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Microbial dynamics and biogeochemistry in the North Pacific Subtropical Gyre

Matthew J. Church
College of William and Mary - Virginia Institute of Marine Science

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MICROBIAL DYNAMICS AND BIOGEOCHEMISTRY IN THE NORTH PACIFIC SUBTROPICAL GYRE

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A Dissertation
Presented to
The Faculty of the School of Marine Science
The College of William and Mary in Virginia

In Partial Fulfillment
Of the Requirements for the Degree of
Doctor of Philosophy

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by

Matthew J. Church

2003

APPROVAL SHEET

This dissertation is submitted in partial fulfillment of
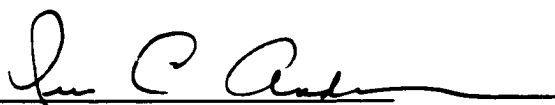
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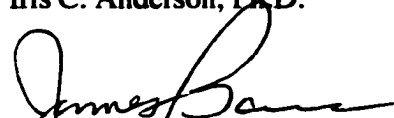
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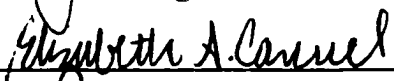
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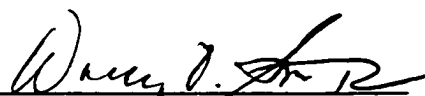
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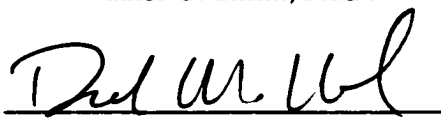
Hugh W. Ducklow
Hugh W. Ducklow, Ph.D.
Committee Chairman/Advisor

Iris C. Anderson
Iris C. Anderson, Ph.D.

James E. Bauer
James E. Bauer, Ph.D.

Elizabeth A. Canuel
Elizabeth A. Canuel, Ph.D.

Walker O. Smith
Walker O. Smith, Ph.D.

David M. Karl
David M. Karl, Ph.D.
University of Hawaii
Honolulu, Hawaii

DEDICATED TO THE MEMORY OF

DR. JAMES M. CHURCH

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ABSTRACT

The research presented in this dissertation describes the influence of planktonic bacteria on upper ocean organic matter dynamics in the North Pacific Subtropical Gyre (NPSG). Analyses of the temporal dynamics in dissolved organic matter (DOM) inventories were coupled to studies investigating the influence of heterotrophic bacterial production (HBP) on upper ocean organic carbon fluxes in the NPSG. Nine cruises to the Hawaii Ocean Time-series field site Station ALOHA revealed that HBP accounted for a large flux of organic carbon in the upper ocean of the NPSG. HBP was significantly enhanced by sunlight, with photoenhancement of HBP accounting for 3.2 mol C m^{-2} yr^{-1}, equivalent to 21% of the annual photoautotrophic production in this ecosystem. These observations suggest that HBP in the upper ocean of the oligotrophic NPSG exerts a large influence on organic matter dynamics in this ecosystem, and that a large fraction of HBP depends on sunlight.

Several experiments were conducted to asses the response of heterotrophic protein production to irradiance at Station ALOHA. The results of these experiments revealed that HBP responded to irradiance similar to the response of photosynthesis to irradiance. Upper ocean HBP increased with light intensity at low light fluxes (<0.200 mmol quanta m^{-2} s^{-1}), but saturated or declined with increasing irradiance. Experiments conducted in the upper and lower photic zone revealed significant photoinhibition of bacterial production in the lower photic zone. Overall, the heterotrophic response was similar to the photosynthetic response, suggesting light-driven HBP could result from mixotrophic growth by the photoautotrophic unicellular cyanobacteria *Prochlorococcus*.

Analyses of dissolved organic matter (DOM) inventories from 1988 to 1999 revealed multiyear increases in the inventories of dissolved organic carbon, nitrogen, and phosphorous (DOC, DON, and DOP) in the upper ocean of the NPSG. During the latter half of the observation period, the rate of DOP accumulation declined, coincident with significant accumulations of DOC and DON. Analyses of bacterial population dynamics between 1992 and 1999, revealed an apparent shift in the abundance of *Prochlorococcus* during the period of observation. Increasing abundance of *Prochlorococcus* coincident with accumulated inventories of DOM suggests that prokaryote population structure directly influences the cycling of organic matter in this ecosystem.

MICROBIAL DYNAMICS AND BIOGEOCHEMISTRY

IN THE NORTH PACIFIC SUBTROPICAL GYRE

INTRODUCTION

Marine biology and ocean carbon cycling

The oceans play a fundamental role in regulating Earth's climate. Strong couplings between atmospheric and oceanic processes promote the transfer of heat and material between the ocean and the atmosphere. The carbon storage capacity of the world's ocean far exceed that of the atmosphere, and the vast oceanic reservoirs of dissolved carbon compounds comprise some of the largest inventories of carbon (Table 1). As a result, there is considerable interest in the whether the oceans may buffer the detrimental impacts of anthropogenic carbon dioxide (CO_2) emissions to the atmosphere.

Understanding the interaction of marine biota and the cycling of mineral elements in the oceans has been the focus of considerable research through the past century. Much of this research has focused on ocean carbon cycling. Carbon transformation in the marine environment occurs via a series of oxidation-reduction reactions that are largely mediated by biological processes. Understanding the factors that influence marine biological activity provides valuable insights into the role of marine biota in carbon cycling and ultimately on global climate regulation.

Nearly half of the global primary production occurs in the oceans, and approximately 80% of this marine production occurs in the open oceans, far removed from terrestrial influences (Martin et al. 1987) (Table 2). The subtropical ocean gyres cover approximately 40% of Earth's surface, and largely because of their massive

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Table 1. Mass of carbon in various Earth reservoirs

Location	Form	Quantity $(10^{15}$ g C)
Atmosphere	CO_2 gas	720
Oceans	total inorganic	40,000
	total organic	700-1,000
	living	1-3
Terrestrial	sedimentary carbonates	>10,000,000
	total organic	2,000
	living	800
	non-living	1,200

Adapted from Falkowski and Raven (1997) and Hansell and Carlson (1998).

Table 2. Primary and new production in various marine environments

Region	Area (10^6 km^2)	Primary Production (g C m^{-2} yr^{-1})[a]	Total Production (10^{15} g yr^{-1})	New Production (10^{15} g yr^{-1})[b]
Open Ocean	326	169	55	3.5
Coastal Zone	37	250	9.0	1.5
Upwelling Areas	0.36	420	0.15	0.03
Total	**363**	**311***	**56**	**7.4**
Ocean Basin				
Pacific	149	132	20	1.3
Atlantic	74	199	15	2.2
Indian	45	143	6.5	1.3
Southern	58	141	8.2	1.5
Total	**326**	**154***	**49.7**	**6.3**

* Mean rate of primary production
[a] compiled from Eppley and Peterson (1979), Martin et al. (1987), Longhurst et al. 1995, and Karl et al. (1996).

surface area, the subtropical gyres play important roles in ocean-atmosphere material exchange (Sarmiento et al. 1993, Bates 2001, Gruber et al. 2002). Considerable research has been devoted to understanding the factors that regulate organic matter production, remineralization, and carbon export in subtropical ocean ecosystems. Despite chronic nutrient limitation of the upper water column in the subtropical oceans, organic matter productivity and carbon export in these ecosystems has a significant role in global carbon cycling (Eppley and Peterson 1979, Martin et al. 1987, Carlson et al. 1994, Emerson et al. 1997).

The biological pump is the principal biological regulator of ocean-atmosphere carbon cycling. The biological pump describes the removal of carbon from the surface ocean to the deep ocean via gravitational settling of particulate organic carbon (Longhurst and Harrison 1989). The net removal of photosynthetically fixed carbon from the surface ocean is regulated by many factors including nutrient input and biological remineralization. Organic matter production in the upper ocean is supported by several nutrient sources. New production defines the total amount of primary production supported by nutrient input (specifically nitrogen (N)) from outside the photic zone (typically NO_3^-) (Dugdale and Goering 1967). Regenerated production describes the amount of primary production supported by regenerated sources of nitrogen (N), mainly in the form of ammonium (NH_4^+). Under steady state conditions, regenerated production maintains organic matter productivity within the ecosystem, but new production represents the carbon available for export.

DOM and the microbial loop in oligotrophic marine ecosystems

One of the most important oceanic pathways of carbon production and export is through the large pool of dissolved organic matter (DOM) (Williams 1990, 2000, Kirchman et al. 1992). In many seasonally impacted oligotrophic ecosystems, the production of microbially resistant DOM compounds represents a significant component of the biological pump (Copin-Montégut and Avril 1993, Carlson et al. 1994). DOM represents as an important intermediate between photoautotrophic and heterotrophic production. The distribution of DOM in the oceans depends on different input and removal processes, with production a function of biologically mediated processes, and removal dependent on both physical and biological processes.

DOM is an operationally defined term to describe organic forms of carbon, nitrogen, and phosphorous that pass through GF/F filters (nominal pore size of 0.7 μm). Dissolved organic carbon (DOC) forms one of the largest bioactive reservoirs of carbon on Earth, accounting for ~7 x 10^{17} g C (Table 2, Hansell and Carlson 1998). Despite the potential relevance of DOC to global carbon cycling very little is known about the specific composition of oceanic DOC or about the processes which transform it. Based on its biological reactivity, DOM is typically classified by the time scales of its persistence in the marine environment. Labile DOM pools turnover on time scales of hours to days, while semi labile pools appear more persistent, turning over on time scales of weeks to months, and refractory pools may resist biological degradation for thousands of years (Williams and Druffel 1987, Bauer et al. 1992, Kirchman et al. 1993).

Photoautotrophic production represents the ultimate source of DOM to the open ocean, but the exact pathways that transform inorganic carbon and nutrients into the large, uncharacterized DOM pool remain unclear. Environmental factors such as grazing, cell death/lysis, and exudation all appear to constitute significant sources of DOM. Removal of DOM from the upper ocean results from both physical mixing and dilution of relatively organic-rich upper ocean waters, and biological utilization of DOM. Heterotrophic bacteria, or more precisely chemoorganotrophic bacteria, are believed to be the dominant consumers of DOM in the oceans (Azam and Hodson 1977, Ducklow and Carlson 1992). These organotrophic bacteria transform DOM in at least two fundamental ways (Figure 1): 1) construction of biomass from nutrient constituents within the DOM pool, and 2) the oxidization of DOM for energy and subsequent regeneration of inorganic nutrients (Ducklow et al. 1986).

Determining the magnitude of carbon flux into heterotrophic bacteria is necessary for understanding the fate of carbon in marine ecosystems. The microbial loop represents the collective processes that result in the transfer of DOM into bacterial biomass, effectively reintroducing carbon and nutrients lost to dissolved non-living pools back into the marine food web (Pomeroy 1974, Azam et al. 1983). However, in many cases, heterotrophic bacterial metabolism serves as a net sink for DOM, remineralizing it back to its inorganic constituents (Ducklow et al. 1986).

Heterotrophic utilization of DOM (and hence DOM reactivity) appear to depend on several factors, but perhaps most importantly on the molecular composition of the DOM substrate. Dissolved free amino acids, nucleotides, and

Figure 1. A simplified schematic describing the pathways of carbon turnover in the

upper ocean of the NPSG. Arrows represent carbon fluxes into each compartment of

the ocean food web. Dashed line represents losses of carbon from the upper ocean.

The dark arrow into DOC reflects the relative importance of this carbon flux in the

NPSG surface waters.

Pathways of primary production in the NPSG
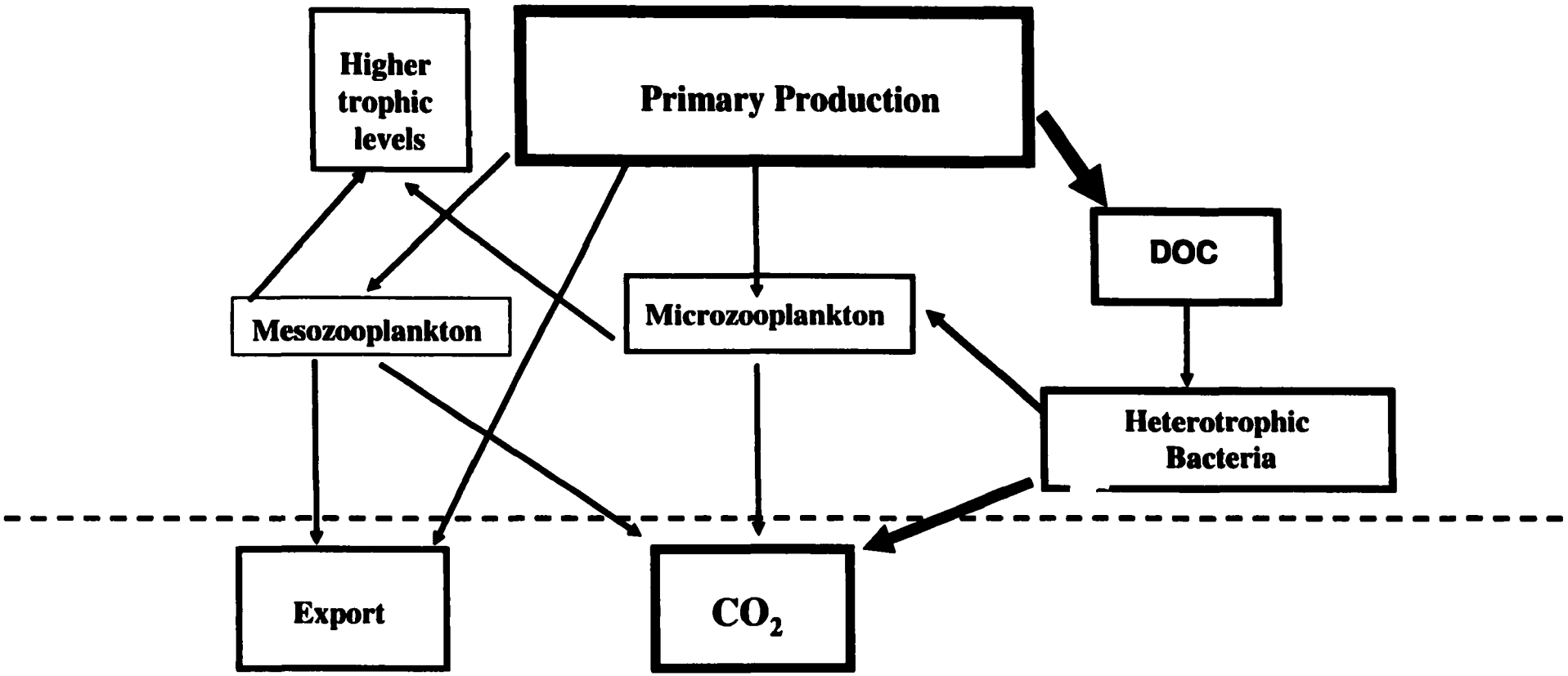
neutral monosaccharides all cycle rapidly in the ocean, suggesting they represent some of the most biologically reactive components of the bulk oceanic DOM pools (Fuhrman and Ferguson 1986, Keil and Kirchman 1999). In some systems, DOM production and microbial utilization can be temporally uncoupled resulting in significant accumulations of labile and semi-labile DOM over time scales ranging from days to months (Carlson and Ducklow 1996, Carlson et al. 1998). In other cases, microbial utilization appears tightly coupled with photoautotrophic production and no measurable labile DOM accumulates (Carlson and Ducklow 1996, Cherrier et al. 1996).

Heterotrophic bacterial growth efficiency (HBGE) controls the magnitude of carbon flux into the microbial loop in marine ecosystems. Conceptually, HBGE describes the proportion of carbon biomass produced relative to the total pool of carbon assimilated for growth. HBGE is defined as:

$$HGBE = HBP/ HBP + Respiration$$

Heterotrophic growth is supported by both catabolic and anabolic pathways; catabolic pathways harvest energy from reduced DOM and produce ATP, while anabolic pathways yield biomass and use ATP. DOM is assimilated for construction of cell constituents (biomass production) and broken down to yield energy. HBGE reflects the relative importance of these intracellular pathways (del Giorgio and Cole 2000). Many factors influence HBGE including the nature and amount of organic substrate available for growth, temperature, and inorganic nutrients (del Giorgio and Cole

1998). In marine systems, HBGE generally ranges from 5-30%, averaging ~20% (del Giorgio and Cole 2000).

By studying the factors that regulate heterotrophic production in the oligotrophic North Pacific Ocean this study aims to evaluate the role of bacterial growth and production on upper ocean organic matter productivity and remineralization. Planktonic prokaryotes, or more generally bacterioplankton, bear the distinction of potentially being the most important, yet poorly understood members of the marine plankton community. The prokaryotes include the *Archaea* and *Bacteria*, although the latter are considerably more numerous in the upper ocean of the NPSG (Karner et al. 2001). Prokaryotes are the most numerous organisms on Earth (Whitman et al. 1998), and they comprise the largest inventories of living carbon in the sea.

The NPSG and Station ALOHA

The North Pacific Subtropical Gyre (NPSG) forms the largest oceanic circulation pattern on Earth (Sverdrup et al. 1946). The NPSG extends between 15-35° N and 135° E to 135° W and covers an area of 2×10^7 km^2. The convergent circulation pattern of the surface ocean restricts the vertical influx of deep, nutrient laden waters to the surface ocean. Moreover, a permanent thermocline establishes a strong physical barrier to vertical mixing that further accentuates nutrient depletion in the upper ocean. The physical isolation of surface waters results in highly oligotrophic conditions characterized by low nutrients, low biomass, and relatively low production.

Early research into biological and chemical properties of the NPSG (nutrients and chlorophyll *a*) revealed weak or non-existent seasonal variability and interannual constancy (McGowan and Williams 1973, Hayward et al. 1983), although the resolution was not sufficient to resolve seasonal patterns. The physical stability observed in the upper ocean was thought to be responsible for the observed spatial and temporal homogeneity in biological communities, where the biomass and production of planktonic assemblages were relatively constant through both space and time. Primary production appeared largely sustained by regenerated nutrients, implying that new production and export fluxes were low (Hayward 1983, Hayward et al. 1987).

Recent studies on the biogeochemistry of the NPSG have been facilitated by the creation of the Hawaii Ocean Time-series (HOT) as part of the US JGOFS program. The research site for the HOT program is Station ALOHA (22°45'N, 158°W); a deep water (>4000 m), open ocean time-series station located approximately 100 km north of the island of Oahu, Hawaii. On a monthly basis, the HOT program visits Station ALOHA and conducts measurements on water column nutrient and carbon inventories and fluxes. The high frequency sampling at Station ALOHA has revealed considerable temporal dynamics in the NPSG biogeochemistry not observed in previous studies. In particular, biogeochemical processes in the NPSG appear tightly linked to large scale climate dynamics. These linkages appear to have altered organic matter production, biomass, and nutrient cycling in the upper ocean (Venrick et al. 1987, Venrick 1993, Karl et al. 1995, 1997, Karl 1999, Karl et al. 2001a, 2002b).

Considerable attention has focused on the role of nitrogen-fixing bacteria as potential sources of new nitrogen to the NPSG and their influence on biogeochemical cycles at Station ALOHA. Nitrogen input to the photic zone by diverse diazotrophic bacteria may support up to half of the new production in this ecosystem (Karl et al. 1997, Zehr et al. 2001, Dore et al. 2003). The introduction of this new nitrogen source to the upper ocean without corresponding inputs of other bioessential elements is hypothesized to have significantly altered the turnover and stoichiometric composition of the dissolved and particulate organic matter pools in the NPSG on decadal time scales (Karl et al. 1997, 2001c).

The upper waters at Station ALOHA are characterized as having persistently low nutrient concentrations, low plankton biomass, and relatively high fluxes of solar radiation. Upper ocean concentrations of $NO_3^-+NO_2^-$ typically range from <1 to 100 nM, while PO_4^{3-} concentrations tend to range between 10 and 100 nM (Figure 2, 3). Inorganic nutrient concentrations remain low throughout the upper 100 m of the water column, increasing with depth near the base of the photic zone (1% surface isopleth) (Figure 2, 3). The resulting N:P ratio of the inorganic nutrient pools approaches zero in the surface waters, and increases to upwards of 10:1 near 150 m depth.

In contrast to the dearth of inorganic nutrients in the upper ocean, organic nutrient profiles reveal that the surface waters are relatively enriched in reduced N and P containing compounds. Concentrations of DON in the upper 200 m of the water column range from ~4-6 μM, and DOP ranges from ~0.1-0.3 μM (Figure 2, 3). Concentrations of both these organic nutrient pools are greatest in the surface ocean,

Figure 2. Mean vertical profiles of dissolved nitrogen species (NO_3^-+NO_2^-, DON)

and chlorophyll a concentrations at Station ALOHA in the NPSG. Profiles are

average concentrations measured at Station ALOHA between 1989 and 2001.

Dashed lines represent the percent surface irradiance penetrating to various depths in

the photic zone.
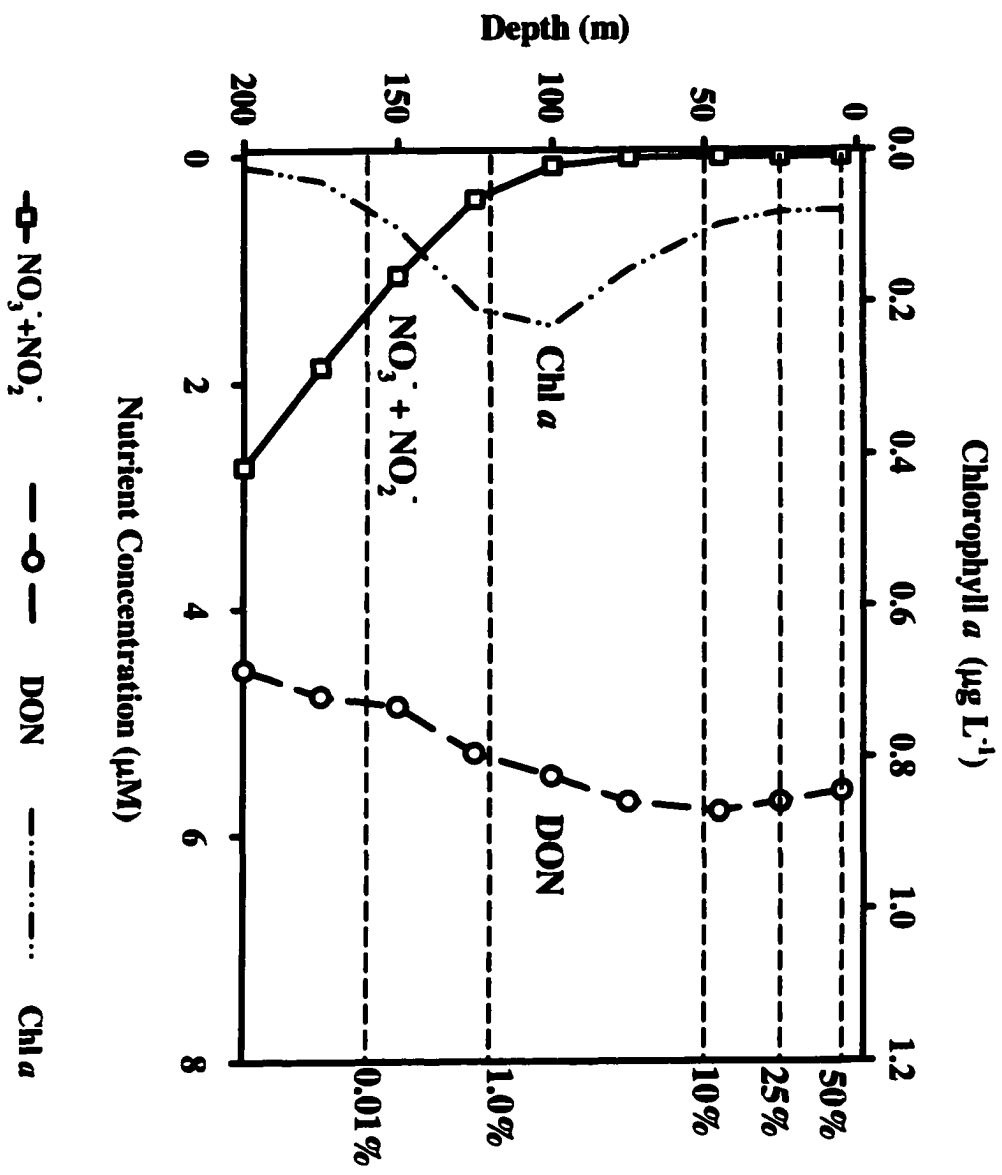
Figure 3. Mean vertical profiles of dissolved phosphorus species (PO_4^{3-}, DOP) and

chlorophyll a concentrations at Station ALOHA in the NPSG. Profiles are average

concentrations measured at Station ALOHA between 1989 and 2001. Dashed lines

represent the percent surface irradiance penetrating to various depths in the photic

zone.
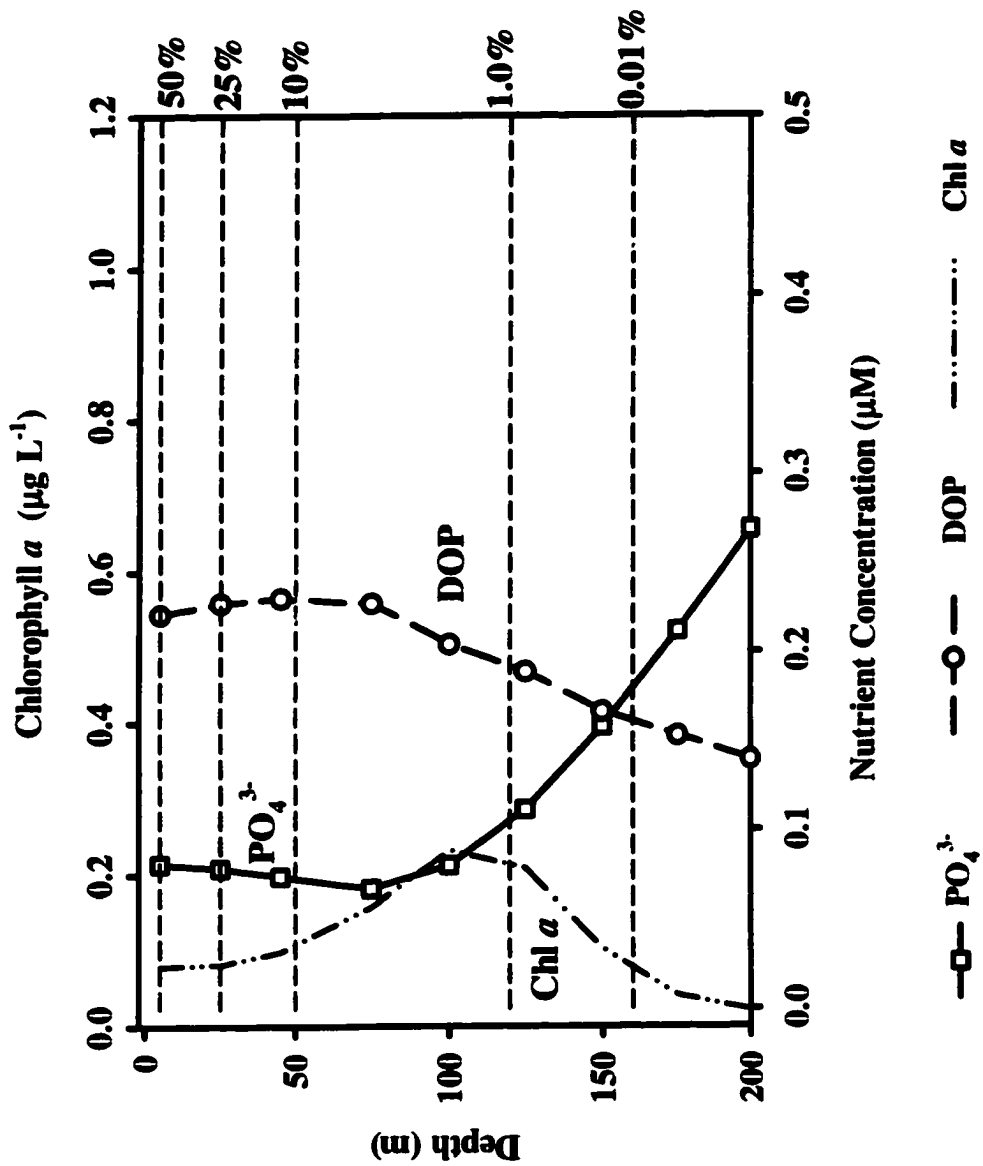
and decrease with increasing water depth. Beneath the nutricline (~100 m), PO_4^{3-}

concentrations are substantially greater than DOP concentrations, but DON is

persistently greater than $NO_3^- + NO_2^-$ throughout the upper 200 m of the water

column. The distribution of DON and DOP in the upper ocean results in a bulk DOM

pool maintaining an N:P ratio of between 25:1 and 32:1, or roughly 30-50% greater

than the Redfield ratio.

Biogeochemical cycles and organic matter production and remineralization in

the upper ocean of the NPSG are largely controlled by bacterial metabolism. At

Station ALOHA, a large portion of organic matter productivity is sustained by

regenerated nutrients. The upper ocean waters appear highly retentive with respect to

carbon and nutrient export; carbon fluxes out of the photic zone average only 6% of

the measured photoautotrophic production (Table 3, Karl et al. 2001d).

Estimates of plankton biomass in the upper water column at Station ALOHA

range from ~113-300 mmol C m^{-2} depending on the methods used to quantify

plankton abundance and concentrations (Table 3). Chl a concentrations in the upper

100 m of the water column are consistently low, ranging from 0.07-0.23 µg chl a L^{-1}.

Vertical profiles of chl a reveal a consistent subsurface maximum located between

~100-125 m, roughly coincident with the 1% surface isopleth (Figure 3). The vertical

position of the deep chlorophyll maximum (DCM) appears largely influenced by

nutrient diffusion from the top of the nutricline and photoadaptation of the plankton

populations to the low light fluxes at the base of the photic zone (Letelier et al. 1993,

Winn et al. 1995).

Table 3. Carbon inventories and fluxes in the upper ocean (0-175 m) at Station ALOHA in the NPSG.

Parameter	Mean Concentration[a] (μmol C L^{-1})	Inventory (mmol C m^{-2})	Method
DIC	2038	356739	Coulometry
DOC	86	15119	High Temp. Combustion
PC	1.8	306	High Temp. Combustion
Chl. Biomass[b]	0.31	54	HPLC chl a
Prochloro.[c]	0.33	58	flow cytometry
Synec.[d]	0.010	1.8	flow cytometry
H. Bacteria[e]	0.31	54	flow cytometry

Fluxes	Mean Rate (mmol C L^{-1} d^{-1})	Primary Production (mmol C m^{-2} d^{-1})	Method
PP	0.24	41.4	^{14}C-in situ production
HBP[f]	7.8×10^{-2}	13.7	^{3}H-Leucine L/D
Particle Export	$1.5\text{-}3.1 \times 10^{-2}$	2.7-5.5	Sediment Traps
DOC Accum.	4.6×10^{-3}	0.82	Regression Analyses

Abbreviations are: DIC –Dissolved inorganic carbon, DOC- dissolved organic carbon, PC- particulate carbon, Prochloro.-Prochlorococcus, Synec.-Synechococcus, H. Bacteria-non-chl a containing bacteria, P.P-primary production, HBP-heterotrophic bacterial production, DOC Accum.- dissolved organic carbon accumulation.
[a]Mean concentrations and rates determined by dividing inventory by 175 m.
[b]based on C : Chl ratio of 30:1 (Karl et al. 1996)   [c] based on 30 fg C cell^{-1} (Bertillson et al. in press)   [d] based on 190 fgC cell^{-1} (Landry and Kirchman 2002)
[e] based on 10 fgC cell^{-1} (Christian and Karl 1994)   [f] based on 1.5 kgC mol^{-1} Leu (Simon and Azam 1989)

The pigmented picoplankton *Prochlorococcus* and *Synechococcus* account for >75% of the photoautotrophic biomass and primary production at Station ALOHA (Liu et al. 1997, Campbell et al. 1994, Karl et al. 2001a, b). Vertical profiles of *Prochlorococcus* reveal relatively homogeneous cell distributions through the upper 75 m of the water column, averaging roughly 2.0 x 10^5 cells ml^{-1}, and declining more than an order of magnitude toward the base of the photic zone (Figure 4). The abundance of the pigmented cyanobacterium *Synechococcus* is substantially lower than *Prochlorococcus*. *Synechococcus* abundance averages 2.0 x 10^3 cells ml^{-1} in the upper 75 m of the water column, and declines approximately an order of magnitude to the base of the photic zone (Figure 4). The non-chl *a* containing cells (which include the obligate heterotrophic and photoheterotrophic *Bacteria*) are greatest in the surface ocean, averaging roughly 4.5 x 10^5 cells ml^{-1} in the upper 75 m of the water column before declining to 2.3 x 10^5 cells ml^{-1} at the base of the photic zone (Figure 4).

The abundance of *Prochlorococcus* is remarkably high at Station ALOHA. Throughout the upper 75m of the water column, *Prochlorococcus* abundance averages 47% of heterotrophic bacterial abundance (Figure 4); by comparison, *Prochlorococcus* accounts for 4-10% of heterotrophic bacterial abundance in the oligotrophic Sargasso Sea. *Prochlorococcus* appears more successful in the upper ocean of the NPSG than in seasonally oligotrophic ecosystems. Examination of the time-series data on bacterial abundance at Station ALOHA reveals that the relative abundance of *Prochlorococcus* has increased ~37% within the past decade (Figure 5). The biogeochemical and ecological consequences of the accumulated cell biomass are still unknown; however, these observations provide additional evidence that the upper

Figure 4. a.) Mean vertical profiles of upper ocean bacterioplankton

(*Prochlorococcus*, *Synechococcus*, and non-chlorophyll containing bacteria) at

Station ALOHA. Profiles are average cell abundances measured at Station ALOHA

between 1991 and 2001. b.) Ratio of chlorophyll containing bacteria

(Prochlorococcus and Synechococcus) to non-chlorophyll containing bacteria at

Station ALOHA. Dashed lines represent the percent surface irradiance penetrating to

various depths in the photic zone.

Figure a: Depth (m) versus Cells ml⁻¹ showing *Prochlorococcus* (square), *Synechococcus* (circle), and Non-chl *a* bacteria (triangle).

Figure b: Pigment Bact.:Non-pigment Bact.
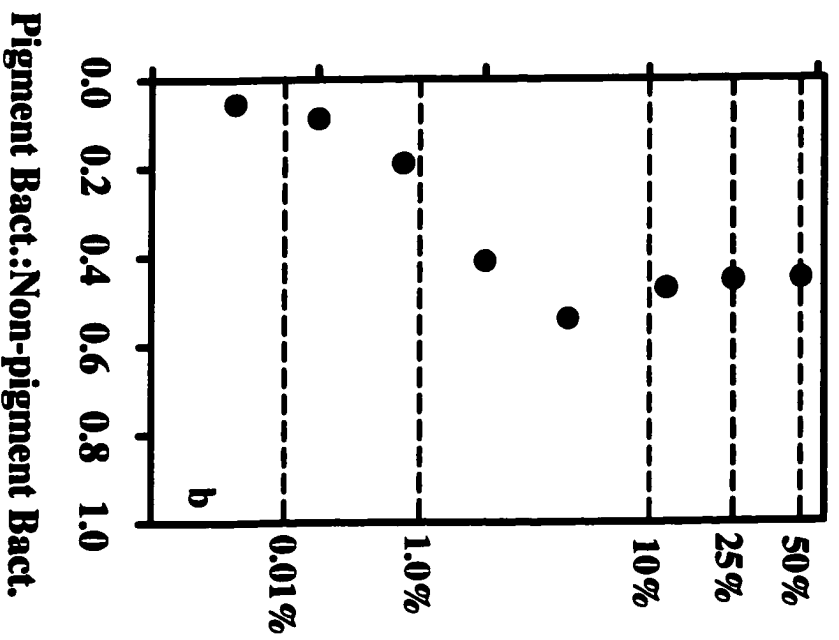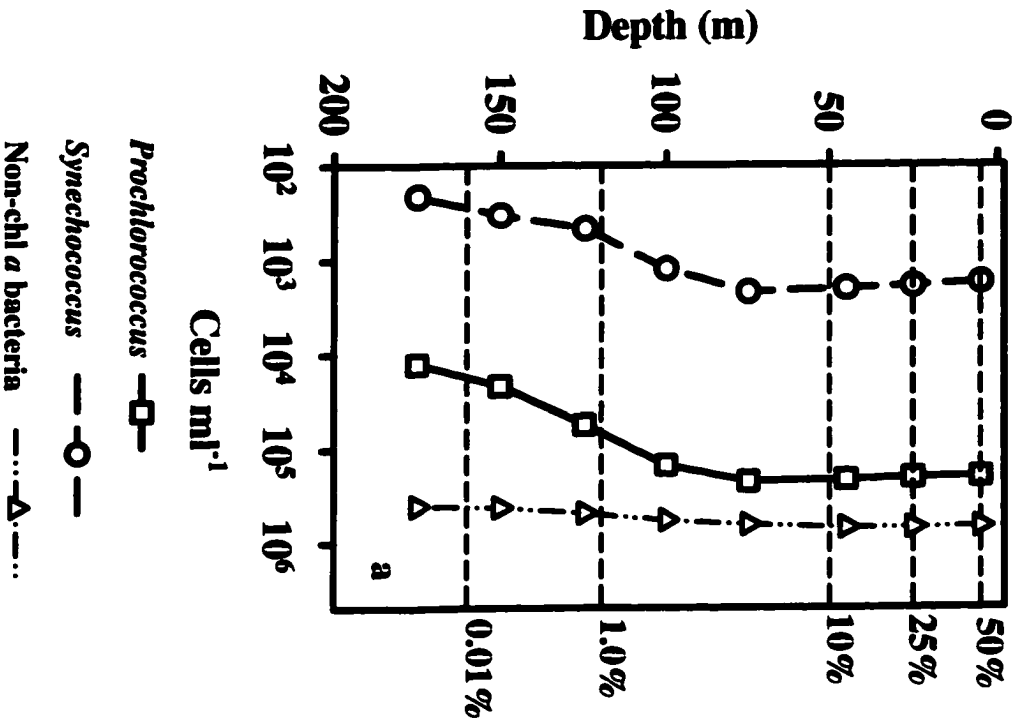
Figure 5. Depth integrated stocks of *Prochlorococcus* relative to non-chlorophyll

containing bacteria at Station ALOHA. Line represents Model I linear regression of

entire data set (1990-2001). Equation shown is least squares linear regression, where

x is years since the beginning of the time series (December 1990).

$$y = 0.010\,x + 0.27 \qquad r^2 = 0.14,\ p<0.0005$$
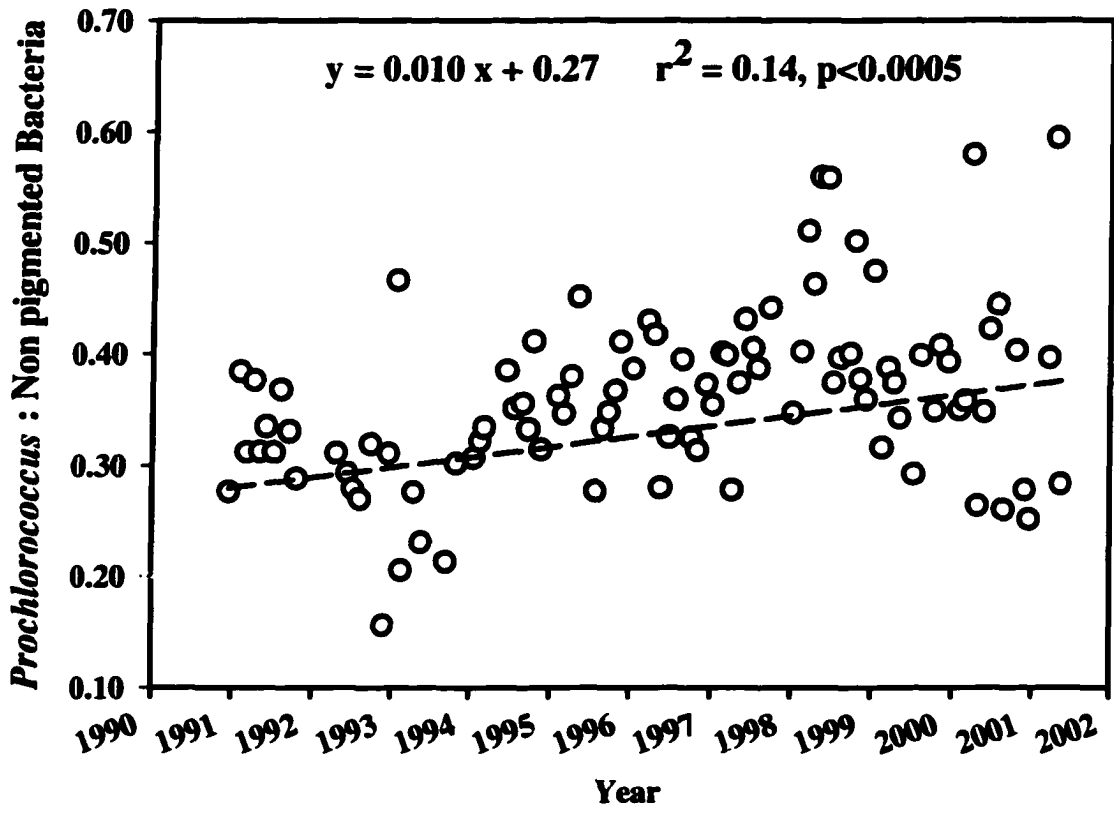
ocean of the NPSG is temporally dynamic over decadal time scales.

The influence of bacterioplankton metabolic diversity on carbon cycling

Our knowledge on the growth and dynamics of bacterioplankton populations has stemmed primarily from two types of research: culture-based studies on isolated microorganisms, and culture-independent approaches to characterize rates of microbial biomass, growth, and diversity. Culture-based approaches use either solid or liquid media to isolate and cultivate representative members of prokaryote assemblages. The utility of these methods are apparent; by understanding the metabolic capacities of the organisms valuable information is obtained about the potential role of the organism in the environment. However, cultivation of marine microbes has proven a daunting task, and to date, fewer than 10% of oceanic bacterioplankton are believed to be in culture.

Development of culture-independent techniques revealed that bacterioplankton are numerous and actively growing in the oceans (Jannasch and Jones 1959, Hobbie et al. 1977, Karl 1979, Fuhrman and Azam 1980). Recent applications of both cultivation-dependent and independent methodologies have continued to improve our understanding of the role of bacterioplankton in ocean biogeochemistry. In particular, the use of flow cytometry to determine bacterioplankton abundance led to the landmark discovery of the most abundant photosynthetic organism on the planet, the unicellular bacterium *Prochlorococcus* (Chishlom et al. 1988).

Application of genomic approaches to the study of microbial diversity has revolutionized the way we view taxonomic and physiological diversity in the oceans. For example, the discovery that three of the most deeply branching domains of life exist in the oceans was possible from sequencing 16S rRNA gene fragments (Giovannoni et al.1990, DeLong 1992, Fuhrman et al. 1992, López-Garcia et al. 2001). Use of molecular methods has provided information about the abundance of prokaryotic groups that live in oceans; perhaps most importantly, the deep and mesopelagic regions of the open ocean appear dominated by planktonic *Archaea* (Karner et al. 2001) and the marine SAR 11 cluster of α-proteobacteria may comprise the most numerically abundant microorganisms on the planet (Rappé et al. 2002, Morris et al. 2003).

Despite these advances in characterizing the types of microorganisms present in the open ocean, recent investigations in the NPSG suggest that our knowledge of the physiological diversity of marine bacteria remains in its infancy. Previous studies that have considered the contributions of bacterioplankton to carbon and energy fluxes in the oceans were largely devoted to understanding the contribution of "autotrophic" and "heterotrophic" microorganisms. However, the recent rediscovery of abundant and diverse groups of aerobic anoxygenic photoheterotrophic (AAnP) bacteria in the upper ocean waters of the NPSG provided evidence that marine microbes can rely on complex physiological strategies for growth (Kolber et al. 2000, 2001, Béjà et al. 2002).

AAnP bacteria capture energy from sunlight for the production of ATP. Unlike oxygenic photoautotrophs, AAnP bacteria do not cleave water as a source of

reductant and therefore do not evolve oxygen as a photosynthetic biproduct.

Photosynthesis in the AAnP bacteria is driven by a single light reaction with

bacteriochlorophyll a (Bchl a) serving as the primary light harvesting pigment. AAnP

bacteria also contain a wide diversity of cartenoids including zeaxanthin, β-carotene,

and spirilloxanthin, which appear to play central roles in the light-harvesting

capabilities of these organisms (Yurkov and Beatty 1998). Photosynthesis by AAnP

bacteria bears similarity to oxygenic photosynthesis; however, the one light reaction

center generates energy (ATP) by cyclic electron flow, and does not produce

reductant in the form of NADH. As a result, AAnP bacteria require exogenous

sources of reductant, typically as reduced organic matter. Thus,

AAnP bacteria are facultative heterotrophs, utilizing DOC as a carbon substrate, but

capable of fixing inorganic carbon when DOC is in limiting concentrations (Kolber et

al. 2001).

In addition to the studies of AAnP bacteria, analyses of large bacterial gene

fragments extracted from upper ocean bacterioplankton assemblages in the NPSG

revealed that members of the SAR 86 cluster of γ-Proteobacteria contained a

previously undocumented phototrophic pathway (Béjà et al. 2000, 2001). These

bacteria use rhodopsin-like molecules (called proteorhodopsin) to capture light and

transform photons into ATP. Upon absorption of light energy, the membrane bound

proteorhodopsin undergoes a conformational shift, establishing a proton-motive force

that drives ATP synthesis. Neither the carbon nor reductant sources of these

proteorhodopsin containing bacteria are known; however, similar light-dependent

proton pumping has been observed in the halophilic archaeon *Halobacterium salinarum*, which depends on organic matter as a carbon and reductant source.

A growing number of observations have demonstrated that various groups of bacteria grow mixotrophically utilizing multiple carbon, energy, and reductant sources (Kaden-Lee and Simonis 1982, Paerl 1991, Montesinos et al. 1997, Kamjunke and Jahnichen 2000.). Facultative photoautotrophy (or mixotrophy) is not uncommon among the photosynthetic plankton, and numerous cultivated photoautotrophic organisms have the ability to supplement or replace light and CO_2 with DOM as the primary energy and carbon sources for growth (Falkowski and Raven 1997). Similarly, facultative heterotrophs supplement or replace their utilization of DOM with sunlight and CO_2 as primary energy and carbon resources (Kolber et al. 2001). This versatility in bacterial metabolism expands the suite of ecological resources available to the bacterioplankton. In fact, the advantages of mixotrophic metabolism in oligotrophic oceans leave one to wonder whether there are roles for obligate heterotrophs or autotrophs in these ecosystems.

Influence of light on bacterial growth at Station ALOHA

The recent rediscovery of AAnP bacteria combined with the discovery of proteorhodopsin bacteria suggest that marine bacteria demonstrate versatile nutritional and metabolic strategies (Karl 2002a). The apparent diversity of bacterial metabolism in the NPSG reinforces the need for investigations that examine the contribution of diverse microbial physiologies to ocean carbon cycling. The relative increases in chlorophyll containing bacteria observed at Station ALOHA (Figure 5)

may result from increasing reliance of *Prochlorococcus* and *Synechococcus* on

mixotrophic growth. The increasingly oligotrophic nature of the upper water column

at Station ALOHA likely selects for biological communities that can selectively

utilize the relatively abundant pools of DOM for nutritional supplement, while

acquiring energy from sunlight. One of the motivating hypotheses of this study is

that simultaneous utilization of DOM and sunlight provides the upper ocean plankton

assemblages with sources of energy, reductant, nutrients, and carbon (Table 4).

A central theme throughout this study will be to evaluate the impact of solar

energy on heterotrophic production in the oceans. The observation that heterotrophic

production in the NPSG was significantly enhanced by sunlight was noted by Karl

(1999), who suggested it likely resulted from tightly coupled photoautotrophic and

heterotrophic processes. The present study sought to determine the importance of

light-enhanced heterotrophic production on upper ocean carbon cycles. Three

mechanisms are presented, all of which would result in light-stimulated bacterial

production (Figure 6). The first mechanism (Figure 6a) is the utilization of sunlight

for energy and DOM as a source of carbon and nutrients by the photoheterotrophic

bacterial assemblages in the upper ocean at Station ALOHA. In this case, the

harvesting of solar energy increases HBGE by decreasing the need for ATP

production by substrate level and oxidative phosphorylation allowing more efficient

retention of substrates into cell biomass. The net result is an increase in HBGE,

effectively decreasing the total carbon flux into heterotrophic bacteria, while

simultaneously increasing productivity.

Table 4. Physiological classifications and nutritional, energy and reductant resources of the upper ocean plankton at Station ALOHA

Metabolism	Carbon	Energy	e⁻ donor
Obligate Photo-			
-heterotroph	DOM	sunlight	DOM
-autotroph	CO_2	sunlight	H_2O
Obligate Chemo-			
-heterotroph	DOM	DOM	DOM
-autotroph	CO_2	reduced inorganic substrate/DOM	inorganic substrate
Facultative Photo/Chemo/Auto/ Heterotroph (Mixotroph)	DOM/CO_2	sunlight/DOM	DOM/H_2O

Figure 6. Schematic depicting possible mechanisms resulting in light-enhanced

heterotrophic bacterial production at Station ALOHA. Included in schematic are

possible sources of energy, reductant (e⁻), and carbon and nutrients (CNP) for the

three possible scenarios. a.) Photoheterotrophic production by newly discovered

proteorhodopsin containing photoheterotrophs (Beja et al. 2001). Energy is derived

exclusively from sunlight, while CNP and e- stem exclusively from dissolved organic

matter (DOM). b.) Mixotrophic metabolism by aerobic, anoxygenic photosynthetic

bacteria (AAnP) Kolber et al. 2000) or *Prochlorococcus* and *Synechococcus*. Energy

is derived from either sunlight or DOM, e⁻ may be either DOM (for the AAnP) or

H_2O (*Prochlorococcus*), and CNP sources may be either inorganic or organic. c.)

Tightly coupled photoautotrophic and heterotrophic production. *Prochlorococcus*

grows as an obligate photoautotroph, utilizing sunlight and inorganic nutrient pools.

Exudation of DOM in the light fuels heterotrophic bacterial production.

a

Energy

DOM

e⁻

C/N/P

Photoheterotrophs
(i.e. Proteorhodopsin
containing bacteria)

b

Energy

CO₂
NO₃
PO₄

CNP

Mixotroph
(i.e. AAnP bacteria
or *Prochlorococcus*)

e⁻ H₂O

CNP
Energy
e⁻

DOM

c

Energy

CO₂
NO₃
PO₄

CNP

Photoautotroph
(i.e. *Prochlorococcus*)

Energy, e⁻
C/N/P

Obligate
Heterotroph

Energy e⁻ C/N/P

DOM
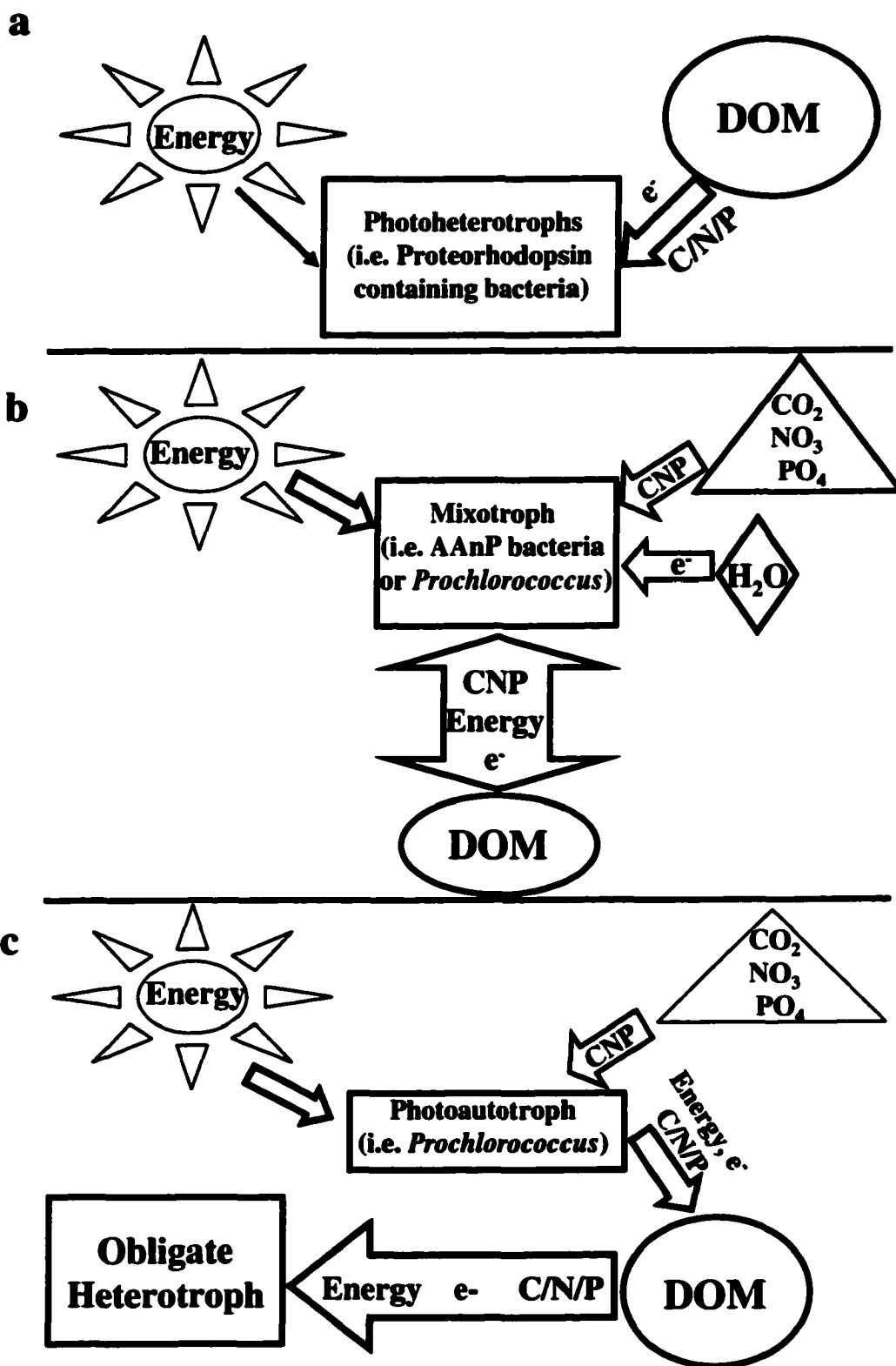
The second mechanism that would result in light-stimulated heterotrophic production is similar to the previously described mechanism, but the bacterial assemblage utilizing light and DOM is different. In this case (Figure 6b) mixotrophic bacteria gain energy by both DOM catabolism and sunlight, while tapping into a suite of reductant, carbon, and nutrient pools that include both organic and inorganic sources. *Prochlorococcus* represents a potentially important mixotrophic population to examine under this scenario.

The final mechanism of light-stimulated heterotrophic production described in this study results from a tightly coupled photoautotrophic-heterotrophic ecosystem (Figure 6c). By this mechanism, photoautotrophic production of labile DOM is directly coupled to heterotrophic utilization of this DOM pool. Under this scenario, carbon fluxes into the microbial loop would increase during the day, coincident with production of labile DOM and decrease at night. Under this circumstance, the daily integrated "light effect" would disappear due to the diel dependence of heterotrophic production.

Based on recent investigations demonstrating the presence of diverse photoheterotorphic bacterial assemblages, and observations that the upper ocean at Station ALOHA has become increasingly oligtrophic in the past decade, mixotrophic and photoheterotrophic production may have become an increasingly important pathway of carbon production in this system. As such, one of the central hypotheses of this work was that in the increasingly oligotrophic NPSG, utilization of multiple modes of nutrition and energy acquisition by the bacterioplankton would be favored.

PROJECT JUSTIFICATION AND DISSERTATION OUTLINE

The motivation for this study stemmed from the desire to understand the

ecological and biogeochemical role of bacteria in the NPSG. The documented

changes in upper ocean biogeochemistry during the past decade provide fertile

ground for examining hypotheses about microbial mediation of organic geochemistry

in the NPSG. The NPSG is a prokaryote dominated ecosystem so constraining rates

of prokaryote production and growth, and inventories of bacterioplankton abundance

provides insight into ecosystem productivity and biomass. By combining time-series

measurements on heterotrophic bacterial production with HOT program

measurements of ocean biogeochemical processes, my objectives were three-fold:

1) Determine the influence of heterotrophic, bacterial production to upper

ocean carbon fluxes.

2) Evaluate the influence of irradiance on heterotrophic production.

3) Describe the temporal dynamics of upper ocean DOM inventories.

A large portion of my dissertation focuses on the role of light-regulated heterotrophic

production in the open ocean.

Section II of this dissertation summarizes measurements of heterotrophic

bacterial production from nine cruises to Station ALOHA between March 2000 and

May 2002. Through quantification of heterotrophic production rates, the relative

importance of heterotrophic bacteria to upper ocean organic carbon fluxes could be

constrained. Section II describes a light-enhancement of heterotrophic bacterial

protein production in the NPSG, and explores possible mechanisms that could result

in light-stimulated heterotrophic production. The results of this section reveal that

light-dependent bacterial production is a significant carbon flux in the upper ocean,

equivalent to half of the heterotrophic bacterial production in the photic zone of the

NPSG.

Section III evaluates the response of heterotrophic bacterial protein production

to irradiance at Station ALOHA. Based on the results of photosynthetron

incubations, the dependence of leucine incorporation on irradiance is evaluated from

several experiments conducted in the upper and lower photic zone. The results from

Section III revealed that the relationship between bacterial protein production and

irradiance differed from response of photosynthesis to the same variable. Overall,

heterotrophic protein production was more responsive to low light fluxes than

photosynthesis; leucine incorporation rates increased significantly at low light fluxes,

typically saturating or declining at light fluxes optimal for photosynthesis. This

section provides the first quantitative assessment of the response of heterotrophic

bacterioplankton protein production to irradiance.

Section IV of this dissertation describes the results of analyses of upper ocean

DOM inventories at Station ALOHA. Several interesting temporal trends emerged

from these analyses including apparent multi-year accumulations of dissolved organic

carbon (DOC), nitrogen (DON), and phosphorus (DOP). In particular, upper ocean

inventories of DOC increased by 303 mmol C m^{-2} yr^{-1}, while DON accumulated at 14

mmol N m^{-2} yr^{-1}. Inventories of DOP also demonstrated long-term accumulation

$(0.73 \text{ mmol P m}^{-2} \text{ yr}^{-1})$, but through the latter period of observations, the rate of DOP

accumulation decreased suggesting net long-term imbalances in the input and

removal of DOP in the upper ocean. The observations from this section provide the

first multi-year evaluation DOM dynamics in the oligotrophic North Pacific Ocean.

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SECTION II

LIGHT-ENHANCED BACTERIAL PRODUCTION

IN THE NORTH PACIFIC SUBTROPICAL GYRE

ABSTRACT

Time-series observations of heterotrophic bacterial production (HBP) revealed

consistent photoenhancement throughout the upper ocean of the North Pacific

Subtropical Gyre (NPSG). Rates of ^{3}H-leucine (Leu) incorporation were significantly

greater in samples incubated under *in situ* light (Leu$_L$) relative to samples incubated

in the dark (Leu$_D$); Leu$_L$ rates were 1.4-1.8-fold greater than Leu$_D$ throughout the

upper water column. Photoenhancement of Leu incorporation was apparent

throughout the upper 100 m of the water column, with relative light-stimulation

greatest near the 1% surface irradiance isopleth. Light-stimulation of Leu

incorporation resulted in a ~50% increase in integrated protein production rates

relative to rates measured in the dark, making HBP one of the largest organic carbon

fluxes in the upper ocean. Linear regression analyses indicated that HBP varied

positively with stocks of *Prochlorococcus*, but was not significantly related to either

primary production or surface PAR fluxes, suggesting *Prochlorococcus* may utilize

both organic and inorganic sources of nutrients for growth. Estimates of organic

carbon fluxes in the upper ocean show that net community respiration ranged from

12.7 to 13.8 mol C m^{-2} yr^{-1}, and bacterial growth efficiencies were as high as 29%.

Overall, light-regulated protein production contributed significantly to organic matter

fluxes at Station ALOHA.

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INTRODUCTION

The growth of planktonic prokaryotes in the oceans plays an important role in global biogeochemical cycling. In large areas of the oceans, the combination of prokaryote biomass production and respiration accounts for the largest biologically mediated flux of carbon in the sea (Falkowski et al. 1994, Azam 1998). The North Pacific Subtropical Gyre (NPSG) covers nearly 40% of the Earth's surface, forming both the largest circulation feature and continuous biome on the planet (Sverdrup et al. 1946, Karl 1999). The NPSG is a prokaryote dominated ecosystem; biomass of the unicellular prochlorophyte *Prochlorococcus* alone accounts for 40-90% of the photosynthetic biomass in the photic zone (Chisholm et al. 1988, Campbell and Vaulot 1993). Rates of both primary and secondary production in this ecosystem are largely dictated by prokaryotic growth (Karl 1999). Therefore, quantifying the factors that influence prokaryote production (both primary and secondary) and remineralization are crucial to understanding the region's role in global carbon cycling.

Heterotrophic prokaryotes serve as the primary consumers and remineralizers of organic matter in the oceans, and therefore exert fundamental control over ocean biogeochemistry. In the photic zone of the NPSG, non-chlorophyll containing (presumably heterotrophic) bacteria dominate picoplankton abundance (Karl 1999,

Karner et al. 2001). Heterotrophic bacterial growth results in at least two dominant

carbon fluxes in the open ocean. First, transformation of dissolved organic matter

(DOM) into living biomass (by HBP) comprises a pathway for carbon to reenter the

marine plankton food web (Pomeroy 1974, Azam et al. 1983, Cho and Azam 1988,

Ducklow 1999). Second, heterotrophic respiration is a primary organic carbon sink in

oceanic systems (Ducklow et al. 1986, Cole et al. 1988, del Giorgio and Cole 1998).

Together, HBP and heterotrophic respiration (HR) account for the total carbon fluxes

required to sustain heterotrophic prokaryote growth.

Measurements of HBP provide insight into the rate that heterotrophic bacteria

utilize organic matter for production of living biomass. The efficiency with which

organic carbon is converted into bacterial biomass is the heterotrophic bacterial

growth efficiency (HBGE). HGBE reflects the gross organic carbon fluxes required

to sustain bacterial growth. HBGE is defined as:

$$HBGE = HBP/HBP + HR$$

Estimates of HBGE in oceanic environments range from 0.01-0.6, averaging ~0.2 in

the open ocean (Carlson et al. 1996, del Giorgio and Cole 1998, Carlson et al. 1999).

Despite a persistent dearth of inorganic nutrients (average NO_3^- and PO_4^{3-}

concentrations are persistently below standard analytical detection limits, ranging

from <1 to 10 nM and 10 to 100 nM in the surface ocean, respectively) observations

from the Hawaii Ocean Time-series (HOT) between 1989-2001 suggest that the

average annual rate of photoautotrophic production (PP) is 15 mol C m^{-2} yr^{-1} (Karl et

al. 2001), nearly three-fold greater than the global average for open ocean ecosystems (Eppley and Peterson 1979, Longhurst et al. 1995). During the same observation period particulate carbon export averaged 1-2 mol C m^{-2} yr^{-1}, resulting in export ratios (e-ratio = measured export / PP) at Station ALOHA ranging between 0.06-0.13 (Karl et al. 2001), suggesting that 87-94% of the organic carbon fixed by photosynthesis was remineralized or retained as dissolved organic carbon (DOC) in the upper water column (Church et al. 2002). These estimates of PP do not include the potentially large contribution of photosynthetically derived carbon partitioned as DOC. For example, Karl et al. (1998) suggested that inclusion of DOC production at Station ALOHA would increase the total organic carbon production as much as two-fold. Assuming a doubling of total organic carbon production (particulate + DOC), 91-97% of total organic carbon produced would be remineralized, reinforcing the importance of bacterial metabolism as one of the largest organic carbon fluxes in the upper ocean.

Living cells depend on a continuous supply of energy for both growth and cellular maintenance (Schegel 1991). Cellular energy derives from metabolism, e.g. the orderly biochemical transformation of substances within the cell. In the past, studies on bacterioplankton growth in the upper ocean have focused on photoautotrophy and chemoheterotrophy as the primary metabolic pathways in the upper ocean. Recent studies suggest that these metabolic classifications may be insufficient to explain the diversity of bacterially-mediated processes driving upper ocean biogeochemical cycles (Fenchel 2001, Karl 2002). In particular, attention has focused on photoheterotrophy as a potentially important form of bacterial metabolism in the open ocean. Photoheterotrophy is defined as the light-dependent uptake and

metabolism of dissolved organic matter (DOM) by phototrophic prokaryotes. Photoheterotrophs are physiologically distinct from chemoheterotrophs (which use DOM as sole energy, carbon, and reductant sources) and photoautotrophs (which use sunlight for energy, inorganic carbon, and water for reductant) in that they produce ATP from sunlight (via photophosphorylation) and gain reducing equivalents and carbon from DOM.

The oceans contain diverse groups of photoheterotophic bacteria, with equally diverse photoheterotrophic metabolic pathways. Kolber et al. (2000, 2001) and Béjà et al. (2002) recently described abundant groups of aerobic anoxygenic (AAnP) bacteria; AAnP are photoheterotrophic bacteria that contain bacteriochlorophyll *a*. Isolated strains of AAnP appear to be predominately heterotrophic, with the majority (>95%) of carbon anabolism being derived from heterotrophic metabolism (Yurkov and Beatty 1998, Kolber et al. 2001). However, AAnP use cyclic photophosphorylation to generate energy, and may also use carbon dioxide as a carbon source for biomass synthesis. Furthermore, they do not dissociate water as a source of reductant for synthesis of NADPH, and therefore do not evolve oxygen during photosynthesis (Yurkov and Beatty 1998).

In addition to the photoheterotrophic AnAP bacteria, another novel, bacterially-mediated, phototrophic pathway was identified by analyses of large gene fragments extracted from upper ocean prokaryote assemblages (Béjà et al. 2000, 2001). Members of the SAR 86 clade of the upper ocean prokaryote assemblage are capable of harvesting light-energy via a non-photosynthetic pathway, employing a rhodopsin-like light harvesting molecule for ATP photoproduction (Béjà et al. 2000,

2001). Although the metabolic pathways utilized by these phototrophic bacteria remain largely unknown, the relatively large pool of oceanic DOM has been suggested as a ready source of both carbon and/or reductant (Kolber et al. 2000, Fenchel 2001, Béjà et al. 2001, Karl 2002). By harvesting light-energy for photophosphorylation, photoheterotrophic metabolism minimizes oxidative phosphorylation, thereby decreasing the overall aerobic respiratory demands of the cells (Yurkov and Beatty 1998). Understanding the potential contributions of these bacteria to upper ocean organic matter production and remineralization will require understanding their metabolic pathways.

Solar radiation influences a myriad of both abiotic and biotic processes in the upper ocean (see reviews by Moran and Zepp 2001, Mopper and Kieber 2002). Many studies have targeted the potentially important influences of UV light (~200-400 nm) on photochemical transformation of DOM in the marine environment. UV light penetrates relatively deep (~30 m) into the upper ocean in oligotrophic marine ecosystems (Smith and Baker 1981), suggesting its influence on microbial processes in these ecosystems may be particularly important.

UV photooxidization appears to impact DOM bioavailability, often producing low molecular weight, partially oxidized substrates (such as carbonyl compounds) that may support a large fraction of the upper ocean microbial metabolic requirements (Kieber et al. 1989, Mopper et al. 1991, Benner and Biddanda 1998). However, several studies have also observed photochemical production of recalcitrant DOM, a process likely attributable to cross-linking of large molecular DOM compounds (Tranvik and Kokalj 1998). In addition, DOM absorption of UV radiation releases

and consumes inorganic and organic nutrient ions such as NH_4^+ and amino acids (Bushaw et al. 1996, Bushaw-Newton and Moran 1999). Any of these photochemically mediated processes can influence the productivity of marine microbes.

In addition to abiotic transformation of organic and inorganic nutrients by solar radiation, solar radiation may also directly influence planktonic growth and productivity in the upper ocean. UV light generally appears detrimental to the growth of planktonic microbes; absorption of UV-B radiation (300-315 nm) results in cross linking of DNA bases and interferes with DNA replication (Jeffrey et al. 1996a). UV-light appears to inhibit bacterial production by 10-70% in the upper 20 m of the water column (Herndl et al. 1993, Aas et al. 1996); however, a significant fraction of the UV-induced photodamage to bacterioplankton cells appears repairable by light-sensitive DNA repair enzymes, reducing the net effect of daytime photochemical damage (Jeffrey et al. 1996b, Booth et al. 2001).

The principal objectives of this study were: 1.) conduct time-series observations on HBP in the NPSG; 2.) examine the potential importance of PAR on bacterial production to upper ocean organic carbon fluxes; and 3.) construct an annual carbon flux budget for the upper ocean at Station ALOHA inclusive of the contribution of HBP and photoenhanced HBP (PHBP). The results of this study indicate that light-stimulated HBP significantly enhanced the flux of carbon to the base of the plankton food web, and influenced the cycling of carbon in the upper ocean.

MATERIALS AND METHODS

Study site and sampling

Sampling for this study was conducted on nine HOT cruises (HOT 114, 115, 116, 118, 119, 124, 135, 136, 137) to Station ALOHA (22° 45' N, 158° 00' W) between February 2000 and May 2002 aboard the R/V Kaimikai-O-Kanaloa (Table 1). Water samples were collected at eight discrete depths (5, 25, 45, 75, 100, 125, 150, 175 m) in the upper ocean at Station ALOHA using a conductivity-temperature-depth (CTD) rosette sampler with 24-PVC bottles. The sampling depths were chosen to match simultaneous determination of rates of ^{14}C-PP.

Rates of heterotrophic bacterial production (HBP) were estimated based on the incorporation of the ^{3}H-amino acid leucine into bulk plankton community proteins (Kirchman et al. 1983). Whole seawater was collected into acid-cleaned, 40 ml polycarbonate centrifuge tubes and inoculated with 20 nmol L^{-1} of leucine (19 nmol L^{-1} nonradioactive leucine + 1 nmol L^{-1} ^{3}H-leucine). Three replicates and one blank were prepared for each treatment (light and dark incubation) from each depth. Polycarbonate (PC) has been shown to effectively screen UV-A and UV-B radiation, but is largely transparent to PAR (Holm-Hansen et al. 1989); the specific spectral absorption of the PC incubation tubes used in this study is shown in Figure 1.

Table 1. Sampling and physical water column properties at Station ALOHA

Cruise	Month/Year	Temperature (°C)	MLD[1] (m)	PAR[2] (mol quanta m^{-2} d^{-1})	k[3] (m^{-1})	EZ[4] (m)
114	April 2000	23.9	52±10	50.2	0.048	96
115	May 2000	24.9	31±8	46.3	0.039	118
116	June 2000	25.6	29±9	58.7	0.057	84
118	August 2000	25.9	62±7	49.4	0.043	108
119	October 2000	25.9	70±10	30.6	0.043	107
124	March 2001	24.1	59±16	43.1	0.039	119
135	February 2002	23.0	89±14	37.1	0.042	104
136	March 2002	23.3	93±51	44.7	0.040	114
137	May 2002	25.0	21±10	51.9	0.040	116

[1] Mixed Layer Depths (MLD) determined by 0.125 unit potential density criterion of Monterey and Levitus (1997).
[2] PAR fluxes at surface ocean as measured by LICOR detector.
[3] Attenuation coefficient of PAR (0-100 m) based on vertical profiles of PAR fluxes.
[4] Photic zone depths equal to depth of 1% surface isopleth.

Figure 1. Absorption spectra of Oak Ridge polycarbonate centrifuge tubes used for this study. Spectra were obtained using a Varian Cary 20 UV-Visible Spectrophotometer. Note strong absorption of UV wavelengths (200-400 nm), and low relative absorption in the PAR region (400-700 nm) of the spectrum.
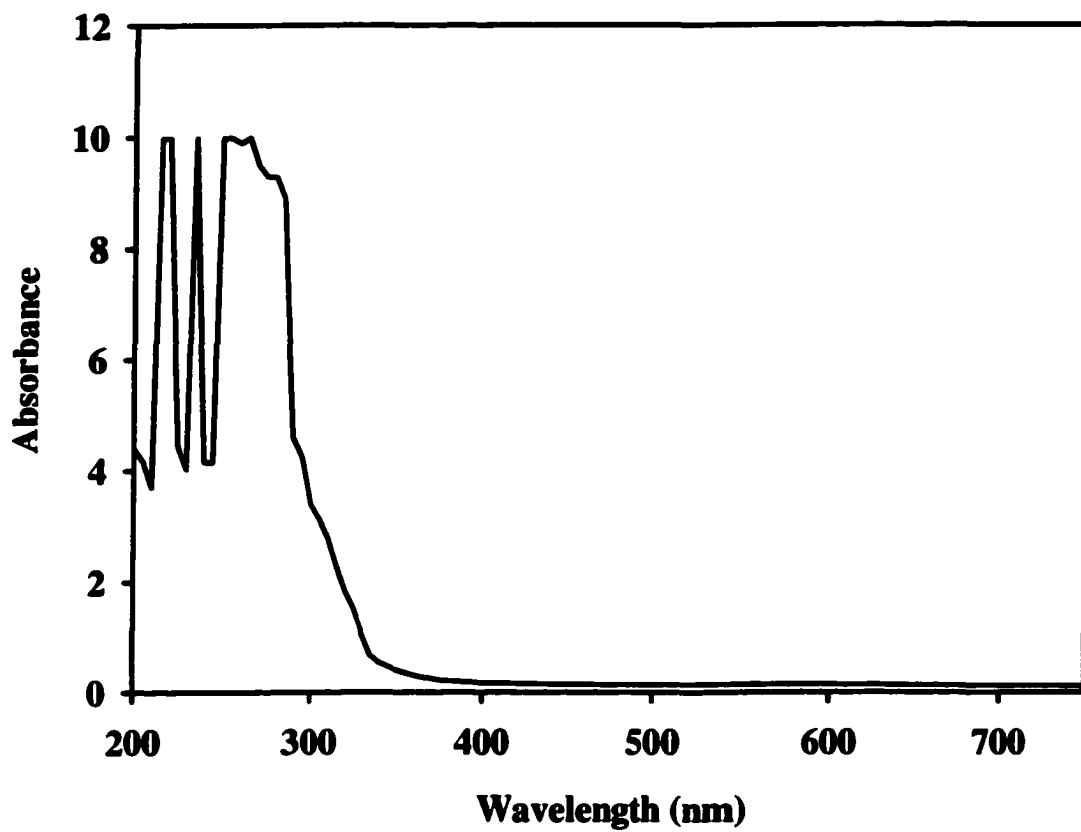
Specific activities of ^{3}H-leucine stocks were 150-180 Ci mmol^{-1} (New England Nuclear, NEN460A). The concentration of Leu added was empirically determined to nearly saturate heterotrophic uptake, thereby minimizing intracellular isotope dilution (Figure 2).

To determine the effect of PAR on HBP, samples were incubated under both *in situ* light and in the dark. HBP assays were incubated alongside ^{14}C-PP assays on a free-floating *in situ* array. Blank Leu treatments were immediately filtered onto a 25 mm, 0.2 μm cellulose acetate filters (Millipore) and stored frozen in 15 ml centrifuge tubes until processed at the laboratory. Triplicate 40 ml subsamples were placed in transparent plexiglass cylinders and hung from a free-floating, surface tethered, *in situ* array; triplicate dark treatments were placed in opaque cloth bags and also hung from the array. Samples were hung at eight depths throughout the photic zone (5, 25, 45, 75, 100, 125, 150, 175 m) and incubated throughout the daylight period (the average incubation time was 12.5 hours). Time-course experiments were conducted to assure that ^{3}H-protein production was linear throughout the period of incubation (Figure 3). To terminate incubations, samples were filtered onto 0.2 μm HA filters and frozen in centrifuge tubes. In the laboratory samples and blanks were processed identically.

Determination of ^{3}H-leucine incorporation rates

After transport to the laboratory, determination of the Leu incorporation rate into proteins and nucleic acids was based on a modification of the Schmidt-Thannhauser procedure (Karl 1982). Proteins were extracted via alkaline hydrolysis

Figure 2. Rate of Leu incorporation into protein as a function of Leu concentration. Samples were collected at 5 m depth in May 2001. Open circles are light incubated samples, closed circles are dark incubated samples. Samples were incubated on the deck of the ship in incubators designed to mimic temperature and light conditions of the surface ocean. Lines are least squares regression: dashed line (light): $y = 74 \ x \ / (3.1 + x)$, $r^2 = 0.87$, P=0.021; solid line (dark): $y = 64x \ / (4.1 + x)$, $r^2 = 0.87$, p= 0.021.
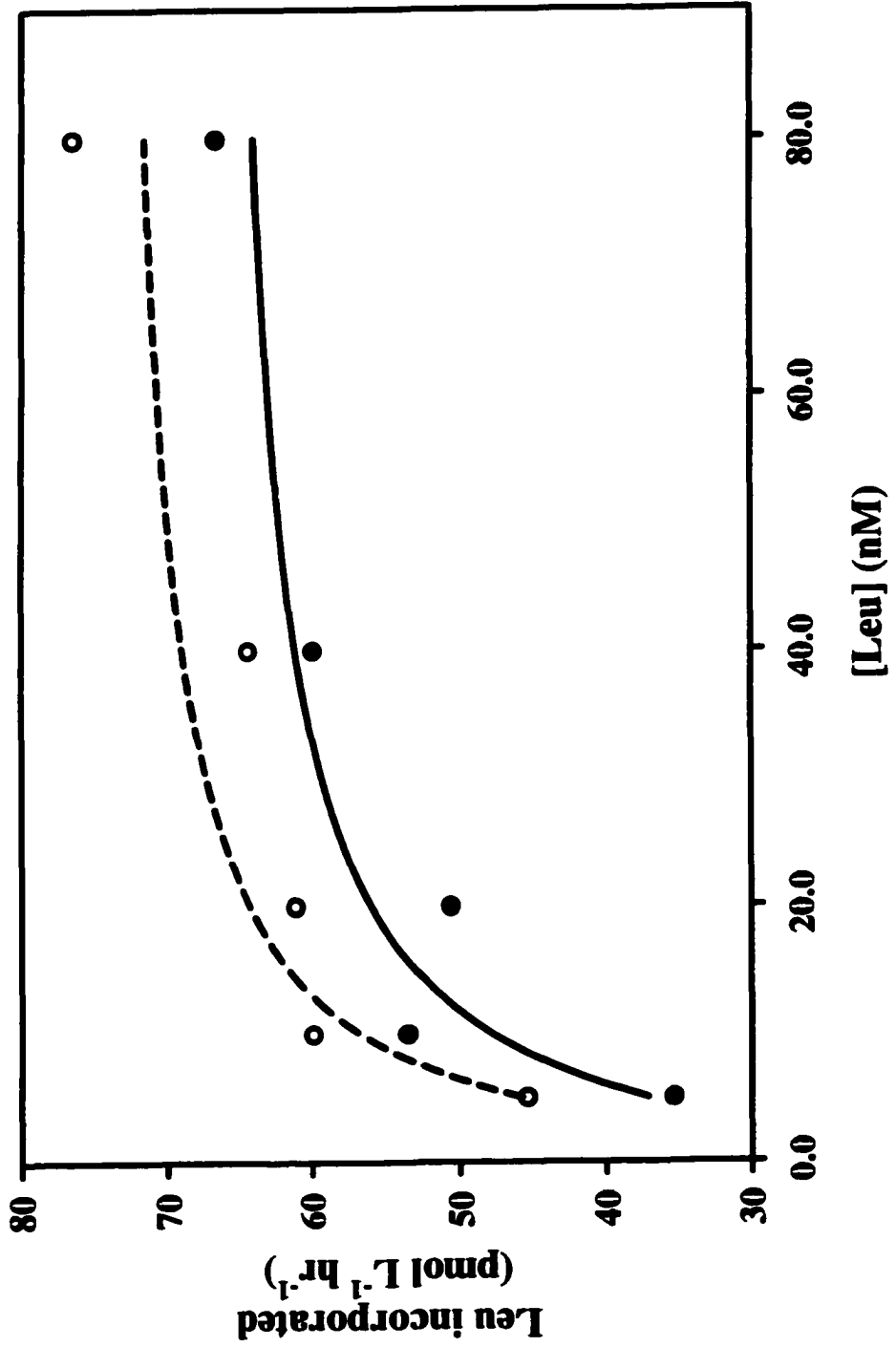
Figure 3. Time course of ^{3}H-leucine incorporation (DPM) into plankton proteins from 5 m depth in April 2000. Temperature of incubation was 24°C; incubator was shaded to mimic light intensity at 5 m depth. Open symbols are samples incubated in the light, filled circles are samples incubated in the dark. Lines are least-square linear regression (Model I) of DPM versus time. Solid line (dark): y = 4318x + 10651, r^2 = 0.93, P=0.001; dashed line (light): y = 9559x + 553, r^2 = 0.94, P<0.005.
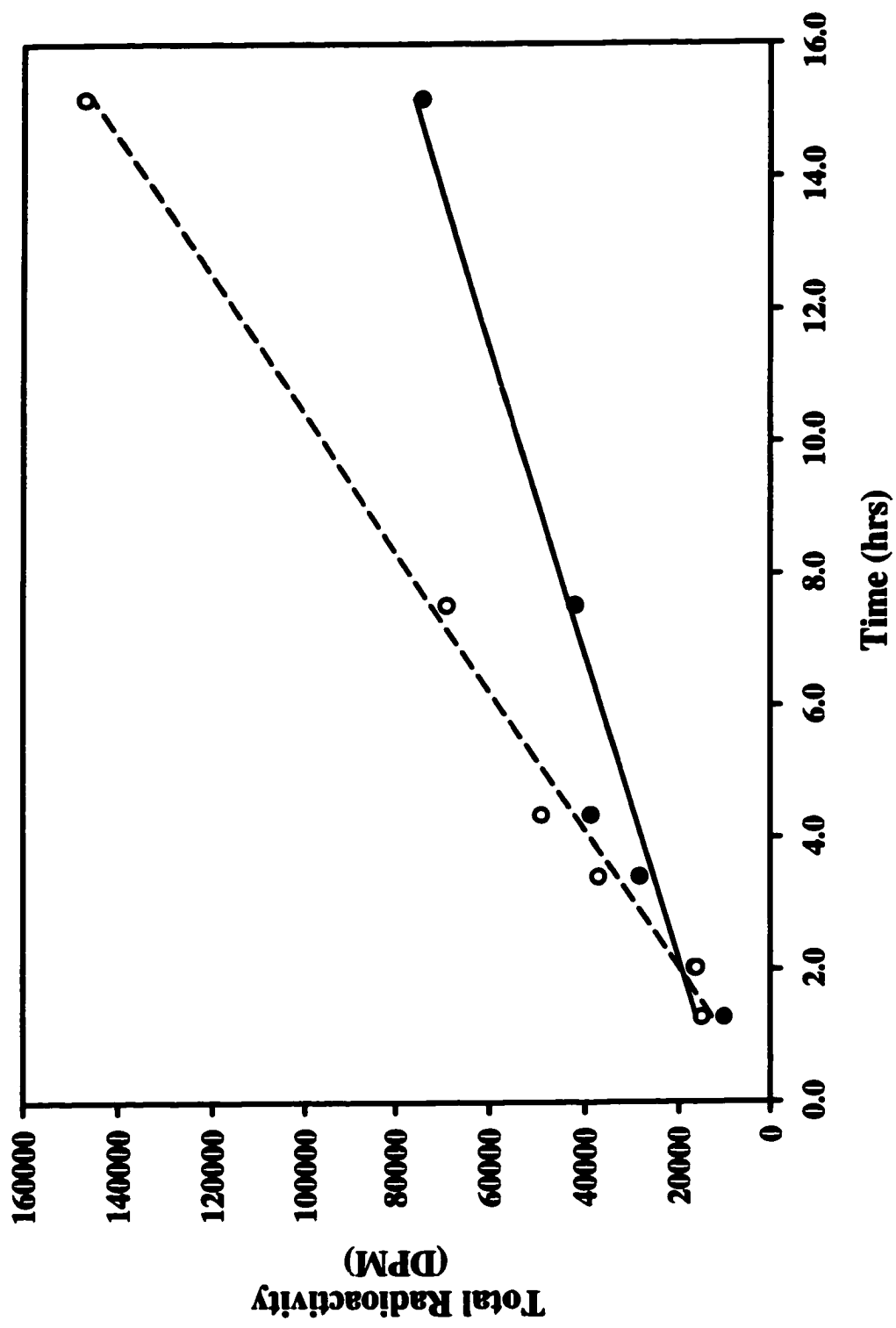
of acid-insoluble macromolecules, and nucleic acids were separated by hot

acidification. Briefly, 0.5 ml of a 2 mg ml^{-1} mixture of RNA, DNA, and protein (as

bovine serum albumin) were added to each sample. Five milliliters of an ice-cold

acetone slurry containing diatomaceous earth (0.5 g diatomaceous earth + 5 ml 100%

acetone) was then added to each sample. The RNA, DNA, protein mixture was added

to aid macromolecular precipitation (Karl 1982). Filters were solubilized in the

acetone slurry for one hour, after which the centrifuge tubes were placed in a

refrigerated (2°C), bench-top centrifuge and spun at 4000 rpm for 10 minutes. Upon

completion of centrifugation, the supernatant was aspirated from each sample, and 5

ml of ice-cold 5% trichloroacetic acid (TCA) was added to each sample test tube.

Samples were vortexed briefly, and placed back into the centrifuge and spun for an

additional 10 minutes. This process was repeated twice with 5% TCA, then twice

with ice-cold 80% ethanol (EtOH). After the second ethanol rinse, centrifuge tubes

were placed in a heating block (80°C) and excess EtOH was evaporated.

When samples were completely dry, 4 ml of 5% TCA were added to each

sample and tubes were placed in a heating block set at 100°C, and samples were

boiled for 30 minutes. Two-ml subsamples were removed for determination of the

relative amount of ^{3}H dissimilated into nucleic acids. Subsamples were placed in 20

ml glass scintillation vials with 10 ml of Aquasol II liquid scintillation cocktail. The

remaining 5% TCA supernatant was aspirated from each tube, and 5% TCA rinses

were repeated two more times, followed by one additional 80% EtOH rinse. Excess

EtOH was evaporated, and 4 ml of 1 M NaOH was added to each sample. Samples

were placed in a water bath at 43°C for 18 hours. Two-ml of base hydrolyzed

proteins were removed from each tube and placed in a 20 ml glass scintillation vial

with 1 M HCl and 10 ml Aquasol II. Samples were counted on TRI-CARB 4640

liquid scintillation counter (Packard Instruments, Co.) using external quench

standards and luminescence correction.

Measured rates of Leu incorporation into proteins (pM Leu hr^{-1}) were

converted to carbon production rates, ignoring isotope dilution. A carbon conversion

factor of 1.5 kg C mol^{-1} Leu (Simon and Azam 1989) was used to calculate carbon

based HBP. Integrated water column production rates were calculated through the

upper 200 m using trapezoidal integration.

Primary production and cell abundance

Estimates of PP were based on depth profiles of photosynthetic rates

measured using the ^{14}C-bicarbonate uptake assays described in Letelier et al. (1996).

Data used in the present study were obtained from the HOT web site

(http://hahana.soest.hawaii.edu/hot). Briefly, seawater from predawn hydrocasts was

subsampled into acid cleaned 500 ml polycarbonate bottles and spiked with ^{14}C-

bicarbonate. A 250 μl subsample was removed from each bottle at the beginning of

the incubation for determination of ^{14}C specific activity. Triplicate samples were

incubated on an *in situ* array. Similar to HBP, samples were incubated for variable

lengths of time depending on the daylight period, averaging 12.5 hours. Experiments

were terminated by filtering seawater onto 25 mm glass fiber filters (Whatman GF/F,

nominal pore size 0.7 μm). Filters were placed in glass scintillation vials and stored

frozen until processed. In the laboratory filters were acidified with 1 ml of 2M HCl and vented for 24 hours, followed by addition of 10 ml Aquasol II scintillation cocktail. Samples were counted on a TRI CARB 4640 liquid scintillation counter. Calculations of photosynthetic production relied on measured total inorganic carbon concentrations for determination of isotopic dilution. Integrated PP was derived by trapezoidal integration of vertical profiles of photosynthetic rates over the top 200 m of the water column.

Prokaryote abundance was enumerated by flow cytometry using the methods described in Monger and Landry (1993) and Campbell and Vaulot (1993). Samples were collected from the same hydrocasts as productivity assays; seawater was collected in 15 ml polypropylene tubes, and then 1 ml was subsampled into Cyrovials (Corning) containing 0.02 ml of 10% paraformaldehyde. Cyrovials were then quick frozen in liquid nitrogen and stored at $-80°C$ until analyzed. Prior to analyses, samples were thawed and stained for 2 hours with the fluorochrome Hoescht 33342 (Monger and Landry 1983). Samples were analyzed for abundance of *Prochlorococcus*, *Synechococcus*, and non-chlorophyll containing prokaryotes (presumably heterotrophic *Bacteria* and *Archaea*) on a Coulter EPICS dual laser (1 W 488 nm and 225 mW UV) flow cytometer. Particle signals were collected as forward light scatter, side scatter, red fluorescence, orange fluorescence, and blue fluorescence, and converted to cell abundance using the software CYTOPC (Vaulot 1989).

Light measurements

Surface ocean water PAR fluxes were determined from a shipboard LICOR LI-200 detector on the days that primary production and bacterial production was measured. Light fluxes were binned into 10 minute intervals and integrated over the daily photoperiod to determine daily PAR fluxes. To determine the variation in light flux with depth, extinction coefficients were calculated from verticai profiles of downwelling irradiance using a Biospherical Instruments Profiling Reflectance Refractometer. Photic zone depths were calculated as the depth of the 1% surface irradiance isolume. The mean mixed layer depth averaged PAR fluxes (I_z*) were calculated as:

$$I_z* = (1/kz) \, I_0 \, (1-e^{-kz})$$

where k is the calculated attenuation coefficient, z was the depth of the mixed layer, and I_0 was the measured incident PAR flux at the surface (Townsend and Spinrad, 1986).

Statistical Analyses

Statistical analyses of the data were performed using Minitab (v. 12.1). Least square linear regressions (Model II) were used for linear correlation analyses among various integrated stocks and fluxes. Data were checked for homogeneity of variance and normality. Differences among mean rates and stocks were analyzed using one-way ANOVA. Significance of all statistical tests were evaluated at the $P<0.05$ level.

RESULTS

Physical environment: temperature, mixing, and light

Samples were collected on nine cruises over a three year period (2000-2002) with at least one sampling in each season. Five of the cruises took place in the spring, two cruises in the summer, and one each during the fall and winter (Table 1). Surface water temperatures at Station ALOHA between January 2000 and June 2002 are shown in Figure 3. Surface water temperatures for the entire 13-year time-series at Station ALOHA were binned by month and the mean monthly climatological cycle is shown in Figure 3b. During this study, surface water temperatures (23.0-26.3°C) were typical of mean monthly climatology (Table 1, Figure 4).

Temporal variability in surface water temperatures strongly influenced the mixed layer depths at Station ALOHA (Figure 4). Mixed layer depths on the nine cruises of this study varied between 21-93 m, with the deepest mixing in February and March (Figure 5, Table 1), typical of the climatological range reported at Station ALOHA (Karl and Lukas 1996). Despite clear seasonality in mixing, mixed layer depths rarely exceeded photic zone depths (1% surface isopleth) (Figure 5).

Surface ocean PAR fluxes (I_0) ranged between 25.6-58.7 mol quanta m^{-2} d^{-1}, with peak fluxes in the summer, and lowest fluxes in the winter (Figure 6). The depth

Figure 4. Temporal variability in surface water temperature (°C) at Station ALOHA.

(a) Surface ocean temperatures from January 2000-June 2002; closed symbols are

cruises sampled during this study. (b) Mean monthly surface water temperatures at

Station ALOHA for the entire HOT observation period (1989-2002). Error bars are

standard deviation of mean monthly temperature.
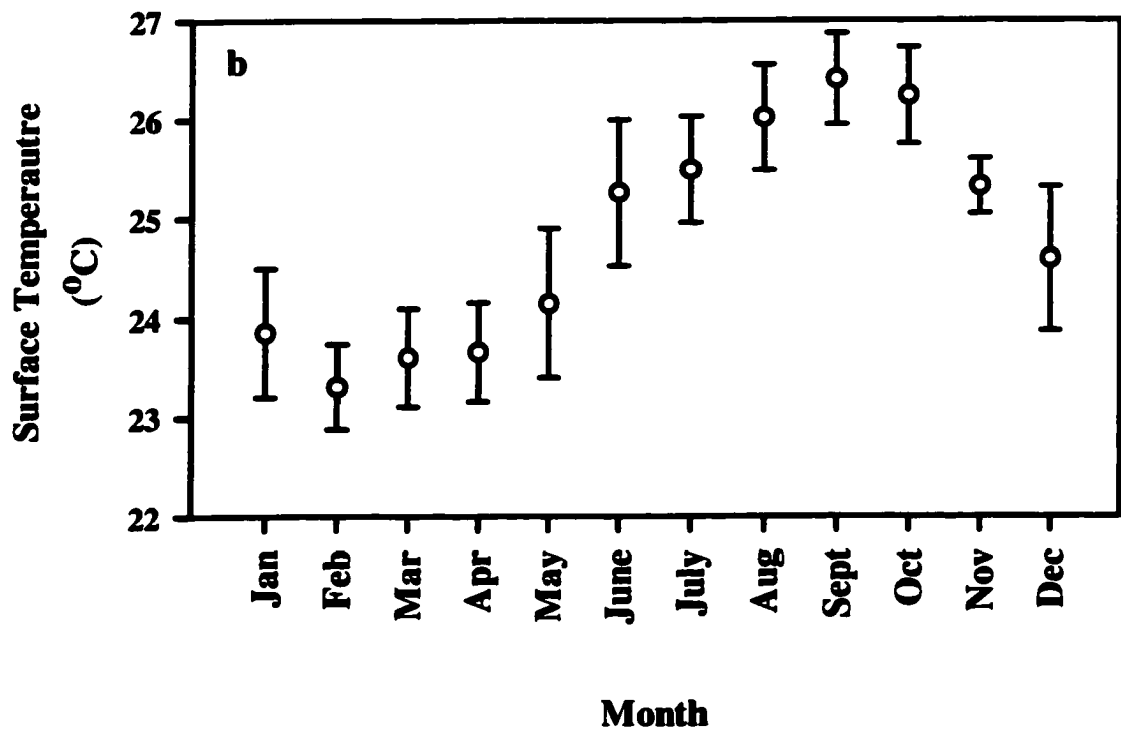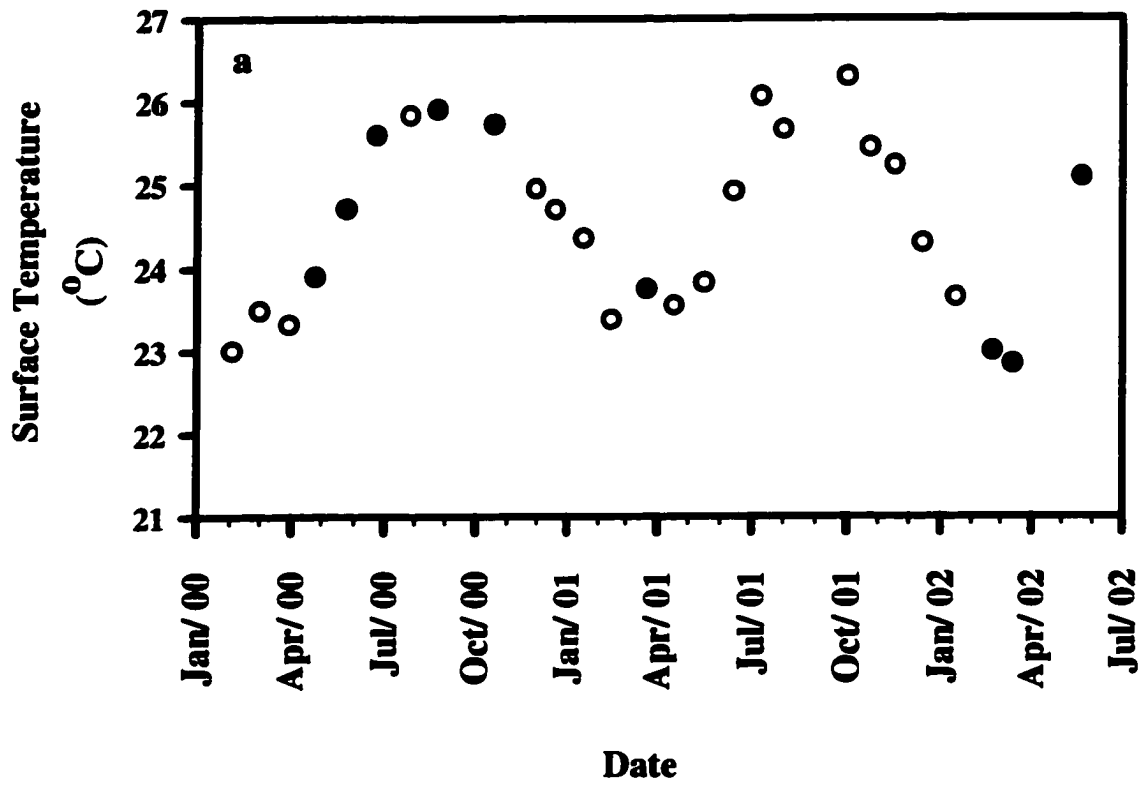
Figure 5. (a) Mixed layer depths (MLD; squares) and photic zone depths (1%

isopleth) at Station ALOHA between January 2000 and June 2002. Photic zone

depths depicted with triangles. Symbols containing closed circles are cruises sampled

during this study. (b) Mean MLD binned by month for entire HOT observation

period (1989-2002). Error bars are standard error of mean monthly MLD. MLD

calculated using the 0.125 unit potential density criterion (Monterey and

Levitus1997).
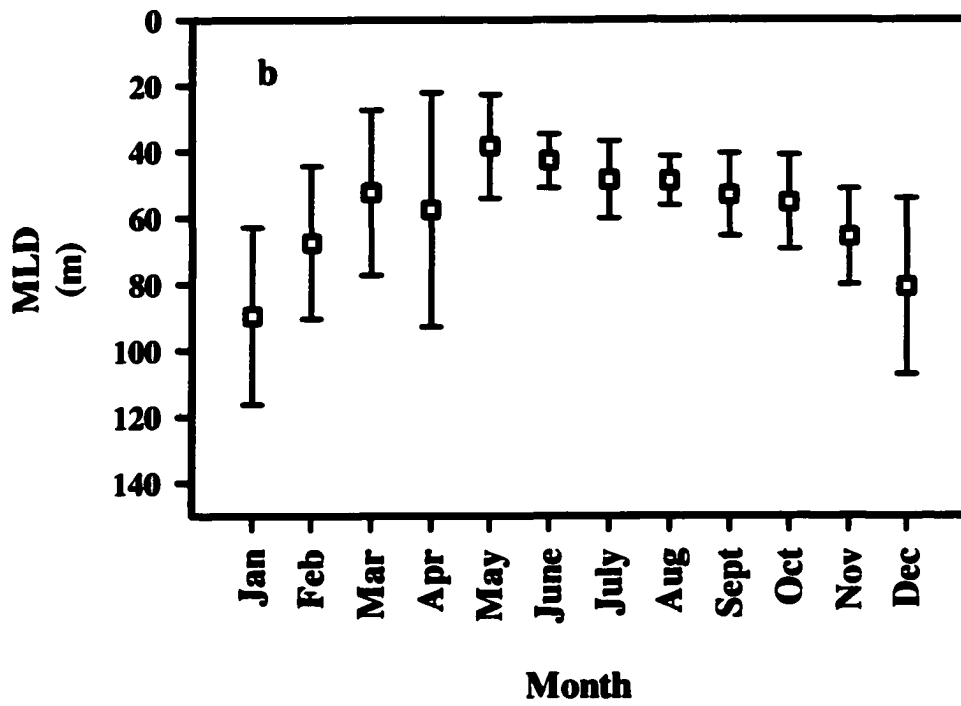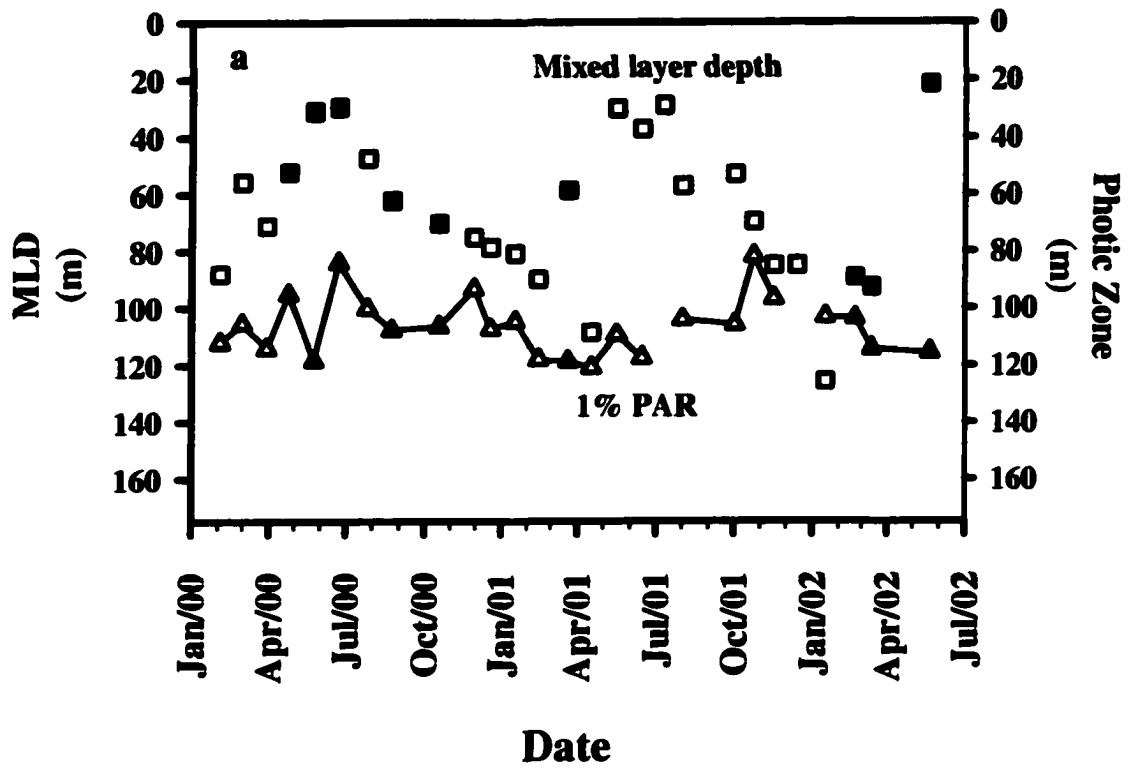
of the 1% isopleth ranged between 82-121 m averaging 106 m (Figure 5, Table 1).

I_z* fluxes ranged 5.9-30 mol quanta m^{-2} d^{-1}, with mixed-layer PAR fluxes maximal in

the summer and lowest in the winter. Relatively large fluxes of PAR penetrated the

mixed layer and provided an energy source to the plankton assemblages growing in

the top of the stratified pycnocline. PAR fluxes at the base of the photic zone ranged

from 0.16-0.59 mol quanta m^{-2} d^{-1} and averaged 0.42 mol quanta m^{-2} d^{-1} (Figure 6,

Table 1). The mean daily photoperiod for the nine cruises sampled in this study was

12.5 hours. Attenuation coefficients (k) during this study were positively correlated

with integrated Chl a (Model II linear regression, $r^2 = 0.55$, p<0.05), suggesting that

pigmented plankton biomass influenced light penetration into the upper ocean. A

large phytoplankton bloom in the upper 45 m of the photic zone (chlorophyll a >30

mg Chl. a m^{-2}) on HOT 116 (June 2000) increased light attenuation in the upper

ocean, and shoaled the base of the photic zone from 120 m to 82 m (Figure 5, 6,

Table 1).

Upper ocean picoplankton distributions

The mean time-averaged vertical distributions of picoplankton (<2.0 μm in

diameter) at Station ALOHA during the period of this study are shown in Figure 7.

Non-chlorophyll containing bacteria were the most abundant bacterial group in the

upper ocean; cell concentrations averaged 4.8 x 10^5 cells ml^{-1} in the surface water,

and declined approximately 60% through the upper 175 m. The abundance of non-

chlorophyll containing bacteria were relatively homogeneous through the upper 45 m,

Figure 6. (a) Fluxes of photosynthetically available radiation (PAR) at Station

ALOHA between January 2000 and June 2002. Triangles are surface fluxes, squares

are average mixed layer fluxes, and circles are fluxes at the base of the photic zone.

Closed symbols are cruises sampled for this study. (b) Attenuation coefficients (k)

during the same observation period.
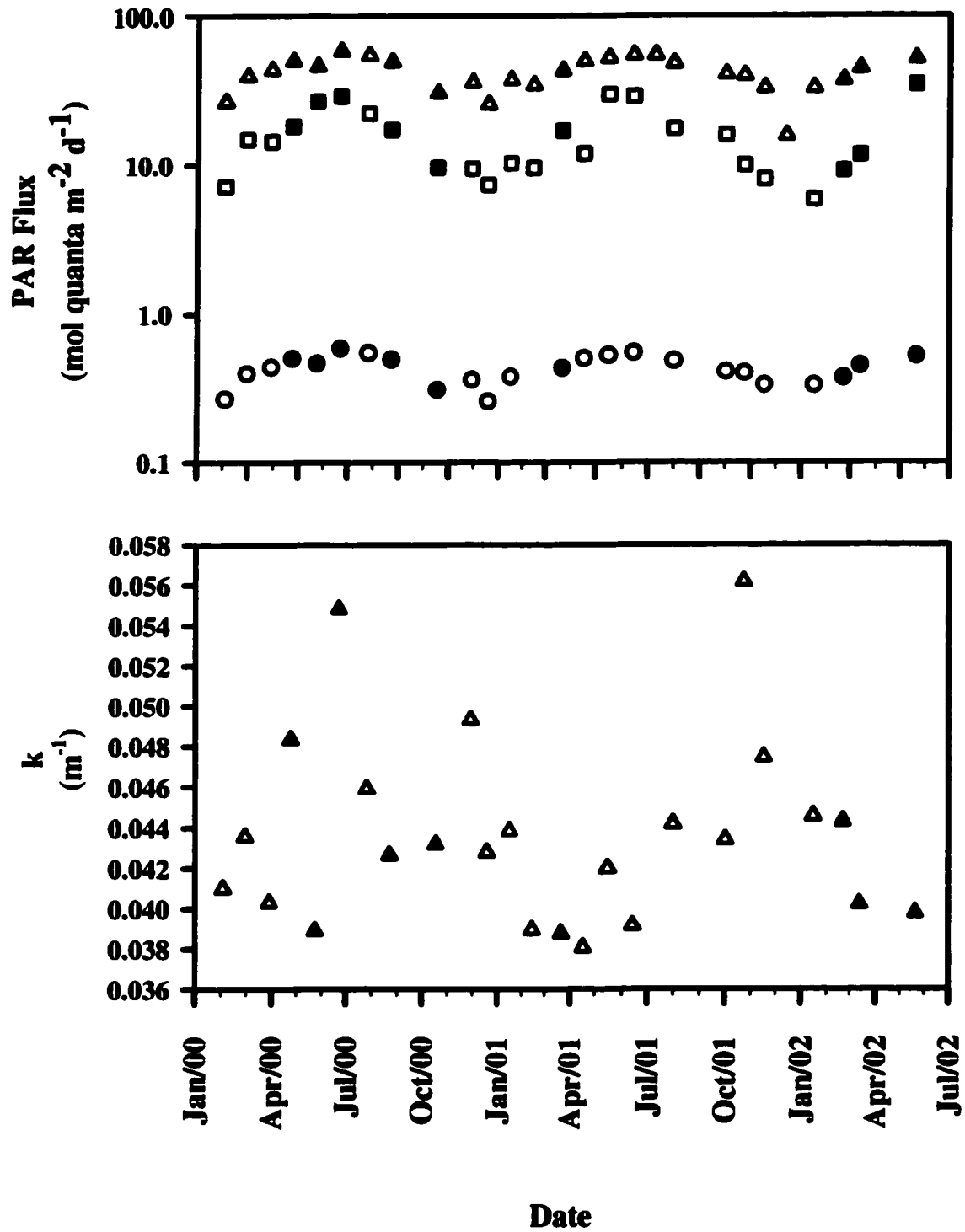
Figure 7. Mean time-averaged vertical profiles of a.) non-chlorophyll containing

bacteria (closed squares), b.) *Prochlorococcus* (open circles), and c.) *Synechococcus*

(open triangles) in the upper ocean at Station ALOHA during this study. Values are

mean abundances for the nine cruises of this study; error bars represent standard

deviation of the mean.
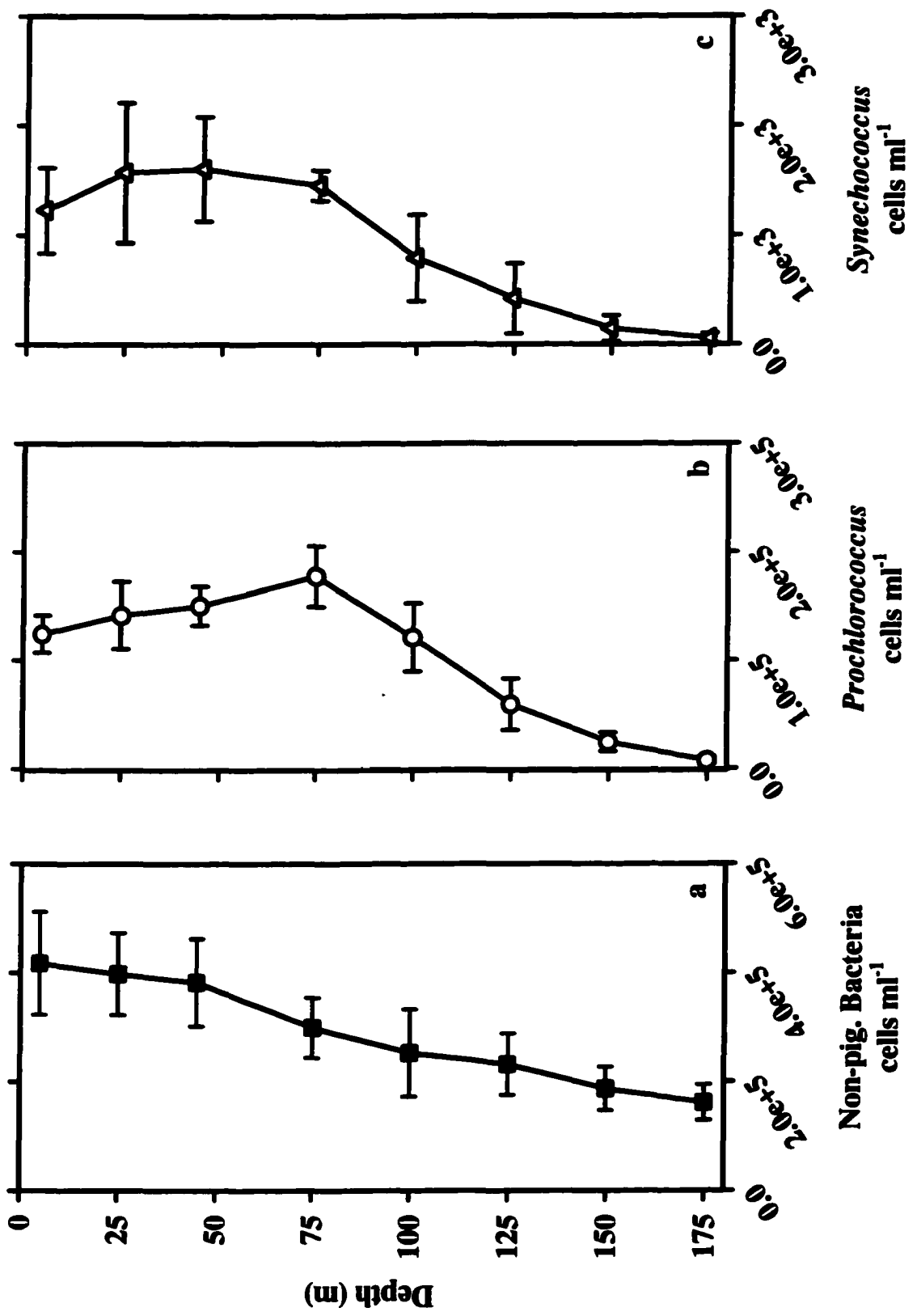
but declined ~30% between 45-75 m, and continued to decrease roughly linearly with increasing depth.

The abundance of the pigmented photoautotroph *Prochlorococcus* was consistently lower than the abundance of non-chlorophyll containing bacteria. Surface water abundance of *Prochlorococcus* was roughly 70% lower than the abundance of non-chlorophyll containing bacteria, averaging 1.7×10^5 cells ml^{-1} (Figure 7). *Prochlorococcus* abundance was greatest in the mid-photic zone (45-75 m), where PAR fluxes were reduced to 0.63-1.8 mol quanta m^{-2} d^{-1} (4.2-14% surface isopleth). Peak *Prochlorococcus* abundance averaged 2.9×10^5 cells ml^{-1} and was only 15% lower than the abundance of non-pigmented bacteria. The unicellular cyanobacterium *Synechococcus* was more than two orders of magnitude less abundant than *Prochlorococcus*. Surface water *Synechococcus* abundance averaged 1.3×10^3 cells ml^{-1}, increasing slightly between the surface water and 45 m, and then declining ~95% to 175 m (Figure 7).

Partitioning of Leu into nucleic acids

The relative proportion of ^{3}H extracted in bulk plankton nucleic acids was typically <10% of ^{3}H found in the protein extracts (Table 2), suggesting only a small fraction of Leu incorporated into heterotrophic bacteria was dissimilated or nonspecifically assimilated into intracellular nucleic acid pools. The relative proportion of Leu in the nucleic acids subfractions tended to increase with depth, but even below the photic zone ^{3}H in nucleic acids was still typically <10% of the counts observed in proteins (Table 2).

Table 2. ^{3}H-activity of nucleic acids extracts and ^{3}H-activity in nucleic acids relative to protein extracts at Station ALOHA.

HOT Cruise Date	Depth (m)	NA Light[a] (DPM ml^{-1})	NA Dark (DPM ml^{-1})	% NA/Pro Light[b]	% NA/Pro Dark (%)
115 May 2000	5	200.83	164.28	0.65	3.21
	25	308.93	151.81	0.86	3.35
	45	252.24	85.21	4.10	3.19
	75	189.01	117.37	3.39	3.20
	100	174.57	94.15	3.76	4.14
	125	135.09	75.94	4.90	4.46
	150	52.75	44.69	8.11	6.81
	175	28.78	40.08	5.21	5.91
118 June 2000	5	73.82	24.58	1.36	0.56
	25	100.71	103.31	1.12	1.87
	45	107.13	57.95	1.52	1.25
	75	55.39	22.12	0.98	2.93
	100	24.49	42.18	0.59	2.46
	125	19.48	30.55	2.19	2.68
	150	23.23	17.06	3.25	3.00
	175	19.25	17.22	3.48	3.73

[a] dpm per ml (^{3}H) of planktonic nucleic acid subfractions. Samples incubated *in situ* in the light and dark.
[b] Percentage ^{3}H in nucleic acids from samples incubated in the light and in the dark relative to ^{3}H in protein subfractions [i.e. (^{3}H nucleic acids/^{3}H PRO) *100].

Figure 8. Vertical profiles of a.) leucine incorporation in the light (Leu_L), b.) leucine

incorporation in the dark (Leu_D), and c.) photoautotrophic production (PP) in the

upper ocean (0-175 m) for all cruises sampled during this study. Symbols used for

each cruise are shown in figure legend.
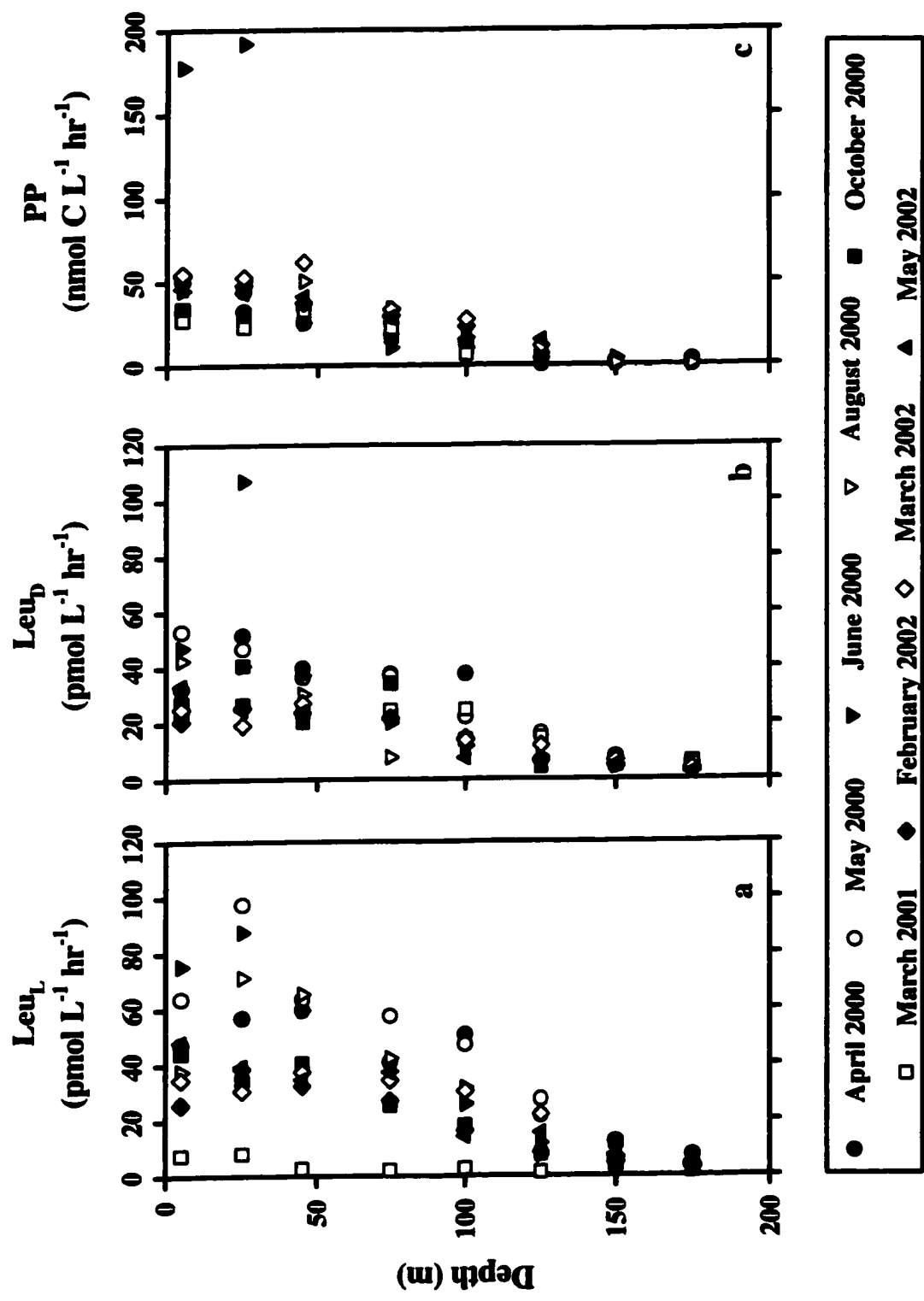
Vertical profiles of Leu incorporation and heterotrophic production

Figure 8 shows vertical profiles of Leu_L, Leu_D and PP for the nine cruises

sampled during this study. Overall, rates of Leu_L and Leu_D decreased significantly

with depth and along the light gradient in the upper ocean (Two-way ANOVA,

$P<0.05$). Leu incorporation rates displayed little temporal variability; on average,

rates of Leu_L were not significantly different throughout the upper 75 m of the water

column among the cruises sampled (One-way ANOVA, $P>0.05$). Similarly, Leu_D

incorporation rates were statistically indistinguishable throughout the upper 75 m of

the water column for all of the cruises sampled in this study (One-way ANOVA,

$P>0.05$).

Leu_L rates demonstrated significant photoenhancement throughout the upper

photic zone (45-100 m) among the cruises sampled for this study (T-test, $P<0.05$);

mean Leu_L rates were 1.5-1.9 fold greater than corresponding Leu_D rates (Figure 9).

Below 125 m Leu_L and Leu_D rates were not significantly different (T-test, $P<0.005$).

Light fluxes at 125 m were <0.5% of the daily surface insolation, and PAR fluxes fell

below 0.22 mol quanta $m^{-2} d^{-1}$. Photoenhancement of bacterial protein production

was apparent on all nine cruises sampled, although occasionally, at discrete depths,

rates of Leu_D exceeded Leu_L. For example, a large phytoplankton bloom in June

2000, concentrated in the upper 45 m, increased Leu_D rates at 25 m by more than two-

fold relative to the mean Leu_D rate at 25 m. During this event, rates of Leu_L did not

increase proportionately as much as Leu_D, resulting in one of the sampling

opportunities where Leu_D rates exceeded Leu_L.

The difference between Leu_L and Leu_D (ΔLeu) provided a quantitative

estimate of the rate of photoenhanced Leu incorporation:

$$\Delta Leu = Leu_L - Leu_D$$

Average ΔLeu rates in the upper 75 m ranged from 13-20 pmol L^{-1} hr^{-1} (Figure 9),

with peak rates found at 45 m. Rates of ΔLeu demonstrated little temporal variance

in the upper 75 m, despite over an order of magnitude decrease in PAR fluxes (1.8-37

mol quanta m^{-2} d^{-1}) (Figure 9).

Conversion of Leu incorporation rates to carbon equivalents emphasized the

importance of light-stimulated HBP to the upper ocean at Station ALOHA. HBP_L

averaged 4.8-7.1 nmol C L^{-1} hr^{-1} in the upper 75 m, 33-67% greater than

corresponding HBP_D. The difference between HBP_L and HBP_D, defined as

photoenhanced heterotrophic bacterial production (PHBP), accounted for 1.7-2.5

nmol C L^{-1} hr^{-1} the upper 75 m of the water column. Significant rates of PHBP were

observed at depths ≤ 125 m, where the mean irradiance was $\geq 0.5\%$ of the daily surface

PAR flux (Figure 10). Proportionately, sunlight had the greatest influence on HBP

rates in the lower portion of the photic zone (45-125 m), where PHBP accounted for

7.4-33% of contemporaneous photoautotrophic production, 35-40% of HBP_L, and 68-

91% of HBP_D (Figure 10).

Similar to Leu_L and Leu_D, mean rates of PP were not significantly different

throughout the upper 75 m of the water column among the cruises sampled in this

study (One way ANOVA, $P > 0.05$). Rates of PP in the top 25 m of the photic zone

averaged 57 nmol C L^{-1} hr^{-1}, decreasing to 24-37 nmol C L^{-1} hr^{-1} between 45-75 m

Figure 9. Mean time-averaged vertical profiles of a.) Leu_L (open circles) and Leu_D

(closed diamonds), b.) $Leu_L:Leu_D$ ratio, and c.) photoenhanced Leu (ΔLeu) during

this study. Symbols are mean values of the nine time-series cruises in this study,

error bars are standard errors of the means (n=9).
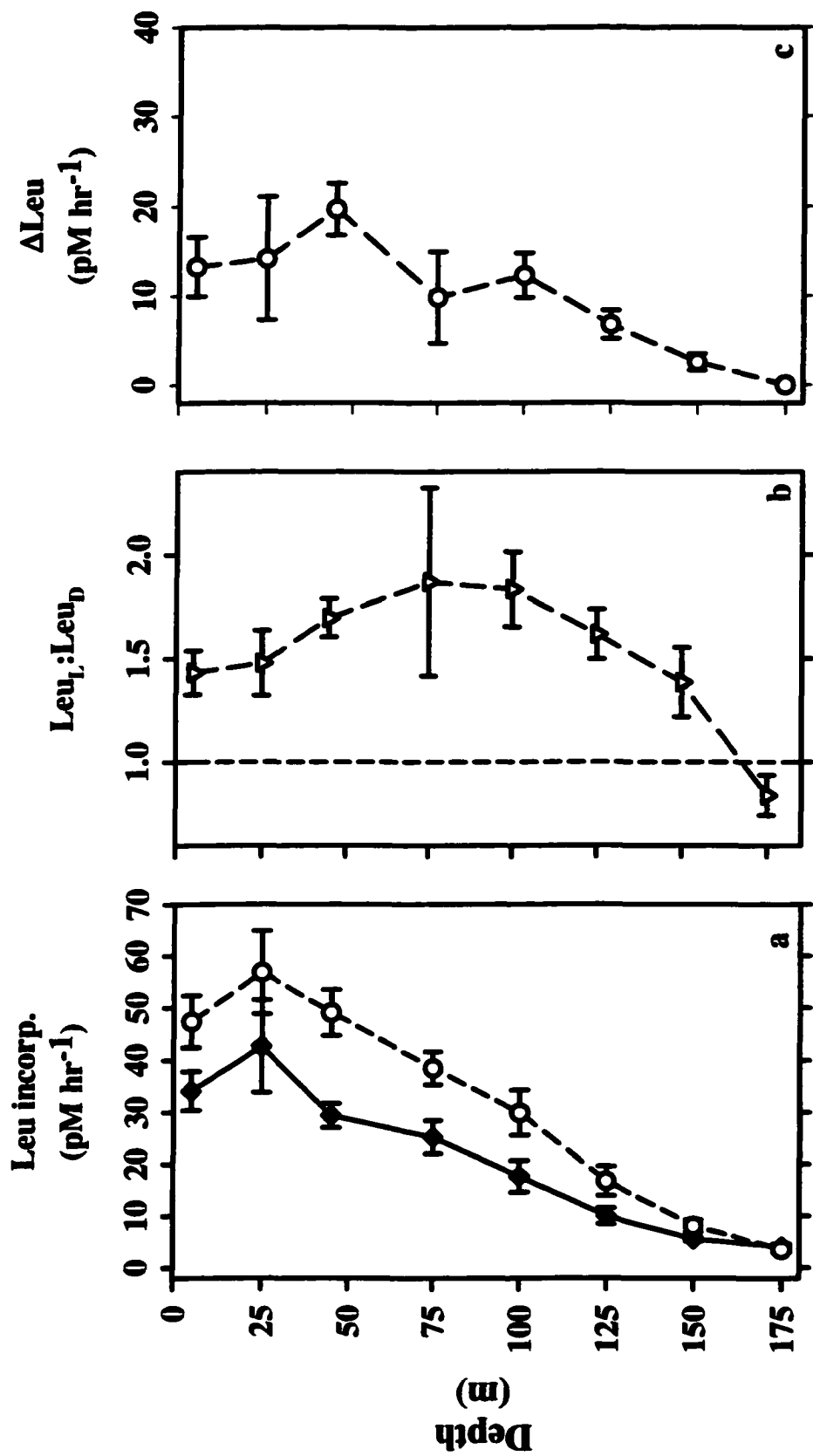
Figure 10. (a) Mean time-averaged vertical profiles of HBP_L (open circles), HBP_D

(closed diamonds), and PHBP (bars) for the nine cruises sampled in this study. Error

bars are standard error of the mean rates. (b) Mean vertical profile of PP (open

squares) and PAR fluxes (open triangles) in the upper ocean at Station ALOHA.

Error bars of PP are standard error of mean rate; error bars of PAR fluxes are standard

deviation of mean flux. (c) Profiles of relative importance of PHBP; closed

diamonds are $PHBP:HBP_D$; open circles are $PHBP:HPP_L$; grey squares are PHBP:PP.
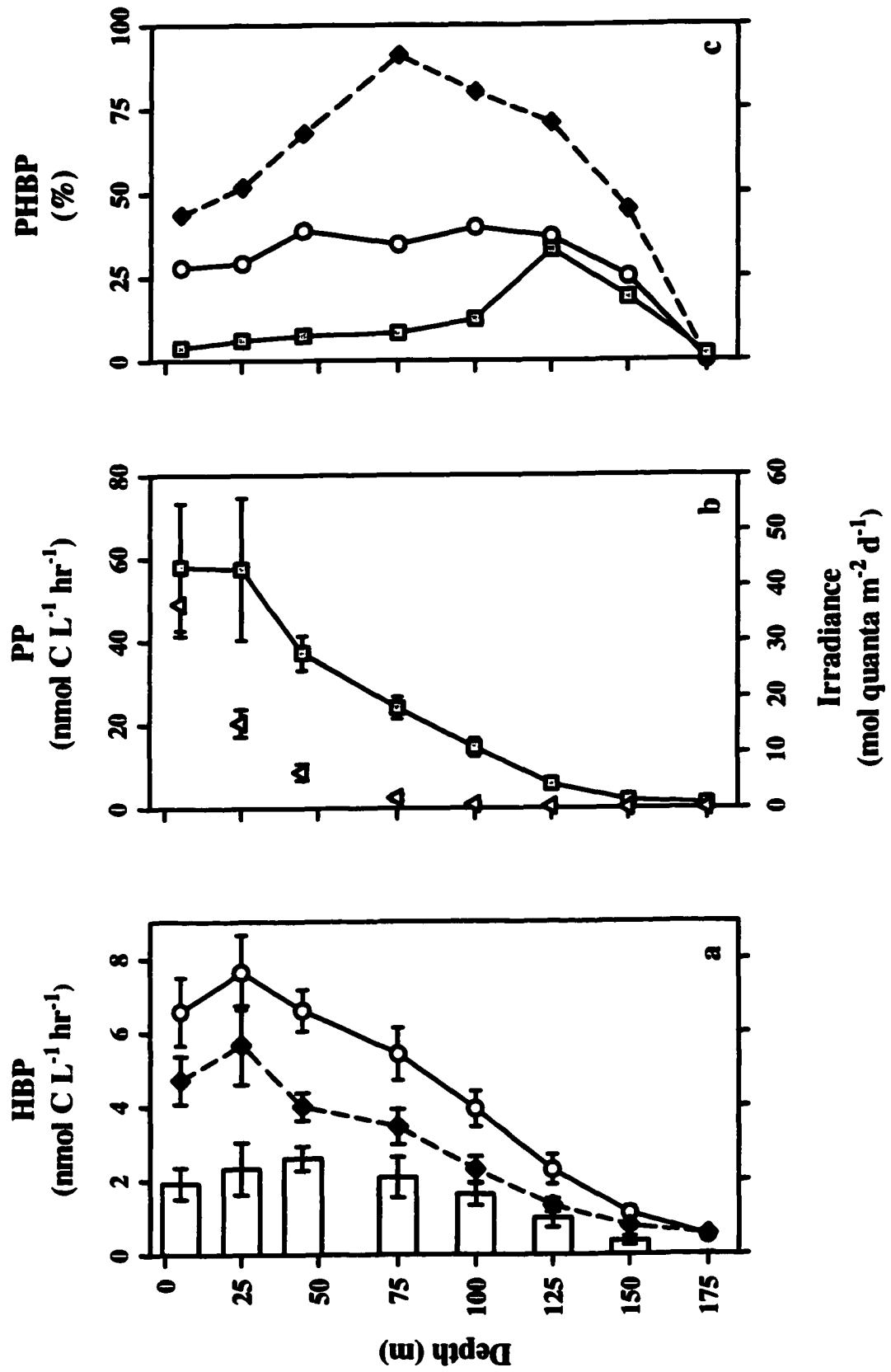
(Figure 10). In June 2000, rates of photoautotrophic production in the top 25 m were more than three times greater than the average upper ocean productivity for the entire sampling period (Figure 8). No significant PP was observed below the 0.22 mol quanta m^{-2} d^{-1} isolume, or the approximate depth of the 0.5% isopleth (>125 m).

Depth integrated production and abundance

Mean depth-integrated (0-175 m) light-enhanced HBP was 50% greater than corresponding HBP_D. Average integrated HBP_L was 0.69 mmol C m^{-2} hr^{-1} while mean HBP_D was 0.46 mmol C m^{-2} hr^{-1}. HBP_L and HBP_D ranged 0.44-1.0 and 0.34-0.62 mmol C m^{-2} hr^{-1}, respectively, and by difference PHBP ranged 0.09-0.41 mmol C m^{-2} hr^{-1} (Table 3, Figure 11). Integrated rates of HBP_L HBP_D, and PHBP were not significantly different between the cruises despite significant differences in PP and PAR fluxes. Both HBP_L and HBP_D were lowest in February 2002 (HOT 135) when *Prochlorococcus* stocks were at their minimum (Table 3), and PHBP was lowest in October 2000 (HOT 119) (Table 3).

The average rate of depth-integrated PP during the observation period was 4.0 mmol C m^{-2} hr^{-1}, with rates ranging from 1.8-7.5 mmol C m^{-2} hr^{-1} (Figure 11). Peak rates of PP were observed in June 2000 (HOT 116), coincident with the large increase in upper ocean chlorophyll (Table 3), and lowest rates were measured in October 2000 (HOT 119) and March 2001 (HOT 124) (Table 3). PHBP and HBP_L were equivalent to 5% and 16% of PP, respectively. Daily total heterotrophic production (HBP_T) was calculated as

$$HBP_T = [(HBP_L * 12 \text{ hours}) + (HBP_D * 12 \text{ hours})]$$

Figure 11. Time-series (February 2000-June 2002) of depth-integrated HBP in the

upper ocean (<175 m) at Station ALOHA. Integrated HBP_L rates are closed bars;

PHBP rates are open bars; error bars are standard deviation of integrated rates. Also

shown are depth-integrated PP (grey squares) and surface PAR fluxes (open circles).

HBP (mmol C m^{-2} hr^{-1})

Date

Irradiance (mol quanta m^{-2} hr^{-1})
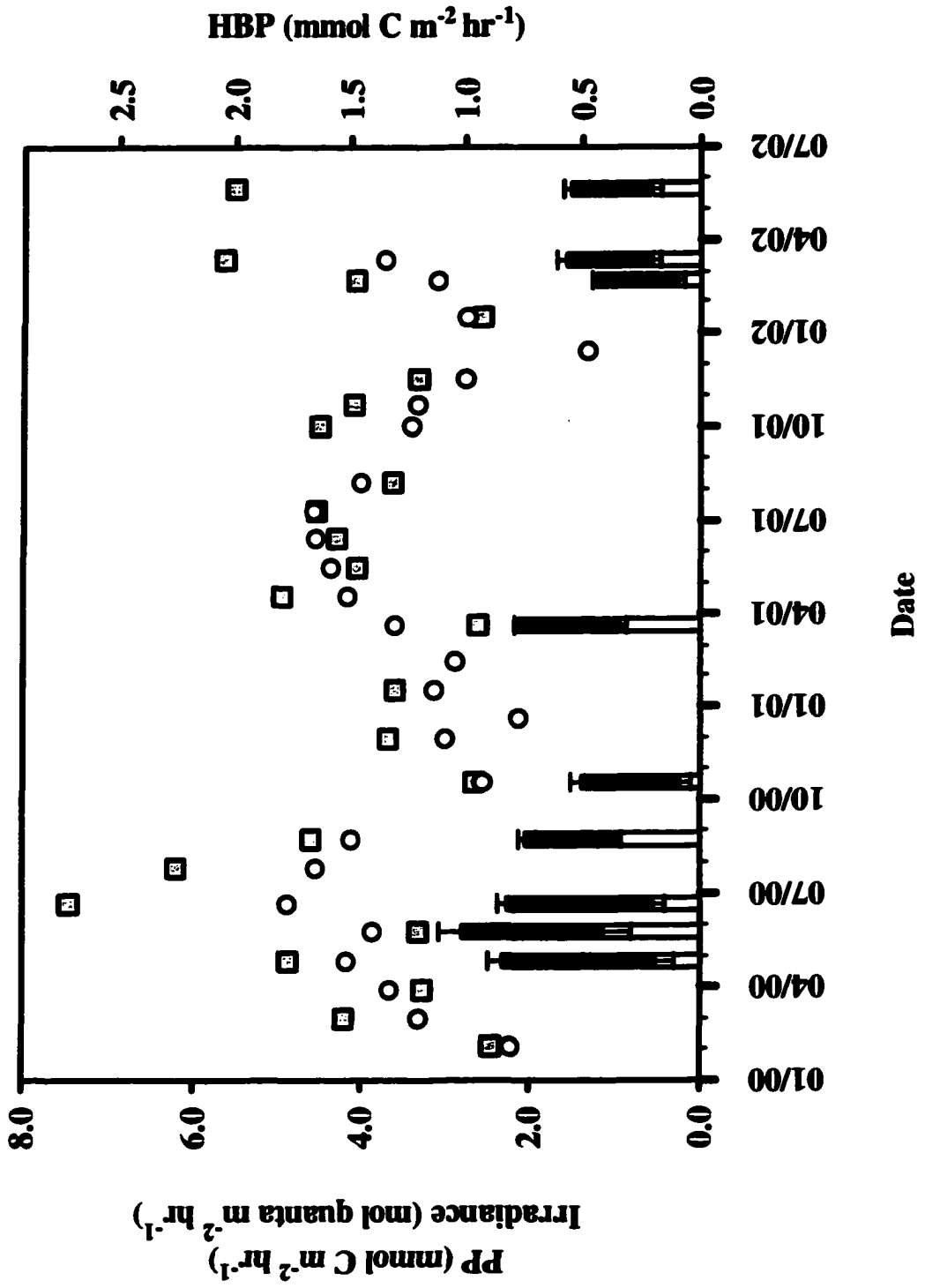
PP (mmol C m^{-2} hr^{-1})

Table 3. Depth-integrated rates and bacterial stocks in the upper ocean (0-175 m) at Station ALOHA.

Date Cruise	Chl a [a] (mg Chl m^{-2})	PP (mmol C m^{-2} hr^{-1})	HBP_L (mmol C m^{-2} hr^{-1})	HBP_D (mmol C m^{-2} hr^{-1})	PHBP (mmol C m^{-2} hr^{-1})	Prochloro (10^{11} cells m^{-2})	Non-pig (10^{11} cells m^{-2})	Syn (10^{11} cells m^{-2})
April 2000 HOT 114	20.7	4.9	0.84 (0.06)	0.64 (0.05)	0.20 (0.076)	187	708	3.05
May 2000 HOT 115	22.9	3.3	1.0 (0.10)	0.60 (0.070)	0.40 (0.12)	274	787	1.35
June 2000 HOT 116	34.1	7.5	0.82 (0.036)	0.62 (0.039)	0.20 (0.052)	328	765	1.12
August 2000 HOT 118	21.8	4.6	0.74 (0.026)	0.38 (0.020)	0.36 (0.033)	161	637	1.23
October 2000 HOT 119	18.4	2.6	0.50 (0.047)	0.42 (0.028)	0.08 (0.054)	131	305	0.745
March 2001 HOT 124	21.3	2.6	0.77 (0.016)	0.43 (0.019)	0.34 (0.025)	211	252	2.79
February 2002 HOT 135	16.5	3.7	0.44 (0.023)	0.34 (0.018)	0.10 (0.029)	106	321	1.46
March 2002 HOT 136	19.6	4.9	0.57 (0.035)	0.36 (0.021)	0.21 (0.041)	na	na	na
May 2002 HOT 137	21.9	5.5	0.55 (0.035)	0.34 (0.031)	0.21 (0.047)	145	253	1.27

Top numbers are depth-integrated rates and stocks, numbers in parentheses are standard deviation of the mean integrated property.
[a] Abbreviations are: Chlorophyll a (Chl. a), photoautotrophic production (PP), heterotrophic bacterial production in the light (HBP_L) and dark (HBP_D), photoenhanced heterotrophic bacterial production (PHBP), *Prochlorococcus* (Prochloro), non-pigmented bacteria (Non-pig), *Synechococcus* (Syn), na –data not available.

assuming nighttime HBP was equivalent to HBP_D. HBP_T averaged 16.2 mmol C m^{-2} d^{-1} with PHBP contributing 3.0 mmol C m^{-2} d^{-1} or ~19% of HBP_T.

Regression analyses

Table 4 shows the results of Model II least-squares linear regression analyses of upper ocean (<175 m) productivity and stocks measured during this study. No significant linear relationships were observed between stocks of *Prochlorococcus* and either non-pigmented bacteria or *Synechococcus*. Both HBP_L and HBP_D were correlated with the integrated abundance of *Prochlorococcus* and non-pigmented bacteria; integrated stocks of *Prochlorococcus* accounted for roughly 60% of the variability in both integrated HBP_L (r^2= 0.79, P=0.04) and HBP_D (r^2=0.77, P=0.05) (Table 4). These results suggest that HBP in this ecosystem was significantly influenced by the abundance of *Prochlorococcus*.

Table 4. Model II least squares linear regression analyses of depth-integrated properties of the upper ocean at Station ALOHA. Top numbers are coefficients of determination of regressions (R^2) and bottom numbers are significance (P) of regression.

Variable	I_0[a]	PP	HBP_L	HBP_D	PHBP	Non-pig Bact	Prochloro	Syn	Chl a
I_0	***	0.67 (0.01)	ns[b]	ns	ns	0.79 (0.03)	0.76 (0.05)	ns	0.81 (0.02)
PP		***	ns	ns	ns	ns	ns	ns	0.74 (0.02)
HBP_L			***	0.81 (0.008)	0.75 (0.02)	0.79 (0.02)	0.80 (0.02)	ns	ns
HBP_D				***	ns	0.81 (0.02)	0.78 (0.02)	ns	ns
PHBP					***	ns	ns	ns	ns
Non-pig. Bact.						***	ns	ns	ns
Prochloro.							***	ns	0.88 (0.004)
Syn.								***	ns
Chl. a									***

[a] Abbreviations are: Incident PAR flux (I_0), photoautotrophic production (PP), heterotrophic bacterial production (HBP), photoenhanced heterotrophic bacterial production (PHBP), non-pigmented bacteria (Non-pig Bact.), *Prochlorococcus* (Prochloro.), *Synechococcus* (Syn.). [b] ns= not significant ($P>0.05$).

DISCUSSION

Motivation and significance

This study was motivated by two fundamental questions: 1) what are the contributions of heterotrophic bacteria to upper ocean organic carbon fluxes in the NPSG, and 2) how does PAR impact heterotrophic bacterial growth? Recent investigations on the structure and functioning of the NPSG microbial food web provided compelling observations that the upper ocean microbial assemblage exerts fundamental control on upper ocean biogeochemistry. In particular, long-term changes in plankton community structure appear to have altered nutrient dynamics and organic matter cycling in the NPSG (Karl et al. 1995, Karl 1999, 2002b, Church et al. 2002). In addition, several groups of upper ocean bacterial assemblages utilize photoheterotrophic production, potentially influencing organic matter cycling in this ecosystem (Fenchel 2001, Kolber et al. 2001, Béjà et al. 2001, Karl 2002). These uncharacterized modes of bacterial phototrophy may comprise an important pathway for biomass production in the ocean (Béjà et al. 2000, 2001, Kolber et al. 2000, 2001).

By characterizing how the HBP responded to light, this study evaluated how PAR fluxes influenced the magnitude of organic carbon flux through the base of the plankton food-web at Station ALOHA. The influence of sunlight on rates of

secondary production were pronounced: PHBP rates averaged 3.0 mol C $m^{-2} d^{-1}$,

nearly equivalent in magnitude to carbon export from the upper ocean (Emerson et al.

1997, Karl et al. 2001), the dominant sink for organic carbon in the this ecosystem.

There are a myriad of photophysiological and photochemical processes that could

result in photostimulation of HBP; this study was not designed to specifically isolate

which of these processes resulted in photoenhancement of heterotrophic production.

However, the results of this study demonstrate that sunlight may exert an important

and previously uncharacterized influence on upper ocean cycling of carbon by

heterotrophic bacteria.

Mechanisms of photoenhanced heterotrophic production

A number of processes potentially contribute to photoenhancement of

heterotrophic protein production at Station ALOHA. These processes may generally

be grouped into abiotic and biotic categories that result in both direct and indirect

photoenhancement of heterotrophic bacterial production. Photoenhancement of

bacterial protein production rates at Station ALOHA likely stems from a combination

of several of these processes.

HBP may be directly enhanced by sunlight if the bacterial assemblages

harnessed light-energy for ATP production, resulting in elevated growth rates and

enhanced rates of protein synthesis. Such direct processes include photoheterotrophy

and mixotrophy. Photoheterotrophic production is the process whereby bacterial

assemblages simultaneously harvest light energy for photophosphorylation and

acquire DOM as a source of both carbon and reductant. Mixotrophy describes the

physiological ability of photoautotrophic plankton to utilize selected organic

compounds.

Alternatively, light-stimulated protein production may have been the result of

several indirect abiotic and biotic processes including close temporal couplings

between photoautotrophic production of labile organic exudates and subsequent

utilization by heterotrophic bacteria (Gasol et al. 1998). Alternatively, photochemical

production of labile DOM could result in light-stimulated heterotrophic productivity

(Lindell et al. 1995, Wetzel et al. 1995, Benner and Biddanda 1998). If protein

production rates demonstrated photoenhancement as a result of heterotrophic uptake

of labile DOM production, Leu incorporation rates might be expected to demonstrate

temporal covariance with PP (the ultimate source of labile DOC). Regression

analyses did not reveal a significant relationship between PP and PHBP or HBP_L,

suggesting that at the time of this study, heterotrophic and photoautotrophic

production were only weakly coupled in the upper ocean at Station ALOHA.

However, because UV light was excluded from incubations conducted in this study,

UV induced photoproduction of labile organic and inorganic nutrients can not be

excluded as a potentially important source of heterotrophic nutrient resources.

Prochlorococcus mixotrophy

Regression analyses revealed several potentially important clues about the

light-driven processes that resulted in enhanced Leu incorporation rates. HBP_L and

HBP_D both displayed a weak but positive relationship to stocks of non-pigmented

bacteria and *Prochlorococcus*, suggesting that these two "groups" of bacterioplankton

influenced HBP. Assuming the majority of non-chlorophyll containing bacteria were heterotrophic (including the diverse assemblages of photoheterotrophic bacteria such as AAnP and rhodopsin-containing bacteria), the observed relationship between these bacteria and HBP was partly expected. However, the relationship between HBP and the abundance of *Prochlorococcus* was somewhat surprising. These results suggest one of two possibilities: either *Prochlorococcus* directly incorporated Leu as a facultative heterotroph, or measured heterotrophic bacterial production rates were directly related to the abundance of *Prochlorococcus*. No significant correlation was observed between stocks of non-pigmented bacteria and *Prochlorococcus*, suggesting that the abundance of non-pigmented bacteria was not specifically coupled to *Prochlorococcus* abundance.

Direct uptake of Leu by both cultivated and natural populations of pigmented picoplankton (including *Prochlorococcus*) has been shown (Lindell and Post 1995, Montesinos et al. 1997, Kamjunke and Jahnichen 2000, Zubkov et al. 2003). The phylogenetic and physiological diversity of *Procholorococcus* isolates indicate that as a whole the genus is highly adaptive. Studies identifying the genetic diversity of upper ocean picoplankton communities suggest that multiple "ecotypes" of *Prochlorococcus* inhabit the upper layers of the subtropical and tropical oceanic waters (Moore et al. 1998, Moore et al. 2002). These physiologically distinct *Procholorococcus* ecotypes are distributed differently throughout the upper ocean, demonstrating adaptive physiologies to maximize nutrient and light availability. Generally, two primary *Prochlorococcus* types have been characterized by their chlorophyll *b* (chl *b*) /chl *a* ratios: the high chl *b*/chl *a* cells adapted to low light

intensities and thus inhabited the lower photic zone, and the low chl $b/$ chl a ecotypes

adapted to high light fluxes and thus appeared largely restricted to the upper photic

zone where light fluxes were much greater (Moore et al. 1998). Consistent with these

adaptations, the high chl $b/$ chl a ecotypes appeared capable of growth on NO_2^-, while

the low chl b/chl a strains appeared incapable of growth on NO_3^- or NO_2^-, growing

instead on reduced source of N such as NH^+_4 and urea (Moore et al. 2002).

Knowledge that *Prochlorococcus* assimilates components of the bulk DOM pool

(such as amino acids) requires re-evaluation of the role that *Procholorococcus* plays

in oceanic carbon cycling.

Based on the differences between light and dark heterotrophic production

measured in this study, facultative heterotrophy (mixotrophy) by *Prochlorococcus*

may have been a substantial component of the photostimulated Leu incorporation

rates. Depth-integrated abundance of *Prochlorococcus* averaged 37% of non-

pigmented bacterial abundance. Assuming *Prochlorococcus* and non-pigmented

bacteria both incorporated leucine at equal rates in the dark, *Prochlorococcus* would

have accounted for 27% of the total Leu incorporation in the dark. If

Prochlorococcus growth rates increased 75-100% under *in situ* light, and non-

pigmented bacterial growth rates were unchanged by light, then *Prochlorococcus*

incorporation of Leu could have increased total Leu incorporation by 39-43% in the

light. The average difference between mean Leu_L and Leu_D measured during this

study was 34%; these rough calculations suggest mixotrophic growth by

Prochlorococcus could have accounted for a large portion of Leu photoenhancement.

During this study, depth-integrated HBP_L, HBP_D, and PHBP were not significantly correlated to either PP or daily PAR fluxes. Karl et al. (1998) demonstrated that net $DO^{14}C$ production at Station ALOHA was maximal in the surface ocean, displaying a vertical structure similar to PP. If photostimulated HBP resulted from indirect coupling of heterotrophic production to photoautotrophic production of labile organic exudates, the photoenhancement effect should scale positively with PP. Vertical profiles of the Leu_L:Leu_D ratio revealed that light-stimulated Leu incorporation was proportionately highest at the base of the photic zone where PP was ~75% lower than rates in the surface waters. These observations provide additional support to the hypothesis that the observed light-stimulated Leu incorporation was governed by direct light-driven processes such as mixotrophic production by *Prochlorococcus*.

The influence of light on bacterial growth in the sea

Previous studies that have examined how light affects heterotrophic bacterial growth in the ocean have generally focused on the impact of UV-radiation on bacterial growth (Bailey et al. 1983, Herndl et al. 1993, Aas et al. 1996, Jeffery et al. 1996). In a study conducted in coastal California waters and in the Gulf of Mexico, Aas et al. (1996) found that bacterial incorporation of Leu was enhanced ~10% upon exposure to PAR. In contrast, these authors noted that PAR appeared inhibitory to ^{3}H-thymidine (TdR) incorporation rates. Studies in high mountain lakes suggested that exposure of bacterial assemblages to PAR had an adverse effect on incorporation of Leu and TdR, reducing rates by 70% (Sommaruga et al. 1997). In contrast,

Helbling et al. (1995) found that exposure of Antarctic bacterial isolates to PAR had little effect on the survival of bacterial populations.

Both UV-A (315-400 nm) and and UV-B (280-315 nm) are rapidly attenuated in seawater due to absorption by dissolved constituents in seawater (mainly DOM); as a result of this rapid attenuation, the biogeochemical influences of UV-radiation are largely restricted to the upper 20-30 m in clear, oligotrophic waters (Smith and Baker 1981, Herndl et al. 1993, Zepp et al. 1995). UV-B radiation is absorbed by numerous several intracellular macromolecules including nucleic acids, proteins, and lipids; absorption of UV-B by these cellular constituents appears largely inhibitory to heterotrophic production (Jeffery et al. 1996). Several studies have also found significant modification of oceanic DOM pools by absorption of UV-light. Most importantly, photochemical oxidation of DOM may convert non-biologically reactive components of the bulk DOM into more labile, readily available substrates (Moran and Zepp 1997, Benner and Biddanda 1998). As such, UV-radiation appears to simultaneously inhibit and stimulate heterotrophic production in the sea.

The methods employed by the present study excluded the influences of UV radiation on bacterial growth and photochemical alteration of the DOM pool, making assessment of the impacts of UV light on the measured rates in this study impossible. However, assuming that UV radiation penetrated to 25 m at Station ALOHA and that UV radiation directly inhibits HBP throughout the upper 25 m of the water column, the potential detrimental impact of UV radiation on HBP can be estimated. Assuming HBP was reduced by 40% throughout the top 25 m of the water column as a result of UV photoinhibition (Herndl et al. 1993) depth-integrated HBP would be reduced by

11-15% in this study. This estimate is likely to overestimate the inhibitory effects of UV-radiation on HBP because it assumes an equal inhibitory influence throughout the waters column; in fact, UV-inhibition of HBP would be expected to decrease as an exponential function of depth, similar to the decrease of UV intensity as a function of depth. While potentially significant, the relative inhibitory influences of UV radiation on HBP in the NPSG appear less than the beneficial influences of PAR found in this study.

Recently, Morán et al. (2001) performed a series of *in situ* Leu incorporation assays in the Mediterranean Sea and at a coastal North Atlantic station to examine how PAR influenced Leu incorporation rates. These investigators observed two distinct effects of light on Leu incorporation: rates were greater in samples incubated in the dark than samples incubated at *in situ* irradiance, and Leu incorporation generally increased with increasing light intensity. Elevated dark Leu assimilation was hypothesized to be the result of increased labile DOC exudation by the photoautotrophic community upon dark confinement. The results of the present study were fundamentally different from the results of Morán et al. (2001); in the NPSG, *in situ* light-energy consistently stimulated rates of Leu incorporation, resulting in 50% more carbon entering the microbial food web compared to incubations conducted in the dark. In the NPSG, PAR appears to directly influence rates of secondary production; the high light fluxes, combined with the relative paucity of inorganic nutrients likely necessitate adaptive metabolic strategies by members of the upper ocean plankton assemblage.

HBP and organic carbon fluxes in the NPSG

Photoheterotrophic and mixotrophic growth by planktonic microorganisms

has been observed in a variety of aquatic environments (Rippka 1972, McKinley

1977, Shiba et al. 1979, Paerl 1991, Kolber et al. 2001). In the open ocean various

groups of bacteria appear capable of photoheterotrophic metabolism, including

members of the cyanobacteria and representatives in the α-, β-, and γ-

Proteobacteria (Rippka 1972, Kolber et al. 2001, Béjà et al. 2002, Zubkov et al.

2003). Cultivated photoheterotrophic bacteria appear capable of growth as strict

photoautotrophs; however, given sufficient organic matter most photoheterotrophic

bacteria become predominately heterotrophic, relying on inorganic carbon as a

subsidy when DOM availability is low (Yurkov and Beatty 1998, Kolber et al. 2001).

At Station ALOHA rates of Leu_L were consistently elevated relative to Leu_D with one

prominent exception, in May 2000 during a large phytoplankton bloom concentrated

in the upper 45 m of the water column, the Leu_D rate at 25 m increased more than

two-fold above the mean rate (Figure 7). Kolber et al. (2001) found that AAnP

bacteria were able to tune their metabolism to maximize growth depending on the

nutrient environment, becoming mostly heterotrophic when organic matter was

available, and switching to facultative phototrophy when necessary.

In the mixed layer of the NPSG, inventories of organic N and P are more than

an order of magnitude greater than corresponding inorganic N and P inventories; thus,

a metabolic adaptation that allows an organism to utilize the relatively abundant N

and P sources found in the DOM pools while simultaneously obtaining energy from solar radiation (as a photoheterotroph or mixotroph), would provide a competitive advantage over a strict photolithotroph.

Using measured rates of Leu incorporation to estimate HBP, the contributions of HBP_L and HBP_D to overall organic carbon fluxes were assessed for the upper 175 m at Station ALOHA (Table 5). Total daily HBP (HBP_T) was estimated by assuming HBP_L represented the daytime rate of HBP, HBP_D represented the nighttime HBP rate, and that the average daytime period was 12 hours. Daily rates of HBP_T were 15.2 mmol C m^{-2} d^{-1}, with 2.9 mol C m^{-2} d^{-1} derived exclusively from light-stimulated production. On an annual basis, HBP_T averaged 5.5 mol C m^{-2} yr^{-1} or 37% of annual PP at Station ALOHA (Table 5). PHBP fluxes averaged 1.1 mol C m^{-2} yr^{-1}, or approximately 20% of HBP_T and 7% of PP. Moreover, fluxes of carbon derived from PHBP were quantitatively similar to particulate carbon export from the surface waters of the NPSG (Table 5).

In an effort to constrain the total flux of carbon required to support bacterial growth (BCD) in this system, a simple one dimensional carbon flux budget was used to balance photic zone organic carbon fluxes and derive community respiration. The model assumes vertical transport is the only significant removal of organic carbon from the surface waters. By balancing organic carbon sources (PP) with organic carbon sinks (export and DOC accumulation) and assuming the remaining organic carbon was remineralized in the upper ocean, community respiration (R) was derived as:

$$R = PP - (\text{Export} + \text{DOC accumulation})$$

Table 5. One dimensional carbon flux budget for the upper ocean at Station ALOHA

Process	Flux $(mol\ C\ m^{-2}\ yr^{-1})$	Flux Range $(mol\ C\ m^{-2}\ yr^{-1})$	Reference
PP	15	5.1-33	Karl et al. 2001
Export (sinking POC flux)	0.9	0.3-1.7	Karl et al. 2001
(mass balance)	2.0	1.7-2.7	Emerson et al. 1998
Accumulating DOC	0.3	-	Church et al. 2002
HBP_L	3.2	1.9-4.4	This study
HBP_D	2.1	1.4-3.2	This study
PHBP	1.1	0.4-2.1	This study
HBP_T[a]	5.3^1	3.2-8.3	This study

Abbreviations used: PP, photoautotrophic production; POC, particulate organic carbon; DOC, dissolved organic carbon; HBP_L, heterotrophic bacterial production (light); HBP_D, heterotrophic bacterial production (dark); PHBP, photoenhanced heterotrophic bacterial production; HBP_T, total heterotrophic bacterial production.

[a] Annual total heterotrophic bacterial production calculated assuming HBP_D represented night time production rate.

R ranged from 12.7-13.8 mol C m^{-2} yr^{-1}, approximately 84-92% of PP, and nearly

three-fold greater than HBP_T. Assuming bacteria were responsible for the majority of

organic carbon remineralization in the upper ocean at Station ALOHA,

BCD approximates 18-19 mol C m^{-2} yr^{-1} and HBGE was constrained to 28-30%.

Inclusion of PHBP into the upper ocean carbon flux budget at Station ALOHA

significantly increases the carbon flux into heterotrophic bacteria and results in

relatively high bacterial growth efficiencies, another indication of the potential

importance of photoheterotrophic growth in this ecosystem.

CONCLUSIONS

Time-series estimates of Leu incorporation provided estimates of the importance of the photoheterotrophic production at Station ALOHA. Photoheterotrophic production may account for as much as 19% of HBP_T, 6% of PP, and may be quantitatively similar to carbon export at Station ALOHA. Integrated HBP_L was 49% greater than HBP_D, suggesting that bacterial metabolism was adapted to utilize the high light fluxes and proportionately large concentrations of DOM (relative to inorganic nutrients) in the upper ocean. Regression analyses revealed that HBP depended on the abundance of *Prochlorococcus*, suggesting *Prochlorococcus* may utilize DOM as a nutrient source in this ecosystem. Understanding the importance of mixotrophic and photoheterotorphic microorganisms to upper ocean biogeochemical fluxes will require investigations targeting these newly discovered metabolic pathways. Regardless of the mechanism of photoenhancement of Leu incorporation, the observation that light-mediated Leu incorporation was roughly two-fold greater than Leu assimilation in the dark indicates that sunlight provides an important source of energy for secondary production in the sea.

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SECTION III

THE INFLUENCE OF LIGHT ON BACTERIAL PROTEIN PRODUCTION IN

THE NORTH PACIFIC SUBTROPICAL GYRE

ABSTRACT

The response of bacterial protein production to irradiance was examined in the North Pacific Subtropical Gyre (NPSG) using photosynthetron experiments. Photoenhancements of ^{3}H-leucine (Leu) incorporation were observed in all experiments. Experiments were conducted in both the upper (5-25 m) and lower photic zone (75-100 m) to determine whether the response of bacterial production to irradiance differed along the depth-dependent light-gradient. Rates of Leu incorporation in the upper photic zone were stimulated 88-153% by light. Leu incorporation typically saturated at light intensities between 160-175 μmol quanta m^{-2} s^{-1} and displayed no significant photoinhibition. Optimal irradiances of Leu incorporation in the upper photic zone were between 183-296 μmol quanta m^{-2} s^{-1}, suggesting the phototrophic bacterial assemblages were adapted to relatively high light fluxes. Rates of Leu incorporation in the lower photic zone were consistently stimulated at low light intensities, but in contrast to the upper photic zone Leu production rates in the lower photic zone were often photoinhibited at light intensities <200 μmol quanta m^{-2} s^{-1}. Heterotrophic protein production responded to irradiance in a manner similar to photosynthesis, facilitating the use of photosynthesis-irradiance models. These results provide the first quantitative analyses of the relationships between heterotrophic protein production and irradiance in the ocean.

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INTRODUCTION

A determination of the factors that constrain bacterial growth and production in the oceans is crucial to understanding the contribution of marine biota to global carbon cycling. In the open oceans that dominate the Earth's surface area, bacterial biomass is one of the largest pools of biogenic carbon in the upper ocean and bacterial growth comprises the primary pathway of both carbon production and remineralization (Williams 1984, Ducklow et al. 1986, Ducklow and Carlson 1992, Karl 1999).

The important role of oxygen-evolving photosynthetic marine bacteria in the tropical and subtropical open oceans is widely acknowledged. The unicellular bacterium *Prochlorococcus* is estimated to be both the smallest and most abundant photosynthetic organism in the world's oceans (Chisholm et al. 1988, Partensky et al. 1999). In the North Pacific Subtropical Gyre (NPSG) *Prochlorococcus* accounts for 60-90% of the primary production and >50% of the total Chl *a* (Campbell and Vaulot 1993, Karl 1999). In oligotrophic open oceans, *Prochlorococcus* has been shown to demonstrate photoheterotorphic production (Zubkov et al. 2003). In addition, other types of photoheterotrophic bacteria may also influence productivity and biogeochemical cycling in the ocean. Members of the relatively abundant SAR 86 clade of marine γ-Proteobacteria possess a light-harvesting protein complex similar in

structure to rhodopsin (Béjà et al. 2000, 2001). When the membrane-bound rhodopsin protein complex absorbs light-energy, it undergoes a conformational change resulting in the establishment of a proton motive force that is coupled to photophosphorylation and ATP production (Béjà et al. 2000). The light-harvesting complexes in these organisms appear non-photosynthetic, that is to say that light-energy does not directly support carbon fixation (Béjà et al. 2001). The metabolic capacity of these organisms is still largely unknown; however, *Archaea* that possess similar rhodopsin-like protein complexes thrive in organic-rich environments as photoheterotrophs.

Another group of recently rediscovered photoheterotrophic bacteria possess both photosynthetic and heterotrophic physiological capabilities (Shiba et al. 1979, 1991, Kolber et al. 2000, 2001, Béjà et al. 2002). The aerobic, anoxygenic photosynthetic (AAnP) bacteria contain a light harvesting complex similar in structure to photosynthetic bacteria; however, unlike cyanobacteria, AAnP bacteria utilize the distinctive bacteriochlorophylls as components of their light harvesting systems (Yurkov and Beatty 1998). Isolated members of AAnP bacteria appear to grow as facultative heterotrophs, utilizing light energy to supplement heterotrophic metabolism (Yurkov and Beatty 1998). When reduced carbon substrates are not available, cultivated AAnP bacteria appear to grow (albeit at relatively low rates) as photoautotrophs (Kolber et al. 2001). The ability to switch physiologies to supplement cell metabolism likely provides a competitive advantage for AAnP bacteria in oligotrophic regions of the open ocean.

Considerable research effort has focused on the mechanisms by which light-energy influences photosynthesis. A number of models have been developed to describe the relationship between photosynthetic production and irradiance. Generally, the relationship appears as a saturation response; photosynthesis increases asymptotically as a function of light intensity to a maximum value (defined as P_{max}), beyond which photosynthetic rates saturate and sometimes decline. Under some circumstances, photosynthesis may be inhibited at high light intensities (photoinhibition), an apparent consequence of photochemical damage to the light-harvesting proteins of the cellular photoreaction centers (Falkowski and Raven 1997). Efforts to model the response of photosynthesis as a function of irradiance (P vs E) have revealed that there are several possible mathematical formulations that accurately describe the relationships (Jassby and Platt 1976, Platt et al. 1980, Sakshaug et al. 1997). Generally, these mathematical models are of two forms: the Monod growth model (similar to Michaelis-Menten enzyme kinetics) (Strickland and Parsons 1984), or models which describe the photosynthetic response to irradiance as an exponential-rise to some maximum value (Jassby and Platt 1976) (Figure 1). The Monod model is:

$$P^B = (P^B_{max} \, E) / (E_k + E) \qquad (1)$$

where P^B is the carbon fixation rate normalized to chlorophyll concentrations, P^B_{max} is the maximum rate of chlorophyll-normalized photosynthesis, E is the independent

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Figure 1. Comparison of three models describing the relationships between photosynthesis and irradiance (P^B vs E). Equation 1 (dotted line) demonstrates the modified Monod model (Strickland and Parsons 1984) (see text for details); parameters are $P^B_{max} = 10$, $E_k = 143$. Equation 2 (long dashes) represents the Jassby and Platt (1976) relationship, parameter values are $P^B_s = 10$, and $\alpha = 0.05$ (see text for description of parameters). Equation 3 is the Platt et al. (1980) model (solid line); parameters are $P^B_s = 10$, $\alpha = 0.05$, and $\beta = 0.005$. Also shown are E_k, P^B_s, and P^B_{max}. Light saturating irradiance (E_k), optimal irradiance (I_m), and maximum photosynthetic rates (P^B_{max}) calculated based on Platt et al. (1980).
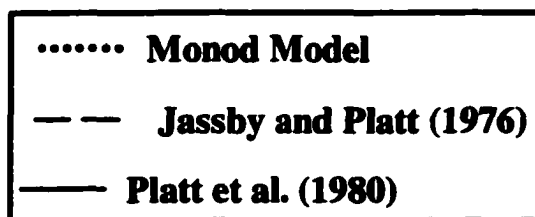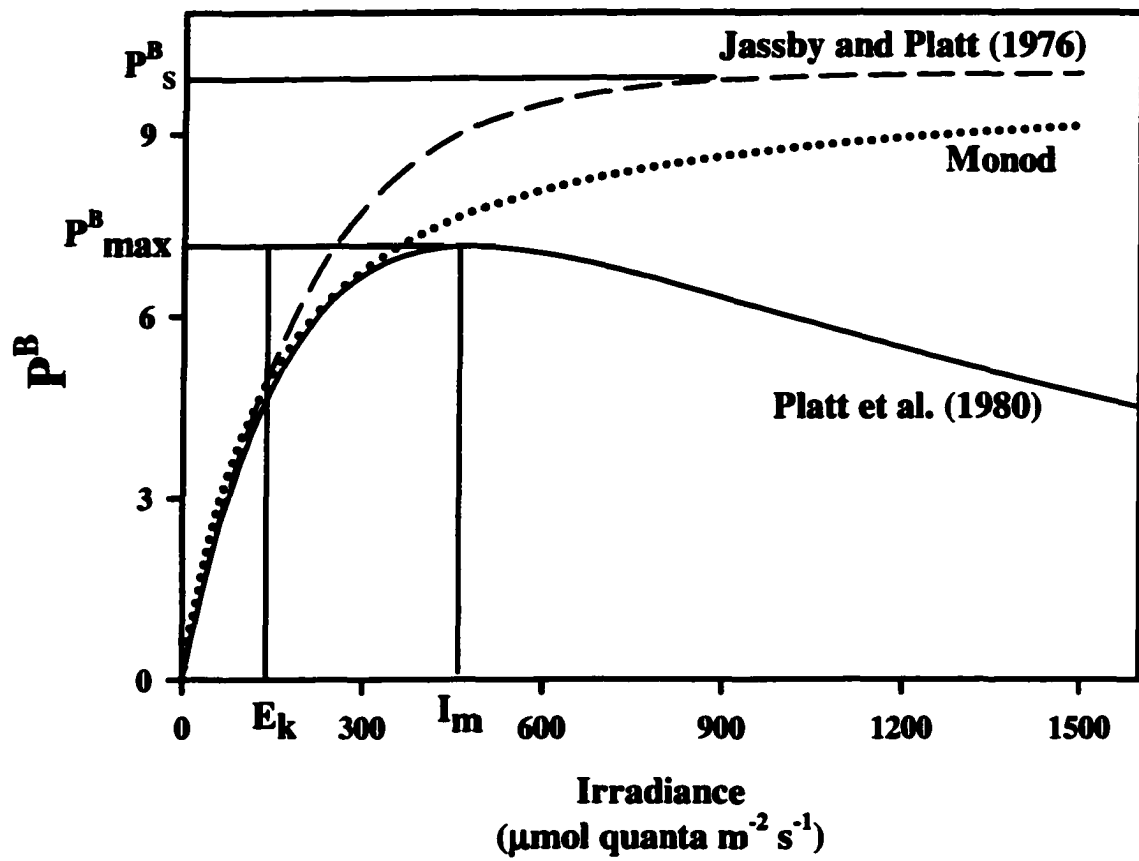
variable (irradiance), and E_k is the light intensity at one-half P_{max} (Figure 1). The exponential model proposed by Jassby and Platt (1976) has the form:

$$P^B = P^B_s [1\text{-exp} (-\alpha E / P^B_s)] \tag{2}$$

where P^B and E are the same as in equation 1, P^B_s is the maximum rate of chlorophyll normalized photosynthesis in the absence of photoinhibition, α is the initial slope of the photosynthetic response at low light intensities; it is a direct measure of light absorption and the maximum quantum efficiency of photosynthesis (Harris 1980, Sakshaug et al. 1997). Both models 1 and 2 appear suitable for describing P vs E data at relatively low light intensities; however, at higher irradiance, an additional parameter may be necessary to constrain the rate that photosynthesis declines due to photoinhibition (β) (Figure 1). Photoinhibition directly influences both P^B_{max} and α. Platt et al. (1980) formulated a model inclusive of the photoinhibitory effect of high light intensities:

$$P^B = P^B_s [1\text{-exp} (-\alpha E / P^B_s)] \text{exp} (-\beta E / P^B_s) \tag{3}$$

where all terms are identical to equation 2 and β is the rate of photoinhibition.

To date, there has been little work on the influence of irradiance on heterotrophic bacterial growth and production in the oceans. Those studies which have included light as a potential determinant of rates of heterotrophic bacterial production (HBP) have generally focused on the harmful impact of UV-radiation on

bacterial growth (Herndl et al.1993, Aas et al. 1996, Jeffery et al.1996, Booth et al. 2001). A recent study conducted in the North Atlantic and Mediterranean Sea described the response of Leu incorporation to photosynthetically available radiation (PAR) (Morán et al. 2001). Leu incorporation rates tended to be greater in samples incubated in the dark than those samples incubated in the light; however, the authors also observed that Leu incorporation rates increased with increasing irradiance between intensities of ~15-1500 μmol quanta m^{-2} s^{-1} (Morán et al. 2001).

Previous observations at Station ALOHA in the NPSG provided evidence that bacterial protein production rates were 30-50% greater in samples incubated *in situ* under natural irradiance relative to *in situ* incubations in the dark. To further investigate the nature of the light-driven protein production, the current study was designed to quantify the response of Leu incorporation to light intensity. Several experiments were conducted at various locations in the NPSG and at different depths throughout the photic zone (1% surface irradiance). In all of these experiments protein production demonstrated a response to irradiance similar to the response of photosynthesis to irradiance.

MATERIALS AND METHODS

Study site and Sampling

This study was conducted during three cruises to the Hawaii Ocean Time-series (HOT) Station ALOHA (22° 45' N, 158° 00' W) during the late winter-early spring of 2002 (February-HOT 135, March-HOT 136, and May-HOT 137). Experimental work for all cruises was performed aboard the R/V Kaimikai-O-Kanaloa. Water was collected in 12 liter polyvinylchloride-bottles mounted to a 24-bottle conductivity-temperature-depth (CTD) rosette from predawn hydrocasts. Upon completion of the hydrocast, water was sampled from the CTD rosette into darkened 2 liter polycarbonate bottles. Bottles were transferred to a radioisotope lab-van where the incubation experiments were conducted.

PAR fluxes were measured at approximately noon on the same day experiments were conducted. PAR fluxes were measured using a Biospherical Instruments Profiling Reflectance Refractometer (PRR 600). Downwelling PAR fluxes (400-700 nm) were measured throughout the upper water column (0-200 m) at 2-3 Hz sampling frequency. The instrument was deployed by off the aft-quarter of the ship using a small crane to extend the instrument away from the ship shadow; the instrument was lowered and raised by hand. Tilt and roll sensors were attached to the PRR instrument, and measurements were excluded from the analyses when the tilt

and roll values exceeded +5° or -5°. Twelve-point running means of the discrete PRR profile were calculated for each profile.

Response of Leu incorporation to irradiance

To examine the response of bacterial protein production to irradiance, photosynthetron incubators similar to those described by Lewis and Smith (1983) were utilized. Samples were exposed to a range of light intensities inside the photosynthetron incubator. Incubations were conducted in 40 ml polycarbonate centrifuge tubes. Samples were placed in wells that had been bored into a metal cooling block atop the incubator. The cooling block had 24 different sample positions; sample tubes were placed into each well and the entire block was placed atop the light source, allowing illumination of samples from below. The photosynthetron used a single 1500 W halogen bulb, which was transmitted through a blue plexiglass shield prior to entering the photosynthetron chamber in an effort to mimic the spectral light quality of the open ocean photic zone (Laws et al. 1990). Neutral density screens were placed in each well of the photosynthetron, producing an array of 24 different light intensities. To keep the samples at or near *in situ* temperatures, the sampling block was plumbed to a large capacity refrigerated water bath and cooled throughout the incubation. Incubations typically lasted 2 hours. Light intensities were estimated by placing a Biospherical QSL-100 PAR-sensor inside a polycarbonate incubation tube and the PAR flux inside each well was measured. Intensities were measured before and after the incubation and the average

intensities were used to determine the light flux. PAR fluxes varied between ~0-1500 μmol quanta m^{-2} s^{-1}.

The response of bacterial ^{3}H-leucine incorporation to irradiance (Leu-E) was determined based on the incorporation of Leu into protein using a modified form of the Kirchman et al. (1983) method. Whole seawater was subsampled into acid-cleaned, 40 ml polycarbonate centrifuge tubes and inoculated with 20 nmol L^{-1} of leucine (19 nmol L^{-1} nonradioactive leucine + 1 nmol L^{-1} ^{3}H-leucine). Specific activities of ^{3}H-leucine stocks were 150-180 Ci mmol^{-1} (New England Nuclear, NEN460A). Samples were capped and placed inside the photosynthetrons. Two time-zero treatments were prepared for each experiment and immediately filtered onto a 25 mm, 0.2 μm mixed-ester HA filters and stored frozen in 15 ml centrifuge tubes until processed at the laboratory. Two replicate dark control samples were placed in an opaque cloth bag and placed into the same water bath used to cool the photosynthetron. To terminate incubations, samples were filtered onto 25 mm 0.2 μm HA filters and frozen in centrifuge tubes. In the laboratory, samples and blanks were processed identically. Details of the laboratory sample processing are given in Section I and described in Karl (1980).

Photosynthesis versus irradiance

Photosynthetic uptake of ^{14}C-labelled bicarbonate was measured during the May 2002 cruise to Station ALOHA using the same photosynthetron incubator used for the Leu-E experiments. Experiments were conducted on water collected at 5 and 75 m depth at 0900 and 1200, respectively. The photosynthesis experiments were

conducted one day after the Leu-E experiments. Water was subsampled into 40 ml polycarbonate incubation tubes and spiked with approximately 400 μCi of ^{14}C sodium bicarbonate. A 250 μl subsample was removed from two different samples and placed into a scintillation vial containing 1 ml of β-phenylethylamine. These samples were used for determination of the specific activity of the ^{14}C-in the 40 ml samples. One time-zero treatment was processed immediately after spiking, and one sample was incubated in the dark to evaluate dark ^{14}C-uptake. Dark controls were subtracted from the resulting photosynthetic rates to correct for dark-mediated inorganic carbon uptake.

At the end of the incubation, the entire 40 ml sample was filtered onto 25 mm, 0.2 μm HA filters. Filters were placed into glass scintillation vials and stored frozen until processed. In the laboratory, filters were acidified with 1 ml of 2M HCl and vented for 24 hours, followed by addition of 10 ml Aquasol II scintillation cocktail. Samples were counted on a TRI CARB 4640 liquid scintillation counter. Determination of photosynthetic production rates used surface water total inorganic carbon concentrations measured at Station ALOHA (http://hahana.soest.hot.edu) to calculate the isotopic dilution ratio. For P-E experiments, photosynthetic rates were normalized to Chl a concentrations. Chl a concentrations were measured by filtering 125 ml seawater samples onto Whatman 25 mm GF/F filters and extracting samples in 90% acetone for one week. Chl a concentrations were determined fluorometrically using a Turner TD-700 fluorometer.

Data Analyses

It was not possible to normalize Leu rates to cell abundance or biomass because the specific populations responsible for the light-enhanced Leu incorporation rates are still unknown; however, differences in the response of Leu incorporation to light intensity appeared relatively small between depths and among cruises sampled (Figure 3); thus facilitating comparison of the model parameters among the different experiments conducted. The measured Leu incorporation rates were fitted to a modified version of the Platt et al. (1980) model based on Priscu (1989), including a term describing dark Leu incorporation (Leu_D) and a term for photoinhibition.

$$Leu = Leu_S \left[1 - \exp(-\alpha E/Leu_S)\right] \exp(-\beta E/Leu_S) + Leu_D \qquad (4)$$

where Leu was the modeled rate of Leu incorporation (pmol Leu L^{-1} hr^{-1}), Leu_S was the maximum Leu incorporation in the absence of photoinhibition (i.e. $\beta=0$) with the same units as Leu, α was the initial slope of Leu incorporation rates at low light intensities (pmol Leu L^{-1} hr^{-1} (μmol quanta m^{-2})s^{-1}), E was the irradiance (μmol quanta m^{-2} s^{-1}), β describes the slope of the curve in the photoinhibited region of the curve with the same units as α, and Leu_D was the incorporation rate in the dark (same units as Leu). Data were fitted by nonlinear least squares regression using an iterative algorithm (Sigma Plot v. 8.0).

In addition to determining the parameters for equation 4, values for the following were also computed following Platt et al. (1980):

$$\text{Leu}_{max} = \text{Leu}_S \, (\alpha/\alpha + \beta)(\beta/\alpha + \beta)^{\beta/\alpha} + \text{Leu}_D \qquad (5)$$

Leu_{max} was the maximum Leu incorporation rate (inclusive of photoinhibition). Note that when $\beta=0$ the function is undefined. Also derived was a term describing the optimal light intensity at which maximum incorporation occurs (I_m).

$$I_m = (\text{Leu}_S/\alpha) \, \ln((\alpha+\beta)/\beta) \qquad (6)$$

Finally, parameters from equation 4 were used to calculate the parameter, E_k, which describes the saturating irradiance of Leu incorporation rates.

$$E_k = \text{Leu}_{max}/\alpha \qquad (7)$$

E_k has been applied as an index of photoadaptation when describing photosynthesis (Platt et al. 1980). Error estimates for Leu_{max}, I_m, E_k were calculated by propagation of standard errors determined by the regression analyses.

Based on the observed Leu responses to irradiance, experiments conducted in the upper ocean were also fitted to a modified version of the Monod growth model. The specific parameters were:

$$\text{Leu} = (\text{Leu}_{max} {}^*E)/(E_k + E) + \text{Leu}_D \qquad (8)$$

where Leu was the rate of Leu incorporation (pmol L^{-1} hr^{-1}), Leu_{max} was the maximal

rate of Leu incorporation (pmol L^{-1} hr^{-1}), E was the measured irradiance (μmol quanta

m^{-2} s^{-1}), and E_k (μmol quanta m^{-2} s^{-1}) was the light intensity of one-half Leu_{max} similar

to the half saturation constant in the Michaelis-Menten equation, and Leu_D was the

rate of Leu incorporation in the dark. The most appropriate model was selected from

significant results of least squares regression analyses based on the coefficient of

determination (r^2) of the regression.

RESULTS

Upper ocean physical characteristics and productivity

Photic zone depths (1% surface isopleth) for the three cruises were 112, 108,

112 m for February, March, and May, respectively. Light fluxes (E^*_z) were measured

at approximately noon on each day of these experiments (Figure 2). Letelier et al. (in

press) used a climatological model to estimate the daily insolation at Station ALOHA;

this model predicts the surface light fluxes at Station ALOHA for March 12 and May

20 (days that experiments were conducted) were 38 and 51 mol quanta m^{-2} d^{-1},

suggesting that cloud cover may have resulted in the lower surface light fluxes

measured in May (Figure 2).

Experiments were conducted in both the upper (5-25 m) and lower photic

zone (75-100 m). Experiments conducted in the upper photic zone were within the

surface mixed layer, while those in the lower photic zone were below the upper

mixed layer. Light fluxes at the depths that the experiments were conducted are

shown in Table 1. Daily PAR fluxes in the upper photic zone ranged from 12-42 mol

quanta m^{-2} d^{-1} while fluxes in the lower photic zone were 0.54-2.6 mol quanta m^{-2} d^{-1}

(Table 1).

Photic zone Chl a inventories ranged from 16.5-21.9 mg Chl a m^{-2}, increasing

between February and May. Chl a concentrations at the discrete depths where

Figure 2. Depth profiles of photosynthetically available radiation (PAR) on HOT cruises 135, 136, and 137 (February, March, and May 2002). Note X-axis is plotted on log scale. PAR was measured at approximately noon using a Biospherical Instruments Profiling Reflectance Refractometer (PRR). Plotted are twelve-point running means of discrete vertical PAR fluxes.
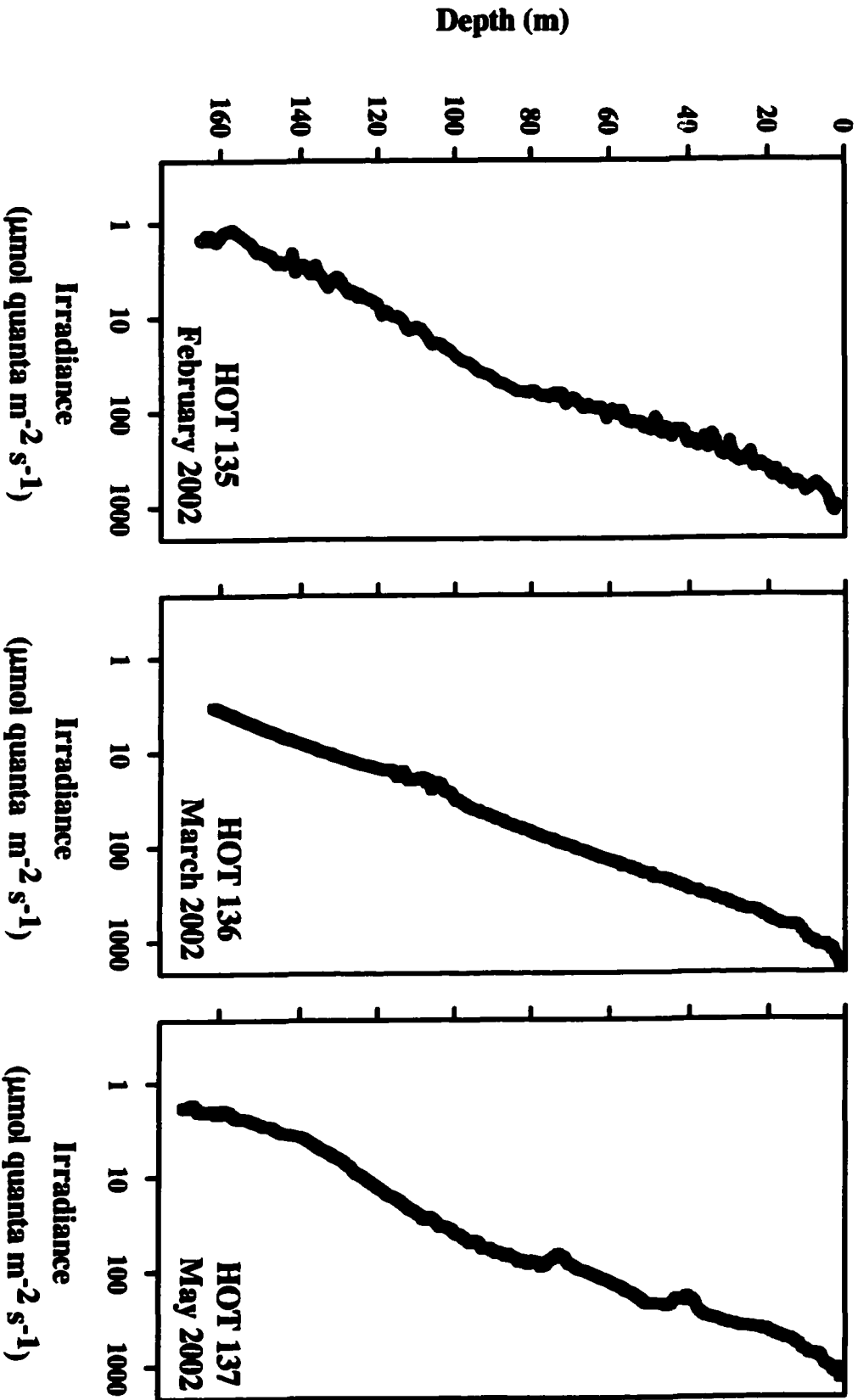
Table 1. Upper ocean properties at Station ALOHA for cruises where experiments were conducted.

Cruise	E_z^a (mol quanta m^{-2} d^{-1})	$E^*_z{}^b$ (μmol quanta m^{-2} s^{-1})	Chl a (μg Chl L^{-1})	Non-pig. (cells ml^{-1})	Prochloro (cells ml^{-1})	Syn. (cells ml^{-1})	Leu$_L$ (pmol L^{-1} hr^{-1})	Leu$_D$ (pmol L^{-1} hr^{-1})	PP (nmol C L^{-1} hr^{-1})
Feb. 20, 2002									
25 m	12	302	0.08	2.5×10^5	1.0×10^5	1.7×10^3	39 (0.02)	26 (4.2)	48
100 m	0.54	25	0.24	2.0×10^4	4.8×10^4	1.5×10^1	17 (0.87)	15 (1.7)	23
March 12 2002									
25 m	16	480	0.06	na^c	na	na	31 (0.48)	19 (0.63)	53
100 m	0.80	31	0.17	na	na	na	30 (1.0)	14 (2.8)	27
May 20 2002									
5 m	42	887	0.05	2.2×10^5	7.8×10^4	8.5×10^2	48 (2.1)	34 (2.6)	46
75 m	2.6	81	0.11	2.3×10^5	1.3×10^5	1.4×10^3	37 (5.5)	22 (3.1)	29

Abbreviations are: Non-pig Bact.-Non pigmented bacteria, Prochloro-*Prochlorococcus*, Syn.- *Synechococcus*, Leu$_L$ and Leu$_D$ are *in situ* daily rates of Leu incorporation from incubations in the light and dark, respectively, PP- photosynthetic production.
[a] Daily PAR fluxes calculated using measured PAR incident on the surface ocean and measured attenuation coefficients.
[b] Maximum PAR flux, based on noon PRR cast.   [c] na, data not available.

experiments were conducted ranged from 0.07-0.24 µg Chl a L^{-1} (Table 1). Chl a was

elevated in the lower photic zone due to the permanent deep chlorophyll maxima

observed at Station ALOHA (Letelier et al. 1996). Upper ocean (0-175 m) rates of

photosynthetic production (PP) ranged from 44-66 mmol C m^{-2} d^{-1}. Photosynthetic

rates at those depths where experiments were conducted ranged from 26.7-52.5 µmol

C L^{-1} hr^{-1} (Table 1).

Daily water column profiles of Leu incorporation and photosynthetic rates for

the three cruises sampled for these experiments are shown in Figure 3. Rates of Leu

incorporation in the light (Leu$_L$) were significantly greater than dark incubated

samples (Leu$_D$) throughout the upper 125 m of the water column during both the

March and May cruises (One-way ANOVA, $p < 0.05$). Rates of photosynthesis in the

upper 75 m of the photic zone ranged from 550-671 nmol C L^{-1} d^{-1} (Figure 3).

Photosynthetic rates were typically greatest in the upper 25 m of the water column,

declining approximately linearly to the base of the photic zone.

Leu incorporation versus irradiance in the upper photic zone

In total, six experiments were conducted to determine the response of Leu

incorporation to irradiance (Leu-E). Three of the experiments were conducted using

water collected in the upper 25 m of the water (Figure 4), and three experiments were

conducted from samples collected in the lower photic zone (75-100 m) (Figure 5).

Leu incorporation increased with irradiance in a non-linear fashion; the measured Leu

rates were fitted to the modified Platt et al. (1980) model (equation 4) and the results

are presented in Table 2. In both March and May of 2002, the estimated optimal

Figure 3. Depth profiles showing daily (daylight period only) Leu incorporation rates measured in the light (Leu_L – open triangles) and dark (Leu_D – closed circles). Also shown are photosynthetic production (PP) (open squares) for HOT cruises 135 (panels a, b), 136 (panels c, d), and 137 (panels e, f). Symbols are mean of three replicates, and error bars represent standard deviations of the means.

Leu incorp.
(pmol L^{-1} d^{-1})

PP
(nmol C L^{-1} d^{-1})
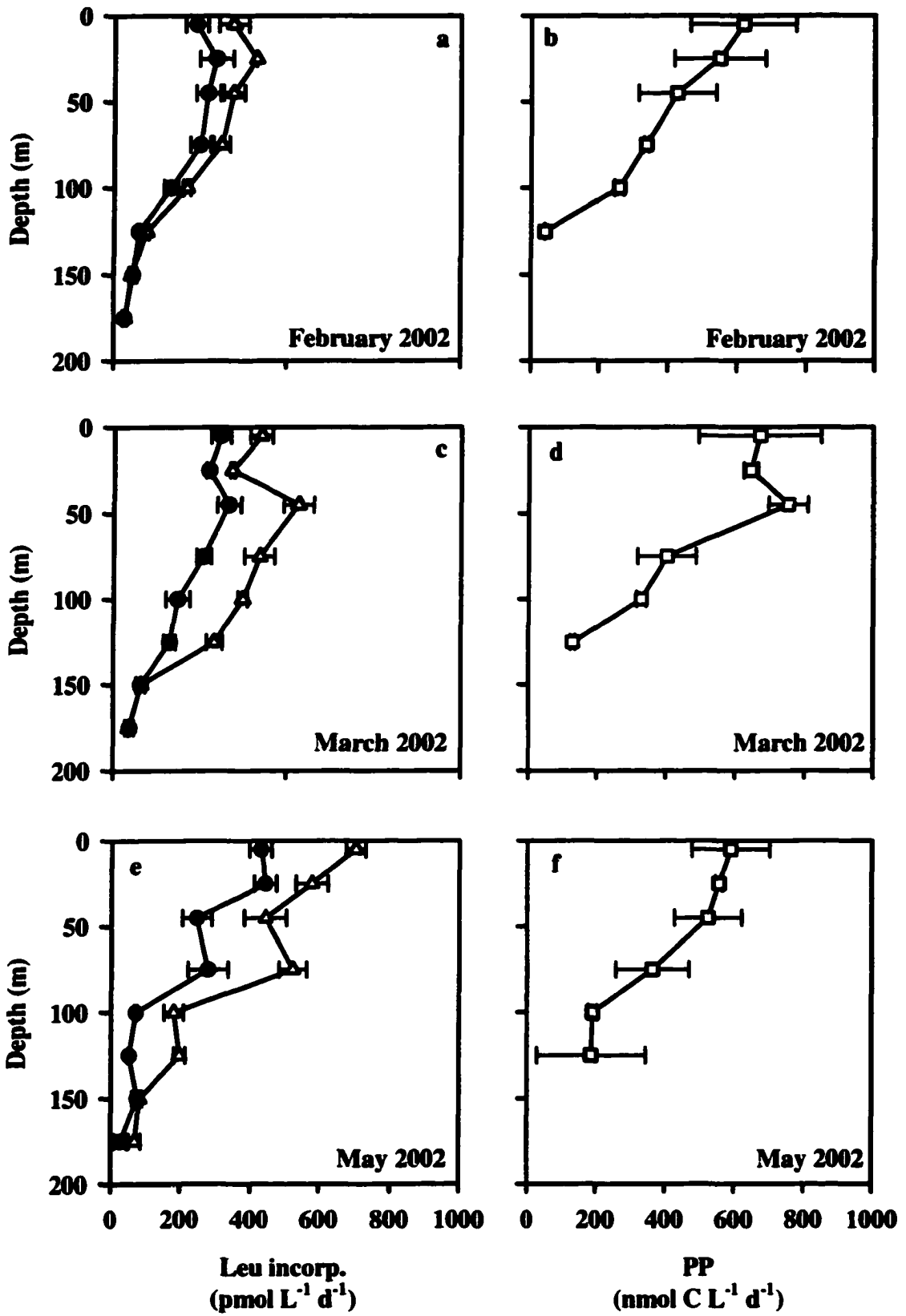
Figure 4. Relationship between Leu incorporation (Leu) and irradiance (E) in the upper photic zone (<25 m) at Station ALOHA (symbols). Fitted lines are results of least squares regression using modified Platt et al. (1980) model (equation 4). Parameters for line fits given in Table 2. Dashed vertical lines represent maximal (noon-time) PAR flux measured at each depth for the three cruises shown.
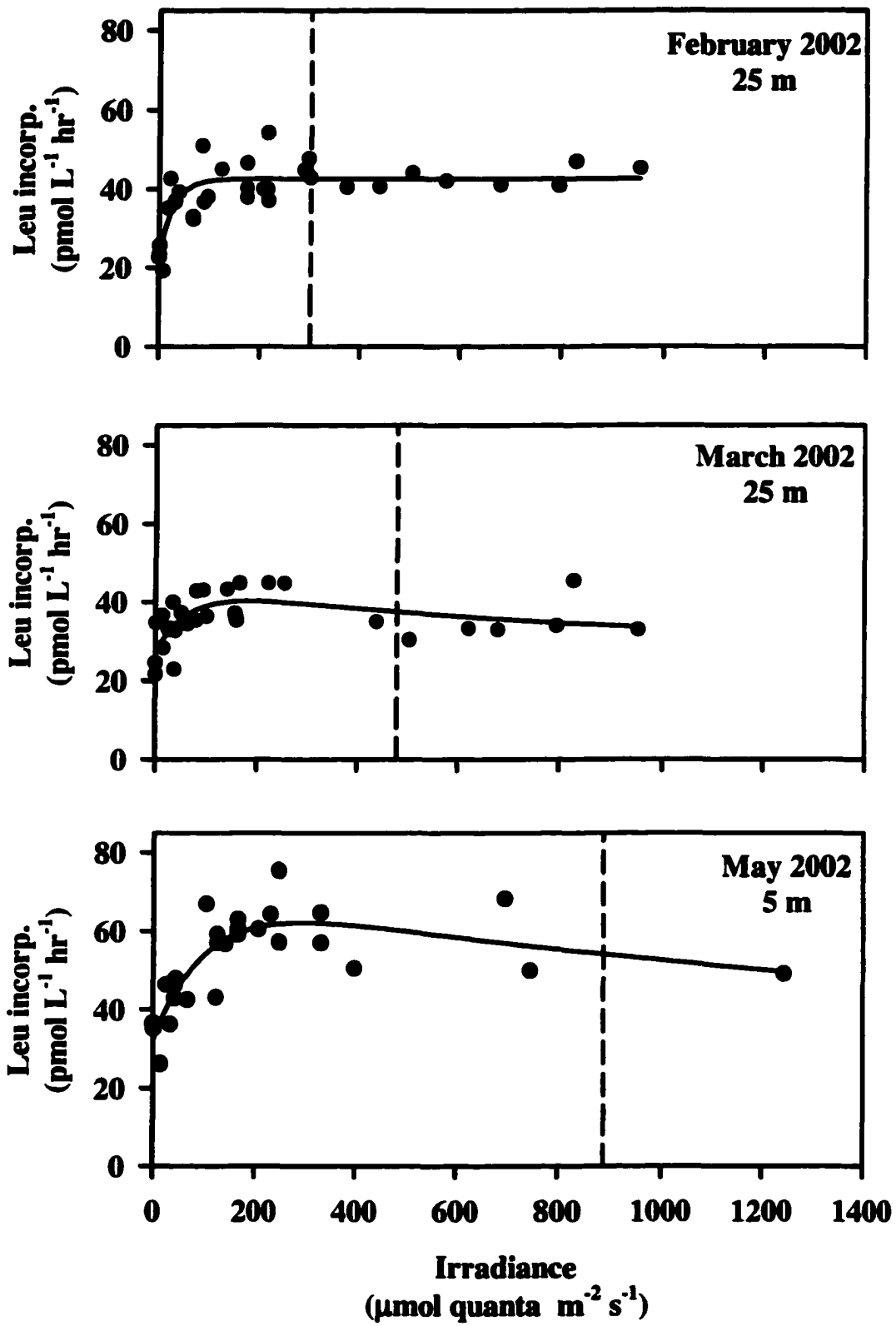
Figure 5. Relationship between Leu incorporation (Leu) and irradiance (E) in the

lower photic zone (75-100 m) at Station ALOHA (symbols). Fitted lines are results

of least squares regression using modified Platt et al. (1980) model (equation 4).

Parameters for line fits given in Table 2. Dashed vertical lines represent maximal

(noon-time) PAR flux measured at each depth for the three cruises shown.
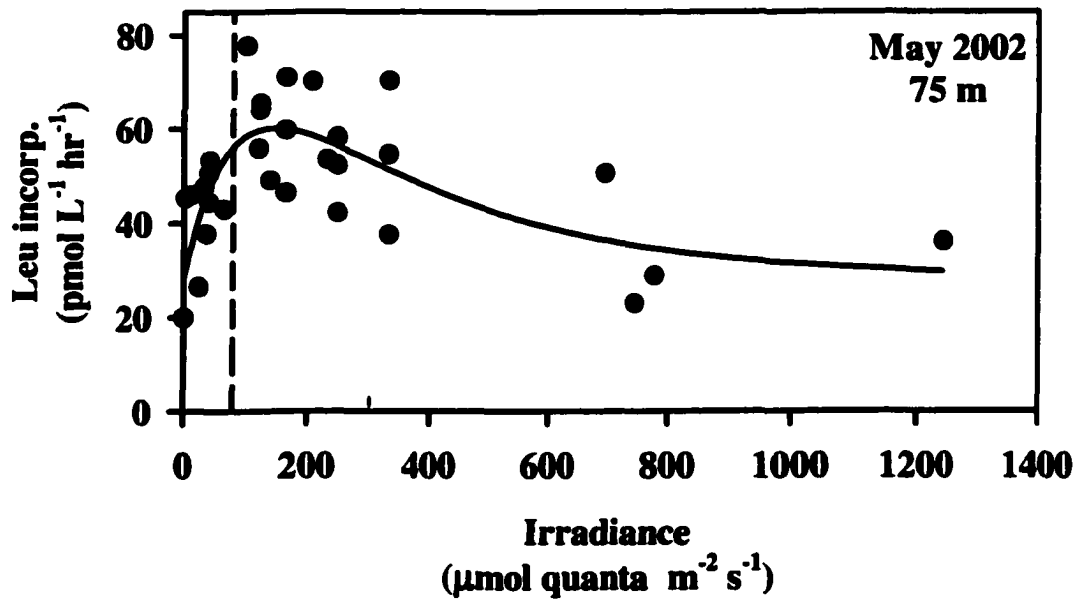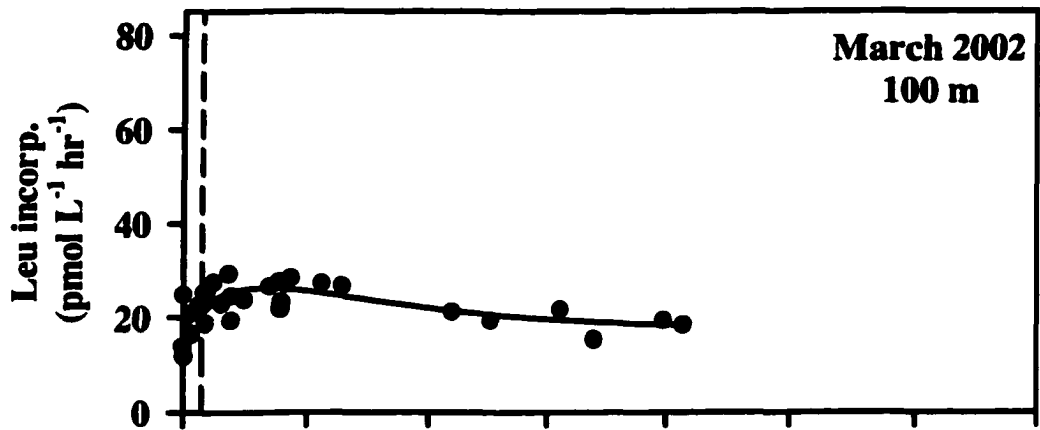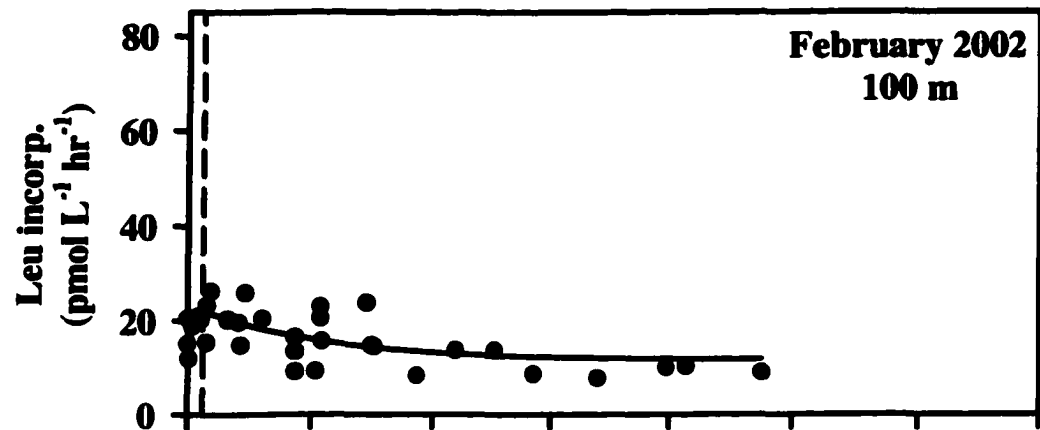
Table 2. Summary of coefficient of determination (r^2) and significance of non-linear regressions describing Leu incorporation as a function of irradiance.

Cruise Depth (m)	Monod Model[a]	Exponential Model[b]
February 2002 25 m	0.62[c] (P<0.0001)	0.62 (P<0.0001)
February 2002 100 m	0.02 (ns)	0.41 (P=0.0015)
March 2002 25 m	0.26 (P=0.015)	0.50 (P=0.0019)
March 2002 100 m	0.17 (ns)	0.43 (P=0.0079)
May 2002 5 m	0.57 (P<0.0001)	0.68 (P<0.0001)
May 2002 75 m	0.25 (P=0.017)	0.54 (P<0.0001)

[a] Monod model describing saturation response of Leu and irradiance, see text for details.
[b] Exponential Model modification of Platt et al. (1980), see text for description.
[c] Top number is coefficient of determination of non-linear least squares regression, bottom number is significance of regression; ns indicates P>0.05.

Table 3. Summary of parameters defined by least squares nonlinear regression of Leu incorporation as a function of irradiance at Station ALOHA. Top numbers are derived parameters; numbers in parentheses are standard errors of parameters.

Cruise Depth (m)	α^a		β^a		$Leu_D{}^b$		$Leu_S{}^b$		$Leu_{max}{}^b$		$I_m{}^c$		$E_k{}^c$	
February 2002 25 m	0.75 (0.33)	A^d	0.00* (0.00)	A	23 (2.8)	A	20 (3.3)	A	-		-		-	
February 2002 100 m	1.2* (1.3)	A	0.062 (0.014)	B	12 (1.9)	B	12 (3.3)	B	22 (3.5)	A	30 (14)	A	18 (28)	A
March 2002 25m	0.25 (0.07)	A	0.017* (0.17)	A	27 (2.3)	A	17 (5.6)	A	40 (5.7)	B	184 (6.8)	A	160 (51)	A
March 2002 100 m	0.20 (0.10)	A	0.062* (0.098)	A	17 (1.7)	B	19* (15)	A	26 (15)	B	133 (18)	A	130 (102)	A
May 2002 5 m	0.36 (0.13)	A	0.025* (0.024)	A	32 (3.8)	C	39 (9.6)	A	62 (9.6)	B	304 (18)	A	175 (70)	A
May 2002 75 m	0.64 (0.31)	A	0.19 (0.032)	B	28 (5.8)	C	64 (12)	A	60 (12)	B	148 (33)	A	94 (52)	A

^{a} pmol L^{-1} hr^{-1} (μmol quanta m^{-2})$^{-1}$ s^{-1} ^{b} pmol L^{-1} hr^{-1} ^{c} μmol quanta m^{-2} s^{-1} ^{d} Letter designations determined by testing differences between cruises and among depths. Parameters with the same letter designation are statistically indistinguishable at P=0.05.   -, undefined function   *, not significant (P>0.05).

irradiances (I_m) of Leu incorporation in the upper photic zone were lower than the measured maximal (noon time) PAR fluxes.

Generally, Leu incorporation in the upper ocean demonstrated a saturation-like response to irradiance, increasing asymptotically with increased irradiance, and remaining roughly constant through the range of irradiances beyond the saturating light fluxes (Figure 4). In both March and May the relationship was best described using equation 4 (Table 3), and in February the Leu-irradiance relationship was described equally well by equation 4 and equation 8 (Table 2). Leu incorporation rates in the upper ocean typically saturated at light fluxes less than 200 μmol quanta $m^{-2} s^{-1}$ (Figure 4, Table 2). The majority (50-68%) of the variance in the response of Leu incorporation to irradiance was accounted for using an exponential model of Leu incorporation at low light intensities, with a weak photoinhibition term at greater light fluxes (Table 3).

During the February cruise, Leu rates increased rapidly at low light intensities, roughly doubling (relative to Leu_D) by 50 μmol quanta $m^{-2} s^{-1}$ (Figure 4). The difference between the maximum measured Leu rate (Leu_{max}) and Leu_D was ~20 pmol $L^{-1} hr^{-1}$, and the initial slope (α) was 0.75 pmol Leu $L^{-1} hr^{-1}$ (μmol quanta $m^{-2} s^{-1}$). No significant photoinhibition was observed for the entire range of irradiances during the February cruise (Figure 4, Table 2). In March, α was three-fold lower than in February and the difference between Leu_D and Leu_{max} was ~23 pmol $L^{-1} hr^{-1}$.

During the May cruise, rates of Leu incorporation increased with irradiance more than in either of the two previous experiments. Leu incorporation rates increased roughly two-fold between 0-200 μmol quanta $m^{-2} s^{-1}$, and light-stimulated

Leu_{max} was roughly 30 pmol L^{-1} hr^{-1} greater than Leu_D (Table 3). Estimated Leu_D incorporation was significantly greater in May than in either February or March (T-test, $P<0.05$), but there were no significant differences in derived estimates of Leu_{max}, or in the saturating light intensities (T-test, $P>0.05$).

There were no significant differences in α between the cruises sampled in this study despite more than a four-fold decrease between February and March (Table 2). Similarly, upper photic zone rates of Leu_{max} were statistically indistinguishable at the 95% confidence level between all three cruises averaging 51 pmol L^{-1} hr^{-1}.

Leu incorporation versus irradiance in the lower photic zone

The responses to of Leu incorporation to irradiance in the deep photic zone were largely similar as those in the upper photic zone, with one notable exception: in two of the three experiments conducted in the lower photic zone, Leu incorporation exhibited photoinhibition at relatively low light intensities (Figure 5). Forty-one to fifty-four percent of the variance in Leu incorporation rates could be described as a function of irradiance when the data were fitted to the exponential model (equation 4) (Table 2). In both February and May, Leu rates in the lower photic zone demonstrated significant photoinhibition, while no significant photoinhibition was observed in the upper photic zone (Figure 5, Table 2). On average, β was 60% greater in the lower photic zone than in the upper water column. Despite significant differences in β, there were no significant differences in the optimal (I_m) and saturating irradiances (E_k) derived from the upper and lower photic zone (T-test, $P>0.05$).

Noontime PAR fluxes at the depths where these experiments were conducted were less than or nearly equivalent to the calculated I_m values suggesting that *in situ* light intensities may have been near or slightly less than the optimal irradiance (in the absence of photoinhibition) for heterotrophic protein production. During the February cruise, E_k was 18 μmol quanta m^{-2} s^{-1} (Table 2) and the maximal PAR flux at 75 m was 25 μmol quanta m^{-2} s^{-1} (Table 1, 2), revealing that the *in situ* Leu incorporation rates may have been weakly photoinhibited. In contrast, E_k values in March and May were greater than measured PAR fluxes, suggesting the *in situ* Leu incorporation rates in the lower photic zone were not photoinhibited (Table 1, 2).

During the February cruise, Leu rates demonstrated no significant enhancement with rates becoming photoinhibited at light fluxes as low as 30 μmol quanta m^{-2} s^{-1}, and saturating at 18 μmol quanta m^{-2} s^{-1} (Figure 5, Table 3). At noon, the irradiance at 100 m was approximately 25 μmol quanta m^{-2} s^{-1}, similar to the saturating irradiance estimated in this experiment (Table 3). The photoinhibitory response of Leu rates in the lower photic zone contrasted the response in the upper photic zone where Leu rates remained roughly constant above ~100 μmol quanta m^{-2} s^{-1} (Figure 6).

During the March cruise, Leu incorporation rates were enhanced by low light fluxes, and Leu rates saturated at irradiances greater than 130 μmol quanta m^{-2} s^{-1} (Figure 5, Table 3). The response of Leu incorporation to low light intensities was very similar to the response in the upper photic zone; Leu rates increased gradually at low light fluxes (α= 0.20 pmol L^{-1} hr^{-1} (μmol quanta m^{-2} $s^{-1})^{-1}$, and never demonstrated significant photoinhibition (Table 2).

Figure 6. Summary of derived parameters from Table 3 for the Leu vs. E

experiments in the upper and lower photic zone. Initial slope (α) of Leu versus

irradiance (a), rate of photoinhibition (β) (b), optimal irradiance (I_m) (c), and

saturating irradiance (E_k) of Leu (d). Error bars are standard error of derived

parameters.

initial slope (α)

Upper

Lower

a.

$$\text{pmol Leu L}^{-1}\text{hr}^{-1} (\mu\text{mol quanta m}^{-2}\text{s}^{-1})^{-1}$$

photoinhibition (β)

Upper

Lower

b.

$$\text{pmol Leu L}^{-1}\text{hr}^{-1} (\mu\text{mol quanta m}^{-2}\text{s}^{-1})^{-1}$$

optimal irradiance (I$_m$)

Upper

Lower

c.

$$\mu\text{mol quanta m}^{-2}\text{s}^{-1}$$

saturating irradiance (E$_k$)

Upper

Lower

d.
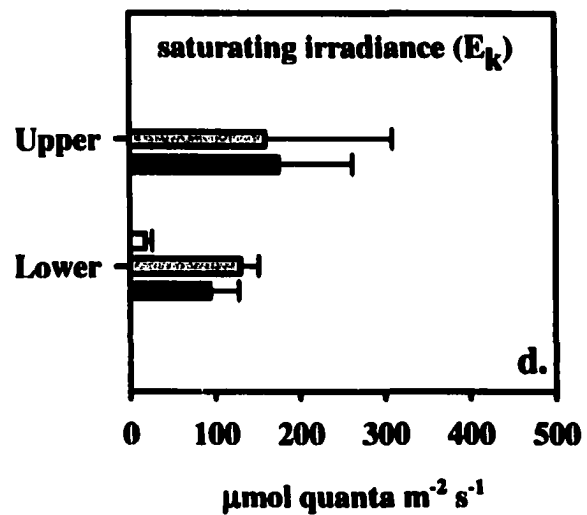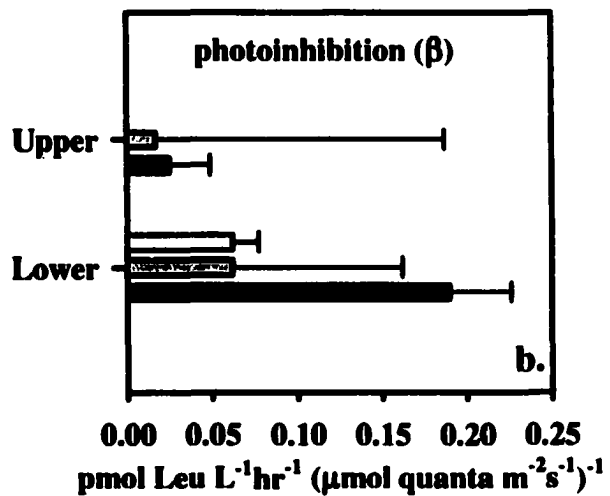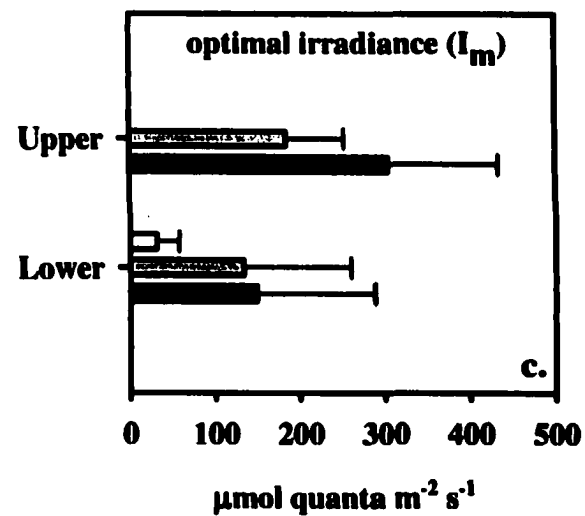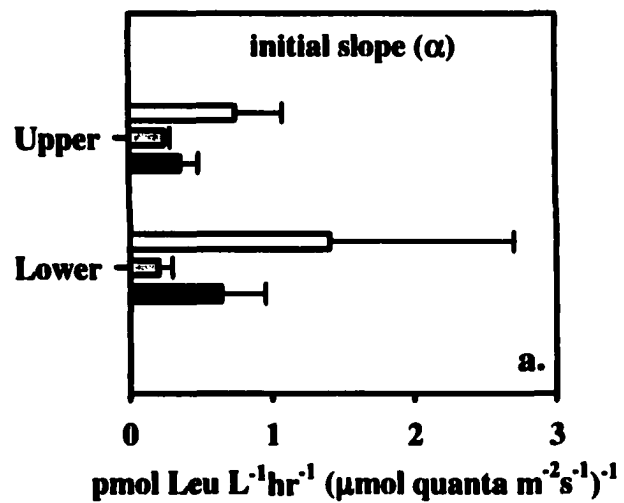
$$\mu\text{mol quanta m}^{-2}\text{s}^{-1}$$

☐ February 2002   ▨ March 2002   ■ May 2002

During the May cruise, Leu incorporation demonstrated a steep initial increase

at low light fluxes (α= 0.64 pmol Leu L^{-1} hr^{-1} (μmol quanta m^{-2})$^{-1}$ s^{-1}) before

saturating at 94 μmol quanta m^{-2} s^{-1} and declining to rates approximately equivalent

to the Leu$_D$ rate (Figure 5, Table 3). There were no significant differences in α, Leu$_D$,

Leu$_{max}$, or E$_k$ between the upper and lower photic zone during the May cruise (T-test,

P>0.05). The large stimulation of Leu incorporation rates by light observed in the

lower photic zone resulted in the largest photoenhancement of Leu incorporation rates

(ΔLeu$_{max}$) of all the experiments (in either the upper or lower photic zone).

Photosynthesis as a function of irradiance

To determine how photosynthesis varied as a function of irradiance, two

experiments were conducted in the upper and lower photic zone during the May HOT

cruise (Figure 7). The results from both experiments were successfully fitted to

equation 3 (Figure 7), yielding estimates of α, β, and P^{B}s; using these parameters, E$_k$,

I$_m$, and P$^B_{max}$ were calculated (Table 4). The normalized photosynthetic rates tended

to increase asymptotically at low light fluxes to 0.53 μmol C mg Chl^{-1} hr^{-1}.

Photosynthetic rates saturated 151 μmol quanta m^{-2} s^{-1} and I$_m$ was 616 μmol C mg

Chl^{-1} hr^{-1} (Table 4). Based on these results, *in situ* photosynthetic rates in the upper

photic zone may have been photoinhibited (noontime PAR flux was ~800 μmol

quanta m^{-2} s^{-1}).

In the lower photic zone the response of photosynthesis to irradiance was

similar to the upper photic zone; however, similar to the response of Leu to

irradiance, photosynthetic rates in the lower photic zone also demonstrated significant

photoinhibition (Table 3). There were no significant differences in P^B_{max}, or α between the upper and lower photic zone; however, both I_m and E_k were significantly lower in the lower photic zone than estimates derived for the upper photic zone (T-test, P<0.05) (Figure 7, Table 4). Based on the noontime PAR flux at 75 m (81 μmol quanta m^{-2} s^{-1}), irradiance in the lower photic zone was likely sufficient to saturate photosynthetic production.

On the one cruise where both the P-E and Leu-E responses were examined (May 2002), both estimates of production responded similarly to irradiance. Direct comparison between the P-E and Leu-E responses is hampered due to the inability to normalize the Leu-E responses to cell abundance or biomass; however, there were notable similarities among the P-E and Leu-E responses. In particular, neither the P-E response nor the Leu-E response demonstrated significant photoinhibition in the upper photic zone, and both the P-E and Leu-E responses were photoinhibited at higher light fluxes in the deep photic zone. Calculated saturating light fluxes for upper photic zone P-E and Leu-E responses were 151 and 175 μmol quanta $m^{-2}s^{-1}$, while estimated E_k values in the lower photic zone were 25 and 94 μmol quanta m^{-2} s^{-1}, respectively.

Figure 7. Relationship of P^B (photosynthesis normalized to chlorophyll) and

irradiance in the upper (top) and lower (bottom) photic zone. Experiments conducted

in May 2002. Fitted lines are least squares non-linear regression using equation 4

(see text), parameters of line fits given in Table 4. Dashed vertical lines represent

maximal (noon-time) PAR flux measured at 5 m and 75 m during May 2002 cruise.

May 2002
5 m

May 2002
75 m
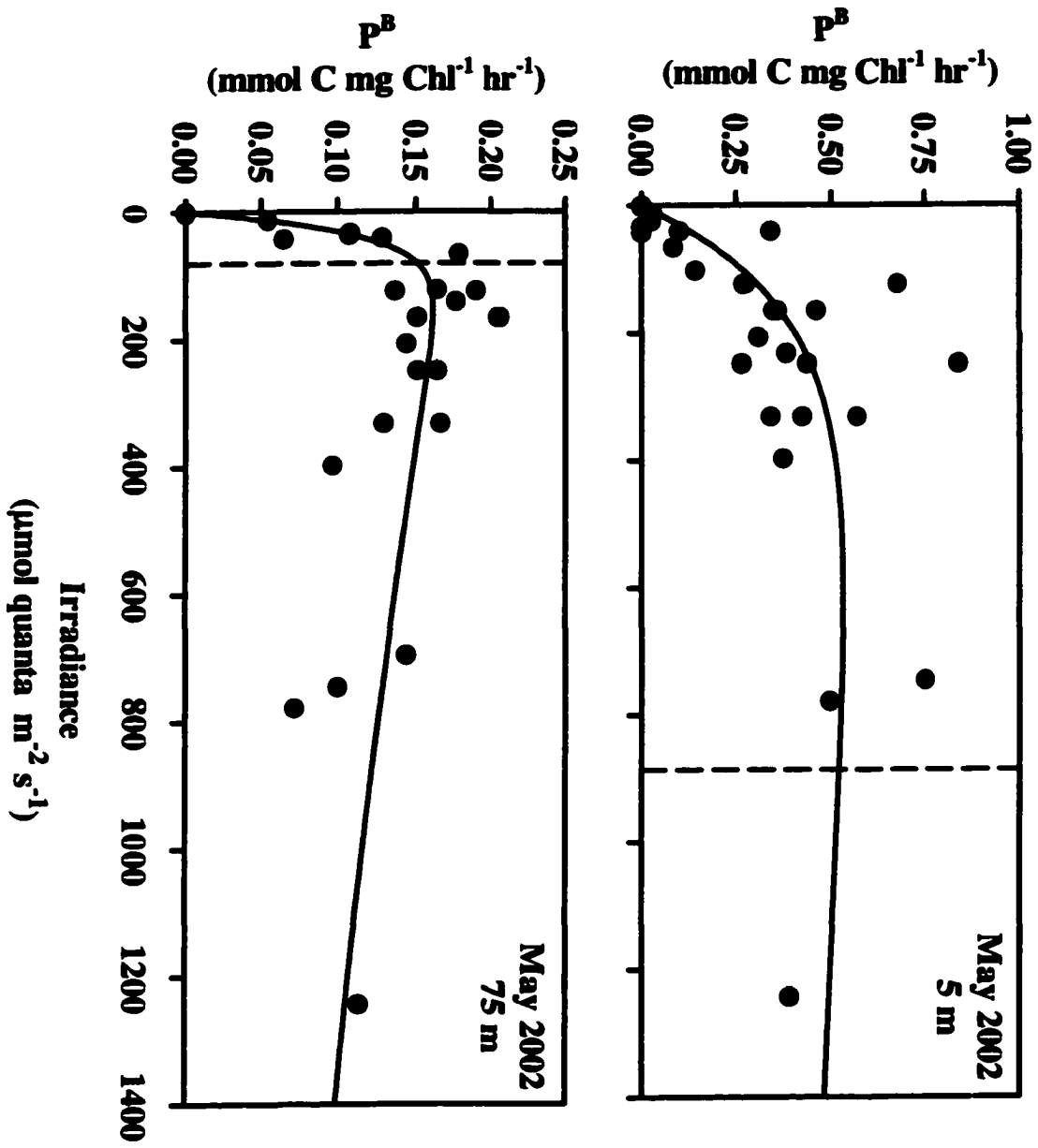
Table 4. Parameters describing photosynthesis as a function of irradiance at Station ALOHA. Top numbers are regression derived parameters, numbers in parentheses are standard errors of derived parameters. Parameters with similar letter designations are statistically indistinguishable at a P value of >0.05.

Cruise Depth (m)	α^a		β		$P^B_s{}^b$		$P^B_m{}^b$		$I_m{}^c$		$E_k{}^c$		R^2
May 2002													
5 m	0.0035	A	0.0001*	A	0.61	A	0.53	A	616	A	151	A	0.64
	(0.0009)		(0.0002)		(0.19)		(0.24)		(158)		(79)		
75 m	0.0048	A	0.0005	B	0.17	B	0.12	A	86	B	25	A	0.77
	(0.0011)		(0.0001)		(0.0013)		(0.030)		(19)		(8.5)		

[a] mmol C mg Chl^{-1} hr^{-1} (μmol quanta m^{-2})$^{-1}$ s^{-1} [b] mmol C mg Chl^{-1} hr^{-1} [c] μmol quanta m^{-2} s^{-1}

* not significant (P>0.05).

DISCUSSION

This is the first study to evaluate the response of heterotrophic bacterial protein production to PAR using short term photosynthetron experiments. Experiments were conducted in both the upper and lower photic zone, providing information on the relationship between protein production and PAR along the depth-dependent light gradient observed in the upper ocean. Experiments conducted in the upper photic zone were within the seasonal mixed layer, while those in the lower photic zone were in the more stratified thermocline.

The results of this study revealed two important features on the nature of light-enhanced HBP in the NPSG. First, the response of Leu incorporation to irradiance was notably similar to the more well-studied relationships between photosynthesis and irradiance, facilitating the use of photosynthetic models to describe the nature of the Leu-irradiance relationship. Second, the response of Leu incorporation to irradiance was similar in the upper and lower photic zone with one exception: unlike the lower photic zone, upper photic zone Leu incorporation did not appear susceptible to photoinhibition.

Six experiments were conducted on three different HOT cruises to assess how Leu incorporation rates varied as a function of irradiance. In addition, two experiments were conducted on one of the cruises in the upper and lower photic zone

to assess the photosynthetic response to irradiance. In all of the experiments Leu incorporation demonstrated significant photoenhancement. The nature of the response varied with depth and between cruises; in total, 41-68% of the variance in Leu was described by a model of Leu incorporation as an exponential function of light at low irradiance, with rates becoming photoinhibited at high light intensities. Generally, the Platt et al. (1980) model accounted for more of the variability in the Leu-E relationship in the upper photic zone (R^2 ranged 50-68%), than in the lower photic zone (R^2 ranged 41-54%). The inability to account for more than 68% of the variance in the Leu-E response led to relatively large error estimates associated with α, Leu_D, and Leu_S. As a result, no significant differences in these parameters were found between experiments conducted in the upper and lower photic zone.

The response of Leu incorporation to light

The rate at which Leu was incorporated into bacterial proteins under varying irradiance was successfully modeled using equation 4. This modification of the Platt et al. (1980) model has also been used to describe the response of NO_3^- and NH_4^+ uptake by phytoplankton to irradiance (Priscu 1989), and the relationship between fixation of atmospheric nitrogen by cyanobacteria to irradiance (Lewis and Levine 1984). The model was more successful in describing the variability in the Leu-E response in the upper photic zone than in the lower photic zone (Table 2).

Based on the experiments conducted in this study, heterotrophic protein production rates in the upper and lower photic zone at Station ALOHA responded in a similar manner to irradiance. These results are somewhat surprising given that the

upper photic zone experiments were conducted in the upper mixed layer of the water column, while the lower photic zone rates were in the physically stable top of the thermocline. Moreover, differences in *in situ* light fluxes between the mixed layer and lower photic zone were more than an order of magnitude lower in the deeper photic zone. Given these conditions, it is somewhat surprising that there were no observable differences in Leu_S, E_k, I_m, or α among the experiments conducted in the upper and lower photic zone. The one parameter which did vary between the upper and lower photic zone was β. In two of the three experiments, Leu incorporation in the lower photic zone displayed significant photoinhibition at light fluxes between 18-94 μmol quanta m^{-2} s^{-1}, while Leu rates in the upper photic zone never demonstrated significant photoinhibition. These results suggest that heterotrophic protein production rates in the upper photic zone were less susceptible to higher light fluxes and the bacterial groups demonstrating photoenhanced Leu incorporation were photoadapted to the ambient light fluxes.

Also somewhat surprising was the lack of difference in α, I_m, and Ek between the cruises sampled during this study. Noon-time PAR fluxes varied between 300-887 μmol quanta m^{-2} s^{-1} in the upper photic zone (Table 1), but despite these large changes in PAR intensity, no significant differences were found between α, I_m, and E_k among the various experiments conducted in this study. Im in the upper ocean varied less than two-fold between cruises (~180-300 μmol quanta m^{-2} s^{-1}), and E_k was relatively constant. These results suggest that at least during the three months of this study, the photic zone phototrophic bacterial assemblage response to irradiance varied very little.

Photophysiological acclimation in the NPSG

A number of marine phototrophic picoplankton demonstrate photophysiological adaptations to response to depth-varying light fluxes in the upper ocean. For example, although *Prochlorococcus* and *Synechococcus* are genetically similar (96% identical 16S rDNA sequences), these cyanobacteria utilize dissimilar light-harvesting strategies and have different physiological responses to light and nutrients (Moore et al. 1995, Urbach et al. 1998, Rocap et al. 2002). At Station ALOHA, *Prochlorococcus* abundance in the upper 100 m of the water column is roughly two orders of magnitude greater than *Synechococcus*. High-light adapted *Prochlorococcus* isolates (surface water) have been grown in light fluxes high enough to completely inhibit growth of the deeper photic zone populations (Moore et al. 1998). *Synechococcus* populations appear capable of adjusting their utilization of spectral light energy to match the available light spectra of the environment (Palenik 2002).

Short-term photoacclimation likely provides a competitive advantage for populations in the upper mixed layer where the available light energy varies depending on the depth of mixing. Similarly, rhodopsin-containing photoheterotrophic bacteria appear to tune their light harvesting protein-pigments to maximize the spectral energy available along the depth-dependent gradient in the upper photic zone (Béjà et al. 2000). The results of the Leu-E experiments demonstrate that the response of bacterial protein production to light was similar in the upper and lower photic zone at Station ALOHA; however, deep photic zone

production appeared more responsive to higher light fluxes than production in the upper photic zone.

Photosynthetic production and irradiance

Previous investigations into the relationship between photosynthesis and irradiance in the NPSG found that upper ocean P^B_{max} values were two to ten-fold greater than estimates from the lower photic zone (Ondrusek et al. 2001); the P-E experiment conducted during the present study revealed no significant differences in P^B_{max} in the upper and lower photic zone (Table 3). The results of the P-E experiment conducted in May (Figure 7) resemble laboratory studies on the photosynthetic responses of *Prochlorococcus* and *Synechococcus* to irradiance. P-E relationships of high- and low-light adapted isolates of *Prochlorococcus* typically demonstrate rapid increases in photosynthesis at low light fluxes, becoming saturated at ~200 µmol quanta m^{-2} s^{-1} (Moore et al. 1995, 1998). In contrast, photosynthetic rates in low light adapted *Prochlorococcus* strains saturate at much lower light fluxes (30-50 µmol quanta m^{-2} s^{-1}) and become completely inhibited at light fluxes greater than ~140 µmol quanta m^{-2} s^{-1} (Moore et al. 1995, 1998). *Synechococcus* isolates appear to require higher light fluxes for optimal growth (~140 µmol quanta m^{-2} s^{-1}) often exhibiting photoinhibition at relatively high light fluxes (>480 µmol quanta m^{-2} s^{-1}) (Kana and Gilbert 1987, Moore et al. 1995).

Primary production at Station ALOHA is dominated by the photosynthetic production of *Prochlorococcus* (Campbell and Valout 1993, Karl 1999). The P-E responses observed in the upper water column are consistent with those expected for

high-light adapted *Prochlorococcus* and *Synechococcus*, but in the lower photic zone,

the P-E relationship appears more consistent with low-light adapted *Prochlorococcus*

populations.

There were a few unique differences in the nature of the P-E relationship

relative to the Leu-irradiance responses. Although the P-E relationship was only

determined on one of the three cruises sampled during this study, both the optimal

and saturating light fluxes of Leu incorporation in the lower photic zone were greater

than those determined for photosynthesis. In addition, Leu incorporation increased

sharply at low light intensities, suggesting heterotrophic protein production may have

been more responsive at low light fluxes than photosynthesis. Leu incorporation rates

tended to increase rapidly under dim light, and saturate at light fluxes photoinhibitory

to photosynthesis.

To compare how *in situ* Leu and photosynthesis rates differed in their

response to light, the *in situ* Leu_L rates and photosynthesis rates were plotted against

the measured daily light fluxes (Figure 8). Photic zone *in situ* profiles of Leu_L and

Leu_D rates were measured on nine different cruises to Station ALOHA between

February 2000 and May 2002. The results were fitted with equation 4 and with

equation 8. Generally, both the modified Monod and the Platt et al. (1980) models

accounted for equal amounts of the variance in the measured Leu and photosynthesis

rates (Figure 8).

In situ Leu incorporation generally appeared more responsive to low light

fluxes than photosynthesis. Maximal Leu rates were ~640 pmol L^{-1} d^{-1}, saturating at

Figure 8. Relationships between *in situ* daily Leu incorporation rates and daily PAR

fluxes (E) on nine different HOT cruises from March 2000-May 2002. Leu

incorporation measured in the light (Leu$_L$), photoenhanced Leu incorporation rates

(ΔLeu=Leu$_L$-Leu$_D$), and photosynthetic production (PP). Lines are the result of fitting

relationships to equation 4 (solid lines) and equation 8 (dashed lines) for Leu rates,

and equation 2 (dashed line), and 3 (solid line) for PP. The equations for the various

relationships are:

a.) Leu = 570*(1-e$^{-1.02E}$) + 68, r^2=0.64, P<0.0001 (solid line);

Leu = [638*E / (0.58 + E)] + 33, r^2= 0.64, P<0.0001 (dashed line).

b.) Leu = 216*(1-e$^{-1.45E}$) + 24, r^2=0.37, P<0.0001 (solid line);

Leu = [243*E / (0.37 + E)] + 6.3, r^2 = 0.35, P<0.0001 (dashed line).

c.) PP = 646*(1-e$^{-0.23E}$), r^2=0.37, P<0.0001 (solid line)

PP = [739*E / (3.5 + E)], r^2=0.38, P<0.0001 (dashed line).
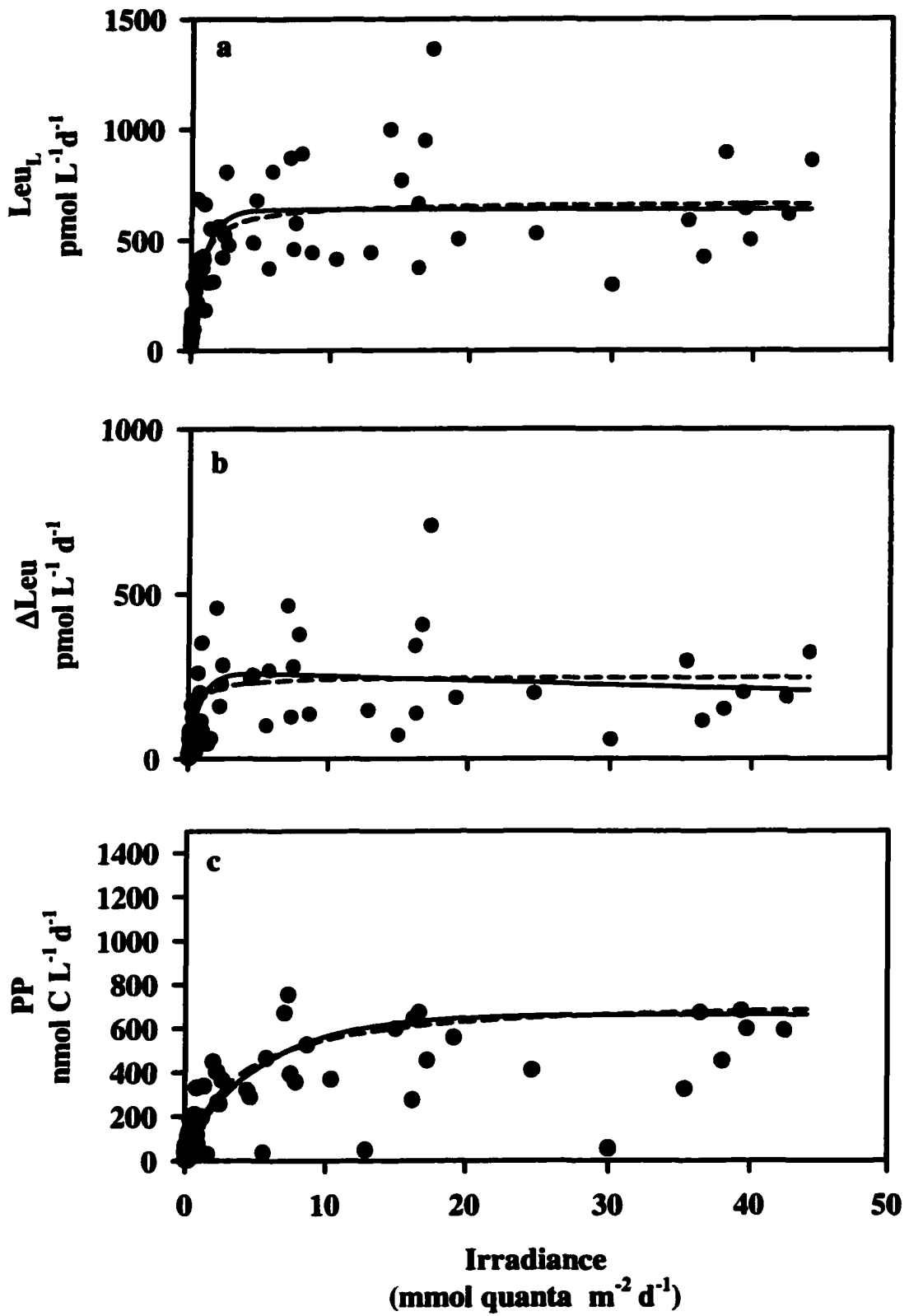
light fluxes of ~1 mol quanta m^{-2} d^{-1}. ΔLeu (Leu$_L$-Leu$_D$) rates also increased rapidly

at low light fluxes, and saturated at similar light fluxes as Leu$_L$. In contrast,

photosynthesis demonstrated a more gradual increase at low light fluxes typically

saturating ~10 mol quanta m^{-2} d^{-1}. Increases in photosynthesis were proportionally

greater than the photoenhanced bacterial production, implying that the relationship

between heterotrophic production and irradiance was fundamentally different than the

photosynthetic response to light.

Possible mechanisms of photoenhanced Leu incorporation

Vertical profiles of Leu incorporation often revealed photostimulation

throughout the upper 125 m of the water column (Figure 3). Although the

mechanisms resulting in the photoenhancement remain unclear, there are several

processes that should be considered. Leu incorporation rates would be expected to

increase in response to sunlight if phototrophic bacterial growth increased in direct

response to light-energy. Bacterial populations that may demonstrate direct light-

stimulated protein production include the AAnP, SAR86-rohodpsin containing

bacteria, and *Prochlorococcus*.

Alternatively, several abiotic photochemical processes could exert also

indirect influence on heterotrophic production. Photoproduction of labile DOM by

UV light has been observed in a number of studies (Kieber et al. 1989, Miller and

Moran 1997); in addition, studies have described UV photoproduction of ammonium

and amino acids from dissolved humic material (Bushaw et al. 1996, Bushaw-Newton

and Moran 1999). While these abiotic photochemical changes to nutrients and DOM

are likely to influence heterotrophic production in the upper ocean at Station

ALOHA, their influence on the response of Leu to irradiance in the experiments

described here should be minimal. All of the experiments conducted in these

experiments were conducted in polycarbonate, thereby eliminating the influence of

UV light on bacterial growth in these experiments. Moreover, the nature of the Leu-E

response observed in these experiments appears inconsistent with abiotic

photochemical modification of organic and inorganic nutrients. If photochemical

transformation of DOM was responsible for the observed effects, photoproduction of

labile nutrient sources would presumably resemble the non-linear response observed

in the Leu-E experiments. The nature of the response of Leu-E observed in these

experiments more likely stemmed from direct light stimulation of heterotrophic

protein production, or resulted from an indirect coupling between photoautotrophic

and heterotrophic production.

The similarities between the P-E and Leu-E responses suggest that

heterotrophic and photosynthetic productivity in the upper ocean are coupled. Such

coupling may result from a tight temporal dependence of heterotrophic production on

photosynthetic production, or could result from direct incorporation of DOM (such as

Leu) by facultative photoautotrophic bacteria like *Prochlorococcus*. Photosynthetic

production and phototrophic bacterial cell division in tropical and subtropical

ecosystems demonstrate clear diel dependence (Gasol et al. 1998, Binder and Durand

2002). Morán et al. (2001) conducted experiments on the response of Leu

incorporation to a range of irradiances (0-1500 μmol quanta m^{-2} s^{-1}) in the

Mediterranean Sea and in the North Atlantic, finding that Leu incorporation tended to

increase with increasing irradiance; however, Leu rates were maximal in samples incubated in the dark. In contrast to Morán et al. (2001), Leu incorporation in the NPSG consistently demonstrated a 1.2-1.9-fold photostimulation relative to dark controls.

If the response of Leu incorporation to irradiance reflected a coupling between photosynthetic DOM production and heterotrophic consumption, then the relationship between gross DOM production (the subsidy of heterotrophic production) and irradiance would be expected to resemble the response of Leu incorporation to irradiance. In a study of dissolved organic carbon (DOC) production at Station ALOHA, Karl et al. (1998) measured peak rates of net $DO^{14}C$ production in the upper photic zone, decreasing with depth similar to PP. Rates of $DO^{14}C$ production were approximately equivalent to PP below ~75 m (Karl et al. 1998). Morán and Estrada (2001) conducted a series of P-E experiments in the oligotrophic western Mediterranean Sea and found that $DO^{14}C$ production and PP differed in their responses to irradiance; in their study, $DO^{14}C$ production was relatively constant over a range of irradiances spanning 0-1500 μmol quanta m^{-2} s^{-1}, while PP was described by the Platt et al. (1980) model. The response of $DO^{14}C$ to irradiance is unknown in the NPSG; however, if the response was similar to responses observed in the Mediterranean, it appears unlikely that the Leu-E results were driven solely by heterotrophic utilization of photosynthetic exudates.

The observed response of Leu incorporation to irradiance rates may be better described by a combination of direct light-stimulated protein production and tight microbial recycling of DOM. Both *Prochlorococcus* and *Synechococcus* have been

shown to utilize nanomolar concentrations of amino acids (Rippka 1973, Cuhel and

Waterbury 1984, Paerl 1991, Berman and Chava 1999, Kamjunke and Jahnichen

2000, Zubkov et al. 2003). The incorporation of Leu by these photosynthetic bacteria

may have substantial ecological and biogeochemical consequences. *Prochlorococcus*

is estimated to be the most abundant photosynthetic organism in the ocean (Partensky

et al. 1999), and *Prochlorococcus* dominates photoautotrophic production and

biomass in the NPSG (Campbell et al. 1993, Karl 1999). Utilization of DOM as a

nutritional subsidy by photic zone populations of *Prochlorococcus* would suggest that

photoautotrophic production in this ecosystem could be partly regulated by

heterotrophic utilization of DOM. Photoheterotrophic production by

Prochlorococcus may significantly impact upper ocean carbon cycling.

The results of Leu-E experiments suggest that if the light-stimulated Leu rates

are the result of mixotrophic growth by cyanobacterial populations in the upper

ocean, *Prochlorococcus* (rather than *Synechococcus*) likely exerted a greater

influence on Leu incorporation rates. $Leu_L:Leu_D$ ratios were largest near the base of

the photic zone (75-125 m) where *Prochlorococcus* abundance is relatively high. The

abundance of *Prochlorococcus* at Station ALOHA occasionally exceeds that of non-

chlorophyll containing, heterotrophic bacteria, and *Prochlorococcus* abundance is

typically two-orders of magnitude greater than the abundance of *Synechococcus*.

Irrespective of whether the photoenhancement of protein production rates resulted

from photoheterotrophic, mixotrophic, or coupled autotrophy and heterotrophy, its

potential impact on ecosystem productivity appears to be substantial and has gone

entirely overlooked in past studies of bacterial production in the open ocean.

By assessing the response of bacterial Leu incorporation to irradiance, this study has shown that light-energy directly controls the rate of bacterial protein production in the NPSG. Moreover, the dependence of heterotrophic Leu incorporation on irradiance appears to differ slightly in the upper and lower photic zone. Low light adapted phototrophic bacteria appear somewhat more susceptible to photoinhibition than cell in the upper mixed layer of the water column. Irrespective of the phototrophic groups responsible for the observed response to irradiance, this study has shown that Leu incorporation exhibits a response to irradiance similar in form to photosynthesis and therefore may be described using models developed for P-E relationships. Use of these models provided the first quantitative look at the impact of sunlight on heterotrophic productivity.

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SECTION IV

MULTI-YEAR INCREASES IN DISSOLVED ORGANIC MATTER INVENTORIES AT STATION ALOHA IN THE NORTH PACIFIC SUBTROPICAL GYRE[†]

ABSTRACT

The inventories and dynamics of dissolved organic matter (DOM) in the surface water at Station ALOHA were analyzed from the Hawaii Ocean Time-series (HOT) data set for the period 1989-1999. Euphotic-zone, depth-integrated (0-175 m) concentrations of dissolved organic carbon (DOC), nitrogen (DON), and phosphorus (DOP) were temporally variable. In particular, during the period 1993-1999, concentrations of DOC and DON increased while inventories of DOP remained unchanged. DOC inventories increased by 303 mmol C m^{-2} yr^{-1}, a value equivalent to approximately 2% of measured primary production (^{14}C method) at this site. DON increased at 11 mmol N m^{-2} yr^{-1}, resulting in a mean molar DOC:DON ratio of 27.5 for the accumulated DOM. Accumulation of DOC and DON without corresponding accumulation of DOP resulted in changes to the bulk organic C:N:P stoichiometry; bulk DOC:DOP ratios increased 16% and DON:DOP ratios increased by 17%. These results indicate that a small fraction of the annually produced organic matter escaped biological utilization on time scales of months to years. More importantly, the accumulated DOM inventories grew progressively enriched in C and N relative to P. Fundamental changes in the North Pacific Subtropical Gyre (NPSG) habitat appear to have altered microbial processes that regulate organic matter fluxes. Considered together, the long-term increases in DOC and DON inventories are consistent with

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previous observations indicating that a recent reorganization of plankton community

dynamics may have altered organic matter cycling in this ecosystem.

INTRODUCTION

Dissolved organic matter (DOM) in seawater constitutes one of the largest exchangeable pools of reduced carbon on Earth so accurate quantification of DOM inventories and fluxes is essential for understanding oceanic carbon cycling (Carlson et al. 1994, Ducklow et al. 1995, Williams 1995). Efforts to quantify carbon fluxes in oligotrophic marine ecosystems indicate that significant fractions of organic carbon may cycle through DOM pools (Carlson et al. 1994, Emerson et al. 1997, Carlson et al. 1998). At Station ALOHA in the North Pacific Subtropical Gyre (NPSG), the dissolved carbon (C), nitrogen (N) and phosphorus (P) inventories in the photic zone represent 98, 95 and 94% of the total organic matter inventories, respectively.

Three primary classes of bulk DOM have been defined based on their temporal lability in the marine environment: (1) labile DOM with turnover times of hours to days, (2) semi-labile DOM that turns over on seasonal to annual time scales, and (3) non-labile DOM inventories that cycle on time scales of hundreds to thousands of years (Kirchman et al. 1993). The ultimate source of DOM in the open ocean is marine primary production so across finite time and space scales DOM production should covary with primary production. However, the exact pathways of DOM production and utilization are still poorly constrained (Ducklow and Carlson 1992). DOM is an operationally defined term used here to describe measured pools

of organic carbon, nitrogen, and phosphorus that pass through a microfine glass fiber filter (nominal pore size of 0.7 μm).

Investigations in the oligotrophic NPSG indicate that recent changes in the autotrophic community structure may have significantly altered the cycling of organic matter in this ecosystem. In particular, the phytoplankton community, once dominated by eukaryotes, appears to have shifted to a phototrophic community dominated by smaller, prokaryotic cells such as *Prochlorococcus* and *Synechococcus*. Karl (1999) describes this reorganization of the phototrophic community from eukaryotes to prokaryotes as a "domain shift". One result of this shift is an apparent doubling of both chlorophyll *a* (Chl *a*) inventories and rates of ^{14}C-primary production (Venrick et al. 1987, Karl 1999, Karl et al. 2001a). In addition, rates of nitrogen fixation and abundance of nitrogen fixing microorganisms are hypothesized to have increased in the past decade, potentially altering the elemental stoichiometry of dissolved and particulate matter inventories in the NPSG (Karl et al. 1995, 1997). Determining the impacts of such an ecological reorganization on organic matter inventories is crucial to assessing how community structure and nutrient cycling define the magnitude of carbon export and sequestration in oceanic ecosystems.

This paper examines the variability of DOM in the surface ocean of the NPSG between 1989-1999. We focus our temporal analyses on the latter period of observations (1993-1999), where profound changes in DOM pool dynamics may reflect the reorganization of the NPSG food web. During this period, the bulk DOM pool underwent significant alteration of varying amplitude and periodicity. Such

alterations appear consistent with previously hypothesized changes in microbial

community dynamics.

MATERIALS AND METHODS

Station Location and Sample Collections

All data were collected at Station ALOHA (22° 45' N 158° 00' W)

approximately 100 km north of Oahu as part of the HOT program. The complete data

sets are available through the HOT World Wide Web site *(http://hahana.soest.hawaii*

edu/hot/ hot_jgofs.html). Water samples for DON and DOP determinations were

collected at approximately monthly intervals from October 1988 to December 1999,

while samples for DOC were collected between January 1993 and December 1999.

Comparative analyses of DOM inventories therefore focus on HOT cruises 44-110

(1993-1999), because these cruises provided complementary measurements of C, N

and P pool dynamics.

Water samples were collected from discrete depths using a 24-bottle CTD

rosette sampler (Karl et al. 2001b), with intensive sampling in the upper 200 m

corresponding to the depths of primary production measurements. For this

investigation, spatial analyses focus on the upper 175 m of the water column. We

define this region as the euphotic zone (EZ), as it roughly corresponds to the depth of

~0.05% surface irradiance (175 m) and below which there is, on average, no net

autotrophic production (Letelier et al. 1996).

Measurements

Descriptions of the methods used in this study can be found in Karl et al.

(2001b) and Hebel and Karl (2001) and are also described in the electronic version of

the "HOT Laboratory and Field Protocols" manual

(http://hahana.soest.hawaii.edu/hot/protocols/protocol.html). Analyses of nitrogen

and phosphorus included total dissolved (TDN, TDP), inorganic (nitrate+nitrite

(N+N), soluble reactive phosphorus (SRP)) and particulate nitrogen and phosphorus

(PN, PP) inventories. DON and DOP were calculated by difference (i.e. DON = TN

– [N+N + PN] and DOP = TP – [SRP + PP]). DOC was calculated as the difference

between TOC and particulate carbon (PC). Ambient EZ inventories of N+N and SRP

reported in Table 1 were derived by the chemiluminescent method of Garside (1982)

and by the MAGIC method described by Karl and Tien (1992), respectively.

Seawater for TOC analyses was subsampled directly into clean, sterile 15 ml

polypropylene tubes (Corning #430052). Samples were immediately frozen (-20°C)

until analyzed in the laboratory. Upon return to the laboratory, TOC samples were

thawed to room temperature, shaken, and acidified with H_3PO_4 (final pH 2.0-2.5).

Samples were purged for ~10 minutes using high-purity oxygen to remove inorganic

carbon. One hundred microliters of sample were injected into the instrument port.

Between 1993-1996, TOC concentrations were measured using a commercially

available Ionics Model 555 TOC analyzer modified with a LICOR Model 6252

infrared detector after removal of total inorganic carbon. Organic matter oxidation to

CO_2 was facilitated by platinum catalysis. Instrument precision (based on the

standard deviation of 3000 m TOC measurements for the 1993-1996 period) was ± 4

μM C. Beginning in January of 1997, TOC concentrations were measured using a commercially available MQ Model 1001 TOC analyzer equipped with the same infrared detector. Similarly, analytical precision of the MQ instrument was ± 4 μM C. Analysis of deep-sea (3000 m) TOC concentrations during the period of transition (1996-1997) between the Ionics instrument and the MQ system revealed no significant baseline offset between the two instruments (Figure 1). Additional details of the operation of the Ionics and MQ instruments can be found in Tupas et al. (1994) and Qian and Mopper (1996), respectively.

Beginning in 1997, certified reference materials obtained from J. Sharp (University of Delaware) were analyzed and used for tracking instrument performance and assessing measurement accuracy. From 1993-1996, baseline blanks were determined by injection of UV-oxidized distilled water into the instrument; beginning in 1997, low-carbon water distributed from J. Sharp's laboratory served as the instrument blank. Blank values were subtracted from the measured seawater TOC values. Standards were prepared from either sucrose or phthalate stocks then analyzed with each sample run.

Complementary samples for PC, PN, and PP were collected during the same period (1989-1999) and the details of the sample collection and analyses can be found in Hebel and Karl (2001). Briefly, seawater was collected from the CTD rosette and subsampled into acid-cleaned polyethylene bottles. Samples were transferred through Tygon tubing containing a 202 μm screen mesh to exclude large particles. For PC and PN analyses, water was pressure filtered onto precombusted 25 mm glass fiber filters (Whatman GF/F). Variable volumes of seawater were filtered depending on

Figure 1. Concentration of TOC at Station ALOHA from 3000 m between 1996-

1998. Closed circles are measurements of TOC using the Ionics Model 555 TOC

analyzer; open triangles are measurements of TOC made using the MQ Model 1001

TOC analyzer. Solid line represents mean 3000 m TOC concentration, while dotted

lines are 1 standard deviation of the mean. No significant difference (ANOVA,

$p > 0.05$) in the mean 3000 m TOC concentration was observed, indicating comparable

analytical baselines between the two instruments.

Sampling Date
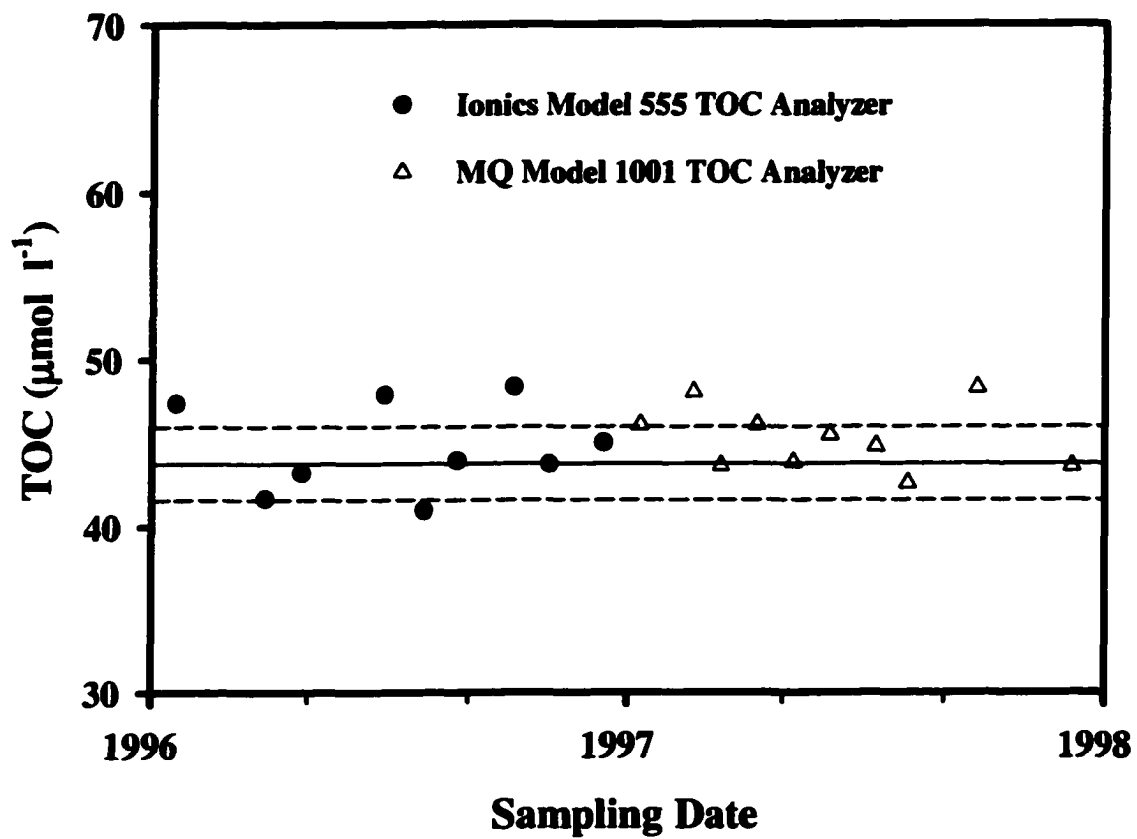
the depth sampled (typically 4 liters in the upper 150 m and 10 liters from water >150 m). After filtration, filters were placed onto 2.5 cm^2 sections of combusted aluminum foil and stored at -20^oC until analyzed in the laboratory by high temperature combustion using commercially available instruments (Hebel and Karl 2001). Unlike PC and PN analyses, GF/F filters used for PP analyses were combusted and HCl-rinsed to reduce the P blank. Following filtration, PP samples were placed in acid-rinsed 12 x 70 mm^2 glass test-tubes, covered with foil and stored frozen. Upon return to the laboratory, PP filters were combusted, acid-hydrolyzed, centrifuged and the supernatant subsampled for SRP analyses as described below.

DON was estimated by subtraction of PN plus N+N from TDN. Samples for DON were collected into 100 ml polyethylene bottles from the CTD sampling rosette and frozen until analysis. N+N samples were analyzed directly on a four-channel Technicon Autoanalyzer II (Armstrong et al. 1966). TDN samples were exposed to high intensity (1200 W) UV radiation for 24 hours at 84 ± 6 °C and analyzed for N+N and NH_4^+ using the Technicon autoanalyzer. At the concentrations reported in this paper, the precision of the TDN measurement is ±7%.

DOP was estimated by subtraction of SRP and PP from TDP. Samples DOP were collected in polyethylene bottles and stored frozen. Prior to analyses, samples were thawed and divided into subsamples for independent measurements of SRP and TDP. SRP was measured using the standard molybdenum blue assay (Murphy and Riley 1962) modified for the Technicon autoanalyzer II system. TDP concentrations were determined using UV-photooxidation of seawater for 2 hours followed by SRP autoanalysis of hydrolysis products using standard procedures (Armstrong et al.

1966). Calculating DOP by difference between TDP and SRP assumes a negligible

dissolved inorganic polyphosphate pool. At the concentrations measured in this

study, the precision of the TDP measurement is ±14%.

Bacterial cell abundance was enumerated by flow cytometry using Hoechst

33342 as the fluorochrome (Monger and Landry, 1993). Briefly, samples were

collected in 15 ml polypropylene centrifuge tubes, and then 1 ml was subsampled into

Cryovials (Corning) containing 0.02 ml of 10% paraformaldehyde (final

concentration 0.2%). The cryovials were quick frozen in liquid nitrogen and stored at

-80°C until analyzed. Prior to analysis, samples were thawed, cells were stained with

Hoechst 33342 for ~2 hours, and then counted using a Coulter EPICS dual laser (225

mW UV and 1 W 488 nm) flow cytometer. Non-pigmented, presumably

heterotrophic, *Bacteria* and *Archaea* were enumerated by stained DNA fluorescence

and forward angle light scattering (FALS), a proxy for cell size, using the software

CYTOPC (Vaulot 1989).

Data Analysis

Areal standing inventories were calculated for the 0-175 m depth range using

trapezoidal integration with linear interpolation between the discrete samples.

Statistical testing was performed using Minitab 4.0. Statistical comparisons were

based on one-way analysis of variance (ANOVA) and, for temporal trends, Model 1

linear regression analyses. The latter tests were performed on the depth integrated

DOM inventories versus time. All ANOVA statistical tests were based on integrated

values from the monthly observations rather than on seasonally or annually averaged

data. Significance was evaluated at the $p<0.05$ level.

RESULTS

Station ALOHA and the NPSG

The prominent physical, chemical and biological water column dynamics at Station ALOHA have been well characterized (see Karl 1999); selected data are summarized in Table 1. Surface water temperatures are perennially warm (>20°C) but seasonally variable. Inorganic major nutrients (NO_3^- and HPO_4^{2-}) are consistently low in the surface water (<0.1 μM), increasing at the top of the permanent nutricline (typically near the 150 m depth horizon) (Karl et al. 2001b). Phytoplankton abundance at Station ALOHA is dominated (>95% by cell number) by the prokaryotes *Prochlorococcus* and *Synechococcus* (Campbell and Vaulot 1993).

The annual integrated rates of primary production were not significantly different ($p > 0.05$, one-way ANOVA) between 1989-1999 with a mean value of 40 mmol C m^{-2} d^{-1} (Table 1). Interannual variability in primary production rates ranged between ~30-45 mmol C m^{-2} d^{-1}, similar in magnitude to seasonal variability. Chl. *a* concentrations always showed a subsurface peak between 100-120 m, with integrated EZ inventories ranging between ~17 and 26 mg Chl m^{-2} (Table 1). Heterotrophic bacterial abundance displayed no significant interannual or seasonal differences ($p > 0.05$, one-way ANOVA) (Table 2).

Concentrations of DOM in the upper 175 m of the water column were highest in the upper 50 m (DOC = 85-105 μM, DON = 4.5-6.2 μM, DOP = 0.15-0.27 μM),

Table 1. Mean Integrated Properties of the Euphotic Zone (0-175 m) at Station ALOHA (1989-1999)

Year	Temperature ($^\circ$C) [a]	$NO_3^- + NO_2^-$ (mmol m^{-2}) [b]	SRP (mmol P m^{-2})	Chlorophyll a (mg Chl a m^{-2})	Primary Production (mmol C m^{-2} d^{-1})
1989 (n=4) [c]	16.8-26.5	—	18.20	26.68	38.18
			7.25	5.44	19.16
1990 (n=8)	17.6-25.9	20.95	19.47	22.14	29.95
		28.58	3.13	7.10	6.89
1991 (n=9)	18.5-27.0	27.88	18.76	21.76	44.00
		12.18	6.86	1.55	9.84
1992 (n=9)	17.2-27.0	31.88	16.66	20.12	44.72
		10.53	4.93	2.55	8.56
1993 (n=6)	16.3-26.6	21.71	17.13	20.13	37.46
		13.05	7.58	4.85	13.80
1994 (n=8)	18.3-26.6	39.43	11.34	22.37	36.10
		18.69	4.08	3.61	8.49
1995 (n=9)	17.1-27.0	43.22	18.18	18.36	43.65
		34.22	6.40	2.46	14.27
1996 (n=10)	17.3-27.7	27.86	13.86	17.19	39.47
		9.34	4.94	3.63	9.83

Year					
1997 (n=9)	16.0-27.1	31.78 19.11	12.04 6.50	19.87 2.47	36.32 9.63
1998 (n=12)	17.3-26.0	36.44 16.32	12.50 3.48	22.44 2.59	42.24 12.55
1999 (n=10)	17.4-26.0	41.70 29.29	15.74 4.46	23.30 3.66	44.91 9.33

[a] Range of water column temperatures (0-175 m).

[b] Top numbers are mean integrated stocks; bottom numbers are standard deviations of the means.

[c] n indicates number of annual observations from which mean integrated stocks were calculated. Typically 8-10 depths were sampled between the surface and 175 m for each observation.

Table 2. Interannual Variation in Integrated (0-175 m) Organic Matter Stocks, Stoichiometric Ratios and Bacterial Abundance at Station ALOHA (1989-1999) [a]

Year	DOC (mmol C m^{-2})	DON (mmol C m^{-2})	DOP (mmol C m^{-2})	Bacteria (10^{11} cells m^{-2})	DOC : DON	DOC : DOP	DON : DOP
1989							
Mean	—[b]	873.6	30.0	—	—	—	29.8
SD		56.9	3.3				4.3
(n=9)[c]							
1990							
Mean	—	739.6	26.8	—	—	—	27.8
SD		103.5	2.4				4.2
(n=8)							
1991							
Mean	—	888.9	33.2	623.8	—	—	27.1
SD		69.7	5.4	87.8			3.2
(n=10)							
1992							
Mean	—	976.5	37.6	564.9	—	—	26.3
SD		27.7	4.2	125.5			3.1
(n=11)							
1993							
Mean	13636.2	924.6	33.7	659.4	14.8	408.6	27.6
SD	859.2	69.6	4.3	248.8	1.0	49.3	2.4
(n=6)							

1994							
Mean	14358.18	913.4	37.3	662.9	15.8	400.0	25.1
SD	1302.0	48.1	5.9	125.7	1.7	112.8	4.8
(n=9)							
1995							
Mean	14750.0	923.4	39.0	651.8	16.1	390.4	24.0
SD	1153.1	100.3	5.6	74.5	2.1	97.6	3.3
(n=9)							
1996							
Mean	15054.8	948.8	34.5	855.6	15.9	439.4	27.7
SD	1446.0	43.4	2.7	161.7	1.8	55.7	2.5
(n=10)							
1997							
Mean	15702.1	968.7	37.8	697.6	16.3	427.7	26.2
SD	942.5	82.3	6.7	134.9	1.8	80.4	3.7
(n=10)							
1998							
Mean	15414.2	999.5	32.5	556.7	15.4	483.4	31.4
SD	1012.3	41.2	4.9	122.4	0.9	78.4	4.9
(n=12)							
1999							
Mean	15621.3	954.5	35.2	645.3	16.4	478.8	29.2
SD	567.5	54.2	9.3	120.1	1.2	154.3	9.8
(n=10)							
Signif. [d]	p<0.001	p<0.001	p<0.001	ns	ns	ns	p<0.05

Regression [e]	DOC = 0.83x + 12200 r^2=0.25 p<0.0001	DON = 0.03x + 840 r^2=0.10 p<0.01	ns	ns	ns	DOC : DOP = 0.04x + 382 r^2=0.09 p<0.05	ns

[a] Values presented are mean annual integrated (0-175 m) inventories of DOC, DON and DOP. Numbers in parentheses are standard deviation of the mean integrated inventory.

[b] — indicates no data available

[c] n indicates number of annual observations from which mean integrated stocks were calculated. Typically 8-10 depths were sampled between the surface and 175 m for each observation.

[d] One-way ANOVA testing interannual differences in specified inventories and ratios. Statistical tests based on integrated inventories from each cruise grouped by year. Significance determined at p<0.05, ns indicates p>0.05.

[e] Model 1 linear regression where x is days since 01 January 1993. ns denotes regression not significant at p<0.05.

Figure 2. Concentration (μmol l^{-1}) versus depth (m) contour plots of: (top) dissolved

organic carbon (1993-1999), (center) dissolved organic nitrogen (1989-1999), and

(bottom) dissolved organic phosphorus (1989-1999), in the upper EZ at Station

ALOHA. The dark solid circles indicate depths and dates of sample collections.

Dissolved organic carbon (μmol l^{-1})

Dissolved organic nitrogen (μmol l^{-1})

Dissolved organic phosphorus (μmol l^{-1})
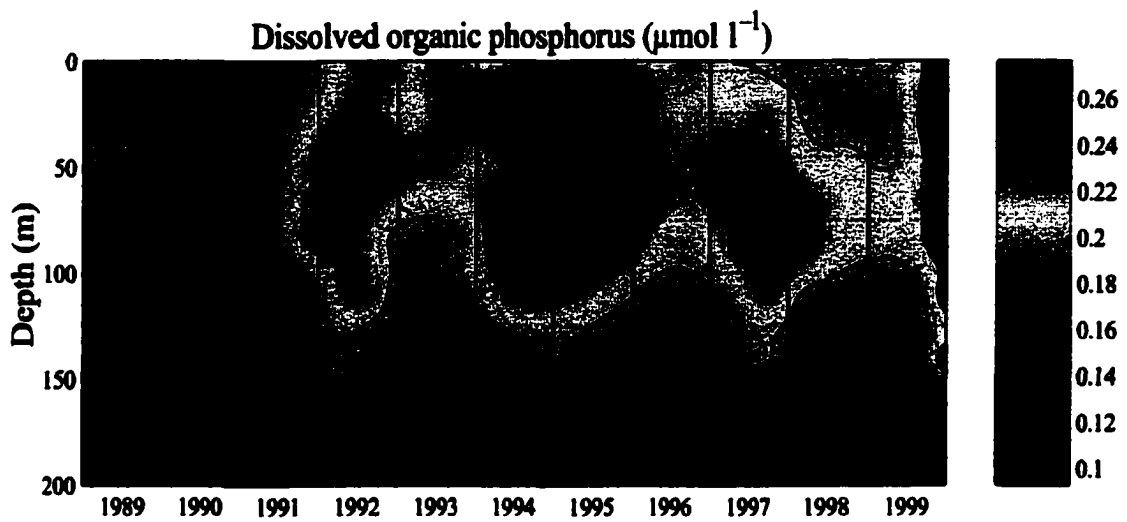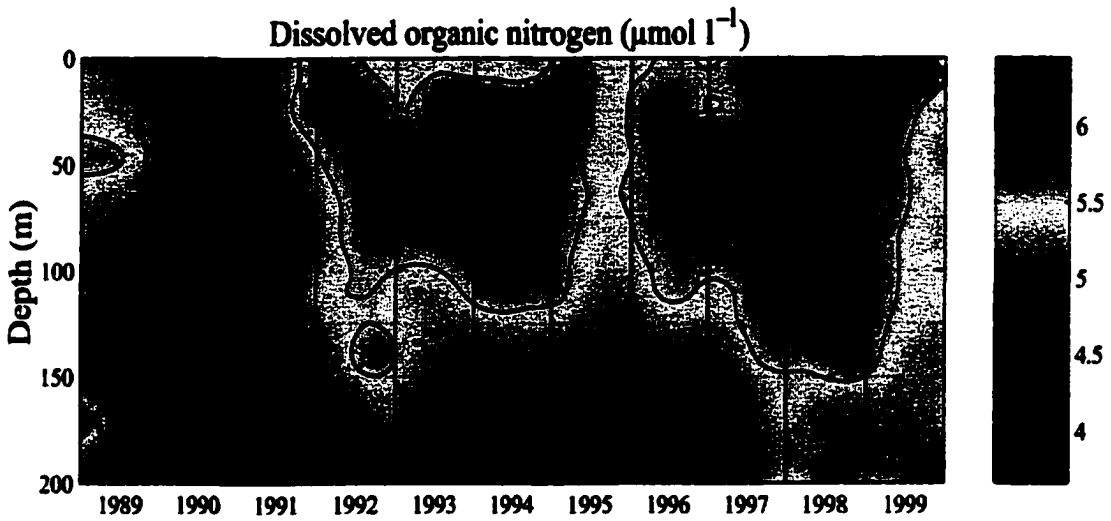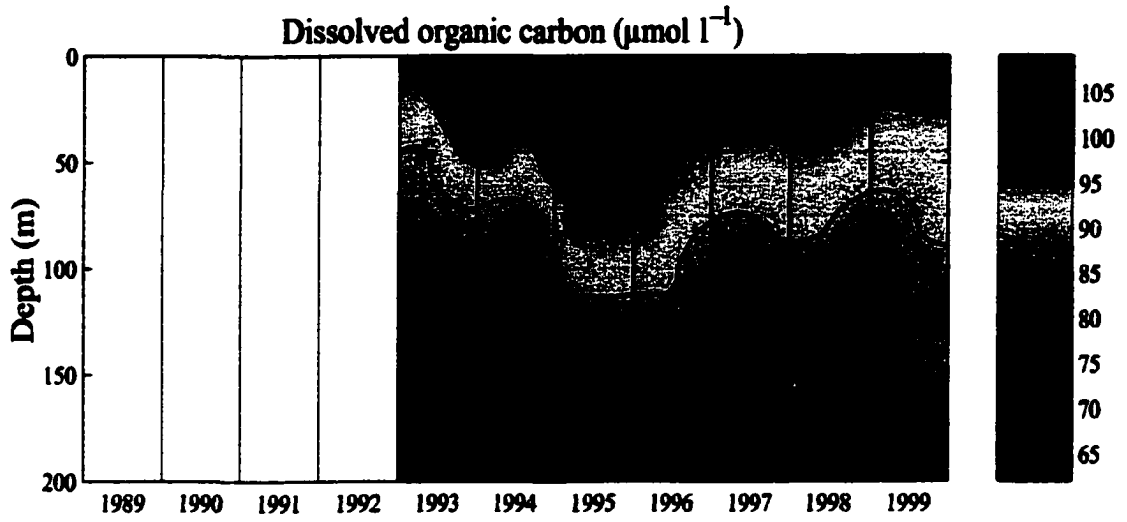
decreasing through the lower portion of the EZ (DOC = 63-93 µM, DON 3.7-6.1 µM,

DOP = 0.10-0.25 µM) (Figure 2). The mean concentration (i.e., standing inventories

divided by 175 m) of DOC in the EZ varied between ~71-98 µM, while DON and

DOP ranged between 3.6-6.5 µM and 0.11-0.30 µM, respectively (Figure 3, Table 2).

Seasonal and Interannual Variability

Integrated inventories of DOM displayed significant variability and several

consistent long-term trends (Figures 3, 4, Table 2). DOC, DON and DOP inventories

fluctuated by as much as 30% on an annual basis (Figure 2, 3, 4); however, no

significant seasonal differences were observed (one-way ANOVA, $p > 0.05$, Figure 3).

From 1993-1999, integrated DOC concentrations in the upper 175 m of the water

systematically increased at a rate of 303 mmol C yr^{-1}, resulting in a ~14% net increase

in EZ DOC inventories, and increasing the mean DOC concentration from ~78 to 89

µM (Figures 3, 4, Table 2). Although DOC inventories accumulated throughout the

entire EZ, the relative increases were most dramatic towards the base of the EZ

(Figure 2). For example, the mean concentration of DOC at the base of the EZ

increased nearly 30% between 1993-1999, compared to ~10% increase in the surface

waters (Figure 2).

DON inventories increased significantly ($p < 0.001$) throughout the decade

long period of observations (1989-1999) (Figure 2, 3c, 4). Consistent with DOC

inventories, no significant seasonal differences in DON inventories were observed

(one-way ANOVA, $p > 0.05$). Interannual increases in DON inventories led to a 15

mmol N m^{-2} yr^{-1} pooling of DON for the full 11-year data set and 11 mmol N m^{-2} yr^{-1}

Figure 3. Mean interannual changes in euphotic zone DOM concentrations (left panels) and seasonal climatology (right panels) based on full data sets. Boxes represent annual and seasonal (quarterly) divisions of DOM stocks. The line inside the box indicates the annual or seasonal mean, while the upper and lower boundaries of each box are the 75 and 25th percentiles, respectively. The capped bars show the 10 and 90th percentiles of the data. **All Seasons** is the total mean stock for the entire data set (DOC 1993-1998, DON and DOP 1989-1999) without seasonal divisions; seasonal classifications were defined as: **Spring**- March, April, May; **Summer**-June, July, August; **Fall**-September, October, November; and **Winter**-December, January, February. Mean EZ concentrations calculated as integral inventories divided by the 175 m depth interval.
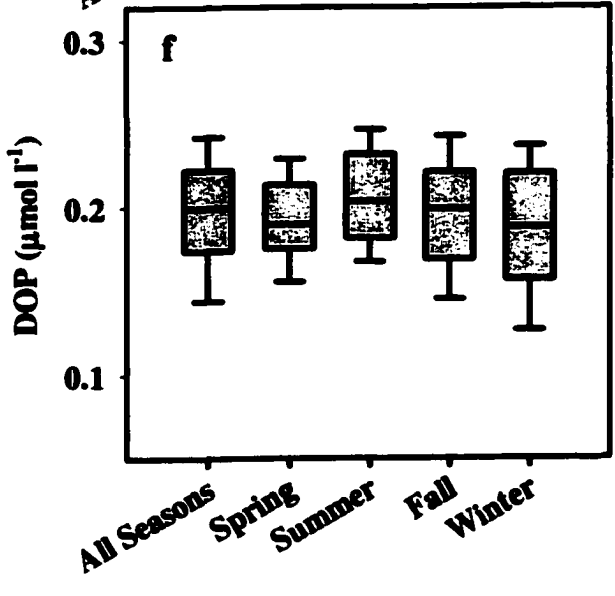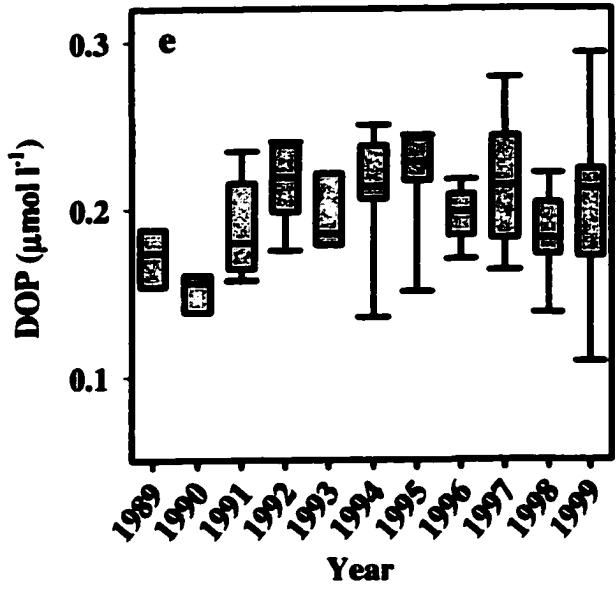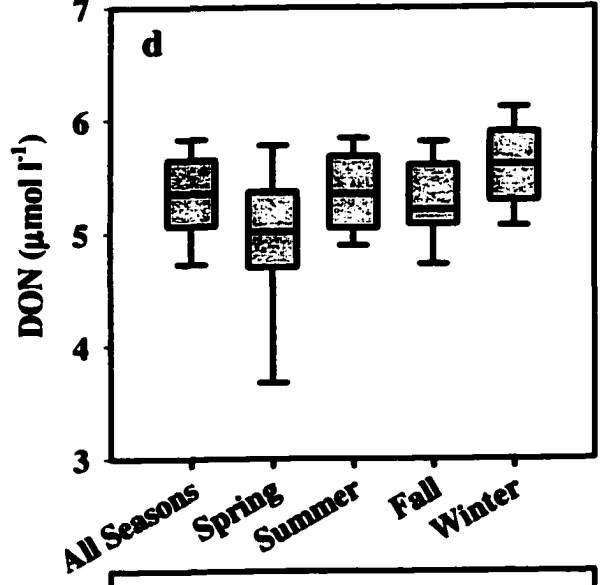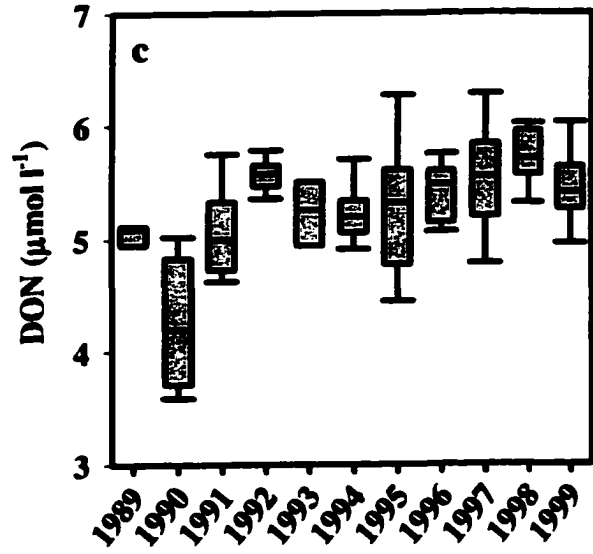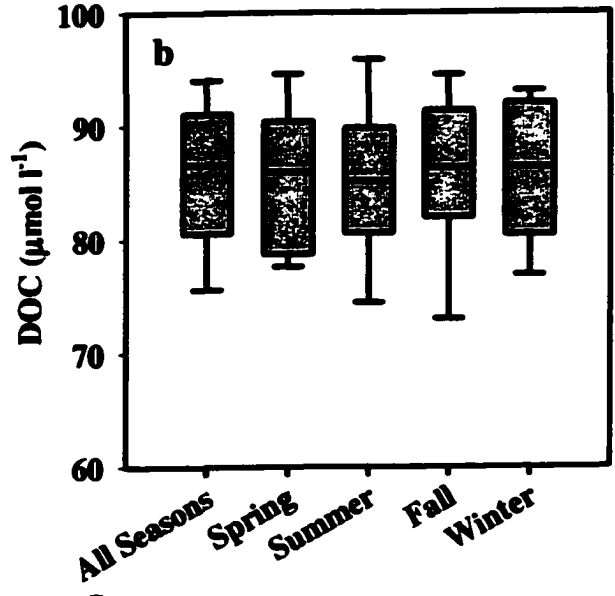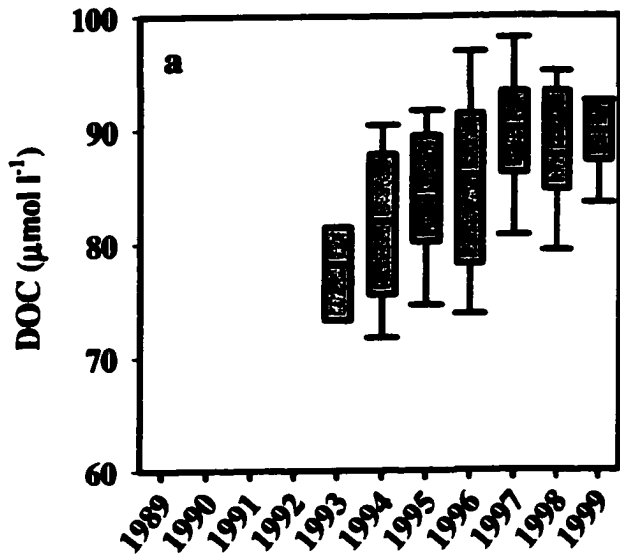
Figure 4. EZ (0-175 m) depth integrated DOM inventories at Station ALOHA.

Triangles are integrated inventories from each cruise; solid trend lines represent 3-point running mean of DOM inventories. a) Dissolved organic carbon, solid line is least squares Model 1 linear regression of cruise data (1993-1999). Equation describing least-squares linear regression (1993-1999) is shown on bottom of panel; x is days since 01 January 1993. b) Dissolved organic nitrogen, solid line is Model 1 linear regression of data (1993-1999) dashed line is Model 1 linear regression of data (1989-1999). Equation on the top of panel describes the regression line for the 1989-1999 period, while equation shown on the bottom of the panel describes the 1993-1999-time period. In both equations x is days since the beginning of the time series: 01 January 1989 (top equation) or 01 January 1993 (bottom equation). c) Dissolved organic phosphorus, regression lines and equations are same as above.
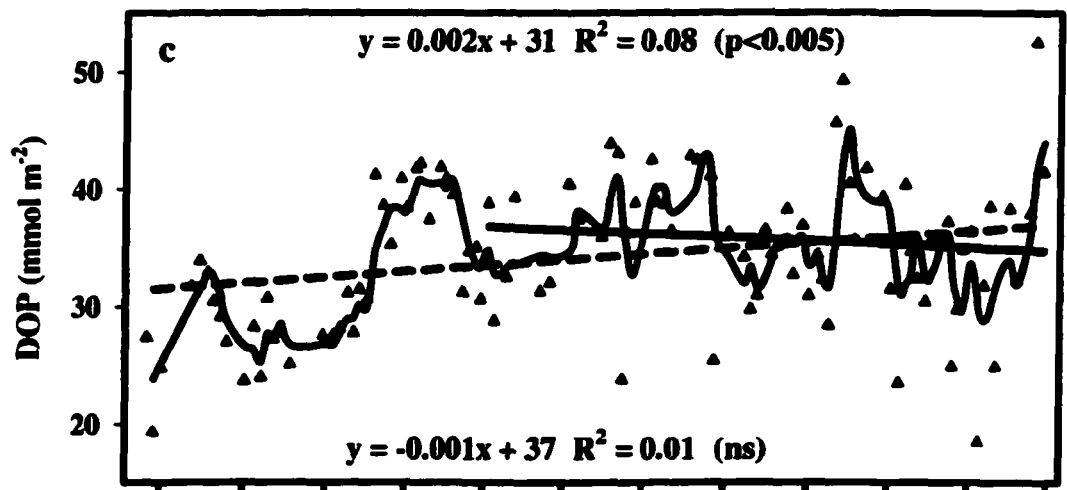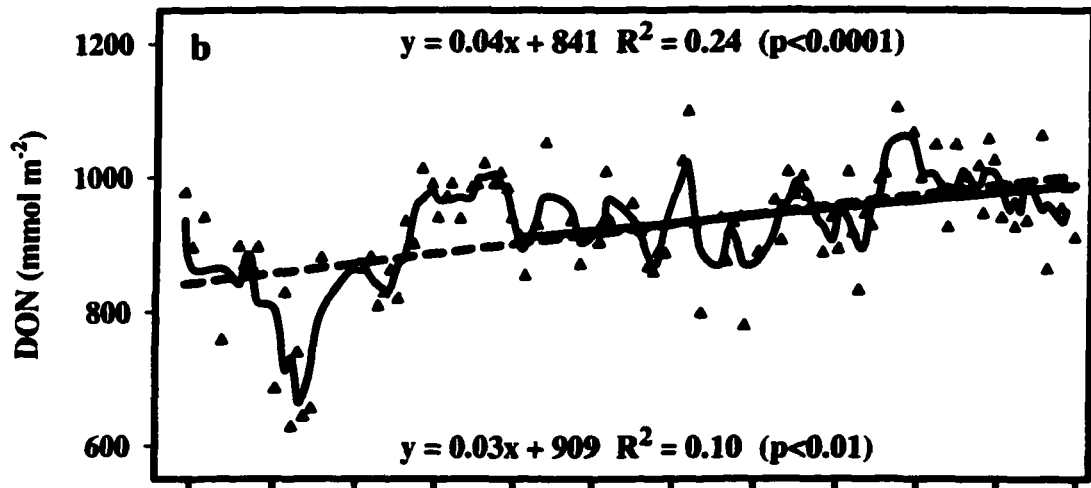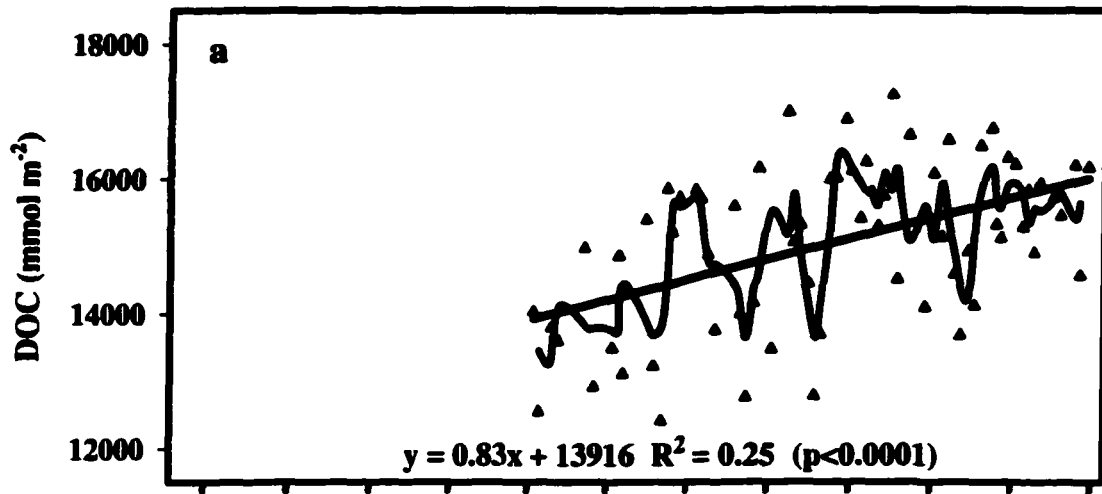
Sampling Date

accumulation for the 1993-1999 period. This consistent accumulation resulted in an overall increase in the bulk DON inventory of roughly 9% and 3%, respectively (Table 2). The 11-year mean integral DON inventory was 918 mmol N m^{-2}. A sharp decline in DON inventories was observed in 1990 when integrated DON inventories dipped to ~740 mmol N m^{-2}, the lowest value during the decade of observations (Figure 4, Table 2). A substantial increase in DON inventories was observed between 1990-1993, increasing inventories from ~740 mmol N m^{-2} to 924 mmol N m^{-2} (Figure 4, Table 2).

Similar to DOC and DON, DOP inventories displayed no significant seasonal trend (one-way ANOVA, p>0.05). DOP displayed a small increase between 1989-1999 at a rate of 0.73 mmol P m^{-2} yr^{-1} (Figure 3, 4). Similar to DON, the largest increase in DOP inventories occurred between 1990 and 1992 (Figure 3, 4). However, unlike DOC and DON inventories, DOP inventories remained relatively constant between 1993-1999, displaying a slight but insignificant decrease through this period (Figure 3, 4).

Variability in DOM stoichiometry

These systematic temporal changes in DOM inventories were reflected in changes in the stoichiometric ratios of the DOM inventories. The most consistent trend was a significant increase in the DOC:DOP ratio through time (Figure 4, Table 2). Increases in DOC enriched the bulk DOM pool in C, relative to P, by ~17%

Figure 5. Interannual changes in the EZ DOM stoichiometry a.) DOC:DON, b.)

DOC:DOP and c.)DON:DOP molar ratios of DOM inventories. Solid circles

represents mean ratio and error bars are standard deviation of the mean for each year

presented. No DOC data exist for the initial period of observation (1989-1992).
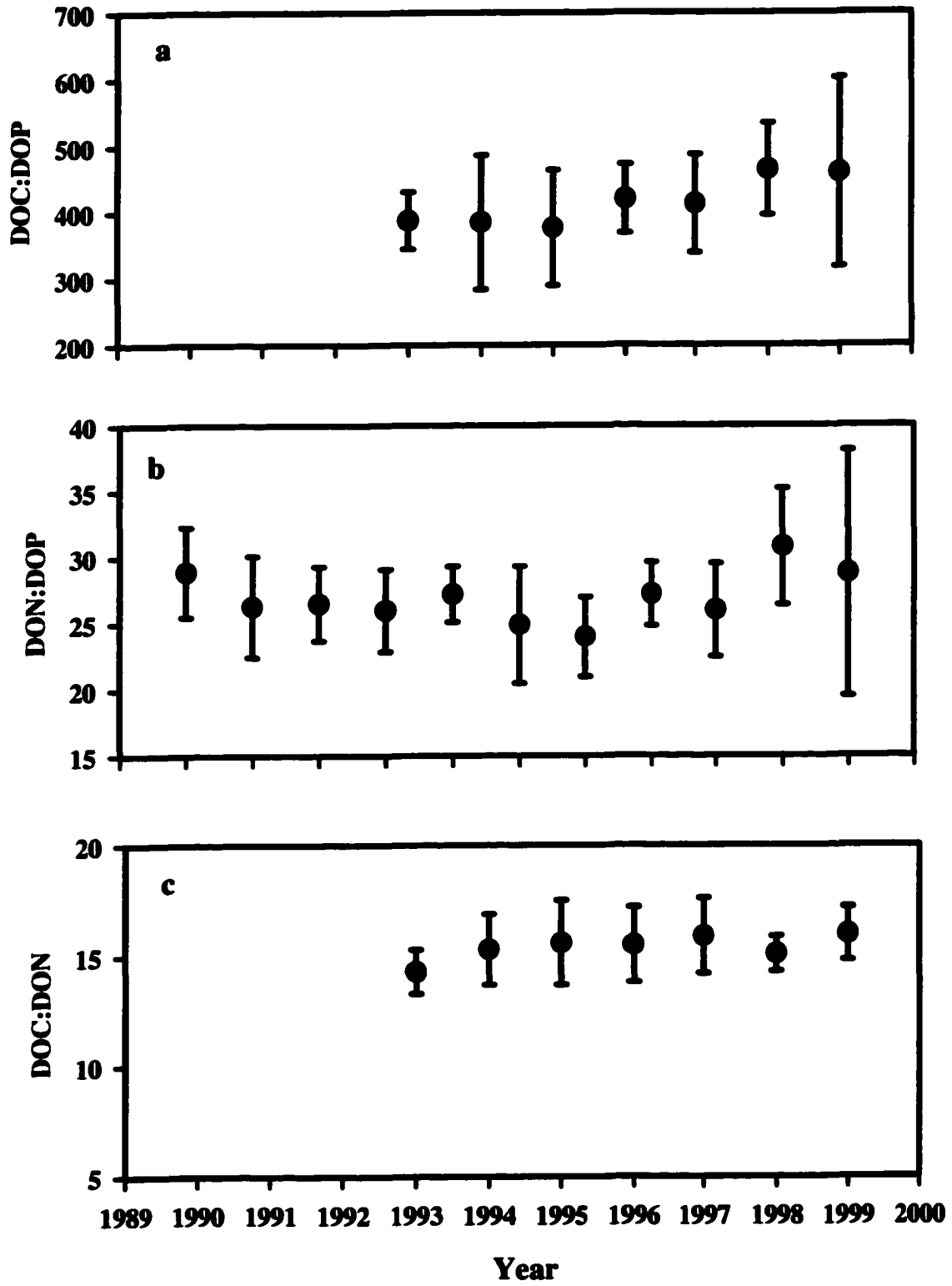
between 1993-1999 (Table 2). The bulk DOC:DOP molar ratios increased from 408 to 478:1 (Figure 5, Table 2). DON:DOP molar ratios also showed slight but insignificant increases from 28:1 in 1990 to 31:1 in 1998, while the bulk DOC:DON molar ratios remained relatively constant, ranging between ~15:1 to 16:1 (Table 2). Interannual accumulation of DOC and DON also resulted in changes to the C:N:P stoichiometry of the accumulated pools of DOM. The DOC:DON ratio of the accumulated DOM was ~66:1, while the DON:DOP ratio of the accumulated DOM was 15:1, and the resulting DOC:DOP ratio was 992:1.

DISCUSSION

DOM Cycling in the NPSG

Examining temporal variability in bulk DOM inventories has broad importance for our understanding of ocean biogeochemistry and microbial ecology. Temporal changes in the elemental composition of bulk DOM reflect alterations in the rates of production or utilization of organic matter in the marine environment. Our observations support previous studies suggesting that the NPSG has undergone fundamental changes in the past decade (Karl 1999, Karl et al. 2001a). In particular, we maintain that multi-year variability in DOM inventories implies that between 1989-1999, the NPSG was undergoing ecologically and biogeochemically important transitions. We argue these transitions may reflect the response of the planktonic community to enhanced nitrogen fixation and shifting phototrophic community composition.

Between 1993 and 1999 we observed a significant accumulation of DOC and DON with no corresponding increase in DOP. The resulting DOC:DOP and DON:DOP molar ratios of the bulk DOM were 478:1 and 29:1, respectively. Total net accumulation of DOC for the 7 year period between 1993-1999 was ~ 2100 mmol C m^{-2}, resulting in more than an 12 µM increase in DOC concentrations over the entire EZ (Figure 2, 3, 4, Table 2). The concentration of DON increased at 15 mmol N m^{-2} yr^{-1}, for a mean net increase of more than ~165 mmol m^{-2} over the eleven year period (Figure 4). The net accumulation of a C and N enriched DOM pool implies

non steady-state dynamics in production and utilization of organic matter in the surface ocean.

Studies examining DOC-production in the NPSG indicate that exudation of photosynthetically produced carbon may account for as much as 50% of the gross primary productivity of the NPSG (Karl et al. 1998). The high DOC production rates estimated from the NPSG may result from several factors acting individually or in concert. First, the predominance of small prokaryotes in the surface water phototrophic communities may result in high DOM production rates (Bjørnsen 1988, Hagström et al. 1988, Jumars et al. 1989). Secondly, DOM production due to nitrogen fixing microorganisms may account for a portion of the accumulated DOM, as both C- and N- rich DOM accumulated (Capone et al. 1994, Gilbert and Bronk 1994). Finally, diminishing inventories of bioavailable P could enhance DOM production while simultaneously decreasing DOC and DON utilization. Based on our analysis of DOM cycling in the NPSG, the processes governing accumulation of DOC and DON appear to operate over multi-year time-scales. A tight seasonal coupling between DOM production and consumption appears superimposed over small (<1% of gross primary production) but significant secular accumulations of DOC and DON. Apparently, the microbial processes responsible for DOM cycling over annual time scales are retentive with respect to DOP and relatively non-conservative with respect to DOC and DON. The net result of these processes is the build-up of C and N-rich DOM over annual-to-decadal time-scales.

Karl et al. (1997) observed significant changes in the dissolved and particulate N and P inventories at Station ALOHA between 1989-1994, a finding they attribute

to enhanced nitrogen fixation. These authors described multi-year increases in DON inventories accompanied by decreases in SRP inventories. Coincident with these trends, Karl et al. (1997) noted increases in DOP inventories, hypothesizing that these increases were due to an enhanced production of biorefractory DOP. Our analyses support Karl et al. (1997), with one exception: towards the latter half of our observation period (1993-1999), integrated DOP inventories remained unchanged. The observed decrease in the rate of DOP accumulation may reflect increased biological demand for P over time and enhanced retention of P by the biota. In support of these observations, P-limited plankton growth is hypothesized to temporally uncouple DOC production and its utilization (Thingstad et al. 1997), consistent with our observed accumulations of DOC and DON.

DOM Variability in Oligotrophic Oceans

The processes responsible for DOM cycling in oligotrophic oceans have received considerable attention over the past decade. In particular, studies evaluating the temporal dynamics of DOC in the oligotrophic Sargasso Sea and the Northwestern Mediterranean (Copin-Montegut and Avril 1993, Carlson et al. 1994, Hansell and Carlson 2001) have revealed an important seasonal dynamic in DOC inventories. The cyclic interaction between stratification of sea-surface waters and increased vernal primary production appears to drive seasonal DOC dynamics in the surface waters of these seasonally variable regimes. Such studies indicated that DOC production and export represent a potentially important permutation of the biological pump in oligotrophic environments (Ducklow et al. 1995).

No consistent pattern of seasonal production and removal of DOC was observed during the 11 years of our study at Station ALOHA. Instead, a multi-year net accumulation of DOC and DON throughout the upper ocean was observed. The seasonally driven process of DOC accumulation observed in the surface waters of the Sargasso and Mediterranean Seas apparently manifests itself over interannual and sub-decadal time scales in the NPSG. The lack of seasonal mixing in surface waters at Station ALOHA results in diffusion-dominated export of DOM rather than the seasonal pumping witnessed in other oligotrophic oceans (Copin-Montegut and Avril 1993, Carlson et al. 1994). The resulting long-term accumulation of DOM reflects multi-year excesses in the balance between production, utilization, and diffusive loss, rather than the seasonal-scale imbalances observed in other oligotrophic oceans.

In their study of temporal DOC dynamics in the oligotrophic Sargasso Sea, Carlson et al. (1994) observed net seasonal mixed-layer accumulations of DOC of $\sim$1.2 mol C m^{-2}. Of this DOC input, approximately 10% turned over on seasonal time scales, and the remaining $\sim$90% was exported to depth with winter convective mixing (Carlson et al. 1994). Similarly, Copin-Montegut and Avril (1993) estimated $\sim$1.5 mol C m^{-2} of the annually produced DOC in the Mediterranean Sea was seasonally exported from the surface ocean. Our analyses indicate net annual accumulation of DOC in the surface NPSG equals $\sim$0.3 mol C m^{-2}, approximately 20% of the amount of DOC annually exported in the Sargasso and the Mediterranean Seas. Moreover, the long-term accumulation of DOC in the EZ was nearly 30% as large as estimated total carbon export from this system (Emerson et al. 1997).

The mean rate of net ^{14}C incorporation into particulate matter in the EZ at Station ALOHA for the 1993-1999 period was 40 mmol C m^{-2} d^{-1} or ~15 mol C m^{-2} annually. Balancing this estimate of net primary production with our estimate of net DOC accumulation of ~0.3 mol C m^{-2} yr^{-1} and calculated total export rates of approximately 1 mol C m^{-2} yr^{-1} (Emerson et al. 1997), indicates that 91% of the net primary production was remineralized in the EZ. The remaining 9% of this net production was exported (7%) below the 175 m depth horizon or cycled on time scales greater than 1 year and accumulated in the EZ (2%). If DOC production rates in the NPSG approximate particle production rates (Karl et al. 1998) gross production in the NPSG could be as high as 30 mol C m^{-2} yr^{-1}. Given this scenario >95% of the gross primary production would remineralized in the EZ, leaving ~1% of this production to accumulate as DOC.

The apparent net rise in DON inventories between 1988-1999 was one of the most intriguing observations in this study. Assuming the 15 mol C m^{-2} measured as the mean annual ^{14}C-production rate had a C:N molar ratio approximating Redfield stoichiometry of 6.6:1, our estimate of annual nitrogen based primary production was ~2.0 mol N m^{-2} with N export below the EZ exceeding 0.15 mol N m^{-2} yr^{-1}. Balancing this production and export with our calculated rise in DON inventories (0.01 mol N m^{-2} yr^{-1}) indicates that 92% of the annually produced organic N was remineralized in the EZ. The remaining 8% of this DON was exported (7.5%) or cycled on time scales greater than 1 year and accumulated in the EZ (0.5%). Based on examination of the dynamics of the DOM inventories at Station ALOHA, it appears that a large fraction (>90%) of the annually produced DOM turns over on

short time scales (< 1 year), while a small fraction (~1%) of unutilized DOM grows

progressively richer in C and N relative to P, and may escape degradation altogether.

Climate Change and Biogeochemical Implications

Changes in the DOM inventories at Station ALOHA may be a reflection of

changes in the plankton community structure driven by basin-scale climate

variability. Investigations on surface water chemistry, physics and biology of the

North Pacific have emphasized the coherence of upper ocean processes with

interannual and decadal scale climate variability (Karl et al 1997, McGowan et al.

1998, Karl 1999, Karl et al. 2001a,b). Modification of surface water circulation and

enhanced stratification may have reduced the frequency of deep mixing events in the

NPSG. Such changes have been attributed to the occurrence and duration of El-Niño

Southern Oscillation (ENSO) events (Karl 1999). The lack of surface water mixing

events that penetrate into the permanent nutricline restricts the delivery of new

nutrients into the surface ocean and maintains the oligotrophic character of the gyre.

The non steady-state changes in bulk DOM inventories significantly altered

the underlying elemental stoichiometry of the bulk DOM inventories. Between 1993-

1999, the average C:N:P signature of bulk DOM inventories at Station ALOHA

increased from ~408:28:1 to 478:29:1 (Figure 5). The DOC:DON ratio of the bulk

DOM pool was more than two times greater than the Redfield ratio of 106:16, and the

resulting DOC:DOP ratio was more than four times richer in C relative to the

Redfield ratio. Between 1993-1999 the bulk DON:DOP ratio grew ~7% while the

bulk DOC:DOP ratio increased by 16% (Figure 6). More importantly, the resulting

C:N:P ratio of the accumulated DOM was 992:15:1, approximately ten times richer in C relative to the Redfield ratio. We are unaware of any other study documenting a systematic change in the bulk oceanic DOM C:N:P ratios over multi-year time scales. Increases in the C:N:P stoichiometry of the bulk DOM suggest either that inputs of C- and N- rich organic matter accelerated during this period or that selective heterotrophic utilization of P rich compounds became more prevalent.

Accumulations of C- and N- enriched DOM, relative to P, are important to quantify for complete understanding of biogeochemical cycling in the world's oceans. If C-and N- rich DOM accumulate under conditions favoring nitrogen fixation, revision of our understanding of carbon transport to the deep-ocean in oligotrophic systems may be required. New production defines the process whereby the removal of particulate and dissolved material from the surface ocean is balanced by input of new nitrogen into the surface ocean (Dugdale and Goering 1967, Eppley and Peterson 1979). If the observed DOM production and utilization imbalances are fueled by nitrogen fixation then new nitrogen input may not necessarily be temporally coupled to vertical export of carbon. The near-surface accumulation of C-and N-rich DOM comprises an alternate pathway to vertical export for multi-year carbon storage. Considered together, such near-surface C and N pooling may require reconsideration of the NPSG's capacity for carbon storage. Unlike the seasonal accumulation and export of DOM witnessed in the oligotrophic Sargasso and Mediterranean Seas, the EZ of the NPSG appears to focus DOM dynamics on interannual to interdecadal time scales. Such behavior reflects multi-year production imbalances and reflects the NPSG capacity for sub-decadal C- and N- DOM storage in the upper water column.

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SECTION V

SYNTHESIS AND CONCLUSIONS

Heterotrophic bacterial production in the NPSG

One of the primary objectives of this dissertation was to evaluate the importance of heterotrophic bacterial production (HBP) to carbon cycling in the upper ocean of the North Pacific Subtropical Gyre (NPSG). Bacterial carbon fluxes were estimated from time-series observations of heterotrophic protein production rates at Station ALOHA. The results of these studies revealed that HBP constitutes a major pathway of organic matter production, equivalent to roughly 15% of the annual primary production, and more than three times greater than particle export in this system.

Carbon production by heterotrophic bacteria in the NPSG was similar to rates measured in other oligotrophic ocean systems including the Sargasso and western Mediterranean Sea (Table 1). Direct comparison between the present study and studies in these other ecosystems is hampered by the observation that sunlight has a strong positive influence on heterotrophic production in the NPSG and its impact is still mostly unknown in these other ecosystems. Nonetheless, comparison with these other systems provides a relative basis for assessing the magnitude of carbon flow into the microbial food web in these different ecosystems.

The establishment of the Bermuda Atlantic Time-Series (BATS) has provided a wealth of information on HBP in the Sargasso Sea. HBP in the photic zone at BATS averages 5.8 mmol C m^{-2} d^{-1} (Carlson et al. 1996), roughly equivalent to HBP measured in the dark in the NPSG. Similarly, rates of primary production in the two ecosystems are roughly equivalent (Karl et al. 2002); however, there are several

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Table 1. Mean photic zone heterotrophic bacterial production in various open ocean ecosystems.

Location	HBP (mmol C m^{-2} d^{-1})	Study	Comments
Sargasso Sea	5.8	Carlson et al. 1996	Nine time-series cruises to BATS, 1991-1993
Equatorial Pacific	19	Ducklow et al. 1995	Two ~1 month cruises in spring and fall of 1992
North Atlantic Spring Bloom	23	Ducklow et al. 1993	Three ~1 month cruises in the spring/summer of 1989
Subarctic North Pacific	4.7	Kirchman et al. 1993	Two cruises in the spring of 199?
Ross Sea, Antarctica	4.6	Ducklow et al. 2001	Six, 1-2 month cruises between 1994-1997
Mediterranean Sea	Light: 4.1 Dark: 8.8	Morán et al. 2001	One cruise in February 2000
North Pacific Subtropical Gyre	Light: 8.3 Dark: 5.1	This study	Nine time-series cruises to Station ALOHA, 2000-2002

fundamental biogeochemical differences between the NPSG and the Sargasso Sea. Most noticeably, the upper ocean at BATS is seasonally transformed by the injection of nutrient-rich deep water during the winter; in contrast, mixed layers at HOT rarely penetrate the nutricline, resulting in nearly continual nutrient deprivation (Karl et al. 2002). In addition, concentrations of inorganic nitrogen (N) are much more variable at BATS than at HOT; NO_3^- + NO_2^- concentrations at BATS range between ~10-1000 μM. Moreover, concentrations of inorganic phosphorus (P) are roughly an order of magnitude lower at BATS than HOT (Cavender-Bares et al. 2001).

The influences of sunlight on HBP in the Sargasso Sea are still largely unknown but several differences in upper ocean plankton community structure between BATS and HOT suggest that HBP might not depend on light to the same extent as in the NPSG. For example, the mean photic zone abundance of *Prochlorococcus* is roughly an order of magnitude greater at Station ALOHA than at BATS. In contrast, *Synechococcus* abundance at BATS can exceed concentrations measured at Station ALOHA by more than an order of magnitude (Cavender-Bares et al. 2001). The relationship between HBP and *Prochlorococcus* observed in the NPSG (Section I) suggests *Prochlorococcus* may be linked to the photostimulated production; if this is the case, then sunlight is likely to play a greater role in HBP at Station ALOHA than at BATS.

The causes of the differences in bacterioplankton population structure among these two oligotrophic ocean systems are unknown; however, BATS consistently experiences wintertime convective mixing and nutrient input while HOT does not, a factor that likely impacts the abundance of the bacterial populations. Despite these

important differences in plankton structure and upper ocean biogeochemistry, both primary and secondary carbon production in these two systems appear roughly comparable.

Studies in the western Mediterranean Sea suggest that HBP may partly depend on sunlight, with HBP ranging between 4-8 mmol C m^{-2} d^{-1} (Table 1, Gasol et al. 1998, Morán et al. 2001). The influence of sunlight on HBP in the Mediterranean Sea appears somewhat different than in the NPSG; HBP in the Mediterranean was consistently lower when measured in the light than in the dark (Table 1, Morán et al. 2001). Generally, the upper ocean biogeochemistry in the Mediterranean Sea is more similar to the Sargasso Sea than to the NPSG. For example, the abundance of chlorophyll containing bacteria in the Mediterranean resembles that at BATS; *Prochlorococcus* accounts for $\sim 10^3$-10^4 cells ml^{-1} (roughly two orders of magnitude lower than at HOT) and *Synechococcus* abundance averages $\sim 10^4$ cells ml^{-1}, or roughly an order of magnitude greater than found in the NPSG. In addition, inorganic N concentrations in the upper ocean are roughly an order of magnitude greater in the Mediterranean than at HOT.

The apparent differences in the light-response of HBP in the NPSG and the Mediterranean Sea provide additional support to the hypothesis that *Prochlorococcus* demonstrates light-stimulated Leu incorporation in response to the dearth of inorganic N available in the upper ocean of the NPSG. This hypothesis is consistent with a recent study demonstrating DON utilization by natural populations of *Prochlorococcus* in the Arabian Sea (Zubkov et al. 2003).

The heterotrophic response to light

One of the most important findings of this study was that Leu incorporation rates in the upper ocean were significantly enhanced by sunlight. The mechanisms responsible for this possible photoenhancement of heterotrophic production remain unclear; however, the results suggest that carbon fluxes into the microbial food web are significantly greater due to abiotic or direct biotic photostimulation of heterotrophic production.
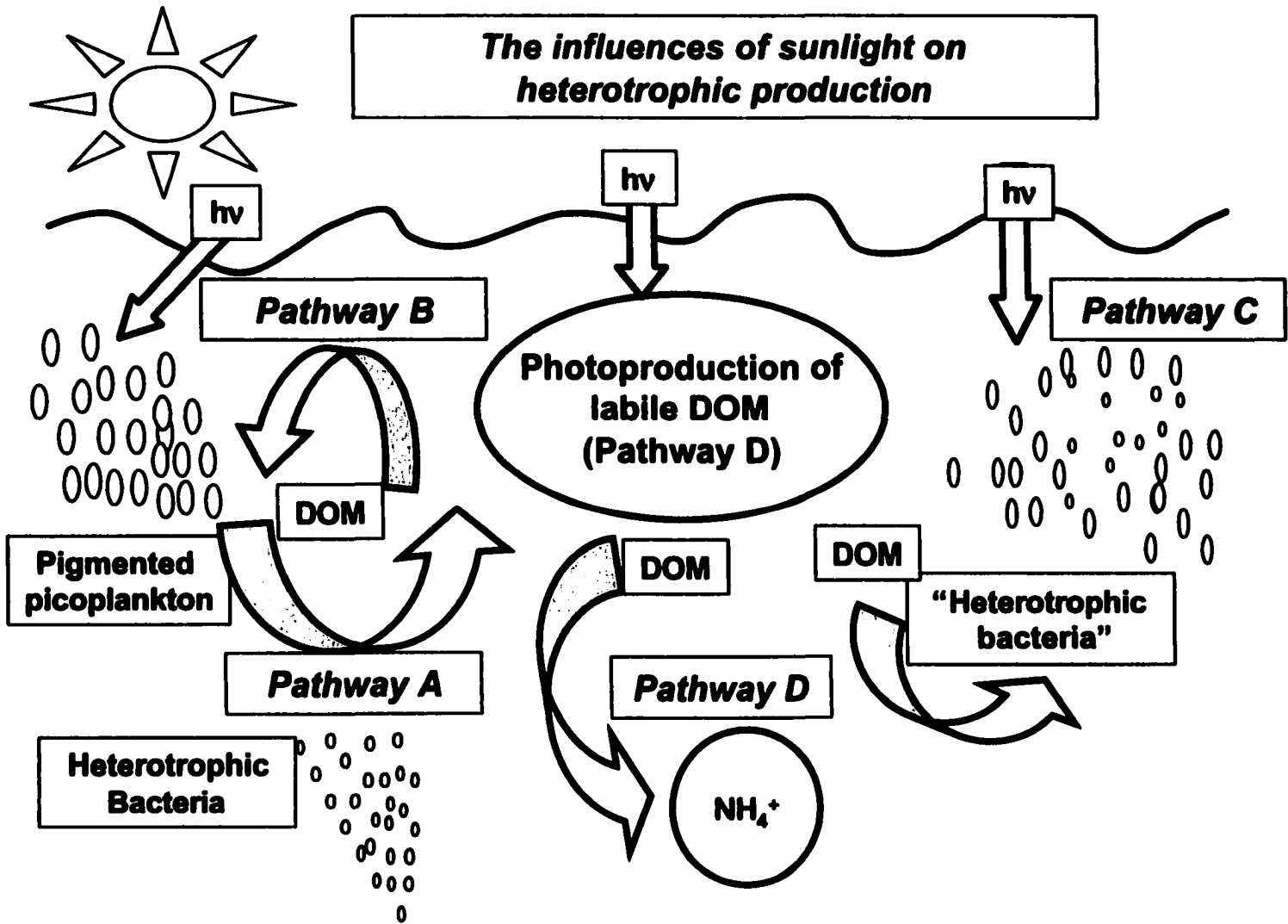
Overall, photoenhancement of Leu incorporation accounted for greater than half of the light-incubated protein production in the upper ocean. Rates of both light- and dark- Leu incorporation were positively correlated with the abundance of *Prochlorococcus* and non-chlorophyll containing bacteria. While the relationship between the non-chlorophyll containing bacteria was not unexpected, the apparent relationship between *Prochlorococcus* and heterotrophic productivity suggests two possibilities: 1) *Prochlorococcus* directly incorporates amino acids, or 2) heterotrophic bacterial growth was indirectly linked to the abundance of *Prochlorococcus*.

Several experiments were conducted in both the upper and lower water column at Station ALOHA to evaluate the nature of the relationship between protein production rates and irradiance (Leu-E) over a range of light fluxes (0-1200 μmol quanta m^{-2} s^{-1}). The results of these experiments suggested two important aspects of the heterotrophic response to irradiance: protein production varied with irradiance in a manner similar to the photosynthetic response to light (P-E); and the response of heterotrophic production to light differed only slightly in the upper and lower photic

zone. Similar to the P-E relationships observed in this study and previous investigations of the NPSG (e.g. Ondrusek et al. 2001), the Leu-E response in the upper photic zone typically exhibited a saturation response, increasing asymptotically at low light fluxes and saturating with increasing irradiance. However, upper photic zone Leu incorporation rates appeared more responsive than photosynthesis at low light fluxes, displaying optimal irradiances between 183-296 mmol quanta $m^{-2} s^{-1}$, compared to ~600 mmol quanta $m^{-2} s^{-1}$ for photosynthesis. In the lower photic zone, Leu incorporation rates often displayed significant photoinhibition at light fluxes greater than 18-130 mmol quanta $m^{-2} s^{-1}$, and photosynthetic rates saturated at 26 mmol quanta $m^{-2} s^{-1}$.

The results from Section I and II suggest a number of potential processes may photostimulate bacterial production in the NPSG (Figure 1). Direct utilization of solar energy by photoautotrophic and photoheterotrophic bacterial assemblages might result in photoenhanced heterotrophic protein production. For example, light-driven photosynthetic production of labile DOM could result in a tight temporal coupling among heterotrophic and photoautotrophic bacteria in the upper ocean (Figure 1, pathway A). There are many examples of tightly coupled plankton dynamics in subtropical oceans ecosystems, with bacterial production often closely synchronized with photoautotrophic production. Presumably HBP at Station ALOHA demonstrates some temporal coordination with primary production (PP); however, regression analyses did not reveal a consistent relationship between PP and HBP (Section I). The time scale of the coupling between PP and HBP may be considerably shorter than

Figure 1. Various light-driven processes that could result in light-stimulated

heterotrophic production at Station ALOHA. Processes include biotic and abiotic

influences of solar energy on upper ocean biogeochemical processes. **Pathway A:**

Light energy fuels photoautotrophic production of labile DOM, triggering

heterotrophic response to increased flux of labile DOM. Pathway A requires a tight

temporal coupling between heterotrophic and photoautotrophic bacterial assemblages.

Pathway B: mixotrophic utilization of DOM by facultative photoautotrophs. An

example of this pathway is the heterotrophic utilization of amino acids such as

leucine to supplement the cellular nitrogen requirements of *Prochlorococcus*.

Pathway C: photoheterotrophic production by rhodopsin-containing or AAnP

bacteria. Utilization of sunlight as an energy source and DOM as a carbon and

nutritional supplement could result in light-stimulated heterotrophic production by

these groups of bacterioplankton. **Pathway D:** Abiotic transformation of DOM by

UV or PAR could photochemically alter upper ocean DOM inventories, producing

NH_4^+ or labile DOM. This process could indirectly result in light-stimulated

heterotrophic production.

The influences of sunlight on heterotrophic production

Pathway B

Photoproduction of labile DOM (Pathway D)

Pathway C

Pigmented picoplankton

DOM

Pathway A

Heterotrophic Bacteria

DOM

Pathway D

DOM

"Heterotrophic bacteria"

NH_4^+

the intervals sampled between cruises in this study. Appropriate evaluation of the temporal coupling between PP and HBP would need to include much higher frequency sampling (i.e. hourly) than was undertaken in this study.

Light-stimulated bacterial production might also result from mixotrophic growth by upper ocean phototrophs. Selective utilization of DOM (like leucine) as a supplement to photoautotrophic production would presumably exhibit a response to sunlight, corrdinated with increased growth by photosynthesis (Figure 1, pathway B). Alternatively, a significant portion of the upper water column bacterial assemblages may grow photoheterotrophically, utilizing light as an energy source and DOM as carbon and reductant sources (Figure 1, pathway C). If this were the case, heterotrophic growth and production would be significantly greater in the light than in the dark.

A third process considered throughout this dissertation is that light-stimulated bacterial production occurred by an indirect abiotic pathway via photoproduction of labile DOM or inorganic nutrients (Figure 1, pathway D). Although not evaluated directly in this study, high light fluxes (particularly UV-A and UV-B) are a characteristic of oligotrophic ocean ecosystems. UV radiation has several possible influences on microbial growth in the upper ocean. Direct absorption of UV light by upper ocean plankton assemblages may damage DNA, and reduce heterotrophic production (Jeffrey et al. 1996). All of the experiments described in this study were conducted in polycarbonate bottles, minimizing the impact of UV light on bacterial production. While not specifically tested, the estimates of HBP reported in this study may overestimate the actual flux of carbon into heterotrophic bacteria due to the

detrimental influences of UV light. However, as described in Section I, the overall influence of UV should be restricted to the upper 30 m of the water column and likely not reduce depth-integrated rates of HBP in the photic zone by more than ~12%.

Heterotrophic production might also demonstrate light-stimulation if photoproduction of bioavailable forms of nutrients from DOM was occurring at appreciable rates in the upper ocean. Photolytic production of both NH_4^+ and amino acids from marine DOM has been observed (Bushaw et al. 1996, Bushaw-Newton and Moran 1999), and could potentially play an important role of introducing labile nitrogen into the upper ocean at Station ALOHA. However, high-energy radiation (i.e. UV light) is believed the major spectral component necessary for photochemical conversion of DOM, so the potential importance of this process was not included in this study.

Based on the analyses of the Leu-E and P-E responses to irradiance (Section II), and analyses of the temporal relationships between heterotrophic production and photic zone inventories of bacterial assemblages (Section I), Leu incorporation in the NPSG appears at least partly regulated by the unicellular cyanobacterium *Prochlorococcus*. Growth in the nutrient-poor upper ocean likely requires that the plankton assemblages utilize the larger pool of DOM as an additional nutrient resource. *Prochlorococcus* is estimated to be the most abundant photosynthetic organism on Earth (Partensky et al. 1999); if the growth of *Prochlorococcus* exerts fundamental control over both primary and secondary production in large areas of the world's oceans, it is imperative that we reevaluate its contributions to global carbon fluxes.

Previous studies have documented long-term, increasingly oligotrophic conditions in the upper ocean of the NPSG. In particular, several studies have hypothesized that decreases in upper ocean concentrations of phosphorus may be linked to the increased abundance of nitrogen-fixing cyanobacteria in the NPSG (Karl et al. 1995, 1997). The paucity of inorganic nutrients in the upper ocean of the NPSG may select for groups of phototrophic prokaryotes that supplement or entirely meet their cellular N and P requirements using the relatively large pool of DOM. The ability to harvest sunlight for energy while obtaining carbon, nutrients, and energy from DOM presumably increases bacterioplankton productivity in this sunlight-rich, nutrient-poor open ocean ecosystem.

Bacterial growth in the NPSG forms an important pathway of carbon flow in the ocean. Moreover, the oligotrophic waters of the subtropical gyres result in a complex microbial food web that obscures explicit distinctions between the contributions of heterotrophic and autotrophic productivities. One of the primary conclusions of this study is that sunlight significantly increased the flux of carbon into bacterial biomass. Whether this process occurred as a result of diverse physiological capabilities of the upper ocean bacterial assemblages, or resulted from photostimulated production of labile DOM or inorganic nutrients, the net result was that roughly half of the measured heterotrophic production in this system appears directly fueled by solar energy.

Climate-induced changes in biogeochemistry in the NPSG

Section III of this dissertation focused on the temporal dynamics of upper ocean DOM inventories in the NPSG, and evaluated how long-term climate-driven changes in ocean biogeochemistry may have impacted organic matter concentrations at Station ALOHA. The results from the analyses in Section III demonstrated that inventories of DOC, DON, and DOP in the upper ocean have all undergone multi-year accumulations; however, toward the latter half of the observation period (1993-1999) DOP inventories ceased accumulating while concentrations both DON and DOC continued to increase. The accumulating DOC was a relatively minor component of upper ocean organic carbon fluxes, accounting for ~1-2% of the annual primary production in this ecosystem. Nonetheless, the pathway may provide a temporary storage for fixed carbon in the upper ocean.

The results of Section III provide support to previous studies that have described recent changes in upper ocean biogeochemistry in the NPSG. Increases in both the abundance and activity of nitrogen-fixing bacteria through the past decade have been ascribed to climate-driven stabilization of the upper water column in the NPSG (Karl 1999, Dore et al. 2003) and the introduction of fixed N has been hypothesized to have begun to drive the upper ocean toward a P limited ecosystem (Karl et al. 1997). In addition, long-term increases in the concentration of chlorophyll *a* and the abundance of phototrophic bacteria are hypothesized to reflect climate-induced alteration of upper ocean biogeochemical processes (Karl et al. 2001). Accompanying these changes in bacterial abundance and activity, upper ocean

organic matter inventories appear to have grown increasingly rich in C and N, relative to P.

Long-term accumulations of DOC and DON in the upper ocean may substantially alter microbial food web processes in the NPSG. In particular, increasing availability of DOC and DON may provide a source of energy and nutrients to sustain heterotrophic or mixotrophic bacterial growth. If the bacterial assemblage were able to utilize this accumulating DOM, the total carbon flux into the bacterial assemblage would likely increase, enhancing remineralization and altering food web dynamics in the upper ocean. However, the observation that DOM is accumulating also suggests that this material resists bacterial degradation over annual time scales. The poor nutritional "quality" of this accumulating DOM is likely to restrict bacterial utilization of this reduced carbon (Thingstad et al. 1997, Carlson and Ducklow 1996).

The impact of light-enhanced heterotrophic production could also substantially alter DOM dynamics in the NPSG. One explanation for the long-term increases in bulk DOM inventories in the NPSG could relate to the diversity of metabolic capabilities expressed among the photic zone bacteria in the NPSG. Both photoheterotrophic and mixotrophic production are important pathways of organic carbon production in this ecosystem, and both of these physiologies are likely to directly impact heterotrophic bacterial growth efficiencies (HBGE) (Yurkov and Beatty 1998, Kolber et al. 2001, Karl 2002). In Section I, a simple budget of organic carbon fluxes revealed that HBGE in the upper ocean at Station ALOHA could be as great as 29%; in comparison, the HBGE in the oligotrophic Sargasso Sea is ~14%

(Carlson et al. 1996). Increased growth efficiencies by photoheterotrophic and mixotrophic bacteria may increase the upper ocean bacterioplankton HBGE, thereby increasing the flux of carbon into the microbial loop relative to obligate heterotrophy.

The vast majority (generally >80%) of DOC consumed by heterotrophic bacteria is used to generate energy to sustain basic cell functions such as transport and construction of biomass (del Giorgio and Cole 2000). Catabolic processes are both the dominant pathways of carbon flow and the primary energy generating mechanisms for obligate heterotrophic bacteria (Figure 2, del Giorgio and Cole 2000). Bacterial catabolism of DOM for energy results in a large respiratory carbon fluxes among natural heterotrophic bacterial assemblages. As a result, HBGE in many marine ecosystems appears relatively low (5-20%) (del Giorgio and Cole 1998). By obtaining energy from the sun rather than metabolizing organic matter for energy, photoheterotrophic bacterial growth is expected to be more efficient than obligate heterotrophic metabolism (Kolber et al. 2001, Karl 2002). By meeting cellular energy demands through harvesting of sunlight, photoheterotrophic bacteria may selectively utilize labile DOM containing bioessential nutrients (particularly N and P) rather than utilizing DOM as an energy source. Such selective DOM utilization might increase the proportion of biosynthetic precursors directly incorporated into biomass, and increase net growth rates (Figure 3).

By reducing organic matter catabolism and efficiently transforming carbon into bacterial biomass, photoheterotrophic or mixotrophic growth has the potential to significantly reduce the carbon demand required by the bacterial assemblages in the

Figure 2. Schematic based on del Giorgio and Cole (2000) displaying the dominant

pathways of energy flow in heterotrophic bacteria. ATP production occurs by

catabolism of DOM and intracellular energy reserves (pathways a and e). ATP is

utilized for cellular functions such as transport (b), anabolic reactions that build

cellular constituents (c), and to maintain cellular constituents (d). The growth rate of

the bacterial population is determined by the rate that carbon enters the anabolic
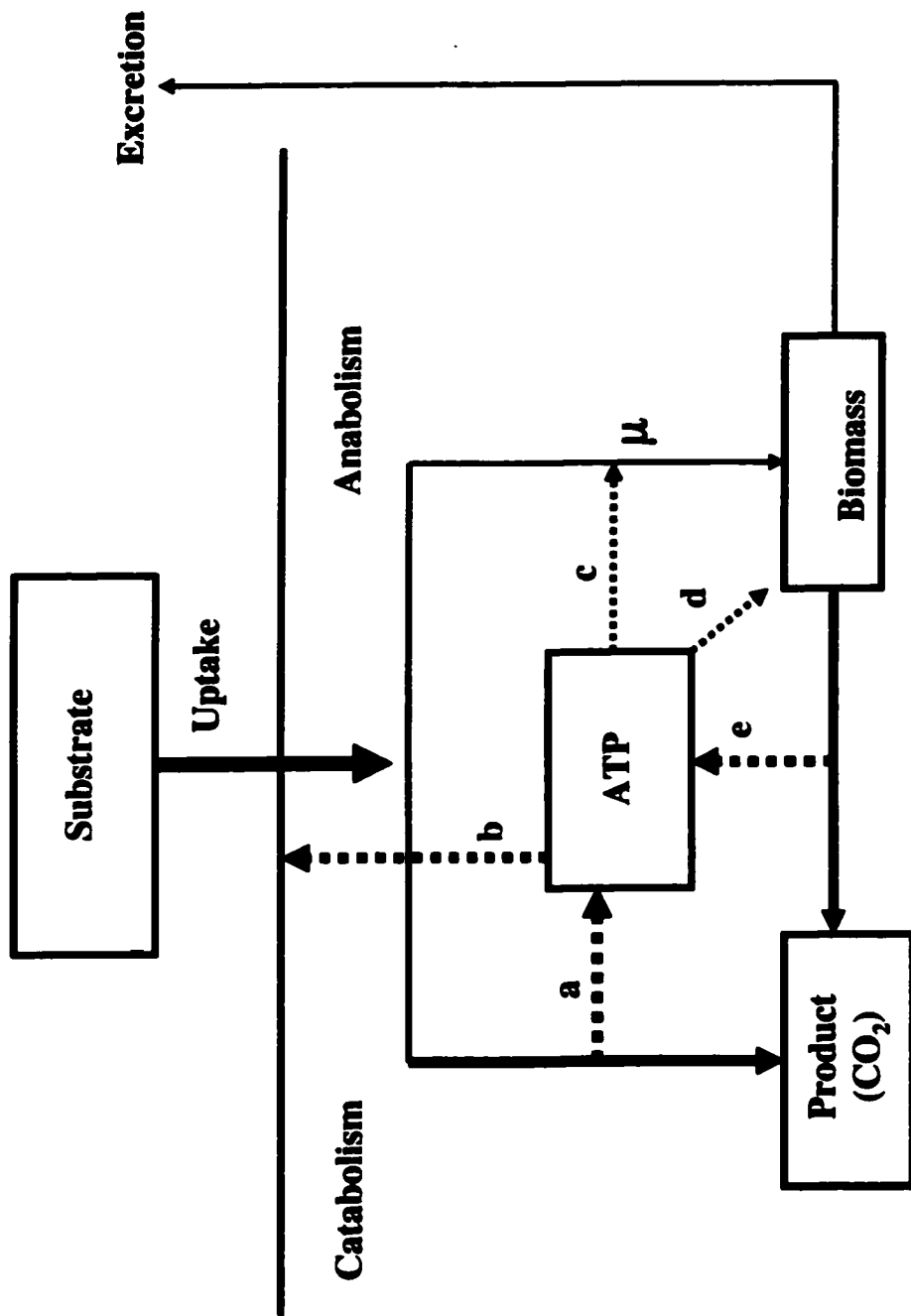
pathways to build biomass.

Figure 3. Schematic of cellular energy generation and partitioning in photoheterotrophic or mixotrophic bacteria. ATP production and utilization pathways are similar to Figure 2, but solar energy provides the dominant source of ATP, with smaller fluxes derived from substrate level and oxidative phosphorylation. Energy gained from sunlight decreases the cells dependence on catabolism as the sole energy yielding processes, and provides additional energy to fuel biosynthesis (anabolic pathways), resulting in a net increase in the growth rates of the population.
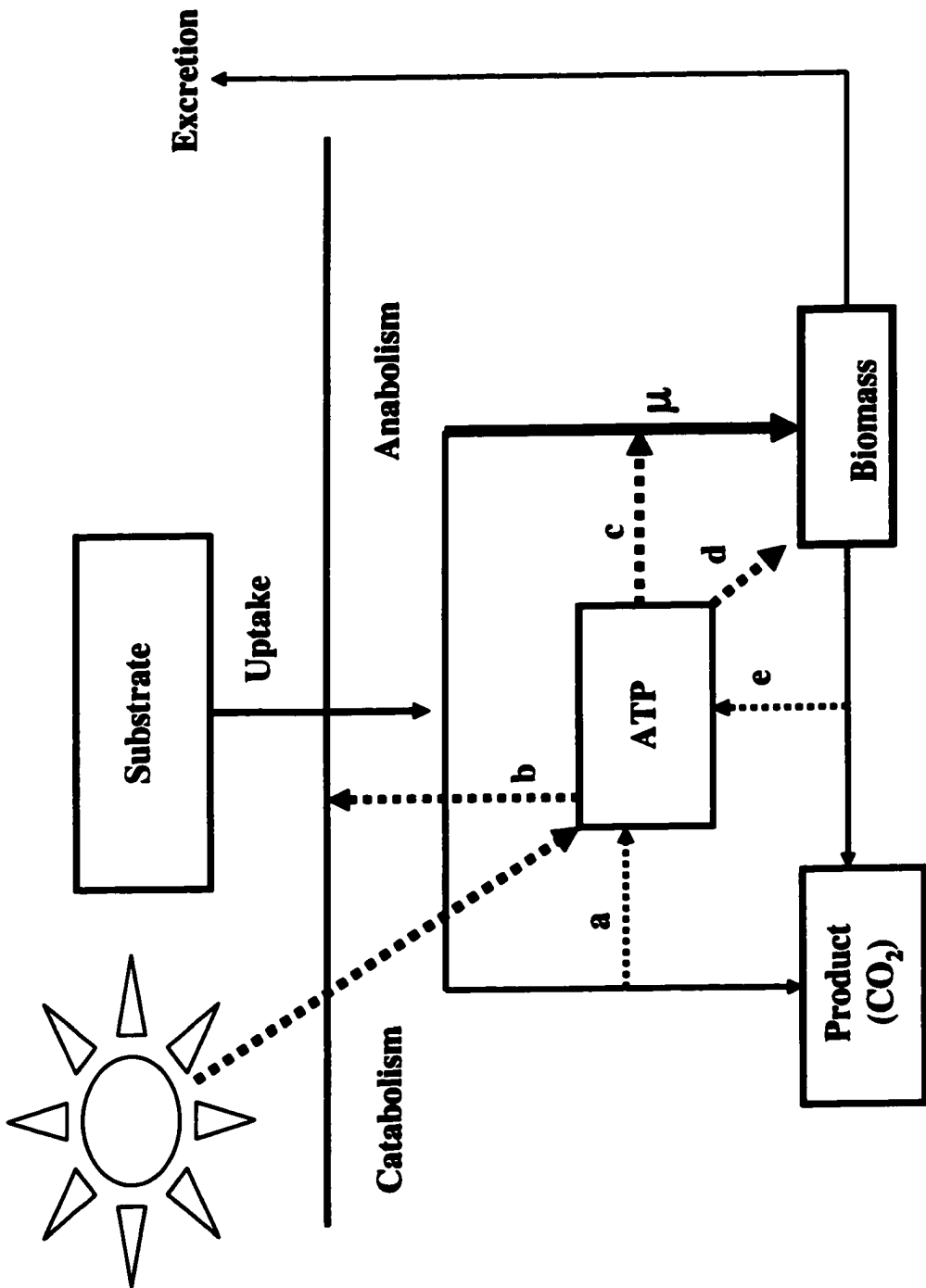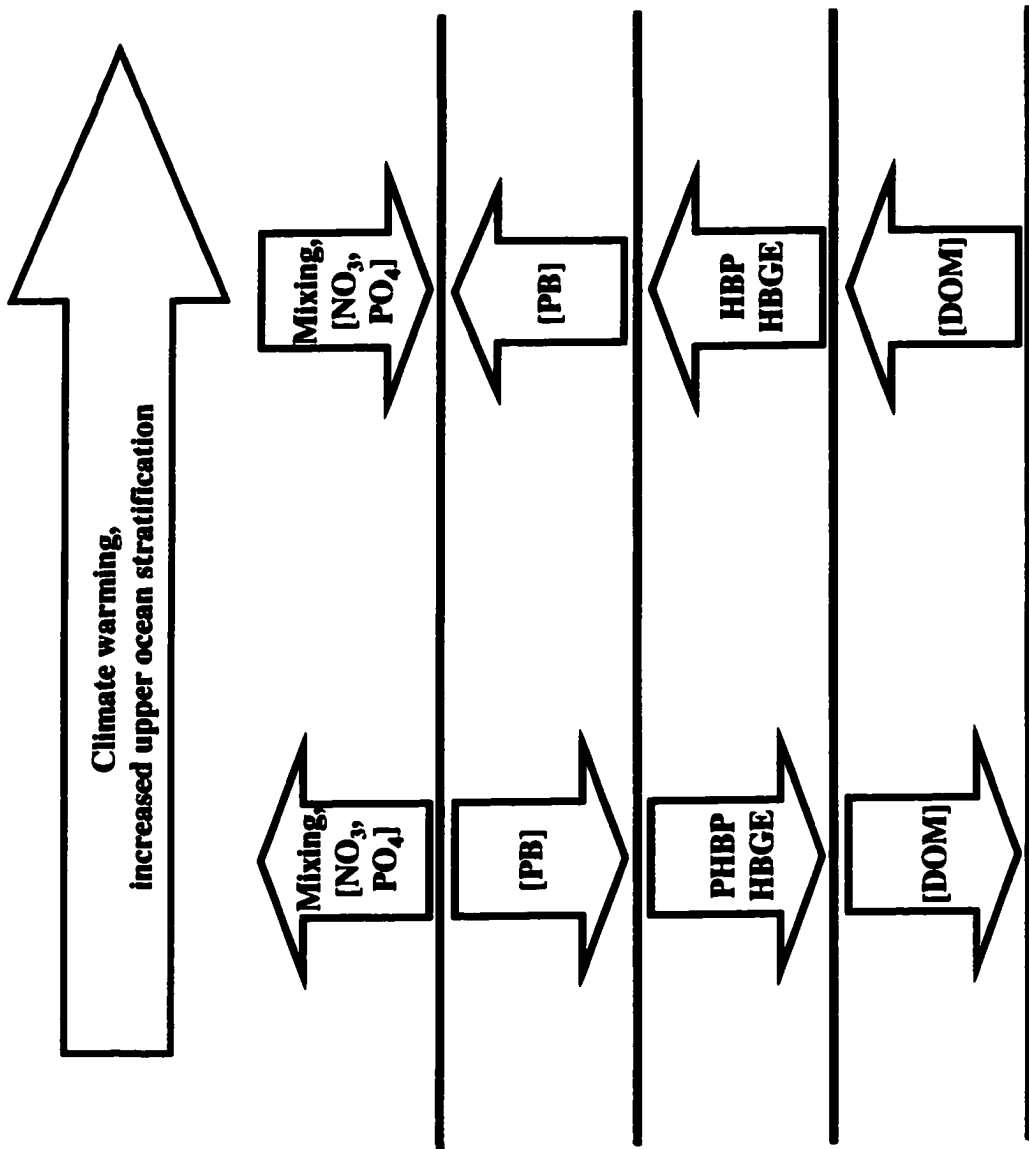
upper ocean. Reduction of the carbon flux through the bacterial assemblages could allow DOM production to outpace utilization, and result in accumulation of carbon-rich DOM in the upper ocean. By harvesting energy from sunlight and using both CO_2 and labile DOM in anabolic processes, mixotrophic production could decrease bacterial dependence on semi-labile or refractory DOM, allowing multi-year accumulations of these pools of DOM.

Climate change and its impact on NPSG biogeochemistry

Ocean-climate models predict that anthropogenic emission of CO_2 may dramatically alter Earth's climate. One potential consequence of increased CO_2 emissions is an increase in the upper ocean temperatures throughout the tropics and subtropics. Such climate-driven changes in upper ocean temperature could presumably impact ocean circulation. One potentially important consequence of ocean warming is stabilization of the upper ocean water. In the NPSG, this process could substantially impact ocean biogeochemistry (Figure 4). In particular, restricted vertical mixing may further exacerbate the isolation of the upper photic zone, enhancing the oligotrophic conditions. Restriction of convective mixing and nutrient input may select for diazotrophic bacteria, and effectively tighten P recycling in the upper ocean. Inventories of DOC and DON would be expected to continue to accumulate due to the activities of N-fixing bacteria, and restricted vertical dilution of upper ocean inventories.

Figure 4. Schematic depicting predicted influence of climate warming and enhanced

stratification of the upper ocean in the NPSG. Deepening of the thermocline due to

warming of upper ocean would restrict vertical nutrient fluxes, and enhance

conditions conducive to the growth of N_2-fixing bacteria. In addition, phototrophic

bacteria (PB) capable of utilizing DOM as a nutrient and energy source may become

increasingly abundant. Mixotrophic and photoheterotrophic production by PB

bacteria would increase heterotrophic bacterial production (HBP) and increase

heterotrophic bacterial growth efficiency (HBGE). Increased utilization of sunlight

as an energy source would decrease the heterotrophic demand for DOM, resulting in

upper ocean accumulations of DOC and DON.

Climate warming, increased upper ocean stratification

Mixing, [NO₃, PO₄]

[PB]

HBP HBGE

[DOM]

Mixing, [NO₃, PO₄]

[PB]

PHBP HBGE

[DOM]

The impact of climate-driven upper ocean stratification could substantially alter heterotrophic production in the NPSG. Increased stratification might favor physiologically diverse bacteria. In particular, organisms capable of utilizing DOM to fulfill cellular nutrient demands, and simultaneously harvesting energy from the sun might be favored over obligate photoautotrophs or strict heterotrophs (Figure 4). Increased productivity by photoheterotrophic and mixotrophic bacterial assemblages may further emphasize the role of the microbial food web in this ecosystem. In total, future changes to the physical structure of the upper ocean may substantially alter nearly every aspect of biogeochemical cycling in the NPSG. Our understanding of the processes that regulate the functioning of the microbial loop in the open ocean provides a basis for forecasting how the oceans will respond to the inevitability of global climate change.

Future Directions

This study may have raised more questions than it has answered. Several important ideas need to be followed up on in order to understand the processes controlling the observations in this study. In particular, understanding the factors that regulate the temporal dynamics in upper ocean DOM inventories may provide insight into the apparent long-term accumulation of DOM in the upper ocean. In addition, careful evaluation of the processes governing the photoenhanced heterotrophic production in this ecosystem is required. By isolating the specific mechanisms that resulted in light-stimulated Leu incorporation, it may be possible to quantify the importance of the various light stimulated heterotrophic pathways described in this

study. Various experimental approaches may be employed to identify the specific

photoheterotrophic bacteria in this system. Such experiments include isolating

Prochlorococcus from the other members of the bacterioplankton assemblage by

cultivation based approaches. Cultivation-based appraoches would allow

determination of the physiological capacilities of the organisms under strictly

controlled laboratory conditions. Such experiments may provide insight into the

interactions of growth, nutrients assimilation, and light. In addition, utilization of

nucleic acid probes to target specific populations of *Prochlorococcus* could be

combined with substrate autoradigraphy, yielding information on whether

Prochlorococcus is capable of amino acid assimilation. Similar methods could be

applied to examine how different photoheterotrophic bacteria (including the AAnP

and rhopsin-containing bacterial populations) influence Leu incorporation rates in the

upper ocean. In addition, RNA-based approaches could be utilized to target the

expression and diversity of photoheterotorphic bacterial amino acid transport systems.

Finally, experimental determinations of heterotrophic growth efficiencies could

provide constraint on the importance of photoheterotrophic and obligate heterotrophic

production and remineralization.

Additional research efforts should also be direct at understanding the factors

that limit heterotrophic utilization of accumulated DOC and DON in the upper ocean

of the NPSG. Experimental studies which examine the factors that limit

heterotrophic utilization of DOM at Station ALOHA could be undertaken and

quantification of DOM production and removal rates might provide insight into the

relatively lability of the accumulated C and N pools. In addition, by understanding

the major forms of bacterial metabolism in the upper ocean may yield clues into how microbial population dynamics influence carbon and nutrient cycling in the upper ocean. There are many questions yet to be answered, but hopefully this research will open new doors of opportunity to advance our understanding of ocean biogeochemistry and ecology.

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VITA

Matthew James Church was born December 27, 1970 by the shores of Lake Erie in

Buffalo, NY. He moved west to Portland, OR. in 1972. He graduated from

Woodrow Wilson High School in June 1989. The following September he attended

The Evergreen State College in Olympia, WA. After completion of his Bachelor's of

Science, he moved to Bermuda where he worked for two years at the Bermuda

Biological Station for Research. In 1996, he left Bermuda, relocating to Gloucester

Point, VA. to attend graduate school at the School of Marine Science at The College

of William and Mary. He earned a Master's in Marine Science in 1999. In March of

2003 he successfully defended his Ph.D.