AN ABSTRACT OF THE DISSERTATION OF

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Title: Phosphorus Physiology and Environmental Forcing of Oceanic Cyanobacteria, Primarily *Trichodesmium* spp.

Abstract approved:

Ricardo M. Letelier Yvette H. Spitz

The biological transformation of dinitrogen gas (N_2) into combined forms (termed N_2 fixation) by certain genera of oceanic cyanobacteria represents the largest incoming flux of nitrogen to the global ocean. As such, biological nitrogen fixation plays a significant role in the regulation of oceanic productivity and the export of carbon and nitrogen out of the sun-lit surface waters. Currently, our knowledge of the biogeochemical and ecological significance of N_2-fixing organisms is restricted by our relative inability to define mechanistically the relevant chemical, biological and physical controls of the production and abundance of biological N_2 fixation in the marine environment. The four chapters that form the main body of this dissertation touch upon specific aspects of the controls of the production and abundance of N_2-fixing organisms, particularly that of the cyanobacterial genus *Trichodesmium*. Chapter II defines the range of intracellular elemental composition exhibited by *Trichodesmium* and identifies phosphorus (P) as a key factor limiting N_2 fixation by populations of this organism residing in specific regions of the Atlantic and Pacific oceans. Chapter III explores vertical migration as a physiological adaptation relevant

to the growth of this same cyanobacterium, *Trichodesmium*. Results from this work indicate that vertical migration may allow a subset of the population to exploit the separation of light and nutrients under stratified oligotrophic conditions, thus potentially supplementing their P requirements for growth. Chapter IV examines time-series records in an attempt to define the physical characteristics of the environment that may regulate surface blooms of cyanobacteria. These analyses indicate that season, sea surface temperature and mixed layer depth are the most constrained predictors of blooms of N_2-fixing cyanobacteria in the North Pacific. Lastly, Chapter V identifies a novel region of the ocean inhabited by N_2-fixing organisms.

Phosphorus Physiology and Environmental Forcing of Oceanic Cyanobacteria, Primarily *Trichodesmium* spp.

by
Angelicque E. White

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APPROVED**:**

Co-Major Professor, representing Oceanography

Co-Major Professor, representing Oceanography

Dean of the College of Oceanic and Atmospheric Sciences

Dean of the Graduate School

I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Angelicque E. White, Author

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At times our own light goes out and is rekindled by a spark from another person. Each of us has cause to think with deep gratitude of those who have lighted the flame within us. – Albert Schweitzer

This dissertation represents a five-year exploration of aspects of the biology and ecology of N_2-fixing cyanobacteria. The genesis of this project was not solely my own, rather it arose from guided conversations with my formal and informal advisors, Ricardo Letelier, Yvette Spitz and Dave Karl. I am foremost thankful to each of these individuals, as they have significantly shaped my work. Ricardo has shared his passion for science with me, inspired me to grow as a scientist, taught me to question more and always encouraged me to follow my interests. Yvette has grounded me, kept me focused and taught me that I can stand on my own. Dave Karl has allowed me to participate in cruises in the Atlantic and Pacific Ocean, funded my travel to international and national conferences and provided helpful criticism and guidance from the beginning of my time at Oregon State. Dave has literally made the ocean accessible to me, and I am most grateful. I would also like to thank all of my co-authors: Ricardo Letelier, Yvette Spitz, Dave Karl, Fred Prahl and Brian Popp. Learning to do research and write as a team is likely one of the most important skills I have gained. Thanks also to Doug Capone and Ed Carpenter for allowing me time at sea and for valuable conversations. I am also appreciative of the time and effort that my committee members (Ricardo Letelier, Yvette Spitz, Barry Sherr and Jim Richman) and graduate representative (Bruce Dugger) have taken to carefully read and evaluate my work along the way. In the last few years, Fred Prahl has been especially helpful to me as both a mentor and a friend. I am appreciative of his willingness to share his knowledge and enthusiasm; he has made me a better scientist and a better person. I have also gained from numerous discussions and interactions with fellow students, particularly Sam Laney, Mike Wetz, Guido Corno and Amanda Whitmire. I also thank Amanda Ashe for all her help in the lab. Finally, I must acknowledge those that provide my life balance. I am blessed to be surrounded by friends and family that

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CONTRIBUTION OF AUTHORS

Ricardo M. Letelier and Yvette H. Spitz provided intellectual contributions and editing for material presented in Chapters 2-4. Ricardo M. Letelier also contributed to the design of Chapter 5. Fred Prahl and Brian Popp aided in the writing, data processing and data interpretation for Chapter 5. Each of the aforementioned is co-authors for the submitted or accepted manuscripts of these respective chapters.

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1 Introduction

"Before a single plant or animal appeared on the planet, bacteria invented all of life's essential chemical systems. They transformed the earth's atmosphere, developed a way to get energy from the sun, devised the first bioelectrical systems, invented sex and locomotion, worked out the genetic machinery and learned how to merge and organize into new and higher collectives. These are ancestors to be proud of."

-Mahlon Hoagland in "The Way Life Works"

"After a few days… my attention was called to a reddish-brown appearance of the sea. The whole surface of the water, as it appeared under a weak lens, seemed as if covered by chopped bits of hay, with their ends jagged. Mr. Berkley informed me that they are the same species (*Trichodesmium erythraeum*) with that found over large spaces in the Red Sea and whence its name of Red Sea is derived. Their numbers must be infinite: the ship passed through several bands of them one of which was ten yards wide and judging from the mud-like color of the water at least two and a half miles long."

-Charles Darwin in "The Voyage of the Beagle"

1.1 Cyanobacteria

Taxa of the domain *Bacteria*, phylum Cyanobacteria are photoautotrophic prokaryotes. Formerly known as blue-green algae because of the color imparted to them by accessory pigment complexes called phycobiloproteins, cyanobacteria are an ancient and diverse group of organisms. Over 2000 species of free-living and symbiotic cyanobacteria of unicellular, colonial and filamentous morphologies have been recorded (Van Der Hoek et al. 1995) occupying the full habitat spectrum from marine to freshwater to terrestrial to the extreme biotopes of glaciers and hot springs. In the open ocean provinces far removed from land (oceanic gyres), the tremendous importance of cyanobacteria has just recently been realized.

Within the last 30 years, researchers have determined that cyanobacteria are dominant players in the cycling of carbon (C) and nitrogen (N) in ocean gyres. By virtue of the sheer magnitude of their numbers [10^4-10^5 cells/ml, (Campbell and Vaulot 1993)], the picoplanktonic cyanobacteria *Prochlorococcus* and *Synechococcus* are now known to be responsible for a significant fraction of marine carbon fixation (Ting et al. 2002). Our understanding of the marine N cycle has also been changed

dramatically in light of the discovery of previously unknown N_2- fixing cyanobacteria (Zehr et al. 2001) and numerous revisions of the growth and nitrogen fixation rates of long known taxa (Karl et al. 2002). The role that certain cyanobacteria, particularly the genera *Trichodesmium*, play in the cycling of elements in oceanic gyres is a central focus of this dissertation.

Trichodesmium are phototrophic, dinitrogen (N_2) fixing (diazotrophs) cyanobacteria, that are geographically resident in the oligotrophic tropical and subtropical regions of the global ocean (Capone 2001; Karl et al. 2002). In these open ocean habitats, the ambient concentrations of reactive N (N_R) are low (typically 100's of nmol L^{-1}). Hence, the capacity to utilize atmospheric N allows diazotrophs such as *Trichodesmium* to fulfill their nitrogen requirements for growth and subsequently contribute new nitrogen to the system via the release of NH_4^+ and dissolved organic matter rich in nitrogen (DON) (Mulholland and Capone 2001). More than just a conduit for inputs of reactive N (N_R), *Trichodesmium* spp. also form large surface blooms, thereby transiently dominating primary productivity and N cycling (Bowman and Lancaster 1965; Capone et al. 1998; Karl et al. 1992). A mechanistic understanding of what controls the growth and abundance of N_2-fixing cyanobacteria such as *Trichodesmium* would further our ability to model the cycling of elements in the marine system.

1.2 The marine N cycle: the role of N_2-fixing cyanobacteria

Nitrogen is an essential nutritional requirement for life, hence the biological availability of this element can limit organic productivity in the marine environment (Zehr and Ward 2002). Nitrogen is delivered to oceans via riverine inputs, atmospheric deposition and biological nitrogen fixation and "lost" or removed via denitrification, the flux of particulate organic matter to the ocean sediments, and nitrous oxide (N_2O) emissions (Galloway et al. 2004). Nitrogen fixation is the biological reduction of atmospheric N_2 to ammonium (NH_4^+) and dissolved organic N

(DON) while denitrification is the reduction of oxidized N species (NO_3^-, NO_2^-) to nitrous oxide (N_2O) and N_2. Figure 1-1 presents the most recent global ocean estimates for each of these N source and sink terms in addition to the primary microbial transformations of the marine N cycle. From the relative magnitude of each flux, it is apparent that the total pool of N in the ocean is principally determined by the balance of N losses via microbial denitrification and N additions from biological nitrogen fixation. Thus, the relative contribution of each of these biologically-mediated processes is a primary control of marine primary productivity.

Currently, estimates of pelagic marine nitrogen fixation are in the range of 87 to 141 Tg N yr^{-1} where the lower value is determined by extrapolation of direct N_2 fixation measurements and the upper value is based upon indirect geochemical estimates. Direct biological rates are typically derived from average daily rates of N_2 fixation by cyanobacteria of the genus *Trichodesmium* (Galloway et al. 2004). These rates are scaled up from point measurements to cover the typical geographical distribution of this organism, that is warm (>25ºC) oligotrophic surface waters (Capone et al. 1997). Geochemical estimates of net N_2 fixation provide another means of accounting for N_2 fixation. On average, particulate matter in the ocean is remineralized by heterotrophic bacteria in fixed elemental proportions of 106 C: 16 N: 1 phosphorus (P). This stoichiometry is classically referred to as the Redfield ratio (Redfield 1958). On a regional or basin scale, deviations in the concentration of nitrate in a water mass relative to the canonical N:P ratio of 16:1, termed N* and calculated as some variant of $NO_3^- - 16PO_4^+$, are used to estimate the net inputs or deficits of nitrate occurring as a result of nitrogen fixation or denitrification respectively. Galloway et al. (2004) have compiled the regional and global N* analyses of Michaels et al. (1996), Gruber and Sarmiento (1997) and others to estimate annual pelagic N_2 fixation rates of 141 Tg N. Benthic nitrogen fixation is estimated to add another 15 Tg N per annum for an upper estimate of oceanic N_2 fixation equivalent to 156 Tg N yr^{-1}.

Comparison of the biological and geochemical estimates of N_2 fixation to the range of estimates for global denitrification (147 to 454 Tg N yr^{-1}), again a difficult term to constrain, indicates that the present day ocean is either near steady state or

alternatively, in a period of net N loss. This dichotomy is largely a consequence of the different time and space scales implicit in each means of estimating global N_2 fixation (Bates and Hansell 2004). Biologically derived estimates represent local rates over periods of days, while geochemically derived estimates typically integrate on the basin scale over considerably longer time periods (Bates and Hansell 2004). Despite these methodological discrepancies, a reconciliation of these independent estimates may be on the horizon. Recent measurements of the abundance of *Trichodesmium* have been revised upwards (Capone 2001; Davis and McGillicuddy Jr. 2006) and novel unicellular cyanobacteria have been discovered to be active N_2 fixers in the pelagic environment (Zehr and Ward 2002). In effect, these recent findings indicate that the global estimates of N_2 fixation are likely toward the upper range of previous estimates. Additional knowledge of the controls of the abundance and growth of N_2-fixing cyanobacteria and denitrifying bacteria in the marine system would help determine the present and future balance of the marine N budget.

1.3 Potential controls of oceanic N_2 fixation

The role of diazotrophs in bioelemental cycling has been the topic of considerable oceanographic research for nearly five decades now (Redfield 1958; Sohm and Capone 2006) yet we still do not completely understand the controls of the abundance and growth of N_2 fixers. Recent revisions of the global marine N cycle (Codispoti 2006; Galloway et al. 2004) stress the necessity of this research in order to establish the present day range of the largest incoming flux of new N to the global ocean, that is diazotrophy. Both phosphorus (P) and iron as well as physical forcings such as sea temperature and wind induced vertical mixing have been suggested to limit the rate of nitrogen fixation in oligotrophic systems (Hood et al. 2004; Sañudo-Wilhelmy et al. 2001).

In terms of nutritional controls, a significant effort has been directed toward understanding the source and requirements of iron (Kustka et al. 2003; Tovar-Sanchez et al. 2006) while *Trichodesmium* P nutrition has just recently begun to be explored

(Fu et al. 2005; Sohm and Capone 2006). In this dissertation, I identify two specific physiological adaptations unique to *Trichodesmium* that may regulate the potential magnitude of production driven by this key bloom-forming genus under the low P conditions indicative of the oligotrophic North Pacific subtropical gyre (NPSG). First, *Trichodesmium* spp. exhibit the physiological capacity to modulate internal P quotas such that this genus can maintain active growth with reduced P quotas and is able to consume P luxuriously, i.e. in greater quantities than necessary for growth. Such physiological plasticity must arise from the internal regulation of quantitatively important P-rich biopolymers and as such it is likely to impact the net productivity of this organism and affect the dynamics and availability of C, N, and P to the remaining pelagic community. Secondly, *Trichodesmium* spp. have the capacity to alter their buoyancy and thus change their vertical water column position via carbohydrate ballasting. If these buoyancy driven vertical migrations allow a migrating colony to reach the depths of the phosphocline, it may facilitate the biological enhancement of elemental fluxes into the euphotic zone via *Trichodesmium*, a scenario termed P-mining (Karl et al. 1997). The research described in Chapters II and III will address these respective physiological adaptations, their potential impacts on elemental cycling and the ecological implications of such. The outcome of this research should help determine the means by which *Trichodesmium* spp. acquire sufficient P to support growth in their native oligotrophic habitat.

Environmental conditions have also been hypothesized to control the abundance and distributions of N_2-fixing organisms (Karl et al. 2002; Zehr and Ward 2002). Specifically, it is oft noted that blooms of *Trichodesmium* (and/or *Richelia*) are confined to calm, well-stratified, warm (typically ≥ ~25° C) surface waters (Capone 2001; Letelier and Karl 1998). The physiological underpinning by which these conditions may favor *Trichodesmium* growth are not well constrained for field populations nor has it rigorously been shown that blooms are in fact restricted to waters with such conditions. In point, a recent paper by Davis and McGillicuddy (2006) reported that the abundance of *Trichodesmium* is not significantly different in the wake of hurricane strength winds than that observed in relatively calm conditions

within the same region. This finding contradicts the notion that wind forcing would have a direct control on *Trichodesmium* growth and abundance. Nonetheless, if relationships between environmental conditions, particularly those that could be retrieved from space (e.g. sea surface temperature, wind speed, sea surface height, surface fluxes of irradiance) could be determined, we would be able to improve our ability to quantify the spatial and temporal distribution of key bloom forming N_2 fixers (*Trichodesmium* and/or *Richelia*). In Chapter IV, I have examined time-series of environmental forcing derived from vessel-based program, mooring data and remote sensing in an effort to define relationships between SST, wind speed, mixed layer depth, sea surface height, integrated irradiance and observed blooms of large cell sized cyanobacteria in the NPSG.

Demarcation of the seasonal and regional distribution of N_2 fixers and the coincident environmental conditions can help to refine our global estimates of nitrogen fixation. Along these lines, a growing body of work suggests that unlike previously thought, N_2 fixation occurs in portions of the surface ocean proximate to denitrification zones (Brandes et al. 1998; Capone et al. 1998; Sigman et al. 2005; Westberry and Siegel 2006). Given that nitrogen fixation is favored by a low N:P ratio (Karl et al. 2002), upwelling of waters depleted in N via denitrification, if followed by stratification and Redfield-type nutrient drawdown, can prime surface waters for nitrogen fixation and thus lead to potential feedbacks for export production (Sigman et al. 2005). In this manner, geographical coupling of N_2 fixation and denitrification would be expected to provide strong counterbalancing feedbacks. As a result of a cruise of opportunity, we have identified the Gulf of California as a region where denitrification and nitrogen fixation are spatially coupled. In Chapter V, we report the first records of N_2 fixation in the central gyres of the Gulf of California. In order to expand these local findings, we have analyzed satellite records of SST and SSH so as to describe the environmental conditions coincident with presumed blooms of N_2-fixing organisms. Again, the aim of this research was to better define the spatial and temporal distribution and controls of N_2 fixation.

1.4 Dissertation aims

In sum, the aim of this dissertation is to contribute to the existing body of knowledge regarding the environmental and biochemical controls of growth and abundance of large cell-sized cyanobacteria, primarily of the genus *Trichodesmium*. This contribution should move the field towards a more accurate budget of the marine N cycle. Chapter II focuses on physiological adaptations of *Trichodesmium* that may generate deviations in the elemental composition of this organism and thus regulate the potential magnitude of primary production. Chapter III describes a numerical model of vertical migration by *Trichodesmium* that can allow for better simulations of the vertical distribution of *Trichodesmium* biomass and thus improve numerical representations of *Trichodesmium* productivity. Chapter IV combines remote sensing of ocean color and environmental forcing with mooring and vessel-based time series in an attempt to define the environmental determinants of large cell sized cyanobacteria blooms in the surface waters of the North Pacific Ocean. Chapter V reports the first recorded measurements of N_2 fixation in the Gulf of California and speculates on the potential controls of diazotrophy in this region.

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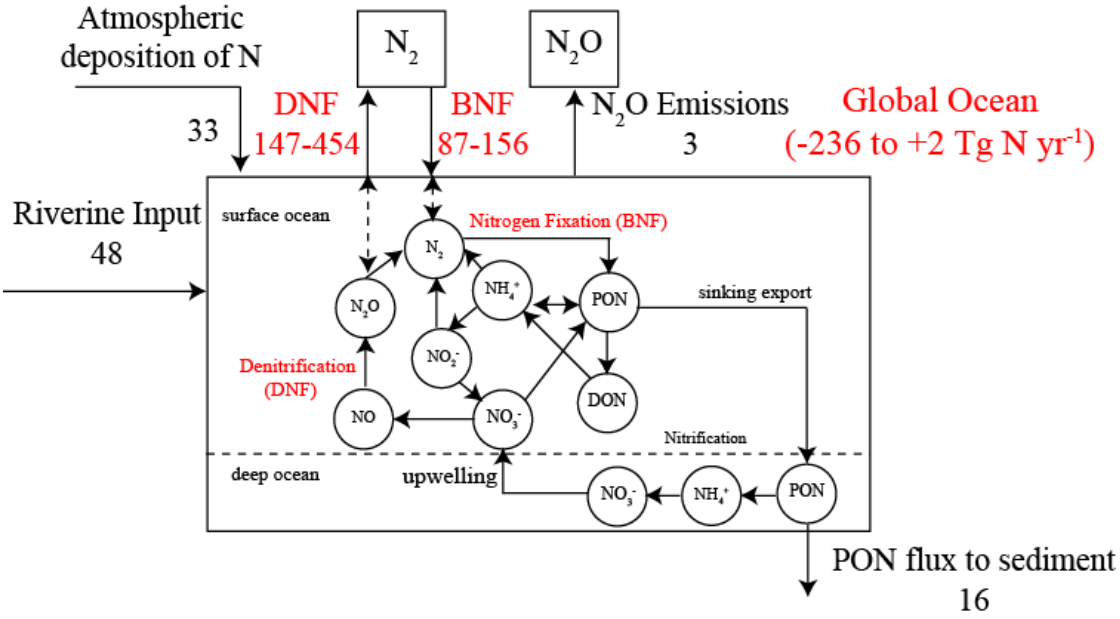

Figure 1-1. Source and sink terms for the global marine N cycle in units Tg N yr^{-1}, derived from Galloway et al. (2004). Denitrification (DNF) and biological nitrogen fixation (BNF) are the largest flux terms in this budget. The primary internal transformations of N within the global ocean are also shown.

Flexible elemental stoichiometry in *Trichodesmium* spp. and its ecological implications

Angelicque E. White, Yvette H. Spitz, David M. Karl and Ricardo M. Letelier

Limnology and Oceanography
343 Lady MacDonald Crescent
Canmore, Alberta T1W 1H5 Canada
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2 Flexible elemental stoichiometry in *Trichodesmium* spp. and its ecological implications

2.1 Abstract

We conducted laboratory experiments to assess the bioelemental plasticity of cultures of *Trichodesmium* IMS101 under phosphorus (P)-replete, P-restricted, and light-limited conditions. The results reveal a high degree of stoichiometric flexibility. Specifically, *Trichodesmium* IMS101 is capable of growth with C: N: P ratios of $C_{585\pm56}$: $N_{90\pm10}$: P_1, approximately six times higher than would be predicted by the Redfield reference ratio (C_{106}: N_{16}: P_1), thus signifying low cellular P quotas relative to carbon (C) and nitrogen (N). Luxury consumption of P occurs rapidly after periods of prolonged P restriction, under both light and dark conditions, resulting in substantial increases in P quotas and reductions of C: N: P ratios ($C_{96\pm8}$: $N_{16\pm1}$: P_1). Comparison of laboratory culture data to our field observations from the Northwest Atlantic and the North Pacific indicate that, while natural populations of *Trichodesmium* exhibit persistently low P content relative to C and N ($C_{290\pm15}N_{53\pm3}P_1$), the highest and lowest C : P and N : P ratios recorded in the laboratory are rarely observed in nature. We have also performed laboratory experiments intended to simulate the energetic and nutritional extremes that would occur as naturally migrating populations of *Trichodesmium* sink out of the euphotic zone into P-rich regions of the upper disphotic zone. The temporal duration of dark survival for this isolate is on the order of 3–6 d, after which time cells are unable to recover from light deprivation. This finding provides a constraint on the temporal scale of vertical migration.

2.2 Introduction

Species of the colony-forming marine cyanobacterial genus *Trichodesmium* have been well described as prominent dinitrogen (N_2) fixing organisms (diazotrophs) in the oligotrophic tropical and subtropical regions of the global ocean (Capone, 2001; Mulholland and Capone, 2001; Karl et al. 2002). The notoriety of this genus has only increased in recent years as revisions of abundance estimates and N_2 fixation rates have emphasized *Trichodesmium* as a significant source of new nitrogen to otherwise nitrogen (N) deficient ecosystems (LaRoche and Breitbarth, 2005). More than just a delivery vehicle for reactive N (N_R), *Trichodesmium* spp. are also noted for their ability to episodically form large surface accumulations, thereby transiently dominating primary productivity and N cycling (Bowman and Lancaster, 1965; Karl et al. 1992; Capone et al. 1998).

Trichodesmium is ecologically significant in oligotrophic oceanic regimes such as the North Atlantic and North Pacific gyres, where the process of biological N_2 fixation has been documented to enhance seasonally the net transport of carbon and nitrogen out of the euphotic zone (Karl et al. 1997; Capone et al. 2005). Given that *Trichodesmium* based productivity is by nature stochastic, a composite understanding of the role of this genus in elemental cycling will require characterization of physiological variability. Specifically, if we can define the full range of physiological and stoichiometric flexibility and begin to understand the underlying functionality driving deviations from average elemental composition, we will improve our capability to predict potential responses of oligotrophic marine ecosystems to the stresses of a changing environment.

The assumption of any fixed elemental stoichiometry for marine biota, e.g., the Redfield reference ratio (Redfield 1958), implies that the internal chemical content of cells is strictly regulated. From this perspective, departures from static elemental ratios require that organisms have the capacity to modulate the relative proportions or the governing transformation rates of intracellular pools of biomolecules. In the North Pacific Subtropical Gyre (NPSG), a fundamental characteristic of increased *Trichodesmium* productivity is a systematic alteration of the particulate and dissolved elemental pools relative to the canonical Redfield ratios ($C_{106:}N_{16:}P_1$) occurring as a

primary result of the excess production of N_R through N_2 fixation (Karl et al. 1997, Dore et al. 2002). Particularly during bloom periods the stoichiometric fluctuations of *Trichodesmium* spp. can be striking; for example, particulate matter collected from surface waters during an August 1989 *Trichodesmium* bloom in the NPSG had an average elemental composition of C_{891}:N_{125}:P_1 (Karl et al. 1992). These ratios indicate intense, community level P-limitation relative to either C or N.

Phosphorus regulation of diazotrophic production, however, is not particularly well understood. Specifically, while it is well known that cyanobacteria can grow with reduced cellular quotas of P (Karl et al. 2002; Bertilsson et al. 2003; Heldal et al. 2003) the minimal nutritional requirements needed to maintain active growth have not been constrained for *Trichodesmium* spp. Moreover, as both natural and cultured populations of *Trichodesmium* spp. have been shown to have a relatively low affinity for inorganic P ($k_P \cong$ 50-200 nmol P L^{-1} for Pacific populations, K. Björkman, pers. comm.; 400 nmol P L^{-1} for *Trichodesmium* IMS101, Fu et al. 2005), it appears that this genus is a poor competitor for DIP in their native oligotrophic habitats. Conversely, uncoupling of growth and P-uptake rates in cultured populations suggests a capacity for the consumption of P in excess of metabolic requirements for growth (Fu et al. 2005). Given that *Trichodesmium* spp. can constitute a considerable fraction of total biomass in oligotrophic systems and can function as a significant year round source of N_R for the pelagic community, we need to define the bounds of elemental composition and the physiological changes that drive these changes in *Trichodesmium* spp. in order to better understand the extent to which this key oceanic diazotroph regulates the flow of elements within the ecosystem. The goal of the research presented below is to characterize the stoichiometric plasticity exhibited by cultured populations of *Trichodesmium* IMS101 in response to environmental fluctuations in energy and nutrient (P) availability in an experimental context. Empirical results are compared to data from in situ sampling of NW Atlantic and North Pacific *Trichodesmium* populations. This research focuses primarily on the physiological processes characteristic of *Trichodesmium* spp. that may generate significant

deviations from mean stoichiometry, i.e., P-sparing, luxurious P uptake and vertical migration.

2.3 Methods

2.3.1 Laboratory culture maintenance

Trichodesmium IMS101 was provided by Dr. Margaret Mulholland (Old Dominion University, ODU) in April of 2002 and has since been maintained at Oregon State University. This strain was originally isolated from coastal Atlantic waters (Prufert-Bebout et al. 1993) and has since been identified as *T. erythraeum* (Janson et al. 1999). Over the course of our experiments, all cultures were grown in batch mode, in order to simulate the rapid growth and decline associated with natural *Trichodesmium* blooms. Cultured populations were maintained on an artificial seawater medium (YBCII) lacking combined nitrogen (N) sources as described by Chen et al. (1996). The typical initial dissolved inorganic phosphorus (DIP) concentration of this media is 50 μmol L^{-1}. Thus, this laboratory strain has long been adapted to DIP concentrations approximately 4-5 orders of magnitude higher than those observed by natural oligotrophic populations. In order to acclimate laboratory cultures to lower DIP conditions, batch cultures were grown in YBCII media with an initial DIP concentration of 0.5 μmol L^{-1}, for three successive 14-day transfer periods. This initial DIP concentration was chosen as a result of preliminary experiments showing 0.5 μmol L^{-1} to be the lowest DIP level capable of sustaining extended batch culture growth. Over the entire course of the experiment, cultures were maintained in a recirculating 24^{o}C water bath under computer-controlled cool white fluorescent lights set on a 12/12 sinusoidal light-dark cycle with a noon maximum (E_{MAX}) of 500 μmol quanta m^2 s^{-1}, sufficiently higher than the typical half-saturation irradiance (E_K) for photosynthesis (300 μmol quanta m^{-2} s^{-1}, LaRoche and Breitbarth, 2005). On three-hour intervals during the light cycle, air was gently bubbled from an aquarium pump,

through sterile filters and autoclaved tubing into each culture bottle through an opening in the cap of the culture bottle in order to redistribute the culture and ensure that CO_2 did not limit growth.

2.3.2 Parent batch cultures

Following the initial acclimation to reduced DIP conditions, 200 mL subsamples of late-exponential growth phase *Trichodesmium* IMS101 were successively transferred to three 2.5 L acid-washed and rinsed polycarbonate culture bottles. Each replicate was diluted to a final volume of 2.3 L with UV-sterilized, artificial YBCII media with an initial DIP concentration of 0.5 μmol L^{-1}. For further reference, these 2.5 L replicates are termed the parent batch culture. Temperature and light were maintained at previously stated levels (12L:12D, E_{max}= 500 μmol quanta m^{-2} s^{-1}, T=24^{o}C). Growth of the parent batch culture was monitored daily within the first hour of the light cycle by Pulse Amplitude Fluorometry (PAM; Model XE-PAM, Heinz Walz GmbH Instruments) so as to record increases in biomass as determined by maximal chlorophyll *a* (Chl *a*) fluorescence (Chl *a* F_M). At the initial point of transfer and at multiple points throughout the growth cycle, each parent batch culture was sampled in duplicate for particulate carbon (PC), particulate nitrogen (PN), particulate phosphorus (PP), and Chl *a* concentrations by filtering aliquots of culture onto combusted (3 h at 450º C) 21 mm Whatman GF/F filters. Cultures were swirled prior to sampling to ensure homogeneity. Duplicate samples were also collected daily for microscopic enumeration of filament concentrations, determination of fluorescence parameters and evaluation of potential heterotrophic bacterial contamination. All filtrations were performed under a low atmospheric pressure differential with acid-washed and rinsed glassware. The filtrate from this sampling procedure was then collected for determination of inorganic nutrient concentrations (phosphate, ammonium, nitrate and nitrite). Following collection, chlorophyll samples were

placed in a liquid nitrogen dewar and all other samples were stored at -20 ºC until time of analysis.

2.3.3 Dark bottles

At three time points (day 5, day 10, and day 14) during the growth of the parent batch cultures, 650-mL subsamples of each of the three parent batch culture replicates were transferred to 1 L acid-washed and rinsed, polystyrene dark bottles. A 5.0 µmol L^{-1} spike of DIP was then added to each dark treatment. For reference, dark bottles (db) are termed db-A (t_i= day 5, *n*=3), db-B (t_i= day 10, *n*=3), and db-C (t_i= day 14, *n*=3) where t_i refers to the batch day on which each culture was initiated. On a daily basis, following transfer to dark bottles, duplicate samples were collected from each db replicate for Chl *a* content, dissolved inorganic nutrients, PC, PN, and PP composition as previously described for the parent batch culture.

2.3.4 Recovery determination

At multiple time points throughout the experiment, 5 mL subsamples were taken from the parent batch cultures and db incubations in order to assess the response of the samples to external P additions (parent batch culture recovery samples) or re-exposure to light (dark bottle recovery samples). Additions of 0.5 µmol L^{-1} DIP were made to each parent batch recovery subsample. Further DIP additions were not made to dark bottle recovery samples. All recovery samples were replaced in the incubator with the parent batch cultures (12L:12D, E_{max}= 500 µmol quanta m^{-2} s^{-1}, T=24^{o}C). Growth of recovery samples was monitored daily via Chl a F_M. Also, selected samples were filtered and processed for chlorophyll-a, PP, PC, and PN determinations. A positive growth rate response to DIP additions was taken as initial confirmation of

P-limitation of the parent batch culture. Growth of db recovery samples was evaluated as a measure of the dark survival capacity of the cells.

2.3.5 Analytical procedures

Changes in biomass concentrations in each db were measured by three independent methods: Chl *a* concentrations, Chl *a* F_M, and microscopic enumeration of individual filaments. Chl *a* concentrations were determined fluorometrically, as described by Strickland and Parsons (1972) using a Turner 10-AU fluorometer (Turner Designs). Chl *a* F_M and the quantum yield of fluorescence ($F_V F_M^{-1}$) were measured using a PAM fluorometer employing all proper blank corrections (Cullen and Davis, 2003) with fresh YBC11 media. Use of Chl *a* F_M as a proxy for biomass is advantageous as it is non-invasive, requires relatively small volumes (≤4-mL) and fluorescence-derived parameters can be used as a general indicator of the physiological state of the culture (Campbell et al. 1998). Preliminary experiments with *Trichodesmium* IMS101 resulted in a significant correlation (r^2= 0.96) between Chl *a* F_M and Chl *a* concentrations when cultures are sampled at equivalent light levels. Filament enumeration was assessed as duplicate, 3 mL samples were fixed in 2.5% final concentration SEM grade gluteraldehyde, stained with 5 μg DAPI mL^{-1} and filtered onto black-stained 0.2 μm Nuclepore membrane filters. Slide mounted filters were kept frozen at -20°C in slide boxes until counts were performed. The entire slide was counted using UV-epifluorescence microscopy for enumeration of individual filaments. Selected slides were also examined for the presence of contaminating heterotrophic bacterial biomass using the protocol of Sherr et al. 2001.

Particulate C and N were analyzed on a Carlo Erba CHNS analyzer (model NA1500) using a cystine (29.99 % C and 11.66% N by weight) reference. Error terms for all elemental ratios were calculated using standard error (SE) propagation techniques (Bevington and Robinson, 2003). Combusted filter blanks processed during laboratory experiments had an average C and N content of 5.7 μg C $filter^{-1}$

(SE= 0.6, n=14) and 0.51 μg N filter^{-1} (SE= 0.16, n=14), respectively (values for filtration of 25 mL medium). The magnitude of this blank correction corresponded to 14.3 ± 0.7% of the PN signal and 23.9 ± 0.9% of the PC signal for individual samples (n=150). Filter blanks (n=11) processed for field samples in the Atlantic accounted for 7.6 ± 0.6% of the PN signal and 18.4 ± 1.1% of the PC signal for individual samples (n=52). For Pacific samples, filter blanks (n=8) represented 12.2 ± 1.2% of the PN signal and 29.8 ± 2.7% of the PC signal for individual samples (n=36).

Particulate P was analyzed according to current Hawaii Ocean Time-series (HOT) program protocols. Filters were heated in pre-combusted, acid-washed / DI rinsed glass test tubes for 4-5 hours at 450ºC in a muffle furnace. Samples were then cooled and immersed in 10 mL of 0.15 mol L^{-1} hydrochloric acid. Extracted samples were analyzed for particulate P using molybdenum blue spectrophotometry as per the protocol of Hebel and Karl (2001). Accuracy was assessed from the analysis of a known dry weight of certified reference material (National Institute of Standards, NIST 1515, orchard leaves, certified 0.159% P by weight). The measured P content of NIST 1515 reference material averaged 0.152% (SE= 0.003%, n=16) for standards used relative to laboratory samples, 0.151% (SE= 0.004%, n=8) for Atlantic reference samples and 0.152% (SE= 0.016%, n=6) for Pacific reference samples. Filter blanks used for laboratory experiments accounted for an average 13.8% (SE= 3.3 %, n=13) of the sample PP signal (n=158). For the field samples, filter blanks represented 11.6% (SE= 1.2%, n=7) of the Atlantic PP signal (n=24) and 14.1% (SE= 1.6%, n=8) of the Pacific PP signal (n=28).

A Technicon AutoAnalyzer II™ was used to measure phosphate and ammonium while Alpkem RFA 300™ components were used to measure nitrate plus nitrite concentrations from sample filtrates. Phosphate concentrations were determined following a modification of the molybdenum blue procedure of Bernhardt and Wilhelms (1967), where phosphate is measured as reduced phosphomolybdic acid employing hydrazine as the reductant. Ammonium concentrations were measured following an indophenol blue method modified from ALPKEM RFA methodology.

The nitrate + nitrite analysis uses the basic method of Armstrong et al. (1967), with modifications to improve the precision and ease of operation (Patton 1983).

2.3.6 Natural populations

Colonies of *Trichodesmium* were collected and analyzed for elemental stoichiometry during two separate cruises, the first in the tropical NW Atlantic (9º30'N -11º21'N 50º05'W-56º31'W) aboard the R/V SEWARD JOHNSON II in April-May of 2003 and the second in the subtropical N Pacific (18º30'N -21º01'N 154º50'-161º00'W) aboard the R/V ROGER REVELLE in July-August of 2003. Both cruises were components of the NSF funded MANTRA (MArine Nitrogen-fixation and Tropospheric Response to Aeolian inputs)/ PIRANA (Potential Influences of Riverine and Aeolian inputs on Nitrogen-fixation in the Atlantic) projects. *Trichodesmium* colonies were collected with either 202-μm mesh nets towed off ship drift near the surface or by oblique tows with 1 m diameter nets towed at depths of 5-30 m. Colonies were selected with a plastic bacteriological transfer loop and rinsed in filtered seawater. At each sampling point, a minimum of 10 colonies per analysis were filtered onto pre-combusted GF/F filters for determination of particulate C, N, and P content. Following collection, all samples were stored at -20 ºC.

2.4 Results

2.4.1 Parent batch culture

The parent batch culture exhibited a typical pattern of lag and exponential growth phases. Over a 14-day period, the specific growth rate (μ) of the culture was equivalent to 0.13 day^{-1} when calculated from Chl *a* F_M and 0.15 day^{-1} when

calculated from filament counts. Specific growth rates based upon particulate elements PC, PN, and PP were 0.19 day^{-1}, 0.20 day^{-1}, and 0.21 day^{-1}, respectively. This disparity in growth rates appears to be due to elongation of filaments over the course of batch culture growth (results not shown). Chl *a* F_M reached maximum values after 10 days, after which time Chl *a* F_M began to decline while filament density plateaued (Figure 2-1). The decline in Chl *a* F_M coincided with a decline in the quantum yield of fluorescence ($F_V F_M^{-1}$) from an average of 0.35 to a minimal value of 0.24 (trend not shown) and was thus taken as an indicator of physiological stress (as in Berman-Frank et al. 2004). Following this decline, on days 11 and 14 of batch culture growth, the response of the batch culture to P-additions was assessed. By all measures, Chl *a* F_M (Figure 2-2A), the growth rate calculated over days 11-14 and days 14-18, as well as PP and Chl *a* concentrations measured at the peak of recovery biomass (day 14 and day 18, respectively) (Figure 2-2B), recovery samples responded positively to P-additions. Positive growth of batch recovery samples was preliminary confirmation of P-limitation of the parent batch culture beginning at day 11.

The temporal change of DIP, PP, and total P (DIP+PP) concentrations in the batch culture is shown in Figure 2-3. PP concentrations increased steadily over the course of batch culture growth, whereas DIP was drawn down to a minimum concentration of 40 nmol L^{-1} (SE=0.001 *n*=6) after only 5 days (Figure 2-3B). This concentration is typical of near surface values at Station ALOHA (Karl and Tien, 1997). The balance of the batch culture P budget was assessed by examining deviations of total P from the total P measured at the initiation of the parent batch culture (TP_i). Figure 2-3A shows that DIP+PP decreased to a minimum at day 5 of the batch culture, indicating loss of P to some undetectable form. Batch culture PP content relative to filament number and PN peaked at day 4 (PP $filament^{-1}$ =0.15 ng P $filament^{-1}$ SE=0.014, *n*=6; PN:PP= 30.1 mol:mol, SE=9.2, *n*=6) after which time normalized PP content declined (Figure 2-3C). Calculated over the period of maximum biomass increases (days 0-4), the specific growth rate of PP was 0.48 d^{-1} while the specific uptake rate of PP $filament^{-1}$ was 0.42 day^{-1}, corresponding to a turnover time of 1.4 to 1.6 days. The lowest normalized PP content was measured at time zero of batch

culture growth (PN:PP= 93.8 mol:mol, SE=24.2, *n*=4; PP filament^{-1} =0.03 ng P filament^{-1}, SE=0.003, *n*=4), consistent with pre-adaptation of cultures to P-restricted growth conditions.

Increases in batch PN and PC are shown in Figure 2-4A. Batch culture PC:PN ratios (Figure 2-4B) did not vary significantly during the course of growth (*t*-tests *p*> 0.1). However, samples taken on day 4 (PC:PN = 8.56, SE= 0.7, *n*= 6) were significantly greater than the Redfield reference ratio of 6.63 (*t*-test *p*= 0.02, df=5). PN normalized to filament biomass reached a maximum after 5 days of growth after which time PN filament^{-1} stabilized (Figure 2-4B). PC quotas per filament peaked at day 5 (PC filament^{-1} = 16.1, SE= 1.2, *n*=6) and then declined, reaching stable levels by day 10 (data not shown). Dissolved inorganic N ($NH_4 + NO_3 + NO_2$) accumulated in the parent batch medium from an initial concentration of 0.08 μmol L^{-1} (SE=0.004 *n*=4) to a maximum concentration of 1.25 μmol L^{-1} (SE=0.06, *n*=6) at day 14, a net increase of 1.17 ± 0.02 μmol N L^{-1} or 3% of the total measured PN increase (38.7 μmol N L^{-1}).

2.4.2 Dark bottles: biomass decline

Subsamples of each parent batch replicate were transferred to dark bottles with added P on day 5 (db-A), 10 (db-B), and 14 (db-C). Figure 2-5 presents the decline in the number of intact filaments per volume in each dark treatment, relative to the growth of the parent batch culture. Biomass change over time was measured by microscopic enumeration of filaments and Chl *a* $F_{M.}$ Chl *a* concentrations were recorded for db-A only, due to inadvertent loss of db-B and db-C samples. For this reason, only Chl *a* F_M and the number of filaments per volume are presented for db analyses. In all of the dark bottles, Chl *a* F_M (Figure 2-6) and filament concentrations (Figure 2-5) remained relatively stable for a period of 2-4 days of dark exposure after which time biomass began to decline rapidly. The average rate of decline derived from filament counts was 15.6% day^{-1} for db-A, 12.8 % day^{-1} for db-B, and 10.4 % day^{-1} for

db-C. The total percentage biomass lost over the course of dark incubations was 94%, 98%, and 83% for db-A, db-B, and db-C respectively, based on filament counts. The total Chl *a* F_M loss and the rate of Chl *a* F_M losses closely parallel the decline of filament counts.

2.4.3 Dark bottles: recovery from nutrient and light stress

Samples taken from the early phases of db incubations displayed positive growth rates when replaced in light conditions (Figure 2-6), regardless of the nutritional state of the cells prior to dark bottle initiation. The average exponential phase specific growth rates of positive response recovery samples from db-A, db-B, and db-C were 0.27 day^{-1}, 0.20 day^{-1}, and 0.28 day^{-1} respectively. Recovery samples taken from days 4-6 of db-A, days 5-7 of db-B, and day 8 of db-C did not increase in biomass in response to reintroduction to light conditions (Table 2-1).

Table 2-1. Results of response of db subsamples to light conditions. Samples that did not display a positive response are indicated by NR (no recovery). Growth rates (μ) were calculated from Chl *a* F_M. Db-A was terminated after sampling on day 6, db-B on day 7 and db-C on day 8, subsequent days are denoted by a dash. At the onset of db incubations, the filament specific P_Q was 0.136 ± 0.05, 0.054 ± 0.039, and 0.070 ± 0.014 ng P $filament^{-1}$ for db-A, db-B, and db-C, respectively.

day	db-A	db-B	db-C
1	not sampled	μ=0.23	μ=0.23
2	μ=0.29	μ=0.25	μ=0.36
3	μ=0.25	μ=0.17	not sampled
4	NR	μ=0.22	μ=0.19
5	NR	NR	μ=0.30
6	NR	not sampled	μ=0.32
7	-	NR	NR
8	-	-	NR

2.4.4 Dark bottles: Elemental concentrations and ratios

The elemental concentrations and ratios presented here represent a mixture of living and non-living particulate material, because there is no straightforward method for separating the two fractions either in cultures or in the field due to sampling constraint. The temporal change of PP, DIP, and total measured P (DIP+PP) concentrations are shown in Figure 2-7A:C. The change in DIP concentrations explains 78%, 79%, and 92% of PP variation for db-A, db-B, and db-C, respectively (regressions not shown). All regressions are significant at the 95% confidence level. Conservation of measured P for dark incubations is addressed in the same manner as that described for the parent batch culture, by examining deviations of total P over time from the initial total measured P. In db-A, total P does not change significantly from the initial total P, indicating that P was conserved over the time course of this dark incubation. In contrast, a net loss of P is observed by the final day of the db-B incubation period and at three time points during the db-C incubation period. These losses indicate conversion of measurable P to a form undetectable by the analytical methods used in this study.

PC and PN concentrations for dark incubations are shown in Figure 2-7D:F. In db-A, PN concentrations decreased by less than 10% (-0.9 μmol N L^{-1}) over the time course of the incubation while PC concentrations dropped by 24%, a net loss of 22 μmol C L^{-1} (Figure 2-7D). These decreases are reflected by a gradually declining PC:PN ratio for db-A (Figure 2-8A). During the time course of db-B and db-C incubations, PN and PC concentrations increased over the first 1-2 days of the incubation period and then steadily returned to near or below initial concentrations (Figure 2-7E:F). The net loss of PN recorded for db-B was 3.4 μmol N L^{-1} (11% of initial values) while PC concentrations fell by 37 μmol C L^{-1} (19% of initial PC), again reflected by a change in PC:PN ratios (Figure 2-8B). With the exception of the initial

time point, db-B and db-C PC:PN ratios were significantly lower than the Redfield PC:PN ratio of 6.63 (Figure 2-8B:C).

Particulate C:P ratios for all db incubations indicate net P uptake relative to C (Figure 2-8A:C) under dark conditions. PC:PP ratios in db-A reflect variation in PC (Figure 2-7D) and a steady increase in bulk PP (Figure 2-7A), resulting in PC:PP ratios ranging from an initial value of 293 (SE= 31, *n*=6) to a minimum of 85 (SE=5.5, *n*=6). In db-B, PC:PP ratios reached a relatively stable minimum (day 1-7 average PC:PP= 99, SE=5.5, *n*=36) after a single day of darkness whereas db-C required 4 days to reach a stable minimum (day 4-8 average PC:PP= 113, SE=3.7, *n*=21). PP per filament also increased over the course of all dark incubations (Figure 2-8D:F). When evaluated over the time period for which cells were able to recover from the light period (days 0-3 for db-A, days 0-4 for db-B, and days 0-6 for db-C) (Table 2-1), specific P uptake rates calculated from the net change in PP filament^{-1} are 0.50±0.09 day^{-1} for db-A, 0.75±0.13 day^{-1} for db-B, and 0.52 ± 0.09 day^{-1} for db-C. These correspond to P turnover times of 1.4 days (db-A), 0.9 days (db-B), and 1.3 days (db-C).

The net accumulation of dissolved inorganic fixed N ($NH_4^+ + NO_3^- + NO_2^-$) during the time course of dark bottle incubations was 0.09 μmol L^{-1}, 1.33 μmol L^{-1}, and 1.91 μmol L^{-1} for db-A, db-B, and db-C, respectively. These increases correspond to 0.8% (db-A), 4.3% (db-B), and 4.7% (db-C) of initial PN concentrations. Dark conditions did not result in the preferential loss of N from the particulate pool relative to C (Figure 2-8A:C), Chl *a* or filament concentration (data not shown), thus the liberated N is likely the result of cell senescence and subsequent cell lysis.

2.4.5 Phosphorus budget considerations

Conservation of measured P in the parent batch and db incubations was addressed by calculating the difference between the concentration of total P (TP) and

initial total measured P pools (TP_i) at each time point. This analysis allowed us to determine whether or not the cultures were ever contaminated with an outside source of P (TP_i -TP > 0) or conversely if P was converted to a form of P that was not analyzed in this study (e.g., DOP or intracellular polyphosphate) (TP_i -TP < 0). No P contamination was detected over the course of this experiment however P losses were observed. Negative values of (TP_i –TP) are termed missing P (P_M).

Given the analytical techniques used in this study, it is possible that the calculated P_M pool was comprised of intracellular polyphosphates and/or dissolved organic phosphorus (DOP). Polyphosphates are known to accumulate in most cyanobacteria (Romans et al. 1994; Grillo and Gibson, 1979). If present and of high molecular weight, they would have not been detected by our analytical methods as high temperature acid hydrolysis is necessary to effectively retrieve the entire polyphosphate fraction of the particulate pool (Karl and Tien, 1992). Our methodology would have only recovered low molecular weight polyphosphate. DOP concentrations were not measured.

Negative values of (TP_i –TP) were observed in the parent batch culture (Figure 2-3A), indicating that a fraction of the P present at the initiation of the batch culture was converted to an undetectable P pool (P_M). In batch culture, calculated P_M concentrations peaked at 33% of total predicted PP on day 5, coinciding with minimal DIP levels (Figure 2-3A). After day 5, the calculated P_M concentrations decreased suggesting that cells began utilizing intracellular polyphosphate pools or DOP to fuel growth once DIP was no longer available, a scenario consistent with the concept of luxury P consumption. The net increase in the concentration of PP over the entire 14-day batch culture, 0.463 μmol L^{-1} (SE=0.025), is statistically balanced by the net drawdown of DIP over this same time period, 0.453 μmol L^{-1} (SE=0.003). Negative values of (TP- TP_i) were also observed at select points during db-B and db-C incubations (Figure 2-7B:C). In contrast to the calculated magnitude of the P_M pool observed in the parent batch culture, db P_M accumulations accounted for less than 13% of total predicted PP concentrations.

2.4.6 Field data

The oceanic regions from which our field samples were collected exhibit similar hydrographic properties. The average surface temperature and salinity of the 9 stations sampled in the subtropical Pacific region were 26.8ºC and 35.1‰ while the 19 stations sampled in the tropical NW Atlantic had a mean temperature of 27.3ºC and mean salinity of 34.6‰. Annual mean surface phosphate concentrations for the Pacific region (~ 20°N 158°W) are typically about 100 nmol L^{-1} (data from HOT climatology) while the Atlantic region (~ 10°N 53°W) ranges from 120-220 nmol L^{-1} (data from NOAA NODC WOA01 climatology). Particulate C-N-P stoichiometry for each sample group (Pacific and Atlantic) is presented in Figure 2-9. The mean stoichiometry of the Pacific group was $C_{302\pm32}$:$N_{56\pm6}$:P_1 and $C_{285\pm33}$:$N_{49\pm3}$:P_1 for the Atlantic sample group. Results of *t*-tests assuming separate variance revealed no significant difference between the PC:PP (p=0.59, df=50), PN:PP (p=0.19, df=50) or PC:PN (p=0.16, df=86) ratios of samples collected from these oceanic regions.

2.5 Discussion

The composite data set presented here allows us to identify ecologically relevant sources of stoichiometric variability, specific to *Trichodesmium* spp., which would permit this genus to alter the bulk elemental composition of dissolved and particulate pools, and thus regulate the flow of elements in otherwise nutrient depleted marine environments. Specifically, results from this study suggest that *Trichodesmium* spp. have very low P requirements relative to C or N. Maintenance of diazotrophic growth with reduced P content would preferentially enhance both the C and N components of dissolved and particulate pools, thus favoring community scale P-limitation. Conversely, cultured populations of *Trichodesmium* previously acclimated to low P conditions appear to have a high capacity for luxury consumption of P. Utilization of P in excess of the requirements for growth would be expected to alter C-

N-P stoichiometry without a concomitant increase in growth rate (Sommer 1985). Lastly, this research addresses basic tenets of the P-mining vertical migration scenario: cell survival and P uptake in the absence of light.

2.5.1 P-sparing

Natural populations of *Trichodesmium* spp. occur in oceanic environments where dissolved phosphate concentrations are extremely low, typically at or near standard detection limits set by standard autoanalyzer technology (50 nmol P L^{-1}), thus leading to widespread speculation of regional scale P-limitation of *Trichodesmium* growth (Fu et al. 2005; Sañudo-Wilhelmy et al. 2004; Karl et al. 1997). It has been suggested that *Trichodesmium* spp. may persist in a prolonged state of P-limitation by decreasing internal P quotas, most likely through a reduction of P-rich cellular components such as RNA, an effect termed P-sparing (Karl et al. 2002). Phosphorus-restricted laboratory populations, those cells harvested from media with the lowest measured DIP and PP filament^{-1} (batch days 0, 10, and 14; mean DIP=0.054 μmol L^{-1}, SE=0.004, n=16), produced biomass with mean elemental stoichiometry of $C_{585\pm56}N_{90\pm10}P_1$, approximately 6 times higher than the Redfield reference ratio (see Table 2-2 for range). If we include P_M in this calculation of P-restricted stoichiometry, the mean ratio is not substantially altered ($C_{574}N_{88}P_1$). Furthermore, neither PN filament^{-1} (Figure 2-4B) nor PC filament^{-1} were elevated during P-restriction reinforcing the conclusion that the high PC:PP and PN:PP measurements are a result of low P quotas rather than C or N storage. Figure 2-10 compares the full range of our empirical data (Figure 2-10B) to the elemental composition of natural populations collected in the tropical NW Atlantic and the subtropical N Pacific (Figure 2-10A). Most apparent is the observation that natural populations are consistently depleted in P relative to C or N with 98% of the samples falling above the typical Redfield C-N-P stoichiometry of $C_{106}N_{16}P_1$.

Table 2-2. Elemental stoichiometry for cultured and natural populations of *Trichodesmium* spp. Data from batch culture measurements in this study cite the range of values for the minimum and maximum measured P quota (PQ) where db values are derived from the time range over which cells were able to recover from light limitation (viable). Data from other sources are referenced.

	N:P	C:P	C:N	[ng P filament^{-1}]
Minimum $P_{Q:}$ (Batch culture: days 0,10, and 14) $[PO_4]$=0.05±0.004 μmol L^{-1}	84 to 94	531 to 668	6.3 to 7.1	0.03 to 0.07
Maximum $P_{Q:}$ (Batch culture: days 4 and 5) $[PO_4]$= 0.10±0.03 μmol L^{-1}	30 to 37	257 to 292	7.8 to 8.6	0.13 to 0.15
Maximum P_Q (db-ABC, viable) $[PO_4]$= 3.8±0.23 μmol L^{-1}	14 to 18	82 to 109	5.3 to 6.8	0.55 to 1.7
IMS101: range of [DOP][1]	4.4 to 156	no data	no data	no data
IMS101: range of [Fe][2]	4.8 to 13	55 to 156	8.9 to 16	no data
IMS101 and GBRTRLI101: range of [DIP][3]	14 to 45	94 to 392	not reported	no data
Lab range	4.4 to 156	55 to 668	5.3 to 16	0.03 to 1.7
NW Atlantic	22 to 103	133 to 558	4.2 to 6.7	no data
N Pacific	17 to 93	87 to 498	4.5 to 6.3	0.02 to 0.44**
Central Atlantic[4]	14 to 62	98 to 416	no data	0.04 to 0.34 **
N Australia[2]	21 to 33	120 to 201	5.4 to 7.3	0.06 to 0.45 **
N Australia[5]	44 to 87	no data	no data	no data
Gulf of Mexico[5]	65.6 to 66.3	no data	no data	no data
NPSG (Station ALOHA)[6]	42 to 52	269 to 330	6.1 to 6.4	0.39 to 0.48
1989 N Pacific Bloom[7]	125*	891*	7.1*	no data
N Australia[8]	14 to 52	79 to 310	no data	no data
NW Atlantic[9]	25 to 182	137 to 943	no data	0.02 to 0.2**
Field range	14 to 182	79 to 943	4.2 to 7.3	0.02 to 0.48

References: [1]Mulholland et al. (2002) [2]Berman-Frank et al. (2001) [3]Fu et al. (2005) [4]Sanudo-Wilhelmy et al. (2001) [5]Villareal and Carpenter (2003) [6]Letelier (1994) [7]Karl et al. (1992) [8]Kustka et al. (2003) [9]Sanudo-Wilhemy et al. (2004), Intracellular Ratios

* Values for bulk particulate matter collected within a *Trichodesmium* bloom

** Using a conversion factor of 182 filaments per colony

In order to interpret this data set in terms of nutrient restriction, we must assume some critical N:P ratio indicating a transition point above which P-limitation can be inferred. Based upon the physiologically feasible range of biomolecular composition for marine phytoplankton, Geider and LaRoche (2002) calculated that the critical N:P ratio for a given species is between 10 and 50 mol:mol. Genera with slow growth rates and high protein content such as *Trichodesmium* would have a critical N:P towards the higher end of this range. Similarly, Kustka et al. (2003) infer a critical N:P of 40 mol:mol from their own experimental data set. If we conservatively take the upper limit of 50 to represent the critical N:P ratio for *Trichodesmium*, our data suggests that 53% of field samples (64% of N Pacific samples; 38% of NW Atlantic samples) were P-limited at the time of sampling. When other field data are considered (Table 2-2), it is apparent that, for virtually every published observation, some fraction of the *Trichodesmium* population is P-limited when a critical N:P of 50 mol:mol is used as the benchmark for P-limitation. Thus, the composite range of field data is consistent with a paradigm of widespread P-limitation of natural populations of *Trichodesmium*. Yet, if we assume that the stoichiometry of P-restricted laboratory cultures sampled during this study approximate a minimal P quota, it becomes evident that the extremes measured in the laboratory are rarely exhibited in nature. Specifically, only 8% of field samples from our own data set (n=4, all from the NW Atlantic), a single observation of a NPSG bloom (Karl et al. 1992) and three observations from the data set of Sañudo-Wilhelmy et al. (2004) fall above the stoichiometric mean exhibited by P-restricted cultures from this study ($>C_{585}N_{90}P_1$).

An obvious question arising from this research is why the end member PC:PN:PP ratios observed in this study are not commonly observed in nature. We can speculate that the elemental composition of particulate matter in natural populations is more tightly constrained as a consequence of Liebig's law of the minimum, such that the low DIP concentrations characteristic of the oligotrophic environs inhabited by *Trichodesmium* spp. will only drive relatively moderate stoichiometric anomalies. Co-limitation by another element, such as Fe, would preclude further stoichiometric

deviation. In this research, the initial Fe concentrations in our media were on the order of 0.5 μmol L^{-1}, whereas Fe concentrations in the oligotrophic oceanic gyres are typically on the nanomolar level (Boyle et al. 2005; Wu et al. 2001). Thus, in this study, the maintenance of such high levels of potentially co-limiting elements ensured that P was the only limiting factor. In natural oligotrophic environments where multiple elements are in low abundance, this scenario is not likely. It is also possible that the extremes observed in the laboratory are more common in nature, albeit masked by the presence of surface adsorbed P. Recent work by Sañudo-Wilhelmy et al. (2004) indicates that 46-91% of total particulate P measured in natural samples of *Trichodesmium* collected from the N Atlantic existed in the form of surface adsorbed P, rather than intracellular P. Given that we did not process our samples in the same manner as these authors, we can only speculate that our values may represent a conservative estimate of intracellular stoichiometry.

2.5.2 Luxury consumption

In contrast to a reduction of nutrient quotas, deviations from mean stoichiometry may also be driven by the consumption and subsequent storage of P in excess of the requirements for growth. In our culture experiments, P-restricted cells (P_Q =0.03±0.003 ng P filament^{-1}) transferred from medium with minimal DIP concentrations (70±10 nmol P L^{-1}) increased their P content (P_Q =0.15±0.01 ng P filament^{-1}) five-fold in a span of 4 days after being exposed to fresh medium containing 500 nmol P L^{-1}. Over this time span, doubling times calculated from net increases in PP filament^{-1} (1.6 days) or PP concentrations alone (1.4 days) were shorter than the doubling times for biomass increase (Chl a F_M= 4.2 days, filament mL^{-1} = 4.2 days, PC=2.0 days, PN= 2.3 days) indicating that P was consumed in excess of the demands for net balanced growth. Luxury consumption is also suggested by the time series of missing P pools observed in the parent batch culture: an

unmeasured P pool (P_M) accumulated rapidly as P-limited cells were transferred to fresh media. At the time point where DIP was drawn down to minimum concentrations, P_M began to be utilized until all P was accounted for by the end of the batch culture incubation period. Intracellular P quotas were enhanced under dark, P-rich conditions ($C_{96\pm8}N_{16\pm1}P_1$) consistent with the research of Fu et al. (2005) who have shown that light is not a requirement for *Trichodesmium* P uptake.

Much like the extreme minima, such levels of P-enrichment are not commonly sampled in the field, whereas only a single sample from our field data set and a few from other field observations (n=1, Sañudo-Wilhelmy et al. 2001; n=1 Kustka et al. 2003) conform to Redfield stoichiometry. Further examination of empirical data from other laboratory studies (Table 2-2) indicates that while *Trichodesmium* can in fact produce particulate matter similar to the typical Redfield ratio of $C_{106}N_{16}P$, this requires exposing cells to P concentrations (5-50 μmol P L^{-1}) orders of magnitude greater than what would be observed in the oligotrophic ocean. Following the work of Geider and LaRoche (2002), Kustka et al. (2003) suggest that *Trichodesmium* populations having PN:PP ratios less than 20 mol:mol would be indicative of populations engaging in luxurious P uptake and storage. Given the exceedingly high levels of DIP required to produce particulate matter with PN:PP ratios < 20 in the laboratory, coupled with the low frequency of field measurements occurring below this threshold, it appears that either significant luxury consumption and P storage are not common in natural oligotrophic populations or that such P-enrichments are infrequently sampled because they are short-lived physiological transients.

2.5.3 Vertical migration and P-mining

The proposed scenario of P acquisition via buoyancy-mediated vertical migration (Villareal and Carpenter, 1990) requires that natural populations transit from high light, P depleted surface waters to the depths of the phosphocline, acquire P in

low light conditions and then respire dense cellular material (e.g., carbohydrate) at rates sufficient to allow a return to the euphotic zone where the cycle can begin anew. Such buoyancy driven vertical migrations may allow this genus to fulfill elemental requirements with nutrients acquired at depth and potentially enhance elemental fluxes into the euphotic zone in the absence of strict physical control. These elemental inputs could become available to the remaining microbial assemblage thereby inducing a successional pattern in the pelagic microbial community. While our data set does not address the rate of buoyant density change, we are able to address other basic assumptions of the P-mining scenario: dark period phosphorus uptake and long-term dark survival.

Evidence for active dark uptake of inorganic phosphorus by *Trichodesmium* is supported by increases in PP and PP filament^{-1} in dark incubations initiated at variable stages of growth. Measured doubling times of PP in the absence of light were between 1.7 and 2.8 days, while PP-based doubling times in light incubations were on the order of 1.4 to 3.3 days. Measurable dark uptake of P by *Trichodesmium* spp. is also corroborated by other research (Fu et al. 2005; Letelier and Karl, 1998). In combination, these results suggest that encounters with the phosphocline could act to supply a substantial portion of the phosphorus required for growth assuming migrating colonies were able to return to the euphotic zone.

Whether or not P assimilated at depth is transported to the surface requires that net density loss rates are sufficient to ensure return transit. In this regard, in regions such as the NPSG, where the average depth of the phosphocline is between 75 and 120 m, the process of vertical migration may involve prolonged exposure to dark or low irradiance conditions. To our knowledge, no prior research has been conducted to assess the dark survival capacity of this genus. Our dark incubation experiments indicate that the IMS101 strain can survive in the absence of light for periods of 3-6 days, dependent upon the physiological state of the cell at the point of dark initiation. Populations transferred during early growth having relatively high intracellular P quotas retained the capacity to recover from light deprivation for a period of 3 days of dark exposure, after which time cells did not respond to light (Figure 2-6A, Table 2-1).

In contrast, samples isolated from P-restricted growth remained viable for 5-6 days (Figure 2-6B-C, Table 2-1). If we speculate that this coastal isolate is representative of *Trichodesmium* in blue water, our results indicate that migrating populations would be able to persist for a minimum period of 3 to 6 days in total darkness and perhaps longer in dim light.

The ability to survive and consume P, in dark, nutrient-rich environments, at rates sufficient to support growth does not confirm vertical migration as a source of P to the euphotic zone, but rather implies that there is potential for this process to occur. The actual feasibility of phosphocline-scale migrations depends on whether the rate of *Trichodesmium* density change is sufficient to facilitate vertical migration to and from the depth of the phosphocline. Direct determination of colony density, via density centrifugation measurements or some comparable method, along with measures of parameters relevant to Stokes equation (e.g., colony size) would more closely constrain the actual range of vertical velocities achievable by natural populations of *Trichodesmium*. These measures will be crucial to a precise understanding of the potential for phosphocline-scale vertical migration in natural populations. Alternately, vertical migration is likely advantageous because movement alone, whether up or down, may enhance P contact and facilitate increased P uptake relative to non-motile cells.

2.5.4 Ecological implications

In the oligotrophic biomes of the world's oceans, *Trichodesmium* spp. are ubiquitous and key for the addition of reactive N. For these reasons, and for their role in elemental cycling, there has been a substantial research effort devoted to understanding the ecology and physiology of this genus (see Karl et al. 2002 and others therein). Nonetheless, there are still many unanswered questions. In regions such as the NPSG where the system has been hypothesized to be in a period of N_R

sequestration and phosphorus control of plankton rate processes (Karl et al. 2001), the physiological plasticity of *Trichodesmium* can have a profound impact on the composition of particulate and dissolved elemental pools and furthermore may play a regulatory role in net export of particulate matter from the upper ocean.

In the laboratory portion of this study, we show that *Trichodesmium* is able to grow with significantly reduced intracellular quotas of phosphorus. Conversely, when exposed to P following periods of P-restriction, cultured populations appear to engage in luxury consumption of P. In sum, this organism is able to appreciably alter its biochemical composition in response to environmental fluctuations in light and nutrient availability. Comparison of these results to stoichiometric observations of natural populations indicates that the extremes of biochemical composition generated in the laboratory are rarely observed in nature. However, analysis of field data establishes *Trichodesmium* spp. as a genus typically possessing low proportions of intracellular P relative to either C or N. The ability to maintain net productivity with reduced P quotas is clearly an adaptation to the oligotrophic habitat. Given that natural populations only rarely appear to be pushed towards their physiological limits with respect to any single nutrient, the additional P-sparing potential of this genus could further elevate the N component of dissolved and particulate elemental pools and ultimately intensify or establish ecosystem scale P-limitation.

Another strategic feature of *Trichodesmium* physiology is the ability to regulate buoyancy and thereby affect bidirectional migrations in the water column. In order to achieve these migrations, *Trichodesmium* adjust their internal concentrations of carbohydrate reserves, and possibly other constituents. The intriguing potential of this process is whether or not these vertical migrations are of amplitudes sufficient to penetrate the phosphocline. If so, *Trichodesmium* would act as vectors for the upward transfer of phosphorus into the euphotic zone, thereby potentially offsetting some proportion of the P-limitation incurred by their own diazotrophic production. Data presented in this study suggest that if these populations were to reach the phosphocline, P-uptake rates in the dark would be sufficient to support accumulation of P. Moreover, we present the first data detailing the dark survival capacity of this

genus, indicating that migrating populations would be able to sustain light deprivation for periods of 3-6 days. It is acknowledged that these results address corollaries of the P-mining scenario. Confirmation of P-mining should be verified by direct determination of the rate of cell density change in field populations.

Our research findings are in line with a growing body of literature illustrating deviations from static estimates of the elemental composition of marine particulate matter (Falkowski, 2000, Sterner and Elser, 2002, Bertilsson et al. 2003 and others). Assumptions of fixed elemental ratios such as the canonical Redfield ratio are widespread as they allow us to conceptualize a rigid relationship between nutrient supply and the productivity of marine ecosystems. The perception of inflexible elemental stoichiometry, however, limits our ability to predict ecosystem responses to environmental variability, a key challenge in contemporary oceanography.

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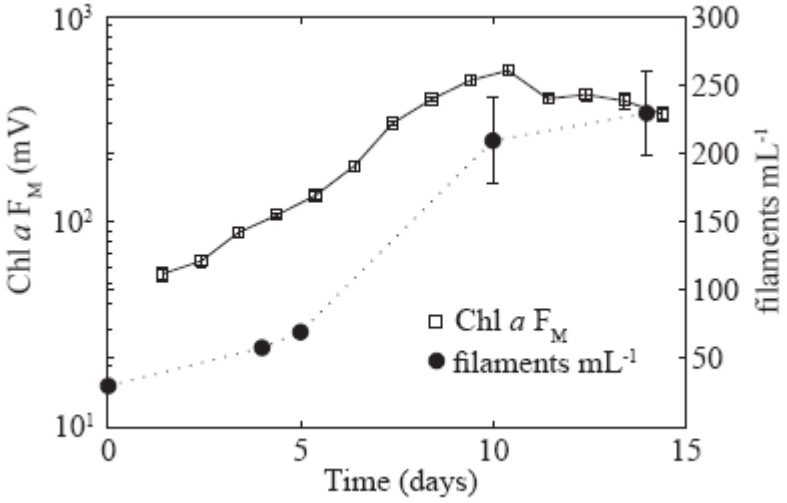

Figure 2-1. Growth curve for batch culture of IMS101 based upon direct microscopic enumeration of filaments and chlorophyll F_M. Dark bottles were initiated on day 4, 10, and 14.

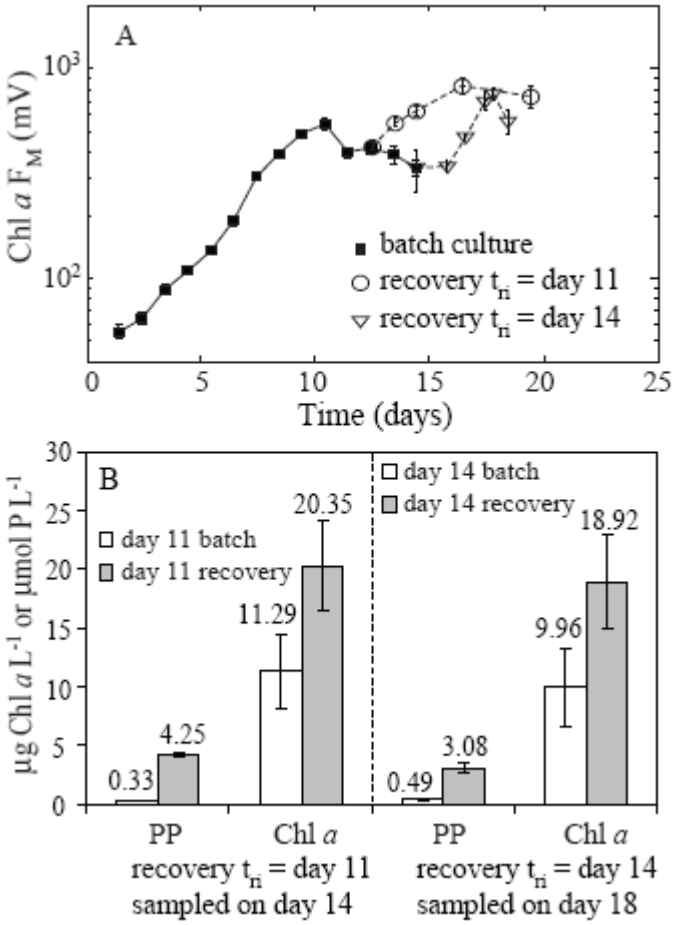

Figure 2-2. Response of batch culture samples to P additions. (A) External P additions made on day 11 (t_{ri}= day 11) and then on day 14 (t_{ri}= day 14) resulted in an increase of Chl *a* F_M. (B) Comparison of PP and Chl *a* concentrations between recovery samples and the batch culture also indicate a positive response to P-additions. The first recovery assessment of PP and Chl *a* compares batch culture and recovery samples sampled on day 14 while the second recovery assessment refers to biomass sampled on day 18. Error bars represent the standard error.

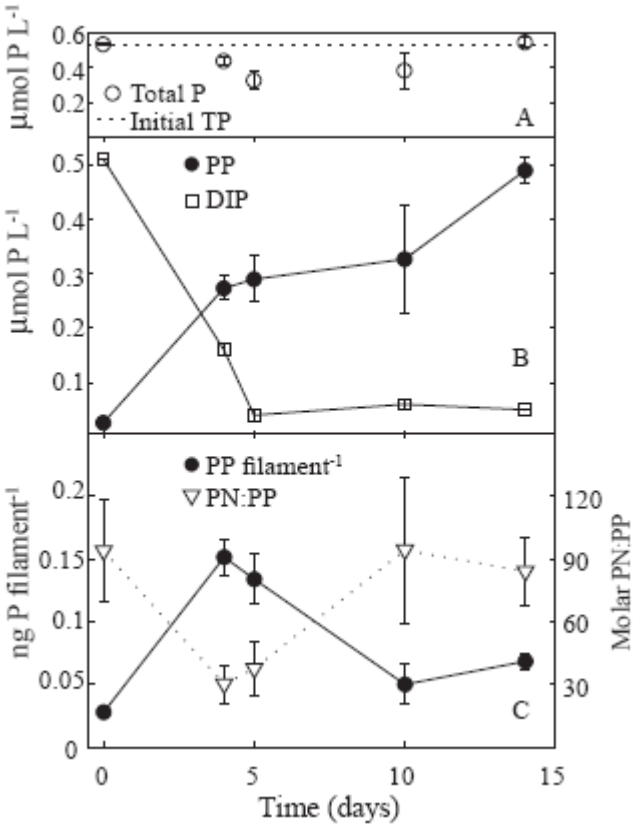

Figure 2-3. P dynamics in the parent batch culture. (A) Total P (DIP+PP) compared with the initial measured total P (initial TP). (B) PP and DIP over 14 d of batch culture growth. (C) Change in measured PP content per filament (filled circles) and PN : PP ratios over the course of the batch culture. All error bars represent standard error.

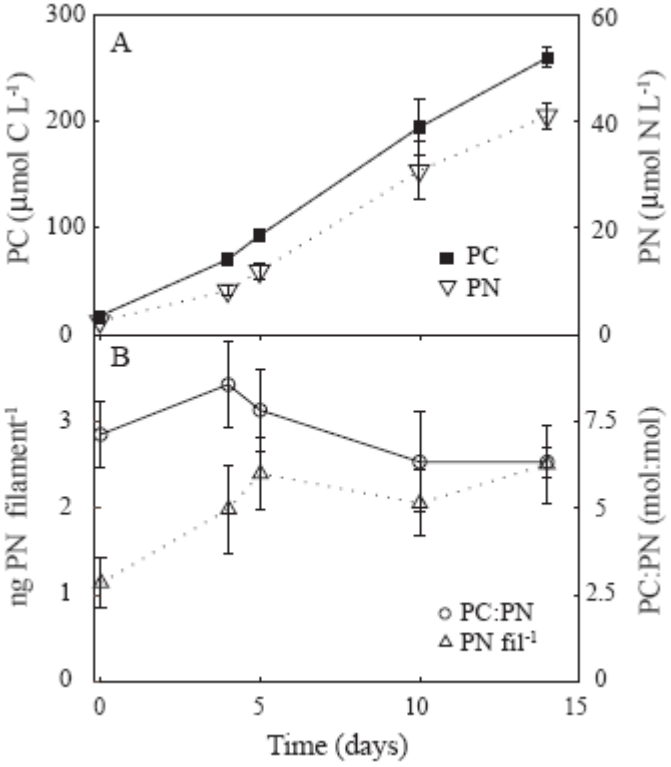

Figure 2-4. (A) Batch culture PN and PC. (B) Batch culture PC:PN ratios and PN filament^{-1}. PC : PN ratios significantly differ from the Redfield reference ratio of 6.63 only on day 4.

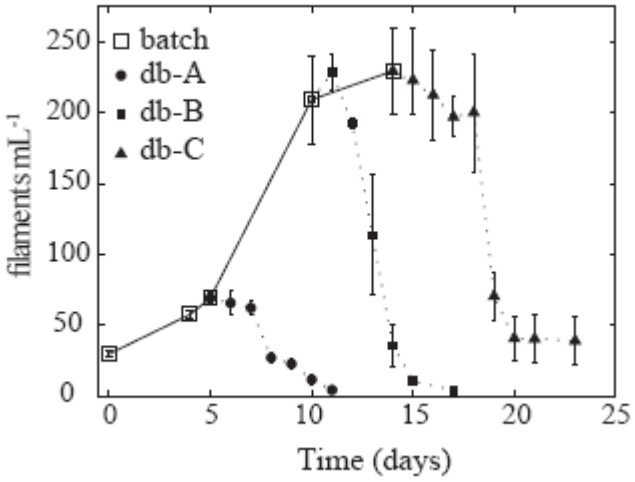

Figure 2-5. Filament count per milliliter for batch culture and dark bottle samples. The total percentage filament biomass lost over the course of dark incubations was 95%, 89%, and 89%, for db-A, db-B, and db-C, respectively.

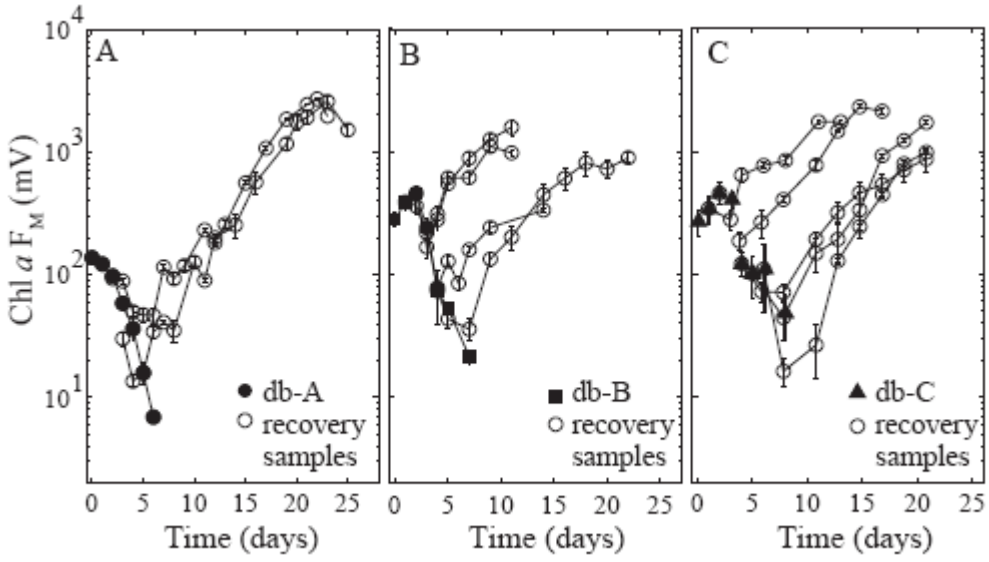

Figure 2-6. Fluorescence based measure of biomass (Chl *a* F_M)for db samples and those taken from (A) db-A, (B) db-B, and (C) db-C that responded positively to reintroduction to light conditions. Samples that did not recover are not shown. These occurrences are cited in Table 2-1.

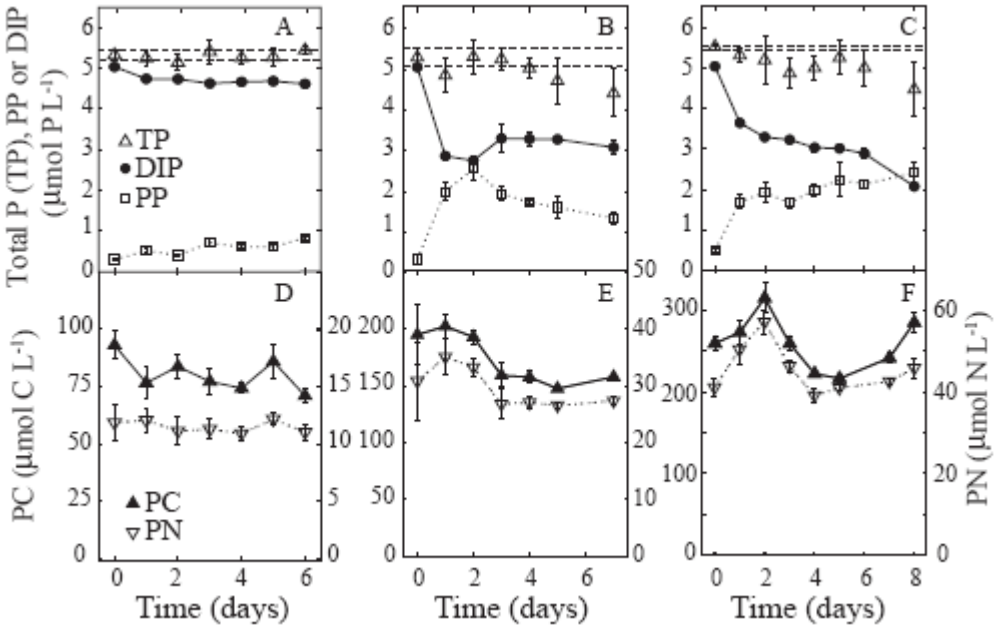

Figure 2-7. (A-C) Temporal change in PP, DIP, and total measured P (TP=DIP+PP) for all dark bottle incubations. Dashed lines represent the standard error of initial total P for each dark bottle. TP is conserved in db-A, while net losses of P are observed by the final day of db-B and at multiple points during the db-C incubation period. (D-F) Temporal change in PC and PN concentrations for all dark bottle incubations.

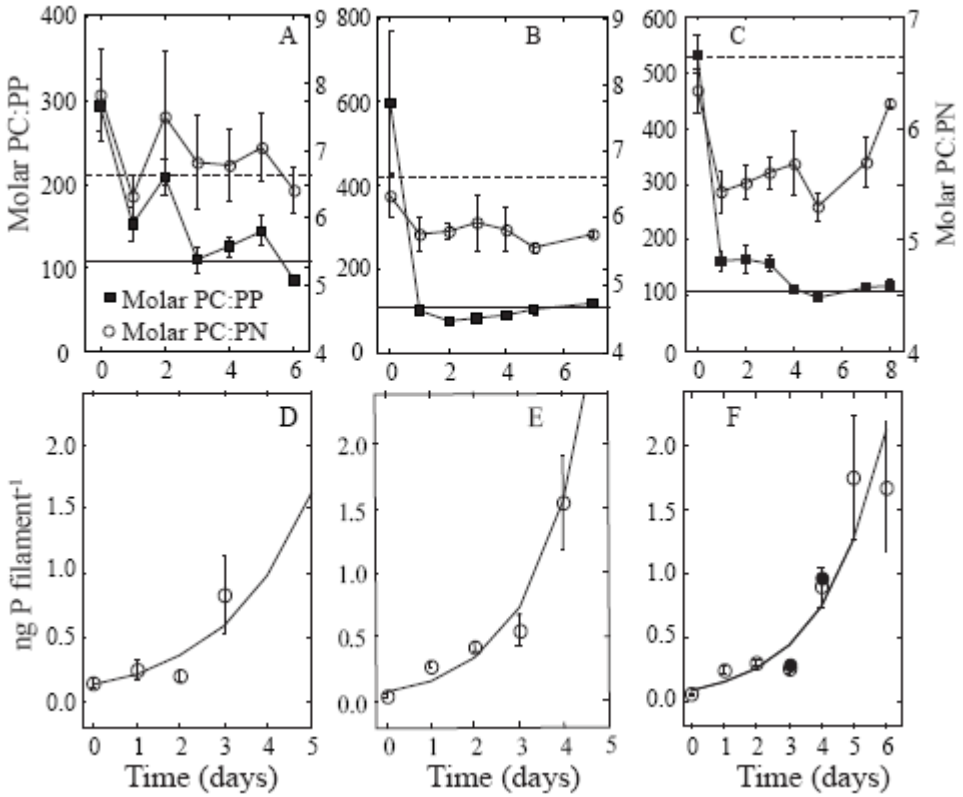

Figure 2-8. PC:PP and PC:PN ratios and P content per filament over time for each dark bottle incubation. (A-C) PC:PP and PC:PN ratios for each dark bottle incubation. In each subplot the solid line represents the Redfield PC:PP reference ratio of 106 while the dashed line represents the Redfield PC:PN reference ratio of 6.63. In all dark bottles, PC:PP ratios approach Redfield stoichiometry, however the slope of this approach is notably more rapid in db-B and db-C. In db-A, PC:PN ratios differ from Redfield stoichiometry only at the initial time point. In contrast, the PC:PN stoichiometry of db-B and db-C are generally lower than the Redfield reference ratio. Note: scales differ for each dark bottle. All error bars represent standard error. (D-F) Phosphorus content per filament in dark incubations shown for the time period during which recovery analyses were positive. Filled circles indicate measured $P + P_M$ per filament.

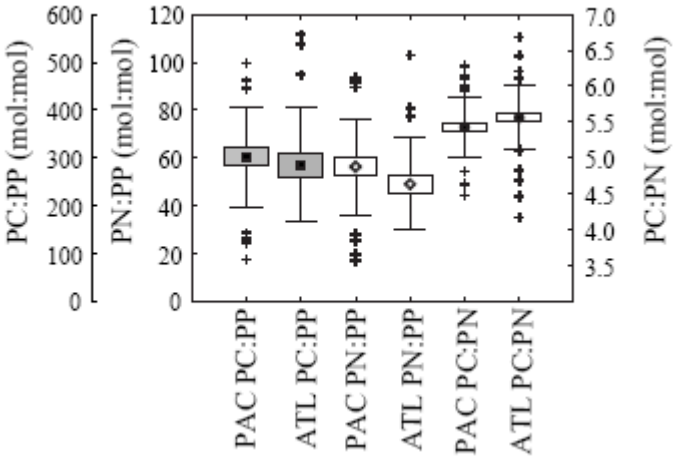

Figure 2-9. Box and whisker plot for elemental stoichiometry of natural populations of *Trichodesmium* from this study. Boxes represent the mean ± standard error. Whiskers indicate standard deviation. Plus signs indicate extreme values, i.e., those data points falling outside of the upper or lower box value ± 6 SE.

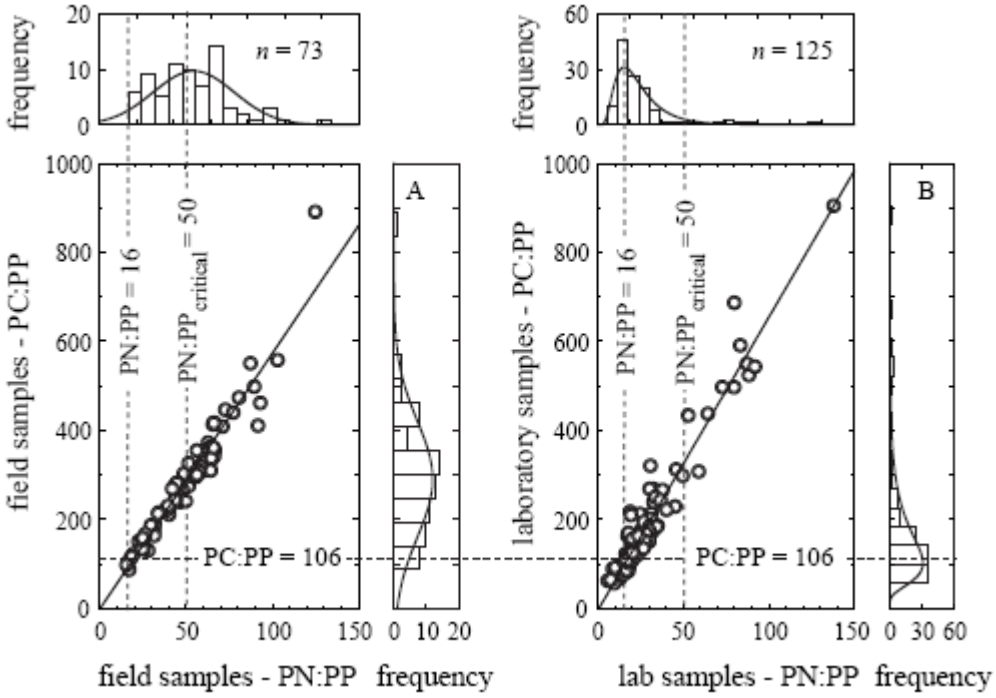

Figure 2-10. PN : PP versus particulate PC : PP for field and laboratory data sets from this study. Dotted lines show the Redfiels PC : PP (106), PN : PP (16) stoichiometry, and the critical PN : PP ratio (PN : $PP_{critical}$ = 50). Histograms for each axis are also shown. Field data conform to a normal distribution [*p* values for the Kolomogorov-Smirnov and Lilliefors tests for normality (Zar 1999) are not significant at the .05 level]. Laboratory data adhere to a log normal distribution.

Modeling carbohydrate ballasting by *Trichodesmium* spp. at Station ALOHA

Angelicque E. White, Yvette H. Spitz and Ricardo M. Letelier

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Nordbünte 23 (+21, 26, 28, 30)
21385 Oldendorf/Luhe, Germany
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3 Modeling carbohydrate ballasting by *Trichodesmium* spp.

3.1 Abstract

We report the development of a numerical model simulating vertical movement of the cyanobacterium, *Trichodesmium* spp. Given a range of physiological parameters derived from the literature, resultant model solutions allow us to explore the ecological significance of vertical migration by *Trichodesmium* colonies in a stratified oligotrophic oceanic system such as the North Pacific subtropical gyre (NPSG). Whereas the dominant type of model solution results in trapping of the migrating colony at the water surface, the next most frequent type of model solution is consistent with colony migration to the average depth of the phosphocline followed by return to the upper euphotic zone, a scenario termed P-mining. Given the temporal phasing of these migrations, our results indicate that while *Trichodesmium* colonies could fulfill their entire P-quota in a single phosphocline scale migration, the minimal colony size required for such a migration would be approximately 1000 μm in radius. Colonies of this size and larger have been observed in nature however neither the frequency of occurrence nor the relative size distributions of natural populations is known. Using published estimates of colony abundance at Station ALOHA (22º 45'N, 158º00'W, in the NPSG), we estimate that upward fluxes of P associated with *Trichodesmium* migration represent as much as 10% of the P-based export flux measured at this site. Inclusion of this simple model into coupled biophysical models should allow for better simulations of the vertical distribution of *Trichodesmium* biomass and thus improved representations of *Trichodesmium* productivity.

3.2 Introduction

3.2.1 Background

The colony-forming marine cyanobacterium *Trichodesmium* has been well described as a prominent diazotroph (i.e. nitrogen fixer, literally "two nitrogen eater") in oligotrophic tropical and subtropical regions of the global ocean (Capone 2001, Mulholland & Capone 2001, Karl et al. 2002). Primarily, this genus is a significant source of new nitrogen to otherwise nitrogen (N) deficient ecosystems (LaRoche & Breitbarth 2005). Furthermore, *Trichodesmium* spp. are well known to form visually conspicuous surface blooms (Bowman & Lancaster 1965, Thona 1991, Karl et al. 1992, Capone et al. 1998). During these bloom phases, *Trichodesmium* may transiently dominate primary productivity and N cycling (Karl et al. 2002).

A particularly striking feature of *Trichodesmium* autoecology is its ability to conduct bi-directional migrations in the vertical plane of the water column. As is common for many cyanobacteria, *Trichodesmium* possess low-density gas vesicles that generate strong positive buoyancy (Walsby 1978). Consequentially, *Trichodesmium* populations are able to accumulate at the sea surface during periods of calm weather. Contrasting the capacity for flotation is the frequent accumulation of *Trichodesmium* biomass in subsurface maxima (Letelier & Karl 1998, Carpenter et al. 2004). Consequently, such occurrences imply that this genus actively regulates its buoyancy, again a common trait of gas-vacuolate cyanobacteria (Oliver 1994). It has been found that *Trichodesmium* counteract the positive buoyancy provided by gas vacuoles via the photosynthetic production of carbohydrates (Villareal & Carpenter 1990). The balance between the production and respiration of these dense biomolecules regulates buoyancy by altering the ballast of the cell, i.e. the difference in density between the cell and the surrounding seawater.

In the North Pacific subtropical gyre (NPSG), one of the enigmas surrounding *Trichodesmium* is how this organism obtains the necessary phosphorus (P) required to support the formation of regular summer blooms (Karl et al. 1992). NPSG *Trichodesmium* blooms occur under calm conditions, after extended summer and early fall stratification periods, indicating that deep mixing events can be eliminated as a potential P supply mechanism. Karl et al. (1992) has suggested that the requisite P could be obtained by vertical migration to the depths of the phosphocline, a scenario

termed P-mining. P-mining requires that *Trichodesmium* migrate to depths of approximately 100 to 150 m to reach the phosphocline, store intracellular P under light-limited, P-rich conditions and subsequently return to the euphotic zone. Shuttling P from the phosphocline would allow migrating colonies to maintain balanced growth in surface waters at the expense of stored P obtained at depth (Karl et al. 1992).

Previous work in the NPSG (Letelier & Karl, 1998) found that rising colonies are more P enriched relative to sinking colonies, a disparity coherent with P acquisition from depth. Furthermore, recent work by Villareal and Carpenter (2003) reports that differences between pooled N:P ratios of rising vs. sinking colonies collected in the Gulf of Mexico were also consistent with the scenario of P-mining by *Trichodesmium*. However, this conclusion was not supported by data collected off North Australia by these same authors. These contrasting results suggest that either P-mining is intermittent in time or this behavior is confined to specific oceanic regions. Direct confirmation of *Trichodesmium* P-mining in the NPSG would require substantial ship time and would thus be labor intensive and costly. For these reasons, we have explored the possibility of *Trichodesmium* P-mining from a modeling perspective, drawing upon the empirical literature regarding carbohydrate ballasting in limnetic cyanobacteria. This theoretical approach is not intended to provide evidence for migratory P-mining. Rather the objectives of this research are to explore the hypothesis of P-mining in the NPSG using realistic parameterizations of buoyant density change to simulate vertical movement of *Trichodesmium* colonies, and to provide insights that could inform future experimental work in the field and laboratory.

3.2.2 Modeling Carbohydrate Ballasting in Cyanobacteria

Several laboratory experiments have been conducted in order to ascribe functionality to the carbohydrate ballasting response of cyanobacteria to light (Kromkamp & Walsby 1990, Visser et al. 1997, Wallace & Hamilton 1999).

Kromkamp and Walsby (1990) developed a model of vertical migration for the freshwater cyanobacterium *Planktothrix aghardii (*formerly *Oscillatoria aghardii*) based on the experimentally determined relationship between the rate of density change and photon irradiance (0-500 μmol photons m^{-2} s^{-1}). Density change as a function of irradiance was best fit with a rectangular hyperbola whereas density loss in the dark was determined to be a linear function of the previous light history (Kromkamp & Walsby, 1990). Kromkamp and Walsby (1992) later applied this empirically derived model to *Trichodesmium* and concluded that colonies could sink to depths of 100-200m. This model application, however, did not take into account water column density structure appropriate for seawater (as opposed to freshwater) nor were alternate parameterizations of density change considered given that the model was established for freshwater analogues. Clayton (2001) applied the model of Kromkamp and Walsby (1992) to *Trichodesmium* using constants suitable for seawater. This study limited analysis to the impact of single parameter variations upon model solutions without examining parameter interactions or accessing the effect of multiple parameter variations. Results emphasized the need for experimental validation of the buoyancy response of *Trichodesmium* to light.

Closely following the experimental design of Kromkamp and Walsby (1990), Visser et al. (1997) established an irradiance-response curve for another freshwater cyanobacterium, *Microcystis* spp. This work investigated the buoyancy response over a much wider range of irradiance (0-1600 μmol photons m^{-2} s^{-1}). *Microcystis* exhibited maximum rates of density change at ~300 μmol photons m^{-2} s^{-1} with strong inhibition of density change occurring at higher irradiances. This light-driven response was best fit with a negative exponential function including inhibition. The rate of density change in the dark was expressed as a linear function of the initial density of the cells prior to dark initiation (Visser et al. 1997). The final empirically derived model of carbohydrate ballasting is that of Wallace and Hamilton (1999). In this study, the authors observed that the density change response of *Microcystis aeruginosa* was quite similar to that exhibited by Kromkamp and Walsby (1990) for *Oscillatoria aghardii*. Considering that multiple cyanobacterial species have been shown to exhibit similar

density change responses to irradiance and that carbohydrate ballasting has been confirmed for *Trichodesmium* spp (Villareal & Carpenter 1990, 2003), we propose to adopt the general structure of these limnological models to explore vertical migration and the possibility for P-mining by *Trichodesmium* spp. in the North Pacific subtropical gyre.

3.3 Model description and assumptions

Previous models describing the rate of density change by gas-vacuolate cyanobacteria indicate that the functional response to irradiance can be modeled with either a rectangular hyperbola assuming no photoinhibition (Kromkamp & Walsby 1990) or a negative exponential function including photoinhibition (Visser et al. 1997). Both of these models are based upon empirical data showing that density increases are driven by photosynthesis, thus the shape of these functions mirror generalized models of photosynthesis (P) versus irradiance (E). Given that the density response of *Trichodesmium* has not been determined, we suggest that any P vs. E relationship shown to be applicable to *Trichodesmium* colonies should be a valid first approximation for light-driven density change by this oceanic cyanobacterium. LaRoche and Breitbarth (2005) have summarized the physiological literature regarding *Trichodesmium* and noted that only four studies (e.g. Li et al. 1980, Lewis et al. 1988, Kana 1993, Roenneberg & Carpenter 1993) have characterized the relationship between *Trichodesmium* photosynthetic rates and photon flux density. While a few studies have observed photoinhibition at moderate irradiances (e.g. Li et al. 1980, photoinhibition detected at $E > 500$ μmol photons m^{-2} s^{-1}), the predominant finding has been that *Trichodesmium* colonies are able to tolerate high irradiances (up to 2700 μmol photons m^{-2} s^{-1}) without apparent signs of photoinhibition (e.g. Lewis et al. 1988, Kana 1993, Carpenter & Roenneberg 1995). Considering these observations, we have chosen to utilize a negative exponential model (similar to Platt et al. 1980) to model the light-driven rate of density change by *Trichodesmium*. This model was

chosen for the reason that, (1) it has been shown to apply to the photosynthetic response of natural populations of colonies (Lewis et al. 1988), (2) it is consistent with previous models of density change in gas-vacuolate cyanobacteria and (3) it can be parameterized to disregard or consider photoinhibition.

The equation for the temporal rate of density change by a single *Trichodesmium* colony (Equation 1) is then written as

$$\frac{d\rho_c}{dt} = c_1 \cdot \left(1 - e^{-PAR_z\, m/c_1}\right) \cdot e^{-\beta\, PAR_z\, m/c_1} - \left(c_2 E_a\right) - c_3 \qquad \text{Eq. 3-1}$$

where the first term represents the light-driven rate of density change and is a function of a rate coefficient describing the maximal rate of density increase (c_1), the initial slope of the density-response curve (m), a photoinhibition parameter (β) and incident PAR at the depth of the colony (PAR_z). The rate of density change over time reflects the accumulation and degradation of excess carbohydrate density and is most sensitive to changes in c_1 and m, by nature of the prescribed functionality. The coefficient c_2 describes the relationship between the integrated value of irradiance accumulated since dawn, E_a (as per Kromkamp & Walsby, 1990), and density loss. The constant, c_3, represents the minimal rate of density loss due to the basal metabolism of carbohydrates. This loss term is independent of irradiance.

The daily cycle of PAR reaching the sea surface (PAR_0) at Station ALOHA (latitude = 22°45'N) is calculated following the classical astronomical formula of Brock (1981) employing the correction of the solar constant by Duffie and Beckman (1980). All model runs begin on July 1, 2005 such that solar forcing is consistent with summer fluxes of irradiance. Cloud-free days are assumed. The photosynthetically active irradiance at the depth z of the colony (PAR_z) is calculated according to the Beer-Lambert law (Equation 2) using a vertical attenuation coefficient (k_{PAR}=0.04 m^{-1} as per Letelier et al. 2004) appropriate for Station ALOHA.

$$PAR_z = PAR_0 \cdot \exp^{(-k_{PAR}\, z)} \qquad \text{Eq. 3-2}$$

During periods of darkness, the first term of Equation 1 goes to zero and the rate of density change becomes a combination of a linear light history term ($c_2 \cdot E_a$) and a constant loss term (c_3). These terms follow the formulation of Kromkamp and Walsby (1990), given that there is no data with which to determine the specific dark period density response of *Trichodesmium*.

3.3.1 Vertical Velocity

Once the density of the colony has been calculated, Stokes' Law is applied to estimate sinking or rising velocity (v) (Equation 3). This calculation is dependent on gravitational acceleration (g), the effective colony radius (r), the difference between the density of the colony (ρ_c) and the density of seawater (ρ_w), the molecular viscosity of the medium (η), the ratio of cell volume to colony volume (A) and a coefficient of form resistance (ϕ). Values or ranges for each of these parameters are listed in Table 3-1. The chosen value of A is specific to the near-spherical colony shape of *Trichodesmium thiebautii* (the so-called puff morphology). Water column density (ρ_w) structure is derived from the density profiles collected during the summer of 2004 at Station ALOHA (Figure 3-1).

$$v = \frac{2}{9} g r^2 (\rho_c - \rho_w) A / (\phi \eta) \qquad \text{Eq. 3-3}$$

Colony density (ρ_c) is constrained by minimum (ρ_{min}) and maximum density (ρ_{max}) values. The range for these parameters reported in the literature, a reference parameter set chosen to apply to *Trichodesmium,* and the range of values used for a sensitivity analysis are presented in Table 3-2.

Table 3-1. Parameter values for Stokes' law. References for each parameter are noted at the bottom of the table.

Parameter	Definition	Parameter range	Units	Source
r	Colony radius	Up to 2500	µm	Capone et al. (1997)[b]
g	Gravitational acceleration	9.8	m s^{-2}	Crowell (1998)
ρ_c	*Trichodesmium* colony density	Variable	kg m^{-3}	See Table 3 for ranges
ρ_w	Seawater density[a]	Variable	kg m^{-3}	See Fig. 1
η	Dynamic viscosity	9.60×10^{-4}	kg m^{-1} s^{-1}	Sverdrup et al. (1946)
A	Cell volume colony volume^{-1}	0.05		Kromkamp & Walsby (1992)
ϕ	Form resistance	1		Kromkamp & Walsby (1992)[b]

[a]Density profile reflects average summertime water column structure at Stn ALOHA; [b]radial or puff colonies of *T. theibautii*

Table 3-2. The model was run for successive parameter combinations, whereas each parameter range tested is consistent with the range of empirically determined values drawn from the literature. Increment values for each parameter are shown in parentheses. From the 3600 model solutions, a single parameter set (of Type 3), termed the P-mining solution, was chosen to represent a parameterization that would facilitate P-mining by *Trichodesmium* in the NPSG.

Parameter	Definition	Units	Literature range[a]	Parameter range	Increment value	P-mining solution
c_1	Max. rate of density increase	kg m^{-3} min^{-1}	0.043 to 0.161	0.033 to 0.170	0.033	0.1
c_2	Previous light history interaction term	(kg m^{-3} min^{-1}) / µmol photons m^{-2} s^{-1}	1.67×10^{-5} to 7.67×10^{-5}	1.53×10^{-5} to 1.38×10^{-4}	6.14×10^{-5}	2.56×10^{-5}
c_3	Min. rate of density loss	kg m^{-3} min^{-1}	0.0165 to 0.023	0.023		0.023
m	Initial slope of density-response curve	(kg m^{-3} min^{-1}) / µmol photons m^{-2} s^{-1}	1.6×10^{-3}[b]	1.76×10^{-4} to 8.78×10^{-4}	1.8×10^{-4}	5.27×10^{-4}
beta	Photoinhibition parameter	Dimensionless	None	0 to 0.06	0.03	0.03
ρ_{min}	Min. colony density	kg m^{-3}	920 to 970	920 to 1010	30	950
ρ_{max}	Max. colony density	kg m^{-3}	1035 to 1065	1020 to 1065	15	1065

[a]Parameter values from Kromkamp & Walsby (1990), Visser et al. (1997), Wallace & Hamilton (1999)
[b]Value from the nonlinear regression analysis of Visser et al. (1997), considered similar to parameter m

3.3.2 Model Assumptions

This model does not include *Trichodesmium* growth, nutrient uptake or loss processes, rather the only physiological processes that are considered are the light-driven rates of accumulation and degradation of excess carbohydrate density. Given the lack of empirical information regarding rates of density change by *Trichodesmium* in response to environmental parameters, we have utilized a wide range of parameter space. Perhaps the greatest sources of error inherent in our model formulation are that

(1) we have considered the effects of physical forcing to be negligible and (2) we have assumed that the only variable biomolecular component of the cell affecting density is carbohydrates. In regards to the first point, *Trichodesmium* spp. typically bloom under calm, stratified conditions, suggesting that our first results are valid when applied to summer populations. Secondly, intracellular concentrations of other biomolecules (e.g. proteins, lipid, polyphosphate) and the nutritional history and composition of the cell may also affect cell density and rates of density change (see review of Oliver 1994). While we acknowledge these points, given the quantity of data that supports light-driven carbohydrate ballasting as a mechanism of buoyancy regulation by *Trichodesmium* (Walsby 1992, Villareal & Carpenter 1990, Letelier 1994) we have reduced the problem so as to present an assessment of vertical migration, as it would be driven by carbohydrate ballasting alone. This approach allows for the prediction of rates of density change, vertical colony velocities, migratory amplitudes and depth distributions of colonies that can be compared to observations from natural populations.

3.4 Results

The model was run using successive parameter combinations of c_1, c_2, m, β, ρ_{min} and ρ_{max} resulting in a total of 3600 unique model solutions. The range of each parameter tested is consistent with the range of empirically determined values drawn from the literature. For all of these model runs the starting depth was 5m, colony radius was 1500 μm, initial colony density was 1025 kg m^{-3} and the initial integrated light history (E_a) was set to 650 μmol photons m^{-2}. The parameter c_3 was held constant at 0.023 kg m^{-3} min^{-1}, thus the parameterizations presented here have analyzed variance of light-dependent parameters, only.

Model solutions (n= 3600) for the full range of parameters cited in Table 3-2 have been sorted into five types (Figure 3-2) based upon the following criteria applied to solutions after a 4-day spin up period. Fifty-nine percent of the model solutions

result in the colony being trapped at the water surface; these solutions are classified as Type 1. Another type of solution (10%) exhibits colonies that return to the water column surface without becoming trapped. The maximum depth of migration for these solutions is less than 85 m; these solutions are classified as Type 2. An additional subset of migrations (25%) also exhibits a return to the surface without trapping, however these colonies migrate to a maximum depth greater that 85 m. These migrations are classified as Type 3. A number of model solutions resulted in migrations that never returned to the water column surface after the spin-up period. Of these solutions without a surface component, 5% (of total) are characterized by migrations with a minimum depth of less than 25 m (Type 4) and 1% (of total) result in trapping of the colony below 25 m (Type 5). The ranges of parameters for each type, the characteristic rates of density change as well as the mean and maximum depths achieved by a migrating colony (radius = 1500 μm) after a 4-day spin up period are presented in Table 3-3. Of the possible types of model solutions (Figure 3-2), Type 3 and a portion of Type 4 solutions (n=698) represent parameterizations of carbohydrate ballasting that could facilitate *Trichodesmium* P-mining at Station ALOHA.

Table 3-3. Parameter range for the five types of model solutions. For all types, the parameter c3 (the min. rate of density change) is equal to 0.023. When solutions encompassed the full range (FR) of a particular parameter, FR is noted (see Table 3-2). c_1: max. rate of density change; c_2: light history interaction term; m: initial slope of the density response curve; β: photoinhibition parameter; ρ_{min}: min. colony density; ρ_{max}: max. colony density. ρ_c: *Trichodesmium* colony density; t: time; z: depth

Type	Total (%)	c_1	c_2	m (10^{-4})	β	ρ_{min}	ρ_{max}	$\Delta\rho_c \Delta t^{-1}$ (kg m^{-3} min^{-1})	Max. z (m)
1[a]	59	FR	FR	FR	FR	FR	1020 to 1065	−0.121 to 0.144	0.02 ± 0.2
2[b]	10	0.07 to 0.17	FR	FR	FR	FR	1035 to 1065	−0.121 to 0.144	47.2 ± 23.8
3[c]	25	0.07 to 0.18	FR	FR	FR	FR	1035 to 1065	−0.116 to 0.144	176.9 ± 64.1
4[d]	5	0.07 to 0.19	FR	FR	FR	FR	1035 to 1065	−0.073 to 0.137	123.1 ± 25.6
5[e]	1	0.10 to 0.17	FR	5.3 to 8.8	FR	FR	1035 to 1065	−0.046 to 0.119	129.5 ± 7.8

[a]Max. depth = 0 m; [b]min. depth = 0 m, max. depth <85 m; [c]min. depth = 0 m, max. depth >85 m; [d]0 m > min. depth < 25 m; [e]min. depth > 25 m

Of the total number of model solutions, 698 (19.4% of the total # of parameter combinations) led to vertical migration to the average Station ALOHA phosphocline depth (125 ± 39m, calculated using a 0.05 μmol surface offset applied to June-August Hawaii Ocean Time-series (HOT) data from 1989-2001; summer data are used as this is when *Trichodesmium* blooms are typically observed). The modeled rate of density change over time was between –0.1062 and +0.1436 kg m^{-3} min^{-1}. From these solutions, we have chosen a single parameter set to illustrate the potential of P-mining. For further reference, this parameter set is termed the P-mining solution (Table 3-2). The rate of density change as a function of light as well as the temporal evolution of colony depth and colony velocity are shown for the P-mining solution in Figure 3-3. Modeled colony velocities (Figure 3-3C) are generally within the range of the velocity measured for *Trichodesmium* spp., 10.8 m day^{-1} (Walsby 1978).

The impact of colony size upon the mean and maximum depth of the P-mining solution is shown in Figure 3-4. This analysis suggests that a colony would need to be approximately 1000 μm in radius to reach the average position of the phosphocline during summer conditions at Station ALOHA. It should be noted that the maximum depth achieved by a colony does not increase steadily as a function of colony radius, rather there are values of increasing colony size that result in a shallower maximum depth (e.g. as observed between 1900 and 2000 μm). This effect is a consequence of colonies having variable light history, whereas the larger colony does not spend enough time in surface waters to incur the photon fluxes that would be required to generate migrations to a depth greater than that of a smaller colony (i.e. values for E_a remain relatively low so that the larger colony will not return to surface thus prohibiting large rates of ballast increase and limiting the maximum depth of migration).

The effect of alterations in water column density structure is presented in Figure 3-5 whereas the model was run using the upper and lower envelope of the data as well as the mean water column density fit derived from Figure 3-1. The major impact of these alterations in water column density structure is a change in the timing of migrations. We have also examined the model solution (using the P-mining

parameter set) for a 150-day runtime in order to assess the potential impact of the seasonal cycle of incoming irradiance upon migrations. Results indicate that the maximum depth of migration decreases by ~50 m in response to a decrease in PAR_0 as the season turns to winter (results not shown).

Additionally, this model has been run with randomized initial conditions in order to simulate a population of colonies with variable buoyancy characteristics. Specifically, 5000 colonies were assigned a randomly generated colony radius between 50 and 1500 μm, a starting depth between 5 and 85m, an initial colony density between ρ_{min} and ρ_{max} and initial E_a in the range of 0 to 650 μmol photons m^{-2} s^{-1}. The model was then run for a 15-day period using parameterizations typical of Type 2, 3 and 4 model solutions. Type 2 and 4 parameters are as follows: c_1= 0.1 (type2&4), c_2= 1.38E-4 (type2) and 1.54E-5 (type4), c_3 = 0.023 (type2&4), m= 5.3E-4 (type2) and 7.02E-4 (type4), β = 0.03 (type2) and 0.0 (type4), ρ_{min}= 950 (type2) and 1020 (type4), ρ_{max}= 1065 (type2) and 1035 (type4). The P-mining solution parameters were used for type 3 runs (Table 3-2). A random day was chosen from each run and the depth of each of the 5000 colonies was binned into 10 m intervals with a time-step of 5 minutes. Figure 3-6 represents the modeled depth distribution at each time-step (*n*=288) over the course of a single day compared to the actual distribution of colonies collected between April and September of 1989-1992 at Station ALOHA (Letelier 1994). The average depth of the mixed layer during summer (~45 m, Letelier 2004) and the phosphocline (125m) are shown in Figure 3-6 for reference. The range of colony concentration at each depth represents the spectrum of colony density that would be encountered over the course of a single day of sampling. These results indicate that vertically migrating cells may accumulate in discrete depth maxima and that the vertical position of these maxima will change over the course of the day.

3.5 Discussion

A common paradigm pertaining to cyanobacteria is that buoyancy regulation may be a physiological adaptation to exploit the separation of light and nutrients (Walsby & Reynolds 1980, Oliver 1994, Pearre 2003). However, this paradigm has been developed for mostly limnological systems where the depth of the nutricline is significantly less than that observed in oligotrophic oceanic habitats such as the NPSG. Given this implied constraint, questions have been raised regarding whether or not it is feasible for oceanic cyanobacteria to conduct the >100 m migrations that would be required to mine for P in the NPSG (see Villareal & Carpenter 2003). While migrations of this scale, accompanied by nutrient shuttling have been confirmed in the NPSG for large mats of the diatom *Rhizosolenia* (Richardson et al. 1998, Villareal et al. 1993) only ancillary data exist for NPSG *Trichodesmium* populations (Letelier 1994). To investigate this possibility, we have constructed a numerical model of *Trichodesmium* carbohydrate ballasting-mediated vertical migration, based upon existing empirical models developed and validated for other cyanobacterial genera. We have applied our results to an analysis of the ecological significance of vertical migration by natural populations of *Trichodesmium* colonies.

Of the 3600 parameter combinations tested, the numerically dominant type of solution was not P-mining, but rather trapping of the colony at the water surface. This type of solution occurred as a consequence of low rates of ballast accumulation ($\leq$ 0.066 kg m^{-3} min^{-1}) resulting in shallower initial migrations and higher rates of ballast respiration occurring in response to a high-irradiance light history (high E_a). The remaining fraction of these surface-trapped solutions occurred as a result of limitation of the maximum colony density to a value (ρ_{max} = 1020) that prohibited the colony from accumulating sufficient ballast to escape the surface. Observation of this type of solution is consistent with the findings by Kromkamp and Walsby (1992) and Clayton (2001), who have also described surface-trapped model simulations for *Trichodesmium* spp. Kromkamp and Walsby (1992) hypothesized that this type of model solution would result as energy generated by photosynthesis is used to fuel nitrogen fixation, thus effectively reducing rates of ballast accumulation. In contrast, Clayton (2001) has proposed that surface-trapping of populations would occur

following prolonged periods of water column mixing where decreased irradiance fluxes would result in lower carbohydrate accumulation rates. As mixing is relaxed, these low-density colonies would rapidly rise to the ocean surface, forming accumulations. Surface accumulations may also be generated by temperature-driven increases in respiration, expressed as an increase in c_3 in this model. In addition to lowered net carbohydrate accumulation rates, our model predicts that limitation of the carbohydrate storage capacity of the cell (expressed as lowered ρ_{max}) could also result in surface accumulations. Each of these hypotheses is independent of whether or not the surface population subsequently experiences de novo growth, i.e. blooms. Experimental determination of the range of de-ballasting rates specific to *Trichodesmium* as well as the boundaries of cell density (ρ_{min}, ρ_{max}) under different conditions (i.e. low light, high temperature, etc.) can be compared to the data presented herein in order to further explore hypotheses regarding the formation of surface blooms, undoubtedly a defining characteristic of *Trichodesmium* auto-ecology.

A considerable percentage (19.4%) of the model solutions (of Type 3 and Type 4 solutions) are consistent with P-mining by *Trichodesmium* such that the maximum depth of migration is ≥125 m. The range of net density change over time for P-mining solutions was between –0.1062 and +0.1436 kg m^{-3} min^{-1}. These parameters represent numerical constraints for the rate of density change that would be required to facilitate P-mining in the NPSG. Further analyses of these results indicate that the observed summertime fluctuations in water column density at Station ALOHA should have little effect on this maximum migration depth (Figure 5). In contrast, colony size significantly impacts model solutions. For P-mining solutions specifically, results suggest that colonies must be on the order of ~1000 μm in radius in order to penetrate the average depth of the phosphocline (Figure 4). Colonies of up this size and larger (i.e. 1000-2500 μm in radius; Capone et al. 1997) have been observed in nature however neither the frequency of occurrence nor the relative size distributions of natural populations is known. This sensitivity highlights the need for information regarding the morphological distribution of *Trichodesmium* populations. For the NPSG, Letelier et al. (1998) reports that the colonial morphology accounts for 12% of

Trichodesmium biomass with free filaments comprising the remaining fraction of the population. Neither the size distribution nor the relative shapes (e.g. fusiform, near-spherical) of these colonies has been determined. This information will be essential to an evaluation of the achievable vertical velocities of natural populations. Nonetheless, we can speculate that the size distribution of *Trichodesmium* colonies would be variable in time. In this case, colony formation and growth itself may facilitate P-mining; such that colonies may only become vectors for the upward flux of P once they reach a critical size (e.g. >1000 μm for near-spherical forms).

P-mining by *Trichodesmium* spp. may act as a purely biological means of transporting P from below the phosphocline into the surface mixed layer, thereby fueling net export. This behavior may also serve to supplement P-requirements for growth. We can approximate these potential P-fluxes by combining results from this model with published rates of colony abundance, P-uptake and P-export. Using the P-mining solution as an example, our model predicts that a migrating colony would spend up to 14 hrs below the average depth of the phosphocline, nearly all of which time the colony would be in the dark (see Figure 3). Using the mean $^{32}PO_4^{-3}$ dark period uptake rate of 0.09 nmol P colony^{-1} hr^{-1} reported by Letelier (1994), we estimate that a migrating colony would consume up to 1.3 nmol P per migration to the phosphocline. Given the mean P quotas exhibited by NPSG populations of *Trichodesmium* (0.91 nmol P colony^{-1}, White et al. 2006), an individual colony could potentially fulfill its entire P-quota during the 12-14 hrs spent below the phosphocline. If we then assume that the depth-integrated concentration of colonies that are able to conduct these migrations is large (2000 colonies m^{-2}, see Figure 4 for reference) and that phosphocline scale migrations occur with a frequency of 0.5 day^{-1} (Figure 3), we calculate that P-mining by *Trichodesmium* could result in P-inputs to the upper mixed layer that would be equivalent to as much as 10% of the mean P-based export flux measured at 150 m (400 μg m^{-2} day^{-1}). While, these back of the envelope calculations illustrate the potential for P-mining by *Trichodesmium* to supplement P-requirements for growth and fuel P-based export, the magnitude of our estimates should be considered upper limits given that we have not considered biomass loss terms.

P-mining is but one predicted type of migration. It may be the case that colony migrations at Station ALOHA more closely match another of the migration types (i.e. Type 1-4), presented here. Speculatively, if the light-driven density response of natural *Trichodesmium* populations were similar to one of the migration types that we described we would expect that this population would exhibit a depth distribution that would also be consistent with model predictions for that type of migration. In order to explore this further, we have compared (Figure 6) the depth distribution of 5000 colonies of randomly generated colony size and variable initial conditions having parameters typical of types 2, 3 and 4 model solutions to the actual depth distribution of *Trichodesmium* colonies collected during April through September of 1989-1992 (Letelier 1994). Specifically, modeled distributions simulate mixed populations of colonies of variable size having a wide range of starting depths, previous irradiance history and initial colony densities with parameters for the rate of density change typical of a single migration type. Comparison of the modeled distributions from each type (2,3 or 4) indicates that type 2 migrations are most consistent with actual colony profiles such that these profiles consist of colonies generally occurring above the surface mixed layer depth. There are a fewer number of observed colony profiles (marked with an asterisk in Figure 6) where a substantial fraction of the population occurs below the mixed layer. These profiles are generally consistent with the depth distributions that would result from simulated migrations where a fraction of colonies would be capable of P-mining (type 3 and 4). Type 3 and 4 distributions, however; appear to overestimate colony abundance in deep layers, suggesting that in nature a smaller fraction of the population would be of the radius required (e.g. 1100 μm for near-spherical colonies) to shuttle P from depth than was randomly generated in our model runs. Regardless of the type of migration simulated, model results indicate that sampling time should have a strong impact upon the perceived depth distributions of natural populations. Given the migratory capacity of *Trichodesmium*, sampling should be conducted at multiple times throughout the day and night and at multiple depths in order to accurately estimate the range of *Trichodesmium* colony profiles.

In conclusion, the output from this model suggests a range of net density change and colony profiles that can be compared to laboratory and field observations in order to more closely constrain the ecological importance of vertical migration by *Trichodesmium*. It should be cautioned, however, that while we have assumed that the timing of vertical migration is related to the light cycle in a diel sense, this does not explicitly imply that migrations occur daily, rather some stimulus may be necessary to drive carbohydrate ballasting. We have already discussed the potential of colony growth in relation to the amplitude of vertical migration; nutrient restriction of growth is another potential means by which the density response of the cell may be altered. It is well documented that nutrient limitation may result in increased carbohydrate accumulation rates (Oliver 1994). The progression of nutrient stress may then lead to the initiation of larger amplitude migrations by nutrient limited colonies, potentially resulting in P-mining.

It should also be noted that while the primary focus of this modeling effort has been an assessment of P-mining, it is likely that vertical migration may serve numerous functions. Specifically, vertical migration should provide a competitive advantage to migrating versus non-migrating colonies (of the same size) as movement alone, whether up or down should enhance P-diffusion close to the cell wall and facilitate increased P uptake relative to non-migrating colonies. Particularly for large colonies, movement may be an effective means to offset the disadvantage of a high surface area to volume ratio. Buoyancy regulation may also allow cells to escape potentially damaging photon fluxes. Following parameter calibration with observations, inclusion of this simple model into 1-D and 3-D models should allow for better simulations of the vertical distribution of *Trichodesmium* biomass and thus better representations of *Trichodesmium* productivity.

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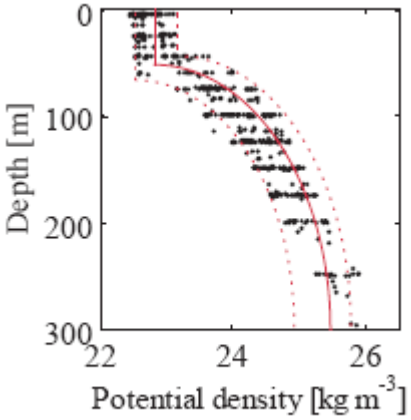

Figure 3-1. Station ALOHA water column density structure. Data are taken from Hawaii Ocean Time-series cruises 159-163 (May – Oct 2004). The data are fit to a piecewise model (solid red line) where potential density is constant (22.854) in the upper 45 m and determined with a 4th degree polynomial fit to the centered and scaled data set for depths greater than 45m. The dotted red lines show the upper and lower envelope of the data. For the lower range, the mixed layer depth is extended to 60 m, while the upper range mixed layer depth is 40 m.

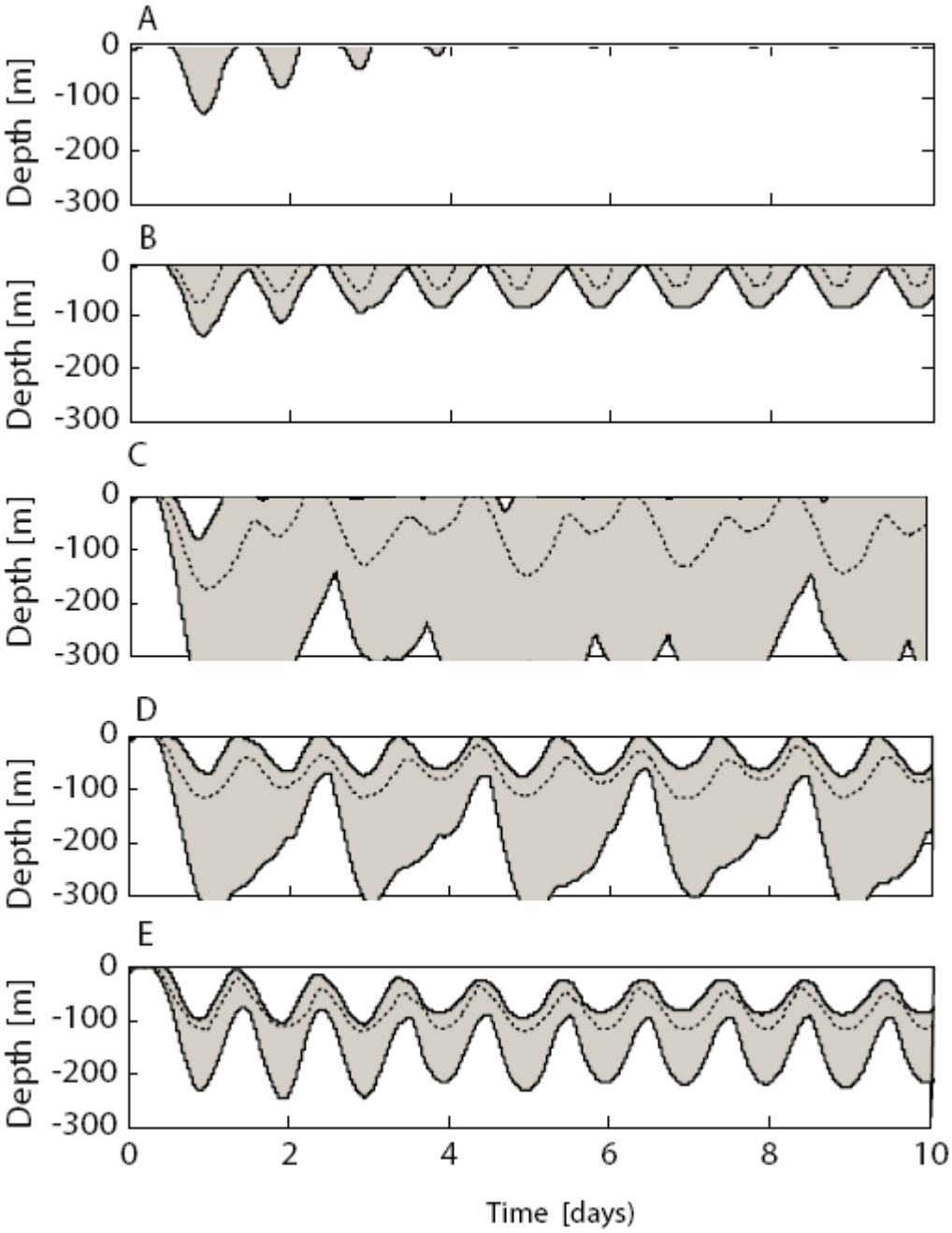

Figure 3-2. The depth envelope ([maximum-minimum depth] = shaded area) of colony depth versus time for model solutions (n= 3600) of Type 1 (A), Type 2 (B), Type 3 (C), Type 4 (D) and Type 5 (E) (see text for criteria). The mean depth for each type is denoted by a dotted line.

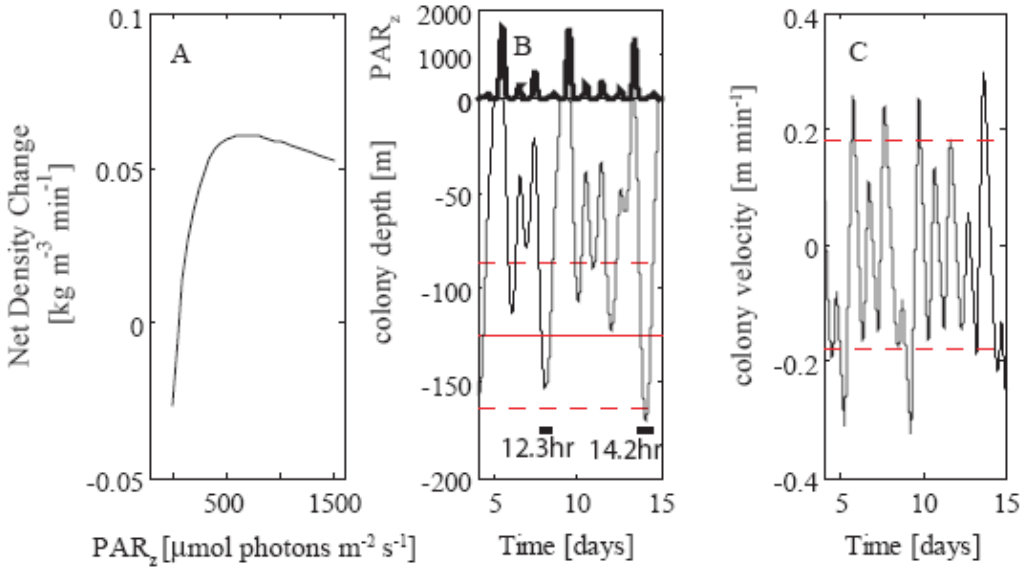

Figure 3-3. P-mining solution showing (A) the net rate of density change as a function of the PAR intensity at the depth of the colony (PAR_z). (B) the temporal evolution of colony depth for a 10-day period relative to PAR_z and (C) the vertical velocity of the colony over this same period. For this model run, as with all of the previous runs, the starting depth was 5m, colony radius was 1500 µm and the initial density of the colony was 1025 kg m^{-3}. The solid line in panel B represents the mean summertime (June-August) phosphocline depth (125 m) at Station ALOHA calculated using a 0.05 µmol L^{-1} surface offset. The dashed lines indicate the mean ± one standard deviation (38.7 m). The duration of two sections of the migration where depth was greater than 125 m are noted in panel B. The dashed lines in panel C represent the maximum vertical velocity recorded for a *Trichodesmium* colony (0.18 m min^{-1}, Walsby, 1978).

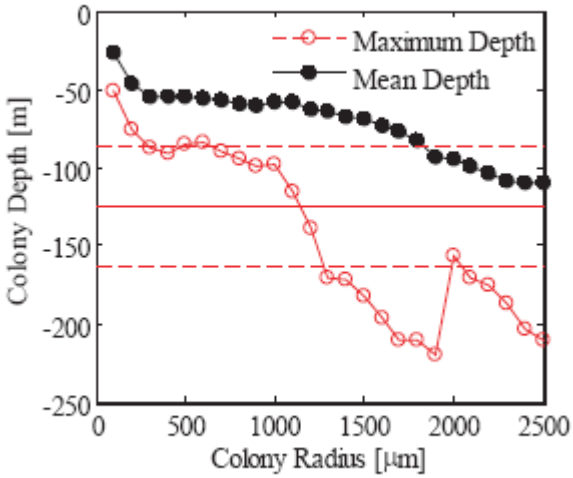

Figure 3-4. The consequence of varying colony size, using the P-mining parameter set, is shown for colony radii of 100 to 2500 μm. These results present the mean and maximum depths achieved by colonies of variable size over a 15-day model run, allowing for a 4-day spin up The solid line represents the mean summertime (June-August) phosphocline depth (125 m) at Station ALOHA. Dashed lines indicate the mean ± one standard deviation (38.7m). The nonlinearity observed in the maximum depth trend reflects the effect of variable light history (see results section).

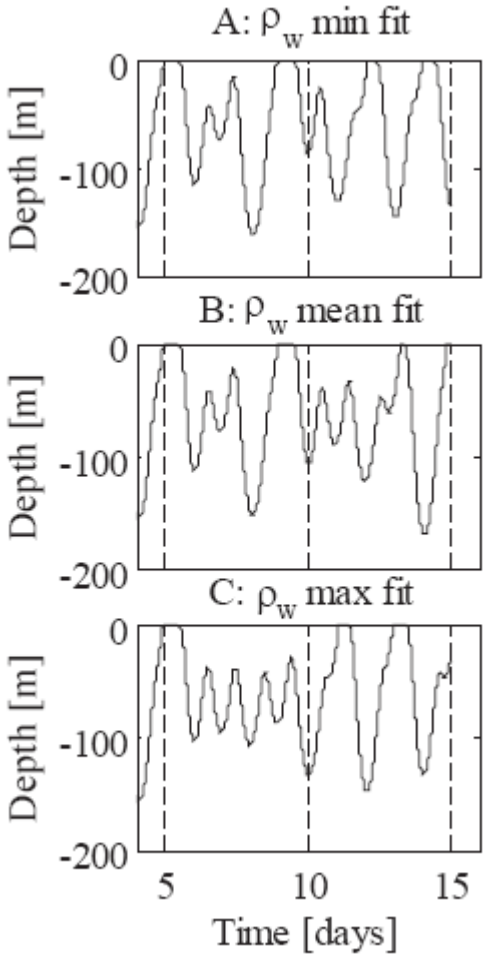

Figure 3-5. The major impact of alterations in water column density (ρ_w) structure is a change in the timing of migrations. Density alterations have little effect on the maximum penetration depth of a colony. Colony depth over time is shown for model runs using the lower envelope of ρ_w (A) the mean ρ_w fit (B) and the upper envelope of ρ_w (C).

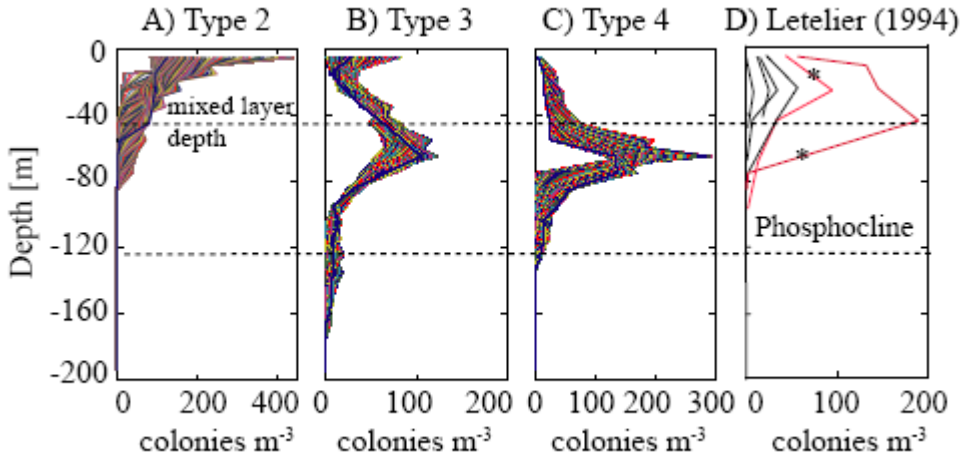

Figure 3-6. Comparison of the actual depth distribution of NPSG *Trichodesmium* colonies collected during the months of April through September, 1989-1992 (Letelier 1994) and the modeled depth distribution of 5000 colonies of random size having randomized initial conditions and parameters consistent with (A) Type 2, (B) Type 3 and (C) Type 4 model solutions (see text for additional run conditions). Model colonies were binned in 10 m intervals and resultant depth profiles of colonies at 5 minute intervals over an entire day (n=288) are plotted for each model type. The range of colonies m^{-3} at each depth then presents the span of colony density that would be encountered over the course of a day. Dashed lines denoted the mean mixed layer depth (MLD, 45m) and phosphocline depth (125m). Observed colony profiles consistent with P-mining are noted with an asterisk in the final panel.

4 Integrating remote sensing, moored and vessel based time-series records to quantify ecological determinants of summer bloom formation in the North Pacific subtropical gyre.

4.1 Abstract

Regularly observed summer to fall surface blooms of the N_2-fixing genera *Trichodesmium* and *Richelia* have a significant impact on biogeochemical cycling in the North Pacific Subtropical Gyre (NPSG). Yet, the environmental forcing of these blooms has not been thoroughly resolved. I have combined remote sensing of ocean color and environmental measures (SST, SSH, wind, integrated irradiance) with the vessel-based time series of the Hawaii Ocean Time-series (HOT) and mooring data derived from the National Data Buoy Center (NDBC) buoy 51001 in an attempt to define the ecological window under which blooms of large cell-sized N_2-fixing organisms increase in abundance in NPSG surface waters. For identified bloom events, these analyses indicate that blooms are confined to SST in the range of 25-27ºC and mixed layer depths less than 70m. Neither wind forcing nor SSH seem to determine increases in diazotroph abundance. Furthermore, blooms do not consistently result in increases in remotely sensed chlorophyll *a*. Additional higher resolution data sets of physical forcing, diazotroph abundance and biochemical properties, sampled on the time-scale of bloom development (days-weeks) will be necessary to define the ecological determinants of N_2 fixation in the NPSG.

4.2 Introduction

The export of biologically produced carbon from the euphotic layer to the ocean interior, the biological pump, is a major component of the earth's carbon cycle. Considering that the oligotrophic subtropical gyres represent ~60% of the global ocean

area and are responsible for a significant fraction of global ocean primary and export production (Emerson et al. 1997), it is apparent that these regions are critical to an understanding of the oceanic carbon cycle and its response to climate change. Since October 1988, the continuous observation and study of the oligotrophic North Pacific Subtropical Gyre (NPSG) by the Hawaii Ocean Time-series (HOT) program has provided a wealth of information documenting seasonal fluctuations and interannual variability in a number of water column properties (Karl and Lukas 1996).

It is now realized that oceanic gyres can account for up to half of the total organic carbon export of the global ocean (Emerson et al. 1997; Lee 2001). For the NPSG specifically, a significant fraction of annual export occurs during the summer in response to regular nitrogen fixation supported blooms developing under stratified water column conditions. Using DIC and $\delta^{13}C$ abundances Quay and Stutsman (2003) estimate that the biologically mediated summer drawdown of DIC at Station ALOHA (22.75ºN, 158ºW) accounts for ~60% of the total organic carbon export in this area. Thus, given that these summer blooms are a predominant control of the oceanic sequestration of atmospheric carbon in the NPSG, a mechanistic understanding of the regulation of these export events would enhance our knowledge of the ocean's role in the cycling of elements.

4.3 Scientific Background and Research Objectives

Contemporary subtropical gyre systems consist of an autotrophic community structure dominated by small cell size phytoplankton assemblages and regenerated production (Letelier et al. 1996). In these stratified, oligotrophic regions, it is thought that only relatively short lived perturbations such as deep vertical mixing events followed by water column stratification, the passage of cyclonic eddies (Letelier et al. 2000), Rossby waves (Sakamoto et al. 2004) and the breaking of internal waves can allow for an uncoupling of autotrophic production and community respiration by lessening nutrient limitation in the euphotic zone. Such uncoupling may lead to

increases in autotrophic biomass and export fluxes. However, large cell size, surface and subsurface phytoplankton summer blooms recorded at Station ALOHA (22°45'N; 158°W) appear to contradict the above scenario (Brzezinski et al. 1998; Karl et al. 2001; Karl et al. 1992). From an ecological perspective, these blooms represent an important shift in our conceptual model of how productivity is enhanced in oligotrophic marine environs. NPSG blooms (Figure 4-1) occur under calm conditions, after extended summer and early fall stratification periods, indicating that deep mixing events are not the dominant mechanism driving bloom formation. Chlorophyll *a* (chl *a*) concentrations during these blooms can reach >40 mg m^{-3} (Karl et al. 1992) and the subsequent carbon (Figure 4-1B) and silicate export represents the largest biogenic flux reaching the benthic environment in this area (Karl et al. 1992; Scharek et al. 1999; Smith et al. 2002). Repeated sampling of bloom events throughout the HOT program has allowed for their characterization as phytoplankton corresponding to the diazotrophic genus *Trichodesmium* and/or the diatom-cyanobacteria symbioses *Rhizosolenia-Richelia and Hemiaulus-Richelia.* All identified taxa possess the capacity fix N_2 (Karl et al. 1997), directly or via symbiosis (Brzezinski et al. 1998).

It has been shown by several authors that surface bloom forming genera *Trichodesmium* and *Richelia* have a significant impact on the biogeochemical cycling and fluxes of elements in the NPSG (Capone 2001; Karl et al. 1992; Venrick 1974). Yet, the physiochemical forcing of these bloom events has not been thoroughly resolved by regular monthly field sampling. Within the past five years, two quantitative bio-optical models have been developed to detect one of the major bloom-forming genera in this region, *Trichodesmium,* via remote sensing (Subramaniam et al. 2002; Westberry and Siegel 2006). Using empirical observations and results from a bio-optical model, Subramaniam et al. (2002) proposed that *Trichodesmium* concentrations of 0.5-3.0 mg chl m^{-3} could be identified from SeaWiFS imagery based on the magnitude of the 490-channel reflectance and the spectral shape of remote sensing reflectance at 443, 490 and 555 nm. Westberry and Siegel (2006) have recently tested this classification scheme using a compilation of coincident

measurements of ocean optical properties and *Trichodesmium* abundances compiled from multiple cruises throughout the subtropical oceans from 1994 to 2005. Application of the Subramaniam et al. (2002) classification scheme failed to identify *Trichodesmium*. Westberry and Siegel (2006) also generated a bio-optical model of their own. This model, however, was unable to distinguish *Trichodesmium* from other phytoplankton at low to moderate chlorophyll values.

Given that direct remote sensing approaches have yet to be successful, at least for *Trichodesmium*, I have tried to quantitatively define the ecological determinants of bloom formation specific to N_2-fixing organisms. Here ecological determinants refer to the physical and chemical forcing that determine bloom versus non-bloom conditions specific to large diazotrophs. In this regard, it is often noted that blooms in the NPSG are coincident with calm, warm, well-stratified and well-lit surface waters (Karl et al. 1997). Many of the forcing agents that are seemingly driving these diazotrophic blooms can be measured remotely from space [e.g. sea surface temperature (SST), photosynthetically active radiance (PAR), sea surface height (SSH)] and/or obtained from local moorings (e.g. wind speed). Given the availability of these data sets, I have examined existing remotely sensed and moored time-series to determine whether or not significant relationships between previously hypothesized physical determinants of bloom formation (e.g. SST, wind speed, PAR, MLD) and episodic increases in the relative abundance of the key-bloom forming genera of the NPSG (*Trichodesmium* and/or *Richelia* symbioses) can be defined. I also seek to identify potential threshold values of these physical forcings in relationship to bloom events. Bloom events are identified using data obtained from monthly HOT cruises to Station ALOHA.

4.4 Data Sources and Bloom Identification

4.4.1 Satellite-derived data

Sea surface temperature (SST)] and chlorophyll *a* (chl *a*) data for the region between 15-30°N and 165-150°W were obtained from the daily, 9-km, level-3

MODIS (MODerate resolution Imaging Spectoradiometer) AQUA data records for the period from July 2002 to present. I have also used the daily 9-km, level 3 SeaWiFS (Sea-viewing Wide Field of view Sensor) chl *a* data for the same region. From these regional data sets, I have extracted values for the ~9 km pixel covering station ALOHA as well as the mean and standard deviation for a 3 x 3 pixel (approximately 27 km x 27 km) region surrounding Station ALOHA.

Sea surface height anomalies (SSHa) for Station ALOHA was obtained from weekly, ⅓º by ⅓º resolution, merged TOPEX/Poseidon and ERS satellite altimetry data. These altimeter products were produced by Ssalto/Duacs and distributed by Aviso, with support from Cnes. Spatially, the ⅓º by ⅓º grid point encompasses Station ALOHA and the 27 x 27 km region for which MODIS derived SST and chl *a* are presented. Contour plots of SSH for the region surrounding Station ALOHA were also generated from the Aviso live access server.

Daily (24-hr averaged) Photosynthetically Active Radiation (PAR) was obtained from SeaWiFS 8-day, 9-km, level-3 data. As was done for SST and chl *a,* data is presented for mean of a 3 x 3 pixel (approximately 27 km x 27 km) region surrounding Station ALOHA. Data were binned by month to obtain monthly mean and standard deviations. Using this PAR data set in addition to mixed layer depths (MLD) determined from HOT cruises, the MLD integrated photon flux was calculated using the Beer-Lambert law (Mann and Lazier 1996), an attenuation coefficient appropriate for Station ALOHA summer conditions (0.04 m^{-1} as per Letelier et al. 2000), and a trapezoidal integration scheme.

4.4.2 Moored data sources

Wind speed and sea surface temperature were obtained from the historical data archives of the National Data Buoy Center (NDBC) buoy 51001. This NDBC buoy is deployed at 23.43º N 162.21º W. Obtained data files reported all meteorological observations at hourly intervals. For the purposes of this analysis, I have further

smoothed these data to daily resolution so that the variability is on the generational scale of the organisms of interest (days).

4.4.3 Shipboard observations

Size-fractionated phycoerythrin, nutrient concentrations and the isotopic composition of the particulate nitrogen pool were obtained from the web-based data extraction program of the Hawaii Ocean Time-series project. For each of these biochemical properties, I present the mean surface ocean (5-25m) values at the near monthly sampling resolution of the HOT program for the full period of phycoerythrin data availability (June 2000 to December 2004). HOT data are used to define instances of cyanobacterial surface blooms. Mixed layer depth (MLD) was calculated using a potential density slope difference of 0.125 (as per Huang and Russell 1994).

4.4.4 Harmonic regression of SST data

To analyze annual variation of SST in the NPSG, I have fit MODIS, NDBC buoy 51001 and HOT SST climatologies to a harmonic regression model. Differences between the model prediction of SST and the actual data were then used to explore potential relationships between SST anomalies (warm and cool) and bloom formation. The model used was a five-parameter regression model (R) composed of a constant plus annual and semi-annual harmonics (1 and 2 cycles per year or a frequency, f, equal to one per 12 months).

$$R = c_1 + c_2\sin(2\pi ft) + c_3\cos(2\pi ft) + c_4\sin(4\pi ft) + c_5\cos(4\pi ft) \quad \text{Eq. 4-1}$$

The fit of each model was assessed by comparing the square of the sample correlation between the estimand and the model estimate (i.e. model skill) to the 95% significance level. All model fits were significant at the 95% significance level. Autocorrelations of model residuals were examined to assess the potential for missed

harmonics or trends. None were found. Neither the MODIS nor the NDBC data records were found to differ significantly from the HOT time-series of SST (t-test assuming separate variance, p= 0.7 and p= 0.12 for MODIS and NDBC respectively when compared over the same time periods)

4.4.5 Bloom identification

Since June of 2000, the HOT program has routinely measured concentrations of the water-soluble cyanobacterial pigment, phycoerythrin (PE), in multiple size-fractions (>0.4 μm, >5 μm, and > 10 μm) at Station ALOHA. Given the relatively large cell sizes of *Trichodesmium* and *Richelia* symbioses (10's – 100's of μm), these organisms would be retrieved in the greater than 10 μm size fraction of PE (PE_{10+}). Thus, for the purposes of identifying surface blooms of N_2-fixing cyanobacteria, I consider the concentration of PE_{10+} in the top 25 meters of the water column at Station ALOHA as a biomarker for *Trichodesmium* and *Richelia* blooms. The composite PE_{10+} data at this oceanic site are log-normally distributed with near annual increases occurring in summer to early fall months (June through October). From the range of these data, I have chosen to define a diazotrophic surface bloom using a threshold PE_{10+} value of 6.7 ng L^{-1}, the equivalent of one positive standard deviation (σ = 5.3 ng L^{-1}) from the mean (μ=1.4 ng L^{-1}). Support for our supposition that these increases in cyanobacterial pigment are associated with diazotrophy is found in the coincident isotopic composition of the particulate nitrogen pool and the relative change of low-level dissolved nitrogen (LLN) and phosphorus (LLP) (Table 4-1).

The ratio of nitrogen isotopes ($^{15}N/^{14}N$ expressed as $\delta^{15}N$) measured in particulate material ($\delta^{15}N_{PN}$) reflects the isotopic composition of the nitrogen source used by biota as well as the biological fractionation that occurs during uptake and assimilation (Mahaffey et al. 2005). In times of appreciable N_2 fixation the biological input of atmospheric N_2 (~ 0‰) would lead to very significantly reduced values of $\delta^{15}N_{PN}$. Alternately, if N_2 fixation were negligible and nitrate was completely depleted in the mixed layer, the average $\delta^{15}N$ of the particulate pool should reflect the $\delta^{15}N$ of

subsurface nitrate (~ 6.5‰, (Wada and Hattori 1991). Using a simple two end-member mixing model having terminal values for N_2-fixing organisms ($\delta^{15}N_{N2}$ = -2‰ as per (Carpenter et al. 1997) and the supply of deep nitrate ($\delta^{15}N_{NO3}$ = 6.5 as per (Wada and Hattori 1991) I have calculated the relative contribution of nitrogen fixation to the particulate matter observed in the surface mixed layer ($\delta^{15}N_{PN\ obs}$) as follows:

$$\text{Estimated contribution from } N_2 \text{ fixation} = \frac{\delta^{15}N_{PN\ obs} - \delta^{15}N_{NO3}}{\delta^{15}N_{N2} - \delta^{15}N_{NO3}} \qquad \text{Eq. 4-2}$$

Using this mixing model, where the $\delta^{15}N_{PNobs}$ measured at the time of the cyanobacterial blooms was typically less than 0‰, I calculate that N_2 fixation supplied 75-92% of the PN in the mixed layer during these bloom events (Figure 4-2).

Additionally, for nearly every bloom identified by PE_{10+} concentrations, a drawdown of LLP and an increase in LLN was observed to occur in the top 25m of the water column at Station ALOHA (Table 4-1). These elemental dynamics are consistent with P utilization in the absence of N uptake, a hallmark of N_2-fixing organisms. Furthermore, as Mulholland et al. (2006, 2004) have shown that *Trichodesmium* release ~50% of their recently fixed N as DIN and DON, the increased LLN concentrations observed to coincide with PE_{10+} blooms may be a result of N excretion. In combination, cyanobacterial pigment, elemental and isotopic data records are consistent with the rapid increase of large-celled diazotrophs, putatively *Trichodesmium* and/or *Richelia*, at the defined dates.

Table 4-1. Dates of large cell-sized cyanobacterial blooms identified from the HOT time-series, PE_{10+} concentrations, the relative change of low-level nitrogen (LLN) and phosphorus (LLP) and the isotopic composition of particulate nitrogen ($\delta^{15}N_{PN}$).

sampling date	mean (stdev) 0-25m PE_{10+} [ng L^{-1}]	% change in LLP [nmol L^{-1}]*	% change in LLN [nmol L^{-1}]*	$\delta^{15}N$ of PN**
6/14/2001	6.8 (3.9)	-11%	61%	-1.30
8/8/2001	18.9 (11.4)	-70%	350%	0.10
10/2/2001	28.1 (11.9)	-8%	56%	-1.01
10/23/2001	49.0 (10.3)	-21%	107%	-0.83
10/7/2002	17.2 (7.0)	-33%	20%	-0.02
7/20/2003	16.4 (13.5)	-106%	0%	-0.18
10/15/2003	75.5 (27.3)	-39%	110%	-0.24
6/16/2004	12.7 (6.5)	-10%	47%	-0.17
7/13/2004	7.1 (0.7)	25%	-32%	0.16

* The percent change of LLP and LLN was calculated from the difference of the mean 0-25m nutrient concentrations measured during the bloom and that measured in the previous month

** Values are for the mean composition of PN in the upper mixed layer

4.5 Results and discussion

Defined bloom events result in increases of chl *a* that are 10-95% higher than measured by the HOT program in previous months (data not shown). These increases are not clearly apparent in the MODIS derived chl *a* time-series. SeaWiFS near bloom time-series, on the other hand, do indicate increases in remotely sensed chl *a*, above monthly means, coincident with the timing of about half of these defined PE_{10+} blooms (see Table 4-2 and Figure 4-3). These sensor based differences are to be expected since (a) MODIS is affected by sun-glint thus this data record has fewer retrieved pixels than SeaWiFS and (b) MODIS values are generally biased low relative to SeaWiFS in oligotrophic waters (by ~0.01 mg m^{-3}) and therefore may not have detected smaller increases in bulk chl *a* that would have been sensed by SeaWiFS. In general, for the bloom cases examined here, bulk phytoplankton pigment does not appear to be a reliable indicator of large-sized cyanobacterial blooms. This finding may in part be due to the fact that *Trichodesmium* in particular is known to

have relatively low cellular chl *a* content (chl a : C ≈ 100 as per Laroche and Breitbarth 2005) hence increases in the abundance of this organism may not translate to large increases in bulk chl *a*.

Table 4-2. Environmental conditions coincident with defined cyanobacterial blooms identified from the HOT time-series.

Sampling date	MODIS/SeaWiFS monthly chl *a* anomaly	SST [^{o}C]*	SST anomaly [^{o}C] monthly/model	MLD [m]	WSPD [m s^{-1}] (relaxtion event within -15d)	SSH
6/14/2001	ND/+0.05	25.2	-0.4 / -0.5	37.1	7.2 (yes)	-11.09
8/8/2001	ND/-0.03	26.0	-0.4 / -0.5	57.0	6.6 (no)	-2.844
10/2/2001	ND/-0.02	26.3	-0.1 / -0.2	52.8	6.7 (no)	-3.928
10/23/2001	ND/+0.02	25.7	-0.7 / -0.6	69.6	7.4 (yes)	-8.355
10/7/2002	+0.01/ -0.001	26.3	-0.2 / -0.2	61.2	9.2 (yes)	8.458
7/20/2003	-0.02/+0.01	26.0	0.0 / 0.0	34.7	8.4 (no)	-2.923
10/15/2003	+.001/-0.01	26.8	0.5 / 0.4	67.0	9.2 (no)	11.54
6/16/2004	ND/+0.05	25.8	0.2 / 0.4	43.2	4.1 (yes)	9.229
7/13/2004	-0.01/+0.03	26.6	0.5 / 0.6	63.1	8.4 (no)	10.53

* SST here is the average of MODIS, HOT and NDCB Buoy 51001

4.5.1 Wind Speed

While the underlying mechanism has never been clear, it is commonly noted that wind speed exerts some control of diazotroph abundance (Karl et al. 2002). *Trichodesmium* blooms in particular are known to dissipate rapidly when winds begin to increase (Karl et al. 2002). This result may either be due to disruption of the colony morphology of *Trichodesmium* (Carpenter and Price 1977) and presumably a concomitant reduction in nitrogen fixation rates or simply a wind-driven deepening of the mixed layer and thus a reduction in the flux of photons available to cells. Alternately, fluctuations in wind speed and subsequent mixing may simply act to dilute the mixed layer population of cells with water devoid of diazotrophs, thus reducing diazotroph abundance in the mixed layer. I have examined the time series records of wind speed as measured at the NDBC buoy 51001 in addition to the time

derivative of this property in order to determine whether the identified blooms were associated with relaxation of wind forcing. Significant relaxation events were observed for a few bloom instances (e.g. 10-15 days prior to the 6/14/01 and 10/7/02 blooms, wind speeds lessened to ~1 m s^{-1}, (see Figure 4-4). In a single bloom instance (6/16/04), a wind relaxation event occurred within days of a sampled bloom. Specifically, wind speed decreased by 2.8 m s^{-1} a day prior to this particular bloom. All other blooms (n=5 of 9), however, were associated with rather constant wind speeds in excess of 4 m s^{-1} having a rate of change fluctuating around zero. Notably these rates are within the range of annual (2001-2005) values (7.1 ± 2.7 m s^{-1}). These findings indicate that wind speed alone is not a consistent indicator of large cell-sized cyanobacterial blooms. In a recent paper, Davis and McGillicudy (2006), using a novel video plankton recorder approach, reported observations of intact colonies of *Trichodesmium* in the North Atlantic in the wake of hurricane strength winds (~200 km hr^{-1}). The abundance of colonies in these rough waters apparently did not differ significantly from that observed in calmer, adjacent waters. In combination with our results, this seems to indicate that previous reports of a negative relationship between wind speed and *Trichodesmium* abundance in the NPSG is not likely due to physical damage to the cells but perhaps due to deepening of the surface mixed layer and regulation of the integrated photon flux available to surface trapped populations and/or governance of growth by SST.

4.5.2 Sea Surface Height

Recent research has reported a significant relationship between *Trichodesmium* abundance and anticyclonic eddies (Davis and McGillicuddy, 2006). I have examined time-series (Figure 4-5) and regional maps of SSH anomalies (Figure 4-6) at Station ALOHA in order to determine whether such a relationship exists for these defined bloom events. Much like the wind speed data, results are mixed. All of the defined 2001 blooms were clearly associated with cyclonic eddies and negative SSH

anomalies. The remaining 2002-2004 blooms were either associated with anticyclonic eddies (10/2002 and 7/2004) or eddy edges. Figure 1-6 provides an example of each type. Unlike the findings of Davis and McGillicuddy (2006), our data do not reveal any relationship between cyanobacterial blooms and SSH anomalies or eddy formations.

4.5.3 Temperature, mixed layer depth and integrated PAR

Temperature control of diazotrophic growth rates has historically been inferred from the geographical constraint of N_2 fixers (*Trichodesmium* and *Richelia*) to subtropical waters having surface temperatures > 20ºC (Karl et al. 2002; Venrick 1974). This relationship may be due to direct temperature control of cyanobacterial physiology or attributed to oceanographic features associated with warm water, such as shallowing of the mixed layer and/or increased mean photon fluxes (Breitbarth et al. 2006). I have examined SST time-series derived from MODIS, NDBC buoy 51001 and HOT cruises in search of temperature thresholds or the predominance of warming events that might be temporally connected (directly or time-lagged) with defined bloom events. At the time of HOT sampling, all of the identified PE_{10+} blooms occurred in 25-27ºC surface waters. While the warmest observed temperatures (~27ºC) were associated with the largest bloom (10/2003), there was no significant relationship between SST at the time of bloom occurrence and PE_{10+} concentrations.

Each of the available SST records (MODIS, HOT and NDBC Buoy 51001) was fit to a harmonic regression model and the anomaly time-series was derived. Anomaly time-series were also calculated as the difference from a monthly mean SST. Blooms occurring in 2001-2002 are associated with negative SST anomalies, when calculated as the difference from a monthly mean or as the difference between the data and a harmonic model fit. Conversely, temperatures corresponding to 2003-2004 blooms are generally higher than monthly means or model estimates (positive SST anomalies). This trend may be explained by the fact that 2001-2002 were within a cool

phase of the Pacific Decadal Oscillation (PDO) while 2003-2004 were years of a positive PDO index (warm phase). Thus, the sign of SST anomalies, as calculated from the harmonic regression of any of the SST data sources presented here, does not appear to impact the formation or intensity of surface blooms such that the magnitude of PE_{10+} concentrations are not significantly different in the cool phase (1999-2002) relative to the warm phase (2003-2005) of the PDO.

Peaks in PE_{10+} concentrations occur in two regions of the annual temperature cycle: (1) in the early summer warming phase, within a month of surface waters having crossed the 25ºC horizon and (2) near the peak of the annual temperature cycle. In the former case, mixed layer depths are shallow (45 ± 12m) whereas in the latter case mixed layer depths are on the order of 62 ± 7m (Figure 4-5). In early summer, these thin mixed layers result from an seasonal increase in the average flux of photons reaching the ocean surface (Letelier et al. 2004). Using monthly mean SeaWiFS PAR data (Figure 4-7) in conjunction with observed mixed layer depths, I calculate MLD-integrated photon fluxes to be equal to 57 ± 2.4 mol photons m^{-2} s^{-1} for the early summer warming phase blooms (June-July) and 41.7 ± 5.2 mol photons m^{-2} s^{-1} for October blooms occurring near the peak of the annual SST cycle. If PAR alone were driving these blooms, one might also expect to see similar blooms occur in spring months when mixed layer depths and PAR are comparable to October values. Such blooms do not appear in the historical record. Thus, I suggest that SST may be driving these early summer cyanobacterial blooms. Recent work by Breitbarth et al. (2006) using cultured populations of *Trichodesmium* IMS-101 provides support for this hypothesis. These authors have found that cultured populations of *Trichodesmium* exhibit optimal growth and nitrogen fixation rates at temperatures of 24°-30°C. Thus these early summer blooms may reflect temperature driven increases in the growth of *Trichodesmium* spp. October blooms are then well above the lower range for optimal *Trichodesmium* growth and thus some control other than SST must be driving these events if they are in fact composed of *Trichodesmium* spp. or likewise if *Richelia* have similar temperature optima.

4.6 Perspective and conclusions

Here, increases in the concentration of the cyanobacterial pigment phycoerythrin in the greater than 10 μm size fraction of surface water samples collected at Station ALOHA as a proxy for diazotrophic blooms. Elemental dynamics (the drawdown of P coupled to an increase in DIN) and the isotopic composition of particulate nitrogen ($\delta^{15}N_{PN}$) corroborate these events as being composed of N_2 fixers. I have combined remotely sensed ocean color and environmental forcing with vessel-based time-series and mooring data in an attempt to define the ecological window under which blooms of large cell sized N_2-fixing organisms increase in abundance in NPSG surface waters. Previous observations of surface blooms of N_2 fixers have correlated these events with calm, warm, well stratified waters (Karl et al. 1992) and more recently with anticyclonic eddies recorded for *Trichodesmium* (negative SSHa, Davis and McGillicudy, 2006). From the data I have analyzed here, neither wind forcing nor SSHa can be shown to be reliable determinants of bloom events (see Table 4-2, Figure 4-4 and Figure 4-6). In regards to wind specifically, three of the blooms were in fact associated with relaxation events occurring within 1-15 days prior to bloom sampling, yet the remainder of events were associated with rather constant wind forcing. This may indicate that the critical threshold required to disrupt growth is not reached at this location. SSHa analyses are even less telling. Blooms appear to develop in cyclonic eddies, anticyclonic eddies and at the edges of eddy formations. As additional biological instrumentation is mounted on gliders, oceanographers may soon see large data sets describing the full evolution and demise of blooms. In this probable future, I would speculate that we may be able to define with greater precision the potential effects of wind forcing and eddies on the growth of functional groups in the NPSG.

In a 2004 paper Hood et al. argue that changes in the mixed layer depth and concentrations of DIN are sufficient to explain large scale patterns of *Trichodesmium* such that *Trichodesmium* will only increase in abundance when the mixed layer is

shallow, integrated photon fluxes are high and other phytoplankton are limited by DIN. While I lack a high-resolution record of DIN dynamics, our analyses do show that PE_{10+} blooms repeatedly occur in early summer, when fluxes of photons to the surface ocean are seasonally high and mixed layer depths are at the shallowest. More recent physiological research with *Trichodesmium* indicates that these early summer blooms may also develop in response to thermal regulation of growth and nitrogen fixation. *Trichodesmium* exhibit maximal growth and nitrogen fixation in the temperature range of 24°-30°C, thus to some degree SST may also regulate the timing of bloom formation, if of course these blooms are composed of *Trichodesmium* and/or *Richelia* have similar thermal responses. Less can be derived regarding what may determine the late fall blooms, although, SST are still within the 25-27ºC range (see Table 4-2). In sum, I can only conclude from these analyses that blooms occur in June-October, are that these blooms are confined to SST in the range of 25-27ºC and mixed layer depths less than 70m. HOT data records do indicate that these blooms increase chl *a* concentrations in surface waters relative to the previous samplings, yet these increases are not sensed by MODIS and only a few of the blooms lead to significant increases in SeaWiFS chl *a*. With our inability to more closely constrain the ecological determinants of N_2 fixers in the NPSG, scenario testing using modeling approaches which include functional representations of nitrogen fixers (e.g. (Fennel et al. 2002; Hood et al. 2004) and competition for nutrients (N&P) may be more competent to identify bloom forcing at present.

Finally, while many studies have been conducted in laboratories and in the field to elucidate the potential environmental controls of *Trichodesmium* abundance, less is known about the regulation of *Richelia intracellularis* since this species has not been successfully maintained in culture. Establishment of viable culture lines of symbiotic and/or free-living *Richelia* would greatly enhance our knowledge of the regulation of this enigmatic organism. This would also allow a comparison of the chemical and physical conditions forcing *Trichodesmium* versus *Richelia*, if they are even distinct. It is my opinion that higher resolution data sets of physical forcing, diazotroph abundance and biochemical properties, sampled on the time-scale of bloom

development (days to weeks) will ultimately be necessary to quantitatively define the ecological determinants of N_2 fixation in the NPSG.

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4.7 Literature cited

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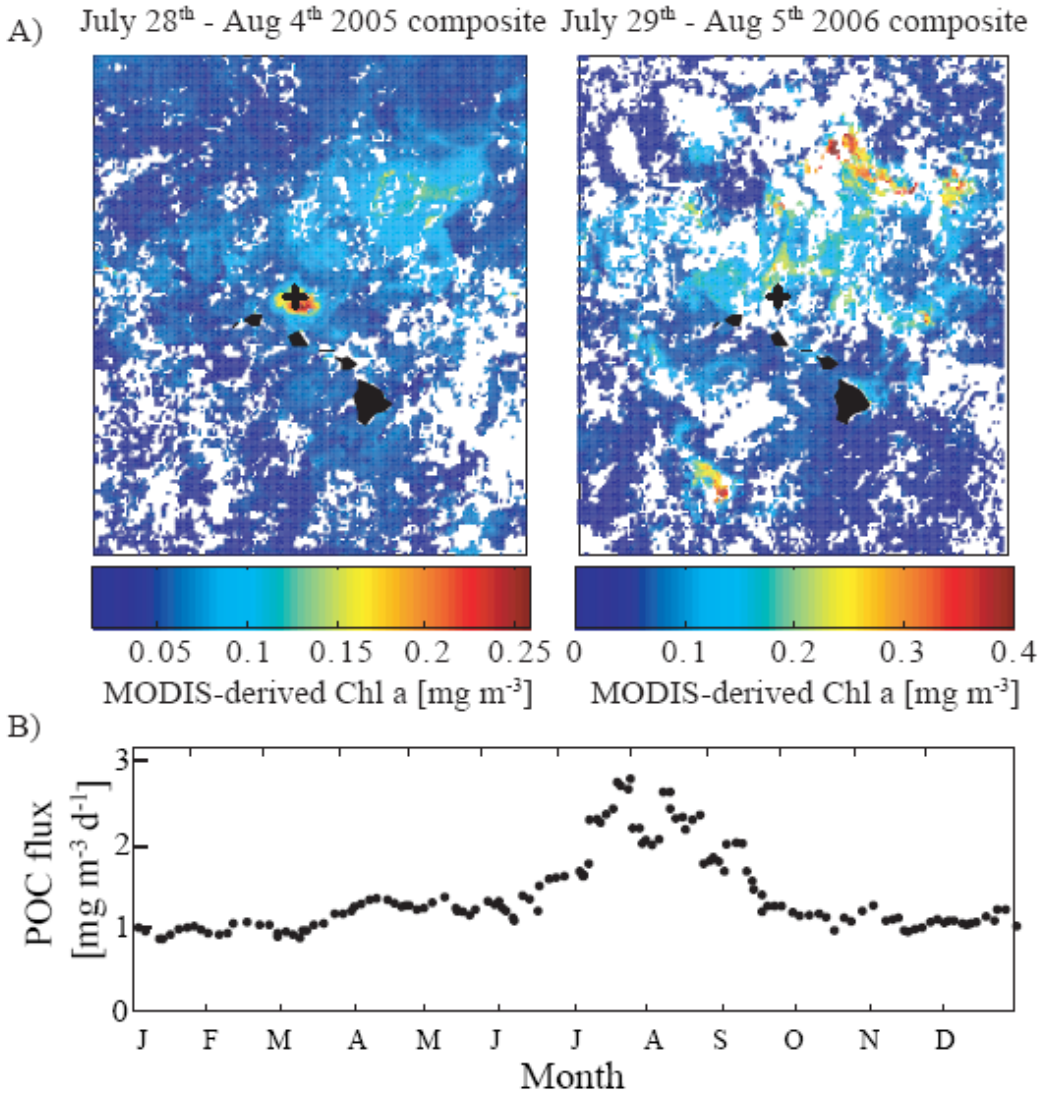

Figure 4-1. (A) Surface expression of summer blooms in the North Pacific in the region of 15ºN to 30ºN, 165ºW to 160ºW detected by MODIS AQUA. The location of station ALOHA is marked by a plus sign. White areas represent clouds while black regions represent land. (B) Climatology of particulate organic carbon (POC) sedimentation rates crossing the 4000 m depth horizon at Station ALOHA. Figure provided by D.M. Karl.

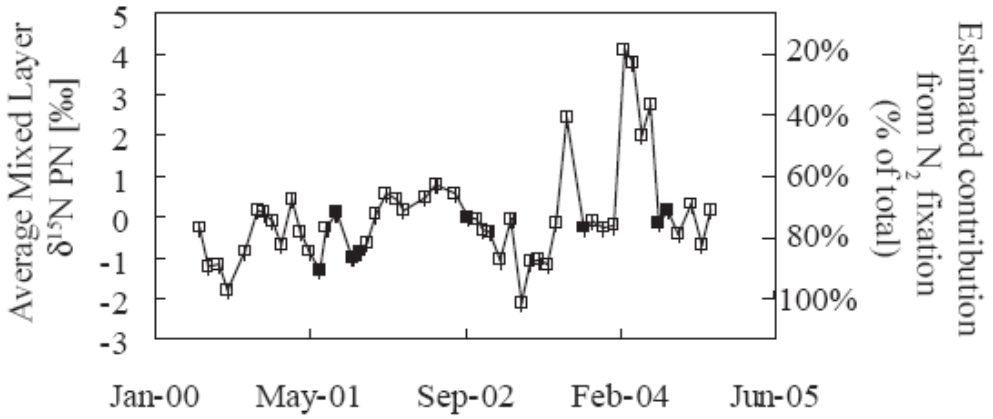

Figure 4-2. The time-series of the mean $\delta^{15}N_{PN}$ in the mixed layer at station ALOHA. Error bars represent the standard deviation and are often smaller than the symbols. Filled symbols correspond to diazotrophic bloom events. The estimated contribution from N_2 fixation was calculated using a two endmember mixing model (see text).

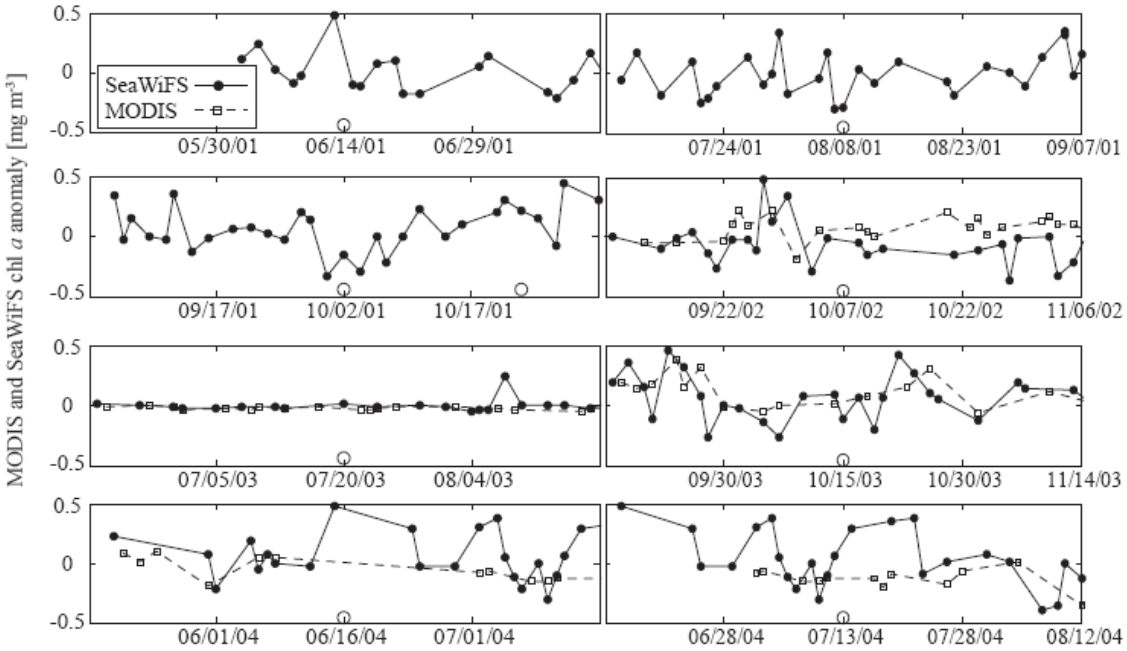

Figure 4-3. Monthly MODIS (2002-2006) and SeaWiFs (2001-2006) chl *a* anomaly time-series for the ±30 day period surrounding defined bloom events. The dates of these blooms are indicated on each plot by circles.

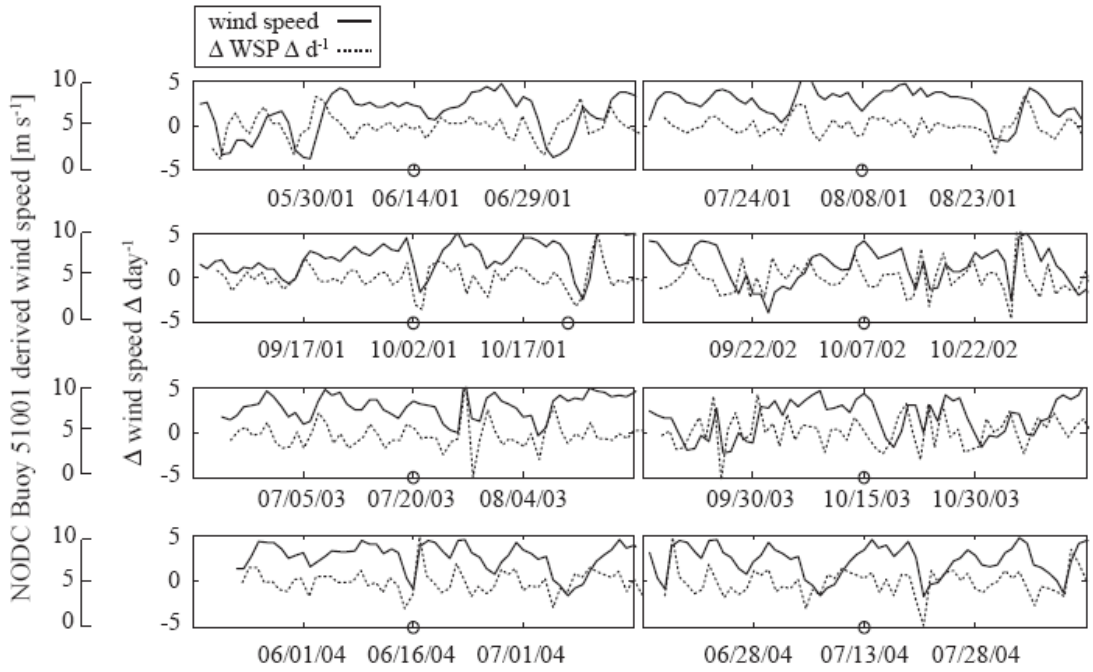

Figure 4-4. NDBC Buoy 51001 derived wind speed and the derivative of this time-series (Δ WSP Δ d^{-1}) for the ±30 day period surrounding defined bloom events. The dates of these blooms are indicated on each plot by circles.

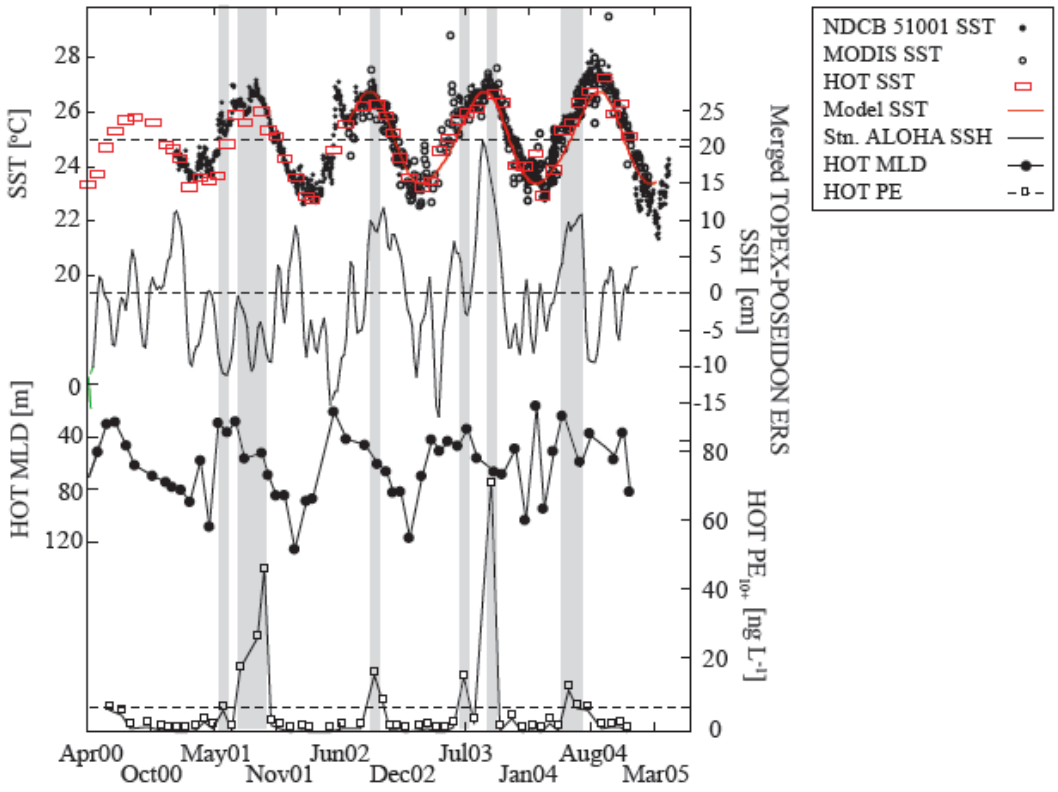

Figure 4-5. Time-series of SST (NDBC Buoy 51001, MODIS and HOT), SSHa obtained from merged TOPEX-POSEIDON and ERS-I altimetry, HOT MLD and the greater than 10 µm fraction of phycoerythrin collected in the top 25m of the water column during HOT process cruises.

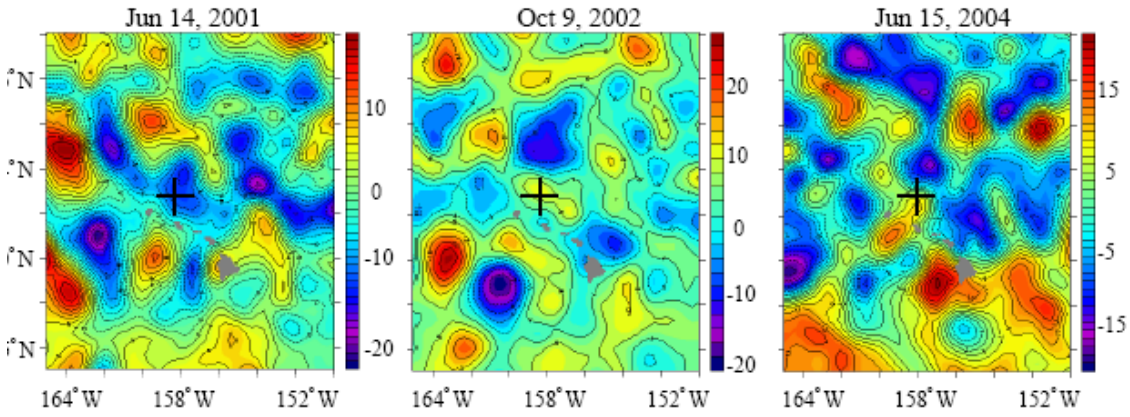

Figure 4-6. Contour plots of SSH anomalies corresponding to the dates of three PE_{10+} blooms. From left to right, these are examples of blooms associated with a cyclonic eddy (negative SSHa), an anticyclonic eddy (positive SSHa) and an eddy edge or boundary. Crosses indicate the location of Station ALOHA. Note that color bar scales differ slightly.

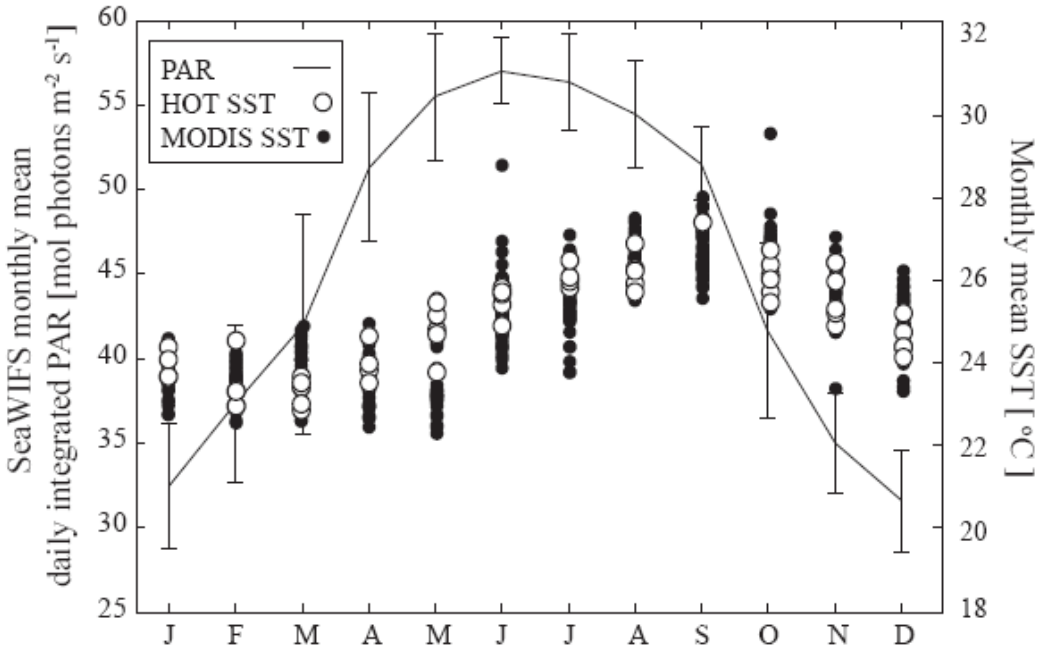

Figure 4-7. Monthly mean daily PAR derived from SeaWIFS and monthly sea surface temperature obtained from MODIS daily 9km resolution SST (2001-2006) and the HOT SST (1989-2004) data sets.

Summer surface waters in the Gulf of California: prime habitat for biological N_2 fixation

Angelicque E. White, Fredrick G. Prahl, Ricardo M. Letelier and Brian N. Popp

Global Biogeochemical Cycles
American Geophysical Union (AGU)
2000 Florida Avenue N.W.
Washington, DC 20009-1277 USA
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5 Summer surface waters in the Gulf of California: prime habitat for biological N_2 fixation

5.1 Abstract

We report significant rates of dinitrogen (N_2) fixation in the central basins of the Gulf of California (GC) during July-August 2005. Mixing model estimates based upon the $\delta^{15}N$ of particulate matter in the surface mixed layer indicate that N_2 fixation provides as much as 35% to 48% of the nitrogen demand of phytoplankton in the central Guaymas and Carmen basins. Microscopic analyses identify the responsible genera as the N_2-fixing endosymbiont, *Richelia intracellularis* with lesser contributions from the large non-heterocystous diazotroph *Trichodesmium*. Analyses of remotely sensed chlorophyll *a* and sea surface temperature indicate that primary production levels are elevated in regions of the GC where oceanographic conditions are ideal in summertime for growth of N_2-fixing organisms. These findings suggest that biological N_2 fixation must be taken into account when assessing past and present nitrogen dynamics in this environmentally important region.

5.2 Introduction

The Gulf of California (GC) is a subtropical marginal sea important as a site of rich biological productivity and as an intermediate in the flow of terrestrial and anthropogenically derived materials to the open ocean. Wind-driven upwelling of nutrient-rich waters (Thunell et al. 1996) and nutrient inputs from continental runoff (Beman et al. 2005) generate strong biological productivity in surface waters during winter and spring. Diatom genera dominate the phytoplankton community during these months causing the region to become a major sink for biogenic silica (Sancetta 1995) and a seasonal mediator for the net transfer of atmospheric carbon to the marine subsurface (Thunell 1998). During the summer, winds relax over the central and

eastern GC promoting upper water column stratification. Phytoplankton growth in these calm, stratified, central regions rapidly depletes surface waters of nutrients, leading to nitrate concentrations in the surface mixed layer (SML) that are typically below the 0.03 μM detection limit set by standard autoanalyzer technology. However, summer phosphate concentrations in the SML generally exceed 0.3 μM, indicating a deficit of N relative to P and hence suggest N-limited growth.

In the central GC, calm, stratified surface waters in summer coupled with low N:P ratios of dissolved nutrient pools represent the ideal ecological conditions for the growth of N_2-fixing organisms (or diazotrophs) (Karl et al. 2002). Despite the observation of blooms of N_2-fixing organisms in the outer entrance zone to the GC [e.g. Mazatlan Bay (Mee et al. 1984)] and observed summer decreases in the $\delta^{15}N$ of sediment trap particulate matter in the central basins of the GC (e.g. Carmen and Guaymas Basin) (Altabet et al. 1999; Thunell, 1988) , neither the presence of N_2-fixing organisms, nor the rate of N_2 fixation has been reported for the GC proper. Additionally, even though there is evidence that summertime primary production is N-limited, export rates of organic nitrogen (N) and carbon (C) out of GC surface waters to the deep sea, measured in sediment traps, are not depressed in the summer months relative to the winter upwelling period (Altabet et al. 1999; Thunell, 1988). In this context, the potential that N_2 fixation may supplement the summer export of N has not been explored in the GC. Here we report significant rates of N_2 fixation occurring in the Guaymas and Carmen basins of the central GC. Microscopic analyses identify the responsible genera as the N_2-fixing endosymbiont, *Richelia intracellularis* and the large non-heterocystous diazotroph *Trichodesmium*. Furthermore, we have analyzed MODIS (MODerate resolution Imaging Spectroradiometer) derived time series of surface chlorophyll *a* (chl *a*) and nighttime sea surface temperature (nSST) for the entire GC region in order to evaluate the occurrence of summer blooms and their spatial distribution, relative to our finding of N_2 fixation in the central GC.

5.3 Methods

5.3.1 Field Data

Field sampling in the Gulf of California (GC) took place between July 23 and August 13, 2005 aboard the R/V New Horizon. The general cruise path cut along the center of the gulf (~111º W) from roughly 22ºN to 30ºN with transect excursions for extended station sampling at 27º01'N 111º25'W (GC-1), 27º30'N 111º20'W (GC-2), 30º6N 113º52'W (GC-3) and 26º4'N 110º7'W (GC-4) (Figure 5-1). Water samples at each of these stations were collected throughout the upper water column with a CTD-rosette, equipped with PVC sample bottles. Nitrate and phosphate concentrations were measured post-cruise following the techniques of Strickland and Parsons (1972) while dissolved silicate was determined according to the method of Armstrong et al. (1967) as adapted by Atlas et al. (1971).

At each of the four extended sampling stations, N_2 fixation and carbon uptake rates were measured using ^{15}N-labelled N_2 and ^{13}C-labelled bicarbonate tracers. The general procedure for these measurements is described in Montoya et al. (1996). Briefly, acid-washed and sterilized silicone tubing was used for transfer of samples from rosette bottles into ~2 L, acid-cleaned and Milli-Q water rinsed polycarbonate bottles. For each incubation depth, duplicate ~2 L volumes were collected for determination of the ambient (time-zero) isotopic composition ($\delta^{15}N$, $\delta^{13}C$) of particles and volumetric concentrations of particulate organic carbon (POC) and nitrogen (PN). All incubation bottles were filled to overflowing before being carefully sealed with a septum cap (Teflon-lined butyl rubber). To each bottle, 0.5 ml of $^{15}N_2$ (99 atom% ^{15}N, Cambridge Isotope Laboratories) was injected using a gas-tight syringe while 0.25 ml of a 0.05 molar $^{13}CO_2$ stock solution (99 atom% $NaH^{13}CO_3$, Cambridge Isotope Laboratories) was added using a separate, plunger-type syringe. Sample bottles were gently mixed and attached to an in situ array for a period of 24 hours. Free-floating array deployment and design followed that described by Prahl et al. (2005). At the end of each incubation period, suspended particles were collected by gentle vacuum

filtration through a 25-mm precombusted (450ºC for 12 h) GF/F filter. Filters were immediately stored at -20ºC in an onboard freezer. Once ashore, samples were acid-fumed, dried overnight at 60ºC and wrapped in tin cups. Analysis of the $\delta^{15}N_{PN}$ and $\delta^{13}C_{POC}$ composition of samples followed the methodology described in *Prahl et al.* (2005).

At select depths at each station, 0.5 L samples were collected for microscopic analysis. This entire volume was filtered onto Irgalan black-stained, 0.2 μm pore diameter Nuclepore membrane filters using gentle vacuum filtration. Each filter was then fixed in 2.5% final concentration SEM grade gluteraldehyde and mounted onto glass slides. All slides were kept frozen at -20°C in slide boxes until counts were performed. For each slide, the entire filter field was counted using UV-epifluorescence microscopy for enumeration of individual diazotrophs.

Both ^{13}C and ^{14}C fixation rates were measured during this cruise. However, only ^{13}C rates are presented since these measurements are available for all stations, while ^{14}C rate measurements (from C. Dupont, Scripps) were only available for stations GC-2 and GC-3. From ^{14}C rate measurements, it was determined that dark bottle rates were 15% of light bottle rates, on average. Thus, a 15% dark correction has been applied to all ^{13}C measurements. For the two stations where concurrent ^{13}C and ^{14}C rates are available, the linear regression of productivity profiles are significant with > 95% confidence (GC-2, $r^2 = 0.91$, $p = 0.04$; GC-3, $r^2 = 0.87$, $p = 0.03$). Volumetric $^{15}N_2$ fixation rates (nmol N L^{-1} hr^{-1}, Eq. 1) were calculated according to Montoya et al. (1996) using the equation

$$N_2 \text{ fixation} = \frac{1}{\Delta t}\left(\frac{A_{PN_f} - A_{PN_0}}{A_{N_2} - A_{PN_0}}\right)\frac{PN_f}{V} \qquad \text{Eq. 5-1}$$

where $A_{N2,}$ A_{PN0} and A_{PNf} are percent abundance ratios (A) for $^{15}N_2$ additions, the PN pool at time zero and the PN pool at the end of the experiment, respectively. The volume (V) for all 24 hr (Δt) incubations was 2.3 L.

5.3.2 Satellite Imagery

Chlorophyll *a* and nighttime sea surface temperature (nSST) data for the region between 22- 32°N and 106-116°W were obtained from the 8-day, 9-km, level-3 MODIS data for the period from July 2002 to December 2005 (Figure 5-1). In each image, black areas represent land or clouds while white is used to depict regions outside the area of interest. All statistical analyses of chl *a* data are calculated from the log transformed data because this property is log-normally distributed and varies spatially and temporally across the GC by over an order of magnitude. The calculated sample mean and standard deviations (sd) are then converted to linear units.

The residual of sea surface height (SSH) for the GC was obtained from the 1º, 5-day TOPEX-POSEIDON altimetry. These data were spatially averaged for the central GC region (26-28ºN, 110-111ºW). Monthly SSH averages represent monthly-binned data for the period from 1/1/1993 to 1/1/2002.

5.3.3 Definition of Summer Bloom Events

We calculated the summer (June, 1 to September, 1) mean (m) and standard deviation (sd) of log-transformed chl *a* for each grid point (~9km resolution) using 44 mapped, 8-day composite images available for the 2002-2005 summer seasons. This data resolution was chosen to provide a general picture of chl *a* and temperature fields in the GC. Using the chl *a*-specific m and sd values, a z-score [(x-m)/sd] was calculated for each grid point (x) at every 8-day summer composite. Summer bloom events are then defined when the z-score at a single location is greater than or equal to 1.0. This means of defining blooms evaluates chl *a* values relative to the temporal summer mean and sd at the spatial scale of the individual gridpoint (~9km). Bloom events are divided between those coinciding with nSST values of < 27°C and those coinciding with nSST values ≥ 27°C in order to conservatively segregate blooms that may be associated with the upwelling of colder, nutrient-rich waters from those

associated with stable water column stratification. Nighttime temperatures are used to eliminate the diurnal variation caused by solar heating at the sea surface.

5.4 Results and Discussion

Changes in the position of the North Pacific high-pressure center relative to the adjacent continental low result in seasonally reversing winds that act as the primary control on circulation and mixing throughout most of the GC (Thunell et al. 1996). In the summer, relatively weak winds from the south generate upwelling along the western margin of the GC that can be seen as regionally high mean chl *a* concentrations coupled to lower mean nighttime sea surface temperature (nSST) values along the interior of the Baja peninsula (Figure 5-1A,C). Mean chl *a* concentrations are also elevated in the waters surrounding the archipelago of midriff islands, where strong tidal mixing brings colder, nutrient-rich waters from depth to the surface. In both cases (upwelling and tidal mixing), nutrient infusions support greater concentrations of phytoplankton biomass in surface waters (Figure 5-1A) (Gáxiola-Castro et al. 1999).

The summer upwelling zones, characterized by higher biomass and higher variability (Figure 5-1B), are contrasted by the relatively warm, lower chl *a* waters of the central to eastern GC below the midriff islands. Field data collected in August 2005 from the central GC revealed shallow mixed layer depths (~15-20m), high SST, and nitrate depletion in surface waters (Figure 5-2). Remote sensing products corroborate these findings. MODIS-derived nSST for the central GC are typically greater than 27ºC (Figure 5-1C) with relatively low variability (Figure 5-1D) indicative of stable water column stratification. Satellite altimetry data further confirm persistent summer stratification in the central GC, whereas high sea surface height anomalies (SSH) generally reflect thermal expansion of the ocean due to surface heating and thus increased stratification. The spatially averaged summer SSH for the

region of 26-28ºN, 110-111ºW indicates increasing elevation of SSH from an average of 22 mm in June to 120 mm in August.

In combination, field observations and remote-sensing products indicate that the central GC is characterized by warm, stratified waters having low concentrations for dissolved inorganic nitrogen in any chemical form (nitrate, nitrite or ammonium), and relatively high inorganic phosphate concentrations. Thus, throughout the central to eastern GC south of the midriff islands, the prevailing summer conditions represent ideal habitat for production of N_2-fixing organisms (Karl et al. 2002).

In July-August of 2005, we sampled surface waters along a latitudinal transect in the center of the GC with extended depth sampling at four stations (GC-1 & GC-2 in Guaymas Basin, GC-3 in the Delfin Basin and GC-4 in Carmen Basin) (Figure 5-1). Depth profiles of $^{15}N_2$ fixation showed high integrated rates of N_2-fixation only occurring at stations GC-2 and GC-4 (Figure 5-3A, Table 5-1). To put these results into the context of oceanic diazotrophy, the rates measured here (GC-2 & GC-4) are comparable to that measured in the subtropical North Pacific (Karl et al. 1997) and the tropical North Atlantic (Capone et al. 2005), regions for which the ecological importance of biological N_2 fixation has been well documented. Using the average C:N ratio for marine plankton (6.6 as per Redfield, 1958), N_2 fixation accounted for as much as 4-6% of depth integrated ^{13}C fixation rates (Table 4-1), with the contributions as high as 10% in the surfacemost waters (Figure 5-3B). In close correlation with ^{15}N rate measurements, epifluorescence microscopy indicated substantial numbers of the endosymbiont *Richelia intracellularis* occurring in association with the centric diatom *Rhizosolenia* at stations GC-2 and GC-4 (Figure 5-3D). Concentrations of *Richelia* were less than 100 L^{-1} in the surface mixed layer at GC-1 and GC-3. The host organism, *Rhizosolenia,* has been described as one of the most abundant and common taxa in the GC during summertime (Kemp et al. 2000; Gárate-Lizárraga et al. 2003); yet to our knowledge, symbioses with *Richelia* have never been reported for this region. Species of the N_2-fixing genus *Trichodesmium* were also observed at each of the stations, however these organisms were not found in abundances greater than 36 filaments L^{-1} or 2 colonies L^{-1}. *Trichodesmium* has only previously been described in

the lagoons of the eastern GC (Gilmartin and Revelante, 1978) and in the outer entrance zone of the GC (Mee et al. 1984).

Table 5-1. Integrated (0-36m) rates of $^{15}N_2$ fixation and ^{13}C fixation at each of the four sampling stations. The Redfield C:N ratio of 6.6 was used to calculate the percent of C fixation that could have been supported by these measured N_2 fixation rates. Surface $\delta^{15}N_{PN}$ measurements were taken from depths of ~ 5m.

Station	Integrated $^{15}N_2$ fixation [μmol N m^{-2} d^{-1}]	Integrated ^{13}C fixation [mmol C m^{-2} d^{-1}]	% of C fixation accounted for by N fixation	Surface $\delta^{15}N$ of PN [‰]
GC-1	20	41	0.3%	9.4
GC-2	132	15	5.8%	5.7
GC-3	23	114	0.1%	10.5
GC-4	250	48	3.4%	8.6

In July of 2004, on a previous cruise to the GC, abnormally high $\delta^{13}C$ values of POC were observed in the SML at our Guaymas basin station (GC-2, $\delta^{13}C$ = -15.0 to -15.5‰, Figure 5-4). These surface values are consistent with the isotopic signature of colonial forms of *Trichodesmium* spp., whose $\delta^{13}C$ values are in the range of -15.2 to -11.9‰ (Carpenter et al. 1997). Surface enrichments of $\delta^{13}C_{POC}$ were not observed in the summer of 2005 (Figure 5-4), consistent with low abundances of *Trichodesmium* and the presence of high concentrations of the endosymbiont *Richelia*, which does not exhibit an anomalously ^{13}C-enriched POC isotopic signal. These data suggest that *Trichodesmium* and *Richelia* may alternately dominate the community structure of the diazotrophic functional group in Guaymas Basin and perhaps other parts of the GC. Details of the timing and specific cause for such a taxonomic shift remain to be discovered.

The $\delta^{15}N$ of PN reflects the isotopic composition of the nitrogen source used by biota as well as the biological fractionation that occurs during uptake and assimilation of this element. In the absence of appreciable N_2 fixation or terrestrial inputs of fixed N, the average $\delta^{15}N$ of the particulate pool should reflect the $\delta^{15}N$ of

subsurface nitrate when there is complete utilization of nitrate in the mixed layer. In the central GC, subsurface nitrate has been preferentially ^{15}N-enriched as a consequence of denitrification, resulting in its isotopically heavy $\delta^{15}N$ signal (~11‰, depth 100-300m (Altabet et al. 1999). If N_2 fixation were occurring in surface waters of the GC, the biological input of atmospheric N_2 (~0‰) would lead to very significantly reduced values of $\delta^{15}N$ in PN. Along our 2005 cruise transect, surface water samples were collected at ~0.5º latitude intervals from the ship's flow-through seawater system for the analysis of the $\delta^{15}N_{PN}$ in suspended particulate materials. PN with low $\delta^{15}N$ values (5.8-7.1‰) were observed in the Northern Guaymas and Carmen basins (Figure 4-3A). These findings suggest that N_2 fixation (~0‰) has contributed significantly to the standing stock of phytoplankton PN in these surface waters..

Regenerated N sources (1.5- 2.0‰) (Altabet 1988) or DON (1 to 2‰) (Abell et al. 1999) may also contribute to some portion of the recorded $\delta^{15}N$ signal. So, while we can not unequivocally identify which N source may be responsible for the relatively light PON found in the central GC basins, our observations of very large numbers of organisms actively fixing N_2 coincident with the regions of low $\delta^{15}N$ and significant measured N_2 fixation rates, lead us to conclude that N_2 fixation has driven these isotopic diversions. Using a simple two end-member mixing model assuming a light (0‰) and a heavy (11‰) isotopic source of N, representing respectively N_2 fixation and a supply of deep nitrate, we estimate that N_2 fixation can account for as much as 35% to 48% of the $\delta^{15}N$ signature of standing stock in the central GC basins.

Altabet et al. (1999) have reported summer minima (5.5-6.6‰) in the $\delta^{15}N$ of PN settling into sediment traps deployed in the Guaymas and Carmen basins between 1990 and 1996. These minima are similar in magnitude to our measured $\delta^{15}N$ values for suspended PN in the SML of these same basins, suggesting potential for the export of primary production derived from N_2 fixation to depth. The summer-derived particulate material reaching the sediment may then record the net effect of surface N_2 fixation and water column denitrification. Altabet et al. (1999) considered this possibility, however they concluded that the episodic summer $\delta^{15}N$ minima could not

be driven by N_2 fixation for the reason that the recorded trap $\delta^{15}N$ minima were intermittent over their ~6yr record, thus requiring that N_2 fixation would have to "turn on" only during certain periods. In other marginal seas such as the Arabian (Capone et al. 1998) and the Red Sea (Post et al. 2002), blooms of large N_2 fixers such as the genera we have described (i.e. *Richelia* symbioses, *Trichodesmium*) are known to occur episodically under stratified summer conditions. Given that biological N_2 fixation requires a high light environment and that diazotrophs are at a competitive disadvantage in the presence of nitrate, it is expected that N_2 fixation would be enhanced in the GC only during summer months when highly stratified, dissolved inorganic nitrogen-poor conditions are common. Shifts in the diazotrophic community structure may further help to explain the interannual variability observed in these sediment trap $\delta^{15}N$ values. Specifically, whereas *Trichodesmium* are strongly buoyant organisms typically resistant to sedimentation, *Richelia-Rhizosolenia* symbioses are packaged in a relatively heavy silica shell, potentially facilitating more rapid export from the SML. Thus, material derived from *Richelia*-supported diatom blooms in summer may be more likely to reach the depth of sediment traps (~650m) and result in $\delta^{15}N_{PN}$ minima than that derived from *Trichodesmium* supported blooms. A logical extension of this problem is to investigate whether or not summer blooms occur in the GC, and if so, are they spatially consistent with our findings of N_2 fixation in the central GC.

To examine the spatial and temporal patterns of phytoplankton biomass in the GC, we have calculated summer (period from June 1 to September 1) maps for the mean (m) and standard deviation (sd) of the 9-km, 8-day resolution MODIS-derived chl *a* in the region of 22-32ºN, 116-106ºW. From these maps, all 9-km pixels within any one 8-day composite (x) with a z-score (x-m/sd) greater than one were defined as a bloom event. Over the four summer periods in the 2002 to 2005 timeframe, we found that, on average, summer bloom events occur in ~10% of the individual time series. Spatially, these events are concentrated primarily in the northern GC with patches occurring in the central regions (Figure 5-3B). We also analyzed the 9-km, 8-day resolution MODIS-derived nighttime sea surface temperature (nSST) in order to

discern the temperature characteristics coincident with summer blooms. Average chl *a* concentrations during these bloom events are 0.79 mg m^{-3}, roughly twice the regionally averaged, mean summer chl *a* concentration (0.38 mg m^{-3}). The average nSST coinciding with summer bloom events is 27.0°C.

In the north and along the western margin of the GC, where upwelling and strong mixing, respectively, are common in summer, we would expect that the majority of the defined bloom events would be associated with lower nSST. Conversely, if biological N_2 fixation were supporting phytoplankton blooms, we would expect these blooms to occur in persistently warm, highly stratified, nitrate-poor surface waters. It may also be possible that summer blooms occurring in warmer waters on the east side of GC could be driven by anthropogenic inputs of N via riverine sources (as per Beman et al. 2005). This latter possibility seems unlikely, as peak irrigation events are isolated to winter and spring months (Beman et al. 2005). Gaxiola-Castro et al. (1999) report that nitrate is non-detectable in surface waters having temperatures greater than 24°C. Our own summer data (Figure 5-2), show that dissolved inorganic nitrogen in either nitrate, nitrite or ammonium form is essentially undetected above 22°C. Thus, in order to evaluate these blooms most conservatively, we chose 27°C as a threshold temperature to indicate the transition between conditions favorable for upwelling of waters enriched in nitrate to the SML, supporting classical phytoplankton blooms, from those bloom events presumed favorable for N_2 fixation.

Figure 5-5B-D presents spatial maps for the percentage of 8-day summer composites (total summer n = 203,786, bloom n = 15,249) that are defined as a bloom (Figure 5-5B) as well as those same blooms segregated according to the nSST threshold of 27°C (Figure 5-5C-D). Blooms co-occurring with nSST < 27°C (n = 7574, mean chl *a* =0.91 mg m^{-3}, mean nSST = 24.7 °C) are spatially consistent with wind-driven upwelling along the western GC boundary and tidal mixing around the archipelago in the northern GC. Conversely, those blooms coinciding with nSST ≥ 27°C (n=7675, mean nSST = 29.3°C, mean chl *a* = 0.68 mg m^{-3}) are presumed to coincide with favorable conditions for biological N_2 fixation (i.e., warm, stratified,

nitrate poor). These analyses indicate that (1) summer blooms occur regionally in ~7.5% of the cloud-free MODIS data record for summer periods from 2002 to 2005, (2) approximately half of the summer GC is characterized by nSST> 27º C, thus approximately half of the defined bloom events that occur coincide with nSST> 27ºC, and (3) these presumed N_2 fixation supported blooms may result in a ~2 fold increase in chl *a* and presumably primary productivity above the regional summer mean. The bloom dynamics of locations coinciding with field sampling stations (GC-1:GC-4) have also been analyzed. These station-specific analyses indicate that bloom occurrences having nSST ≥ 27°C occur in 5%, 19%, 0% and 16% of the MODIS data record at GC-1, GC-2, GC-3 and GC-4 locations, respectively (Figure 5-6). These findings are consistent with our field data showing that the lowest $\delta^{15}N$ values for surface PN and the highest measured integrated rates of N_2 fixation were found at stations GC-2 (5.7‰, 132 μmol N m^{-2} d^{-1}) and GC-4 (8.5‰, 250 μmol N m^{-2} d^{-1}) (Table 4-1). Bloom events were not detected in the ±8 day timeframe corresponding to our sampling dates (Figure 5-6). However, z-scores for chl *a* concentration were elevated at GC-1 (z-score = 0.82) and GC-4 (z-score = 0.75) in the 8-day composite preceding our sampling dates (Figure 5-6). In summary, these analyses suggest that N_2 fixation supported blooms most commonly occur in the central GC (specifically, GC-2 and GC-4), albeit the presence of high concentrations of N_2 fixers does not always result in significant increases in satellite-derived chl *a*.

Both satellite analyses (Figure 5-5D) and direct measurements (e.g. measured N_2 fixation at GC-2 but not at GC-1) suggest spatial patchiness of N_2 fixers in the central and eastern GC. One potential explanation for this perceived patchiness is that N_2 fixation may turn ‘on’ and ‘off’ in response to an external input of eolian-supplied limiting nutrient, such as iron. During the summer months in the GC, convective thunderstorms deliver large inputs of terrigenous material, primarily derived from the Sonoran desert (Baumgartner et al. 1991). These iron-rich aeolian inputs may act to stimulate patches of diazotrophic growth throughout the central GC. Support for this hypothesis lies in the work of Kemp et al. (2000) who analyzed laminated sediment cores from Guaymas basin and found that Rhizosolenid diatoms are commonly

concentrated at the top of the summer terrigenous lamina. While *Richelia* are not preserved in these laminated sediments, it would be intriguing to extract organic matter from the Rhizosolenid diatom tests in these sediment layers for the analysis of $\delta^{15}N$ composition (as per Robinson et al. 2005) in order to determine whether these sediment strata are also associated with ^{15}N-depleted PN and enhanced N_2 fixation.

5.5 Conclusions

Our composite analyses have provided evidence that significant rates of N_2 fixation occur in the GC, with satellite proxies confirming increases in primary productivity in the high temperature, nitrate-poor, phosphate-replete waters of the central and eastern margins. Rate measurements were reinforced by microscopic analyses showing high concentrations of *Richelia intracellularis* at stations GC-2 and GC-4. While the measured N_2 fixation rates and diazotroph abundances were substantial, the net influx of biologically usable N to the system is probably not sufficient to alleviate nitrogen limitation imposed by the low N:P composition (<10 mol N:mol P) for dissolved nutrients introduced from depth to surface waters. Similarly, even though nitrate concentrations in the SML were below detection limits, the measured $\delta^{15}N_{PN}$ values did not reflect the full signal for N_2 fixation (~0‰), rather surface $\delta^{15}N_{PN}$ values indicated only an ~40% contribution from N_2 fixation to the isotopic composition of standing particulate matter. In terms of daily primary production, $^{15}N_2$ fixation rates accounted for as much as 10% of ^{13}C fixation rates. These composite results indicate that (1) N_2 fixation rates may have been higher prior to our measurements such that the $\delta^{15}N_{PN}$ reflects the integrated history of diazotrophy at each station and that (2) the predominant fraction of summer production is likely supported by microbial recycling of N sources and/or utilization of dissolved organic N. Alternatively, given that *Rhizosolenia* has a reputed ability to migrate vertically in the water column (Villareal et al. 1996) and that the summertime SML is quite shallow (15-20m), *Rhizosolenia* may acquire additional N via vertical migration to the depths

of the nutricline. Despite these unanswered questions, it is now apparent that N_2 fixation plays a significant role in the summer ecology of the GC. Additionally, the remote sensing approach we have advanced in this study, while it may not be capable of confirming the occurrence of N_2 fixation, could also be applied to other regions where biogeochemical indicators of N_2 fixation (low $\delta^{15}N$ values or high N* (Gruber and Sarmiento, 1997) coincide with seasonally warm stratified conditions (e.g., Mediterranean Sea (Ribera d'Alcalá et al. 2003) and the eastern Tropical Pacific (Sigman et al. 2005) in order to estimate the spatial and temporal extent of N_2 fixation associated events.

Our findings are also in line with a growing body of work suggesting that seasonal N_2 fixation occurs in parts of the surface ocean proximate to regions of intense subsurface denitrification (Westberry and Siegel, 2006; Sigman et al. 2005; Brandes et al. 1998; Capone et al. 1998). While the GC itself is not renowned as a site of localized denitrification, the California Undercurrent brings a supply of suboxic, denitrifying waters from the eastern tropical North Pacific, which intrude into the central GC at depths of 500-1000m (Liu and Kaplan, 1989). Denitrification, the microbial process by which N electron acceptors (NO_3^-, NO_2^-) are reduced to N_2 to facilitate organic matter degradation, is energetically favorable in low O_2 environments. Hence, denitrification occurs in oxygen minima zones where aerobic respiration of biological material raining from sunlit surface waters has depleted dissolved oxygen levels. Locally, the intensity of denitrification influences the concentration of inorganic N and thus the N:P ratio of dissolved nutrients that are delivered to surface waters via upwelling. Given that nitrogen fixation is favored by a low N:P ratio (Karl et al. 2002), upwelling of denitrified waters, if followed by stratification and Redfield-type nutrient drawdown, can prime surface waters for nitrogen fixation and thus lead to potential feedbacks (positive and negative) for export production, the maintenance of the suboxic conditions that favor denitrification (Sigman et al. 2005) and the magnitude of dissolved N:P ratios that are generated in the denitrification zone. In addition to the GC, geographical coupling of N_2 fixation and denitrification has also been shown to occur in the Arabian Sea (Brandes et al.

1998; Capone et al. 1998) and the eastern tropical Pacific (Westberry and Siegel, 2006; Sigman et al. 2005).

In the central GC, the coherence between the $\delta^{15}N$ values measured in our study and previous reports of summer $\delta^{15}N_{PN}$ minima (Altabet et al. 1999) occurring in sediment trap records for Carmen and Guaymas basins, support N_2 fixation as a mechanism for the net export of particulate material. Episodic fluxes of materials derived from N_2 fixation would provide organic matter to fuel denitrification in subsurface waters and dampen the impact that this process has on the magnitude of ^{15}N-enrichment in residual nitrate. In combination with this effect, passage of such ^{15}N-depleted material through the O_2 minimum zone to the sediment record would attenuate the $\delta^{15}N$ of PN that we now tie simply to denitrification intensity. Given the implications of the phenomenon we now identify on global and regional N budgets and the paleoceanographic interpretation of sediment records, further study of the GC region and other locales (e.g. the Arabian Sea and the ETP) where N_2 fixation and denitrification may be tightly coupled is necessary. Potential results from this effort would almost certainly help to refine our current understanding of the past and present marine nitrogen cycle.

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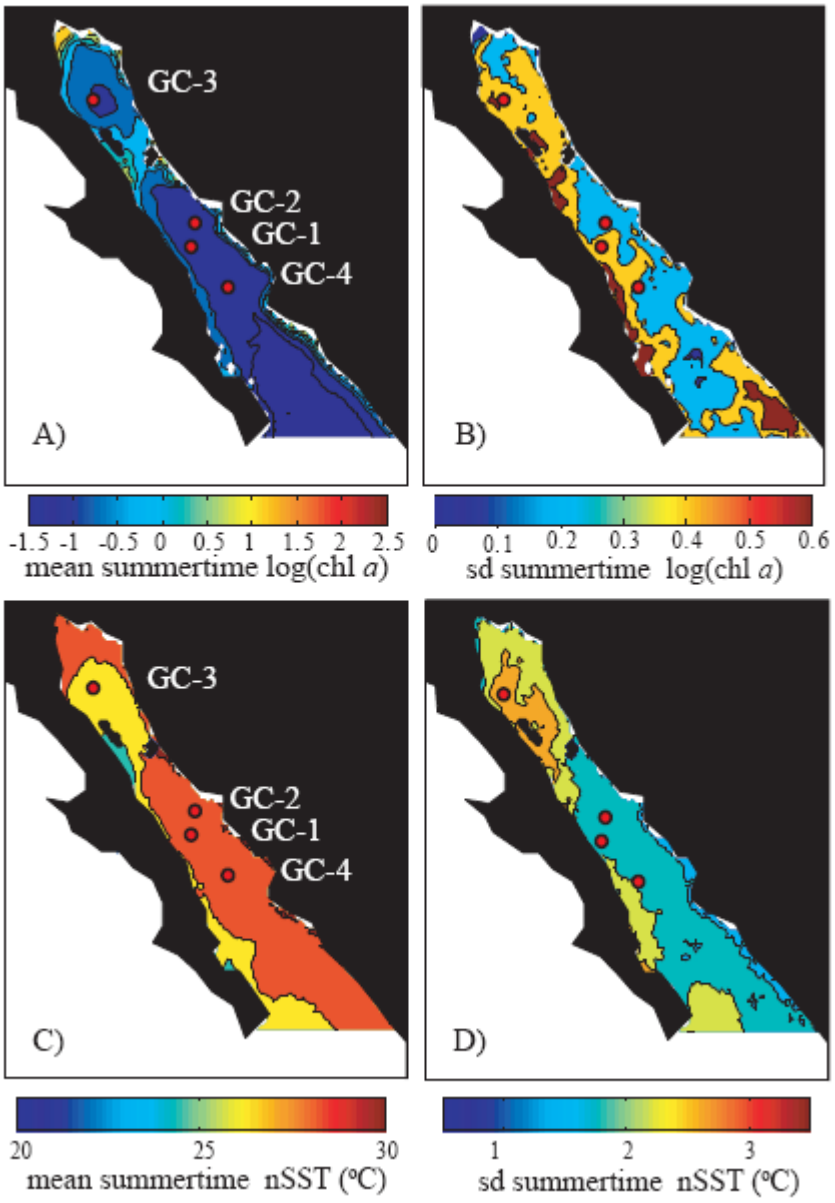

Figure 5-1. Mean and standard deviation (sd) fields for surface chl a (A-B) and nighttime SST (C-D) in the GC calculated for summertime periods (June 1 to September 1) in the years from 2002 through 2005. The spatially averaged mean summertime chl a is 0.38 mg m-3 while the average nighttime SST is 28.3°C. Contour intervals for each panel are as follows: (A) 0.02 (B) 0.05 (C) 2.0 and (D) 0.5. The locations of four sampling stations (GC-1:GC-4) are denoted as red circles in panels A-D.

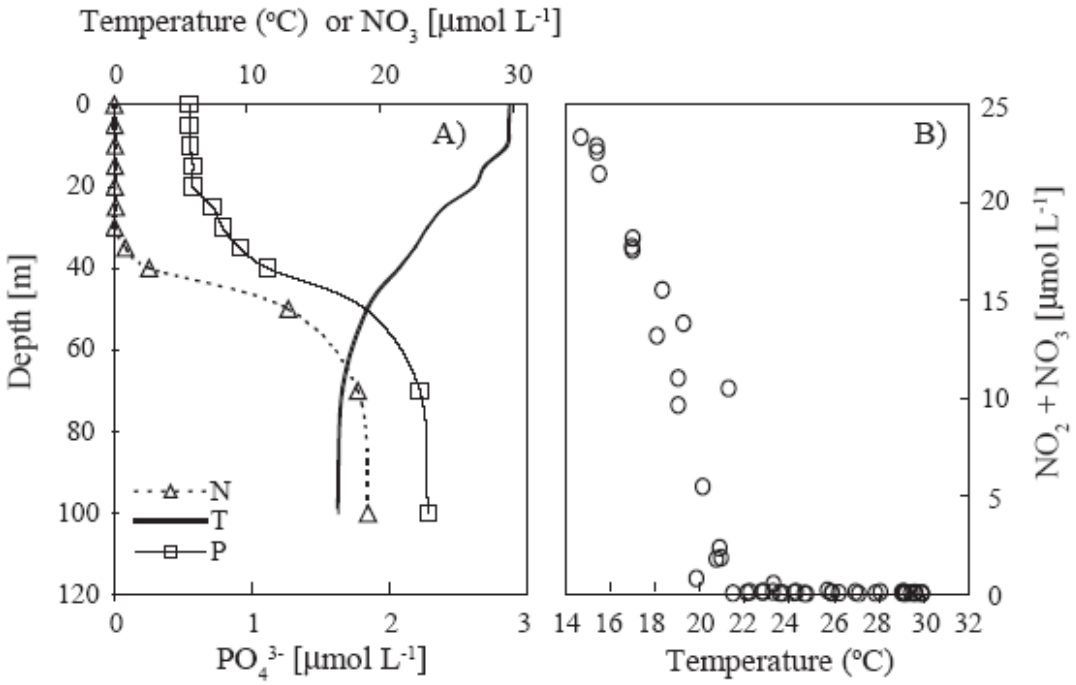

Figure 5-2. (A) Depth profiles of nitrate, phosphate and temperature measured in Guaymas Basin in August, 2005 (B) Nitrate plus nitrate (N+N) concentrations as a function of water temperature for samples collected in July-August of 2005. N+N concentrations are typically below the detection limits of standard autoanalyzer technology at temperatures greater than 22ºC.

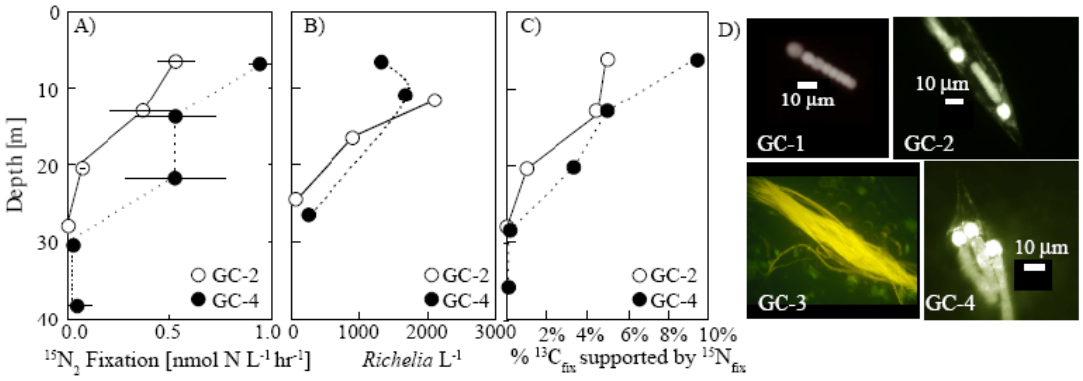

Figure 5-3. (A) Results of measurements of $^{15}N_2$-fixation rate assays from 24hr free-floating incubations indicate significant N_2 fixation occurring at two of the four sampled locations, the first in Guaymas Basin (GC-2) and the second in Carmen Basin (GC-4). $^{15}N_2$ fixation rates were low (≤0.09 nmol N L^{-1} hr^{-1}) throughout the upper water column at stations GC-1 and GC-3. (B) Depth distributions of *Richelia* heterocysts L^{-1} for stations GC-2 and GC-4. Concentrations of *Richelia* were less than 100 L^{-1} at GC-1 and GC-3. *Trichodesmium* were observed at all stations, albeit in low abundances. (C) The percentage of ^{13}C fixation that can be accounted for by $^{15}N_2$ fixation as a function depth. N_2 fixation rates were converted to C fixation rates using measured values for the POC:PN ratio, which were not significantly different from Redfield stoichiometry. (D) Select epifluorescence images from each station. Free trichomes of *Richelia intracellularis* (GC-1), *Richelia-Rhizosolenia* symbioses (GC-2 and GC-4) and *Trichodesmium* spp. (colony shown for GC-3) were observed at all stations, however abundance of N_2 fixers was greatest at GC-2 and GC-4.

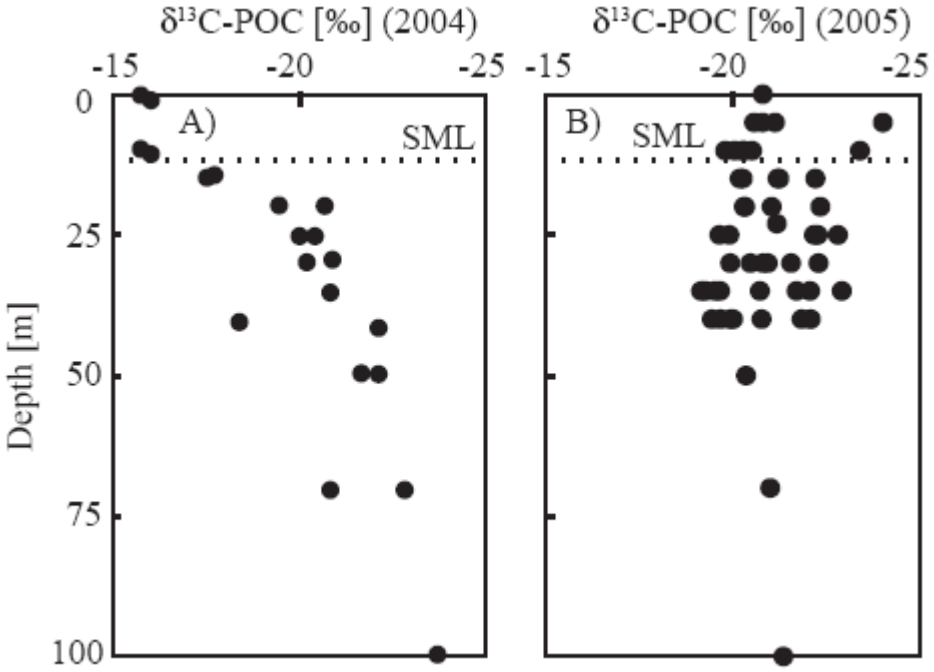

Figure 5-4. Isotopic analyses of $\delta^{13}C$ composition of particulate organic carbon (POC) indicate transitions in community structure at the Guaymas Basin station (GC-2). (A) In July 2004, $\delta^{13}C_{POC}$ values were highly enriched in the surface mixed layer (SML, dotted line), transitioning to more typical values (-21.5 ‰) at depth. In contrast, (B) the $\delta^{13}C_{POC}$ data in the summer of 2005 from all stations were relatively more uniform with depth (~ -21.5 ‰) and showed no apparent enrichment in the SML.

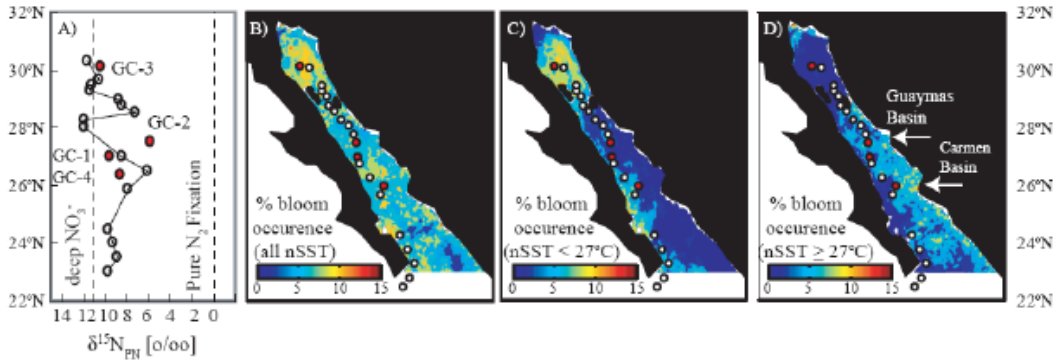

Figure 5-5. (A) The $\delta^{15}N$ of surface water PN samples as a function of latitude for all transect (open circles) and extended station (filled circles, GC-1, GC-2, GC-3, and GC-4) locations. The dashed lines indicate the $\delta^{15}N$ value expected for deep water nitrate [~11‰, (Altabet et al. 1999)] and measured in PN at the bottom of the photic zone for these sites, and the $\delta^{15}N_{PN}$ expected for pure N_2 fixation (0‰). (B-D) Percentage of the total number of summer MODIS chl a composites (8 day) having a z-score ([x-m]/sd, see legend for Figure 6 and text for more description) greater than 1 (e.g. a bloom) and having (B) any retrieved nSST value (C) nSST < 27°C and (D) nSST ≥ 27°C. The location of transect (open circles) and extended station sampling sites (filled circles) are overlain on all of bloom maps.

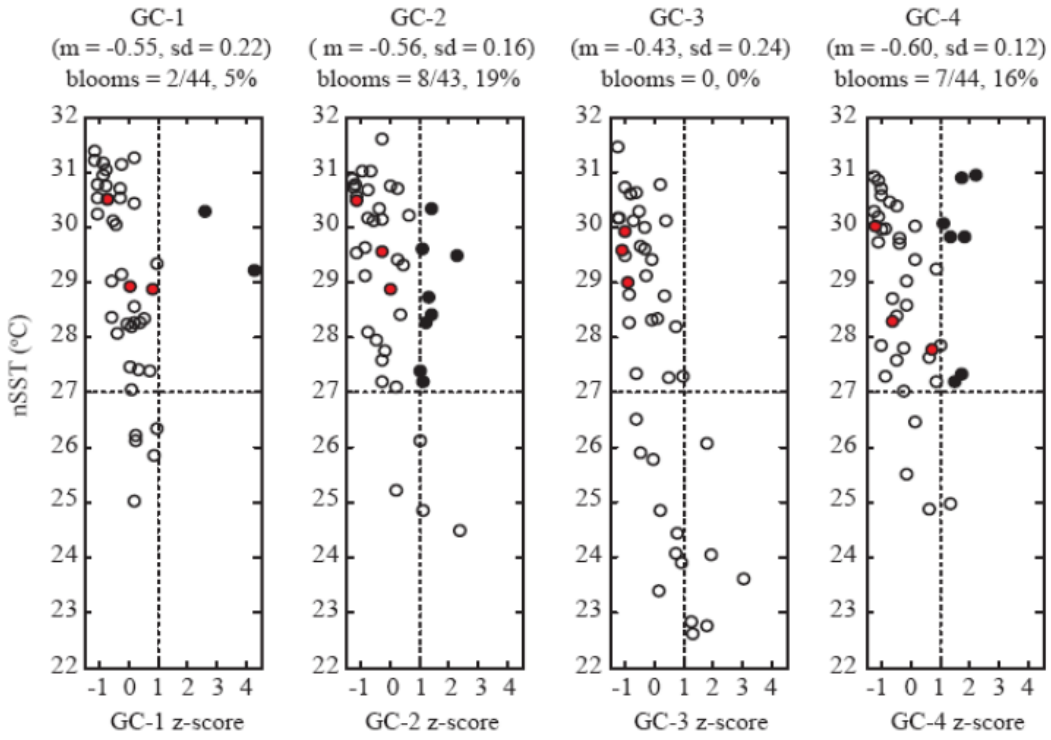

Figure 5-6. The z-score [(x-m)/sd] calculated for each 8-day summer composite at each station location versus the nSST for the same 9-km pixel of an 8-day composite. Bloom events defined to be consistent with biological nitrogen fixation (nSST> 27ºC) are noted as dark circles. At this resolution, blooms were not evident in the ±8 day period (n=3) corresponding to our sampling dates (red circles). The overall summer log(chl a) mean (m), the standard deviation (sd), and the percentage of bloom events relative to the total number of cloud-free composite summer images are noted in the title of each graph.

6 Conclusions and future directions

"The problem . . . is to understand the proper domain of each abstraction rather than becoming its prisoner" -Levins and Lewontin (1980)

Diazotrophy represents the largest incoming flux of nitrogen (N) to the global ocean. Yet, present day estimates of N_2 fixation rates vary widely within and between regions (Galloway et al. 2004). The uncertainty associated with this input term in part reflects our incomplete knowledge of the factors regulating diazotrophic physiology. The production of diazotrophic cyanobacteria, like that of any other planktonic organism, is ultimately controlled by the interactions between cells and the chemical environment, the intracellular coupling of elements, and environmental forcing as defined by atmospheric and oceanic physics. The four chapters that form the main body of this dissertation touch upon specific aspects of the chemical, physiological and physical controls of the production and abundance of N_2-fixing organisms in the marine habitat.

A few specific conclusions can be drawn from this research. First, natural and cultured populations of *Trichodesmium* exhibit a large range of elemental composition, which is largely due to variable internal phosphorus (P) content. On average though, similar to other predominant marine cyanobacteria (e.g. *Synechococcus* and *Prochlorococcus*) *Trichodesmium* can be defined as a genus having very low P requirements relative to either carbon (C) or N. Such streamlined P content is likely to be advantageous in the P-spare habitat occupied by this genus. *Trichodesmium* also appear to have the capacity to store P in excess of the requirements of growth when this element is abundant in the external environment. Despite these adaptations, the elemental composition of natural populations sampled in regions of the Atlantic and Pacific indicates that P-limitation of *Trichodesmium* growth is widespread. Additional research is needed to define the relationship between the relative intracellular P content of *Trichodesmium* and net growth or N_2 fixation

rates in nature. Nonetheless, our findings are in concert with a number of recent publications highlighting dissolved inorganic phosphate (DIP) as a key determinant of the input of N to the marine system (Krauk et al. 2006; Sañudo-Wilhelmy et al. 2001; Sohm and Capone, 2006). Krauk et al. (2006) in particular, present data showing that P content directly impacts N_2 fixation rates of *Trichodesmium* in laboratory cultures. Additionally, similar to our own findings, Krauk et al. (2006) report variable yet low P content in natural populations of *Trichodesmium.* So while it is apparent that the availability of DIP can control N_2 fixation rates, additional research is necessary to determine the physiological and ecological meaning of the highly variable elemental stoichiometry observed in nature.

Secondly, vertical migration cannot be ruled out as a means of P acquisition by large colonies of *Trichodesmium*. From results of a model of light-driven carbohydrate ballasting, we can conclude that (1) shallower migrations (to depths < 85m) more closely match the observed depth distributions of *Trichodesmium* in the NPSG (2) only large colonies, greater than ~1000 μm in radius, would be able to achieve the vertical velocities necessary to reach the average depth of the phosphocline in the NPSG and (3) if P-mining were to occur, colonies would be able to fulfill their entire P quota in a single migration. In sum, model results indicate that if P-mining does occur in nature, it is likely limited to a subset of a *Trichodesmium* population at any one time, yet the ecological and biochemical implications would be significant. While a model approach does not allow for definitive statements on the ecological role of vertical migration by *Trichodesmium*, it does allow us to identify measurable parameters that could inform future field or laboratory investigations of this enigmatic feature of *Trichodesmium* autoecology. Specifically, model sensitivity analyses identify colony size, the rate of ballast loss in the dark and the minimum and maximum achievable colony density as important variables determining migration amplitude. This study has hopefully set the stage for future research regarding the role of vertical migration in elemental cycling and the regulation of *Trichodesmium* production in oligotrophic ocean regimes.

Thirdly, moving from physiological measurements to environmental forcing, we find that N_2 fixation supported phytoplankton blooms in the NPSG are confined to the period of June through October, when SST are in the range of 25-27ºC and mixed layer depths less than 70m. Neither wind forcing nor sea surface height seem to determine increases in the greater than 10 μm size fraction of the cyanobacterial pigment, phycoerythrin, a proxy for diazotroph abundance. Furthermore, blooms do not consistently result in increases in remotely sensed chlorophyll *a*. From these data, we can coarsely outline the ecological window under which blooms of large cell sized cyanobacteria occur in the NPSG. Unfortunately, no predictive relationships could be ascertained. These findings are based upon monthly snapshots of an ecosystem state, such that we can never be certain what stage of a bloom is being observed (i.e. bloom development, peak or crash). Additional high-resolution data regarding cyanobacterial concentrations in surface and subsurface waters will be necessary to define mechanistically the environmental conditions associated with bloom events.

Lastly, we have recorded the presence of the N_2-fixing organisms *Richelia intracellularis* and *Trichodesmium* in the central gyres of the Gulf of California, a region where these organisms were previously not documented. From this finding we can conclude that the geographical distribution of N_2-fixing organisms is wider than previously believed. Satellite observations of chl *a* and SST have allowed us to temporally extend these regional analyses and further conclude that increases in phytoplankton biomass and presumably primary productivity are a regular summer feature in the warm, stratified nitrate poor, phosphate-replete waters of the central and eastern GC.

6.1 Perspective and future directions

More than a century of oceanographic research has focused on various aspects of the physiology and ecology of one particular diazotrophic genera, *Trichodesmium,* with the number of peer-reviewed publications increasing exponentially with time

(Figure 6-1). Despite this impressive body of work and the steady stream of new knowledge, still much is unknown about the chemical, physical and biological controls of the growth and abundance of N_2-fixing organisms, especially with respect to the bloom forming diazotroph *Richelia intracellularis*. Progress on this front will inevitably require that contemporary oceanographers continue to develop experimental tools and methodological approaches that allow us to integrate our findings across scales, from the gene to the cell to the organism to the global ocean and back to the gene. This will be a difficult but necessary task if we are to understand the complex interactions and feedbacks between the living and non-living compartments of the ocean system and ultimately predict their responses to an ever changing environment.

In this research, I have tried to address subsets of the regulation of the growth and production of planktonic biota. Future work will be required to scale up these findings in order to allow for a more holistic conceptualization of the interactions between cells and the chemical environment, the environment and the intracellular coupling of elements, and cells and environmental forcing. It is these coupling that ultimately determine the biogeochemical impacts of marine diazotrophs. With this task in mind, in this final section of my dissertation, I will discuss two major challenges that arise from my work. The first challenge is to assess P-regulation of oceanic diazotrophy with full consideration of the adaptations of the organism to its environment. Specifically, we should quantitatively determine the entire suite of P-sources available to *Trichodesmium* (active uptake of DIP, utilization of intracellular P storage compounds, mining of the phosphocline via vertical migration and utilization of dissolved organic phosphorus sources) relative to the production of organic C and N. Also, since *Trichodesmium* colonies are often found in direct association with a wide variety of other organisms (Sheridan et al. 2002), the communal nature of the colony morphology must be accounted for in studies of elemental cycling. Understanding how adaptive physiology couples the cycling of elements at the organismal level, for *Trichodesmium* and other marine N_2 fixers, is directly relevant to our ability to conceptualize the role of N_2 fixers in global C and N cycles.

A second major challenge will be to tease apart the variable climatological and biological controls of each of the dominant bloom-forming N_2 fixers (*Richelia*-diatom symbioses versus *Trichodesmium*). While these genera appear to occupy the same geographical range, *Richelia* has not been successfully maintained in culture and we thus lack a rich understanding of its physiology. Nonetheless, given that *Trichodesmium* are free-living and that *Richelia* occur in symbioses with relatively heavy silicified organisms, each of these bloom types may have very different consequences for the cycling of elements within the upper ocean and are as a result likely to differentially impact primary productivity and the export of C and N from the surface ocean. Thus the second and final challenge I address will be to evaluate diazotrophy in light of the inherent redundancy of this functional group.

6.1.1 Future Directions: P regulation of diazotrophic growth

> "While carbon, hydrogen, oxygen and nitrogen are the elements of biological frameworks, phosphorus is the instrument of manufacture."
>
> - F.G. Pautard (1978)

In this dissertation, among other results we have demonstrated that the intracellular composition of field populations is highly variable, with mean stoichiometry tending towards indices of P-limitation. Before these results can be scaled to the regional or global scale, the relationship between relative intracellular P content and the production of organic matter by *Trichodesmium spp.* needs to be defined. Moreover, as pointed out by a recent study of the marine cyanobacterium *Synechococcus* and *Prochlorococcus*, it is likely that it is not simply total P content but rather the intracellular fractionation of P that governs organic matter production (Bertilsson et al. 2003). Thus, future research will need to evaluate which cellular P components are being regulated and how such regulation will affect *Trichodesmium* productivity. This will hopefully permit the formulation of a mechanistic model

relating the availability of dissolved inorganic P to the production of C and N by *Trichodesmium*.

Despite popular skepticism, several lines of evidence, including our own model simulations, indicate that vertical migration by colonies of *Trichodesmium* to the depths of the phosphocline cannot be ruled out as a mode of P acquisition. To expand our understanding of this enigmatic facet of *Trichodesmium* autoecology, some key experiments need to be performed in the field, ideally in multiple ocean basins. Specifically, it will be necessary to determine the rate of change of colony density in response to light and nutrient availability (ballast and energy storage), measure vertical velocities and morphological distributions, and evaluate potential correlations between buoyancy status, elemental ratios and productivity. This information would help define the actuality of vertical migration as a means of enhancing P fluxes to the euphotic zone and supplementing the P requirements for diazotrophic growth. Alternately, such experiments could confirm vertical migration by *Trichodesmium* or any other capable bloom-forming genera (i.e. *Rhizosolenia* and *Hemiaulus*) as a means of enhancing diffusional fluxes to migrating cells, a mechanism to escape damaging irradiance encountered in surface waters or all of the above.

A complete understanding of P control of diazotrophy must also assess DOP utilization. *Trichodesmium* spp. have long been known to be capable of hydrolyzing dissolved organic P (DOP) compounds (Yentsch et al. 1972). However, the extent to which this process complements DIP uptake, fulfills P requirements, and contributes to active growth in field populations has yet to be determined. Evidence that *Trichodesmium* are utilizing DOP in nature comes from direct measures of enzyme activity (Mulholland et al. 2002) and from analyses of gene expression (Dyhrman et al. 2006). Additionally, in culture, isolated strains of *Trichodesmium* (IMS101& GBRTRLI101) do not exhibit appreciable growth in batch mode when initial DIP concentrations are less than about 0.2 μM (Fu et al. 2005). In sum, these data imply that oligotrophic populations are actively exploiting DOP to fulfill growth requirements. A complete analysis of P regulation of diazotrophic growth will need to

assess the degree to which each mode of nutrient acquisition fulfills P requirements and impacts net growth rates of *Trichodesmium*. Parameterizations of each these unique physiological adaptations (DOP utilization, vertical migration and flexible stoichiometry) could ultimately be incorporated into coupled biophysical models of oligotrophic regions in order to explore the potential relationships between organismal biochemistry and the cycling of elements within oceanic ecosystems.

Finally, a thorough assessment of elemental cycling by *Trichodesmium* must account for the presence and potential activity of associated organisms. It is well documented that colonies of *Trichodesmium* can harbor a diverse microbial and metazoan community including bacteria, pennate and centric diatoms, dinoflagellates, ciliates, and the juveniles and nauplii of harpacticoid copepods (Sheridan et al. 2002). Some of these associations are certain to influence the physiological status of *Trichodesmium* as essential nutrients and metabolites are exchanged between the host cells and the assemblage of associated organisms (O'Neil and Roman 1992). The degree to which these nutrient interactions impact *Trichodesmium* growth and net productivity will be necessary to constrain in a composite analysis of the role that *Trichodesmium* play in the cycling of elements within oceanic systems.

6.1.2 Future directions: discrimination of the biological and climatological conditions favoring *Trichodesmium* versus *Richelia* bloom formation

Much of the research in this dissertation has focused on specific aspects of *Trichodesmium* physiology that may allow this organism to rapidly increase in abundance in its native oligotrophic habitat. When possible we have also focused on the role of the endosymbiotic cyanobacterium *Richelia,* also known to be an important bloom-forming diazotroph in oligotrophic marine systems (Carpenter et al. 1999; Venrick 1974). The most salient difference between these organisms is that *Richelia* occur in symbiotic association with the diatoms *Rhizosolenia* and *Hemialus* while *Trichodesmium* are free-living. This disparity alone indicates that these organisms may occupy different biochemical niches. Despite efforts by a number of research groups,

Richelia has not been successfully maintained in culture, thus we lack experimental knowledge of the ecophysiology of this organism. Nonetheless, for the reasons stated above, it cannot be assumed that each of these genera impacts primary productivity and elemental cycling to the same degree or in the same manner. Particularly, given that *Richelia* reside within the relatively heavy silica shells of their host diatom, blooms of this organism may be more likely to result in particle export than blooms of *Trichodesmium*. Secondly, while the geographical range of these organisms appears to be similar (Karl et al. 2002), we cannot be certain how climatological conditions differentially impact *Richelia* versus *Trichodesmium* bloom formation. Our own findings in the Gulf of California indicate that these genera alternately dominate community structure. Likewise, reports of blooms in the Atlantic and Pacific indicate occurrences of one bloom type versus the other rather than increases in both genera. To better assess the importance of global N_2 fixation, we have to recognize the redundancy of this functional group and design sampling strategies or observational platforms capable of identifying the differential determinants of each bloom type (diatom-*Richelia* symbioses or *Trichodesmium*).

A combination of remote sensing, stationary mooring and buoy platforms and glider technologies may soon prove adequate to address this challenge. As outlined in Chapter V, a rather impressive array of atmospheric and oceanic forcing can now be derived from remote-sensing and near real-time satellite telemetry of data collected at oceanic mooring and buoys. If a combination of these data, perhaps even those presented in Chapter V, were used as bio-indicators for the potential development of N_2 fixation supported blooms, gliders could be deployed on an on-call basis, to "smartly" sample events rather than mean conditions. For example, consider a scenario where profiling moorings indicated significant shallowing of the mixed layer (< 70m) or warming of the surface ocean (> 25ºC). Perhaps, concurrent remote sensing would also indicate increases in chl *a* obove mean summer levels. These conditions, or event cues, could be considered to be a notice of sorts, such that gliders mounted with biological sensors could then be deployed to sample the environment of interest. This sort of smart sampling strategy would capture a more complete spatial

and temporal image of bloom events that would ever be recorded by scheduled, 2-3 day sampling.

Emergent genetic technologies may also help define differential abundance of the primary groups of N_2-fixing organisms. Rather than examining size-fractionated cyanobacterial pigments which cannot discriminate between genera, monthly sampling of DNA (or complementary DNA) sequences as part of the HOT sampling program or even glider based sampling programs, would allow for analyses of community composition of N_2-fixing genera. In recent years, primers and probes have been constructed for phylotype-specific gene sequences of highly conserved protein components of nitrogenase, the enzyme that catalyzes the reduction of atmospheric N_2 (Zehr et al. 2003). Amplification of these gene sequences using quantitative polymerase chain reaction techniques (e.g. Church et al. 2005) can allow for a robust estimate of the spatial differences in the abundance of gene sequences characteristic of *Trichodesmium*, *Richelia* and unicellular diazotrophs. Thus, high resolution sampling of environmental conditions, if combined with appropriate genetic technologies could also allow for the differentiation of the response of each of the major N_2-fixing genera to environmental forcing. This sort of adaptive sampling is a component of what I see as the probable future of oceanography, where integration of traditional and novel approaches to sampling and monitoring the environment will facilitate a more complete understanding of the complex feedbacks between the environment and oceanic biota.

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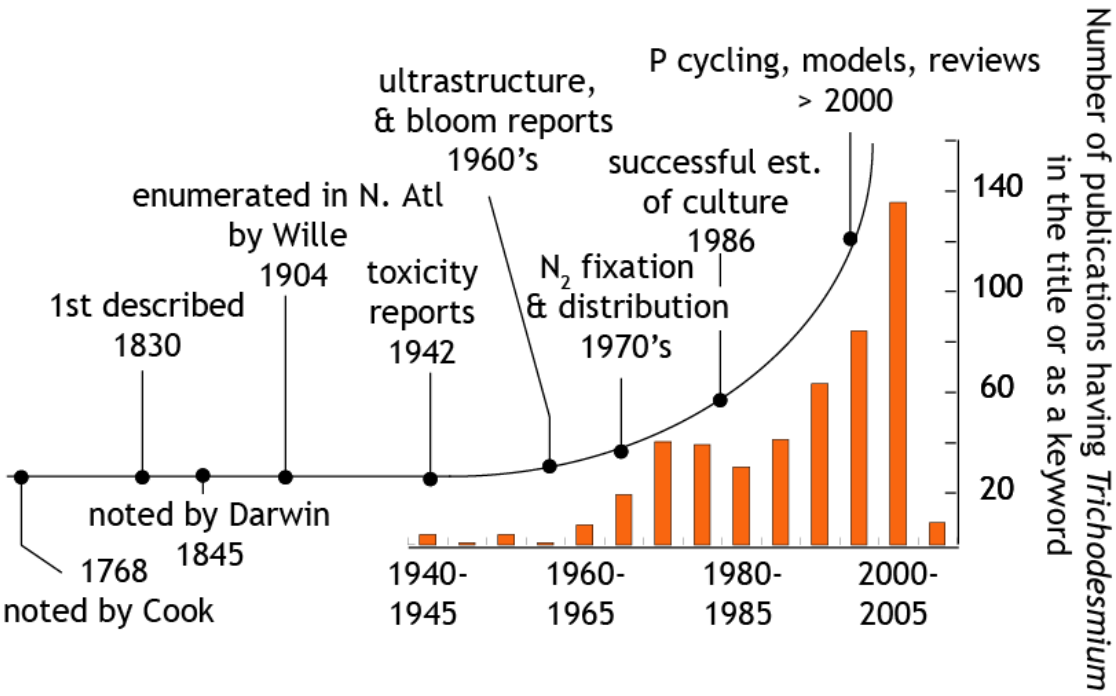

Figure 6-1. Histogram of the number of peer-reviewed publications having *Trichodesmium* in the title or as a keyword. These data are derived from my own literature searches using a variety of on-line citation indices. Significant research findings or topics are denoted along the timeline.

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