27 and 262 for the 0.2-2 µm size class, and 25 and 253 for the > 2 µm size class, consistently greater than the measured downwelling irradiance at these depths (Fig. 4B).

Throughout the upper 45 m of the euphotic zone, rates of $P_{\text{Chl max}}$ derived from the > 2 µm plankton size fractions were more temporally variable than the 0.2-2 µm size fractions. For example, at 25 m, $P_{\text{Chl max}}$ for the > 2 µm size fraction varied ~8-fold over the course of this study, while $P_{\text{Chl max}}$ in the 0.2-2 µm plankton size fraction varied ~2-fold (Fig. 5A). In the lower euphotic zone, temporal variability in $P_{\text{Chl max}}$ was generally less pronounced than observed in the upper euphotic zone, with $P_{\text{Chl max}}$ at 125 m varying ~4- and 2-fold, respectively in the > 2 µm and 0.2-2 µm size fractions (Fig. 5B). In an effort to identify factors influencing the measured and derived photophysiological properties, we examined time-dependent changes in $P_{\text{Chl max}}$, $E_k$ and $\alpha$ relative to several
measured environmental properties. Least squares linear regression analyses revealed that 
$E_k$ among both size-fractions demonstrated dependence on downwelling irradiance in the 
lower euphotic zone (Model II least-squares linear regressions; $> 2 \mu m$: $r^2 = 0.77$, $p < 
0.001$; $0.2-2 \mu m$: $r^2 = 0.66$, $p < 0.001$). $P_{\text{Chl}}^\text{max}$ derived for the $> 2 \mu m$ size fraction were 
also related to downwelling irradiance in the lower euphotic zone ($r^2 = 0.35$, $p = 0.006$). 
In contrast, $\alpha$ in the $0.2-2 \mu m$ size fraction demonstrated dependence on downwelling 
irradiance and temperature in the lower euphotic zone ($r^2 = 0.45$, $p = 0.003$ and $r^2 = 0.24$, 
$p = 0.045$, respectively). Moreover, $\alpha$ in the $0.2-2 \mu m$ size fraction demonstrated a weak 
but significant relationship to temperature in the lower euphotic zone. However, in the 
upper euphotic zone, least squares linear regression analyses failed to identify statistically 
significant relationships between the derived P-E parameters and seawater temperature, 
downwelling PAR, or concentrations of nutrients (N+N and SRP) (Model II least-squares 
linear regression analyses, $p > 0.05$).

DISCUSSION

By examining temporal and vertical variations in the responses of $^{14}$C-bicarbonate 
assimilation to variations in irradiance, we sought to identify potential differences in the 
photophysiological behavior of different size classes of phytoplankton in the oligotrophic 
NPSG. Such differences could exert an important influence on ecosystem productivity and 
play roles in driving temporal variations in plankton community structure. The results of this 
study highlighted several features regarding phytoplankton physiology and photosynthetic 
carbon fixation in the NPSG. Over a nearly 5 year period of near-monthly sampling at
Station ALOHA, we found that Chl \( a \) normalized rates of carbon fixation by phytoplankton captured onto 2 \( \mu \)m filters were always greater than the Chl \( a \) normalized carbon fixation rates measured in the 0.2-2 \( \mu \)m size class. In addition, in the well-lit upper ocean the P-E derived maximum rates of photosynthesis, \( P_{\text{Chl max}} \), were significantly greater for the larger (> 2 \( \mu \)m) phytoplankton size fraction than in the smaller (0.2-2 \( \mu \)m) size fraction. Finally, the P-E derived parameters \( P_{\text{Chl max}}, \alpha \) and \( E_k \), were highly variable in time, particularly among the > 2 \( \mu \)m size classes, suggesting phytoplankton within this size fraction may be more sensitive to variations in irradiance than the smaller picophytoplankton.

Our findings that small (0.2-2 \( \mu \)m) phytoplankton dominated concentrations of Chl \( a \) and rates of carbon fixation at Station ALOHA are consistent with previous investigations in this ecosystem (Campbell and Vaulot 1993, Letelier et al. 1993, Anderson et al. 1996, Campbell et al. 1997). The \textit{in situ} rates of \(^{14}\text{C}-\text{bicarbonate assimilation and } P_{\text{Chl}} \text{ values for both plankton size-fractions demonstrated dependence on temporal and vertical variability in downwelling PAR. By fitting these observations to the Platt et al. (1980) model, the derived } in situ P_{\text{Chl opt}} \text{ from the } > 2 \mu \text{m size-fraction averaged } 11.2 \pm 0.8 \text{ mg C (mg Chl)}^{-1} \text{ h}^{-1}, \text{ while } in situ P_{\text{Chl opt}} \text{ derived from the 0.2-2 } \mu \text{m size-fraction averaged } 3.0 \pm 0.2 \text{ mg C (mg Chl)}^{-1} \text{ h}^{-1}. \text{ A similar analysis, using the HOT } in situ \text{ }^{14}\text{C}-\text{bicarbonate assimilation rates derived from samples filtered onto glass fiber filters (nominal pore size 0.7 } \mu \text{m), revealed that the } in situ P_{\text{Chl opt}} \text{ during our study period (2004-2009) was } 5.8 \pm 0.2 \text{ mg C (mg Chl)}^{-1} \text{ h}^{-1} \text{ (data not shown). Letelier et al. (1996) reported } P_{\text{Chl opt}} \text{ values from plankton captured on glass fiber filters ranged between 4.2 and 9.5 mg C (mg Chl)}^{-1} \text{ h}^{-1} \text{ at Station ALOHA. Such results}
suggest that changes in phytoplankton size structure might impact $P_{Chl}^{opt}$ of the bulk community.

Previous investigations of phytoplankton size structure and productivity in other oceanic systems also indicate the dominance of picophytoplankton (0.2-2 $\mu$m) both in terms of biomass and primary production (Li et al. 1983, Magazzu and Decembrini 1995, Buck et al. 1996, Gasol et al. 1997, Marañón et al. 2001, Ignatiades et al. 2002, Fernandez et al. 2003, Teira et al. 2005, Poulton et al. 2006). Several studies in the tropical and subtropical Atlantic Ocean have reported that the relative contribution of large (> 2 $\mu$m) phytoplankton to Chl $a$ was less than their contribution to primary production, implying the Chl $a$-normalized rates of carbon assimilation ($P_{Chl}$) were significantly greater in the > 2 $\mu$m size phytoplankton than for the < 2 $\mu$m picoplankton (Marañón et al. 2001, Fernandez et al. 2003, Poulton et al. 2006). Consistent with these reports from other regions, our study in the oligotrophic NPSG revealed elevated rates of $P_{Chl}$ in the > 2 $\mu$m size phytoplankton, suggesting larger cells in this habitat may be more photosynthetically efficient (on a per unit Chl $a$ basis) in carbon fixation than picophytoplankton.

Analyses of size fractionated P-E responses yielded information on time and space variability in euphotic zone photosynthesis and highlighted differences in the photosynthetic behavior of the two phytoplankton size fractions examined. For example, consistent with prior studies (Ondrusek et al. 2001, Church et al. 2004) comparison of the P-E derived estimates of $E_k$ relative to measured downwelling irradiance indicated that rates of photosynthesis were light-saturated throughout the well-lit upper ocean, where PAR flux varied between 128-562 $\mu$mol quanta m$^{-2}$ s$^{-1}$. In this light-saturated region of the upper
euphotic zone, maximum Chl $a$ normalized rates of carbon fixation ($P_{\text{Chl}}^{\text{max}}$) by the larger phytoplankton size fraction were significantly greater than those observed among the smaller size fraction. Thus, while our study confirmed that smaller phytoplankton (0.2-2 µm) consistently dominate carbon fixation in this ecosystem, size fractionation of in situ rates of $^{14}$C-bicarbonate assimilation and the P-E derived estimates of $P_{\text{Chl}}^{\text{max}}$ both indicate that throughout the high-light, nutrient poor upper ocean waters, on a per unit Chl $a$ basis, the relatively rare larger phytoplankton (> 2 µm) appear more efficient at carbon fixation than more dominant smaller celled organisms. In addition, the relatively high temporal variability observed in $\alpha$ suggests that although these larger phytoplankton comprise a relatively small portion of the total biomass of the NPSG, members of this larger size class appear capable of rapid photophysiological acclimation.

Based on information on the intracellular turnover of Chl $a$, Falkowski (1981) estimated a theoretical upper limit for $P_{\text{Chl}}^{\text{max}}$ of 25 mg C (mg Chl)$^{-1}$ h$^{-1}$. In our study, $P_{\text{Chl}}^{\text{max}}$ values ranged between 0.9 and 4.6 mg C (mg Chl)$^{-1}$ h$^{-1}$ for the 0.2-2 µm size fraction and between 1.2 and 14.2 mg C (mg Chl)$^{-1}$ h$^{-1}$ in the > 2 µm size fraction. Several previous studies conducted in regions of the oligotrophic ocean have argued that relatively low rates of $P_{\text{Chl}}^{\text{max}}$ were a consequence of nutrient limited plankton growth (Platt et al. 1992, Hood 1995, Marañón and Holligan 1999). Marañón and Holligan (1999) examined the P-E parameters in the surface waters of the North Atlantic Subtropical Gyre, finding that $P_{\text{Chl}}^{\text{max}}$ values ranged between 4 and 7 mg C (mg Chl)$^{-1}$ h$^{-1}$. These authors observed much greater $P_{\text{Chl}}^{\text{max}}$ values in more nutrient-enriched temperate waters and within upwelling regions in Atlantic, suggesting nutrient availability plays an important role in controlling variability in
Our findings would be consistent with the hypothesis that larger phytoplankton in the NPSG are capable of more rapid growth than the abundant picophytoplankton, but that the biomass and productivity of these larger cells are restricted by the availability of nutrients.

It’s important to note that the relatively small sample volumes (15 ml) utilized for P-E studies likely results in under representation of less abundant, larger phytoplankton cells and colonies such as chain-forming diatoms and *Trichodesmium* that are known to be important components of the NPSG plankton community. Undersampling of these organisms could contribute to the relatively high variability observed in the photophysiological parameters. Moreover, our separation of Chl *a* and rates of production on the basis of cell size likely reflects the contributions of many different phytoplankton taxonomic groups, so the resulting temporal variability in P-E parameters could reflect compositional changes in phytoplankton community structure within each size fraction rather than physiological changes in any one group.

In addition, several other important caveats need to be considered when utilizing Chl *a* normalized rates of production as proxies for understanding phytoplankton physiology and growth. We did not attempt to constrain variations in phytoplankton C : Chl *a* ratios as part of this study; however, laboratory studies suggest C : Chl *a* ratios can vary more than 10-fold, with changes in this ratio attributable to various environmental factors that influence phytoplankton life histories, including light, nutrient concentrations and temperature (Goldman 1980, Geider 1987, Geider et al. 1997, MacIntyre et al. 2002). Winn et al. (1995) found that the fluorescence per unit cell of *Prochlorococcus* populations dwelling in the
upper euphotic zone at Station ALOHA varied 2-fold between winter and summer. For additional constraint on biomass normalized primary production (analogous to specific growth rate) by picophytoplankton at Station ALOHA, we examined flow cytometric measurements of *Prochlorococcus* and *Synechococcus* cell abundances conducted by the HOT program between 2005 and 2007. These measurements, combined with estimates of cellular carbon content of these cells (Bertilsson et al. 2003), provided additional constraint on C-biomass associated with these picophytoplankton. The resulting specific growth rates derived from these analyses ranged from 0.15 to 0.37 d\(^{-1}\) in the upper euphotic zone and from 0.05 to 0.47 d\(^{-1}\) in the lower euphotic zone. We also observed a significant positive relationship between concentrations of N+N and the derived growth rate estimates (Least square linear regression, \(r^2 = 0.64, p < 0.001\)) within the lower euphotic zone. Such results suggest time-varying changes in nutrient availability, such as those associated with perturbation of isopycnal surfaces due to internal waves or mesoscale physical dynamics, could exert important control on variability in phytoplankton growth.

The finding that when normalized to Chl \(a\) larger cells appear more productive than smaller cells could also reflect uncertainties associated with use of the \(^{14}\text{C}\)-methodology to measure carbon fixation. In particular, ecosystems where microbial food webs impose tight coupling in growth and removal on picoplanktonic cells, turnover of photosynthetically fixed carbon by these smaller cells would be expected to be rapid. Moreover, several studies suggest that respiration per unit cell volume decreases with increasing phytoplankton size (Laws 1975, Banse 1976, Tang and Peters 1995). Similarly, if a greater fraction of photosynthetically fixed carbon by picophytoplankton passes to the dissolved organic carbon
pool compared to larger cells (Bjørnsen 1988), filtration-based approaches could underestimate carbon fixation by these small cells. As such, the measured rates of $^{14}$C-based carbon fixation by small phytoplankton cells in our study could more closely approximate net than gross rates of production.

Comparison of our P-E derived values of $E_k$ to measured downwelling PAR in the lower euphotic zone indicated that the photosynthetic production by both phytoplankton size fractions was light limited when PAR fluxes were less than $\sim$3 mol quanta m$^{-2}$ d$^{-1}$. At these subsaturating light intensities, rates of carbon fixation by both size fractions demonstrated a near linear response to changes in irradiance. Moreover, the slope of this response ($\alpha$) was comparable among both size fractions. In the dimly-lit region of the lower euphotic zone, both $P_{\text{Chl}}^{\text{max}}$ and $E_k$ of larger size-fraction were significantly lower than observed in the well-lit regions of the euphotic zone. Such results are consistent with physiological acclimations to growth at low irradiance (MacIntyre et al. 2002) whereby phytoplankton can increase the size of the light-harvesting antenna and/or increase the number of photosynthetic reaction centers (Falkowski & Owens 1980; Dubinsky et al. 1986). Laboratory studies indicate that variability in the electron turnover rate of the photosynthetic unit (PSU) forms a major determinant on $P_{\text{Chl}}^{\text{max}}$ and $E_k$ (Moore et al. 2006), with the number of photosynthetic reaction centers inversely related to the electron turnover rate of the PSU (Sukenik et al. 1987). As a result, the observed decrease in $P_{\text{Chl}}^{\text{max}}$ and $E_k$ in the lower euphotic zone in the present study may reflect acclimation through changes to the number of reaction centers, rather than increases in the size of the light harvesting antenna.
We also utilized linear regression analyses to try and identify prominent environmental factors influencing photosynthesis at Station ALOHA. These analyses revealed that $E_k$ in the lower euphotic zone was significantly correlated with downwelling irradiance, suggesting that these light-limited cells react rapidly to \textit{in situ} changes in irradiance. However, our results also indicate that the two size-fractions of phytoplankton appear to have different strategies of photoacclimation. In the larger size fraction, $P_{\text{Chl}}^{\text{max}}$ was significantly correlated with downwelling irradiance while $\alpha$ was not significantly correlated with downwelling irradiance, suggesting that the larger phytoplankton adjust the turnover rate of photosynthetic electron transport instead of varying the functional absorption cross-section of photosystem II ($\sigma_{\text{PSII}}$) during photoacclimation (Falkowski and Raven 2007). In contrast, $\alpha$ in the smaller size fraction was significantly correlated with downwelling irradiance while no significant correlation was found between $P_{\text{Chl}}^{\text{max}}$ and downwelling irradiance; such results may indicate that picophytoplankton are better able to adjust $\sigma_{\text{PSII}}$ in response to changes in irradiance.

Our results indicated that the initial slope $\alpha$ in the < 2 $\mu$m size class displays significant vertical variations, while $\alpha$ of the > 2 $\mu$m size class is more constant with depth. Such results are consistent with laboratory studies by MacIntyre et al. (2002), who found that $\alpha$ in many microalgae (mostly > 2 $\mu$m) demonstrated little (< 20%) phenotypic variability; in contrast, $\alpha$ among cyanobacteria (< 2 $\mu$m) tended to decline with increasing irradiance. These authors attribute such changes to plasticity in the phycobiliprotein to Chl $a$ ratio. The initial slope $\alpha$ is proportional to Chl $a$ specific light absorption coefficient ($a^{\text{Chl}}$) which varies among species due to different pigment compositions and packaging effects.
Since phycobiliproteins dominate light absorption in the cyanobacteria and phycobilinprotein to Chl $a$ ratios decrease with increasing irradiance (Kana et al. 1988, Moore et al. 1995), $a_{\text{Chl}}$ in the cyanobacteria would be expected to decline with increasing irradiance. Thus, the observed increase in $\alpha$ among the 0.2-2 $\mu$m size fraction in the lower euphotic zone could be explained by higher phycobiliprotein to Chl $a$ ratios among the picocyanobacteria which dominate the phytoplankton community at Station ALOHA. Moreover, depth-dependent changes in phytoplankton community structure, such as those accompanying the transition between vertical separated and physiologically distinct ecotypes of *Prochlorococcus* (Moore et al. 1995, Malmstrom et al. 2010) could also control the observed vertical differences in $\alpha$.

In conclusion, our research suggests that different size classes of phytoplankton demonstrate significant temporal and vertical variability in photophysiology in the oligotrophic NPSG. Our findings revealed large variability in photosynthesis associated with larger phytoplankton assemblages providing potential insight into processes controlling the formation of large-celled phytoplankton blooms in the NPSG (White et al. 2007, Dore et al. 2008, Fong et al. 2008, Wilson et al. 2008). However, the specific factors controlling variability in the efficiency of carbon fixation remain unclear, as do the mechanisms that permit larger sized phytoplankton cells to accumulate during summertime bloom events. Future studies focused on defining processes that underlie the apparent decoupling in cell removal (e.g., predation, sinking or viral attack) from those that enhance phytoplankton growth and the efficiency of carbon fixation would help lend additional mechanistic insight into the dynamics underlying observations from this study.
REFERENCES


Eppley RW (1972) Temperature and phytoplankton growth in the sea. Fish Bull 70:1063-1085


Table 1. Summary of euphotic zone characteristics at Station ALOHA during the study period (2004-2009). Depicted are median and range (in parentheses) of temperature, downwelling irradiance (PAR), percentage downwelling irradiance relative to surface irradiance, nitrate+nitrite (N+N), soluble reactive phosphorus (SRP) at depths where rates of $^{14}$C-bicarbonate assimilation were measured and P-E experiments were conducted. Data binned into two seasons, winter (November to April) and summer (May to October). BD indicates N+N concentration below detection limit (1 nmol l$^{-1}$).

<table>
<thead>
<tr>
<th>Season</th>
<th>Depth (m)</th>
<th>Temp ($^\circ$C)</th>
<th>PAR (mol quanta m$^{-2}$ d$^{-1}$)</th>
<th>Irradiance (%)</th>
<th>N+N (nmol l$^{-1}$)</th>
<th>SRP (nmol l$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Winter</td>
<td>5</td>
<td>24.1 (22.9 - 26.1)</td>
<td>20.2 (9.3 - 34.4)</td>
<td>80.1 (76.9 - 82.4)</td>
<td>3 (1 - 8)</td>
<td>40 (8 - 92)</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>24.0 (22.8 - 26.1)</td>
<td>8.0 (3.9 - 15.8)</td>
<td>33.2 (24.4 - 37.9)</td>
<td>3 (BD - 9)</td>
<td>41 (7 - 76)</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>23.9 (22.7 - 26.1)</td>
<td>3.4 (1.7 - 7.3)</td>
<td>13.6 (9.4 - 17.5)</td>
<td>3 (BD - 14)</td>
<td>35 (7 - 79)</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>23.7 (22.6 - 25.3)</td>
<td>0.9 (0.4 - 2.3)</td>
<td>3.6 (1.9 - 5.5)</td>
<td>3 (BD - 8)</td>
<td>37 (9 - 72)</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>22.9 (21.8 - 24.3)</td>
<td>0.3 (0.1 - 0.9)</td>
<td>1.2 (0.5 - 2.1)</td>
<td>16 (2 - 600)</td>
<td>48 (16 - 99)</td>
</tr>
<tr>
<td></td>
<td>125</td>
<td>21.8 (20.6 - 23.3)</td>
<td>0.1 (0.04 - 0.3)</td>
<td>0.4 (0.1 - 0.8)</td>
<td>377 (3 - 1019)</td>
<td>94 (22 - 173)</td>
</tr>
<tr>
<td>Summer</td>
<td>5</td>
<td>26.0 (23.8 - 26.8)</td>
<td>35.1 (19.5 - 44.0)</td>
<td>80.2 (75.6 - 83.0)</td>
<td>4 (BD - 8)</td>
<td>53 (4 - 118)</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>25.9 (23.4 - 26.7)</td>
<td>14.1 (7.3 - 18.4)</td>
<td>33.0 (24.6 - 39.3)</td>
<td>3 (1 - 7)</td>
<td>56 (4 - 117)</td>
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</tr>
<tr>
<td>45</td>
<td>25.7 (22.6 - 26.7)</td>
<td>5.9 (2.8 - 8.7)</td>
<td>13.8 (8.0 -18.6)</td>
<td>3 (1 -7)</td>
<td>41 (6 - 95)</td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>23.8 (21.6 - 26.1)</td>
<td>1.6 (0.6 - 2.8)</td>
<td>3.7 ( 1.5 - 6.1)</td>
<td>3 (1 - 9)</td>
<td>26 (8 - 90)</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>22.7 (21.1 - 24.3)</td>
<td>0.5 (0.2 - 1.1)</td>
<td>1.2 ( 0.4 - 2.4)</td>
<td>4 (2 - 80)</td>
<td>40 (11 - 84)</td>
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</tr>
<tr>
<td>125</td>
<td>22.1 (20.0 - 23.3)</td>
<td>0.2 (0.04 - 0.44)</td>
<td>0.4 (0.1 - 0.9)</td>
<td>70 (4 - 477)</td>
<td>76 (25 - 156)</td>
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</tr>
</tbody>
</table>
Table 2. Summary of photophysiological properties at Station ALOHA (2007 - 2009) derived from P-E experiments. Depicted are median and range (in parentheses) of derived P-E properties for two size classes of phytoplankton. Units for $P_{\text{max}}$, $P_{\text{Chl max}}$, $\alpha$, $\beta$ and $E_k$ are ($\mu$g C L$^{-1}$ h$^{-1}$), (mg C (mg Chl)$^{-1}$ h$^{-1}$), ((mg C (mg Chl)$^{-1}$ h$^{-1}$)) (µmol quanta m$^{-2}$ s$^{-1}$)$^{-1}$), ((mg C (mg Chl)$^{-1}$ h$^{-1}$)) (µmol quanta m$^{-2}$ s$^{-1}$)$^{-1}$), (µmol quanta m$^{-2}$ s$^{-1}$), respectively.

<table>
<thead>
<tr>
<th>Depth</th>
<th>$P_{\text{max}}$</th>
<th>$P_{\text{Chl max}}$</th>
<th>$\alpha$</th>
<th>$\beta$ (x 10$^3$)</th>
<th>$E_k$</th>
</tr>
</thead>
<tbody>
<tr>
<td>25m</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 2 µm</td>
<td>0.12 (0.06 - 0.26)</td>
<td>7.2 (1.7 – 14)</td>
<td>0.04 (0.01 - 0.07)</td>
<td>0.4 (-2 - 5)</td>
<td>175 (37 - 319)</td>
</tr>
<tr>
<td>0.2-2 µm</td>
<td>0.20 (0.08 - 0.26)</td>
<td>3.3 (1.9 - 4.5)</td>
<td>0.03 (0.01 - 0.06)</td>
<td>0.1 (-1 - 4)</td>
<td>124 (37 - 286)</td>
</tr>
<tr>
<td>45m</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 2 µm</td>
<td>0.09 (0.05 - 0.34)</td>
<td>5.8 (2.5 – 14.2)</td>
<td>0.02 (0.02 - 0.06)</td>
<td>0.2 (-0.8 - 2)</td>
<td>137 (111 - 235)</td>
</tr>
<tr>
<td>0.2-2 µm</td>
<td>0.12 (0.05 - 0.25)</td>
<td>2.0 (0.9 - 3.5)</td>
<td>0.02 (0.01 - 0.02)</td>
<td>0.1 (0 - 1)</td>
<td>107 (88 - 178)</td>
</tr>
<tr>
<td>75m</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 2 µm</td>
<td>0.14 (0.13 - 0.49)</td>
<td>6.4 (2.8 - 13.4)</td>
<td>0.04 (0.03 - 0.08)</td>
<td>3 (1 - 13)</td>
<td>162 (64 - 253)</td>
</tr>
<tr>
<td>0.2-2 µm</td>
<td>0.20 (0.08 - 0.51)</td>
<td>2.1 (1.8 - 4.6)</td>
<td>0.02 (0.01 - 0.02)</td>
<td>1 (0.1 – 5)</td>
<td>167 (95 - 262)</td>
</tr>
<tr>
<td></td>
<td>&gt; 2 μm</td>
<td>0.2-2 μm</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>125m</td>
<td>0.06 (0.03 - 0.1)</td>
<td>2.9 (1.3 - 5.9)</td>
<td>0.06 (0.03 - 0.1)</td>
<td>2 (0.4 - 10)</td>
<td>44 (25 - 104)</td>
</tr>
<tr>
<td></td>
<td>0.19 (0.1 - 0.34)</td>
<td>2.4 (1.5 - 3.7)</td>
<td>0.05 (0.02 - 0.1)</td>
<td>5 (1 - 11)</td>
<td>40 (27 - 111)</td>
</tr>
</tbody>
</table>
Figure 1. A) Size-fractionated Chl a concentrations, B) size-fractionated in situ primary production, C) size-fractionated P<sub>Chl</sub> relative to downwelling PAR (October 2004 to October 2007) where filled circles depict > 2 µm size fraction and open circles are 0.2-2
μm size fraction. The daily rate was converted to hourly rate by dividing the daylength.

Lines depict least-squares regression fits to the measured rates using the Platt et al. (1980) model. The parameters describing the line fit are: $P_{\text{max}} = 1.1$, $\alpha = 0.7$, $\beta = 0.002$, $r^2 = 0.52$, $p < 0.0001$, for the > 2 μm size fraction; $P_{\text{max}} = 2.6$, $\alpha = 3.2$, $\beta = 0$, $r^2 = 0.67$, $p < 0.0001$, for the 0.2-2 μm size fraction (penal B). The parameters describing the line fit are $P_{\text{Chl opt}} = 11.5$, $\alpha_{\text{Chl}} = 3.0$, $\beta = -0.002$, $r^2 = 0.57$, $p < 0.0001$, for the > 2 μm size fraction; $P_{\text{Chl opt}} = 3.2$, $\alpha_{\text{Chl}} = 0.9$, $\beta = 0.002$, $r^2 = 0.55$, $p < 0.0001$, for the 0.2-2 μm size fraction (penal C).

Units for $P_{\text{max}}$, $\alpha$, $P_{\text{Chl opt}}$, $\alpha_{\text{Chl}}$ are (μg C L$^{-1}$ d$^{-1}$), ((μg C L$^{-1}$ d$^{-1}$) (µmol quanta m$^{-2}$ d$^{-1}$)$^{-1}$), (mg C (mg Chl)$^{-1}$ h$^{-1}$), and ((mg C (mg Chl)$^{-1}$ h$^{-1}$) (µmol quanta m$^{-2}$ d$^{-1}$)$^{-1}$), respectively.
**Figure 2.** A) Depth integrated (0-125 m) Chl $a$ concentrations, B) *in situ* rates of $^{14}$C-bicarbonate assimilation, and C) $^{14}$C-bicarbonate assimilation normalized to Chl $a$ concentrations ($P_{\text{Chl}}$) for the two size fractions. The daily rate was converted to hourly rate by dividing the daylength. Filled bars represent $> 2 \, \mu m$ size fraction and open bars represent 0.2-2 $\mu m$ size fraction.
Figure 3. Photosynthesis-irradiance (P-E) curves from the A) upper euphotic zone (25 m) and B) deep euphotic zone (125 m) at Station ALOHA (December 2009); > 2 µm size fraction depicted by filled circles and 0.2-2 µm size fraction shown as open circles. C) Histogram of coefficients of determination ($r^2$) derived from least-squares regression analyses of P-E measurements using the Platt et al. (1980) model. Filled bars represent > 2 µm size fraction and open bars represent 0.2-2 µm size fraction.
Figure 4. A) Relationships between $P_{\text{Chl max}}$ for the two phytoplankton size fractions and downwelling irradiance. Symbols represent the time-averaged $P_{\text{Chl max}}$ and downwelling irradiance at the four depths where P-E experiments were conducted; > 2 µm size class
depicted by filled circles and 0.2-2 µm size class depicted with open circles; error bars represent the maximum and minimum values. B) $E_k$ values of two size fractions (> 2 µm in circles, 0.2-2 µm in triangles) relative to downwelling irradiance measured at approximately noon on each cruise; samples collected from the upper 45 m of the euphotic zone depicted by open symbols, samples collected from 75 m and 125 m depicted by filled symbols; error bars represent standard deviation of each $E_k$ and downwelling PAR determined for each cruise; solid line depicts the 1:1 ratio.
Figure 5. Time-series of the $P_{\text{Chl}}^{\text{max}}$ of two size fractions and *in situ* $P_{\text{Chl}}$ at 25 m (A) and 125 m (B); > 2 $\mu$m size fraction depicted by filled bars, 0.2-2 $\mu$m size fraction open bars. $P_{\text{Chl}}$ represented by grey diamonds. Error bars are standard errors associated with each $P_{\text{Chl}}^{\text{max}}$ value.
Chapter 3

Temporal and vertical variability of eukaryotic phytoplankton community structure
in the North Pacific Subtropical Gyre

ABSTRACT

Eukaryotic phytoplankton have been shown to play important roles in regulating new production and particle export in the oligotrophic North Pacific Subtropical Gyre. In this study, we utilized near-monthly time series observations (2007-2009) on chromophytic algal rbcL gene diversity and abundances together with high performance liquid chromatography (HPLC) measurements of diagnostic pigment biomarkers to examine dynamics in the eukaryotic phytoplankton population structure at Station ALOHA (22°45’ N, 158° W). In addition, examination of rbcL genes associated with sinking particulate matter provided insight into the time-varying contributions of selected phytoplankton taxa to particle export in this ecosystem. Polymerase chain reaction (PCR) amplification, cloning and sequencing of form 1D chromophyte rbcL genes revealed a phylogenetically diverse assemblage of chromophytic algae, including representatives of various genera of diatoms, pelagophytes, prymnesiophytes and dinoflagellates. Use of a pigment algorithm to estimate the contributions of selected algal groups to total Chl a concentrations suggested that three major groups of chromophytic phytoplankton (diatoms, prymnesiophytes and pelagophytes) were a large fraction (25-45%) of total Chl a concentrations in the lower euphotic zone (75-125 m). Quantitative PCR amplification of rbcL genes of these three major groups of phytoplankton revealed that the population
structure of these assemblages was highly variable in time. Diatom rbcL genes were typically the most abundant among these groups, with elevated abundances often occurring in the upper euphotic zone (0-45 m) during the summer. Abundances of prymnesiophyte and pelagophyte rbcL genes often increased in the lower regions of the euphotic zone (75-125 m) during fall and winter months. Analyses of the upper ocean (150 m) sediment trap samples suggested that diatom rbcL gene flux was often greatest in the summer, when particulate carbon export was maximal. In contrast, sinking fluxes associated with prymnesiophytes and pelagophytes tended to be greatest in the spring and fall. Our study suggests that in the summer, diatoms are prominent contributors to sinking vertical fluxes, while in the winter and spring, prymnesiophytes and pelagophytes become greater contributors to particle export.
INTRODUCTION

Subtropical ocean gyres occupy ~40% of Earth’s surface area. The North Pacific Subtropical Gyre (NPSG) is the largest of these open ocean habitats (Sverdrup et al. 1942, Karl 1999), extending from approximately 15° N to 35° N latitude and from 135° E to 135° W longitude. Persistent stratification of the upper ocean of the NPSG reduces wind-driven convective mixing, and limits vertical delivery of nutrients to the upper ocean (Dore et al. 2002, Karl 2002). The surface waters of the NPSG are characterized by low nutrient concentrations, deep penetration of photosynthetically available radiation, low plankton biomass, and dominance of plankton biomass by photosynthetic cyanobacteria such as *Prochlorococcus* and *Synechococcus* (Campbell and Vaulot 1993, Letelier et al. 1993, Anderson et al. 1996, Malmstrom et al. 2010).

Although much of the research on phytoplankton dynamics in the NPSG has emphasized the important role of picoplankton as key contributors to productivity and biomass (Campbell and Vaulot 1993, Karl et al. 2001, Church et al. 2006), largernano- and microplankton also play important roles in regulating ecological and biogeochemical dynamics in this ecosystem. Over the course of nearly 20 years (1968-1985), microscopic evaluations of phytoplankton assemblages (taxa larger than 5 µm) conducted at Station Climax (28°N, 155°W), in the northern regions of the NPSG, provided detailed information on vertical and time-variability in phytoplankton community structure (Venrick 1982, 1988, 1990, 1992). These studies identified two vertically separated phytoplankton assemblages (Venrick 1982): one assemblage of photosynthetic plankton appeared largely relegated to the high-light, low-nutrient regions of the upper ocean,
while the other assemblage typically associated with the deep chlorophyll maximum layer (DCML), where nutrient concentrations increase, but penetration of light becomes more temporally variable and often limiting to phytoplankton growth (Venrick 1982, Letelier et al. 2004). More than 230 phytoplankton species were identified and listed as part of these early studies, including 101 diatom species and 47 species belonging to the prymnesiophytes (Venrick 1982).

In the summers of 1994 and 1996, Venrick (1997, 1999) compared phytoplankton species collected at Station ALOHA (22o45’ N, 158o W) to those previously observed at Station Climax. These studies revealed that similar genera and species inhabited both of these open ocean locations, including flora associated with shallow and deeper euphotic zone waters (Venrick 1997, 1999). Research at Station ALOHA indicates that various groups of eukaryotic phytoplankton play seasonally variable roles in plankton ecology in the NPSG. Analyses of phytoplankton community structure based on high performance liquid chromatography (HPLC) separation and analyses of selected algal pigments indicated that prymnesiophytes and pelagophytes can be dominant contributors to eukaryotic phytoplankton biomass (Letelier et al. 1993, Anderson et al. 1996, Karl et al. 2002). In a study focused on prymnesiophyte ecology at ALOHA, Cortés et al. (2001) identified upwards of 125 distinct species of coccolithophores, finding that abundances generally increased in the spring and fall. Scharek et al. (1999a) investigated the abundance of diatoms during 11 cruises (1994-1995) to Station ALOHA, finding that two lightly silicified diatom species (*Hemiaulus hauckii* and *Mastogloia woodiana*) increased in abundance in July 1994 within the well stratified mixed layer; moreover, these diatoms
were identified as key contributors to material export from the upper ocean to the deep sea.

The purpose of the current study was to investigate the temporal variability of eukaryotic phytoplankton dwelling among the suspended particles, and those caught in sediment traps (150 m depth) presumably contributing to sinking organic matter flux. We investigated population dynamics associated with eukaryotic phytoplankton based by examining time-variability in selected algal pigments and through assessment of \textit{rbcL} gene diversity and abundances. Our results highlight the vertical and temporal variability in population structure of the eukaryotic algal assemblages in this oligotrophic environment. Moreover, QPCR amplification of \textit{rbcL} genes revealed that contributions of prymnesiophytes and pelagophytes to particle export tended to increase during the spring and fall, while the contributions of diatoms often increases in the summer.

**MATERIALS AND METHODS**

**Upper ocean and sediment trap sampling and analyses**

Sampling for this study was conducted on near-monthly HOT cruises to Station ALOHA over approximately two years (October 2007 to December 2009). Seawater samples were collected from 8 discrete depths in the upper ocean (5, 25, 45, 75, 100, 125, 150, 175 m) using 12 liter polyvinyl chloride bottles attached to a conductivity-temperature-depth (CTD) rosette sampler. Ten liters of seawater was subsampled into polyethylene carboys and pressure filtered onto 47 mm diameter 2 µm porosity polycarbonate filters for subsequent extraction of planktonic DNA. These filters were
preserved in a buffer containing 0.1M EDTA, pH 8.0; 1% sodium dodecyl sulfate and stored at -80°C.

Samples for subsequent extraction of DNA were also collected from particle interceptor sediment traps (collection depth of 150 m) on each cruise to Station ALOHA. These traps were filled with a 0.2 µm filtered sodium-chloride seawater brine solution (50 g NaCl l⁻¹ amended to surface seawater), and deployed for ~2.5 days on a free-drifting, surface tethered array. Upon recovery of the trap array, the overlying low-density seawater was removed by siphoning and the trap solution was pressure filtered onto 47 mm diameter 2 µm porosity polycarbonate filters and frozen in the same buffer previously described.

**DNA extraction, PCR amplification and sequence analyses**

DNA was extracted from the filters using a combined cetyltrimethylammonium bromide (CTAB)-chloroform method (Zhang and Lin 2005). Briefly, after approximately 12 hours of incubation at 55°C in a 200 µg ml⁻¹ proteinase K solution (0.5 ml), samples were mixed with 165 µl pre-warmed 10% CTAB and incubated for 10 min at 55°C. An equal volume of chloroform (99.8%, HPLC grade) was then added to this solution; samples were vortexed for 1 min and centrifuged at room temperature at 13000 x g for 10 min. The supernatants were purified and eluted using a Genomic DNA Clean & Concentrator kit (Zymo Research).

We utilized chloroplast form 1D rbcL genes to examine phylogenetic relationships among chromophytic phytoplankton. The rbcL gene encodes the large subunit of RuBisCO, the enzyme catalyzing the initial step of carbon fixation in the
Calvin-Benson cycle. The RuBisCO protein has several structural forms (Watson and Tabita 1996), and these differences are reflected in variations in \( rbcL \) gene phylogeny (Tabita et al. 2007). Many eukaryotic algae and cyanobacteria possess a form of the RuBisCO enzyme termed form 1, which includes at least four distinct taxonomic lineages termed forms 1A, 1B, 1C and 1D (Tabita 1999). Previous studies have documented the utility of form 1D genes for distinguishing phylogenetic relationships among chromophytic phytoplankton (Paul et al. 1999, 2000, Mann et al. 2001, Wawrik et al. 2002, 2003).

PCR-primers specific to form ID \( rbcL \) genes (forward primer, 5’-GATGATGARAAYATTAACTC-3’; reverse primer, 5’-ATTTGDCACACAGTGDATACCA-3’, Paul et al. 2000) were used to amplify a 554-bp gene fragment from the DNA extracts obtained in this study. The PCR mix consisted of 2 µl plankton DNA extracts, 31 µl of nuclease free water, 5 µl of Ex Taq Buffer (TaKaRa), 4 µl of 2.5 mM dNTP mix, 4 µl of each 10 µM forward and reverse primers, and 1.5 U of ExTaq polymerase (TaKaRa). Total PCR reaction volumes were 50 µl. Thermal cycling conditions were: 3 min at 95°C, followed by 40 cycles of 1 min at 95°C, 1 min at 52°C, and 1 min 30 s at 72°C, with a final extension at 72°C for 15 min. The resulting PCR products were visualized on an ethidium bromide-stained 1.2% agarose gel. PCR amplicons were excised and purified using with the QIAquick Gel purification kit (Qiagen®). The purified PCR products were ligated into pGEM-T Easy Vectors and transformed in \textit{Escherichia coli} JM109 competent cells following the manufacturer’s instructions (Promega®). Plasmids containing the PCR amplified \( rbcL \) gene fragments
were extracted and purified prior to sequencing using an ABI 3100 Gene Analyzer (Applied Biosystems®). Sequences were edited using BioEdit and imported into an aligned \textit{rbcL} gene sequence database using ARB (Ludwig et al. 2004).

**Primer design and QPCR amplification**

More than 350 marine phytoplankton form 1D \textit{rbcL} sequences obtained from GenBank (NCBI) and from our own environmental clone libraries were used to design and evaluate the specificity of quantitative PCR (QPCR) primers targeting phylotypes found at Station ALOHA. Three primers sets were developed to target form 1D \textit{rbcL} genes from clones phylogenetically clustering among diatoms, prymnesiophytes and pelagophytes, respectively (Table 1). The abundances of \textit{rbcL} genes from these major chromophyte taxa were estimated using QPCR, where QPCR reactions included: 12.5 µl 2x SyberGreen Master Mix (Applied Biosystems), 5.5 µl of nuclease free water, 2 µl each of 10 µM forward and reverse primers, 1 µl of 10 mg ml\(^{-1}\) Bovine Serum Albumin (BSA, BioLabs) and 2 µl of DNA extract. QPCR reaction conditions were: 94.0°C for 15 min; 40 cycles of 15 s at 94.0°C, 30 s at 48.0°C, 35 s at 72.0°C, followed by extension at 72°C for 7 min. Melt curves were run between 44.0 – 95.0°C with the resulting amplicon dissociation products detected at 1.0°C intervals. Standards for the QPCR reactions consisted of serial 10-fold dilutions of plasmids containing targeted DNA fragments. QPCR reactions were conducted in duplicate for each environmental DNA sample and for each standard. Specificities of the QPCR primers were evaluated by comparing the amplification cycle threshold between plasmids containing the intended target \textit{rbcL} insert and non-target controls.
Upper ocean physical and biogeochemical measurements

Incident solar irradiance (400-700 nm) was measured on each HOT cruise using a LI-COR LI-1000 data logger and cosine collector. In addition, daily vertical profiles of downwelling photosynthetically active radiation (PAR) were conducted at approximately noon on each HOT cruise using a Profiling Reflectance Radiometer (PRR; Wetlabs). Vertical profiles of temperature, salinity, and pressure were obtained using a SeaBird conductivity-temperature-depth (CTD) sampler described by Karl and Lukas (1996). High-sensitivity measurements of nitrate + nitrite (N+N) were determined as described by Dore and Karl (1996). Particulate carbon flux was collected and determined as described by (Karl et al. 1996). Primary production of the upper 125 m of the ocean was determined as described by Letelier et al. (1996).

Chl $a$ and photosynthetic accessory pigments were measured by high performance liquid chromatography (HPLC) according to the procedures described in Bidigare et al. (2002). We utilized several photosynthetic carotenoid pigments as a diagnostic indicators of diatoms (fucoxanthin), prymnesiophytes (19’-hexanoyloxyfucoxanthin, abbreviated as 19-hex), and pelagophytes (19’-butanoyloxyfucoxanthin, abbreviated as 19-but). The contribution of these major eukaryote phytoplankton taxa to total Chl $a$ in the lower regions of the euphotic zone (75-125 m) was calculated based on the measured HPLC-determined pigment concentrations and applying the modified algorithms (Table 2) from Letelier et al. (1993). The 19-hex to 19-but ratio in prymnesiophytes was re-estimated for this study based data from four cultures of prymnesiophytes (Jeffery and Wright 1994),
and diatom contributions to Chl $a$ were calculated based on laboratory studies on fucoxanthin and 19-but concentrations (Mackey et al. 1996).

RESULTS

**Upper ocean biogeochemical characteristics**

Consistent with the well-resolved climatology at Station ALOHA, the euphotic zone during this period appeared to generally form two vertically segregated habitats: a high-light, nutrient-poor upper euphotic zone (0-45 m) that supported the majority of primary production; and a low-light, nutrient-enriched lower euphotic zone (75-125 m) that included the deep chlorophyll maximum layer (DCML). On average, concentrations of N+N were consistently low (< 4 nM) in the well-lit regions of the euphotic zone (0-45 m), with nutrient concentrations increasing sharply below 75 m (Fig. 1). Concentrations of Chl $a$ were low in the upper 45 m (averaging 103 ± 35 ng L$^{-1}$), increasing through the mid-euphotic zone (75-125 m), before declining again toward the base of the euphotic zone where light fluxes decreased to <1% of the surface irradiance (Fig. 1). Rates of primary production were elevated in the upper euphotic zone, ranging between 2.5 and 10.8 $\mu$g C L$^{-1}$ d$^{-1}$, with production decreasing with depth (Fig 1).

Over the period of this study, sea-surface temperatures varied between 22.9$^{\circ}$C and 26.6$^{\circ}$C with euphotic zone temperatures relatively homogenous during the winter (varying < 2$^{\circ}$C), with the upper ocean warming and becoming more stratified during the summer and fall months (Fig. 2). Light flux at the sea surface varied approximately 4-fold during the study (ranging 17 to 47 mol quanta m$^{-2}$ d$^{-1}$), with downwelling irradiance
varying as much as 12-fold (0.03-0.37 mol quanta m\(^{-2}\) d\(^{-1}\)) in the lower regions of the euphotic zone (125 m). Deepening of isolumes in the late spring and early summer coincided with periods of lower concentrations of N+N in the dimly region of the euphotic zone, while N+N concentrations increased during wintertime periods when isolumes shoaled upwards (Fig. 2).

Concentrations of Chl \(a\) in the upper euphotic zone (0-45 m) ranged between 42 and 215 ng L\(^{-1}\) (averaging 103 ng L\(^{-1}\)), while concentrations in the lower euphotic zone (75-125 m) varied between 81 and 374 ng L\(^{-1}\) (averaging 234 ng L\(^{-1}\)) (Fig. 3). Chl \(a\) concentrations typically peaked between 100 and 125 m, where light decreased to < 2% of the surface flux. The DCML generally deepened in the late spring and early summer, and shoaled in late summer and fall, consistent with seasonal variations in light availability (Fig. 3). Concentrations of fucoxanthin, an accessory pigment produced by diatoms, did not display clear vertical maxima, with concentrations demonstrating episodic variations in the upper and lower regions of the euphotic zone (Fig. 3). Concentrations of 19-hex, and 19-but were generally low in the upper euphotic zone, increasing with depth where concentrations of both pigments tended to be greatest coincident with the DCML (Fig. 3). In the lower euphotic zone, 19-hex and 19-but displayed similar temporal dynamics with elevated concentrations in winter, late spring and fall months (Fig. 3).

Taxonomic assignment of Chl \(a\) concentrations based on a modified pigment algorithm (Letelier et al. 1993) indicated that on average, the three major eukaryotic phytoplankton groups examined in this study contributed to 25-45\% of the total Chl \(a\)
concentrations measured in the lower euphotic zone (75-125 m), with prymnesiophytes contributing 20.8% (± 2.3%), pelagophytes 8.8% (± 2.2%) and diatoms 1.5% (± 0.8%) respectively (Table 3). A significant positive relationship was observed between Chl a contributed by prymnesiophytes and the contribution of pelagophytes (least-square linear regression, $r^2 = 0.42$, $p < 0.01$). Moreover, there was a significant positive relationship between Chl a and the contribution of prymnesiophytes to Chl a in the lower euphotic zone (least-square linear regression, $r^2 = 0.48$, $p < 0.001$). No significant relationship was found between Chl a contributed by diatoms and Chl a contributed by the other groups of phytoplankton examined in this study (least-square linear regression, $p > 0.1$). There were no clear temporal patterns in the Chl a contributed by these three phytoplankton taxa; however, the relative contribution of pelagophytes to total Chl a often increased in fall and winter relative to other times of the year (Table 3).

**rbcL gene clone libraries**

Two hundred eighty eight rbcL gene sequences were obtained from clone libraries of PCR amplified environmental DNA extracted from samples collected throughout the euphotic zone (0-150 m) and from upper ocean sediment traps. These environmental rbcL sequences clustered with four major phytoplankton clades of form 1D rbcL genes including various genera of diatoms, pelagophytes, prymnesiophytes, and dinoflagellates (Fig. 4). One hundred twenty five of these rbcL gene sequences clustered at 89-96% identity with more than 12 known diatom genera (Fig. 4); the majority of these sequences clustered among diatoms belonging to the genera *Chaetoceros*, *Corethron*, *Pseudonitzschia* and *Thalassiosira* (Fig. 4). In addition, we retrieved 16 sequences
believed to derive from diatoms belonging to the genera *Hemiaulus*. Seventy-five *rbcL* gene sequences clustered (88-100% identity) among 7 known genera of prymnesiophytes, including *Emiliania, Phaeocysitis, Umbilicosphaera, Calyptrosphaera, Helicosphaera, Chrysochromulina* and *Gephyrocapsa*. Sequences most closely related (91-100%) to *Emiliania* were retrieved exclusively from samples collected in the upper euphotic zone (0-45 m), while sequences phylogenetically clustering among *Chrysochromulina* were all retrieved from samples collected in the lower euphotic zone (75-125 m). In total, 71 sequences clustered (87-99% identity) among pelagophytes belonging to the genera: *Pelagomonas, Pelagococcus* and *Aureococcus*. Sequences most similar to *rbcL* genes belonging to *Pelagomonas* were exclusively retrieved from the lower euphotic zone (75-125 m) and from sediment traps, while sequences clustering with *Aureococcus* were only retrieved from samples collected in the upper euphotic zone waters (0-45 m) and in sediment traps (Fig. 4).

A total of 108 *rbcL* gene sequences were retrieved from samples collected using upper ocean sediment traps, with 45 of these sequences clustering with *rbcL* gene sequences from pelagophytes. The majority (31) of these pelagophyte sequences were most closely (91-99%) related to the *rbcL* gene sequence of *Pelagomonas calceolate*, a 2-4 µm diameter flagellated cell (Daugbjerg and Andersen 1997). Thirty six sequences derived from the sediment trap samples were phylogenetically related to diatom *rbcL* gene sequences, including diatoms belong to the genera: *Thalassiosira, Rhizosolenia, Nitzschia, Pseudonitzschia*, and *Cylindrotheca*. Finally, 26 sequences retrieved from the sediment trap samples clustered among *rbcL* gene sequences from prymnesiophytes; the
majority (16) of these sequences clustered with *Emiliania huxleyi* and *Chrysochromulina* spp.

**Vertical and temporal variability in rbcL gene abundances**

QPCR analyses of form 1D rbcL gene abundances provided insight into the vertical and temporal dynamics associated with three major phytoplankton taxa in this ecosystem: diatoms, pelagophytes, prymnesiophytes. In the upper euphotic zone (0-45 m), diatoms were often the most abundant of rbcL gene groups examined (Fig. 5). rbcL gene abundances of diatoms ranged between $2 \times 10^4$ and $2 \times 10^6$ gene copies L$^{-1}$, with peak abundances occurring in July 2008 and July 2009. rbcL gene abundances of prymnesiophytes ranged between $6 \times 10^3$ and $4 \times 10^5$ gene copies L$^{-1}$, and were generally elevated in the fall and winter (Fig. 5). Gene abundances of pelagophytes were the most variable and often lowest of the taxa examined, with rbcL gene abundances ranging between $2 \times 10^2$ and $6 \times 10^5$ gene copies L$^{-1}$ in the upper euphotic zone (Fig. 5). Depth integration of the upper euphotic zone (0-45 m) gene abundances provided insight into population dynamics of these three taxa in the well-lit region of the euphotic zone (Fig. 6). rbcL gene inventories of all three taxa in upper euphotic zone fluctuated as much as 23-fold between the near-monthly samplings. None of the taxa examined demonstrated significant seasonality in rbcL gene abundances (one-way ANOVA, $p > 0.05$) in the upper euphotic zone. Diatom rbcL gene abundances were highest in July of both years. Peak abundances of prymnesiophytes occurred in February and December of 2009, while abundances of pelagophyte rbcL genes were greatest in May 2009, undergoing a ~14-fold increase relative to the previous month (Fig. 6).
In the lower euphotic zone, diatom \textit{rbcL} genes were generally the most abundant of the taxa examined, ranging between $7 \times 10^2$ and $3 \times 10^6$ gene copies L$^{-1}$. Gene abundances of the prymnesiophytes and pelagophytes in the lower euphotic zone were generally elevated in the fall and winter, decreasing in the summer (Fig. 5, 6). Abundances of prymnesiophyte \textit{rbcL} genes peaked ($> 4 \times 10^5$ gene copies L$^{-1}$) in the lower euphotic zone (75-125 m) during the fall and winter months. These periods of elevated gene abundances occurred when temperatures and light intensities ranged between 21-24$^\circ$C and 0.1-1 mol quanta m$^{-2}$ d$^{-1}$, respectively. Similarly, elevated \textit{rbcL} gene abundances ($> 10^5$ gene copies L$^{-1}$) of the pelagophytes were almost exclusively localized to depths and times when seawater temperatures ranged between 21$^\circ$ and 23$^\circ$C. Depth integration of \textit{rbcL} gene abundances in the lower euphotic zone (75-125 m) revealed that \textit{rbcL} abundances associated with diatoms, prymnesiophytes and pelagophytes increased sharply in February 2009 (Fig. 6); during this event \textit{rbcL} gene abundances of all three taxa increased 15, 16 and 32-fold, respectively relative to samples collected in the previous month. In addition, there was a significant positive correlation between \textit{rbcL} gene abundances of prymnesiophytes and pelagophytes (least-square linear regression, $r^2 = 0.71$, p < 0.001), consistent with the positive correlation found between the contributions of prymnesiophytes and pelagophytes to Chl \textit{a} derived based on the pigment algorithm.

In the fall and winter months, diagnostic accessory pigments concentrations and \textit{rbcL} gene abundances of the three phytoplankton taxa generally demonstrated similar vertical distributions (Fig. 5); however, during the well-stratified summer months when
*rbcL* gene abundances were often greater in the upper ocean, pigment concentrations remained elevated near the depth of the DCML (Fig. 5). Notably, *rbcL* gene abundances of prymnesiophytes were significantly higher in fall and winter months than those in summer months in the DCML (100-125 m) (one-way ANOVA, p < 0.05), despite no significant differences among seasons in prognostic accessory pigments for these phytoplankton taxa in the lower euphotic zone (75-125m) (Fig. 5).

**Vertical flux of *rbcL* genes**

We also examined temporal variability in chromophyte phytoplankton export based on QPCR amplification of selected form1D *rbcL* genes associated with sinking particulate material. Diatom *rbcL* gene fluxes were highly time-variable, fluctuating as much as 29-fold (3 x 10⁶ - 8 x 10⁷ gene copies m⁻² d⁻¹) over the period of observations. While there was no significant seasonality in diatom *rbcL* gene export (one-way ANOVA, p > 0.05), diatom *rbcL* gene flux was often elevated in June, July and August, relative to other months (Fig. 7). Periods of elevated diatom flux coincided with periods when particulate carbon (PC) fluxes were elevated (Fig. 7), resulting in a weak but significant relationship between diatom *rbcL* gene flux and PC flux at 150 m (least-square linear regression, r² = 0.24, p < 0.05).

Gene fluxes associated with prymnesiophytes and pelagophytes demonstrated significant temporal covariance (least-square linear regression, r² = 0.91, p < 0.001), with elevated fluxes in the spring (April) and late fall (October to December) (Fig. 7). Moreover, vertical export of prymnesiophyte and pelagophyte *rbcL* genes were significantly greater in the fall relative to the summer or winter (one-way ALOHA, p <
Export of prymnesiophyte and pelagophyte derived rbcL genes varied 128-fold and 429-fold, respectively, during the study period, suggesting greater temporal variability in the contributions of these taxa relative to diatoms. Although diatom rbcL genes were often the most abundant in the euphotic zone, their contribution to rbcL gene flux out of the euphotic zone was relatively low compared to rbcL gene fluxes of prymnesiophytes (Fig. 7).

DISSCUSION

Our study utilized both molecular and pigment-based approaches to evaluate the population structure and temporal and vertical variability in chromphytic phytoplankton population structure in the NPSG. Modification and application of a previously developed pigment algorithm to evaluate contributions of major taxa to total Chl a (Letelier et al. 1993), suggested that three major taxa of eukaryotic phytoplankton (prymnesiophytes, pelagophytes and diatoms) contributed to 25 and 45% of the total Chl a in the lower euphotic zone (75-125 m). On average, the pigment-based model indicated prymnesiophytes, pelagophytes and diatoms contributed 21%, 9% and 2%, respectively to the total Chl a in the lower euphotic zone at Station ALOHA. Such results are similar to estimates reported by Letelier et al. (1993) using data collected between 1989 and 1991. Moreover, in a study comparing HPLC pigment and electron microscopy-derived determinations of phytoplankton community structure in the Sargasso Sea and the NPSG, Andersen et al. (1996) concluded that prymnesiophytes and pelagophytes were among the
most abundant eukaryotic phytoplankton, contributing 30-60% to total euphotic zone Chl $a$.

Pigment-based approaches indicate pelagophytes and prymnesiophytes are important components of eukaryotic phytoplankton biomass in various regions of the open sea including the subarctic North Pacific (Obayashi et al. 2001, Suzuki et al. 2002), South Pacific Ocean (DiTullio et al. 2003), Indian Ocean (Not et al. 2008), Mediterranean Sea (Barlow et al. 1997, Marty et al. 2002), and the Arabian Sea (Barlow et al. 1999). During the North Atlantic Spring bloom, Barlow et al. (1993) estimated prymnesiophytes contributed 20-40% of the Chl $a$ based on multiple regression analysis of accessory pigments. Similarly, Claustre and Marty (1995) estimated that pelagophytes and prymnesiophytes comprised important components of phytoplankton biomass in the lower euphotic zone of the tropical North Atlantic (21°N, 31°W) during both the spring and fall. In the equatorial Pacific Ocean, Bidigare and Ondrusek (1996) estimated the contributions of these two phytoplankton taxa to total Chl $a$, finding prymnesiophytes and pelagophytes comprised 30-40% and 10-20% of the total Chl $a$, respectively, during two survey cruises in summer and winter.

al. 2008, Rodríguez-Martínez et al. 2009). Such 18S rRNA gene studies often reveal that oceanic plankton include diverse assemblages of largely uncultivated organisms that cluster among the six super-groups of unicellular eukaryotes (Adl et al. 2005). However, in many cases, 18S rRNA gene clone libraries are dominated by non-photosynthetic phylotypes, including those clustering among the Alveolata, Radiolaria and Stramenopiles. For example, Countway et al. (2007) found that the majority of sequenced clones from both shallow and deep waters in the North Atlantic clustered among Alveolate lineages. Similarly a study of picoeukaryotic diversity in the Sargasso Sea observed ~ 67% of the sequenced phylotypes clustered within the Alveolata (Not et al. 2007). In another study, Massana et al. (2006) determined the abundance of uncultured marine Stramenopiles using fluorescent in situ hybridization, finding that several lineages of presumed bacterivorous heterotrophic flagellates were widespread and abundant.

Given our interests in examining temporal and vertical variability in phytoplankton assemblages in the NPSG, we utilized chloroplast *rbcL* genes as molecular biomarkers to specifically target photosynthetic eukaryotes. By examining diversity in form 1D *rbcL* genes, we were able to develop and apply group-specific QPCR primers to obtain additional information on the gene abundances of three groups of chromophytic algae. Several previous studies have applied reverse-transcription PCR (QRT-PCR) assays to examine temporal and spatial variability in patterns of *rbcL* gene transcription by diatoms, haptophytes, *Prochlorococcus* and *Synechococcus* (Wawrik et al. 2002, 2003, John and Paul 2007), but to our knowledge this is the first study to examine time-variability in gene abundances by these major phytoplankton groups.
Cloning and sequencing of PCR amplified rbcL genes from the euphotic zone at Station ALOHA revealed numerous sequences phylogenetically related to diatoms, prymnesiophytes, dinoflagellates and pelagophytes. Notably, nearly half of the total clones (180) clustered among diatom rbcL genes, suggesting these organisms were prominent components of eukaryotic plankton in this oligotrophic habitat. Such results were further supported by our QPCR amplification results indicating that diatom rbcL genes were often the most abundant of the groups examined in both the upper and lower euphotic zone. Phylotypes clustering among the prymnesiophytes and pelagophytes contributed 27% and 13%, respectively, to the total sequences from the euphotic zone clone libraries. In general, the identity of environmental rbcL clones ranged from 87% to 100% in our study which is similar to the identity range (81-99%) of form 1D clones in Paul et al. (2000).

QPCR analyses of rbcL gene abundances provided insight into the relatively large temporal variability underlying population dynamics associated with diatoms, haptophytes and prymnesiophytes. Notably, diatoms often dominated the rbcL gene abundances among the three groups examined, even in the lower regions of the euphotic zone. These results, contrast those of the pigment-based model, which suggested diatoms were minor contributors to total Chl a in the lower euphotic zone. The discrepancy in the two approaches could be explained by various factors, including: 1) diatoms may have a larger number of rbcL gene copies relative to Chl a compared to prymnesiophytes and pelagophyte, or 2) the pigment-based model relies on accessory pigment to Chl a ratios derived from relatively few cultured species in each taxa, and thus may underestimate
pigment ratios for the diverse species growing in a fluctuating environment. In addition, to date there is relatively little known regarding variability in *rbcL* gene copies per cell ratios for most phytoplankton. Thus, the observation that diatom *rbcL* gene abundances were often greater than prymnesiophyte and pelagophyte abundances does not necessarily indicate higher diatom cell abundances relative to these other taxa.

No significant seasonal variability was observed in either *rbcL* gene abundances or accessory pigment concentrations in the lower euphotic zone for the three phytoplankton taxa examined. The similar temporal trends between the accessory pigments (19-hex and 19-but) and *rbcL* gene abundances of prymnesiophytes and pelagophytes in the lower euphotic zone suggested that the two biomarkers (*rbcL* genes and accessory pigments) co-varied temporally in the low light regions of the euphotic zone. Moreover, the significant positive correlation between Chl *a* contributed by prymnesiophytes and pelagophytes in the lower euphotic zone hints that these two groups of phytoplankton may respond to time-varying environmental forcing (i.e. nutrients or light) in a similar manner. Although, the *rbcL* gene determinations and pigment biomarker measurements generally agree in the lower euphotic zone, both methodologies provide different views of the vertical distributions of these phytoplankton groups, particularly during the summer months. Based on water samples collected from Station ALOHA during a single cruise in April, Andersen et al. (1996) found good agreement between cell counts and pigment-based estimates of prymnesiophytes in the upper euphotic zone, with increasing disagreement between the two methods in the lower euphotic zone. These depth-dependent differences were suggested to result from vertical
variations in accessory pigment to Chl a ratios (Anderson et al. 1996). In the present study, the observed differences in vertical distributions between the rbcL gene abundances and related accessory pigments could derive from several factors, including: light/depth-dependant variations in amount of accessory pigments per cell, or variations in rbcL gene copies per cell for the different taxa.

Interestingly, we observed peak abundances (> 4 x 10^5 gene copies L^-1) of prymnesiophytes rbcL genes occurred in the lower euphotic zone (75-125 m) in winter and fall during the study period, when water temperatures dropped to 21 – 24°C and light fluxes fell to 0.1 – 1 mol quanta m^-2 d^-1 (~ 2.3-23 µmol quanta m^-2 s^-1). These results are consistent with the observations of Cortés et al. (2001), who found peak coccolithophore cell densities (> 3 x 10^4 cells L^-1) at Station ALOHA occurred at depths where temperature and light varied between 20-25 °C and 2-25 µmol quanta m^-2 s^-1. In addition, we observed pelagophyte rbcL gene abundances were elevated in the lower regions of the euphotic zone during the winter and fall. To date there have been relatively few studies examining vertical or temporal dynamics associated with pelagophytes; however, based on analyses of 19-but to 19-hex ratio, Claustre and Marty (1995) suggested that pelagophytes are more abundant in the DCML and contribute significantly to new production near the bottom of the euphotic zone. The apparent predominance of prymnesiophytes and pelagophytes in the lower euphotic zone near the top of the nutricline suggests these phytoplankton taxa rely on nutrients supplied from below the euphotic zone. The observed increases in their abundances in the winter and fall suggests population dynamics of these taxa could be closely tied to seasonal variation in nutrient
delivery to the euphotic zone, including processes attributable to wind-driven mixing or reduction in light flux to the lower euphotic zone in the fall (Letelier et al. 2004). In contrast, the frequent increases in diatom \textit{rbcL} gene abundances during the warm summer months suggests nutrient demands of upper ocean diatoms may be closely linked to seasonal increases in N\textsubscript{2}-fixation (Dore et al. 2002, Church et al. 2009).

In addition to examining variability in euphotic zone phytoplankton diversity and \textit{rbcL} gene abundances, we also sought to examine the contributions of these phytoplankton groups to particulate export. Clone libraries built from sediment trap-derived materials revealed that although diatom phylotypes dominated euphotic zone clone libraries, these phylotypes were a relatively small component of the \textit{rbcL} gene flux. In contrast, pelagophyte \textit{rbcL} clones were heavily represented in the sediment trap clone libraries compared to the euphotic zone suspended particle samples, suggesting small phytoplankton taxa could play important role in particle export in the oligotrophic open ocean (Richardson and Jackson 2007). In the subtropical North Atlantic, Amacher et al. (2009) compared 18S rRNA gene clone libraries from suspended particles to clone libraries constructed from sinking particulate flux caught in sediment traps and found that diatoms were major contributors to suspended particulate material, while radiolarians and alveolates appeared to dominate sediment trap clones. Interestingly, our study in the subtropical North Pacific and the Amacher et al. (2009) study in the North Atlantic both suggest larger plankton taxa (e.g., diatoms) may not have higher contribution to particle flux compared to small plankton taxa (e.g., pelagophytes).
Although vertical fluxes of diatom \( rbcL \) genes in the upper ocean (150 m) did not display significant seasonality over the course of the two year observation period, we did observe a significant correlation between the downward flux of diatom \( rbcL \) genes and particulate carbon fluxes. Such results are consistent with previous studies documenting the importance of diatom blooms in fueling export when the upper ocean waters of the NPSG are warm and thermally stratified (Scharek et al. 1999a,b, Dore et al. 2008, Church et al. 2009). In contrast, \( rbcL \) gene fluxes of prymnesiophytes and pelagophytes were elevated in the spring and fall. The elevated gene fluxes in fall coincided with periods where \( rbcL \) gene abundances of these two groups were elevated in the lower euphotic zone. The elevated gene fluxes associated with prymnesiophytes and pelagophytes in April 2009, suggests biomass that accumulates in the lower euphotic zone through the winter undergoes sedimentation in the early phases of the spring. A previous study examining alkenone production and export at Station ALOHA (Prahl et al. 2005) observed pronounced export events in sediment traps at 2800 m in the early winter and summer during a two-year study. Two major genera of prymnesiophytes, \( Emiliania \) and \( Geophyrocapsa \), are recognized as important alkenone-producers (Conte et al. 1998, Cortés et al. 2001). The time lag between export events observed in the upper ocean and deep sediment traps could be explained by slow sedimentation rate of the cells, or reflect under collecting (in time) of the upper ocean traps of episodic export events.

In conclusion, this study utilized both molecular and pigment-based approaches to elucidate temporal dynamics in major groups of chromophytic algae in the NPSG. Our results indicate that diatoms were usually most abundant in the upper euphotic zone in
summer months and had higher contribution to the particle export in summer. Abundances of prymnesiophytes and pelagophytes were elevated in fall and winter months in the lower euphotic zone. Such results suggest their growth may have been mainly supported by nutrient supply from below the euphotic zone, with the major contribution of these groups to the particle export occurring in the early spring and fall. The significant temporal correlation between abundances of prymnesiophytes and pelagophytes in the lower euphotic zone and vertical export suggests these two major groups of eukaryotic phytoplankton could share physiological characteristics and play similar roles in carbon export in this ecosystem.

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Table 1. Oligonucleotide primers utilized for quantitative PCR analysis of selected form 1D rbcL genes.

<table>
<thead>
<tr>
<th>Targeted phylotypes</th>
<th>Forward primer (5’ – 3’)</th>
<th>Reverse primer (5’ – 3’)</th>
<th>QPCR efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diatoms</td>
<td>5’-GATGATGARAAYATTAACCT-3’</td>
<td>5’-GTAAACTDGCCADKNCATTTC-3’</td>
<td>85 - 92</td>
</tr>
<tr>
<td>Prymnesiophytes</td>
<td>5’-GGTTTCTACAACACTYTWYW-3’</td>
<td>5’-ATTTGDCCACAGTGDATACCA-3’</td>
<td>84 - 92</td>
</tr>
<tr>
<td>Pelagophytes</td>
<td>5’-CRACACWTTATTARAGACTAAG -3’</td>
<td>5’-ATTTGDCCACAGTGDATACCA-3’</td>
<td>85 - 95</td>
</tr>
</tbody>
</table>
Table 2. Pigment algorithms used to estimate contribution to total Chl a by selected groups of chromophytic phytoplankton.

Pigments abbreviations are: 19’-hexanoyloxyfucoxanthin (19-hex), 19’- butanoyloxyfucoxantin (19-but). P represents the 19-hex to 19-but ratio in prymnesiophytes and C represents the 19-hex to 19-but ratio in pelagophytes.

<table>
<thead>
<tr>
<th>Algal group</th>
<th>Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diatoms</td>
<td>$[\text{Chl } a]<em>{\text{diatom}} = 0.8 ([\text{fucoxanthin}] - 0.14 [19-\text{but}]</em>{\text{pel}})$</td>
</tr>
<tr>
<td>Prymnesiophytes</td>
<td>$[\text{Chl } a]<em>{\text{pry}} = 1.3 [19-\text{hex}]</em>{\text{pry}}$</td>
</tr>
</tbody>
</table>
| Pelagophytes | $[\text{Chl } a]_{\text{pel}} = 0.9 [19-\text{but}]_{\text{pel}}$

$[\text{hex}]_{\text{pry}} = (P / (P - C)) \ast ([19-\text{hex}]_{\text{total}} - [19-\text{but}]_{\text{total}} \ast C)$

$[\text{but}]_{\text{pel}} = (P / (P - C)) \ast ([19-\text{but}]_{\text{total}} - [19-\text{hex}]_{\text{total}} / P)$

$P = [19-\text{hex}]_{\text{pry}} / [19-\text{but}]_{\text{pry}} = 65.44$

$C = [19-\text{hex}]_{\text{pel}} / [19-\text{but}]_{\text{pel}} = 0.14$
Table 3. Seasonally averaged ± SD of total Chl \( a \) (T Chl \( a \)) stocks in the lower euphotic zone (75-125 m) and the derived contributions to total Chl \( a \) by prymnesiophytes (Chl \( a_{\text{pry}} \)), pelagophytes (Chl \( a_{\text{pel}} \)) and diatoms (Chl \( a_{\text{dia}} \)). Also depicted are the derived relative contributions (as % of total Chl \( a \)) by the selected algal taxa (Chl \( a_{\text{pry}} \) %, Chl \( a_{\text{pel}} \) % and Chl \( a_{\text{dia}} \) %) based on modified pigment algorithm of Letelier et al. (1993).

<table>
<thead>
<tr>
<th>Season (months)</th>
<th>T Chl ( a ) (µg m(^{-2}))</th>
<th>Chl ( a_{\text{pry}} ) (µg m(^{-2}))</th>
<th>Chl ( a_{\text{pry}} ) %</th>
<th>Chl ( a_{\text{pel}} ) (µg m(^{-2}))</th>
<th>Chl ( a_{\text{pel}} ) %</th>
<th>Chl ( a_{\text{dia}} ) (µg m(^{-2}))</th>
<th>Chl ( a_{\text{dia}} ) %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Winter (Jan - Feb, ( n = 4 ))</td>
<td>10564 ± 735</td>
<td>2425 ± 606</td>
<td>22.8 ± 4.2</td>
<td>997 ± 397</td>
<td>9.3 ± 3.1</td>
<td>210 ± 40</td>
<td>2.0 ± 0.3</td>
</tr>
<tr>
<td>Spring (Mar - May, ( n = 3 ))</td>
<td>11891 ± 2617</td>
<td>2458 ± 609</td>
<td>20.7 ± 3.2</td>
<td>843 ± 165</td>
<td>7.2 ± 1.3</td>
<td>187 ± 66</td>
<td>1.7 ± 1.0</td>
</tr>
<tr>
<td>Summer (Jun - Aug, ( n = 6 ))</td>
<td>12424 ± 1505</td>
<td>2556 ± 277</td>
<td>20.6 ± 1.0</td>
<td>963 ± 212</td>
<td>7.7 ± 1.4</td>
<td>158 ± 85</td>
<td>1.3 ± 0.8</td>
</tr>
<tr>
<td>Fall (Sep - Dec, ( n = 8 ))</td>
<td>13119 ± 922</td>
<td>2627 ± 159</td>
<td>20.1 ± 1.0</td>
<td>1297 ± 257</td>
<td>9.9 ± 2.1</td>
<td>155 ± 108</td>
<td>1.2 ± 0.9</td>
</tr>
</tbody>
</table>
Figure 1. Time-averaged concentrations of Chl a, N+N and rates of primary production (PP) in the upper ocean (0-150 m) at Station ALOHA. Dashed lines depict the range of 1% light level during the study period.
Figure 2. Contour plots of temperature (A), irradiance (B), N+N concentrations (C) in the upper 150 m at Station ALOHA during this study (October 2007 - December 2009).
Figure 3. Contour plots of concentrations of Chl a (A), fucoxanthin (B), 19’-hexanoyloxyfucoxanthin (C), and 19’-butanoyloxyfucoxanthin (D) in the upper 150 m at Station ALOHA during this study.
Figure 4. Neighbor-joining phylogenetic tree of form 1D rbcL gene sequences. Sequences retrieved the upper ocean (0-45 m), lower euphotic zone (75-150 m), and floating sediment traps (150 m) at Station ALOHA depicted by pyramids, upside down pyramids and squares, respectively. Numbers inside each symbol indicate the number of clones sequenced. Polygons represent grouping of rbcL sequences sharing > 85% identity. Numbers inside polygons indicate the total number of sequenced clones clustering among each group. Bootstrap values of > 50% are indicated at the tree nodes; form 1A rbcL gene sequence of Prochlorococcus marinus (Strain CCMP1375) utilized as the out-group.
Figure 5. Depth profiles of $rbcL$ gene abundances of diatoms (A), prymnesiophytes (B) and pelagophytes (C) at Station ALOHA. Also depicted are concentrations of fucoxanthin (D), 19'-hexanoyloxyfucoxanthin concentration (E) and 19'-
butanoyloxyfucoxanthin (F) at Station ALOHA. Open circles represent average summertime (June, July and August) gene abundances or pigment concentrations, and filled circles represent average values from fall and winter months (September - February), error bars indicate standard deviations of seasonal means.
**Figure 6.** Depth-integrated *rbcL* gene abundances at Station ALOHA during the study period. Gene abundances of diatoms (panels A and B), prymnesiophytes (panels C and D) and pelagophytes (panels E and F) in the upper euphotic zone (0-45 m) (panels A, C and E) and in the lower euphotic zone (75-125 m) (panels B, D and F) during the study period.
Figure 7. Temporal variability in particulate material fluxes at 150 m at Station ALOHA. Depicted are particulate carbon (A), diatom *rbcL* gene (B), prymnesiophyte *rbcL* gene (C) and pelagophyte *rbcL* gene (D) fluxes.
Chapter 4

Diatom dynamics in the oligotrophic North Pacific Subtropical Gyre

ABSTRACT

In low nutrient open ocean ecosystems, diatoms generally constitute minor components of plankton biomass, despite playing important roles in particle export and new production. To date, however, understanding of temporal and spatial variability in open ocean diatom population structure (diversity and abundances) remains limited, in part due to under sampling of these habitats. In this study, we characterized temporal and vertical dynamics in diatom population structure and the contribution of specific diatom genera to sinking particle fluxes at near-monthly time scales over a 2 year period (October 2007-December 2009) at Station ALOHA (22°45’ N, 158° W) in the North Pacific Subtropical Gyre (NPSG). Polymerase chain reaction (PCR) amplification, cloning and sequencing of diatom \textit{rbcL} genes provided insight into the phylogenetic structure of diatom populations in this ecosystem. Moreover, quantitative PCR amplification of \textit{rbcL} genes from five major diatom genera (\textit{Chaetoceros}, \textit{Pseudonitzschia}, \textit{Nitzschia}, \textit{Rhizosolenia} and \textit{Hemiaulus}) provided insight into time variability in diatom abundances. Diatoms belonging to the genera \textit{Pseudonitzschia} and \textit{Nitzschia} were often the most abundant of the \textit{rbcL} phylotypes examined. Gene abundances of diatoms belonging to the genera \textit{Hemiaulus} and \textit{Rhizosolenia} increased 11- and 8-fold, respectively during late spring and summer months compared to previous months. Additional analyses of \textit{nifH} gene abundances from heterocystous N\textsubscript{2} fixing...
cyanobacteria previously reported to occur as symbionts of diatoms belonging to the genera *Hemiaulus* and *Rhizosolenia* suggested that the increases in diatom *rbcL* gene abundances often coincided with periods when heterocystous diazotroph abundances were elevated. Additional analyses of diatom *rbcL* gene and heterocystous cyanobacterial *nifH* gene abundances from upper ocean (150 m) and deep sea (4000 m) sediment trap collections indicated that *Hemiaulus*, *Chaetoceros* and *Rhizosolenia* and their presumed diazotroph symbionts contributed to particulate matter export to meso- and bathypelagic waters. Our study provides new insight into temporal dynamics in diatom population structure and the contributions of specific diatom genera to vertical fluxes in the oligotrophic open ocean.
INTRODUCTION

Throughout much of the world’s oceans, the growth of diatoms couples the ocean’s carbon and silica cycles (e.g., Dugdale et al. 1995, Smith et al. 1996, Falkowski et al. 1998, Kemp et al. 2000). In large regions of the world’s oceans, diatoms generally comprise minor components of plankton biomass (Letelier et al. 1993, Scharek et al. 1999a); however, various lines of evidence suggest that episodic and seasonal-scale changes in diatom population dynamics play central roles in fueling net production and particulate matter export to the deep sea (Smetacek 1985, Dugdale et al. 1995, Nelson et al. 1997, Yool and Tyrrell 2003, Benitez-Nelson et al. 2007). For example, approximately monthly scale sampling of the oligotrophic North Pacific Subtropical Gyre (NPSG) by the Hawaii Ocean Time-series (HOT) program indicates that diatom blooms can be a regular features of the late summer (Dore et al. 2008). In some cases, these blooms are intense enough to be detected by ocean color satellite measurements of the near-surface ocean in this region (White et al. 2007, Wilson et al. 2008).

Prior studies on diatom diversity, abundance, and productivity in the NPSG suggest high spatial and temporal variability in these phytoplankton assemblages (Venrick 1982, 1988, 1990, 1997, 1999; Scharek et al. 1999a, b). Observations by Venrick (1982) indicated two vertically separated diatom assemblages, including several genera often found in the high-light, low-nutrient regions of the upper euphotic zone, and other genera most frequently dwelling in the low-light, nutrient-enriched waters of the lower euphotic zone. Venrick’s work (1988, 1990) revealed that the high-light, low-nutrient diatom assemblages included members of the genera *Nitzschia, Mastogloia* and
*Hemiaulus*, while the deeper low-light, high-nutrient assemblage included species of the genera *Thalassiosira, Pseudonitzschia, Nitzschia* and *Chaetoceros*. Abundances of some of the key species within these genera (e.g., *Hemiaulus hauckii, Mastogloia woodiana, Nitzschia bicapitata*) were reported on occasion to exceed 10³ cells L⁻¹ (Venrick 1982, 1988). Scharek et al. (1999a,b) investigated temporal variations in diatom abundance and export at Station ALOHA, finding low and seasonally invariant abundances of diatoms associated with the deep chlorophyll maximum layer (DCML) of the lower euphotic zone. However, these authors suggested that diatom growth in the well-lit, nutrient-depleted regions of the upper euphotic zone appeared to comprise a more important component of carbon export than diatoms growing in the DCML (Scharek et al. 1999a, b).

Superimposed on seasonal scale changes in diatom population dynamics, mesoscale and submesoscale physical processes also have been shown to influence diatom growth, abundance and community structure. Brzezinski et al. (1998) observed a diatom bloom dominated by *Hemiaulus hauckii* and *Mastogloia woodiana ca 200 km north of Station ALOHA; uplift of isopycnal surfaces during their sampling suggested the presence of a cyclonic eddy coincident with this bloom. Benitez-Nelson et al. (2007) examined a wind-forced cyclonic eddy in the lee of the Hawaiian Islands, observing a bloom of large diatoms (> 20 µm) with key species from the genera *Rhizosolenia* and *Chaetoceros*. At Station ALOHA, Fong et al. (2008) sampled a large phytoplankton bloom associated with an anticyclonic eddy that was dominated by several genera of diatoms (including *Hemiaulus* spp., *Chaetoceros* spp. and *Rhizosolenia* spp.), in addition to various N₂ fixing cyanobacteria.
Diatom-associated cyanobacterial symbioses appear to play a significant role in the ecology and biogeochemical dynamics associated with diatoms in oligotrophic waters of the subtropical gyres. Blooms of *Rhizosolenia* spp. containing endosymbiotic cyanobacteria *Richelia intracellularis* have been reported on several occasions in the NPSG (Mague et al. 1974, Venrick 1974, Dore et al. 2008, Fong et al. 2008). Similarly, in the subtropical North Atlantic, Carpenter and colleagues (1999) investigated an extensive bloom of *Hemiaulus hauckii* diatoms hosting *R. intracellularis* and estimated that the N supplied via the N$_2$ fixation symbioses exceeded nitrate flux from below the euphotic zone. Gómez et al. (2005) reported the occurrence of large numbers of the *Chaetoceros-Calothrix* consortia in the western Pacific Ocean. These three diatom-cyanobacteria symbioses (*H. hauckii* and *Richelia* spp., *Rhizosolenia* spp. and *Richelia* spp., *Chaetoceros* spp. and *Calothrix* spp.) have also been described at Station ALOHA. Foster and Zehr (2006) linked symbionts associated with *Rhizosolenia* spp., *Hemiaulus* spp. and *Chaetoceros* spp. to the three previously identified heterocystous nifH phylotypes reported from Station ALOHA: Het 1, Het 2 and Het 3, respectively (Church et al. 2005a,b). Although, these diatom-cyanobacteria symbioses appear to contribute significantly to biogeochemical dynamics in the NPSG, the temporal and vertical relationships between presumed symbiotic cyanobacteria and their diatom hosts has not been extensively investigated in this region.

The present study examined time-variance in upper ocean diatom abundances, together with evaluation of the types of diatoms associated with sinking particulate matter fluxes at Station ALOHA based on quantitative PCR amplification of diatom rbcL genes.
and microscopic enumeration of diatoms belonging to several genera. In addition, we examined temporal variability in \textit{nifH} gene abundances derived from presumed diatom-associated heterocystous cyanobacteria. Our results suggest that differences in the temporal dynamics of specific groups of diatoms could play an important role in regulating the exchange of material between the upper ocean and the deep sea.

MATERIALS AND METHODS

\textbf{Sampling, microscopy and sediment trap analyses}

Sampling for this study was conducted on near-monthly HOT cruises to Station ALOHA over a two year period (October 2007 to December 2009). Seawater samples were collected from 8 discrete depths in the upper ocean (5, 25, 45, 75, 100, 125, 150 and 175 m) using 12 liter polyvinyl chloride bottles attached to a conductivity-temperature-depth (CTD) rosette sampler. Ten liters of seawater was subsampled into polyethylene carboys and pressure filtered onto 47 mm diameter 2 µm porosity polycarbonate filters for subsequent extraction of planktonic DNA. These filters were preserved in a buffer containing 0.1M EDTA, pH 8.0; 1% sodium dodecyl sulfate and stored at -80°C.

Samples for subsequent enumeration of diatom cells by epifluorescence microscopy were collected from the same depths where DNA samples were collected. Seawater (12 liters) for microscopy samples was gravity filtered directly from the rosette sampling bottles onto 47 mm diameter 2 µm polycarbonate filters contained in inline filter holders. While still contained within the filter holders, filters were fixed with 2% paraformaldehyde, then mounted onto glass microscopy slides (50 x 75mm, GOLD
SEAL®) with Type FF immersion oil (Cargille Laboratories) and covered (Fisherbrand 45 x 50 mm cover slips). Slides were frozen at –20°C until cells were enumerated in the laboratory. Abundances of several major genera of diatoms (*Hemiaulus*, *Pseudonitzschia/Nitzschia, Chaetoceros, Rhizosolenia*) were enumerated by epifluorescence microscopy (Zeiss Observer Z1).

Samples for subsequent microscopic isolation of individual diatom cells or colonies were collected using a hand-towed 20 µm mesh-size plankton net towed for 15 minutes in the near-surface ocean. Contents of the net’s cod-end were placed in 500 ml polycarbonate bottles and diatoms were handpicked using an inverted microscope (Zeiss Observer Z1) at 100X or 400X magnification. Single diatom cells or chains of colonial diatoms cells were isolated using a glass-micropipette; cells were rinsed three times in 0.2 µm filtered seawater and transferred to 0.2 ml thin walled polypropylene tubes. Samples were preserved at -20°C for subsequent molecular analyses.

To identify diatoms associated with sinking particulate material, samples were collected from particle interceptor sediment traps (150 m) on each cruise to Station ALOHA. Sediment traps for subsequent extraction of DNA were filled with a 0.2 µm filtered sodium-chloride seawater brine solution (50 g NaCl l⁻¹ amended to surface seawater). Traps were deployed for ~2.5 days on a free-drifting, surface tethered array. Upon recovery of the trap array the trap solution was pressure filtered onto 47 mm diameter 2 µm porosity polycarbonate filters and frozen in the same buffer previously described.
In addition to samples collected using upper ocean particle interceptor sediment traps, we analyzed sediment trap samples collected from 4000 m over a full year (September 1993-September 1994) using McLane ParFlux (McLane Research, Model MK 7-21) bottom-moored sediment traps deployed at Station ALOHA. Samples were preserved in a saline (5 g NaCl L⁻¹), buffered (0.1% borate) formalin solution (3% final concentration), and stored at 4°C since the time of collection. Samples had been processed and split as described in Scharek et al. (1999b). For this study, 15 ml of each trap sample cup (totaling 21 cups) was centrifuged (3600 g) for 20 minutes; supernatants were filtered onto a 25 mm diameter 0.2 μm pore size polycarbonate filter (Millipore). This filter was then put into the 15 ml tube containing the pellet of the same sample and 1 ml of the EDTA-SDS buffer described above.

**DNA extraction, PCR amplification and sequence analyses**

DNA was extracted from the filters using a combined cetyltrimethylammonium bromide (CTAB)-chloroform method described by Zhang and Lin (2005). Briefly, after a 12 hour incubation at 55°C in a 200 μg ml⁻¹ proteinase K solution (0.5 ml), samples were mixed with 165 μl pre-warmed 10% CTAB and incubated for 10 min at 55°C. An equal volume of chloroform (99.8%, HPLC grade) was then added to this solution; samples were vortexed for 1 min and centrifuged at room temperature at 13000 x g for 10 min. The supernatants were purified and eluted using a Genomic DNA Clean & Concentrator kit (Zymo Research).

We utilized chloroplast *rbcL* genes to examine phylogenetic relationships among diatoms and as QPCR gene target to estimate abundances of diatoms in this ecosystem.
The *rbcL* gene encodes the large subunit of the Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) enzyme, the protein that catalyzes the initial step of carbon fixation in the Calvin-Benson cycle, and *rbcL* genes have been shown effective molecular markers for phylogenetically distinguishing relationships among diatoms (Mann et al. 2001, Evans et al. 2007). PCR-primers specific to form ID *rbcL* genes (forward primer, 5’-GATGATGARAAYATTAACTC-3’; reverse primer, 5’-ATTTGDCCACAGTGD ATACCA-3’, Paul et al. 2000) were used to amplify a 554-bp gene fragment from the DNA extracts obtained in this study. The PCR mix consisted of 2 µl plankton DNA extracts, 31 µl of nuclease free water, 5 µl of Ex Taq Buffer (TaKaRa), 4 µl of 2.5 mM dNTP mix, 4 µl of each 10 µM forward and reverse primers, and 1.5 U of ExTaq polymerase (TaKaRa). Total PCR reaction volumes were 50 µl. Thermal cycling conditions were: 3 min at 95°C, followed by 40 cycles of 1 min at 95°C, 1 min at 52°C, and 1 min 30 s at 72°C, with a final extension at 72°C for 15 min. The resulting PCR products were visualized on an ethidium bromide-stained 1.2% agarose gel. PCR amplicons were excised and purified using with the QIAquick Gel purification kit (Qiagen), and cloned using the pGEM-T Easy Vector system (Promega). Plasmids containing the PCR amplified gene fragments were sequenced with an ABI 3100 Gene Analyzer (Applied Biosystems) and sequences were edited and using BioEdit and imported into an aligned *rbcL* gene sequence database using ARB (Ludwig et al. 2004). Based on *rbcL* gene sequences obtained as part of this study together with *rbcL* sequences obtained from GenBank (NCBI), a new diatom-specific reverse PCR primer (5’-GTAAACTDGCCADKNCATTTC-3’) was designed, which when used together
with the forward primer described by Paul et al. (2000), amplified a 508-bp fragment of diatom \textit{rbcL} genes. DNA extracts were PCR-amplified with these diatom-specific primers following the same thermal cycling conditions previously described.

Diatom \textit{rbcL} genes were also amplified from hand-picked, isolated diatom cells or chain-forming diatom colonies. These handpicked diatom isolates were amplified using a nested PCR strategy, whereby 20 µl of nuclease free water was added to PCR tubes containing the diatom cell(s), and tubes were incubated at 100°C for 10 min. PCR reagents were added directly to each tube containing the handpicked, heat-treated cells. For the initial round of PCR, forward and reverse Form ID \textit{rbcL} gene primers described by Paul et al. (2000) were utilized. Following the initial round of PCR, 2 µl of the first-round PCR reaction was added to 50 µl of a second PCR reaction; these additional PCR reactions utilized the diatom-specific \textit{rbcL} gene primers designed as part of the present study. The resulting PCR products were cloned and sequenced as previously described. Sequences were imported into ARB and aligned to the previously described \textit{rbcL} gene database for subsequent phylogenetic analyses.

**QPCR amplification and primer specificity**

Abundances of diatom \textit{rbcL} genes from selected phylotypes were determined by QPCR. QPCR primers were designed to amplify \textit{rbcL} genes from 5 phylogenetically distinct groups of diatom sequence-types that were broadly represented in our clone libraries; these groups included diatoms from the following genera: \textit{Chaetoceros}, \textit{Pseudonitzschia}, \textit{Nitzschia}, \textit{Rhizosolenia} and \textit{Hemiaulus}. The QPCR assays largely focused on characterizing diatoms at the genus level, except for a single set of QPCR
primers designed to target sequences clustering among both *Pseudonitzschia* and *Nitzschia* genera (Table 1). QPCR reactions included: 12.5 µl 2x SyberGreen Master Mix (Applied Biosystems), 5.5 µl of nuclease free water, 2 µl each of 10 µM forward and reverse primers, 1 µl of 10 mg ml⁻¹ Bovine Serum Albumin (BioLabs), and 2 µl DNA extract. QPCR reaction conditions were: 94.0°C for 15 min; 40 cycles of 15 s at 94.0°C, 30 s at 48.0°C, 35 s at 72.0°C, followed by extension at 72.0°C for 7 min. Melt curves were run between 44.0-95.0°C with the resulting PCR products detected at 1.0°C intervals. Standards for the QPCR reactions consisted of serial 10-fold dilutions of plasmids containing PCR amplified *rbcL* genes from the targeted group of diatoms. QPCR reactions were conducted in duplicate for each environmental DNA sample and for each standard. Specificities of the QPCR primers were evaluated by comparing the amplification cycle threshold (Ct) between plasmids containing the intended target *rbcL* insert and non-target controls (plasmids containing non-target *rbcL* inserts; Table 2).

**Upper ocean light and biogeochemical measurements**

Incident solar irradiance (400-700 nm) was measured on each HOT cruise using a LI-COR LI-1000 data logger and cosine collector. Satellite-derived sea surface height anomalies (SSHA) in the vicinity of Station ALOHA were obtained from the Archiving, Validation, and Interpretation of Satellite Oceanographic (AVISO) data server (http://las.aviso.oceanobs.com/). High-sensitivity measurements of nitrate plus nitrite (N+N) were determined using the chemiluminescence method as described by Dore and Karl (1996). Particulate carbon and nitrogen was determined as described by Hebel and Karl (2001). Particulate silica (PSi) was collected onto 47 mm 0.8 µm polycarbonate
filters and analyzed colorimetrically after time-course carbonate digestion (DeMaster 1981, Scharek et al. 1999a).

RESULTS

Upper ocean biogeochemical characteristics

Daily integrated incident light flux at Station ALOHA (October 2007-December 2009) varied approximately two-fold during this study with elevated fluxes during the mid-summer months, decreasing into the fall and winter (Fig. 1A). In contrast, 0-125 m integrated N+N inventories were low in the late spring and summer months and elevated during the fall and winter months (Fig. 1A). The depth of the upper ocean mixed layer (based on the 0.125 kg m\(^{-3}\) change in potential density from the surface ocean, Levitus 1982) during this study varied between 16 and 111 m, with the upper ocean most stratified during spring and summer months (mixed layer depths between April and September averaged 40 ± 15 m) and deepening in fall and winter (averaging 82 ± 22 m between October and March) (Fig. 1B). Analyses of the SSHA record in the vicinity of Station ALOHA during the study period revealed 6 occasions where the SSHA deviated by more than one standard deviation (± 8.2 cm) from the long-term (1992-2010) mean for this region. Three of these 6 events were periods of strong positive SSHA (December 2007, August 2008 and August 2009) and 3 corresponded to periods of strong negative SSHA (April-May 2008, May 2009 and December 2009). During this study, depth-integrated upper ocean (0-45 m) inventories of PSi varied approximately 3-fold, with elevated concentrations during the summer months, while PSi inventories in the lower
regions of the euphotic zone (100-150 m) varied approximately 2-fold, with no clear seasonal dynamic (Fig. 2).

**Sequences derived from rbcL gene clone libraries**

In total, 324 partial rbcL gene clones were obtained from PCR amplified DNA extracts collected from discrete depths in the upper ocean (0-175 m), and upper ocean (150 m) and deep ocean (4000 m) sediment traps. Diatom rbcL gene sequences clustered with more than 14 known diatom genera (based on 85% sequence identity) (Fig. 3). There were 132 sequences retrieved from the water column samples and these sequences clustered with 12 diatom genera. From the 85 sequences retrieved from floating sediment traps collections the majority (67%) clustered with rbcL gene sequences from cultivated diatom strains belonging to the genera *Thalassiosira*, *Rhizosolenia* and *Pseudonitzschia* (Fig. 3). A total of 107 rbcL gene sequences were retrieved from clone libraries constructed from the bottom-moored sediment traps (4000 m); the majority of these sequences (56%) clustered among diatoms belonging to the genera: *Chaetoceros*, *Corethron*, *Pseudonitzschia* and *Cylindrotheca* (Fig. 3). In addition to sequences clustering among these diatom genera commonly found in the NPSG, we retrieved several sequences most similar based on BLAST analyses to rbcL genes from pennate diatoms belonging to the genera *Sellaphora* (diatoms in this genus were formerly classified as part of the genus *Navicula*) and centric diatoms belonging to the genus *Aulacoseira*. However, microscopic identification, isolation and PCR amplification of diatoms identified as *Hemiaulus membranaceus* and *Hemiaulus hauckii* collected in this study suggested these sequences derived from diatoms of the genus *Hemiaulus*. To date,
there are no publicly available *rbcL* gene sequences from cultivated diatoms belonging to this genus. In total, we retrieved 19 *rbcL* gene sequences (11 and 7 from the euphotic zone and bottom moored sediment traps, respectively) from our environmental DNA extracts that shared 88-99% identity to these microscopically isolated *Hemiaulus rbcL* gene clones.

Fourteen of the diatom *rbcL* gene clones clustered most closely (92-96% identity) with diatoms belonging to the genus *Pseudonitzschia*, while another 4 sequences clustered with the diatom genus *Nitzschia*. A single set of QPCR primers were designed to target diatom *rbcL* genes that clustered among *Pseudonitzschia* and *Nitzschia* (Table 1 and Fig. 3). In addition, QPCR primers were designed to target the 12 sequences that clustered most closely (90-92% identity) with diatoms belong to the genus *Chaetoceros*, with another set of QPCR primers designed to amplify the 10 *rbcL* clones most closely related (89-96% identity) to *rbcL* genes from diatoms belonging to the genus *Rhizosolenia* (Table 1 and Fig. 3). Notably, 9 of the 10 gene sequences clustering among *Rhizosolenia* were retrieved from DNA extracted from floating sediment trap samples. Finally, two sets of QPCR primers were designed to amplify distinct phylogenetic clusters of diatoms that grouped among hand-picked, microscopically identified diatoms of the genus *Hemiaulus* (these phylotypes were termed *Hemiaulus* 1 and 2).

**Vertical and temporal variability in diatom *rbcL* gene abundances**

QPCR based determinations of diatom *rbcL* gene abundances revealed unique temporal and vertical differences in the distributions of phylotypes examined as part of this study. In general, diatom *rbcL* abundances were elevated in the upper euphotic zone.
(0-45 m) and in the lower regions of the euphotic zone (125-150 m), with abundances often lower between 75 and 100 m (Table 3). As a result of these vertical distributions, \textit{rbcL} gene abundances were depth integrated to examine temporal variability of these phylotypes in the upper (0-45 m) and lower (100-150 m) euphotic zone. \textit{rbcL} gene inventories of all the diatom phylotypes in both regions of the euphotic zone fluctuated as much as 11-fold between the near-monthly samplings. Moreover, none of the phylotypes demonstrated significant seasonality in \textit{rbcL} gene abundances (one-way ANOVA, p > 0.05) in either the upper or lower euphotic zone.

Gene abundances derived from the \textit{Pseudonitzschia/Nitzschia} genera were often the most abundant of the phylotypes examined (Fig. 4). Depth-integrated (0-45 m) upper ocean \textit{rbcL} gene abundances of members of these genera typically ranged between $6 \times 10^8 - 2 \times 10^{10}$ gene copies m$^{-2}$ (Fig. 4A), decreasing to $2 \times 10^8 - 9 \times 10^9$ gene copies m$^{-2}$ in the lower euphotic zone (Fig. 4B). Peak abundances of the \textit{Pseudonitzschia/Nitzschia} genera occurred in October of 2007 and 2008 (Fig. 4, 5); however, abundances varied widely between the two years of this study (Fig. 4, 5) with depth-integrated (0-45 m and 100-150 m) \textit{rbcL} gene abundances 6- and 9-fold greater (on average) in 2008 than in 2009.

There was considerably temporal variability in gene abundances associated with organisms targeted by the \textit{Chaetoceros} QPCR primers, with inventories varying 57- and 102-fold in the upper (0-45 m) and lower (100-150 m) euphotic zone, respectively (Fig. 4, 5). \textit{rbcL} gene abundances from this group did not demonstrate obvious seasonality, with abundances in the upper euphotic zone (0-45 m) varying between $1 \times 10^8 - 9 \times 10^9$ gene
copies m$^{-2}$ (Fig. 4C), decreasing only slightly in the lower euphotic zone (ranging $6 \times 10^7$ – $6 \times 10^9$ gene copies m$^{-2}$). Chaetoceros gene abundances in both the upper and lower euphotic zone were greatest in October 2007 and February 2009.

Abundances of the Hemiaulus and Rhizosolenia phylotypes tended to be lower than the other phylotypes examined as part of this study; however, gene inventories of both these groups demonstrated sharp increases in the upper euphotic zone waters between May and July of 2008 and 2009, coinciding with periods of elevated PSI inventories (Fig. 4, 5). In contrast, in the lower euphotic zone (100-150 m), gene abundances of Hemiaulus and Rhizosolenia tended to be greater in the fall and winter months than in the summer months (Fig. 4, 5). Depth integrated (0-45 m) $rbcL$ abundances of the Hemiaulus phylotype varied between $1 \times 10^8$ – $6 \times 10^9$ genes m$^{-2}$, with peak abundances observed in June 2008 and July 2009 (Fig. 4E). Phylotypes whose $rbcL$ gene sequences were most closely related (92-99% identity) to gene sequences from hand-picked, microscopically identified isolates of Hemiaulus hauckii (termed Hemiaulus 2) were the dominant Hemiaulus phylotypes, particularly during the summer periods when $rbcL$ gene abundances of these phylotypes increased. Although, $rbcL$ genes associated with the Rhizosolenia phylotype were the least abundant of all the groups examined as part of this study, gene abundances of this group increased sharply in the upper euphotic zone in the late spring and summer of 2009 (Fig. 4G).

The resulting QPCR-derived diatom $rbcL$ gene abundances were compared to microscopy-based diatom cell counts on three cruises to Station ALOHA. This comparison revealed significant relationships between the QPCR derived diatom $rbcL$
gene abundances and microscopy-based cell counts of specific diatom genera (Fig. 6).

The resulting relationships indicated that on average the \( rbcL \) gene copies per cell for the \textit{Pseudonitzschia/Nitzschia, Hemiaulus (Hemiaulus 1 plus Hemiaulus 2), Chaetoceros and Rhizosolenia} phylogenotypes were \( 26 \pm 4, 273 \pm 22, 320 \pm 44 \) and \( 110 \pm 10 \), respectively.

Diatoms belonging to the genera \textit{Rhizosolenia, Hemiaulus and Chaetoceros} have been previously described in symbiotic associations with \( \text{N}_2 \) fixing cyanobacteria (Villareal 1991, Carpenter et al. 1999, Subramaniam et al. 2008). Based on \( nifH \) gene phylogeny, several studies have identified at least three phylogenetically distinct heterocyst-forming \( \text{N}_2 \) fixing cyanobacteria at Station ALOHA (Church et al. 2005, 2008; Foster and Zehr 2006); these groups, termed Het 1, Het 2 and Het 3, have been reported associated with the diatoms of the genera \textit{Rhizosolenia, Hemiaulus and Chaetoceros}, respectively (Foster and Zehr 2006). We examined temporal dynamics associated with the Het 1, Het 2 and Het 3 \( nifH \) phylotypes from the same samples used to amplify diatom \( rbcL \) genes. Gene abundances of these three \( nifH \) phylotypes tended to be greatest during the summer months (Fig. 7). Moreover, over the course of this study, we found a significant positive relationship between the 0-45 m depth integrated \textit{Hemiaulus rbcL} gene abundances and Het 2 \( nifH \) gene abundances (least squares linear regression, \( nifH \) gene abundance = 0.032 X \( rbcL \) gene abundance – 8 x 10\(^6\), \( r^2 = 0.62 \), \( p < 0.001 \)). Notably, during the summer of 2009, Het 2 \( nifH \) gene abundances in the upper euphotic zone (0-45 m) increased 10-fold (July 2009) coincident with 11-fold increases in \( rbcL \) gene abundances of \textit{Hemiaulus} phylotypes. In contrast, there were no significant relationships
observed between the Het 1 and Het 3 nifH phylotypes and the presumed symbiotic diatoms phylotypes.

**Vertical flux of diatom rbcL genes**

We also examined temporal variability in the export of diatom phylotypes associated with sinking particulate material caught in both upper ocean (150 m) and deep-sea (4000 m) sediment traps. Vertical fluxes of the total diatom rbcL genes retrieved from upper ocean (150 m) sediment trap collections varied 21-fold during the period of this study (3 x 10^6 – 6 x 10^7 gene copies m^-2 d^-1). Although there were no significant seasonal differences in the export of any of the diatom rbcL genes (one-way ANOVA, p > 0.05), rbcL gene fluxes of *Pseudonitzschia/Nitzschia* and *Rhizosolenia* phylotypes were often elevated in the summer (June, July and August) relative to other months (Fig 8). The resulting proportion of *Rhizosolenia* rbcL gene flux relative to euphotic zone standing stocks (0-150 m) ranged 0.8-2.7% d^-1 in the summer months. In comparison, the flux to standing stock ratio for the *Pseudonitzschia/Nitzscha* phylotypes ranged 0.1-0.5% d^-1, increasing ~8-fold in the summer months compared to other seasons. Although the *Pseudonitzschia/Nitzschia* phylotypes were often the most abundant phylotypes found throughout the euphotic zone, their contribution to rbcL gene fluxes out of the upper ocean were relatively low. In contrast, despite relatively low stand stocks, rbcL gene fluxes of the *Hemiaulus* phylotype generally dominated diatom rbcL gene sinking fluxes, with peak fluxes occurring in July 2008 and lowest in January 2009. On average, rbcL gene fluxes by the *Chaetoceros* phylotype were 4-fold higher in 2008 than their fluxes in 2009 (Fig. 8). Vertical fluxes of the Het 1, Het 2 and Het 3 nifH phylotypes were greatest
in the summer; ranging $5 \times 10^4 – 5 \times 10^6$, $2 \times 10^4 – 1 \times 10^6$ and $3 \times 10^5 – 9 \times 10^6$ gene copies m$^{-2}$ d$^{-1}$ for Het 1, 2 and 3 groups, respectively.

QPCR amplification of DNA extracted from particulate material collected from deep ocean (4000 m) bottom-moored sediment traps provided insight into temporal patterns associated with diatom export to the deep sea. Samples were analyzed from approximately a 1 year collection period (September 1993 – September 1994) where each trap cup sampling interval was ~17 days. The resulting $\textit{rbcL}$ gene abundances indicated summer maximum of diatom fluxes coincided with peak export of PSi and particulate organic carbon (POC) flux in the mid- to late summer months. Diatom $\textit{rbcL}$ gene fluxes to the deep sea were dominated by contributions from the \textit{Hemiaulus} and \textit{Chaetoceros} phylotypes, with the \textit{Rhizosolenia} phylotype comprising a much lower component of the gene flux (Fig. 9).

\section*{Discussion}

We examined temporal and vertical variability in diatom community structure over a two year period based on near-monthly sample collections from Station ALOHA in the oligotrophic North Pacific Ocean. Variability in the population structure of several major diatom groups was examined based on PCR and QPCR amplification of $\textit{rbcL}$ genes. Several studies have described the use of $\textit{rbcL}$ genes as suitable molecular markers for assessing phylogenetic relationships among chromophytic phytoplankton, including diatoms (Pichard et al. 1997, Paul et al. 1999, Wyman et al. 2000). Such molecular based approaches may help overcome potential misidentification of morphologically similar but
genetically distinct groups of diatoms (Geiser et al. 1998, Sarno et al. 2005, Lundholm et al. 2006). We found that QPCR-derived estimates of \( \text{rbcL} \) gene abundances were significantly correlated with microscopy-based determinations of the abundances of specific groups of diatoms, suggesting our analyses of \( \text{rbcL} \) gene variability served as a suitable proxy to examine temporal and vertical changes in diatom abundances in this ecosystem. Moreover, by combining microscopy and QPCR based approaches our results provided insight into the variability in \( \text{rbcL} \) gene copies per cell among the different diatom groups examined in this study. In general, the centric diatoms examined in this study (\textit{Rhizosolenia, Hemiaulus, Chaetoceros}) contained 5 to 13-fold higher \( \text{rbcL} \) gene copies per cell compared to the pennate diatoms (\textit{Pseudonitzschia} and \textit{Nitzschia}). Together with our results indicating relatively high abundances of \textit{Pseudonitzschia} and \textit{Nitzschia} \( \text{rbcL} \) genes, these results suggest that these genera were numerically dominant members of euphotic zone diatom assemblages during the study period. Such results are consistent with the previous studies on diatom population dynamics in the North Pacific Subtropical Gyre (Venrick 1982, 1990, 1997).

Our results also provide insight into temporal and vertical dynamics associated with diatom population structure in the oligotrophic ocean. Our study suggests that even in low nutrient, relatively physically stable habitats such as the NPSG, diatom populations are dynamic in time. For example, gene abundances of \textit{Hemiaulus} and \textit{Rhizosolenia}, two previously reported bloom-forming genera of diatoms in the NPSG, demonstrated sharp increases during the summer months when the upper ocean was warm, well-lit and depleted in nutrients. Moreover, these summertime periods of elevated
abundances of *Hemiaulus* and *Rhizosolenia* coincided with periods when symbiotic heterocystous cyanobacteria tended to be more abundant. A weak but significant correlation between *Hemiaulus* diatom *rbcL* gene abundances and *nifH* gene abundances derived from the presumed endosymbiotic N$_2$-fixing cyanobacteria *Richelia* spp. (Het 2 phylotype) known to associate with *Hemiaulus* (Foster and Zehr 2006) was observed in the upper ocean perhaps reflecting specificity in this host-symbiont relationship (Foster and Zehr 2006).

Together with previous studies on diatom dynamics in this ecosystem, our work suggests diatom population structure in the NPSG is dynamic in time, and that variability in diatom assemblage composition serves as an important control on fluxes of material to meso- and bathypelagic waters. Moreover, our results support previous studies that indicate temporal variability in diatom dynamics the well-lit, low nutrient regions of the euphotic zone differ from dynamics occurring in the dimly-lit but nutrient-enriched portion of the upper ocean (Scharek et al. 1999 a,b). We found that periods when diatom export from the upper ocean was greatest tended to occur coincident with or closely following periods when diatom abundances in the upper euphotic zone were elevated. Analyses of sediment trap samples collected from 4000 m revealed that diatoms belonging to upper ocean bloom forming genera *Hemiaulus* and *Rhizosolenia* were important contributors to summertime periods of elevated PSi and POC export to the deep sea. Moreover, *nifH* gene abundances derived from N$_2$ fixing heterocystous cyanobacteria often underwent sharp increases during the summer when *Hemiaulus* and *Rhizosolenia* diatoms were more abundant and PSi inventories were elevated. These
results, together with our analyses of temporal dynamics associated with sinking flux of diatoms and N\textsubscript{2} fixing cyanobacteria to the deep sea, support the hypothesis that introduction of nitrogen to the upper ocean via symbiotic N\textsubscript{2} fixing microorganisms forms an important component of diatom-driven new production in this ecosystem.

Convective supply of nitrate to the upper ocean of the NPSG is limited by persistent thermal stratification; however, relatively frequent mesoscale perturbations appear to supply event-scale injection of nitrate into the lower euphotic zone (Johnson et al. 2010). Such mesoscale forcing may form an important control on variability in phytoplankton productivity and carbon export in oligotrophic oceans, in part through supply of nitrate to the well-lit upper ocean (McGillicuddy et al. 1998, McNeil et al. 1999, Letelier et al. 2000, Sakamoto et al. 2004, Benitez-Nelson et al. 2007). However, recent studies in the NPSG suggest that mesoscale events can also favor the growth of N\textsubscript{2} fixing microorganisms (Fong et al. 2008, Church et al. 2009). N\textsubscript{2} fixation appears to fuel a major (36–69\%) fraction of new production in the NPSG (Karl et al. 1997, Dore et al. 2002), and thus understanding the influence of mesoscale perturbations on N\textsubscript{2} fixation could be critical to constraining rates of new production in this ecosystem. Church et al. (2009) suggested that elevated N\textsubscript{2} fixation rates were often associated with periods of positive SSHA, such as those accompanying anticyclonic eddies. In the present study, we observed that early summer blooms of *Hemiaulus* were often preceded by strong negative SSHA features, such as those accompanying the passage of cyclonic eddies through the ALOHA sampling region. The mechanism(s) coupling such physical forcing and plankton dynamics remains unknown (White et al. 2007, Church et al. 2009), but our
results provide additional evidence that mesoscale and submesoscale physical processes appear to play important roles in supporting diatom blooms in the oligotrophic open ocean. Isopycnal uplift, frontogenesis and nonlinear Ekman pumping associated with mesoscale and submesoscale dynamics have been suggested as potential physical processes that could introduce waters enriched in $\text{PO}_4^{3-}$ and $\text{Si(OH)}_4$ relative to nitrate to the upper ocean (Church et al. 2009, Calil and Richards 2010); such processes could thereby serve to stimulate the diatom-heterocystous cyanobacteria consortia in this ecosystem. Taken together, we suggest much of the observed dynamic in diatom population structure and diatom-driven new production may derive from variability in sources of fixed nitrogen to the upper ocean, with various genera of diatoms ($\text{Hemiaulus}$ and $\text{Rhizosolenia}$) utilizing nitrogen supplied via N$_2$ fixation, while other genera ($\text{Pseudonitzschia}$ and $\text{Nitzscha}$) relying to a greater extent on nitrate introduced to the upper ocean by event-scale mixing or upwelling events.

In addition to examining time-dependent changes in upper ocean diatom $\text{rbcL}$ and diazotroph $\text{nifH}$ gene abundances, we also examined the contributions of these microorganisms to particulate export. Although we observed no significant seasonality in the vertical fluxes of any of the diatom or N$_2$ fixing cyanobacteria phylotypes in the upper ocean (150 m), analyses of trap material collected from the deep sea (4000 m) suggested that the export of these microorganisms imparts a distinct seasonal signal on material fluxes in the deep sea. We observed elevated gene fluxes of all the diatom groups examined in deep sediment trap samples during summer months compared to other seasons, despite the same groups of diatoms not displaying clear seasonality in
upper ocean (150 m) sediment trap collections. The apparent discrepancy between the two sediment trap records could reflect sampling artifacts, whereby large export events have a shorter lifetime than the roughly monthly sampling resolution employed by the HOT shipboard program for the upper ocean sediment trap collections. Moreover, the time lag between when a bloom occurs and its subsequent export may be longer than the 2.5 days collecting period of the upper ocean sediment trap collections. Thus, the upper ocean traps could be under collecting (in time) episodic export events. In addition, there remains considerable uncertainty in the quantitative recovery of particles by both upper ocean and bottom-moored sediment trap collectors (Gardner 1980, 1985, Butman 1986, Buesseler 1991, Michaels et al. 1994, Siegel et al. 2008). Alternatively, the resulting discrepancies between the upper ocean and deep trap record might reflect seasonal variability in mesopelagic remineralization and silica dissolution, thereby imparting a seasonally variable signal on particle delivery to the deep ocean.

More than 10 years of bottom-moored (4000 m) sediment trap samples at Station ALOHA have revealed an annually recurring export of PSi during the summer (Dore et al. 2008). By examining time-dependent delivery of diatoms to 4000 m from a single year (1993-1994) of these same sediment trap collections, our results provide new insight into the dynamics underlying the seasonality in diatom export in this ecosystem. The temporal dynamics of diatom production and flux in higher latitudes is characterized by a major bloom and export event in spring (Sverdrup 1953). Kemp et al. (2000) suggested that sedimentation of diatom biomass accumulated during summer and triggered by the fall/winter mixing can be a major contributor to export production. Our results suggest
the diatom export in the NPSG follows or coincides with episodic increases in diatom biomass during the early to mid summer months. Assuming settling speeds of diatom aggregates of 100 to 150 m d\(^{-1}\) (Diercks and Asper 1997), our results suggest diatom abundances might be greatest approximately one month prior to the period of peak export during late June and July, consistent with the observed increases in *Hemiaulus* spp. in this study. Ocean color satellite data suggest that phytoplankton biomass often increases during the late summer in the NPSG (Wilson et al. 2008); however, our study suggests specific groups of diatoms may bloom during the early summer months at depths below that which satellite sensors detect (Villareal et al. 2011), and that blooms of specific groups of diatoms contribute significantly to the PSi and POC flux into the deep ocean.

REFERENCES


Scharek R, Latasa M, Karl DM, Bidigare RR (1999a) Temporal variations in diatom abundance and downward vertical flux in the oligotrophic North Pacific gyre. Deep-

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Table 1. Oligonucleotide primers utilized for PCR and QPCR analysis.

<table>
<thead>
<tr>
<th>Group name</th>
<th>Forward primer (5’ – 3’)</th>
<th>Reverse primer (5’ – 3’)</th>
<th>QPCR efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diatoms</td>
<td>5’-GATGATGARAAYATTAACTC-3’</td>
<td>5’-GTAAACTDGGCCADKNCATTTC-3’</td>
<td>-</td>
</tr>
<tr>
<td>Pseudonitzschia/Nitzschia</td>
<td>5’-GATGATGARAAYATTAACTC-3’</td>
<td>5’-GAACCTTTTACTTCKCC-3’</td>
<td>92</td>
</tr>
<tr>
<td>Hemiaulus 1</td>
<td>5’-GATGATGARAYATTAACTC-3’</td>
<td>5’-CCATRKTACCAGCAGTGATG-3’</td>
<td>95</td>
</tr>
<tr>
<td>Hemiaulus 2</td>
<td>5’-GATGATGARAYATTAACTC-3’</td>
<td>5’-CCATAGTTCTGCGGTGATG-3’</td>
<td>95</td>
</tr>
<tr>
<td>Chaetoceros</td>
<td>5’-GATGATGARAYATTAACTC-3’</td>
<td>5’-GCAGCAGTTACGTATAAG-3’</td>
<td>89</td>
</tr>
<tr>
<td>Rhizosolenia</td>
<td>5’-GATGATGARAYATTAACTC-3’</td>
<td>5’-GCTTTAGCCTATTACACCAGC-3’</td>
<td>94</td>
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</tbody>
</table>
Table 2. Specificity of oligonucleotide QPCR primers. Gene abundances: rbcL gene abundances added to each reaction and gene abundances detected by QPCR analysis; ns: no significant amplification after 40 QPCR cycles.

<table>
<thead>
<tr>
<th>Primer sets</th>
<th>Targets</th>
<th>Gene abundances (10^4 rbcL copies)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Added</td>
</tr>
<tr>
<td><strong>Pseudonitzschia /Nitzschia</strong></td>
<td>Pseudonitzschia clone</td>
<td>28.5</td>
</tr>
<tr>
<td></td>
<td>Nitzschia clone</td>
<td>17.2</td>
</tr>
<tr>
<td></td>
<td>Hemiaulus 1 clone</td>
<td>5.9</td>
</tr>
<tr>
<td></td>
<td>Hemiaulus 2 clone</td>
<td>30.3</td>
</tr>
<tr>
<td></td>
<td>Chaetoceros clone</td>
<td>19.6</td>
</tr>
<tr>
<td></td>
<td>Rhizosolenia clone</td>
<td>1.7</td>
</tr>
<tr>
<td><strong>Hemiaulus 1</strong></td>
<td>Hemiaulus 1 clone</td>
<td>5.9</td>
</tr>
<tr>
<td></td>
<td>Pseudonitzschia clone</td>
<td>28.5</td>
</tr>
<tr>
<td></td>
<td>Nitzschia clone</td>
<td>17.2</td>
</tr>
<tr>
<td></td>
<td>Hemiaulus 2 clone</td>
<td>30.3</td>
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<td>19.6</td>
</tr>
<tr>
<td></td>
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<td>1.7</td>
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<tr>
<td><strong>Hemiaulus 2</strong></td>
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<td>Pseudonitzschia clone</td>
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</tr>
<tr>
<td></td>
<td>Hemiaulus 1 clone</td>
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<td></td>
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<td></td>
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<td></td>
<td>Hemiaulus 2 clone</td>
<td>30.3</td>
</tr>
<tr>
<td></td>
<td>Chaetoceros clone</td>
<td>19.6</td>
</tr>
</tbody>
</table>
Table 3. Median euphotic zone (0-175 m) rbcL gene abundances of the specific groups of diatoms at Station ALOHA (October 2007 – December 2009). Range of rbcL gene abundances depicted in in parentheses.

<table>
<thead>
<tr>
<th>Depth (m)</th>
<th><em>Pseudonitzschia</em> /Nitzschia (copies L⁻¹)</th>
<th><em>Chaetoceros</em> (copies L⁻¹)</th>
<th><em>Hemiaulus</em> 1+2 (copies L⁻¹)</th>
<th><em>Rhizosolenia</em> (copies L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>6 x 10⁴ (5 x 10³ – 3 x 10⁵)</td>
<td>3 x 10⁴ (4 x 10³ – 9 x 10⁴)</td>
<td>3 x 10⁴ (8 x 10² – 1 x 10⁵)</td>
<td>1 x 10³ (1 x 10² – 6 x 10³)</td>
</tr>
<tr>
<td>25</td>
<td>6 x 10⁴ (5 x 10² – 4 x 10⁵)</td>
<td>6 x 10⁴ (3 x 10³ – 2 x 10⁵)</td>
<td>3 x 10⁴ (4 x 10³ – 1 x 10⁵)</td>
<td>1 x 10³ (3 x 10² – 2 x 10⁴)</td>
</tr>
<tr>
<td>45</td>
<td>5 x 10⁴ (3 x 10³ – 1 x 10⁶)</td>
<td>5 x 10⁴ (2 x 10³ – 3 x 10⁵)</td>
<td>2 x 10⁴ (5 x 10³ – 3 x 10⁵)</td>
<td>2 x 10³ (2 x 10² – 3 x 10⁴)</td>
</tr>
<tr>
<td>75</td>
<td>3 x 10⁴ (5 x 10² – 6 x 10⁵)</td>
<td>2 x 10⁴ (1 x 10² – 3 x 10⁵)</td>
<td>1 x 10³ (1 x 10² – 1 x 10⁵)</td>
<td>1 x 10³ (1 x 10² – 8 x 10³)</td>
</tr>
<tr>
<td>100</td>
<td>2 x 10⁴ (3 x 10³ – 2 x 10⁵)</td>
<td>2 x 10⁴ (1 x 10² – 2 x 10⁵)</td>
<td>2 x 10⁴ (4 x 10² – 6 x 10⁴)</td>
<td>9 x 10² (6 x 10 – 1 x 10⁴)</td>
</tr>
<tr>
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<td>----</td>
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<tr>
<td>125</td>
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<tr>
<td></td>
<td>$(5 \times 10^2 - 6 \times 10^5)$</td>
<td>$(1 \times 10^3 - 1 \times 10^5)$</td>
<td>$(4 \times 10^2 - 2 \times 10^5)$</td>
<td>$(2 \times 10^2 - 9 \times 10^3)$</td>
</tr>
<tr>
<td>150</td>
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<td>$1 \times 10^4$</td>
<td>$8 \times 10^2$</td>
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<td>$(2 \times 10^3 - 2 \times 10^5)$</td>
<td>$(4 \times 10^2 - 5 \times 10^4)$</td>
<td>$(2 \times 10^3 - 1 \times 10^5)$</td>
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<td>175</td>
<td>$5 \times 10^3$</td>
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<td>$9 \times 10^3$</td>
<td>$2 \times 10^2$</td>
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<tr>
<td></td>
<td>$(2 \times 10^2 - 2 \times 10^4)$</td>
<td>$(4 \times 10^2 - 5 \times 10^4)$</td>
<td>$(2 \times 10^2 - 2 \times 10^4)$</td>
<td>$(7 \times 10 - 4 \times 10^3)$</td>
</tr>
</tbody>
</table>
Figure 1. Temporal dynamics of sea surface irradiance, nitrate+nitrite (N+N), mixed layer depth (MLD) and sea surface height anomalies (SSHA) at Station ALOHA (October 2007 – December 2009). (A) Surface PAR (open circles) and 0-125 m integrated N+N concentrations (filled circles); (B) MLD (gray circles) and SSHA (short dashed line).
Figure 2. Temporal dynamics of upper ocean (0-45 m) (panels A, B) and lower euphotic zone (100-150 m) (panels C, D) particulate silica (PSi) inventories.
Figure 3. Neighbor-joining phylogenetic tree of PCR amplified rbcL gene sequences from the upper ocean (0-45 m open circle, 100-150 m filled circle), floating sediment traps (150 m, open square) and bottom-moored sediment traps (4000 m, filled square) at Station ALOHA. Numbers inside polygons indicate the total number of clones clustering among each group. Numbers in parentheses indicate the total number of sequenced clones sharing > 95% identity with the representative clones. Diatom groups targeted by QPCR are depicted in bold and in polygons (sharing > 85% identity). The trees in boxes include the sequences of these groups. Open triangles represent the sequences from hand-picked diatom cells. Trees were bootstrapped 1000 times, and bootstrap values of > 50% are indicated at the nodes. rbcL gene from Emiliania huxleyi (accession no. AB043631) was used as an outgroup.
Figure 4. Temporal dynamics of depth integrated $rbcL$ gene inventories of *Pseudonitzschia/Nitzschia* (panel A, B), *Chaetoceros* (panel C, D), *Hemiaulus* (panel E, F) and *Rhizosolenia* (panel G, H) phylotypes at Station ALOHA (October 2007 – December 2009). Open bars represent 0-45 m depth integrated $rbcL$ gene abundances (panel A, C, E, G); filled bars represent 100-150m depth integrated $rbcL$ gene abundances (panel B, D, F, H).
Figure 5. Temporal dynamics (October 2007-December 2009) in depth-integrated $rbcL$ gene abundances in the upper (0-45m) and lower euphotic zone (100-150m) of *Pseudonitzschia/Nitzschia* (panel A, B), *Chaetoceros* (panel C, D), *Hemiaulus* (panel E, F) and *Rhizosolenia* (panel G, H) phylotypes at Station ALOHA. Open circles represent 0-45 m depth integrated $rbcL$ gene abundances (panel A, C, E, G); filled circles represent 100-150m depth integrated $rbcL$ gene abundances (panel B, D, F, H).
Figure 6. QPCR-derived \textit{rbcL} gene abundances and diatom cell counts based on microscopic enumerations at Station ALOHA. Continuous line is the least-squares linear regression; open circles represent \textit{Rhizosolenia}, open triangles represent \textit{Hemiaulus}, open squares represent \textit{Chaetoceros}, and open diamonds represent \textit{Pseudonitzschia/nitzschia} abundances.
Figure 7. Temporal dynamics of 0-45 m depth integrated *nifH* gene abundances for selected heterocystous cyanobacteria at Station ALOHA (October 2007-December 2009). Gene abundances of Het 1 (panel A, B), Het 2 (panel C, D), and Het 3 *nifH* phylotypes (panel E, F).
Figure 8. Temporal variability in the vertical flux of diatom rbcL genes at 150 m at Station ALOHA (January 2008 to December 2009). (A) rbcL gene flux of *Pseudonitzschia/Nitzschia*, (B) rbcL gene flux of *Rhizosolenia*, (C) rbcL gene flux of *Hemiaulus* and (D) rbcL gene flux of *Chaetoceros* phylotypes.
Figure 9. Vertical fluxes of diatom \textit{rbcL} genes (A) and particulate silica (PSi) and particulate organic carbon (POC) (B) at 4000 m at Station ALOHA. Each symbol represents the mean flux over approximately 17 days.
Chapter 5

Conclusions

The goal of this dissertation was to investigate photosynthetic characteristics and population dynamics of eukaryotic phytoplankton assemblages in the NPSG. I have developed three projects (outlined in the proceeding chapters) to address this goal. In Chapter 2, size-dependent photosynthetic variability was evaluated at Station ALOHA. The results suggest that the larger phytoplankton (> 2 µm in size) are capable of more rapid carbon fixation than the abundant picophytoplankton. As such, nutrient delivery to the upper ocean (by either physical or biological processes), could permit larger phytoplankton to grow faster than the numerically dominant picoplankton, eventually resulting in expansion of larger phytoplankton biomass. Although the size-fractionated study provides potential insight into bloom dynamics in the oligotrophic open ocean, it remains unclear what processes drive the episodic shifts in upper ocean plankton dynamics or whether compositional shifts in the primary producer assemblage are accompanied by changes in photophysiology. Our understanding of the photophysiological variability is limited by the size-fractionation approach due to the great diversity of phytoplankton in each size-fraction (> 2 µm and 0.2-2 µm). Future studies could apply novel methods (e.g., flow cytometry, electro-microfluidics) to isolate specific groups of phytoplankton such as picoeukaryotes, pennate diatoms, unicellular diazotrophs and examine their photophysiological characteristics. In addition, further research is needed to measure the size-fractionated/group specific photosynthetically
usable radiation (PUR) in order to examine the variation of maximum quanta yield and to improve the modeling of primary production in this ecosystem.

My third and fourth chapters were focused on the temporal and vertical variability of eukaryotic phytoplankton community structure in the NPSG. \textit{rbcL} gene based QPCR assays were developed to investigate the abundances of prymnesiophytes, pelagophytes and diatoms in both the upper ocean and sediment traps. My results suggest that prymnesiophytes and pelagophytes rely on nutrients supplied from below the euphotic zone, while elevated diatom abundance in summer may be largely supported by symbiotic N$_2$-fixation in the upper euphotic zone. Although \textit{rbcL} gene abundances could serve as proxies to examine the temporal and vertical changes in phytoplankton abundances in the upper ocean, an uncertainty of this approach is that \textit{rbcL} genes may be detected within detritus or non-living particles, especially in the sediment trap samples. It is still unknown how much of the \textit{rbcL} genes retrieved from the sediment trap material derived from dead cells or how rapidly \textit{rbcL} genes are remineralized. Further study is necessary to assess these questions based on incubation experiments of sinking material. Moreover, culturing-based approaches could be utilized to examine the physiological characteristics of the key phytoplankton species in this ecosystem, including bloom-forming diatoms, to better understand the formation and demise of phytoplankton blooms in the NPSG. Future studies on the effects of top down control (grazing and viral attack) on phytoplankton dynamics in the NPSG would yield additional insights into findings emerging from this study.

This study indicates that long-term time-series observations are essential studies on the temporal and spatial dynamics of phytoplankton and their contribution to carbon
export. However, monthly cruises may not adequately resolve event-scale dynamics underlying phytoplankton blooms in the open sea. QPCR assays developed in this study could be integrated onto automated in situ biological sensing platforms such as the environmental sample processor (ESP) and provide a rapid and frequent means to examine the temporal dynamics of phytoplankton in this ecosystem. High frequency measurements of biogeochemical parameters via floats and gliders will also help us understand the mechanism driving the temporal variability of marine phytoplankton. The future of marine phytoplankton ecology will depend on methodological advances that enable us to isolate and culture key species, to study their cellular physiology and metabolic rates, to monitor real-time population dynamics and to model phytoplankton productivity via in situ and remote sensing.