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Ecosystem Structure and Dynamics in the North Pacific Subtropical Gyre: New Views of an Old Ocean

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Lisa Lum <ljlum@hawaii.edu>

Fwd: Ecosystems 20th anniversary - invitation to contribute

1 message

David Karl <dkarl@hawaii.edu> Wed, Jul 6, 2016 at 8:25 AM
To: Lisa Lum <ljlum@hawaii.edu>

---------- Forwarded message ----------
From: **Steve Carpenter** <srcarpen@wisc.edu>
Date: Sun, Mar 8, 2015 at 6:00 AM
Subject: Ecosystems 20th anniversary - invitation to contribute
To: David Karl <dkarl@hawaii.edu>
Cc: Monica <turnermg@wisc.edu>

Dear Dave,

It is hard for us to believe that Ecosystems is now publishing Volume 18. With strong support from the scientific community and an outstanding editorial board, Ecosystems has earned a place among the top tier of journals within ecology. The journal provides an outlet for some of the best research in ecosystem science, bridging fundamental ecology and environmental problem solving. Ecosystems reaches a broad international audience, and published studies address ecosystem-level questions of general interest while spanning a wide range of systems and scales.

To recognize and celebrate the journal's 20th anniversary year, we are inviting an exciting set of articles and special features that reflect on current and future directions in ecosystem science. These high-profile articles will be published in 2017 and distributed throughout the issues that compose Volume 20. Several articles published in Volume 1 have been very influential and addressed topics that remain active and important today. In other cases, new directions that have emerged during the past 20 years are ripe for synthesis and prognosis.

In 1999 you published a highly cited paper in Ecosystems on the dynamics of the North Pacific ecosystem. Since that time, there has been progress in developing long-term perspectives of change in ocean ecosystems, yet we still have a long way to go. Emerging problems of climate change, ocean acidification, food web change, pollution and other factors make the need for a long-term perspective even more urgent. We are pleased to invite you to prepare an article for publication during the 20th anniversary year that updates insights from long-term observations of change in ocean ecosystems, synthesizes developments in the field since that time, and lays out future directions and research priorities. You are uniquely qualified to write this paper because of your extensive experience in observing both ocean ecosystems as well as the people and institutions that study them.

Papers should follow the usual guidelines for manuscript length, and we encourage you to think "outside the box" and be pithy and provocative. We envision the collection of invited papers influencing directions in ecosystem ecology over the next 20 years. Many invited papers and special-feature articles are among the most highly cited papers that have been published in Ecosystems. We anticipate substantial interest and attention to this article.

The deadline for manuscript submission is 15 March 2016, which allows well over one year for your ideas to develop and mature. You are welcome to include coauthors. Manuscripts will follow the usual format for articles presented on the Ecosystems website. Of course, manuscripts will be peer reviewed, and this invitation is not a guarantee of publication. So that we can move forward with our planning, we would appreciate your reply to this invitation by 20 March 2015. We hope you will welcome this opportunity to develop and frame your views on the future of our field.

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Sincerely,

Steve Carpenter and Monica Turner
Co-editors in Chief, Ecosystems

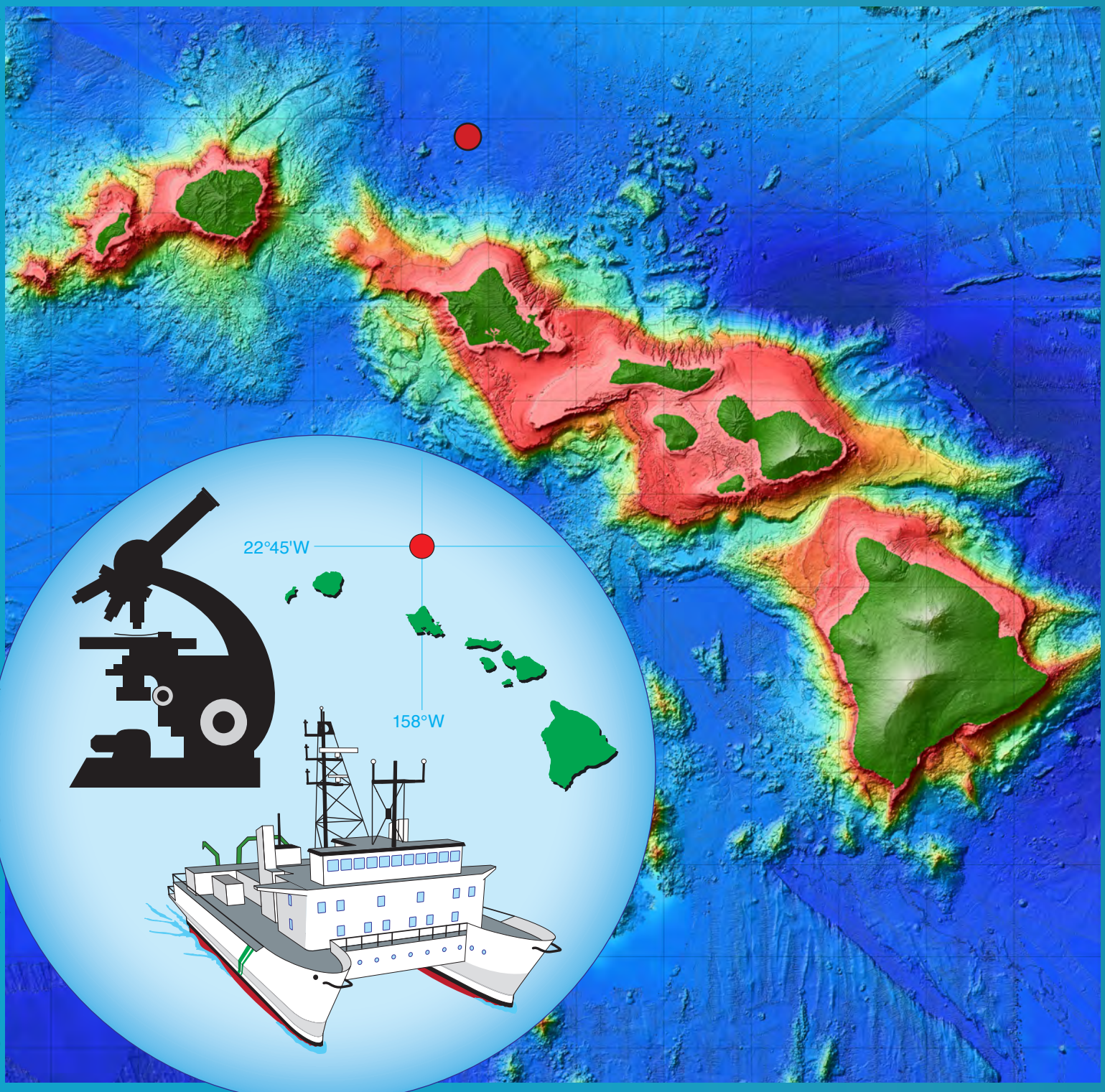
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****** Steve Carpenter, Director, Center for Limnology, U.Wisconsin-Madison, http://limnology.wisc.edu ******

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Ecosystem Structure and Dynamics in the North Pacific Subtropical Gyre: New Views of an Old Ocean

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ABSTRACT

The North Pacific Subtropical Gyre (NPSG) is one of the largest biomes on Earth. It has a semi-enclosed surface area of ~2 x 10^7 km^2 and mean depth of nearly 5 km, and includes a broad range of habitats from warm, light-saturated, nutrient-starved surface waters to the cold, nutrient-rich abyss. Microorganisms are found throughout the water column, and are vertically stratified, due to their physiological tolerances to temperature, light and pressure and by their genetically determined metabolic capabilities. Despite the global significance of the NPSG for energy and matter transformations and its role in the oceanic carbon cycle, it is grossly undersampled and not well characterized with respect to ecosystem structure and dynamics. Since October 1988, interdisciplinary teams of scientists from the University of Hawaii and around the world have been investigating the NPSG ecosystem at Station ALOHA (A Long-term Oligotrophic Habitat Assessment), a site chosen to be representative of this expansive oligotrophic habitat, with a focus on microbial processes and biogeochemistry. At the start of this comprehensive field study, the NPSG was thought to be a "climax" community with a relatively stable plankton community structure and relatively low variability in key microbiological rates and processes. Now, after nearly three decades of observations and experimentation we present a new view of

this old ocean. Our revised paradigm is built on the strength of high-quality time-series observations, on insights from the application of state-of-the-art –omics techniques (genomics, transcriptomics, proteomics and metabolomics) and, more recently, the discoveries of novel microorganisms and metabolic processes. Collectively, these efforts have led to a new understanding of trophic dynamics and population interactions in the NPSG. The conceptually simple producer-consumer model has yielded to a more complex paradigm of hypothesized flows of energy and matter in this oligotrophic marine ecosystem. A comprehensive understanding of the environmental controls on microbial rates and processes, from genomes to biomes, will be required to inform the scientific community and the public at large about the potential impacts of human-induced climate change. As the surface waters of the NPSG become warmer, more stable and more enriched in carbon dioxide (CO_2), the energy and matter pathways will likely be altered with an overall reduction in their ability to sequester carbon. The pace of new discovery, and the importance of integrating this new knowledge into conceptual paradigms and predictive models, is an enormous contemporary challenge with great scientific and societal relevance.

INTRODUCTION

In 1999, one of us (Karl 1999) published a "mini review" in volume #2 of *Ecosystems* that summarized the state of knowledge on microbial and biogeochemical variability in the North Pacific Subtropical Gyre (NPSG). At that time, the Hawaii Ocean Time-series (HOT) program had already amassed one of the most comprehensive data sets for any open ocean ecosystem (10 years of approximately monthly observations), and was beginning to erode the "chronic undersampling" barrier that existed at that time. Because previous zonal and meridional transects across the NPSG had documented weak horizontal gradients in many physical,

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chemical and biological characteristics, relative to other regions of the ocean (Venrick 1971; Hayward and McGowan 1985), serial observations at a strategically located sampling site might be scaled up to the larger region of interest (Karl 1999). This basic premise underlies the establishment of Station ALOHA (A Long-term Oligotrophic Habitat Assessment) at 22°N 45'N, 158°W (Figure 1) as a NPSG benchmark (Karl and Winn 1991; Karl and Lukas 1996). The establishment of a deep-water, open ocean station would also provide an opportunity to test novel methods and instrumentation, and to serve as a natural laboratory for hypothesis-testing, experimentation and ocean science education and training. These data sets would eventually be used to develop a predictive capability for global environmental change, by elucidating the pathways and controls on physical and biological processes that govern the fluxes of carbon (C) into and from the ocean, especially the key roles of microorganisms.

Recently, the HOT program has grown in scope and stature by the addition of several complementary, trans-disciplinary research programs that are co-located at Station ALOHA (see below). In November 2015, the American Society for Microbiology designated Ocean Station ALOHA as a "milestones in microbiology" site in recognition of historic and visionary accomplishments. While our general understanding of microbial and ecosystem dynamics has greatly advanced over the past few decades, in part due to the research conducted at Station ALOHA, there remains a number of unresolved questions, puzzles, enigmas and important scientific challenges. This review will focus on four complementary themes: (1) the fundamental role of sunlight in the structure and function of the NPSG; (2) microbial diversity, including genes and genomes, trophic structure and population interactions; (3) productivity, including solar energy capture, links to major bioelement cycles and export to the deep sea; and (4) variability, including the time-space continuum, diel processes, mesoscale eddies and

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potential impacts of human-induced climate change. The review concludes with some thoughts on future challenges and opportunities.

NPSG Research: From the *HMS Challenger* to HOT, C-MORE and SCOPE

The worldwide voyage of the *HMS Challenger* (1872-1876) is generally considered by science historians to mark the beginning of modern oceanography. In July 1875, on the Yokohama, Japan to Honolulu leg of the expedition, the *HMS Challenger* tracked due east from Japan into the NPSG, then south to Hawaii. Soundings were made and samples were collected throughout the NPSG. These samples were used to measure the chemical composition of seawater, including organic matter content and dissolved carbon dioxide (CO_2). At that time, there was a greater interest in planktonic microorganisms that were fossilized in the deep-sea sediments than in obtaining an understanding of the contemporary populations in the upper portion of the water column where solar energy is harvested by photosynthesis. Key microbial processes that are now known to be crucial for marine ecosystems, such as nitrogen (N_2) fixation (the conversion of dinitrogen gas to ammonia) and nitrification (the conversion of ammonia to nitrate), were not yet known to science. Despite the pioneering work of Louis Pasteur (1822-1895) and Robert Koch (1843-1910) on infectious diseases, vaccination and microbial fermentation – collectively the "First Golden Age of Microbiology" – it would be another two decades before the German biologist Bernard Fischer would publish his landmark treatise *Die Bakterien des Meeres* (*Bacteria of the Sea*), a monograph that stimulated field investigations of marine microbes, including viruses, and eventually led to the modern discipline of microbial oceanography (Karl and Proctor 2007).

A major benchmark in NPSG research was the Pacific sector investigations conducted during the worldwide Danish *Galathea* deep-sea expedition (1950-1952). On 29 March 1952, at a

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station approximately 200 km NE of the current site of Station ALOHA, Steemann Nielsen made the first modern measurements of rates of the photosynthetic production of organic matter using a novel ^{14}C tracer method (Steemann Nielsen 1956; Steemann Nielsen and Jensen 1957). The general availability of this radioactive isotope of C following World War II (^{14}C was discovered in 1940 by a University of California research team led by Martin D. Kamen; Ruben and Kamen 1940), resulted in the rapid discovery and explicit understanding of C assimilation pathways in photoautotrophic microorganisms in the sea, and contributed enormously to our understanding of the marine C cycle. Based on these initial studies, and extensive subsequent work by Maxwell Doty of the University of Hawaii and others, primary production in the NPSG was reported to be very low (100-200 mg C m^{-2} d^{-1}) with negligible seasonal and interannual variability. So began the modern era of the study of microbial dynamics in the NPSG.

Starting in the late 1960s, and for a period of approximately two decades, scientists from the Scripps Institution of Oceanography conducted a series of major expeditions to the NPSG (Hayward and others 1983). Collectively their observations from the "Climax-region" (centered at 28°N, 155°W) provided an unprecedented view of the NPSG habitat and served as the basis for the extant paradigm of ecosystem structure and function as the HOT program began. The ^{14}C-based primary production data obtained from the Climax study region between 1968-1980 generally confirmed the anticipated low rates of photoautotrophic C fixation (Hayward and others 1983), and reinforced the existing perception that the expansive low nutrient surface regions of anticyclonic ocean gyres were aquatic analogues of terrestrial deserts. However, the coarse sampling frequency (18 expeditions conducted between 1971-1985 with 70% in summer months of June-Sept) was unable to resolve seasonal or interannual variability.

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From January 1969 to June 1970, scientists from the University of Hawaii conducted 13, 2-day research cruises to the deep-water (4,760 m) hydrostation Gollum (22°10'N, 158°W) where they measured key physical and chemical variables, including ^{14}C-based primary production and concentrations of particulate organic matter (POM; Gordon 1970; 1971). However, like most ocean time-series programs that have ever been established, this one also ended before it could have a major impact on the discipline.

In 1986, a biogeochemical time-series station was established in the NE sector of the NPSG (33°N, 139°W) as one component of the VERtical Transport and Exchange (VERTEX) program. For an 18-month period beginning in October 1986, investigations were conducted to determine the seasonality and controls of C export from the upper ocean relative to contemporaneous primary production. These were among the first downward particle flux measurements made in the NPSG. Significant temporal variability was observed in both particle production and export, but no clear relationships or physical controls on these biogeochemical processes could be discerned (Knauer and others 1990; Harrison and others 1992).

In response to a growing awareness of the ocean's role in climate and global environmental change, a comprehensive, interdisciplinary research project was established in 1986 under the *International Geosphere-Biosphere Programme (IGBP): A Study of Global Change*. A year later, the Joint Global Ocean Flux Study (JGOFS) was created as a core project of IGBP with a research focus on the oceanic C cycle, its sensitivity to change and its role in regulating the atmosphere-to-ocean CO_2 balance (Brewer and others 1986). To achieve these stated goals, JGOFS sponsored the establishment of long-term, time-series observations at strategic sites, including Station ALOHA in the NPSG (Karl and Lukas 1996; Karl and others 2003a).

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The immediate successes of the HOT program brought attention to the poor state of knowledge about the sea around us, and catalyzed the establishment of similar programs at strategic coastal and open ocean locations worldwide. In 2006, a U.S.-National Science Foundation center of excellence in microbial oceanography was established at the University of Hawaii in collaboration with six partner institutions. The Center for Microbial Oceanography: Research and Education (C-MORE) was literally built on a "HOT foundation" and provided additional intellectual assets and opportunities for hypothesis-testing, experimentation and education and training. The co-location of these two major field programs served to consolidate the importance of Station ALOHA as a site to advance the discipline of microbial oceanography. Between 2006-2016, C-MORE scientists made numerous discoveries of novel microorganisms, metabolic pathways and population interactions. They also facilitated an understanding of the time-space continuum by conducting higher frequency temporal sampling (hourly to weekly) than was possible to achieve in the HOT program structure, and expanded regional analyses using ships and remotely-operated vehicles to better understand mesoscale variability (10-100 km) in microbial dynamics in the NPSG (also see *Variability* section).

In 2014, a second complementary field-based research program was established at Station ALOHA. The Simons Collaboration on Ocean Processes and Ecology (SCOPE) is an international research program with a mission to characterize the flows of energy and matter through NPSG microbial assemblages. The primary stated goal for this planned, decade-long program is to quantify the inputs to, and outputs from, the upper ocean (0-200 m) at Station ALOHA. Principal areas of scientific interest include assessments of total solar energy capture (by all processes), microbial C production and respiration, controls on grazing and viral lysis, and the fluxes and fates of exported C and energy. Since its inception in October 1988, field

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research at Station ALOHA has helped to create and sustain a high level of excitement about the role of microbial life in the sea (Karl and Church 2014).

Station ALOHA: A Proving Ground for Microbial Oceanography

For more than a century, oceanographers have recognized and defined specific biomes, with generally predictable physical, chemical and biological features. Longhurst (1998) has formalized the classification of a coastal boundary biome and three major oceanic zones (polar, westerlies and trades). A key physical feature of the trades biome, which extends from approximately 30°N to 30°S (~45% of the global ocean), is the presence of a seasonally stable pycnocline/thermocline caused by the radiation balance of positive downward heat flux across the sea surface. The broad trades biome can be further divided into distinct equatorial and gyre habitats, the latter representing some of the most nutrient-depleted regions on our planet, aquatic analogues of terrestrial deserts. However, unlike the dry deserts on land, the aquatic counterparts, and especially the NPSG, are teeming with complex, metabolically active and functionally diverse microbial communities that are well adapted for life in these relatively extreme environments. Because large anti-cyclonic gyres analogous to the NPSG are also found in the South Pacific Ocean, the Indian Ocean and both hemispheres of the Atlantic Ocean, they collectively represent an important biome for marine C cycle research, and need to be better understood if we hope to predict the global impacts of environmental variability and climate change.

Research at Station ALOHA has had a major impact on how we study, interpret and model microbial processes in oligotrophic gyre ecosystems. Many of the general characteristics of the NPSG habitat were known prior to the establishment of Station ALOHA (Karl and Lukas 1996), but other key features were either poorly understood or not yet discovered. For example, the

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three major groups of microorganisms that are numerically dominant members of the NPSG ecosystem (i.e., *Prochlorococcus* spp., the SAR11 clade of Alphaproteobacteria and planktonic archaea) were all discovered after the establishment of Station ALOHA in 1988 (Karl and Church 2014). While marine viruses were discovered in the late 1970s (Torrella and Morita 1979), it was not until a decade later that quantitative estimations were available (Bergh and others 1989). It is probably fair to say that without proper consideration of these dominant microorganisms, the NPSG ecosystem paradigms prior to 1988 could not have accurately described even the most basic aspects of energy and matter fluxes in the sea. Furthermore, the origins of the marine –omics revolution post-date the establishment of Station ALOHA, e.g., the first bacterial genome sequence (Fleishmann and others 1995), the first marine metagenome (Venter and others 2004) and the first marine metatranscriptome (Frias-Lopez and others 2008), so we had scant knowledge of microbial diversity, population structure and dynamics or controls on gene expression when the HOT program began.

Open ocean ecosystems like those represented by Station ALOHA are characterized by warm surface waters (>24°C) throughout the year that establishes a permanent low density cap, isolating the upper layer (~0-100 m) from the water column below. This stratified upper zone has excess solar energy, but is chronically inorganic nutrient depleted (Karl and Church 2014). In contrast, deeper waters (>100 m) have limited or no solar energy, but an excess of inorganic nutrients. This observed physical separation between excess light in surface waters and excess nutrients at depth leads to a condition of extreme oligotrophy with low concentrations of chlorophyll *a* (<100 μg m^{-3}), relatively low biomasses of living photoautotrophic and chemoorganotrophic microorganisms and relatively high proportions of organic detritus (i.e., dissolved and non-living particulate organic matter). For example, the average concentrations of

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dissolved organic C (DOC), particulate organic C (POC) and biomass C (including both autotrophic and heterotrophic microorganisms) at Station ALOHA for the 0-50 m and 200-1000 m depth regions are 93, 2.3, 0.8 mmol C m^{-3} and 48, 0.43, 0.05 mmol C m^{-3}, respectively, so nearly all the organic matter in the sea is non-living (Karl and Dobbs 1998). Furthermore, a liter of surface seawater at Station ALOHA typically contains only a few nanomoles of nitrate but has ~3-5 micromoles of dissolved organic nitrogen (DON). The chemical composition, bioavailability and turnover time of this relatively large DON pool is mostly unknown. Equally large pools of dissolved organic phosphorus (DOP) are also present year round in surface waters at Station ALOHA. For DOP, a large proportion (>80%) of this dissolved pool is readily assimilated by microorganisms (Björkman and others 2000) and, therefore, must be continuously produced in order to replenish the pool. The mechanism by which these dissolved nutrient pools are sustained is not well known despite the obvious importance of DOC, DON and DOP for controlling energy flow in these pelagic ecosystems.

Detailed descriptions of the seasonally-resolved, basic habitat characteristics have been summarized elsewhere (Karl 1999; Karl and others 1996, 2001a, 2008; Church and others 2013; Karl 2014a; Karl and Church 2014) and will not be repeated here, other than to emphasize the fundamental role of sunlight in structuring communities and ultimately controlling most microbiological rates and processes (see below). The complete Station ALOHA data set is publicly available at *http://hahana.soest.hawaii.edu/hot/hot_jgofs.html* along with a listing of the >650 scientific papers based on field observations, methods development and applications, and physical-biogeochemical model simulations of ecosystem processes.

Light: The "Staff of Life"

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Simply stated, light is life. All ecosystems on Earth, all biogeochemical cycles and all evolutionary processes are ultimately solar powered. Consequently, a comprehensive understanding of ecosystem structure and function must include a detailed analysis of rates and controls on solar energy capture and transformation (Karl 2014b). In the NPSG, the major portion of solar energy is harvested by phytoplankton via "green plant-like photosynthesis" termed oxygenic phototrophy (OP). Light-driven oxidation of water provides electrons and hydrogen ions that partially conserve the solar energy absorbed by chlorophyll *a* to catalyze the production of a variety of reduced inorganic and organic molecules, and produce free oxygen (O_2). It has been estimated that approximately 5 x 10^{14} W of solar energy are captured per year to support global oceanic primary production, a value that is nearly two orders of magnitude greater than the energy expended by the human-based economies of the world (Kolber 2007). However, field observations of primary production in the NPSG (summarized below for Station ALOHA) indicate that less than 1% of the total solar energy flux is actually harvested by marine phytoplankton due to nutrient limitation and correspondingly low biomasses of photosynthetic microorganisms. A recent analysis of solar energy capture by photosynthetic pigments at Station ALOHA for the period Jan 2006 – Dec 2012 found that the daily absorption of photons was similar in summer and in winter (0.46 ±.10 and 0.45 ± 0.12 mol photons m^{-2} d^{-1}, respectively) despite seasonal changes in total solar energy flux (Letelier and others 2016). At greater depths (>100 m) summertime rates exceeded winter by nearly a factor of two. Assuming a constant photosynthetic yield of 0.1 mol O_2 evolved per mol quanta absorbed by photosynthetic pigments, the authors estimated that depth-integrated (0-200 m) GOP ranged from 64 (winter) to 79 mmol O_2 m^{-2} d^{-1}.

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Both the inherent optical properties of light in the ocean (absorption and scattering) and the pathways of solar energy capture during OP are well known (Falkowski and Raven 2007; Kirk 2011). However, new aspects of microbial phototrophy in the sea continue to be discovered. For example, since the HOT program began in 1988, two novel, unexpected pathways of solar energy capture, that differ significantly from OP, have been reported. These new pathways of solar energy capture, aerobic anoxygenic phototrophy (Kolber and others 2000; 2001) and proteorhodopsin phototrophy (Béjà and others 2000; 2001), are most likely facultative mechanisms to supplement the energy demands and to improve the growth rates/efficiencies of otherwise obligate chemoorganoheterotrophic metabolisms in select marine bacteria and archaea. These profound discoveries could fundamentally alter our views of life in the sea, but we currently lack a quantitative understanding of the rates, controls and ecological significance of these alternate energy capture pathways in nature (Karl 2007, 2014b).

The "euphotic zone" (literally, well lit) is defined as that portion of the water column where there is sufficient light energy for net photosynthesis (Figure 2); the minimum energy flux is termed the photosynthetic compensation irradiance. At Station ALOHA, the compensation irradiance for OP is 0.054 mol photons m^{-2} d^{-1}, which is equivalent to ~0.11% of surface 400-700 nm radiation (also termed photosynthetically available radiation [PAR]) and 1% of surface 475 nm (blue) light based on comparisons of the light versus dark assimilation of ^{14}C-labeled bicarbonate (Laws and others 2014; Figure 2). The corresponding compensation depth varies depending on temporal changes in water clarity, but is on average 173 m (±7 m) at Station ALOHA (Letelier and others 1996). It is not known whether aerobic anoxygenic phototrophy or proteorhodopsin-based phototrophy can occur beneath the OP compensation depth. Opposing seasonal variations in surface PAR (~50% greater in summer than in winter) and water column

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PAR attenuation coefficient (~20% greater in winter than in summer) combine to yield an ~30 m depth displacement of the 1% light level from 120 m in summer compared to 90 m in winter (Letelier and others 2004; Figure 2). The top of the nitricline, defined as the shallowest depth at which the nitrate concentration gradient exceeds 2 μmol m^{-4}, also migrates upward in winter in response to the decreasing photon flux. This complex interaction of seasonal light, nutrients and phytoplankton result in a significant increase in chlorophyll *a* concentration and deepening of the deep chlorophyll maximum layer (DCML) each summer (also see *Variability* section). Letelier and others (2004) estimated that there is a net removal of at least 36 mmol m^{-2} of nitrate in the 90-200 m depth each summer leading to both an increase in chlorophyll *a* (phytoplankton biomass) and enhanced POM export from the lower euphotic zone.

PAR continues to decrease beneath the photosynthetic compensation depth and even at 800 m many marine organisms can sense and respond to this dim light. Indeed, some zooplankton and fish migrate from near the surface of the ocean to depths of ~1000 m on a daily basis in response to downwelling irradiance (Plueddemann and Pinkel 1989). A recent field study at Station ALOHA documented diel vertical migration of 0.5-2 mm sized zooplankton from depths as great as 1000 m during daylight hours to surface waters at night. The day-to-night differences due to migrant zooplankton was 260 mg and 21 mg (dry weight) m^{-2} for the upper 0-100 m and 700-1000 m depth intervals, respectively (Hannides and others 2013). Because the migrant zooplankton feed in the surface at night and respire, defecate and are subject to predation at depth, diel vertical migrations represent a net downward flux of C and energy at Station ALOHA (Al-Mutairi and Landry 2001; Hannides and others 2009).

In addition to solar energy, bioluminescence (biologically produced light) is ubiquitous in the marine environment. However, bioluminescence is also solar powered since it derives from

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chemical reactions in cells and tissues that ultimately owe their existence to sunlight. Bioluminescence has a variety of functions, including quorum sensing and cell signaling, predation (offense) and predator avoidance (defense), mating and many other essential functions (Haddock and others 2010). Below about 600 m in the NPSG, the mean photon flux from bioluminescence exceeds downwelling sunlight (Bradner and others 1987; Figure 2). Bioluminescence decreases with depth reflecting a general decrease in total living biomass but is still detectable even at the greatest ocean depths (Figure 2). In addition, there is a very dim glow of Cherenkov radiation from electrons emitted in the decay of radioactive potassium (^{40}K; ~120 photons cm^{-2} sec^{-1}), though we have no precedent for ^{40}K-based biological function.

Finally, light is also an important tool in microbiological oceanography since many of the instruments employed, especially those capable of *in situ* observations, are based on passive or active (light-stimulated) light detection. This includes irradiance meters, transmissometers, scatterometers, spectrophotometers, fluorometers, optodes, flow cytometers and satellite-based radiometers, to name a few. These have all been used at Station ALOHA to measure the concentrations of solutes and particles and to track the metabolic activities of selected populations and communities.

Diversity: The "Spice of Life"

It has been said that "variety is the spice of life, that gives it all its flavour" (William Cowper 1785, *The Task*). A hallmark of the NPSG is its diversity, which is due in part to its age and extensive evolutionary diversification. The diversity metrics of greatest interest to ecologists and microbial oceanographers include taxonomic/phylogenetic diversity, metabolic/trophic diversity and habitat/niche diversity. All three of these related properties contribute significantly to the

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spice of life and combine to maximize the flow of energy, as well as to confer resilience to environmental variability.

During the pioneering Climax program, comprehensive investigations were undertaken on the phylogenetic diversity of planktonic organisms – both eukaryotic phytoplankton and mesozooplankton. These studies revealed the presence of a coherent, high-fidelity assemblage of co-occurring mesozooplankton species that were unique to the NPSG, and presumably well adapted to the oligotrophic conditions of the ecosystem (McGowan 1974). Although not known at that time, and still poorly understood, each of these unique species probably supports a unique microbiome of associated bacteria and other microorganisms that are both internal and external to the individuals of that population. These mesozooplankton-associated microbial assemblages live within environments that are fundamentally distinct from each other and from the free-living microorganisms in the sea around them. The microbiomes of vertically migrating zooplankton must be able to withstand relatively large and rapid changes in temperature and pressure, or else grow fast enough to re-establish each day. Some, but not all, of these zooplankton-associated microbes are obligate symbionts; others are opportunists, parasites or disease-causing pathogens. Beyond mesozooplankton microbiomes, it is important to emphasize that all living organisms, including microorganisms, also contain unique, perhaps essential, co-existing microorganisms that sustain host species integrity and function in the ecosystem. With advances in DNA sequencing technologies and a general awareness and interest in environmental microbiome ecology, we can anticipate rapid progress in this area over the next few decades.

Phytoplankton, broadly defined here as “obligate” or facultative photoautotrophic microorganisms (including both prokaryotic and eukaryotic forms) play a fundamental role in solar energy capture and organic matter production in the sea. Consequently, there has been

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great interest in phylogenetic diversity of phytoplankton in the NPSG, and several recent discoveries have led to an ever-evolving understanding of the taxonomic diversity and evolutionary histories of NPSG phytoplankton. These research efforts can be divided into three major phases: (1) microscopic analysis of eukaryotic phytoplankton with a primary focus on the phylogeny of diatoms as an important taxonomic group in the NPSG (pre-1980), (2) the discovery and exploration of picophytoplankton, small (<2-3 μm) eukaryotic and prokaryotic phototrophs that fundamentally changed our view of the size structure and trophodynamics of the NPSG (1980-2000) and (3) the development and ecological application of novel DNA sequence-based methods to reveal genetic diversity and evolutionary relationships between and among the diverse phytoplankton assemblages in the NPSG. These specific phases of research coincided with advances in technology including improvements in sampling, isolation and culturing methodologies, as well as the development of advanced instrumentation for the analysis of phytoplankton assemblages (e.g., electron microscopes, flow cytometers, DNA sequencing and bioinformatics).

Venrick (1971, 1982, 1990b) produced the first comprehensive analysis of eukaryotic phytoplankton diversity in the NPSG. Her pioneering research revealed the presence of high fidelity associations of diatoms and other phytoplankton species (Venrick 1971). Two distinct recurring groups of phytoplankton were observed in the upper and lower portions, respectively, of the water column separated by a region of rapid transition at approximately 100 m (Venrick 1982). This transition depth approximates the boundary between a light-saturated, nutrient-limited upper euphotic zone and a deeper light-limited region where nutrients begin to accumulate. Subsequent field observations during six summer cruises over a 12-year interval (1973-1985), revealed 245 and 231 unique phytoplankton species in shallow (0-100 m) and deep

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(100-200 m) portions of the water column within the Climax study region (Venrick 1990b). For each zone, 21 species accounted for ~90% of the individuals, along with a large number of rare species. The most remarkable conclusion of this exhaustive field study was that the rank orders of abundance of the major phytoplankton groups were similar over the >1-decade observation period (equal to >1000 generation times for an average phytoplankton species), and that the surface populations separated by time were more similar to each other than the surface versus deep for any given year (Venrick 1990b). This relative stability in eukaryotic phytoplankton community structure was preserved despite a near doubling of the total depth-integrated chlorophyll *a* over this same period of time (Venrick and others 1987; Figure 3). It was hypothesized that this low interannual variability in phytoplankton species composition must reflect a relatively stable habitat structure. Because the Climax region is approximately 650 km to the northeast of Station ALOHA, Venrick (1997) also compared the larger eukaryotic phytoplankton species structure (rank order of abundance) in August 1994 at Station ALOHA to her historical data set from Climax. The general physical features of both habitats were similar (e.g., water column stability and concentrations of nutrients and chlorophyll *a*) as were the vertical distributions of the shallow and deep water phytoplankton assemblages. Nevertheless, two notable species were observed in much higher abundances at Station ALOHA. The first species, *Phaeocystis* cf. *pouchetti* a colonial prymnesiophyte which was found in high abundance at 5 m at Station ALOHA, is known to form massive blooms in a variety of marine environments. The second important species was the filamentous, N_2-fixing cyanobacterium, *Trichodesmium* spp. which is also known to form blooms under certain environmental conditions (Venrick 1997). It could not be determined from that study whether these differences were due to spatial or temporal (or both) separation (also see *Variability* section).

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Our understanding of life in the sea around us is often limited by the methods available or employed. In the case of Venrick's pioneering research on phytoplankton diversity and community structure, the analyses were biased toward the larger size classes of phytoplankton that were at that time thought to be the most numerous and important. In her study, whole water samples were collected and preserved in buffered formalin. Subsamples (~250 ml) were allowed to settle for 48 hr, and the concentrate was transferred to an Utermöhl settling chamber and allowed to settle for an additional 48 hr. Three fractions of the settled sample were enumerated using a phase-contrast inverted microscope: (1) the entire sample (100% of total) at 100-125x for large diatoms and dinoflagellates, (2) every 6th row (16.7% of total) at 250-312x for smaller cells such as coccolithophorids and (3) 1 diameter at 400-500x (0.9% of total) for even smaller cells. More than 250 categories of phytoplankton were enumerated including individual species, species complexes and subspecies (Venrick 1982). These details are important since this comprehensive, pioneering analysis of NPSG phytoplankton did not include the picophytoplankton (0.2-2 μm) that we now recognize as a dominant component of the NPSG ecosystem (see below). However, no one could have predicted the pace, scope and fundamental implications of the discoveries of the next decade (1980-1990). The intersection of new methods, new career opportunities and the recruitment of a cadre of creative individuals, the realization that marine microbial ecology must be conducted "at sea" and new funding opportunities for oceanography led to the creation of the new discipline of microbial (microbiological) oceanography (the first use of this term may have been Karl and Winn 1984).

The picophytoplankton "revolution" can be traced back to two independent field reports. Waterbury and others (1979) used 0.2 μm Nuclepore filters and a 100-W halogen lamp-equipped eipfluorescent microscope to visualize and enumerate small phycoerythrin-containing

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cyanobacteria in water samples collected from the Arabian Sea, Peruvian coastal waters and the Gulf Stream. A large number (10^4-10^5 per ml) of small (0.8-2 μm) unicellular cyanobacteria were observed in all samples, leading the authors to conclude that these small unicellular cyanobacteria are not only abundant but also geographically widespread and, therefore, may be important components of coastal and open ocean ecosystems (Waterbury and others 1979). The cells that were observed using epifluorescence microscopy were similar to a small, coccoid cyanobacterium previously isolated (and cultured) by R. Guillard off South America that was assigned to the genus *Synechococcus*. This important discovery of the marine picophytoplankton concluded with the equivocation: "*The ecological importance of these cyanobacteria as major primary producers in the world's oceans is still to be assessed*."

Johnson and Sieburth (1979), independently working in the North Atlantic Ocean, reported the presence of a diverse assemblage of small (<2 μm) chroococcoid cyanobacteria in the open sea. Transmission electron microscopy (TEM) confirmed that these cells were prokaryotic, contained photosynthetic thylakoids and, hence, were likely to be photosynthetically active under *in situ* conditions. Three basic cell types differed with respect to mean size (ranging from 0.4 μm to 1.0 μm), membrane structures and other key morphological characteristics. The larger "Type I" cells were able to be cultured; the other two morphotypes were not. The authors speculated that these picophytoplankton may be a major food source for oceanic protozooplankton and that some species might even fix N_2 or perform other key ecosystem services (Johnson and Sieburth 1979). They concluded their discovery paper with a rather prescient statement: "*These cells may play a significant role in oceanic primary productivity*." Indeed Iturriaga and Mitchell (1986) demonstrated that coccoid cyanobacteria were also very abundant in the NPSG, and that they accounted for a majority of the ^{14}C-based primary

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production. They also used radiolabeled cells and microautoradiography to track the consumption of photosynthetic picoplankton into protistan grazers, thereby confirming an active and efficient microbial loop.

In a subsequent report using the same TEM analysis of thin sections of natural microbial assemblages, Johnson and Sieburth (1982) reported a diverse assemblage of eukaryotic picoplankton, including a prasinophyte-like cell that was only 0.5-1.0 μm in diameter, the smallest phototrophic eukaryote ever reported. Attempts to culture these organisms failed to recover most of the observed cell types, so further taxonomic and physiological characterization was not possible.

Shortly thereafter, a novel and abundant group of unicellular cyanobacteria containing both chlorophyll *a* and *b* was discovered in the North Atlantic Ocean and, subsequently, in other marine habitats (Chisholm and others 1988; Li and Wood 1988; Olson and others 1990). Although Lewin (1976) had previously reported a new division of algae, *Prochlorophyta*, based on his discovery of a group of symbiotic photosynthetic prokaryotes containing both chlorophyll *a* and *b*, the discovery of free-living, plankton *Prochlorococcus* was a major benchmark in the history of microbial oceanography. Gieskes and Kraay (1983) had previously reported the presence of high concentrations of "red-shifted" chlorophyll *a* in <1 μm particles collected from the tropical Atlantic ocean. This pigment was later shown to be divinyl chlorophyll *a*, a diagnostic molecular biomarker for *Prochlorococcus*. Sadly, the pioneering discovery of Gieskes and Kraay (1983) took nearly three years to pass peer review, based on the "submitted" and "accepted" dates for this paper.

Prochlorococcus is now recognized as the most abundant photoautotroph in tropical and subtropical marine environments worldwide, including Station ALOHA. This discovery, and

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subsequent research on physiological variability (Moore and others 1998), genetic diversification (Coleman and Chisholm 2007) and niche partitioning in space and time (Bouman and others 2006; Johnson and others 2006) has led to a radically different food web and energy flow paradigm for the NPSG than was present when the HOT program began. A recent review of *Prochlorooccus* reveals the deeply-rooted evolutionary diversity, genomic hypervariability and broad physiological capacity of what is now termed a "federation" of coexisting cells (Biller and others 2015). There is enormous cell-to-cell genomic diversity (Kashtan and others 2014), and it is not entirely clear, either for *Prochlorococcus* or for other microorganisms, how the diversified pan-genome is sustained and whether co-existing *Prochlorococcus* cells occupy the same niche or not. Since the function of many "genes" is not yet known, a determination of precise metabolic function or ecological role is not possible at the present time. Although we know that *Prochlorococcus* is an important photosynthetic organism in the NPSG, it may not be an obligate photolithoautotroph, and recent taxon group-specific measurements conducted at Station ALOHA have shown significant light-dependent assimilation of leucine and adenosine-5'-triphosphate with cell-specific rates that are on par with non-pigmented, heterotrophic bacteria (also see *Productivity* section).

At Station ALOHA, the pico- and nano-sized phytoplankton components are now routinely analyzed using flow cytometry (Campbell and Vaulot 1993; Campbell and others 1994) and epifluorescence microscopy (Pasulka and others 2013). *Prochlorococcus* dominates both in terms of cell numbers and biomass, accounting for nearly half of the total phytoplankton C in the upper 100 m of the water column (Campbell and others 1994; Pasulka and others 2013). While the cell numbers are fairly accurate, especially using state-of-the-art flow cytometers, extrapolation to biomass-C is less certain and is wholly dependent on the C per cell or C per cell

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biovolume employed for the extrapolation. Pasulka and others (2013) used 32 fg C per cell to convert *Prochlorococcus* cell numbers to biomass, but a broad range of values (16 ± 1 to 53 ± 9; summarized in Buitenhuis and others 2012) has been reported in laboratory cultures. Our own direct estimates of *Prochlorococcus* cell size at Station ALOHA suggest that wild populations may be much smaller (~0.55 μm diameter) than laboratory cultures and mean cell size changes significantly within the euphotic zone (larger at greater depths). Consequently, the biomass of the total *Prochlorococcus* assemblage, and therefore total phytoplankton biomass, is not well constrained. Spatial and temporal changes in cell numbers may, or may not, indicate changes in biomass.

Pasulka and others (2013) also present data on picoeukaryotes, both autotrophic (i.e., pigmented) and non-pigmented cells, and larger size classes of phytoplankton (both nano [2-20 μm) and micro [20-200 μm] plankton) which together account for approximately 50% of the total phytoplankton biomass at Station ALOHA (with the previously stated caveats regarding conversion of cell numbers to biomass-C). The phototrophic protist assemblage was dominated by prymnesiophytes and other small flagellates. Larger sized autotrophic dinoflagellates and diatoms were also present, but each contributed only ~1% to total phytoplankton biomass. This comprehensive study included a nearly 5-year, approximately monthly, data set revealing numerous trends in population structure with depth and season (Pasulka and others 2013).

In addition to these microscopic-based analyses, Li and others (2013) recently employed a molecular-based method to quantify chromophytic phytoplankton (diatoms, pelagophytes, prymnesiophytes and dinoflagellates) using sequence analysis of form ID *rbcL* genes. The observed *rbcL* gene clone libraries revealed 4 major clades of chromophytic algae consisting of a diverse assemblage of genera with most taxa predicted from the Climax data set, but also many

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novel clones. This pioneering use of a genetic biomarker for diatoms confirmed the presence of vertically segregated species assemblages (Venrick 1982, 1997), and the more recent reports of the role of prymnesiophytes and pelagophytes at Station ALOHA (Andersen and others 1996; Cortés and others 2001). The Li and others (2013) study also confirmed the year-round presence of diatoms and related chromophytic phytoplankton at Station ALOHA, despite their very low percent abundance in the total phytoplankton assemblage at Station ALOHA. These field observations demand a better understanding of the respective niches of macrophytoplankton, nanophytoplankton and picophytoplankton in the dynamics of the NPSG (also see *Productivity* section).

More recently, Rii (2016) examined the diversity of eukaryotic picoplankton populations at Station ALOHA using 18S rRNA gene fragment analysis over a 2-year period (2011-2013). The supergroup Alveolata comprised a majority (~58%) of total operational taxonomic units (OTUs) recovered from the euphotic zone (0-175 m), followed by Stramenopiles (~10%) and Rhizaria (~9%). Within the Alveolata category, Syndiniales Group I and II, both known to be parasitic, were dominant (Rii 2016). Non-photosynthetic, heterotrophic picoplanktonic OTUs accounted for approximately 16% of the total.

Based on these collective efforts at Station ALOHA, we now know that the picophytoplankton size fraction (approximately 0.2-2 μm), rather than the macrophytoplankton size fraction (20-200 μm), is the dominant component of the photoautotrophic community both in terms of numbers and biomass. This is a fundamentally different paradigm than the one reported from the Climax-era data sets. It is difficult to comprehend that the extensive field-based efforts in the NPSG prior to 1980 could have missed such a fundamental and ubiquitous portion of the marine food web, especially considering the very high level of intellect and

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creativity within selected field-based research groups (e.g., The Food Chain Research Group of the Institute of Marine Resources at Scripps Institution of Oceanography; Strickland 1972). Indeed, this situation led one of us to hypothesize that there may have been a systematic shift in the size structure and composition of the phytoplankton assemblages in the NPSG beginning about 1976 (Karl 1999; Karl and others 2001a). Evidence was provided for a hypothesized "domain shift" from larger eukaryotic phytoplankton to dominance by smaller prokaryotic populations with altered food web structures, new and export production processes and fishery yields. It was further hypothesized that these changes may have been the result of climate variability, especially enhanced stratification of near-surface waters and decreased concentration of nutrients, both of which would select for small phytoplankton species. Observed changes in pigments, rates of primary production and N_2 fixation may be additional manifestations of changing ocean habitats and communities in the NPSG (Karl and others 2001a).

The diversity of phytoplankton collected from the NPSG can be experimentally manipulated simply by the addition of nutrient-rich deep seawater to surface ocean assemblages (McAndrew and others 2007; Figure 4). Within just a few days following modest additions of macronutrients (i.e., 1-3 μM nitrate, 0.1-0.3 μM phosphate) the phytoplankton assemblage shifts from a pico-sized (<2 μm), *Prochlorococcus*-rich assemblage to one dominated by large (>10 μm) diatoms with attendant changes in net community metabolism (McAndrew and others 2007). More recently, Alexander and others (2015) used eukaryotic metatranscriptome analysis to assess the phytoplankton response to nutrient loading at Station ALOHA. Replicated shipboard blooms, stimulated by deep seawater additions, revealed that diatoms and haptophytes significantly up-regulated their metabolic fingerprints relative to controls, whereas dinoflagellates showed little response (Alexander and others 2015). Furthermore, the variable transcript allocation ratio,

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which defines functional group strategies, differed for the two taxonomic groups with diatoms exhibiting an r-trait strategy of growth consistent with their role in oligotrophic ocean blooms. Growth co-limitation by nutrients, metals and vitamins appeared to ultimately control biomass production in their shipboard experiments. The magnitude of the response to nutrient perturbation, and the resultant size spectrum and taxonomic structure of the phytoplankton assemblage vary seasonally, with largest changes occurring in summer (Mahaffey and others 2012). These experimental results indicate that the selection for, or against, a *Prochlorococcus*-dominated assemblage might be related to both the rate of nutrient delivery and the time of year, as well as to longer time-scale changes in habitat structure (Karl and others 2001a; also see *Variability* section). Furthermore, Karl and Letelier (2008) have hypothesized that sustained upwelling of water with a relatively low nitrate:phosphate ratio (as occurs in the NPSG) could lead to a two-stage phytoplankton bloom. Stage one would select against *Prochlorococcus* in favor of diatoms until nitrate is depleted. During stage two, N_2-fixing microorganisms would be selected for, and *Prochlorococcus* would return as a co-occurring and dominant phytoplankton species. An ecosystem with an excess of phosphate relative to nitrate, that is dominated by *Prochlorococcus* where N_2-fixing cyanobacteria provide a significant source of new N, is an accurate description of the contemporary NPSG phytoplankton assemblage and consistent with the predictions of the domain shift hypothesis (Karl and others 2001a). However, a rigorous ecosystem-scale test of the domain shift hypothesis has not yet been possible (White and others 2010), and few sample archives exist for retrospective analysis using appropriate modern tools. On much longer time scales (centuries), it appears that N_2-fixation may be selected for or against, based on hypothesized changes in nutrient dynamics. Sherwood and others (2014) recently reported variations in the N isotopic ratios preserved in the skeletons of long-lived deep-

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sea corals that are consistent with a centennial-scale trend of increased N_2-based export production in the NPSG. A follow-on study of amino acid-specific C isotopic records of the same deep-sea coral specimens constrained the start of the N_2 fixation epoch to approximately 1850 (McMahon and others 2015). These reports provide additional evidence for historical oscillations of periods where either eukaryotic phytoplankton or N_2-fixing cyanobacteria (presumably with *Prochlorococcus*) are dominant primary producers.

So far we have presented information only on the photoautotrophic (i.e., pigmented) component of the extremely diverse microbial assemblage of the NPSG ecosystem. Arguably, the primary producer assemblage is the most critical component of any biome, marine or terrestrial, because of its unique ability to harvest solar energy and to reduce inorganic C to organic matter. However, the sustained activity of photoautotrophs depends critically on co-existing, heterotrophic microorganisms. For example, the continued availability of macronutrients (e.g., ammonia, nitrate, phosphate) requires a diverse assemblage of chemoheterotrophic microorganisms to complete the inorganic-to-organic-to-inorganic cycles of C, N and phosphorus (P). As discussed later in this review, approximately 95% of the organic matter that is produced each day at Station ALOHA is locally recycled, so there is an inextricable and life-sustaining linkage between and among the microbial producers and consumers in the surface waters of the NPSG. Furthermore, many phytoplankton species, especially nano- (2-20 μm) and micro- (>20 μm) eukaryotes require specific vitamins, amino acids or other growth factors that likely derive from co-existing phototropic and chemotrophic prokaryotes, and many "phytoplankton" may be facultative or even obligate mixotrophs (also see *Productivity* section). These "plastidic" (i.e., containing chlorophyll *a*) protists are members of a much larger and diverse group that play a key role in the microbial loop with a broad range of

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functions, from controls on net community production via photosynthesis to controls on net community production via grazing and respiration (Sherr and others 2007; Caron 2016).

In the NPSG and in other marine ecosystems worldwide, most of the phylogenetic diversity derives from the non-photosynthetic components of the ecosystem. Metagenomic surveys, including pioneering work at Station ALOHA (DeLong and others 2006), have revealed an unexpected wealth of genetic variation within the heterotrophic microbial assemblage including many new genes, protein families and a large number of "genes" with yet unknown function. These microbial community metagenomes are stratified along the ocean's depth continuum from the sea surface to the abyss, defining vertically structured microbial assemblages with significant depth-dependent gene repertoires that broadly reflect the changing habitat conditions and microbial lifestyles (DeLong and others 2006). Not unexpectedly, there are unique sets of genes from both the euphotic zone (0-130 m) and the abyss (500-4000 m) that define distinct microbial assemblages analogous to the vertical separation of unique upper and lower euphotic zone phytoplankton species in the NPSG discussed previously. A recent study based on the analysis of bacterial small subunit ribosomal RNA (SSU rRNA) amplicon and shotgun metagenomic sequence analyses followed changes in microbial taxonomic and functional gene diversity and representation at Station ALOHA over a 2-year period (Aug 2007- Sept 2009; Bryant and others 2015). The most dominant microorganism, SAR11 (*Candidatus* Pelagibacter), represented approximately 20% of the total bacterial metagenomic reads at 25 m, after *Prochlorococcus* genes were removed. Other dominant taxonomic groups in the metagenomic libraries included SAR116, SAR86 and SAR406 clusters, and *Roseobacter* all at >1% relative abundances, as well as a large number of less abundant taxa. Furthermore, this study revealed dynamical changes in

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microbial diversity that correlated with seasonal changes in light, and aperiodic perturbations following periods of wind-driven mixing (Bryant and others 2015).

Finally, recent laboratory-based experiments have documented that some *Prochlorococcus* strains grow much better (more rapidly, more efficiently) in the presence of specific chemoheterotrophic bacteria (Morris and others 2008; Sher and others 2011). This symbiotic association is hypothesized to result from the ability of bacteria to remove free radicals that would otherwise be deleterious to the survival of *Prochlorococcus*. Scavenging of hydrogen peroxide may be an important role for such co-existing bacteria, resulting in more efficient growth of *Prochlorococcus* (Morris and others 2011). This and other syntrophic relationships may be partly responsible for the inability to obtain pure (axenic) cultures of many marine phytoplankton. In retrospect, the pure culture approach, guided by Koch's postulates, may have been ill-conceived for an exploration of phylogenetic diversity of NPSG microbial assemblages.

Recently, Yoshimi Rii defended her Ph.D. dissertation entitled "*Ecology of photosynthetic picoeukaryotes in the oligotrophic ocean: Diversity, activity, and dynamics*." This 2016 publication serves as a contemporary bookend to Elizabeth Venrick's pioneering Ph.D. dissertation published in 1969, "*The distribution and ecology of oceanic diatoms in the North Pacific*." It is quite remarkable to consider how much our knowledge base has expanded and evolved during the past half-century.

Productivity: The "Pulse of Life"

Ecologists often use the term "carbon and energy" in the context of system dynamics. However, energy fluxes are rarely measured and even many key C cycle processes are difficult to assess directly leading to equally uncertain extrapolation to the coupled fluxes of associated bioelements like N, P, sulfur and oxygen. Furthermore, while bioelements are recycled, energy

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is unidirectional and is eventually dissipated as heat. Our current state of knowledge is as much determined by the suite of available methods as it is by our conceptual paradigms (Azam 2001). In addition to methodology, it is very important to use precise terminology when discussing these key ecosystem processes (Williams 1993; Chapin and others 2006), and to fully appreciate the uncertainties and caveats in the extrapolation of field measurements to desired state variables and fluxes, especially if bottle incubation methods are employed (Karl and Dore 2001). Nowhere is this more important than in the assessment of ecosystem function, especially the rates, mechanisms, pathways and controls on primary production. In oligotrophic marine ecosystems like the NPSG, we have only a limited number of methods with sufficient sensitivity, reproducibility and accuracy to measure the fluxes of C and associated bioelements. Selected methods, for example, changes in O_2 concentration during dark incubation of a water sample, provide an estimate of a central function – i.e., respiration – that is common to all living cells. Other methods track a specific trait, metabolic process or function (e.g., the rate of N_2 fixation). Separation of activity or function by size or, more recently, by flow cytometric cell sorting (Lomas and others 2011), can provide additional information on selected groups of microorganisms.

In their most basic assessments, gross primary production (GPP) is the conversion of light energy to chemical potential energy (plus a small amount of heat), while microbial community respiration (MCR) is the conversion of chemical energy to heat (Williams and others 1979; Platt and others 1984; Williams 1993). While it would be ideal if we could track fluxes of energy through marine food webs from GPP to MCR, limitations of current methodologies preclude such assessments at the present time. So ecologists have used either O_2 or C, or both, as practical surrogates for energy (Williams 1993). The major limitation in this approach is the

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interconversion of O_2-based to C-based measurements, and vice versa. For example, the photosynthetic quotient (PQ), defined as the molar ratio of the rate of O_2 production to CO_2 reduction is fairly well constrained in laboratory cultures (usually measured in log phase) of marine phytoplankton (e.g., PQ = 1.0-1.3, with systematic variation based on the oxidation state of nitrogen (i.e., ammonia versus nitrite versus nitrate; Davies and Williams 1984). Laws (1991) reported a comprehensive analysis of theoretical PQs based on balanced chemical equations for the production of proteins, polysaccharides, lipids and nucleic acids from inorganic precursors (CO_2, water, phosphate, sulfate and either nitrate or ammonia) and previously measured macromolecular compositions of microalgal biomass. His analysis provides upper and lower constraints of 1.4 ± 0.1 and 1.1 ± 0.1 for obligate photolithoautotrophic growth on nitrate and ammonia, respectively (Laws 1991). However, in nature, phytoplankton may produce a broad range of metabolites and may have growth strategies and constraints that allow PQs to deviate significantly from these theoretical limits. For example, the production of highly oxidized (e.g., glycolic acid or hydrogen peroxide) or highly reduced (e.g., dimethyl sulfide, methane or hydrogen) materials would lead to anomalously low or high, respectively, PQs in natural assemblages of photoautotrophic microorganisms. Furthermore, the relative growth rate of the cells under *in situ* conditions will impact the biomass C:N ratio and, hence, the PQ of photosynthesis. Finally, C- or energy-source mixotrophy among photoautotrophs would lead to a conceptual mismatch between obligately photolithoautotrophic and chemoorganoheterotrophic models of plankton metabolism.

This discussion so far has focused on the interconversion of O- and C-based measurements of GPP, but other elemental currencies can be, and have been, used in ecosystem analyses. In a seminal paper by Dugdale and Goering (1967) the concept of new and regenerated primary

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production was introduced using a N-based currency. The authors defined "new" production as that portion of GPP that was supported by newly available nitrogen (i.e., nitrate-N and N_2-N) whereas ammonia-N supported production was termed "recycled" production (Dugdale and Goering 1967). Conveniently, ^{15}N-labeled nitrate/ammonia could be used in field experiments to measure the new and regenerated proportions and, hence, the factional equivalence of nitrate-based assimilation (e.g., f-ratio = [nitrate assm] / [total N assm]). Furthermore, new production was equated to net community production (NCP), when expressed in N-based currency. Eppley (1989) suggested that the f-ratio could be predicted from measurements of nitrate concentration (Figure 5); however, this empirical relationship lacks predictive value at Station ALOHA where surface nitrate concentrations are consistently ≤10 nmol l^{-1}. Two major assumptions, noted by Dugdale and Goering (1967), but since largely ignored, were that both N_2-fixation ($N_2 \rightarrow$ ammonia) and upper ocean nitrification (ammonia → nitrate) rates were negligible. At Station ALOHA, we now know that both of these assumptions are invalid. Local euphotic zone recycling of ammonia to nitrate appears to be rapid (Dore and Karl 1996; Dore and others 1998) as does the quantitative role of N_2-fixation as a source of new N to the ecosystem (Karl and others 1997; Dore and others 2002; Böttjer and others 2016; see below), so the conceptually and methodologically straightforward analysis of new versus regenerated production can no longer be supported for oligotrophic habitats such as Station ALOHA. Indeed based on our current understanding, much of the surface ocean nitrate at Station ALOHA is probably recycled N (ammonia → nitrate) and a yet unknown portion of ammonia may be new N ($N_2 \rightarrow$ ammonia). This requires us to re-evaluate the new versus recycled paradigm for oligotrophic ecosystems like the NPSG.

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A NPSG Productivity Controversy with Important Implications: Shulenberger and Reid (1981) first reported that the ephemeral subsurface O_2 supersaturation that develops in the NPSG each summer could be used to constrain the net rate of O_2 production over the lifetime of this feature. Based on Climax region observations, their derived estimates of net O_2 production (NOP) greatly exceeded the measured ^{14}C rates, and suggested that primary production may have been systematically underestimated in the NPSG (Shulenberger and Reid 1981). This challenge of one of the most widely used methods in oceanography and the *Science* magazine editorial that followed ("*Are the oceans deserts blooming?*"; Kerr 1983) led to a systematic study of primary production during the Plankton Rate Processes in Oligotrophic Oceans (PRPOOS, pronounced "purpose") program in summer of 1984. Meanwhile, the Shulenberger and Reid (1981) claim was challenged by Platt (1984) on both theoretical and analytical grounds, and a series of heated debates, numerous scientific reports and rebuttals followed (e.g., Reid and Shulenberger 1986; Platt and Harrison 1986). This eventually led to a reconciliation that biological dynamics in the NPSG are intermittent rather than steady and, therefore, must be properly averaged in time and space (Platt and others 1989). The controversy concerning the rate of primary production in the NPSG did not abate (Hayward 1991), and interest in a scientific resolution of the enigma and the need for a better understanding of the role of the ocean in the global C cycle helped to justify the JGOFS program, and HOT.

The primary production controversy also led to the development of a novel method to distinguish biological versus physical contributions to O_2 supersaturations in the upper water column (Craig and Hayward 1987). This approach was based on precise determinations of both O_2 and argon (Ar), the latter as a biologically inert surrogate for O_2. Because the Ar and O_2 solubilities are similar, a precise estimation of contribution of air injection could be made, and

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the independent roles of biological versus physical processes determined (Craig and Hayward 1987). Based on field observations at two stations in the NPSG in summer 1985 during the PRPOOS expedition, the authors found that 68-75% of the total O_2 supersaturation was due to biological production, thereby supporting the previous claims of high rates of NOP in this region.

While the Craig and Hayward (1987) contribution was not the final word on this controversy, and did not resolve the corresponding enigma of identifying mechanisms of nutrient supply to sustain high rates of NOP, it did provide a new method (the so-called O_2:Ar ratio technique) for unambiguous determination of the biological and physical contributions to O_2 supersaturations in the sea. This non-incubation technique integrates over space and time so it cannot be compared directly to instantaneous rates obtained using *in vitro* techniques. These time-averaged estimates are useful for determining NOP and, hence, the mean metabolic state of the ocean at Station ALOHA (Emerson and others 1993, 1995; Juranek and Quay 2005; Hamme and Emerson 2006; Quay and others 2010; also see below, and *Variability* section). Because the O_2:Ar method provides a snapshot of ocean metabolism, a model must be applied to obtain the mean rate generally averaged over the residence time of O_2 in the mixed-layer (order of 1-2 weeks at Station ALOHA; Juranek and Quay 2005). Another novel non-incubation method, referred to as the "triple O_2 isotope" (also termed $^{17}\Delta$), requires precise measurements of the $^{18}O/^{16}O$ and $^{17}O/^{16}O$ isotope ratios of dissolved O_2 in the near surface ocean and can be used to constrain GOP (Luz and Barkan 2000). A comprehensive review of the triple O_2 isotope method, including theory and a summary of global ocean applications, has recently appeared (Juranek and Quay 2013). As with the O_2:Ar method for estimation of NOP, the $^{17}\Delta$ approach yields a time-averaged estimate of GOP.

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Finally, time-dependent changes in O_2 concentrations using moorings, floats and autonomous underwater vehicles have also been used to model and constrain rates of GOP, NOP and MCR in the NPSG (Riser and Johnson 2008; Nicholson and others 2008, 2015; also see *Variability* section). While these non-incubation techniques all require model assumptions, the major advantage is that they do not require a bottle incubation that might otherwise lead to systematic bias in the estimation of microbiological rates (Karl and Dore 2001). Another major advantage of non-incubation methods is the potential for long-term remotely-sensed observations. However, non-incubation methods require the use of models to assess the impacts of mixing and entrainment of O_2 from beneath the surface mixed layer and air-sea gas transfer and O_2 diffusion on the measured ratios. A major limitation of all O_2-based techniques, *in vitro* and non-incubation, is the inability to attribute activity to individual cells or populations, as for ^{14}C-based methods (see below). Also, if it is necessary to convert O_2-based estimates to C currency, PQ and RQ must be assumed or constrained by additional measurements or observations.

Station ALOHA ^{14}C-based Primary Production: Since October 1988, we have measured primary production using the traditional ^{14}C-method which provides an estimate of the rate of POC production. This estimate is certainly less than GPP, but probably greater than NCP (Marra 2009; Pei and Laws 2014). For example, we know that a portion of the recently fixed ^{14}C is excreted as DOC, some of which could be immediately (i.e., during the time scale of the incubation) remineralized by heterotrophic microorganisms back to $^{14}CO_2$ (Figure 6). In addition, protistan grazing on ^{14}C-labeled phytoplankton and heterotrophic bacterial cells, viral lysis, phytoplankton respiration, death and autolysis also contribute to losses of fixed, radioactive C (Figure 6). For selected periods of time (Oct 1988 – July 1997, Oct 2004 – Oct 2007 and April 2010 – Oct 2012) we tracked variability in both ^{14}C-POC and ^{14}C-DOC production at Station

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ALOHA. We initially discovered that the type of filter used for the analysis impacted the measured ^{14}C-POC determination (Karl and others 1998). Enigmatically, the coarser of the two filters (glass fiber filters with nominal porosity of 0.7 μm compared to polycarbonate membrane filters with nominal porosity of 0.2 μm) retained the larger amount of ^{14}C, a phenomenon that we demonstrated was due to adsorption of ^{14}C-DOC onto the glass fiber filters rather than a greater retention of ^{14}C-POC (Karl and others 1998). This overestimate of ^{14}C-POC was variable with depth and time, but averaged ~30% of the total ^{14}C activity. Furthermore, additional ^{14}C-DOC, not retained by the glass fiber filter, was also present in significant but variable amounts (Karl and others 1998). More recent results confirmed the filter discrepancy, and provided a more comprehensive analysis of vertical and temporal trends in the dynamics of ^{14}C-POC and ^{14}C-DOC pools at Station ALOHA (Viviani and others 2015). The most striking result was the variability observed in the ratio of ^{14}C-DOC to total ^{14}C production, indicating that the pathways controlling the distributions of recently fixed carbon are also variable (Viviani and others 2015). Since DOC and POC have fundamentally different fates in oligotrophic oceanic ecosystems, this apparent temporal decoupling of C and energy flow is likely to have major impacts on fundamental ecosystem processes such as the net metabolic balance (GPP versus MCR) and C sequestration via the biological C pump. During the initial course of study, it was also determined that repeated counting of the glass fiber filters over a 30-day period often resulted in a proportional increase in ^{14}C activity that was attributed to the desorption of ^{14}C-DOC over time, leading to higher ^{14}C counting efficiencies (Karl and others 1998). Hence, this time-dependent increase in detected radioactivity may be an index of the relative importance of ^{14}C-DOC production over our entire 27-year data set (Figure 7). Our analysis revealed significant variability ranging from negligible (<5%) change in ^{14}C-activity to a 3-month period in 2002

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where the change exceeded 100% (Figure 7). The observed periods of low ^{14}C-DOC production are as interesting as those when ^{14}C-DOC appears to be abundant, since both trends reflect fundamental changes in C cycle pathways, probably including the ratio of net:gross primary production. Based on these results, we conclude that the NPSG is a dynamic system with respect to the initial processing of recently fixed CO_2, and furthermore that populations reliant on DOC as a primary resource may need to adapt to a feast-or-famine existence in response to a variable supply of recently produced DOC.

The nearly three decades-long ^{14}C data set from Station ALOHA is the most extensive of its kind for any location in the NPSG. Compared to historical estimates of ^{14}C-primary production obtained from the Climax program (1965-1980), the rates observed at Station ALOHA are approximately two to three times higher (Figures 3 and 8). While the basic ^{14}C method was similar for both data sets, vast improvements have been made in sampling and incubation procedures such that direct comparisons may not be valid. However, the high ^{14}C production rates post-1980 are consistent with a greater concentration of chlorophyll *a*, indicative of a higher phytoplankton biomass and productivity during the HOT program era at Station ALOHA (Figure 3).

Daily rates of ^{14}C-based primary production at Station ALOHA range from 175 to 1,100 mg C m^{-2} d^{-1}, with a 26-year mean value of 525.5 mg C m^{-2} d^{-1} (SD = 135.6 mg C m^{-2} d^{-1}), but with significant seasonal and interannual variability (Figure 8). The highest ^{14}C-based production rates in each year of the 26-year observation period, with the exception of 1992, occurred in summer (June-Aug) and the lowest rates, again except for 1992, occurred in winter (Dec-Feb; Figure 8). The start of the new millennium (i.e., 2000) stands out as the year with the lowest wintertime production and the highest summertime production, for the entire observation period.

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The seasonal variations are due in large part to predicted/measured variations in PAR (see below and Figure 2). However, the large interannual variations for a given season (e.g., summer of 1990 versus summer of 2000, or winter of 1992 versus winter of 2000) cannot be attributed to PAR alone and must have additional controls, most likely variations in macronutrient fluxes, trace metals or vitamins, as well as mesoscale physical processes (see also *Variability* section). None of these processes are well constrained at Station ALOHA despite the enormous scientific efforts invested in the HOT, C-MORE and SCOPE programs to date. For example, while macronutrient concentrations (nitrate + nitrate [N+N], hereafter nitrate, and soluble reactive phosphorus [SRP], hereafter phosphate) are routinely measured each month, we do not measure nutrient fluxes, and the low concentrations of nitrate are probably as much a manifestation of rapid uptake as they are of low supply. Nevertheless, the average 0-30 m nitrate and phosphate concentrations are time variable; for example, nitrate and phosphate concentrations both range over two orders of magnitude, but do not co-vary (Figure 9). While nitrate concentrations are greatest in late winter and early spring and at a minimum in summer, phosphate concentrations are highest in spring-summer and lowest in fall-winter. More importantly, there are very large interannual variations in 0-30 m nitrate and phosphate concentrations with generally higher values in the periods 1989-1991, distinct minima from 1996-2006 and increasing concentrations since 2009 (Figure 9). The latter increasing trend is especially enigmatic and does not appear to result in higher rates of primary production or higher phytoplankton biomasses, as would be predicted from our current paradigm of this nutrient-controlled ecosystem. In addition to nitrate and phosphate concentrations we also routinely measure DON and DOP which we know can also be used to support GPP though the bioavailabilities of these pools are not well known. DON and DOP concentrations are much larger than the corresponding nitrate and phosphate pools, in the

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case of DON by three orders of magnitude (Figure 10). Moreover, the mean N:P ratio (25.5) of the organic pools is greater than the Redfield ratio of 16:1, whereas the mean nitrate:phosphate ratio (0.05) is much lower. Since we generally use the Redfield ratio as a reference point to determine whether an oceanic ecosystem is N- or P-limited, this assessment clearly depends on the bioavailabilities of the organic pools (Karl and others 2001b; Figure 10). In addition to N and P, dissolved iron is present throughout the year at Station ALOHA with evidence for a seasonal cycle that is controlled by Asian dust deposition as a primary source (Fitzsimmons and others 2015). A companion study using long-lived thorium isotopes to trace lithogenic metals determined that iron in the surface waters at Station ALOHA has a residence time of 6-12 months and is, therefore, highly dependent upon annual dust deposition events to sustain primary production (Hayes and others 2015). We currently have little information on the bioavailability of dissolved iron. Vitamins are not routinely measured at Station ALOHA.

The 25-year ^{14}C-based climatology for surface ocean (5 m) primary production shows a maximum rate in the May-June period, consistent with increasing PAR throughout the spring-summer period (Figure 11). However, ^{14}C-based primary production decreases in July, followed by an increase from August to mid-September, even though PAR continues to decrease. We interpret this to be a stratification-induced nutrient (nitrate) limitation in mid-summer, followed by a predictable late summer period of N_2 fixation-based new production. The high-light, N-deficient NPSG is an ideal habitat for the proliferation of N_2-fixing, phototrophic microorganisms. Although nitrate is chronically depleted, enhanced stratification during summer and higher N export rates (see below) combine to create a niche space for the N_2-fixing microbes at Station ALOHA. While N_2 fixation occurs throughout the year, average rates of N_2 fixation are greatest in late summer – early fall (Böttjer and others 2016), and we conclude that is the

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reason for the higher than expected (based strictly on PAR) ^{14}C-based production in the HOT program climatology (Figure 11). However, sustained N_2 fixation-based new production requires sources of phosphorus and iron, as well as other trace elements and organics, so eventually production is controlled by other factors (in addition to PAR) and primary production decreases again in late fall.

Marumo and Asaoka (1974) observed five groups of prokaryotic N_2-fixing phytoplankton in the NPSG, including *Trichodesmium thiebautii*, *T. erythraeum, Oscillatoria* sp., *Katagnymene (Trichodesmium) spiralis* and *Richelia intracellularis*. The latter can exist either in free-living form or as an ecto- and endo-symbiont with various eukayotic phytoplankton, especially diatoms (Venrick 1974). Unlike most other phytoplankton in the NPSG, *Trichodesmium* sp. and *Richelia* are often observed to "bloom" in near-surface waters of the NPSG, especially in summer months, thereby supporting the hypothesis that their N_2-fixing capabilities are a keystone metabolic process under certain conditions (also see *Variability* section). Although the potential for N_2 fixation by *Trichodesmium* and *Richelia* has been known for many decades, the actual ecological role and quantitative importance of N_2 fixation to the economy of the NPSG was poorly known at the start of the HOT program in large part due to the episodic nature of these blooms and lack of direct measurements of N_2 fixation. A major, unexpected discovery at Station ALOHA radically changed the way we viewed N_2 fixation in the NPSG. Zehr et al. (2001) reported the existence of small (<10 μm), unicellular N_2-fixing microorganisms based on *nifH* gene sequence analysis. Nitrogenase gene fragments from the pico/nano (0.2-10 μm) size class were amplified, cloned and sequenced to establish their phylogenetic relationships relative to each other and to other well-known N_2-fixing microorganisms. Initial analysis revealed two major groups of cyanobacteria, termed "unicellular cyanobacteria A and B" (UCYN-A and UCYN-B,

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respectively; Zehr and others 2001). UCYN-B is a cluster of organisms (*nifH* gene sequences), that include *Crocosphaera* (Zehr and others 2001), and UCYN-A has been shown to be a very unusual, symbiotic microorganism with a streamlined genome and possibly occupying a unique niche in the NPSG (Zehr and others 2008; Tripp and others 2010; Thompson and others 2012). Furthermore, the detection of *nifH* gene transcription confirmed that these unicellular groups were actively fixing N_2 at Station ALOHA. We now recognize that the small, unicellular cyanobacteria are probably the most important N_2 fixers in the contemporary NPSG (Karl and others 2008; Church and others 2009), but that aperiodic blooms of larger cyanobacteria or diatom-*Richelia* associations can also occur under certain environmental conditions (also see *Variability* section).

By comparison to ^{14}C-based primary production, total euphotic zone (0-200 m) chlorophyll *a* inventories are relatively constant, both on seasonal and interannual time scales (Figure 3). However, systematic seasonal variations have been reported for chlorophyll *a* concentrations in both the upper mixed-layer and the deep chlorophyll maximum layer (DCML) at Station ALOHA. Chlorophyll *a* in the mixed-layer is highest in winter (Winn and others 1995), whereas at the DCML chlorophyll *a* is highest in summer (Karl and others 2002; Letelier and others 2004). For mixed-layer assemblages, this is a manifestation of photoadaptation, rather than a result of higher phytoplankton biomass (i.e., the C:chl *a* ratio is lower in winter than in summer). In the DCML, the higher chlorophyll *a* concentrations in summer are a result of net phytoplankton growth (Letelier and others 2004).

The higher ^{14}C-based primary production rates in summer coupled with lower chlorophyll *a* concentrations leads to a summertime maximum in the photosynthetic assimilation number (AN), with peak values (~8; Figure 11). This peak value is ~35% of the theoretical maximum

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value of 25 g C g chl a^{-1} hr^{-1} based on a turnover time for the photosynthetic unit of 1 millisecond (Falkowski 1981). If we include ^{14}C-DOC production, as previously discussed, or use more direct estimates of GPP (e.g., O_2-based assessments, see below) then the light-saturated (5 m) ANs at Station ALOHA approach the theoretical maximum, indicating that these surface dwelling phytoplankton assemblages are harvesting solar energy at the highest per capita (chlorophyll *a* normalized) rate possible. The standing stock of phytoplankton cells at Station ALOHA, in turn, is ultimately controlled by nutrient availability and mortality. Aperiodic delivery of new nutrients leads to higher phytoplankton biomass, and a higher rate of solar energy capture until the nutrients are depleted. Therefore, NPSG phytoplankton blooms must ultimately be triggered by allochthonous nutrification of the upper euphotic zone (Dore and others 2008; also see *Variability* section).

Shortly after the discovery of picophytoplankton in the late 1970s, it was shown that they are not only abundant but also metabolically active, accounting for 20-80% of ^{14}C-based primary production in selected oceanic regions (Li and others 1983). Liu and others (1997) measured growth and mortality of *Prochlorococcus* using phased cell cycle analysis of natural assemblages at Station ALOHA. DNA replication begins after sunset, followed by cell division with maximum daily *Prochlorococcus* cell abundance occurring at ~0400 hr (Liu and others 1997). The authors assumed a cell quota of 53 fg C, and used the cell division rates to estimate daily euphotic zone, depth-integrated *Prochlorococcus* production of 620 and 743 mg C m^{-2} d^{-1} for samples collected in September 1993 and July 1994, respectively (Liu and others 1997). These values exceeded the ^{14}C-based primary production from these same HOT cruises (541 and 584 mg C m^{-2} d^{-1}) by 15-30%. The authors then applied a conversion factor of 1.55, based on previous results from a study in the North Atlantic Ocean (Bender and others 1992), and

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concluded that *Prochlorococcus* production accounted for 74% and 82%, respectively, of the GPP at Station ALOHA. Although this assessment by Liu and others (1997) is dependent on the validity of the conversion factors used, it was the first to evaluate the importance of *Prochlorococcus* at Station ALOHA.

Subsequent analyses of the role of picoplankton (including both prokaryotes and eukaryotes) were conducted using post-incubation size fractionation of ^{14}C-POC. This approach assumes that the POC:DOC ratios, respiration rates and grazing rates of the <2 μm and >2 μm fractions are equivalent. Based on a 5-year study at Station ALOHA, picophytoplankton (0.2-2 μm size fraction) accounted for 91±2% of the chlorophyll *a* and 74±7% of the ^{14}C-production over the 0-125 m depth-integrated portion of the euphotic zone (Li and others 2011). These results support the main conclusion of Liu and others (1997) regarding the importance of *Prochlorococcus* in solar energy capture in the NPSG.

Li and others (2011) also examined the photophysiological characteristics of both size fractions and found that the larger phytoplankton (>2 μm) had significantly higher light-saturated ANs (11.2±0.8 g C g chl a^{-1} h^{-1} versus 3.0±0.2 g C g chl a^{-1} hr^{-1} for the 0.2-2 μm size fraction; Li and others 2011). As discussed previously, the larger eukaryotes (>2 μm size fraction) appear to be able to sustain higher rates, yet they contribute minimally to daily primary production, except under bloom conditions (also see *Variability* section). External controls on nano- and macro-sized phytoplankton production, including nutrient limitation of biomass accumulation due to competition with picoplankton and grazing pressures, must be important processes at Station ALOHA. More recently, Rii and others (2016) examined the vertical and temporal variability of picophytoplankton productivity over a 1-year period (May 2012 – May 2013) at Station ALOHA using both size fractionation and flow cytometric sorting of ^{14}C-labeled cells. Their results

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confirmed the relative importance of picophytoplankton assemblages, with the 0.2-3 μm size fraction accounting for ~68-83% of total ^{14}C-based primary production. Phytoplankton group-specific productivities for *Prochlorococcus*, *Synechococcus* and photosynthetic picoeukaryotes (PPE) averaged 39%, 2% and 11% of the total ^{14}C-POC (Rii and others 2016), not accounting for ^{14}C-DOC or other loss processes. This 1-year study indicates that *Prochlorococcus* may be less important (<40% of total ^{14}C-based primary production) than the earlier conclusion of Liu and others (1997; >100% of total ^{14}C-production), but is still a very active component of the Station ALOHA microbial food web. Despite their lower cell abundance, the PPE fraction sustained cell-normalized rates of ^{14}C-production that were 25- to 90-fold greater than *Prochlorococcus* (Rii and others 2016). So it appears that the larger picoplankton, as well as the nano- and macro-sized phytoplankton, have much greater growth potential than the smallest picoplankton cells, yet none of these groups achieves biomass dominance under "normal" environmental conditions at Station ALOHA. The contemporary NPSG appears to be a perfect niche for *Prochlorococcus*.

The uptake and assimilation of organic matter by *Prochlorococcus* (Zubkov and others 2003; Church and others 2004, 2006; Michelou and others 2007) has important implications for conceptualizing microbial food webs in oligotrophic regions such as the NPSG. First and foremost, *Prochlorococcus* production (the rate of total biomass-C increase per unit time) could theoretically exceed *Prochlorococcus* primary production (that portion of biomass-C increase derived from CO_2 fixation), with impacts on estimation of PQ, AN and other physiological and ecological attributes. Church and others (2004) were the first to report a light dependence of ^{3}H-leucine incorporation at Station ALOHA. The rates of ^{3}H-leucine incorporation responded to changes in irradiance in a manner that was similar, but not identical, to that of ^{14}C-bicarbonate with maximum light:dark assimilation ratios approaching a factor of 2 at saturating light

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intensities for whole water sample incubations. The authors hypothesized that facultative photoheterotrophy by *Prochlorococcus* may be commonplace at Station ALOHA (Church and others 2004). A follow on time-series study (15 cruises over a 5-year period) of photostimulated ^{3}H-leucine incorporation revealed a persistent enhancement in the light, with light:dark incorporation ratios increasing from 1.3±0.2 in the upper 25 m of the euphotic zone to 1.7±0.2 in the 45-125 m depth range; rates of ^{3}H-thymidine incorporation were not affected by sunlight (Church and others 2006). Both high- and low-light adapted *Prochlorococcus* ecotypes encode putative ABC amino acid transporters (summarized by Yelton and others 2016) which provides additional evidence for their dual role as primary producers and photoheterotrophs at Station ALOHA.

More recently, results using flow cytometric sorting of *Prochlorococcus* cells following incubations with specific radiolabeled organic molecules has confirmed light stimulation of incorporation and, therefore, photoheterotrophy at Station ALOHA. Duhamel and others (2012) demonstrated light-enhanced uptake of dissolved adenosine-5'-triphosphate (ATP) for substrates labeled at either the terminal P group ([γ-^{33}P]ATP) or the adenine moiety ([2,8-^{3}H]ATP). The light:dark ratios of [γ-^{33}P]ATP for *Prochlorococcus* in the upper euphotic zone (0-45 m) averaged 7.84 (and for selected samples was >10), indicating an important role for light energy in the assimilation of organic matter (Duhamel and others 2012). Analysis of [γ-^{33}P]ATP uptake as a function of ATP concentration indicated that the light enhancement of assimilation was observed over the range of added substrate concentrations (0-55 nmol l^{-1} ATP added), but that the light-dark incorporation ratio decreased from >10 at lowest ATP to a factor of approximately 3 at the highest concentrations. Despite the light-stimulated uptake by *Prochlorococcus*, their total uptake represented only 1-18% of the total, due in large part to the dominance of non-

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pigmented (presumably heterotrophic) bacteria (NPB; Duhamel and others 2012). A subsequent kinetic analysis of [γ-^{33}P]ATP uptake by *Prochlorococcus* and NPB confirmed that NPB had lower uptake constants (Km) and higher maximum uptake rates (Vmax) than *Prochlorococcus*, suggesting that competition with NPB for ATP, and possibly other organic substrates, at Station ALOHA may be intense (Björkman and others 2012).

Björkman and others (2015) recently compared the light-stimulation of *Prochlorococcus* for assimilation of ^{14}C-bicarbonate and ^{3}H-leucine in the upper water column (0-175 m) at Station ALOHA over a 3-yr period. Short-term (2-hr) incubation experiments were conducted using shipboard photosynthetrons capable of precise manipulation of light intensities from a few to ~2,000 μmol photons m^{-2} sec $^{-1}$, and longer-term (dawn-to-dusk) *in situ* experiments conducted on free-drifting arrays. ^{3}H-leucine incorporation by *Prochlorococcus* was stimulated by light throughout the water column, with depth-integrated (0-175 m) light:dark ratios ranging from a minimum of 1.4 in April 2009 to a maximum of 17.6 in Nov 2008 (Björkman and others 2015). By comparison, the incorporation of ^{3}H-leucine by NPB was light-independent. Significant cruise-to-cruise differences were observed in the photoheterotrophic activity of *Prochlorococcus*, with greater ^{3}H-leucine incorporation, higher light:dark ratios and higher per cell specific ^{3}H-leucine incorporation during periods defined as "bloom conditions" where total chlorophyll *a* concentrations greatly exceeded the HOT climatology for that month (Aug 2007, Aug 2010, Nov 2008; Björkman and others 2015). Remarkably, the maximum light-stimulated cell-specific rates of ^{3}H-leucine incorporation by *Prochlorococcus* exceeded incorporation rates by NPB throughout the 3-year period of observation, and even under dark incubation conditions the cell-specific incorporation rates of ^{3}H-leucine by *Prochlorococcus* were still 30-50% of those measured for NPB (Björkman and others 2015). Following a previous report by Zubkov and

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others (2004) for the differential assimilation of radiolabeled methionine by low versus high red fluorescing (LRF and HRF, respectively) *Prochlorococcus* populations, Björkman and others (2015) also sorted LRF and HRF cells following a photosynthetron experiment (Figure 12). Their results clearly showed ecotype differentiation for *Prochlorococcus* inhabiting the DCML at Station ALOHA. Both the maximum extent of light-stimulation and the degree of high light inhibition were greatest for the HRF *Prochlorococcus* population. NPB from the DCML had very low cell-specific ^{3}H-leucine incorporation rates, and once again displayed no light stimulation of incorporation (Figure 12). Based on this comprehensive study, and the field research that preceded it, it now appears almost certain that *Prochlorococcus* is responsible for most, if not all, of the light-stimulated incorporation of ^{3}H-leucine as initially hypothesized by Church and others (2004) a decade earlier.

Björkman and others (2015) also conducted simultaneous ^{14}C-bicarbonate and ^{3}H-leucine uptake experiments to begin to determine how "heterotrophic" the *Prochlorococcus* assemblage at Station ALOHA might be. Their analysis suggested that leucine-C is equivalent to ~0.6% of total ^{14}C-bicarbonate uptake. Since *Prochlorococcus* can also assimilate other amino acids, ATP, sugars, peptides and possibly other organic substrates at nanomolar concentrations under *in situ* conditions (e.g., glucose; Muñoz-Marin and others 2013), a quantitative resolution of mixotrophy in *Prochlorococcus* and other phototrophs will require novel experimental approaches and continued access to the sea for *in situ* experimentation.

Finally, based on a recent survey of marine metagenomic data from samples collected by the *Tara Oceans* expedition, the potential for *Prochlorococcus* mixotrophy appears to be cosmopolitan, but variable among the distributed ecotype populations and possibly related to loss of specific transporter genes during the process of genome streamlining (Yelton and others

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2016). A mixotrophic lifestyle may be the rule for all microorganisms in the sea, not just *Prochlorococcus*. Mixotrophy is well known in marine protists (Caron 2016), and the discovery of proteorhodopsin phototrophy in marine bacteria (Béjà and others 2000) documents fundamental and complementary roles for solar energy and chemical potential energy for oceanic metabolism both at Station ALOHA and in similar oligotrophic marine environments. A recent modeling study concluded that the flows of C and energy are more efficient through a "mixotrophic food web" than one comprised of obligate phototrophs and chemotrophs (Ward and Follows 2016). This new knowledge of oligotrophic ocean mixotrophy and other unusual lifestyles must be incorporated into our contemporary C and energy flow paradigms (Karl 2014b; Worden and others 2015).

Use of Alternative In Vitro Primary Production Methods at Station ALOHA: In addition to ^{14}C-bicarbonate, a number of additional methods have been used to estimate rates of primary production in the NPSG, including both incubation and non-incubation O_2-based approaches. The O_2 light-dark bottle production-consumption method (Gaardner and Gran 1927) actually pre-dates the ^{14}C-method, but until analytical improvements were introduced in the early 1980s (Williams and Jenkinson 1982; Williams and others 1983; Williams and Purdie 1991), this approach lacked the sensitivity required to measure the small O_2 changes relative to a large background concentrations (>200 μmol O_2 l^{-1}) within the NPSG. The O_2 light-dark bottle method has not been routinely employed at Station ALOHA, but a limited time-series data set does exist. Williams and others (2004) measured the O_2-based NOP and MCR to derive estimates of GOP during a 1-year period of observation (May 2001 – May 2002). This study was designed to test the hypothesis that oligotrophic areas of the ocean, like the NPSG, support higher rates of O_2 consumption than O_2 production (negative NOP; del Giorgio and others 1997;

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Duarte and Agusti 1998; Williams 1998). An unexpected result from the Station ALOHA field study confirmed that the euphotic zone (0-150 m) appeared to be in metabolic deficit throughout the year and, hence, supported the enigmatic "net heterotrophy" hypothesis for Station ALOHA. The calculated O_2 shortfall was 9 ± 1.7 mol O_2 m^{-2} yr^{-1}, an amount that was equivalent to ~40% of the measured annual GOP (Williams and others 2004). Either the methods and extrapolations used are invalid or our conceptual understanding is incomplete (or both), because the euphotic zone of the NPSG exports O_2 to the atmosphere and organic matter to the mesopelagic zone and, therefore, must be a net autotrophic system (Karl and others 2003b). Williams and others (2004) discussed several possible interpretations, both analytical and conceptual. They concluded that the inability to reproduce the precise light field experienced by phytoplankton cells using fixed-depth, 12-hr incubations and the possibility of temporal intermittency of GOP compared to MCR were the most tenable explanations. While their data set supported the hypothesis of net heterotrophy, they found no evidence for either an internal reserve within the ecosystem or an external subsidy of organic matter of a magnitude that would be required to sustain a long-term deficit (Williams and others 2004). Short-lived pulses (days to weeks) of net autotrophy have been observed at Station ALOHA (Emerson and others 2002; Karl and others 2003b), and these events may be undersampled in the monthly HOT time-series (Karl and others 2003b; also see *Variability* section). Williams and others (2004) concluded that MCR measurements may be more constant and, therefore, yield a more accurate time average constraint on system productivity than GOP. Based on this analysis, the authors concluded that 28 mol C m^{-2} yr^{-1} (330 g C m^{-2} yr^{-1}; assuming a PQ of 1.1) is a reasonable estimate of GOP at Station ALOHA (Williams and others 2004). The recent and lively debate regarding the metabolic state of

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oligotrophic subtropical ocean gyres (Ducklow and Doney 2013), emphasizes the importance and urgency of resolving this enigma (also see *Variability* section).

A major analytical advance came with the introduction of the ^{18}O-H_2O method, which provided a direct estimate of GOP (Bender and others 1987; Grande 1988; but also see Gazeau and others 2007 for potential limitations). GOP, as determined by the ^{18}O-H_2O method, measures total O_2 production, so correspondence between GOP and C-based estimates of GPP will depend on the fate of the O_2 (Bender and others 1999). One path to O_2 consumption in phytoplankton involves light via either the Mehler reaction or photorespiration; another is dark respiration via a number of metabolic pathways (Bender and others 1999).

An initial comparison of the three *in vitro* techniques (^{14}C, O_2 light/dark and ^{18}O) for samples collected in the NPSG and incubated under *in situ* conditions, showed that the two O_2-based techniques yielded similar rates of GOP, whereas the ^{14}C-based rates were ~60-100% of the O_2 methods (Grande and others 1989). Assuming that there are no systematic errors with the two O_2-based methods, then simultaneous measurements can provide information on NOP (NOP = ΔO_2 following a 24-hr *in situ* incubation period), microbial community dark respiration (MC(D)R = ΔO_2 following a 12-hr *in situ* incubation in opaque bottles), microbial community light respiration (MC(L)R = ^{18}O-GOP minus net O_2 production following a 12-hr *in situ* incubation in the light) and total MCR (MC(D)R plus MC(L)R). More recently, Ferrón and others (2016) have described a modification of the ^{18}O-H_2O method that employs a membrane inlet mass spectrometer (MIMS) instead of the more traditional isotope ratio mass spectrometer (IRMS). This now makes it possible to process samples at sea and may lead to an improvement of our understanding of the controls on primary production. The new method was validated at Station ALOHA, and the MIMS- and IRMS-based methods compared favorably for both

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analytical precision and accuracy (Ferrón and others 2016). The *in vitro* ^{18}O method was also expanded to measure net O_2 changes (NOP) during the incubation procedure (by O_2:Ar; see below) and to derive MCR from the differences between ^{18}O-GPP and NOP, all from the same incubation bottle (Ferrón and others 2016).

Comparisons of Primary Production Methods: Several recent comparisons of ^{14}C-based and O_2-based estimations of phytoplankton productivity have been conducted at Station ALOHA. For the purpose of this review we will focus primarily on the *in vitro* methods, namely ^{14}C and ^{18}O-H_2O. Additional data for the assessment of NOP from Seaglider surveys and for model-based GOP and MCR estimation are presented in the *Variability* section.

Juranek and Quay (2005) were the first to measure *in vitro* GOP using ^{18}O-H_2O at Station ALOHA, although the method had been used previously during the Plankton Rate Processes in Oligotrophic Oceans (PRPOOS) field program at the Climax site (Grande and others 1989). Between Feb 2002 and Aug 2003, Juranek and Quay (2005) compared GOP to the ^{14}C-based estimates on four HOT cruises using similar sampling and *in situ* incubation protocols, with the exception of bottle-type (^{14}C – 500 ml polycarbonate; ^{18}O – 120 ml quartz). Profiles of ^{18}O-based GOP were converted to C currency, using a conservative PQ = 1.25, for a direct comparison to the ^{14}C-based estimates. On average, volumetric rates measured using ^{18}O exceeded those for ^{14}C except in Feb 2002 when the two profiles were indistinguishable (Figure 13). When there was a difference between the two methods, it was greatest in the light-saturated mixed-layer. The differences decreased with increasing water depth and the rates became indistinguishable by approximately 100 m (Figure 13). The ^{18}O:^{14}C ratios for the two winter months (Feb 2002, Feb 2003) were also lower than for the summer/fall months (Oct 2002 and Aug 2003; Figure 13). These temporal (perhaps seasonal) differences in the net:gross ratio are

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consistent with variable relative ^{14}C-DOC production rates (Figure 7). Indeed Feb 2002 was among the lowest relative ^{14}C-DOC:^{14}C-POC ratios and Oct 2002 was the highest ^{14}C-DOC:^{14}C-POC relative ratio (based on the time-dependent change in radioactivity, discussed previously) for the 26-year observation period at Station ALOHA. The 0-100 m, depth-integrated rates of ^{14}C for Feb 2002 and Oct 2002 were nearly identical (520±40 and 560±40 mg C m^{-2} d^{-1}, respectively), but the fates of the recently fixed C appears to be fundamentally distinct. The ratio of net:gross primary production might vary with phytoplankton community structure or growth and grazing rates, though we lack a comprehensive understanding at the present time.

A follow-on study, also conducted at Station ALOHA in conjunction with HOT program scientists, compared the *in vitro* ^{14}C and ^{18}O methods over a nearly 2-year period (Mar 2006 – Feb 2008; Quay and others 2010). As expected, the ^{18}O-based estimates of GOP exceeded the ^{14}C-based rates. As for the previous Juranek and Quay (2005) study, the ^{18}O:^{14}C ratios varied systematically with depth (higher in mixed layer and indistinguishable at 100 m) and season (higher in summer than in winter). There was a greater seasonality in ^{18}O-GOP, with summertime (May-Sept) rates exceeding wintertime (Nov-Mar) by 32%, compared to ^{14}C-production rates which varied by 18% between seasons (Quay and others 2010). The estimated annual ^{18}O-GOP and ^{14}C-based rates for the period of observation were 71±16 mmol O_2 m^{-2} d^{-1} and 38±6 mmol O_2 m^{-2} d^{-1} (455±75 mg C m^{-2} d^{-1}), respectively, for the 0-100 m portion of the water column. If the measured mixed layer ^{18}O-GOP rates are converted into C currency (using PQ = 1.1), then the implied ANs (C fixation rate per unit chlorophyll *a*) are at or near the theoretical maximum for light-saturated phytoplankton, implying a maximum efficiency of solar energy conversion throughout the year.

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Both of these pioneering studies at Station ALOHA also estimated GOP using $^{17}\Delta$ and NOP using the O_2:Ar method. The triple oxygen isotopic method in both studies yielded much higher GOP with euphotic zone depth-integrated estimates ranging from 70-185 mmol O_2 m^{-2} d^{-1} (Juranek and Quay 2005) and 140±58 (Quay and others 2010). If these non-incubation methods provide a more accurate estimate of GPP, then the implied ANs would exceed the theoretical maximum values observed in extensive laboratory studies conducted to date. Nicholson and others (2012) analyzed the dynamics of the O_2 isotopes at Station ALOHA using a one-dimensional mixed-layer model. Their results suggested that entrainment of high $^{17}\Delta$ thermocline water was the largest potential source of error, and may have accounted for the anomalously high estimates that have been reported (Quay and others 2010; Nicholson and others 2012). Furthermore, if primary production at Station ALOHA greatly exceeds the ^{14}C-based method, then any comparison or calculation using ^{14}C data would also be in error (e.g., below for comparisons of particulate C export to ^{14}C-based primary production). Unfortunately since the ratio of GOP:^{14}C is temporally variable, there is no simple correction factor that can be applied to the 26-yr HOT program data set. It is sobering to learn that after more than 60 years of measuring primary production by the ^{14}C-bicarbonate method, we still do not have an accurate assessment of how much solar energy is converted into organic matter by the phytoplankton assemblages in the NPSG.

Biological Carbon Pump: The ocean's biological C pump is an integral component of the global C cycle and is largely responsible for long-term sequestration of CO_2 into the meso- and abyssopelagic zones (Volk and Hoffert 1985). The biological C pump is fueled by solar energy, through the process of photosynthesis in the euphotic zone. The primary mechanism that connects surface processes to the deep sea is the gravitational settling of organic particles and, to

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a lesser extent, the net downward diffusion of DOM and active vertical migrations of macrozooplankton. While export processes are responsible for the long-term sequestration of CO_2 into the deep sea, the mechanisms of the biological C pump are not well understood (Karl and others 2003a; Figure 14). Most biogeochemical models predict that the strength and efficiency of the biological C pump are dependent upon the structure of the phytoplankton community with larger cells (e.g., diatoms) supporting a more active biological C pump (Legendre and Le Fèvre 1989; Karl and others 2003a). However, picoplankton can also contribute to C export (Richardson and Jackson 2007). A recent study of C export at Station ALOHA using size class-specific molecular lipid and isotopic markers has documented the role of submicron particles as an important component of the biological C pump (Close and others 2013). The authors hypothesized that continuous disaggregation and reaggregation of POM during the export process (i.e., Figure 14, center mechanism) could transfer very small particles to great ocean depths.

The downward flux of particulate C (PC), measured at the base of the euphotic zone and expressed as a proportion of contemporaneous primary production, is termed the export ratio (e-ratio; Baines and others 1994). Cross-ecosystem analyses from a broad range of oceanic habitats suggest that the e-ratio is a positive, non-linear function of total euphotic zone primary production, with values ranging from less than 0.1 for oligotrophic regions to >0.5 in highly productive coastal regions (Suess 1980; Pace and others 1987). Buesseler and Boyd (2009) have recently argued that the e-ratio reference point should be set at the depth of the compensation irradiance for photosynthesis (the depth where NPP=0) rather than a fixed depth. As discussed later in this review, the depth of the compensation irradiance is itself variable (also see Figure 2), so in practice it may be difficult to achieve this otherwise excellent recommendation (also see

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Variability section). The low e-ratios in oligotrophic ocean regions like Station ALOHA suggest that most of the recently produced organic matter is locally recycled within the microbial food web, and this intensive remineralization process provides the recycled nutrients necessary to sustain photosynthesis. Consequently, the energy subsidy for macrofauna within the euphotic zone, and for all micro- and macro-organisms in the vast aphotic zone of the sea (>200 m, including the seabed) is dependent upon the chemical energy, initially derived from sunlight, that escapes the upper portion of the water column either through passive (particle sinking and downward diffusion of dissolved organic matter) or active (vertical migrations) processes.

Since the beginning of the HOT program in 1988, we have measured the flux of particulate matter from the base of the euphotic zone using free-drifting particle interceptor traps (PITs; Knauer and others 1979). PC fluxes over the 24-year observation period range from 1-5 mmol C m^{-2} d^{-1} with a mean value of 2.32±0.81 mmol C m^{-2} d^{-1} (Figure 15). The flux time-seies reveals a sub-decadal variability with lower fluxes during the 1991-1996 and 2013-2014 periods, for reasons that are not well understood. Particulate N (PN) fluxes are highly correlated with PC fluxes throughout the time-series with an average molar C:N ratio of 8.0 (Figure 15). Despite this comprehensive particle flux data set, there appears to be no simple relationship between ^{14}C-based primary production and PC export (Figure 16). The e-ratios range from 0.012 to 0.149 (mean 0.057±0.022), but do not appear to be uniquely controlled by ^{14}C-based primary production, as predicted by current models. Because the ^{14}C-based production can underestimate GPP by up to a factor of 2-3, the mean e-ratio at Station ALOHA may actually be less than 0.05 (assuming the sediment trap fluxes are accurate), indicative of a very efficient remineralization-intensive system.

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Another key aspect of the biological C pump is the depth-dependent, flux attrition commonly seen in particulate matter flux profiles (Figure 17). Although there is an extensive data set of PC export at 150 m, as well as PC export at a reference depth of 4,000 m, observations on particulate matter fluxes and on the rates and mechanisms of depth-dependent attrition of sinking particles at Station ALOHA between the base of the euphotic and the seabed are poorly known (Figure 14). Based on observations from the Vertical Transport and Exchange (VERTEX) program in the 1980s, the prediction of a non-linear loss of sinking particles due to the combined effects of disaggregation, dissolution and organic matter remineralization by the combined effects of microorganisms associated with the particles or particle consumption by mesopelagic zooplankton and fish was presented by Martin and others (1987; Figure 17). They summarized their open ocean flux observations using log-log transformation and a normalized power function in the form:

$$F_z = F_{100} (z/100)^{-b}$$

where F_z = flux at a given reference depth (z), F_{100} = log-log intercept (also equivalent to the flux at 100 m), z is depth in meters and exponent b is the log-log slope of the flux versus depth relationship (also defines the flux attrition with depth). This generalized equation has been used extensively to predict element fluxes and to constrain subeuphotic zone respiration and nutrient regeneration rates (e.g., Sarmiento and Gruber 2006). Buesseler and Boyd (2009) have recently suggested that the ratio of PC flux at the depth of the compensation irradiance to PC flux 100 m below that depth (termed T_{100} in their paper) might be more useful than the Martin equation because T_{100} is a precise quantification of the biological C pump in the upper mesopelagic zone where the flux attrition is greatest.

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Limited flux profile data from Station ALOHA collected at reference depths of 150, 300 and 500 m for a 5-year period (Oct 1988 – Nov 1993; Karl and others 1996) have been modeled using the proposed normalized power function. The Station ALOHA flux attrition profile for this observation period was similar to that predicted by Martin and others (1987), and for a subsequent VERTEX data set collected in oligotrophic North Pacific waters (Knauer and others 1990; Figure 16). The PC fluxes projected for the 150 m reference depth were 35.5, 31.2 and 28.7 mg C m^{-2} d^{-1} and the flux exponents (b) were 0.858, 0.800 and 0.818, respectively, for the Martin and others (1987), Knauer and others (1990) and Karl and others (1996) field observations. Based on these three data sets, one might assume that PC fluxes are well constrained and the processes controlling them well understood, but they are not. For example, initial observations from the VERTEX program showed evidence for subeuphotic zone particle flux anomalies (Karl and Knauer 1984a, 1984b), including apparent injections of new particles at depth, that would tend to negate any straightforward statistical analysis such as the "Martin curve." Indeed when Station ALOHA PC flux data from 150 m and 300 m are compared on a cruise-by-cruise basis, we see a broad range of 300 m : 150 m flux ratios rather than convergence on a common value as predicted by the Martin and others (1987) flux attrition model (Figure 18). Some 300 m POC fluxes exceeded the contemporaneous fluxes at 150 m, and at other times the 300 m POC fluxes were much lower than the model prediction (Figure 18). A condition of equal POC fluxes at 150 m and 300 m could be explained by very rapidly sinking particles (where microbial decomposition or removal by grazers is minimized) or by space-time decoupling of PC production and PC flux where the particles collected at 150 m and 300 m may have different sources. In contrast, flux periods where the calculated scaling factor (the exponent b) for flux attrition with depth is greater than predicted from the Martin equation might imply either more

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rapid remineralization of sinking particles as a result of lower sinking rates, greater rates of dissolution and disaggregation or greater rates of microbial decomposition and removal by zooplankton and fish (processes presented in Figure 14). These field observations and the broad range of possible interpretations lead to the main conclusion that the structure, mechanisms and controls of the biological C pump at Station ALOHA are not well understood. While the apparent decoupling of surface particle production and euphotic zone particle export may be the result of temporal decoupling of the two processes, it seems highly unlikely that we are undersampling any major ecosystem process related to surface ocean production and export of particles from the euphotic zone given the 26-yr-long data set and approximately monthly temporal resolution. These uncertainties in the ocean's biological C pump remain as major enigmas with important consequences to the global C cycle and the long-term sequestration of CO_2 in the sea.

A major experiment to revisit the carbon export paradigm and to assess the attrition of sinking particulate matter through the upper mesopelagic zone was conducted at Station ALOHA in summer 2004 (Buesseler and others 2007). The VERTIGO (Vertical Transport In the Global Ocean) program was both comprehensive in scope and rich in data return (see Buesseler and Lampitt 2008 for introduction to special VERTIGO issue of *Deep-Sea Research II*). Among other goals, VERTIGO sought to investigate several key assumptions of the biological C pump at two contrasting North Pacific sites (Station ALOHA and K2 in the North Pacific subarctic gyre). Their experimental design included the first large-scale use of neutrally buoyant sediment traps (NBST) and compared their performance to the VERTEX/HOT style PITs. The NBST PC export fluxes (at 150 m) measured during the VERTIGO expedition compared favorably with both the VERTIGO PIT measurements and with the HOT program flux climatology (Buesseler

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and others 2008). The e-ratio was 0.11 and 0.13 for the two separate deployments, but VERTIGO primary production was just 180-220 mg C m^{-2} d^{-1}, a factor of 2-3 lower than the HOT program summertime climatology, for reasons that are unclear (VERTIGO primary production was measured using on-deck incubators; Steinberg and others 2008). The most striking result was a higher than expected flux attrition; the VERTIGO average b value for two separate deployments at Station ALOHA was 1.33±0.15 compared to the HOT 5-year mean of 0.82. These field results suggest that the PC flux measured at the 500 m reference depth was just half of that predicted by the Martin equation. However, based on the PC flux profile variations observed at Station ALOHA (Figure 18), use of any single monthly profile in biogeochemical modeling may be ill-advised. We need to determine the mechanisms that lead to these temporal variations so that we can improve our models of the biological C pump.

Steinberg and others (2008) compared the loss of sinking PC in the upper mesopelagic zone (150-1000 m using the VERTIGO power function equation) to estimates of the metabolic requirements of mesopelagic zone bacteria and zooplankton. The latter were extrapolated from *in vitro*, deckboard ^{3}H-thymidine measurements (and assumptions of bacterial growth efficiency) and zooplankton biomass measurements (and assumptions of carbon consumption rates and assimilation efficiencies). The results indicated that the mesopelagic C demand by bacteria exceeded that of zooplankton by up to 300%. More importantly, the collective demand for C was 4- to 6-fold greater than the loss of sinking PC, even with the higher than expected flux attrition coefficient (Steinberg and others 2008). If these PC flux and C demand estimations are correct, then our understanding of C and energy inputs to the mesopelagic zone need to be improved. Steinberg and others (2008) suggested that diel vertical migrations of zooplankton feeding at the surface and carnivory at depth could be a major pathway to deliver C to the

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mesopelagic. Burd and others (2010) conducted a sensitivity analysis of the assumptions employed in these assessments and found that the predictions are highly sensitive to the values used for bacterial growth efficiencies, which are poorly constrained at present for the mesopelagic zone. Recently, Hannides and others (2013) employed a novel amino acid compound-specific isotope (^{13}C and ^{15}N) approach to examine the dynamics of sources of suspended particles and zooplankton at Station ALOHA. Differences between "source" amino acids and "trophic" amino acids can be used to identify the origin of particles from trophic interactions. This comprehensive study presents a new view of the complex interplays between sinking and suspended particulate matter, and between diel migrants and permanent residents in the mesopelagic zone. They concluded "that 62-88% of the N sustaining midwater zooplankton is surface derived, obtained through the combined processes of consumption of sinking particles, carnivory of vertical migrants or direct feeding in surface waters at night" (Hannides and others 2013). A better understanding, both conceptual and quantitative, of mesopelagic zone processes is clearly needed.

Since 1992, we have also measured the flux of organic matter to the deep sea using bottom-moored sediment traps positioned at 2,800 m and 4,000 m. These C flux data sets establish constraints on food delivery to the deep-sea sediments. Results from the ongoing deep-sea sediment trap experiment at Station ALOHA have documented a large, rapid and predictable summertime (15 July – 15 Aug) pulse of POM, a feature that has been termed the summer export pulse (SEP; Karl and others 2012; Figure 19). This unexpected SEP was comprised of "fresh" organic matter that must have been derived from an annually recurrent event in the euphotic zone that produced rapidly sinking particles. There is no evidence for the presence of a similar, regularly timed feature in either primary production or in the 150 m PC export observations

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(Figure 19). Consequently, we conclude that the SEP is more likely caused by a change in flux attrition (the b exponent in the Martin equation) through the water column during the late summer period, possibly controlled by particle sinking rates, rather than an enhancement of total particle flux (Karl and others 2012). If this interpretation is correct, then the SEP periods that enrich the seabed must also be periods that starve the mesopelagic zone of C and energy.

Microscopic examination of the collected POM during SEP periods revealed a preponderance of diatom species that are known to harbor N_2-fixing endosymbiotic cyanobacteria. Indeed the SEP period coincides with a sharp peak in the total flux of *nifH* genes dominated by Het 1-3 phylotypes that are diagnostic of the suspected heterocystous endosymbiotic cyanobacteria. Furthermore, the ^{15}N isotopic composition of the POM collected during the SEP was depleted relative to other times of the year, a characteristic that is consistent with N_2-based new production in surface waters. Perturbations in the strength and efficiency of the biological C pump at Station ALOHA have previously been attributed to stochastic environmental variability (e.g., pulses of atmospheric deposition of trace metals, storm-induced deep mixing and mesoscale eddies), but none of these processes has the regularity to explain the Station ALOHA SEP. We hypothesized that photoperiodism, specifically the reduction in day length following the second "ALOHA noon" on 5 July (the second time of the summer when the sun is directly overhead at Station ALOHA, which lies just to the south of the Tropic of Cancer), triggers a genetically controlled cascade of physiological and behavioral changes (e.g., aggregation, gamete formation, release of chemical messengers) in selected diatom species leading to a massive and predictable export event following the "turning of the sun" near the end of summer (Karl and others 2012).

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Much of the organic matter delivered from the euphotic zone during SEP periods is deposited on the seabed where it fuels the sedimentary food web. Seabed metabolism has been measured at Station ALOHA on 6 cruises between Dec 1997 and Jan 1999 using a free vehicle grab respirometer that autonomously measures O_2 consumption of a sediment sample collected at a water depth of 4,730 m (Smith and others 2002). Sediment O_2 consumption ranged from a minimum in Feb 1998 of 0.16 mmol O_2 m^{-2} d^{-1} to a maximum of 0.34 mmol O_2 m^{-2} d^{-1} in September 1998. The latter period of elevated sediment respiration followed the 1998 SEP. However, based on extrapolations using a reasonable RQ, the authors concluded that the organic matter measured in deep-sea sediment traps at Station ALOHA can supply only ~65% of the required energy demand (Smith and others 2002). So it appears that our current understanding of deep-sea C and energy fluxes may be as incomplete as it is for euphotic zone particle production and zone export processes.

Finally, Emerson (2014) has recently presented a comprehensive status report on annual NCP and the biological C pump in the world ocean, including Station ALOHA. Based on a variety of observations, model simulations and large-scale geochemical constraints, his analysis revealed an annual NCP of 2.5±0.7 mol C m^{-2} for Station ALOHA. Mean annual PC export at 150 m accounts for less than half of this projected estimate. Emerson (2014) concluded that the downward flux of DOC at Station ALOHA, coupled with upper mesopelagic zone respiration, is a dominant mode of C export from the euphotic zone. He estimates that two thirds of the organic matter degraded in the 110-210 m depth zone is supplied by DOC rather than by sinking particles. This is a fundamentally different view of the major C and energy export pathway than the one dominated by sinking particles including the enigmatic SEP (Martin and others 1987; Karl and others 1996). Because the HOT program routinely measures PC flux at 150 m, rather

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than the 100 m reference depth used by Emerson (2014), the measured PC fluxes at 100 m should be extrapolated to the same reference depth (using the Martin curve) or, better yet, measured in the field. Recent PC flux profiles at Station ALOHA have shown a larger PC export at shallower reference depths, so the role of particles relative to dissolved matter as a vehicle for C and energy export may need to be reassessed. Clearly, the gravitational settling of particulate matter is a more rapid and efficient means to fuel mesopelagic and abyssal biomes than the downward diffusive or advective flux of DOC. There is an urgent need for additional field observations and improved modeling of the ocean's biological C pump.

Variability: The "Uncertainty of Life"

The adage, nothing is constant but change, is an excellent description of habitat and ecosystem variability in the NPSG. Though often viewed as independent variables, there is an inextricable linkage between spatial and temporal variability in the sea (Stommel 1963; Figure 20). This complex time-space continuum defines the "uncertainty of life" for all seascapes and their inhabitants.

Time is so fundamental to Earth system processes, including but not limited to evolution, that it is sometimes taken for granted, or worse, ignored (Karl 2010). Nevertheless it is very difficult to observe long-term temporal changes in most marine ecological processes and, especially, to distinguish natural variability from potential effects of human-induced climate change. Rudnick and Davis (2003) demonstrated that for systems where the signal-to-noise ratio is low, as for most biogeochemical processes at Station ALOHA, short time-series records (<20 yr) are insufficient to statistically resolve recurrent trends. Although there may be certain key leading indicators as crucial transition points or thresholds are approached (Carpenter and others 2011), unambiguous detection of multi-year (or longer) changes in NPSG phytoplankton diversity and

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productivity will require sustained observations for periods of multiple decades to discern systematic temporal trends. This is the primary justification for extended time-series programs like those based at Station ALOHA.

In contrast to the temporal scales of ecosystem variability, contemporary spatial variability in selected oceanic processes may be more easily documented, for example, using satellite remote sensing imagery of sea surface temperature, winds, topography or color. However, a comprehensive understanding of the controls for most biogeochemical rates and mechanisms will still require access to the sea, sample collection and *in situ* experimentation, as well as the application of ocean-basin-scale, coupled physical-biogeochemical models. In this section we present three case studies of ecosystem variability at Station ALOHA. The first example explores high frequency (hourly-daily) changes in the light field at Station ALOHA that ultimately impact primary production and all ecological processes that are tightly coupled to photosynthesis (e.g., NCP, MCR, export). The second example demonstrates how mesoscale (10's to 100's km) ocean processes lead to variations in N_2-fixation with coupled impacts on new and export production. The final example examines human-induced environment variability (both space and time) and potential future states of the NPSG, with a focus on oligotrophic ocean acidification, stratification and the potential impacts on nutrient delivery pathways.

Diel Processes: Within the broad ecologically-relevant domain of time (Figure 20), there is probably no more significant scale than the one that occurs day-after-day, decade-after-decade, millennium-after-millennium, namely the day-night cycle. During each 24-hour period since photosynthetic life first appeared on the planet ~3 billion years ago, there has been a precise diel variation in solar radiation with nearly half of each day (at the latitude of Station ALOHA) being devoid of life-supporting solar energy. So it seems only logical that organisms, especially

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phototrophic microorganisms and all other populations that are tightly linked to solar energy capture via photosynthesis, have evolved metabolic strategies and behaviors to deal with the epitome of a feast-or-famine existence (e.g., day versus night). Complex phytoplankton "clock" mechanisms, including net energy storage (daytime) and net energy dissipation (nighttime) processes, phased cell division and cell-to-cell signaling are but a few examples of the spectrum of highly evolved responses to diel variability in solar radiation.

Cell division in *Prochlorococcus* is in synchrony with the light-dark cycle, even at ocean depths where light intensity does not permit maximum growth rates (Vaulot and others 1995). At Station ALOHA, there is a systematic progression from initial growth phase (G_1), to DNA synthesis phase (S) to late growth phase (G_2) with cell division occurring from about sunset through midnight (Liu and others 1997). This phased cell division results in up to a 2-fold diel change in *Prochlorococcus* cell abundance, which in steady-state must be balanced by corresponding losses due to grazing, viral lysis and cell death. Liu and others (1995) conducted a series of *Prochlorococcus* growth and mortality experiments at Station ALOHA. They concluded that mortality due to protozoan grazing accounted for between 20-116% of daily growth, but generally growth exceeded grazing (Liu and others 1995). More recently, Ribalet and others (2015) have documented a tightly coupled day/night synchrony between *Prochlorococcus* cell production and mortality rates in the NPSG. They observed a peak in mortality ~6 hr after peak production, with essentially no mortality during daylight hours. These processes combine to result in a strong diel phasing in the flux of C and energy through the microbial food web via *Prochlorococcus* population dynamics.

While the timing of the maximum and minimum solar energy flux at the sea surface is highly predictable, the actual solar energy flux to organisms inhabiting the euphotic zone is not. This is

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due to a number of stochastic properties including the presence/absence of clouds and even cloud type, the rate and intermittency of turbulent mixing of the surface ocean (i.e., the mixing rate within the so-called mixed-layer) and a variety of isopycnal vertical displacement mechanisms, including internal waves, tides and near-inertial oscillations (Letelier and others 1996; Karl and others 2002). Ferris and Christian (1991) have reviewed potential phytoplankton responses to a changing light environment. While we routinely measure "mixed-layer depths" at Station ALOHA, defined by one of several criteria that examine the near-surface density profiles to infer water stability, we rarely measure actual mixing rates. If the mixing rates within the mixed-layer are greater than a few meters per hr, then there may be a significant impact on estimated *in vitro* rates of primary production since we incubate samples at fixed water depths. Marra (1978) has shown that vertical movements, similar to those that might occur for rapid ocean mixing, can lead to significant changes in the photophysiology of phytoplankton and to variations in photosynthesis, relative to samples incubated at fixed depths. Recent measurements of upper ocean mixing at Station ALOHA in Aug-Sept 2012 using a novel method based on pigment dynamics (specifically, xanthophyll cycle pigments of chromophytic phytoplankton) estimated a mean turbulent transport velocity of 0.41 (±0.10) cm s^{-1}, or a mixing timescale of 2-4 hr for the ~30-40 m mixed-layer (Bidigare and others 2014). So in addition to evolutionary pressures to maximize the use of solar energy over a 24-hr period, phytoplankton and other micro- and macroorganisms that are dependent on recently fixed C must also be able to cope with high frequency variations in energy flux. A systematic, quantitative assessment of the impact of mixed-layer dynamics has not yet been conducted for Station ALOHA. Due to these uncertain effects of mixing on *in* vitro rate measurements, non-incubation techniques, such as temporal changes in O_2 concentration, O_2:Ar ratios or $^{17}\Delta$, might provide the most accurate estimates of

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the time-averaged product (GOP/NCP) within the mixed-layer. However, the current uncertainties in several model assumptions ultimately limit the accuracy of rate estimations by non-incubation techniques.

Recently, Hamme and others (2012) and Tortell and others (2014) independently reported diel changes in the O_2:Ar saturation in productive marine environments. These observations violated the primary assumption of the "traditional" O_2:Ar method that assumes NOP is constant over the resident time of O_2. Ferrón and others (2015) also reported measurable diel and day-to-day variations in the O_2:Ar saturation ratios, and used these data to estimate mixed-layer GOP, MCR and NOP at Station ALOHA. During a 14-day observation period (11-25 Mar 2014), they reported mixed-layer integrated GOP rates ranging from 59 to 131 mmol O_2 m^{-2} d^{-1}. The GOP:^{14}C ratios averaged 2.7±0.1 mol O_2 : mol C during the observation period (Ferrón and others 2015). Whereas the NOP values using the traditional method of analysis were always positive (range = 7.7-14.4 mmol O_2 m^{-2} d^{-1}), suggesting a net autotrophic system, the diel approach documented a much greater dynamic range of NOP from -20 mmol O_2 m^{-2} d^{-1} (net heterotrophy) to +42 mmol O_2 m^{-2} d^{-1} (net autotrophy), indicating large day-to-day variations for the O_2 balance in the mixed layer of Station ALOHA (Ferron and others 2015).

In addition to turbulent mixing, other vertical displacement mechanisms may be critical for phytoplankton, especially those assemblages within the DCML where short-term vertical displacements may be >60 m within a single inertial period (~31 hr at Station ALOHA; Figure 21). During a representative HOT cruise (HOT-83; May 1997), where a 50-m vertical displacement of the DCML was observed, the calculated daily-integrated solar radiation flux to the DCML phytoplankton on 5 consecutive days varied by nearly an order of magnitude as a result of the combined effects of clouds and inertial motions of the water column (Karl and

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others 2002; Figure 21). Other HOT cruises had smaller, or larger, day-to-day variations in solar radiation as a result of this mechanism, but neither the vertical displacement of the DCML (Figure 21) nor cloud cover are predictable from month to month. Furthermore, since the inertial period of oscillation is longer than one day (~31 hr at the latitude of Station ALOHA), the timing of the maximum upward and downward excursions of the DCML advance by approximately 7 hr from one day to the next. This results in an approximately 4-day oscillation in the optimal light field for photosynthesis when the maximum upward excursion coincides with local apparent noon. The diel variability in solar energy flux has numerous ecological consequences beyond photosynthesis, *per se*. For example, we have already presented evidence for light-dependent assimilation of organic matter by *Prochlorococcus*, and for light-dependent DOC production by the phytoplankton assemblage as a whole. Consequently, rates of photoheterotrophy and energy flux to chemoorganoheterotrophs will also be impacted by these high-frequency variations in solar energy flux.

Recently del Valle and others (2015) reported a strong diel cycle of dissolved methionine (dMet) at Station ALOHA with highest concentrations (28-40 pM) in late afternoon, followed by complete removal (<0.17 pM) at night. These large daily variations indicate a temporal offset between net production and net removal of dMet, a sulfur-containing amino acid with multiple metabolic functions. Cell-specific and microbial group-specific dMet assimilation by natural microbial assemblages, measured using ^{35}S-dMet and flow cytometric cell sorting, revealed a major role of high nucleic acid (HNA) bacteria as the mechanism for nighttime removal; however, *Prochlorococcus* and low nucleic acid (LNA) bacteria also contributed to the total dMet assimilation, especially at night (del Valle and others 2015). These observations are consistent with the results of a Station ALOHA microbial community transcriptome analysis that

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documented a relative overabundance of transcripts for amino acid transport and metabolism during the night for both *Prochlorococcus* and selected heterotrophic bacterial taxa (Poretsky and others 2009). The processes leading to net production of dMet during daylight hours are likely tied to photosynthesis and daily variations in solar energy flux, but metabolic details are lacking at the present time.

Ottesen and others (2014) have reported a high-resolution (2-hr intervals over a 3-day period), multispecies diel transcriptome of the microbial assemblage (0.22-5 μm size fraction) at Station ALOHA for samples collected in the mixed-layer in Sept. 2011. Their analysis revealed a pattern of oscillating diel rhythms for all three domains of life (Bacteria, Eukarya and Archaea). While the diel periodicity in *Prochlorococcus* transcriptomes was expected, gene expression oscillations in heterotrophic bacteria at Station ALOHA were not. Furthermore, the enigmatic temporal patterns of different heterotrophic bacterial taxa were staggered, and displayed what the authors described as "waves of gene transcription" within co-existing populations of microorganisms (Ottesen and others 2014). They concluded that diel processes might control both the tempo and mode of organic matter and energy transformation in the sea. While no mechanistic explanation for the coupled diel transcription patterns is available at the present time, these field observations emphasize the inextricable linkages between solar energy capture by phototrophs and energy dissipation by heterotrophs, consistent with a highly evolved and finely tuned microbial food web at Station ALOHA.

Mesoscale Spatial Variability and the Role of Eddies: The HOT program, by design, routinely collects data at a fixed geographical location (11-km radius circle centered at 22° 45'N, 158°W), whereas the ocean is in constant motion. This Eulerian sampling design cannot resolve spatial variability and assumes that horizontal changes in water column properties from hydrocast-to-

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hydrocast over a 3-4 d period of observation each month are small relative to the longer time-scales of interest (seasonal to decadal). In Aug 1989, a massive bloom of the N_2-fixing, filamentous cyanobacterium *Trichodesmium* was reported near Station ALOHA (Karl and others 1992). Surface ocean concentrations of chlorophyll *a* and particulate C, N and ATP were three orders of magnitude greater than those observed less than 10 km away at Station ALOHA. An aerial photographic survey of the sea surface conducted by the U.S. Coast Guard indicated that this massive bloom was intermittently distributed over an approximately 10^3 km^2 region slightly to the west of Station ALOHA. Blooms of *Trichodesmium* and other N_2-fixing microorganisms are common in the NPSG in late summer, and Dore and others (2008) estimate that summer bloom at Station ALOHA may be responsible for up to 38% of annual N_2 fixation. They also concluded that the blooms may be inextricably linked to physical and biological processes controlling phosphorus flux into the euphotic zone during the summer and preceding winter (Dore and others 2008). While rates of N_2 fixation were not measured during the 1989 bloom, elevated concentrations of dissolved pools of fixed N (nitrate and ammonia) were an order of magnitude greater than at Station ALOHA (Karl and others 1992). The authors concluded that the N_2-fixing microorganisms, rather than stochastic deep mixing event, had fundamentally altered the background state of the NPSG euphotic zone and shifted it from a N-limited to a P-limited system (Karl and others 1992).

In July 2005, another major bloom of N_2-fixing cyanobacteria was sampled at Station ALOHA on 26 July 2005, but in between the scheduled monthly HOT cruises. The bloom was not observed on either the July or Aug 2005 HOT cruises (Fong and others 2008). The occurrence and potential ecological impacts of these two major blooms of N_2-fixing microorganisms are, therefore, neither recorded in the HOT program data archive nor completely

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understood with regard to biogeochemical dynamics and consequences. However, they certainly stand out as anomalies from the mean ecosystem state at Station ALOHA.

While the 1989 and 2005 *Trichodesmium* blooms observed in the vicinity of Station ALOHA were unexpected due to the extreme oligotrophy of the NPSG, their presence and that of other blooms like them (see Dore and others 2008) suggest either an active mechanism for the physical concentration of phytoplankton cells or in the delivery of nutrients to support the production and accumulation of biomass, or both. Furthermore, the physical process(es) that create and sustain these phytoplankton blooms must themselves be variable in time, space or, more likely, both. Prior to the advent of satellites, oceanographers had to rely on shipboard observations of spatial variability in oceanic processes (Hayward and McGowan 1985; Venrick 1990a), and there was no methodology available for synoptic regional or ocean basin scale assessments of sea surface properties to unambiguously resolve space and time. This all changed with NASA's launch of Seasat on 28 June 1978, and during the next 106 days this pioneering Earth-orbiting satellite mission collected more information on the ocean physics than had been collected in the period since the HMS *Challenger* expedition more than a century ago, and possibly in the entire history of the discipline. For the first time, oceanographers could observe and study the "weather of the sea" (Krom and others 1993) including, but not limited to the dynamics of mesoscale (10-100 km) features called eddies. The key instrument, a synthetic aperture radar (SAR), provided the capability to detect and map mesoscale eddies based on precise measurements of altimetry, the distance between the satellite and the sea surface below. These topographic maps of the sea revealed a complex mosaic of eddies, some with relatively high sea surface heights (indicative of warmer water) and others with relatively low sea surface heights (indicative of cooler water). The success of Seasat inspired a generation of international satellite missions with advanced

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observing capabilities that have led to an improved understanding of processes in the NPSG. The previous view of the generalized NPSG basin scale topography, based on ship-based observations taken over many years (Wyrtki 1975), has been replaced with a new view, largely based on contemporary SAR imagery. Whereas the ship-based observations revealed systematic seasonal fluctuations in dynamic height associated with variations in upper ocean temperature with an annual amplitude of ~6 dyn-cm (Wyrtki 1975), the synoptic view from satellites reveals a complex, time-space continuum of mesoscale features (Figure 22). Chelton and others (2011) present a comprehensive, global ocean analysis of eddies based on 16 years (Oct 1992 – Dec 2008) of sea surface height (SSH) observations that identified 35,891 distinct features with individual lifetimes ≥16 weeks. Eddies are ubiquitous (with notable exception of a few "eddy deserts") and variable in terms of size, amplitude, polarity (cyclonic versus anticyclonic) and propagation dynamics (Chelton and others 2011). This impressive census and description of mesoscale eddies provides a robust intellectual framework for studies of their role in biogeochemical dynamics in the NPSG.

Another pioneering satellite, the Coastal Zone Color Scanner (CZCS), was also launched in 1978. CZCS was a multi-channel scanning radiometer designed as a "proof-of-concept" to map the color of the surface ocean to a depth of approximately 25 m (also see *A Sea of Change* section). Thus began the age of ocean color radiometry, that eventually led to much more sophisticated instrumentation to assess the distributions and dynamics of marine phytoplankton in the NPSG (e.g., Sea-Viewing Wide Field-of-View Sensor [SeaWiFS]; Moderate Resolution Imaging Spectroradiometer [MODIS]; Ocean Color and Temperature Sensor [OCTS]; MEdium Resolution Imaging Spectrometer [MERIS]; McClain 2009; Yoder and others 2010). The principal data product derived from ocean color radiometry is chlorophyll *a* concentration, the

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primary photosynthetic pigment found in all marine phytoplankton. The accuracy of chlorophyll *a* estimation depends on the algorithm used including sensor calibrations, and atmospheric radiance and colored DOM (C-DOM) corrections (Sauer and others 2012). Large teams of scientists and engineers are involved in data processing to ensure quality; post-mission re-analysis of the remotely sensed data is sometimes required as algorithms improve or new understanding is obtained. For example, a revised algorithm for chlorophyll *a* detection in oligotrophic waters like those at Station ALOHA has recently appeared (Hu and others 2012). Although chlorophyll *a* is the primary data product for ocean color radiometry, these satellite-based observations have also been used widely in models to derive regional or global estimates of primary production (Behrenfeld and Falkowski 1997), the biological C pump (Siegel and others 2014) and NCP (Westberry and others 2012; Li and Cassar 2016).

In February 1991, just prior to the September 1992 launch of the TOPEX-Poseidon altimeter mission, Chiswell (1996) deployed an array of inverted echo sounders at Station ALOHA to provide continuous measurements of dynamic height. During the next three years, he observed large fluctuations with peak-to-peak amplitudes of up to 27 cm and timescales of ~100 days (Chiswell 1996). Since that pioneering experiment, the satellite-based Sea Surface Height (SSH) anomaly (height relative to the long-term reference level) and satellite-based chlorophyll *a* concentrations have been used extensively in the vicinity of Station ALOHA to map phytoplankton distributions and dynamics. A major limitation is the inability of satellite-based radiometers to detect chlorophyll *a* below the first optical depth (approximately 25 m in the clearest ocean waters). At Station ALOHA, the statistical relationship between 0-25 m depth-integrated chlorophyll *a* and 25-200 m depth-integrated chlorophyll *a* is weak, and the ratio of chlorophyll *a* in the mixed-layer to chlorophyll *a* in the lower portion of the water column varies

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on both seasonal and interannual time scales (Figure 23). Consequently, subsurface phytoplankton blooms go undetected by satellite remote sensing. Furthermore, while we can use synoptic, remotely sensed data to examine patterns between SSH and chlorophyll *a*, we are unable to determine the physical and chemical events leading up to a phytoplankton bloom or the ecological consequences of a pulsed C and energy input of solar energy without complementary ship-based measurements, sample collections and experimentation. Nevertheless, the satellite-based observations have already changed our view of spatial and temporal variability in the NPSG, a seascape previously considered to be relatively homogeneous in space and time.

Mesoscale features including eddies, fronts and planetary waves create unique, but ephemeral, habitats that select for or against specific microbial taxa and metabolic functions. Foremost among the potential habitat variations is the transient change in light or nutrients due to vertical displacements in the density field, enhanced turbulent mixing or enhanced stratification of the upper water column. For example, the interactions of winds with episodic open ocean eddies can lead to intense upwelling and the formation of large blooms of diatoms in oligotrophic regions (McGillicuddy and others 1999, 2007).

In 1997, a physical-biogeochemical-optical mooring (Hawaii Air-sea Logging Experiment; HALE ALOHA) was deployed in the vicinity of Station ALOHA. Continuous observations of upper water column (0-150 m) temperature, spectral distribution of downwelling irradiance and phytoplankton-induced fluorescence at 25 m depth were obtained for an approximately 5-month period that bracketed 4 HOT program cruises (H-80, Feb 16-20 to H-83, May 5-9). The temperature time-series showed evidence for a strong upwelling event in March-April 1997 that displaced the thermocline by ~120 m (Letelier and others 2000). Analyses of concurrent remote sensing imagery (NSCAT and TOPEX/Poseidon) suggested that the observed upwelling event

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was a result of simultaneous strong wind divergence and the passage of a cyclonic (cold) eddy through the region of Station ALOHA. The upwelling event was detected at the HALE ALOHA mooring as a rapid increase in the concentration of C-DOM indicating the upwelling of deep water (Letelier and others 2000). Simultaneous measurements of nitrate, using an autonomous Osmo Analyzer, confirmed the appearance of cold, nitrate-enriched water at the 180-m reference depth for that instrument (Sakamoto and others 2004). At the onset of the upwelling event, there was also an immediate increase in the chlorophyll *a* fluorescence efficiency (CFE) at 25 m, an observation that was consistent with the upward displacement of low-light-adapted, photosynthetic microbial assemblages into a higher light regime (Letelier and others 2000). After a few days, the chlorophyll *a* concentrations at 25 m more than doubled between 21 March and 1 April presumably as a result of net phytoplankton growth due to the combined changes in nutrients and light (Letelier and others 2000). Measurements of total gas pressure and O_2 concentrations were also measured at a reference depth of 50 m during the same 1997 HALE ALOHA mooring mission. This gas tension device (GTD) also detected a rapid cooling, beginning in early March, as a result of the passage of the eddy. There was an immediate increase in N_2 gas concentration, followed shortly by an increase in O_2; former caused by upwelling and the latter a result of nutrient-induced net biological production of O_2 (Emerson and others 2002). Based on the assumption that the measured increase in O_2 at the 50-m reference depth (there was only a single GTD on the HALE ALOHA-I mooring) occurred over a 50-m mixed layer, the estimated NOP for this single eddy event was equivalent to ~40% of the annual estimated NOP at Station ALOHA (Emerson and others 2002). While there may be numerous uncertainties in the space-time scaling of events such as the one encountered near

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Station ALOHA in March 1997, the local impact of these mesoscale eddies on biogeochemical dynamics is undeniable.

Church and others (2009) conducted a 3-year study (2004-2007) of approximately monthly measurements of N_2-fixing microorganism abundances, community structures and rates of N_2 fixation in relation to mesoscale physical forcing at Station ALOHA. This comprehensive analysis used a variety of data sets including ship-based HOT program observations, moored platform measurements (HALE ALOHA and Woods Hole Hawaii Ocean Time-series Station, WHOTS) and satellite-derived SSH anomalies measured at 1/3° by 1/3° resolution from the Archiving, Validation and Interpretation of Satellite Oceanography (AVISO) data archive, which represents merged data products from TOPEX/Poseidon, ENVISAT and Jason-1 missions. Temporal fluctuations in near-surface water temperature and potential density were synchronized over a broad range of SSH anomalies from -12 cm to +15 cm (Figure 24; Church and others 2009). On average, a 1 cm variation in SSH anomaly corresponded to an ~2.3 m vertical displacement of the position of the 24.6 potential density surface. During the 3-year period of observation, 18 significant SSH anomaly events, defined as greater than or less than 1 standard deviation of the long-term (1992-2007) mean for this region, were detected; 10 were positive SSH anomalies and 8 were negative SSH anomalies (Church and others 2009). Monthly HOT cruises coincided with 8 of the positive SSH events. Rates of N_2 fixation were variable over the 3-year observation period, but were on average higher in summer than in winter. More importantly, three of the four highest mixed-layer rates of N_2 fixation (>5 μmol N m^{-3} d^{-1}) occurred during cruises with strong positive SSH anomalies (≥11 cm), indicative of warm, anticyclonic eddies (Figure 25; Church and others 2009). The lone exception was the previously mentioned July 2005 N_2-fixation bloom that was associated with a weaker, but still positive (~4

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cm), SSH anomaly in a decaying anticyclonic eddy (Figure 24; Fong and others 2008). Equally important, measurements of the size distribution of N_2 fixation in all four of these events indicated that large cells, >10 μm, were the most active. Quantitative polymerase chain reaction (QPCR) analyses confirmed that the larger size fraction included *Trichodesmium*, as well as several other heterocystous N_2-fixing phylotypes (Church and others 2009). These results from Station ALOHA were consistent with a trans-Atlantic survey documenting elevated concentrations of *Trichodesmium* associated with positive SSH anomaly regions (Davis and McGillicuddy 2006). However, SSH anomaly alone was a poor predictor of N_2 fixation rates at Station ALOHA, and moderate rates (>2 μmol N m^{-3} d^{-1}) of N_2 fixation were often associated with cyclonic eddies. A more recent and expanded analysis (June 2005 – Dec 2013) of the relationships between N_2-fixation and mesoscale features at Station ALOHA confirmed the important role of anticyclonic eddies in regulating N_2 fixation, especially during summer months (Böttjer and others 2016), yet there is still much to learn about the dynamics and ecological consequences of these and related mesoscale phenomena.

Nicholson and others (2008) were the first to use Seagliders and an O_2 mass balance approach to quantify NCP. They conducted three separate surveys from Feb to Nov 2005 of a 50 km by 50 km "bowtie"-shaped pattern centered at Station ALOHA. Dissolved O_2 was measured from 0-1000 m on ~1100 profiles. This field experiment constituted the most comprehensive coverage ever achieved at Station ALOHA. In addition to obtaining the detailed seasonal progression of the subsurface O_2 supersaturation feature, they also mapped several mesoscale features, four of which displayed isopycnal shoaling consistent with cyclonic flow. Based on these observations and a 1-dimensional model that included net turbulent diapycnal mixing and deep water entrainment, they estimated upper and lower bounds for NOP production of 0.9 to 1.5

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mol O_2 m^{-2} yr^{-1} (Nicholson and others 2008). An important result of this impressive year-long study was the observed intermittency of NOP and the role of mesoscale variability in altering the rates of O_2 production. During all four periods of isopycnal shoaling, biological production was elevated and the deep euphotic zone became a net autotropic system; the opposite, net heterotrophy, was observed when isopycnals were depressed (Nicholson and others 2008).

A follow-on Seaglider study at Station ALOHA mapped primary production in the mixed-layer at Station ALOHA by tracking diel cycles in O_2 concentration during a 110-day, 2 Seaglider mission in summer 2012 (22 May – 8 Sept). Optode-based measurements provided sufficient stability and precision to quantify daily cycles of O_2 concentrations with average amplitudes of ~0.6 μmol O_2 d^{-1}. As expected, O_2 reached a minimum concentration near sunrise and a maximum concentration near sunset (Figure 26). The observations were fitted to an idealized photosynthesis versus irradiance curve that allowed the authors to estimate the daily mixed-layer GOP and MCR. GOP averaged 1.8±0.7 mmol O_2 m^{-3} d^{-1}, which was approximately 3 times greater than the ^{14}C-based estimates that were obtained periodically over this same observation period. There were both seasonal and short-lived peaks (>4 mmol O_2 m^{-3} d^{-1}; Figure 26), once again emphasizing temporal variability in plankton rate processes, for reasons that are not well understood at the present time. NOP over the 110-day experiment was 500 mmol O_2 m^{-2} for the mixed-layer (which averaged 57 m in depth), and was <5% of the GOP measured during this period (Nicholson and others 2015), again emphasizing the fact that Station ALOHA is a remineralization-intensive ecosystem (Nicholson and others 2015).

Finally, an intensive field campaign at Station ALOHA from 8 July to 11 September 2012 that included complementary observations using ships, Seagliders, moorings, profiling floats and satellites, was designed to provide higher frequency temporal and broader spatial coverage than

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is possible for the approximately monthly HOT measurement program (Wilson and others 2015). This multi-phase expedition, termed Hawaii Ocean Experiment: DYnamics of Light And Nutrients (HOE-DYLAN) sought fundamental information on microbial ecosystem processes based on a near daily sampling of the upper water column over the 3-month period. This expedition coincided with the passage of an anomalous, low sea surface (0-40 m) salinity feature that was most pronounced from 6 Sept onwards. This minimum salinity feature was also detected using autonomous profiling floats, but efforts to determine the size, origin and dynamics of this prominent feature were unsuccessful. There was also an absence of mesoscale eddies at Station ALOHA during this extended time period, which is quite unusual for this region of the NPSG (Chelton and others 2011). Among other biogeochemical characteristics, the low salinity feature coincided with decreased abundances of microorganisms and low rates of primary production compared to the HOT program climatology, and a prolonged period of net heterotrophy (negative NOP) based on measurements of O_2:Ar (Wilson and others 2015). The geolocated (Station ALOHA) Eulerian-type sampling design of this study made it impossible to separate time from space, or to obtain a full understanding of controls on microbial biogeochemical processes or the ecological consequences thereof. An ambitious follow-on study using two research vessels was conducted in a Lagrangian sampling design by tracking surface velocity program (SVP) drifters that moved with an anticyclonic eddy that had been pre-selected for this comprehensive field experiment. This major expedition corresponded to the final C-MORE cruise and the inaugural SCOPE cruise. Although many of the samples that were collected are still being analyzed, this C-MORE/SCOPE expedition represented a major step forward in the investigation of mesoscale features near Station ALOHA. A long-term 4-

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dimensional field study with sample return will probably be required to fully understand these complex systems, and hopefully this will be possible to achieve during the next decade.

A Sea of Change: The ocean is constantly changing on multiple time and space scales, and a proper consideration of this natural environmental variability is important for the interpretation of ecosystem dynamics at Station ALOHA, and elsewhere in the world's oceans. However, recent assessments have documented an additional source of environmental variability, namely the impacts of a burgeoning human population with energy and resource demands that are beginning to change fundamental properties of the coupled ocean-land-atmosphere systems of our planet. The most recent global assessment conducted by the Intergovernmental Panel on Climate Change (IPCC) has concluded "Human influence on the climate system is clear" (Stocker and others 2013; Stocker 2015). The future ocean, circa 2100, will be warmer, less saline, more acidic, less oxygenated and less productive than the contemporary ocean (Reid and others 2009; Gruber 2011; Pörtner and others 2014). These changes in the physical habitat will lead to altered marine ecosystems, including accelerated species extinctions, expanded oxygen minimum zones, changes in nutrient cycles and shifts or expansions in the ranges of many species (Doney and others 2012). Marine ecosystem services, including food resources, climate regulation and biogeochemical process (e.g., biological C pump) will all be degraded, relative to the pre-industrial period (Pörtner and others 2014). Time-series observation programs like HOT will continue to be important sentinels to detect ecosystem change and to track leading indicators as critical thresholds or tipping points are approached. Comprehensive modeling efforts based on accurate paradigms of ecosystem structure and function will be key for extrapolating contemporaneous observations and experimental data into the future.

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Station ALOHA is poised to play a major role in both the detection and interpretation of ecosystem change and in the continued refinement of our understanding of the response of the NPSG to human-induced habitat variability and change. Indeed, the 28-year (and counting) ALOHA CO_2 observation program is one of the longest and most robust to track ocean acidification in the open ocean (Figure 27; Dore and others 2009; Church and others 2013; Bates and others 2014). Ocean acidification is a manifestation of the increasing burden of CO_2 in the atmosphere as a result of the combined effects of fossil fuel combustion, deforestation and other human activities. The elevated concentrations of atmospheric CO_2 equilibrate with the surface ocean and in the NPSG lead to increases in the partial pressure of CO_2 (pCO_2) in the surface ocean and corresponding decreases in pH (Figure 27). While the process of open ocean acidification is now well documented and understood, the potential impact on ocean ecosystems is not. A number of credible laboratory studies on the impacts of enhanced pCO_2 and lower pH on phytoplankton productivity, metabolism and specific function (e.g., N_2 fixation) have led to conflicting results. The acute exposure design for most experiments conducted to date, where an untreated control sample is compared to one that receives excess CO_2 (usually added as CO_2 gas or by addition of hydrochloric acid and bicarbonate during a pre-assessment equilibration step) may be difficult to interpret in the context of gradually changing habitats and multiple stressors. Furthermore, the response of a diverse ecosystem like the NPSG may be fundamentally more difficult to observe and predict than the behavior of selected species due to unpredictable ecological and metabolic consequences, including adaptation, evolution and the creation of new ecological niches.

A limited set of field experiments (May 2012 and Sept 2012) on the effects of increases in seawater pCO_2 on N_2-fixing microorganisms has been conducted at Station ALOHA. There was

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no systematic, short-term (24-72 hr) response of either ^{14}C-based primary production or rates of N_2 fixation to abrupt changes in pCO_2 from control sample concentrations of ~390 ppm to experimental concentrations in excess of 1,000 ppm (Böttjer and others 2014). These results suggest that naturally occurring phytoplankton assemblages at Station ALOHA, including the very important N_2-fixing species, are relatively resilient to large perturbations in pCO_2. These field results are consistent with similar experiments conducted in the South Pacific Subtropical Gyre (Law and others 2012), but conflict with several laboratory studies conducted with monospecific N_2-fixing cyanobacteria, including *Trichodesmium*, which documented enhanced rates of N_2 fixation with increased pCO_2 (e.g., Fu and others 2008; Hutchins and others 2013). Recent field studies on isolated *Trichodesmium* colonies conducted at Station ALOHA found no consistent effect of elevated pCO_2 on C or N_2 fixation during 11 independent, short-term manipulation experiments (Gradoville and others 2014). The field results from Station ALOHA support the general null hypothesis presented several years earlier (Joint and others 2011), "major biogeochemical processes in the oceans other than calcification will not be fundamentally different under future higher CO_2 / lower pH conditions." Additional laboratory and field work, designed to investigate the cumulative impacts of multiple stressors (Bopp and others 2013; Boyd and others 2015), are urgently needed to improve our understanding and predictive modeling of microbial processes in the future, changing ocean.

An additional change in NPSG habitat structure that is likely to have major impacts on microbial processes, including productivity, food web structure and export, is enhanced upper water column stratification resulting from the combined effects of increased temperature and decreased salinity. Oligotrophic ocean habitats like the NPSG owe their existence to permanent density-driven stratification which serves to insulate the near-surface waters from the lower

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portion of the water column, and to reduce the flux of life-sustaining nutrients. In a series of reports beginning with Gregg and Conkright (2002), satellite-based time-series observations were used to explore the dynamics of global patterns in chlorophyll *a* in surface ocean waters. The first analysis used data from both the CZCS (1979-1986) and SeaWiFS (1997-2000) records to compare the derived chlorophyll *a* concentrations over the ~2-decade-long observation period. Both seasonal and interannual changes were observed in selected regions, but the mid-ocean gyres (including the NPSG) appeared to exhibit only limited change (Gregg and Conkright 2002). In a subsequent report, Gregg and others (2003) used an improved re-analysis of the blended chlorophyll *a* data sets along with other input parameters in the Vertically Generalized Production Model (VGPM; Behrenfeld and Falkowski 1997) to examine patterns in primary production. Their results suggested that primary production had decreased globally by 6.3%, from 45.3 Pg C yr^{-1} to 42.5 Pg C yr^{-1}, with a change of -9.3% for the NPSG over the decade-long observation period (Gregg and others 2003). The authors hypothesized that warming of the sea surface had led to enhanced stratification and lower rates of nutrient entrainment, as predicted earlier by climate warming simulations (Sarmiento and others 1998). A follow-on study using a 6-year (1998-2003) SeaWiFS time-series (Level-3, Version 4, monthly, 9 km data) reported that the NPSG chlorophyll *a* had decreased by 16.3% and sea surface temperature had increased by 0.31°C per year over the observation period (Gregg and others 2005). An independent analysis of a blended (8 months of Japanese OCTS plus 72 months of SeaWiFS) 7-year ocean color time-series, both using vicarious calibrations with the Marine Optical Buoy (MOBY; Eplee and others 2001) concluded that the most oligotrophic portion of the NPSG, defined as those regions where chlorophyll *a* is ≤0.07 mg m^{-3}, is expanding (McClain and others 2004). These provocative conclusions (i.e., lower chlorophyll *a* concentrations, lower primary production and greater aerial

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extent of the most oligotrophic portion of the NPSG) were qualified with caveats concerning the relatively short duration of the observation period and the need for additional time-series data to unambiguously characterize and interpret these fundamental ecosystem changes. Furthermore, technical issues related to inconsistencies in color sensor design, calibration and operational lifetime, as well as data processing algorithms, makes it very difficult to compare results across multiple satellite missions (Gregg and Casey 2010). Nevertheless, these pioneering studies stimulated additional research to verify these trends and to predict the potential ecological and biogeochemical consequences of habitat changes in the NPSG.

Enter Polovina and others (2008), with a new analysis of a 9-year time-series of SeaWiFS data (Jan 1998 – Feb 2007, 9-km pixel resolution, July 2007 reprocessing by NASA – version 5.2) along with satellite-derived, 1°x1°, monthly resolution sea surface temperature. Their analysis of these time-series observations confirmed that the most oligotrophic regions of the NPSG (≤0.07 mg chlorophyll *a* m^{-3}) were expanding at an annual rate of ~2.2%, and had a cumulative growth of more than 3 million km^2 over the nearly one-decade period of observation (Polovina and others 2008; Figure 28). Based on predictions from a coupled climate-ocean biogeochemical model, the NPSG could expand ~30% by 2100 (Polovina and others 2011); however, the authors were quick to point out that their results were model-dependent (Sarmiento and others 2004; Henson and others 2009). The expansion observed in the ocean color imagery was mostly restricted to an area between 120°W and the dateline, and nearly encircled the region near Station ALOHA (Figure 28). The authors concluded that warming and enhanced surface ocean stratification had resulted in decreased phytoplankton biomass and productivity in the NPSG (Polovina and others 2008).

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An updated, 16-year (1998-2013) analysis of chlorophyll *a* (using combined SeaWiFS and MODIS data records and two different algorithms) and estimates of net primary production (using the Carbon-based Production Model-2 [CbPM2]; Westberry and others 2008) have confirmed the decreasing trends for both chlorophyll *a* (-0.570±0.050 and -0.577±0.049 μg Chl *a* m^{-3} yr^{-1}, for the STD and OCI algorithms, respectively) and production (-7.354±0.420 mg C m^{-2} yr^{-1}) in the NPSG (Signorini and others 2015). However, neither the near-surface chlorophyll *a* (Figure 28) nor the total rates of ^{14}C-based primary production measured at Station ALOHA (Figure 3) during this same time period show any significant decreases. These major discrepancies between the computer models and the field observations were not discussed, but ultimately do need to be understood.

Boyce and others (2010) have combined satellite-derived chlorophyll *a* data (available only since 1979) with a longer term, historical data base of shipboard chlorophyll *a* and ocean transparency measurements for the period 1899-2009. Their comprehensive analysis indicated declines in global phytoplankton, including the North Pacific Ocean, at a mean rate of ~1% per year over the past century. They also attributed these trends to climate change, namely an increase in sea surface temperature (Boyce and others 2010). Despite these century-long projections, both chlorophyll *a* and primary production have increased in the NPSG during the period 1960-present (Figure 3; Karl and others 2001a) so it is unclear which of these trends is more accurate, and why.

A basic premise in all of these analyses and interpretations of decade-scale changes in the NPSG is that sea surface warming leads to enhanced stratification and a reduction in the supply of nutrients from beneath the thermocline (Boyce and others 2010). Changes in stratification can also alter mean phytoplankton cell size (Polovina and Woodworth 2012), and phytoplankton

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species diversity (Bouman and others 2011), and, hence, the structure and function of the microbial food web including the efficiency of the biological C pump. As discussed previously, at Station ALOHA we observe the highest primary production in summer when the sea surface temperature is warmest and the water column is most stratified (Figures 8 and 11). Furthermore, we have also shown that both strong and weak mixing of the water column at Station ALOHA can lead to enhanced primary production, but for fundamentally different reasons. The former is due to entrainment of nutrients and the latter is due to the selection for enhancement of N_2-fixing microorganisms that release the system from chronic N limitation. Of course, enhanced phytoplankton production under conditions of increased stratification can only be a temporary condition because other nutrients, possibly phosphate or iron, would then ultimately control primary production and export. At Station ALOHA, the total dissolved P pool is approximately 200 μmol P m^{-3} (Karl and others 2001a). Assuming that this entire pool is bioavailable, it could support at most a net production of 240 mg C m^{-3} before the pool is exhausted.

Surface ocean stratification can also have additional consequences on the ecosystem dynamics. For example, increased surface ocean irradiance within a more stratified and shallower mixed-layer will lead to increased photolysis of selected compounds with either positive (e.g., partial photolytic degradation of recalcitrant organic matter leading to greater bioavailability) or negative (e.g., production of reactive oxygen species such as hydrogen peroxide) impacts on microbial processes. In addition to warming-induced surface ocean stratification, other factors, such as the strength of local and remote winds or the advective supply of nutrients can also lead to temporal changes in satellite-derived ocean color, complicating any straightforward relationships between sea surface temperature and ocean color on interannual time scales (Lozier and others 2011). A careful analysis of data from Station

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ALOHA revealed that stratification and productivity were not strongly correlated over the observational time period (1988-2008; Dave and Lozier 2010). The authors conclude that in the absence of any strong relationship between stratification and productivity at this well-studied oligotrophic site, the observed linkages between sea surface temperature and satellite-based chlorophyll *a*, independently reported by numerous investigators, "appears to be tenuous" (Dave and Lozier 2010). In an attempt to offer their own explanation for the lack of any correlation between surface ocean stratification and productivity, Dave and Lozier (2010) suggest that since the strength of the seasonal signal is much greater than the interannual variability, the observational time-series is simply too short to yield any consistent relationship. This sobering assessment is supported by a recent comparison of three state-of-the-art biogeochemical models that concludes a satellite-based chlorophyll *a* time-series of ~40 years in length will probably be needed in order to distinguish a global warming trend from natural variability (Henson and others 2010).

Finally, Siegel and others (2013) also concluded that ocean ecosystems may be too complex to ever hope to use a single index, like satellite-based "standard chlorophyll *a* retrieval," as an index of phytoplankton dynamics in the surface ocean. They argue that the proper interpretation of the chlorophyll *a* signal has at least two components: phytoplankton biomass and physiological changes due to changing mixed-layer depths (and hence, light), and for ecosystems such as the NPSG, photophysiological processes control chlorophyll *a* variability (Siegel and others 2013). Observational record length, time period, and algorithm employed for data analysis will all impact the conclusions reached, the potential ecological implications and the projections of future ocean states in a changing world. The possibility of climate-driven expansion of the most oligotrophic regions of the NPSG is clearly in the realm of Walter Munk's

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definition of an inconvenient sea truth: *too fundamental to be ignored, too incomplete to be understood* (Munk 2009).

Future Challenges and Opportunities

The assessment of ecosystem dynamics in the NPSG must be considered a work in progress. If history is our guide, it will likely take many more decades of observations, hypothesis testing, field experimentation and modeling before a comprehensive understanding of the complex interactions and controls of key microbial and biogeochemical processes is likely to emerge. As succinctly stated by Munk and Wunsch (1982) in their crystal-ball-like prospective on "Observing the ocean in the 1990s," to understand the ocean one must observe it on a basin-wide scale with adequate time and space resolution. Chronic undersampling remains a fact of life in microbial oceanography, and even decades-long observation programs like HOT are inadequate to answer some of the most basic questions regarding microbial processes in the sea and their response to climate change. Continued access to the sea will be essential.

During the past several decades there has been remarkable progress in our understanding of NPSG ecosystem dynamics, in part, as a result of research conducted at Station ALOHA. The HOT program, founded in 1988, provided a foundation for other complementary research programs (C-MORE, SCOPE) and a locus for trans-disciplinary investigations of the NPSG. However, many basic ecological questions, some presented in this review, remain and future discoveries will undoubtedly create new research opportunities and refined mechanistic models. The concept of the 'ocean microbiome' is a rapidly advancing frontier in microbial oceanography (Moran 2015; Kujawinski and others 2016). Through detailed studies of microorganisms, genes, proteins and metabolites we are beginning to learn more about functions and controls of major biogeochemical processes in the sea. Future progress in this area will

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require new analytical capabilities and novel approaches for the analysis and visualization of these large, complex datasets, as well as proper training of the next generation of microbial oceanographers who will need to convert these enormous streams of information into knowledge. One major challenge is the identification of 'hypothetical' genes, those open reading frames with unknown biological function. In microbial genomes and metagenomic surveys conducted to date there is always a large percentage (30-50%) of hypothetical genes. A recent analysis of the essential gene set for the model cyanobacterium, *Synechococcus elongates* PCC7942, found that only 718 of the organism's 2723 total genes were essential for growth under laboratory conditions and of those 718 essential genes approximately 15% were hypothetical (Rubin and others 2015). Even the minimal gene set for microbial life consisting of 473 genes (Syn 3.0) contains 149 genes with unknown biological function (Hutchison and others 2016). We will eventually need to determine the functions of all hypothetical genes (essential and non-essential) before we have the complete 'parts list' for life in the sea.

Finally, a major challenge for both science and society is the inextricable linkages between human activities and NPSG ecosystems. Since the beginning of the Anthropocene, human-induced environmental change has begun to affect even the most remote ocean regions. In his assessment of the threat of sea level rise, Wyrtki (1990) concluded that "the effects of a doubling world population within the next 40 years will have more disastrous consequences for our environment than potential rise of sea level." The demands for energy, natural resources, food and water have irreversibly impacted natural ecosystems, including the NPSG. For example, the 'great Pacific garbage patch' is a region of the eastern North Pacific Ocean between Hawaii and California where floating debris is concentrated by gyre-scale circulation processes (Moore and others 2001; Goldstein and others 2013). Plastic, which enters the ocean via numerous transport

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pathways, is a major component of the garbage patch and has received a great deal of attention in recent years. Millimeter scale 'microplastics' are of greatest environmental concern because of their potential toxicity and their ability to enter the marine food web through ingestion (Law and Thompson 2014). A recent NPSG field investigation of plastic debris revealed the presence of diverse assemblages of microorganisms as well as metazoan taxa (Bryant and others 2016). The bacteria inhabiting plastic were taxonomically and functionally distinct from the surrounding picoplankton. The presence of genes encoding chemotaxis, secretion systems and cell-to-cell interactions indicated adaptations for a surface associated lifestyle (Bryant and others 2016). High concentrations of chlorophyll *a* and high rates of GOP indicated that the plastic-associated microbial assemblages were photosynthetically active, and the presence of *nifH*-genes suggested that they may also be fixing nitrogen to partially satisfy their N requirements. Another human impact on NPSG ecosystems is deposition of reactive nitrogen derived from anthropogenic emissions, especially from northeastern Asia (Kim and others 2014). This new and growing N flux pathway will lead to an imbalance in the N:P stoichiometry of surface waters and eventually to changes in the structure and function of microbial assemblages. Over time the NPSG will become phosphorus-controlled and, probably, more oligotrophic than it is at the present time, despite the enhanced delivery of a vital nutrient. As Revelle and Suess (1957) warned, "human beings are now carrying out a large-scale geophysical experiment of a kind that could not have happened in the past, nor be reproduced in the future." There is great urgency to obtain a more comprehensive, mechanistic understanding of the NPSG, including the role of microorganisms. This is the major contemporary challenge of the sea.

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FIGURE CAPTIONS

Figure 1. Map of the North Pacific Ocean showing the location of Station ALOHA (22°45'N, 158°W) within the NPSG. The background colors depict summer chlorophyll *a* distributions as determined by the SeaWiFS satellite mission (*oceancolor.gsfc.nasa.gov/SeaWiFS/*) with blue regions corresponding to the lowest concentrations (<0.1 mg m^{-3}) and green-yellow-red corresponding to higher concentrations (>1 mg m^{-3}). The white contours represent mean annual surface ocean phosphate concentrations (μmol l^{-1}) and emphasize the correspondence between chlorophyll *a* and nutrient concentrations.

Figure 2. Various light fluxes at Station ALOHA. **[A]** Photosynthetically Available Radiation (PAR) versus depth displayed as percent surface PAR for winter and non-bloom summer conditions. Inset on left shows annual variation in surface PAR. Inset on right shows variation in the depth (m) of the 0.415 mol photons m^{-2} d^{-1} isolume as a function of surface PAR (season). The two open symbols on the right, well above the trend line, are from summer blooms. Redrawn from Letelier and others (2004). **[B]** Winter and summer mean daily rates of ^{14}C-based primary production as determined by difference uptake in the light and in the dark at Station ALOHA. **[C]** Meso- and abyssopelagic light fluxes showing sunlight, bioluminescence and the estimated "glow" from radioactive potassium (^{40}K) dissolved in seawater. The bioluminescence data are presented as minimum (background) and median intensity of light flashes detected using a calibrated 5-inch EMI photomultiplier with a 0.1 sec time constant. Redrawn from Bradner and others (1987).

Figure 3. Composite time-series plots of chlorophyll *a* concentrations and rates of primary production for samples collected during the months of May-Oct in the NPSG over the past half-century. **[A]** Euphotic zone depth-integrated chlorophyll *a* as determined by the standard fluorometric method. The solid and dashed lines for the Station ALOHA-era data set represents the mean ±1 SD (mean = 23.76, SD = 4.05, n = 131). **[B]** Euphotic zone depth-integrated rates of primary production as determined by the standard ^{14}C method. Most of the data prior to the Station ALOHA-era were collected at or near the Climax study region and, where appropriate, represent the mean ±1 SD for multiple observations for a given cruise, season or study. Horizontal bars indicate the duration of multi-cruise data sets ("C" indicates Climax program; see Karl and others 2001a). The solid and dashed lines for the Station ALOHA-era data set represents the mean ±1 SD (mean = 577.4, SD = 129.1, n = 127).

Figure 4. Microbial dynamics following deep seawater nutrient amendment of surface samples collected in the NPSG. **[A]** Chlorophyll *a* (left) and chlorophyll *c* (right) concentrations versus incubation time following the addition of 5% and 10% (by volume) additions of deep (700 m) seawater. **[B]** Oxygen flux at the end of the 100-hr incubation period. Shown are gross and net primary production (GPP and NCP, respectively) and microbial community respiration (MCR). **[C]** The addition of nutrients leads to a shift from a *Prochlorococcus*-dominated community with a negligible NCP to a demonstrably net autotrophic community that is dominated by diatoms as depicted (redrawn from McAndrew and others 2007).

Figure 5. Variation in f-ratio (the ratio of new production to total production) with nitrate concentration. Data are from a number of studies off southern California with Station ALOHA

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data shown in shaded portion with f-ratios significantly below the prediction for low nitrate concentrations. Redrawn from Eppley (1989).

Figure 6. Conceptual view of the complex interactions in a ^{14}C-based primary productivity incubation experiment. Depicted are various inorganic and organic C pools (boxes), ^{14}C labeling pathways and recycling mechanisms (arrows) that occur after ^{14}C-bicarbonate is added to a sample of Station ALOHA seawater and incubated under *in situ* conditions. Redrawn from Karl and others (1998).

Figure 7. Change in ^{14}C activity during Whatman GF/F filter storage for 30 days in liquid scintillation counting cocktail. Values shown are mean differences ±1 SD for all 0-100 m depth samples for a given month. The solid line is the 3-point running mean.

Figure 8. **[Top]** Euphotic zone depth-integrated primary production as determined by the ^{14}C method. The solid horizontal line represents the 26-year mean value of 525.6 mg C m^{-2} d^{-1}. **[Bottom]** Primary production data presented as a frequency histogram with the mean and ±1 SD (525.6 and 125.6 mg C m^{-2} d^{-1}, respectively) shown as solid and dashed vertical lines. The inset on upper left shows the seasonal mean values [Spring (S) = Mar-May, Summer (S) = June-Aug, Fall (F) = Sept-Nov, Winter (W) = Dec-Feb]. The inset on right presents annual mean (diamond), and for each year the red and blue solid circles are mean summer and mean winter values, respectively. Data extended from initial presentation in Karl and others (2002).

Figure 9. Average nitrate **[Top]** and phosphate **[Bottom]** concentrations in the upper 0-30 m of the water column at Station ALOHA. The solid horizontal lines represent the 26-year mean values of 3.0 nmol l^{-1} and 58.9 nmol l^{-1} for nitrate and phosphate, respectively.

Figure 10. Dissolved inorganic and organic nitrogen and phosphorus in the upper water column (0-30 m) at Station ALOHA. The primary plot shows dissolved organic N (DON) versus dissolved organic P (DOP), and nitrate versus phosphate relative to the Redfield reference molar ratio of 16N:1P. The inset expands the nitrate vs. phosphate concentrations. The means, SDs and number of observations are: nitrate (3.0 nmol l^{-1}, 3.0 nmol l^{-1}, 232), phosphate (58.9 nmol l^{-1}, 35.7 nmol l^{-1}, 232), DON (5.66 μmol l^{-1}, 0.62 μmol l^{-1}, 116) and DOP (0.22 μmol l^{-1}, 0.36 μmol l^{-1}, 116).

Figure 11. Primary productivity variability in the light-saturated surface ocean (5 m) at Station ALOHA based on 26 years of approximately monthly observations. **[Top, Left]** ^{14}C-based primary production (shown as mean ±1 SD) binned by month along with clear sky, surface PAR (bold red lines ~30-55 mol quanta m^{-2} d^{-1}; see Figure 2). **[Right]** Also shown is the late summer (Jul-Sept) trend, binned into 2-week periods. **[Center]** Chlorophyll *a* presented in the same format, showing a systematic, photoadaptive change in chlorophyll *a* concentration in response to PAR. **[Bottom]** Assimilation number (g C g chl *a*$^{-1}$ hr^{-1}) in same format, showing the increasing trend in late summer.

Figure 12. Light-dependent assimilation of ^{14}C-bicarbonate **[Top]** and ^{3}H-leucine **[Bottom]** by High and Low Red Fluorescent (HRF and LRF, respectively) *Prochlorococcus* cells and non-

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pigmented bacteria sampled from the deep chlorophyll maximum layer at Station ALOHA. Redrawn from Björkman and others (2015).

Figure 13. Primary production at Station ALOHA as assessed using both the ^{14}C and ^{18}O methods. Each data point is the mean of three replicates. ^{18}O data are scaled to C using a photosynthetic quotient of 1.25. The horizontal dashed lines denote the base of the mixed-layer. Redrawn from Juranek and Quay (2005).

Figure 14. Schematic representation of the multiple possible fates of sinking particulate organic matter (POM) and transformations back to carbon dioxide (CO_2). Processes include **[Top, Left]** microbial mineralization of sinking POM to carbon dioxide (CO_2), **[Center, Left]** POM solubilization, hydrolysis, fragmentation, aggregation/flocculation, assimilation and microbial remineralization to CO_2 and **[Bottom, Left]** ingestion, egestion, molting/death and metazoan remineralization to CO_2. These metabolic processes consume oxygen (O_2) and increase the total dissolved inorganic carbon concentration of the mesopelagic zone (200-2000 m) as shown on right with data collected at Station ALOHA. Most likely, all of these processes contribute to the net remineralization and attrition of POM flux with depth.

Figure 15. **[Top]** Particulate carbon (PC) and particulate nitrogen (PN) fluxes measured at the 150 m reference depth at Station ALOHA during a 25-year observation period (1989-2014). The blue line depicts approximately monthly observations and the dark black line is the 3-point running mean value. **[Bottom]** PC flux versus PN flux for the observation period with the mean C:N (8.0) value shown by the regression line. The inset shows variations in the PC:PN flux ratio over the 24-year period.

Figure 16. **[Left]** Relationships between ^{14}C-based primary production and particulate carbon (PC) flux at 150 m for the 24-year observation period (1989-2013). The horizontal lines represent the mean value for each data set (mean primary production = 525.6 mg C m^{-2} d^{-1}; mean PC flux = 27.9 mg C m^2 d^{-1}). **[Right]** PC export flux at 150 m versus ^{14}C-based primary production for all data and for winter (Dec-Feb) and summer (Jun-Aug) observations only. The dashed red lines depict the export ratios (e=0.01 and 0.20) that bound the Station ALOHA data set. While summer rates of primary production and export are greater than winter rates, the e-ratios do not vary with season (mean winter = 0.058, mean summer = 0.057).

Figure 17. **[Left]** Particulate carbon (PC) flux versus water depth for the open ocean composite data set of Martin and others (1987) and **[Right]** for two additional NPSG data sets (VERTEX, Knauer and others 1990; Station ALOHA). Redrawn from Martin and others (1987) and Karl and others (1996).

Figure 18. Contemporaneous particulate carbon (PC) flux at 300 m relative to PC flux at 150 m during a 7-year period at Station ALOHA. The solid horizontal line at PC flux ratio = 0.57 is the predicted flux based on the ALOHA curve (see Figure 16, right). The data points in shaded portions of the plot highlight several unusually high or low PC flux ratios (see text for more details).

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Figure 19. **[A]** Euphotic zone depth-integrated primary production climatology with a 3-point running mean trend line, **[B]** dissolved oxygen (45 m) with solid blue symbols ≤100% air saturation and solid red symbols >100% air saturation showing summertime net O_2 production, **[C]** particulate carbon (PC) flux climatology at 150 m reference depth with a 3-point running mean trend line and **[D]** PC flux at 4000 m reference depth showing the summer export pulse (SEP) of organic matter to the seabed.

Figure 20. A "Stommel" plot showing the broad range of spatiotemporal variability in selected physical and ecosystem processes in the sea. The diel scale of variability, depicted by the horizontal dashed line, impacts all horizontal space scales. The rectangles show the approximate time and space scales for a variety of sampling/observation platforms used to study the NPSG. Adapted from Stommel (1963) and Dickey (1991).

Figure 21. **[Top]** Short-term (~1 day) isopycnal vertical displacements of the deep chlorophyll maximum layer (DCML) at Station ALOHA during the 26-year observation period 1989-2014. **[Bottom]** The mean and ±1 standard deviation (38.95 m ± 15.72 m) is shown along with the frequency distribution binned into 5-m intervals.

Figure 22. **[Top]** Dynamic topography contour map of the North Pacific surface water in dyn-cm relative to 1000 dbar based on historical shipboard observations. Arrows indicate the direction of geostrophic flow. Redrawn from Wyrtki (1975). **[Inset]** Satellite-based sea surface height (SSH) anomalies (cm) for the region surrounding Station ALOHA (shaded portion) in summer 2016. Data product courtesy of Colorado Center for Astrodynamics Research.

Figure 23. Vertical distributions and dynamics of chlorophyll *a* (chl *a*) at Station ALOHA. **[A]** Mid-euphotic zone (25-200 m) depth-integrated chl *a* versus near surface (0-25 m) chl *a* concentrations for the 26-year observation period. **[B]** Seasonal variability in the surface (0-25 m) : deep (25-200 m) euphotic zone ratio, **[C]** Interannual variability in the mean surface (0-25 m) : deep ratios and **[D]** Vertical profiles of chl *a* concentrations for summer (Jun-Aug) and winter (Dec-Feb) periods at Station ALOHA. The lower mean concentrations of chl *a* in the surface, the higher mean chl *a* concentrations in DCML and the deeper position of the DCML in summer are all caused by a greater flux of solar energy (also see Figure 2).

Figure 24. **[Top]** Relationship between ocean temperature measured at 140 m and satellite determined sea surface height (SSH) anomaly. Solid line is least squares regression and dashed lines indicate the 95% confidence interval. **[Center]** Relationship between potential density measured at 135 m and SSH anomaly, as above. **[Bottom]** Relationship between vertical position of the 24.6 isopycnal surface and SSH anomaly for the period 1992-2007, as above. Redrawn from Church and others (2009).

Figure 25. Bubble plots depicting relationships between near-surface ocean rates of nitrogen fixation, temperature and SSH anomalies. **[Top]** Whole seawater and **[Bottom]** <10 μm size fraction. Bubble units are μmol N m^{-3} d^{-1}. Redrawn from Church and others (2009).

Figure 26. **[Left]** Seaglider-based oxygen saturation anomaly (%) averaged over the upper 40 m (above) and through the upper 100 m (below) for the month of July 2012 at Station ALOHA.

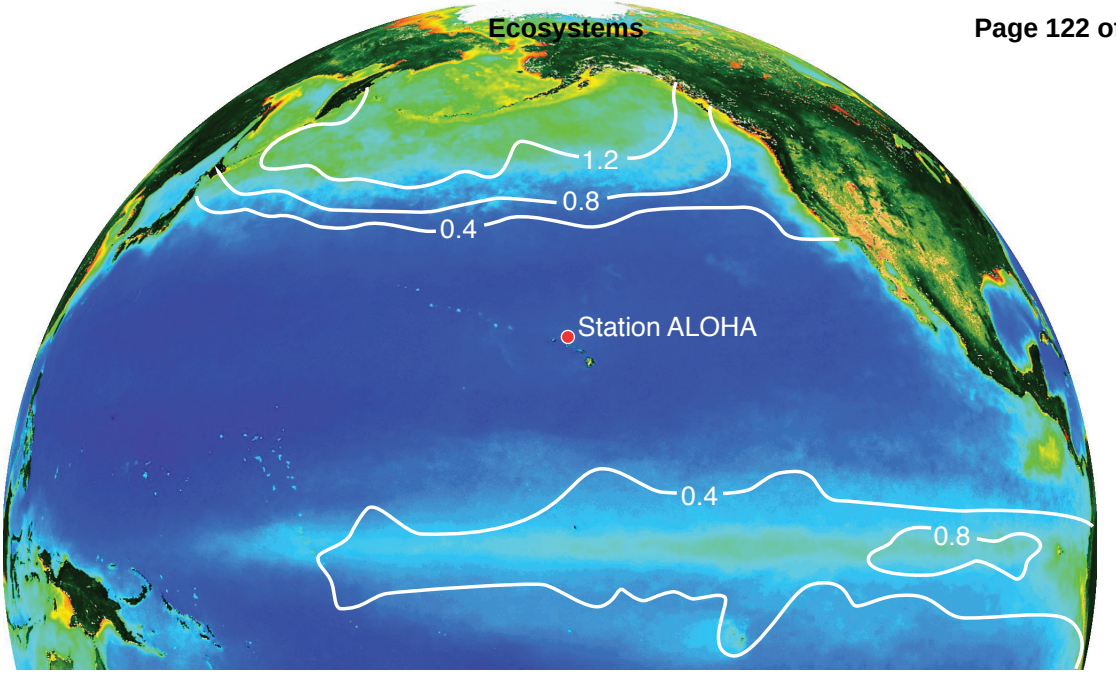
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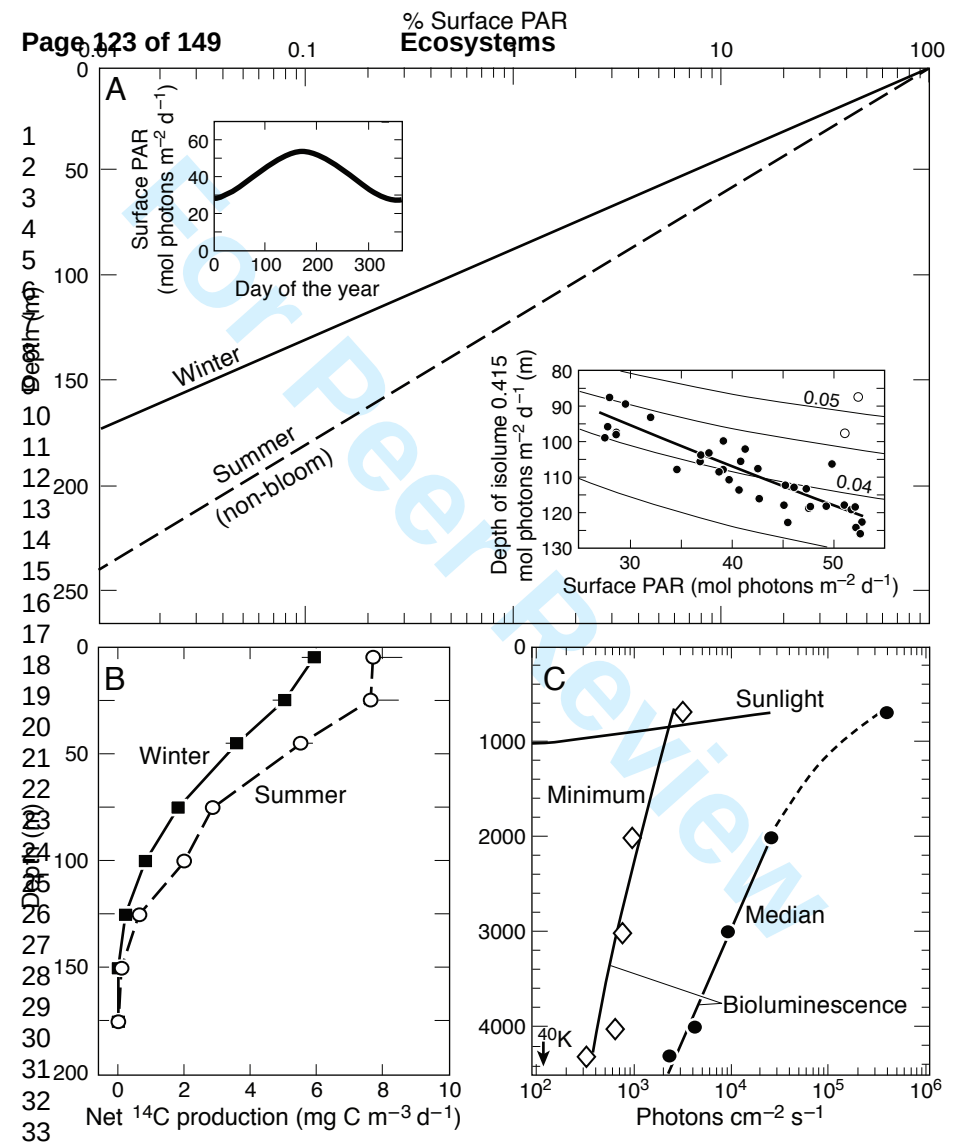
Yellow bars (above) show daylight hours. Mixed layer depth using a density threshold of 0.03 kg m^{-3} (white line) and 0.125 kg m^{-3} (black line) are also presented in the lower plot. **[Right]** Mean value of gross primary production (GPP) for the 0-40 m portion of the water column. Black line shows a 9-day running median with shaded regions corresponding to the 25^{th}-75^{th} percentiles of the running median value. Upward pointing arrowheads are Seaglider ascents and downward pointing arrowheads are Seaglider descents. Red and blue data symbols were collected using Seaglider #146 and #148, respectively. Redrawn from Nicholson and others (2015).

Figure 27. **[Top]** Time-series of partial pressure of carbon dioxide (pCO_2) as determined from total dissolved inorganic carbon and total alkalinity for near-surface water (0-30 m) samples collected at Station ALOHA. The wet air pCO_2 from Mauna Loa Observatory (The "Keeling" curve) is also presented for reference. **[Bottom]** Station ALOHA pH values measured and calculated from carbonic acid system measurements (as above). Redrawn and extended from Dore and others (2009) and Church and others (2013).

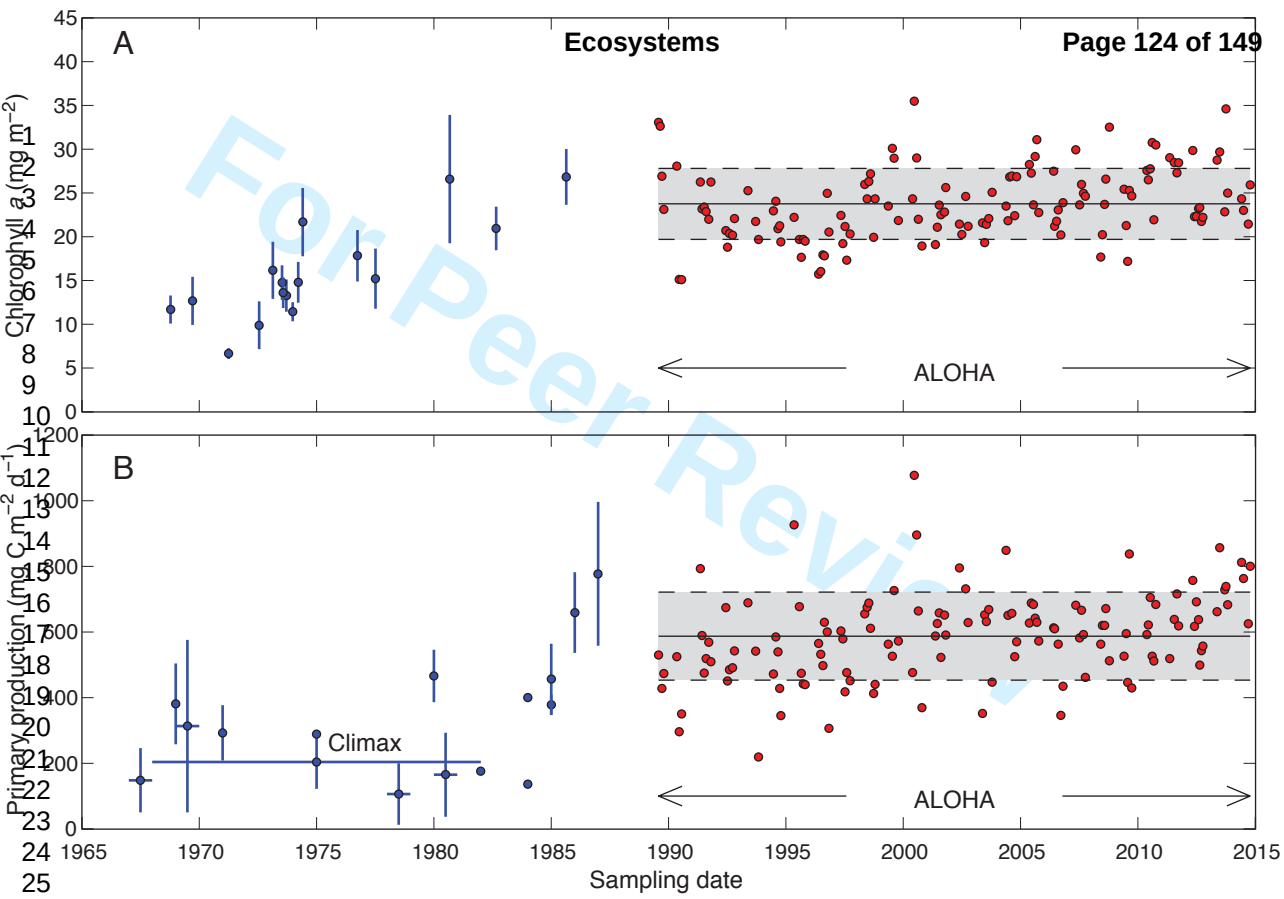
Figure 28: **[Top]** Changes in satellite-based chlorophyll *a* (chl *a*) concentrations in the NPSG over the period 1998-2006. Colored areas indicate regions with surface chl *a* ≤0.07 mg m^{-3} for the two-month means of Dec 1998 and Dec 1999 versus Dec 2005 and Dec 2006. The blue and red regions depict those areas where low (<0.07 mg m^{-3}) chl *a* concentrations are observed in 1998-1999 only or 2005-2006 only, respectively; the grey regions are below the 0.07 mg chl *a* m^{-3} threshold for both time periods. Redrawn from Polovina and others (2008). **[Bottom]** Time-series observations of surface (5 m) chl *a* concentrations at Station ALOHA during this same 9-year period, showing seasonal and interannual variability around the long-term mean value of 0.089 mg chl *a* m^{-3}.

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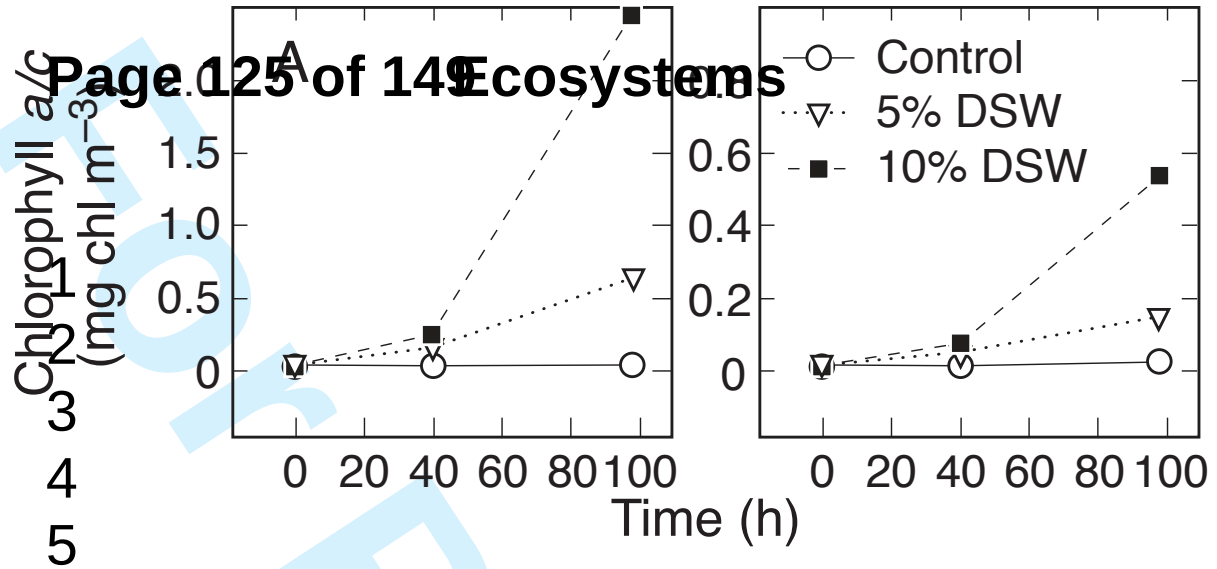

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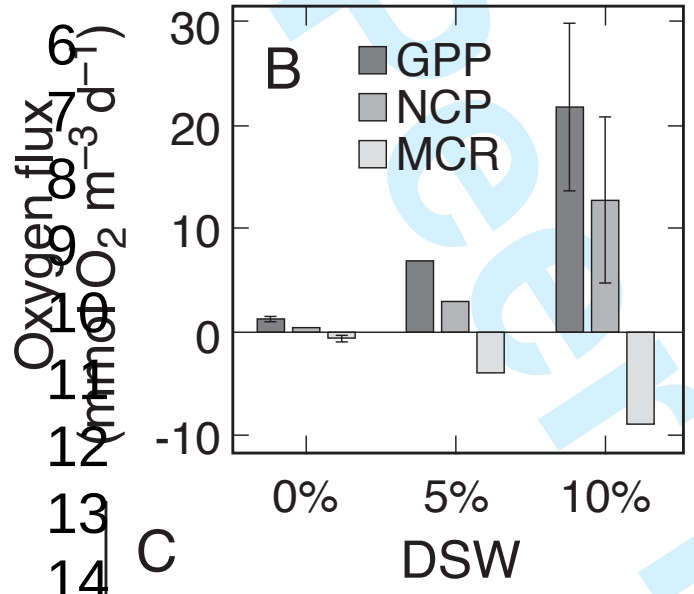


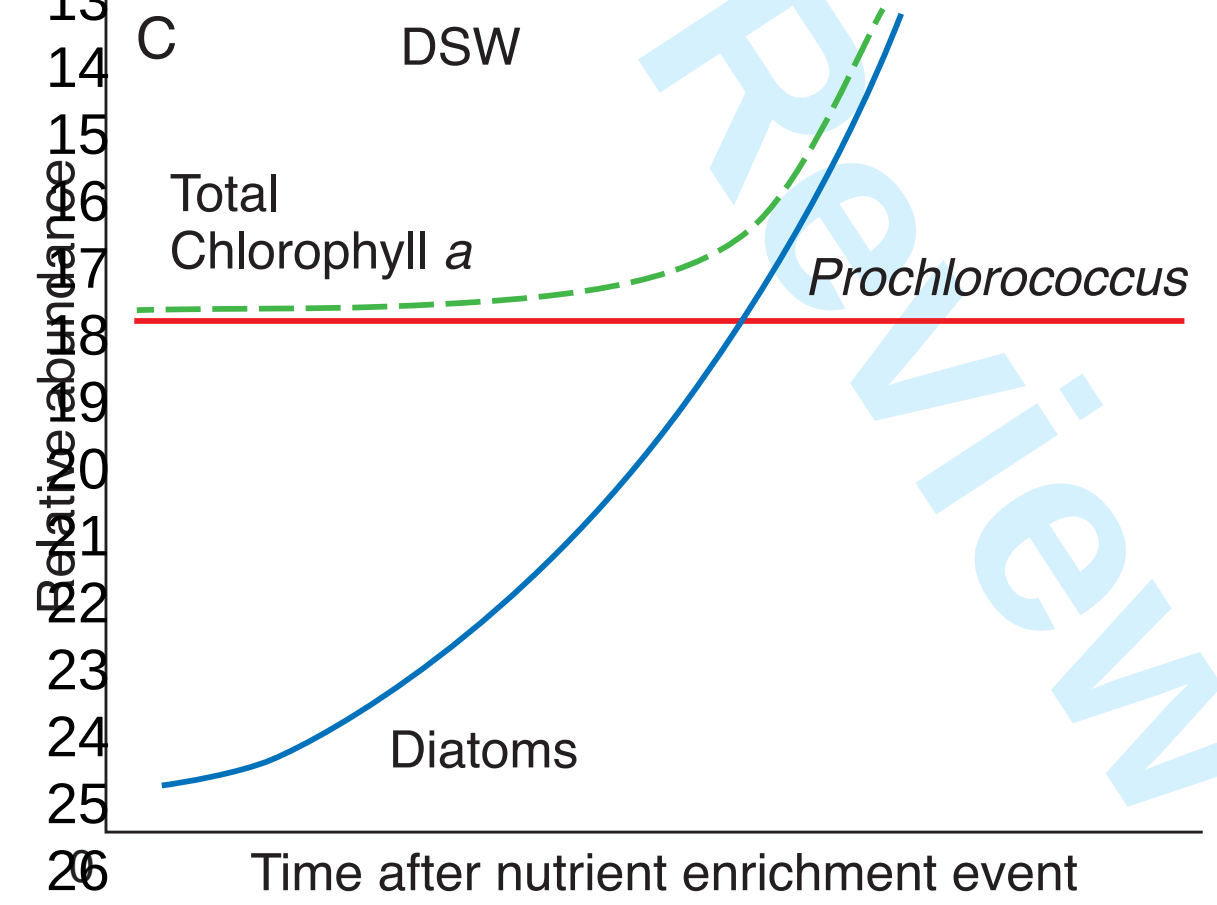
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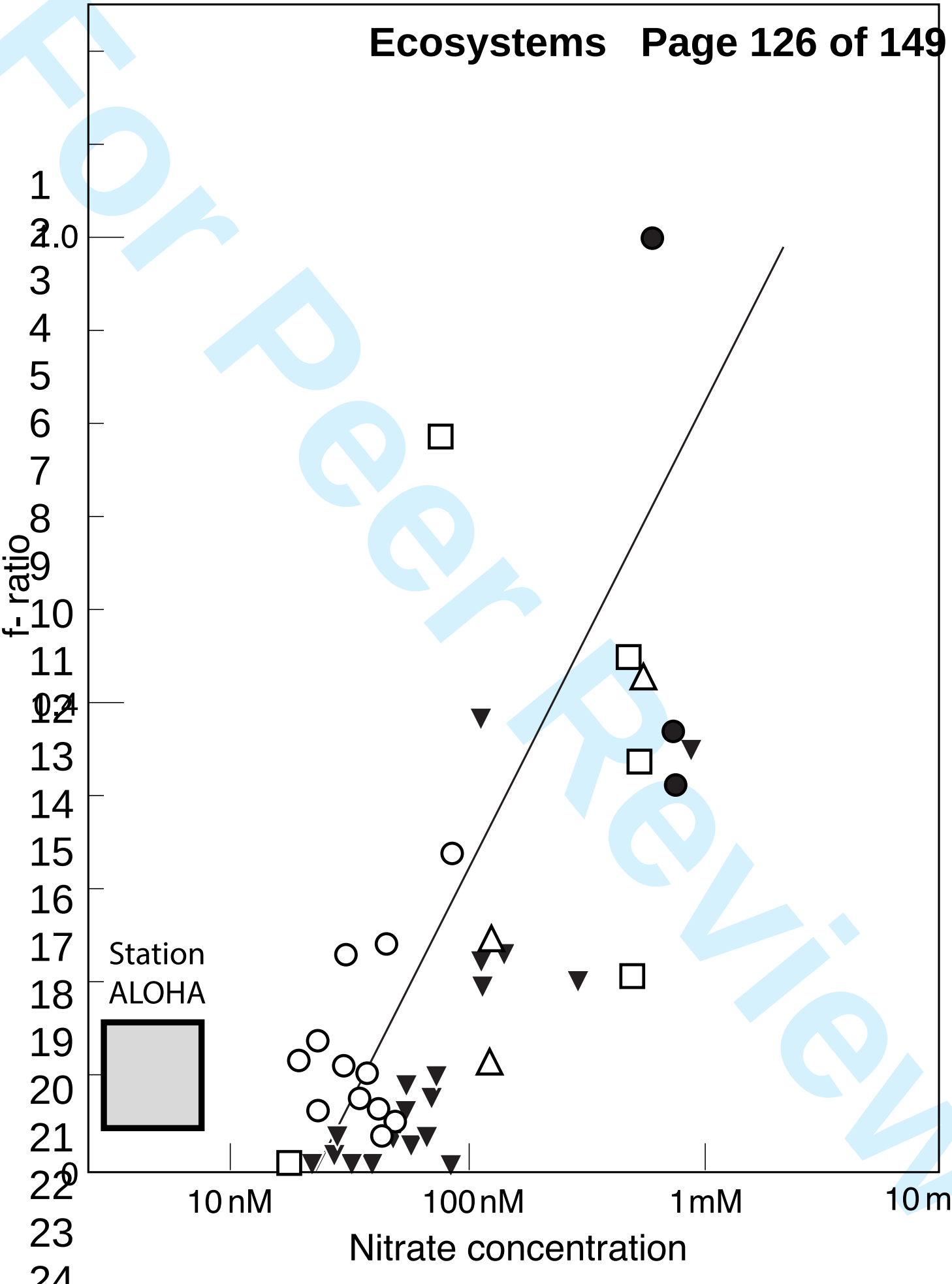
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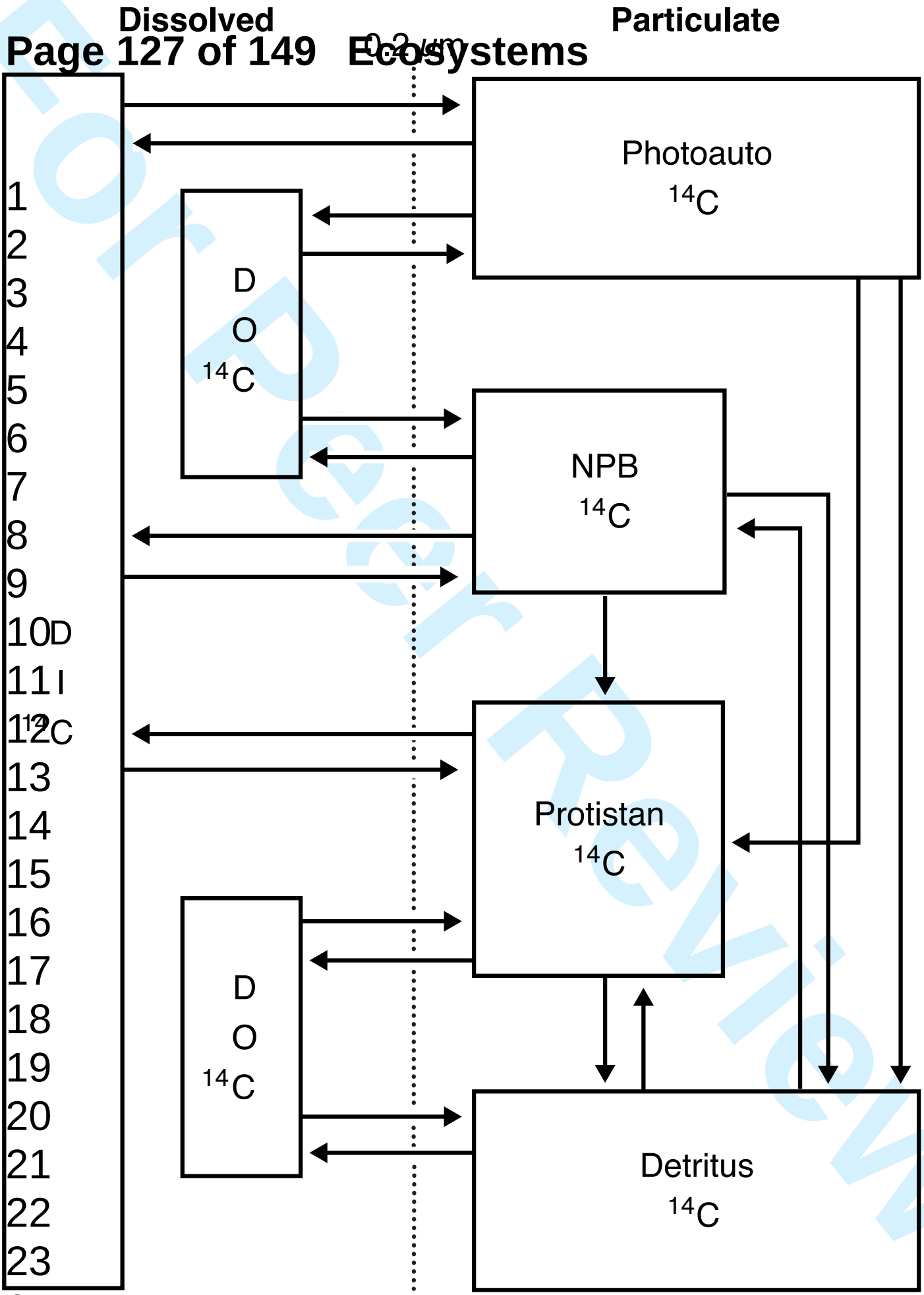




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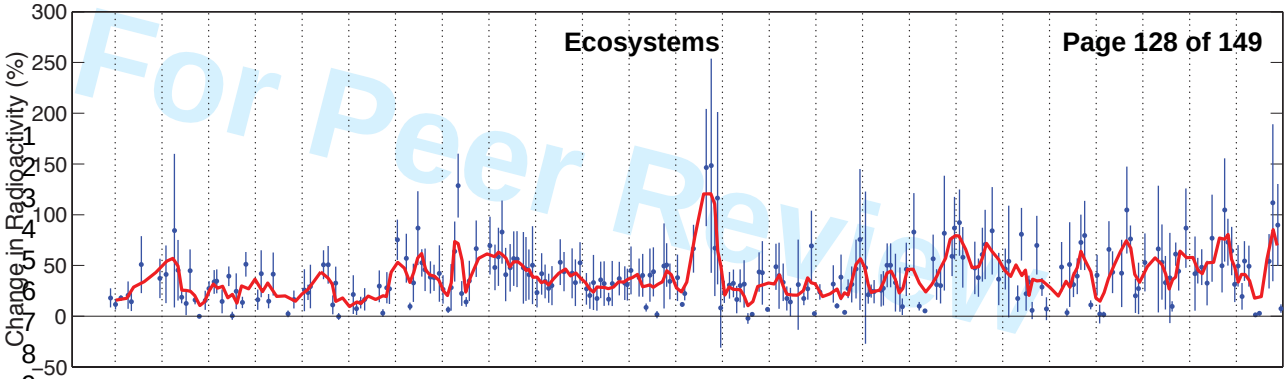


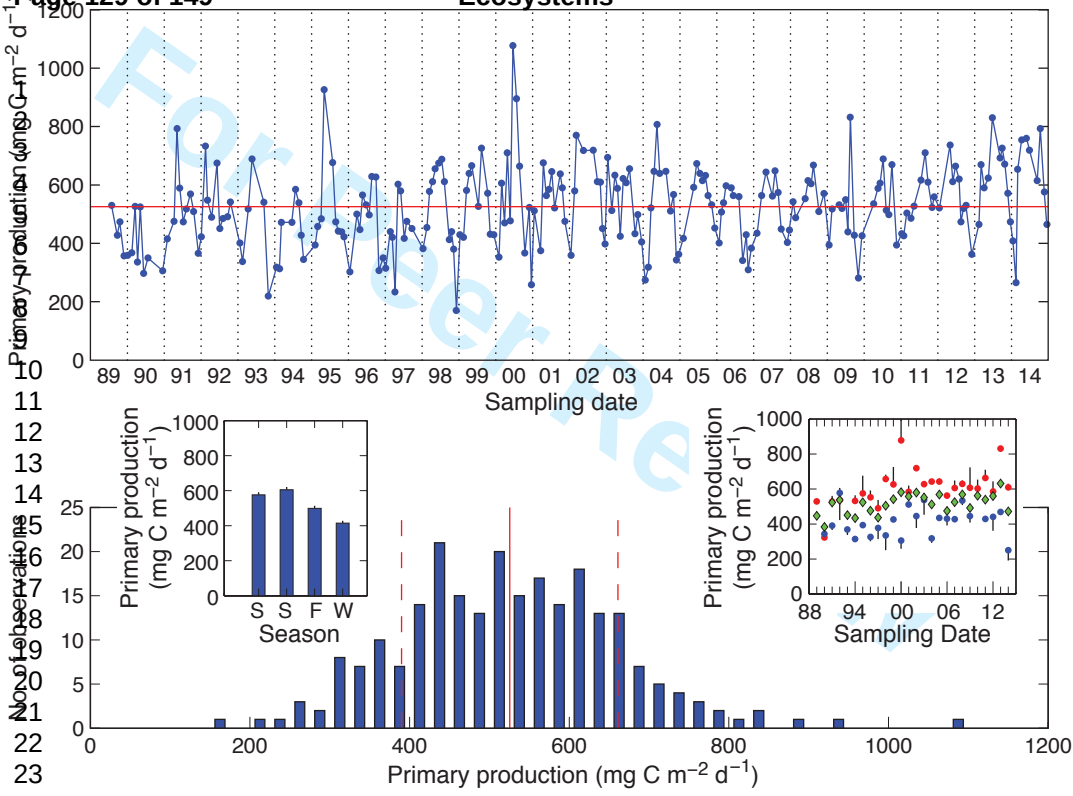
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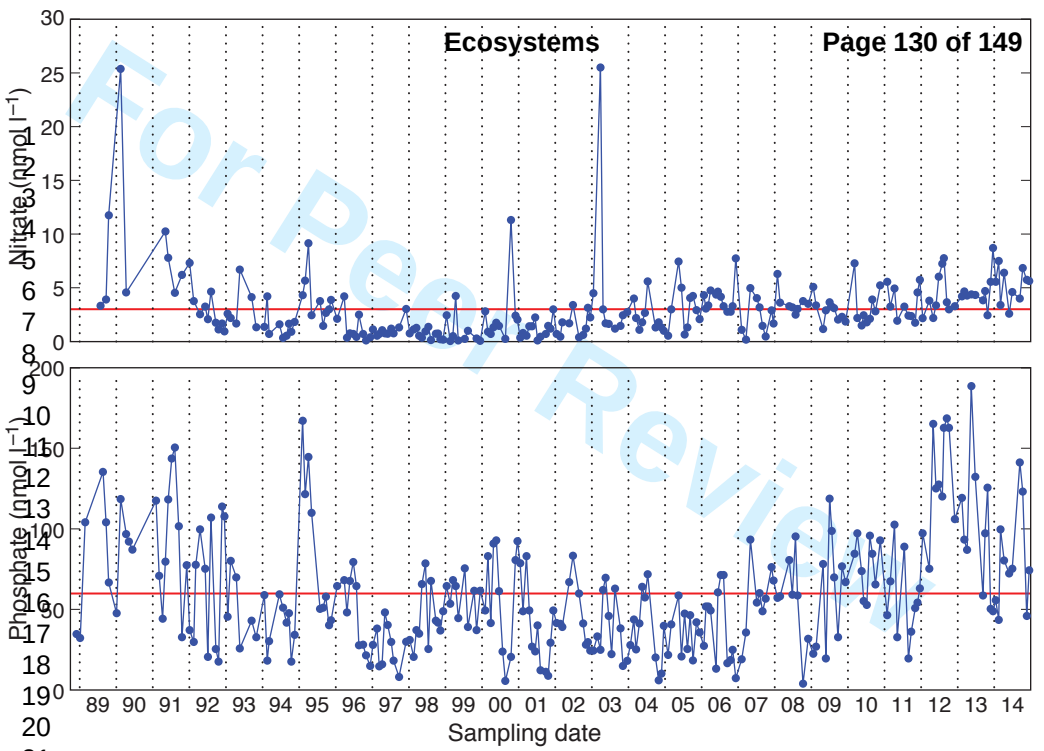
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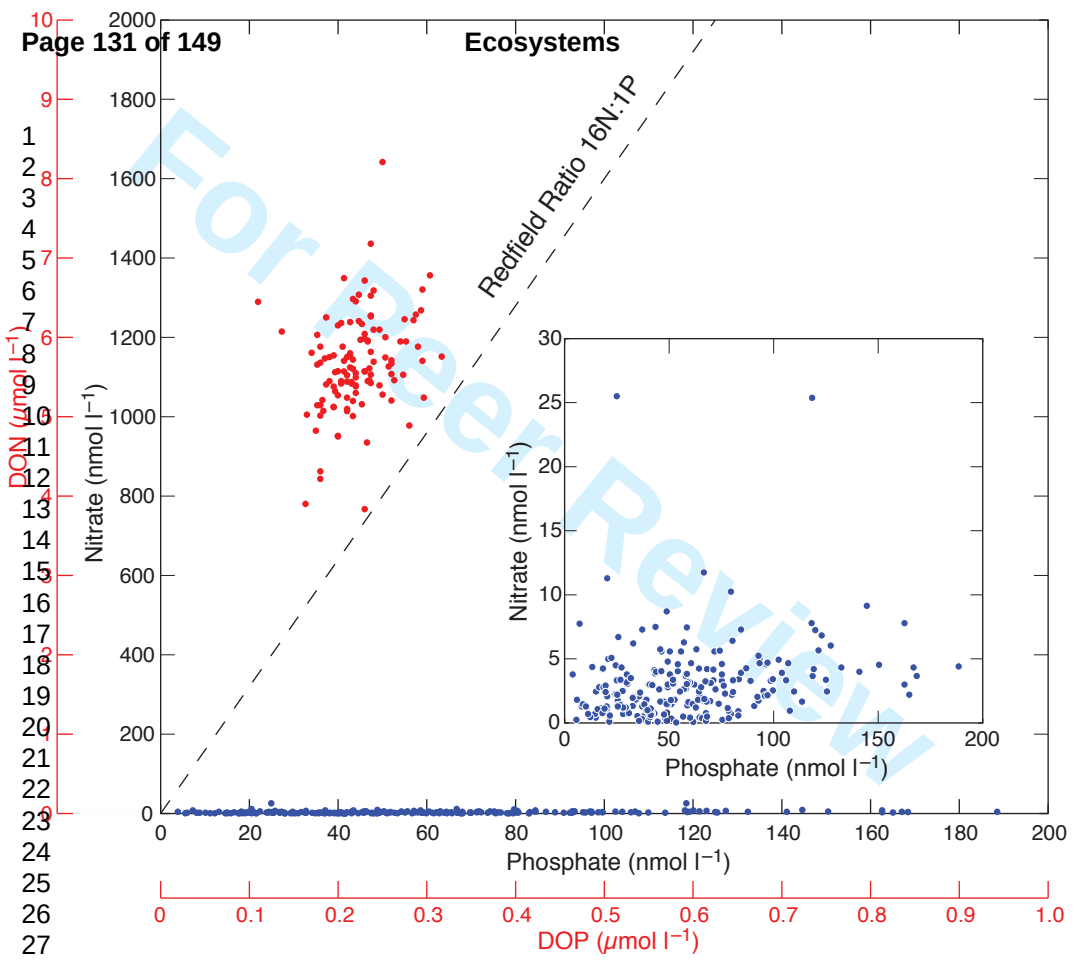




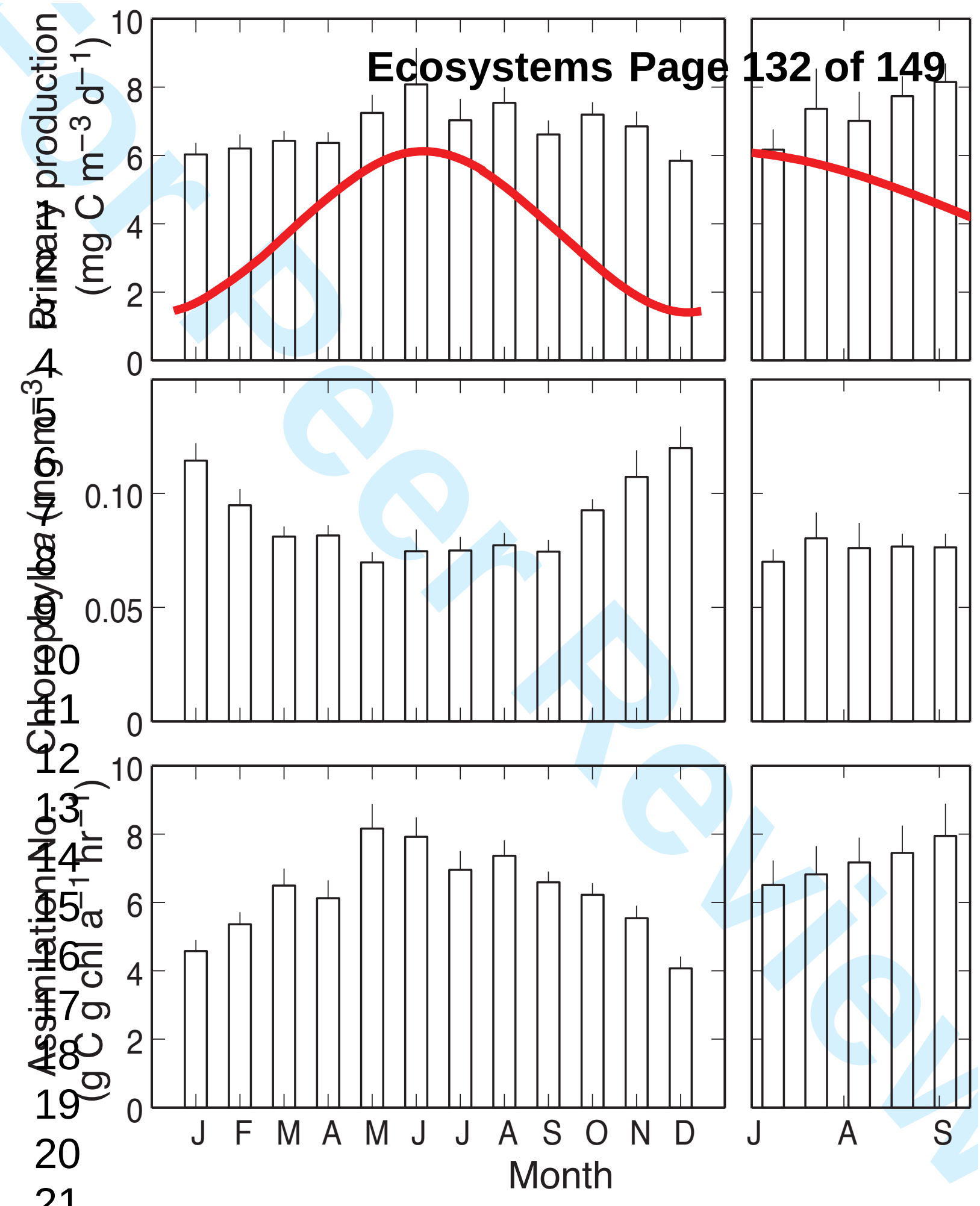
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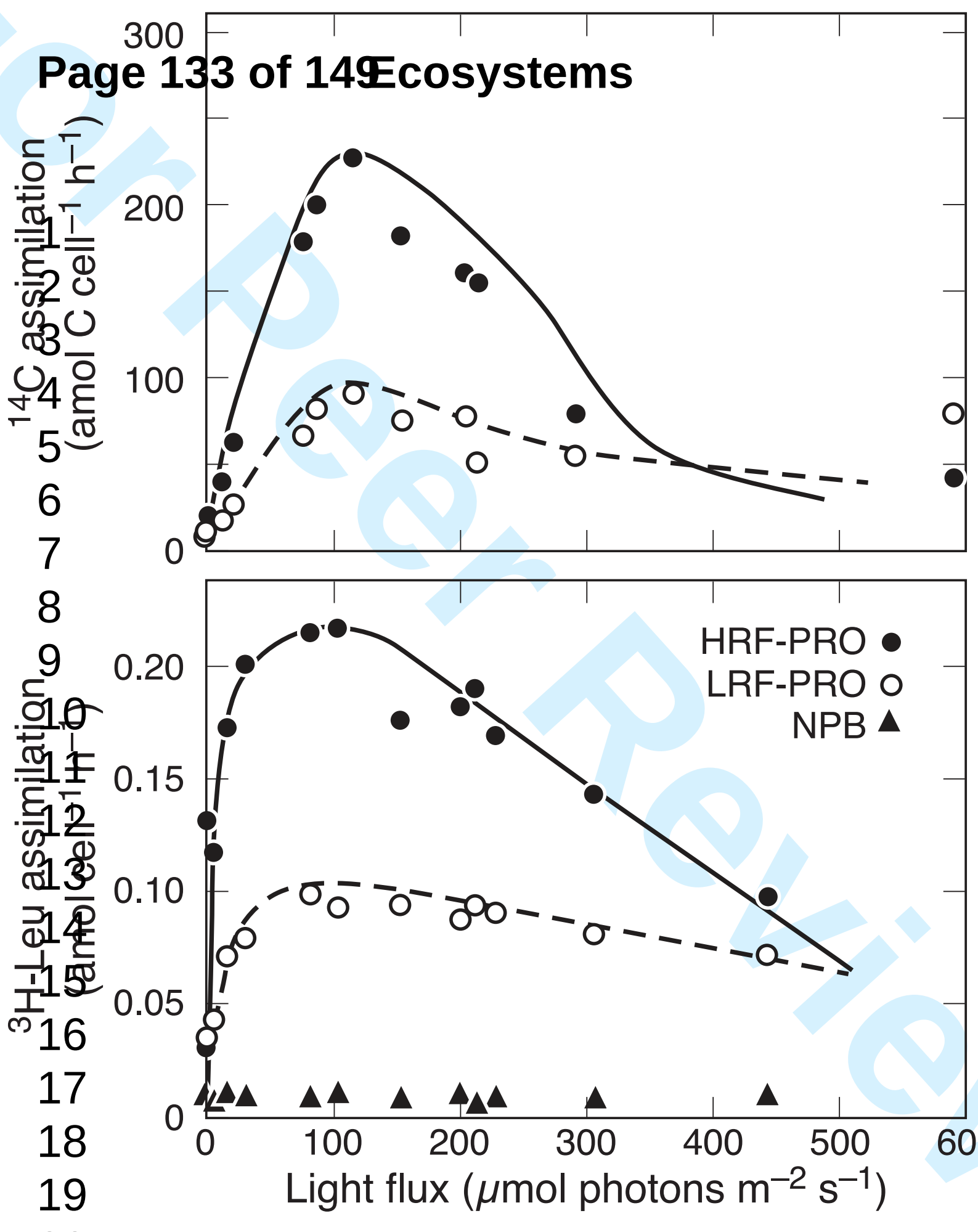


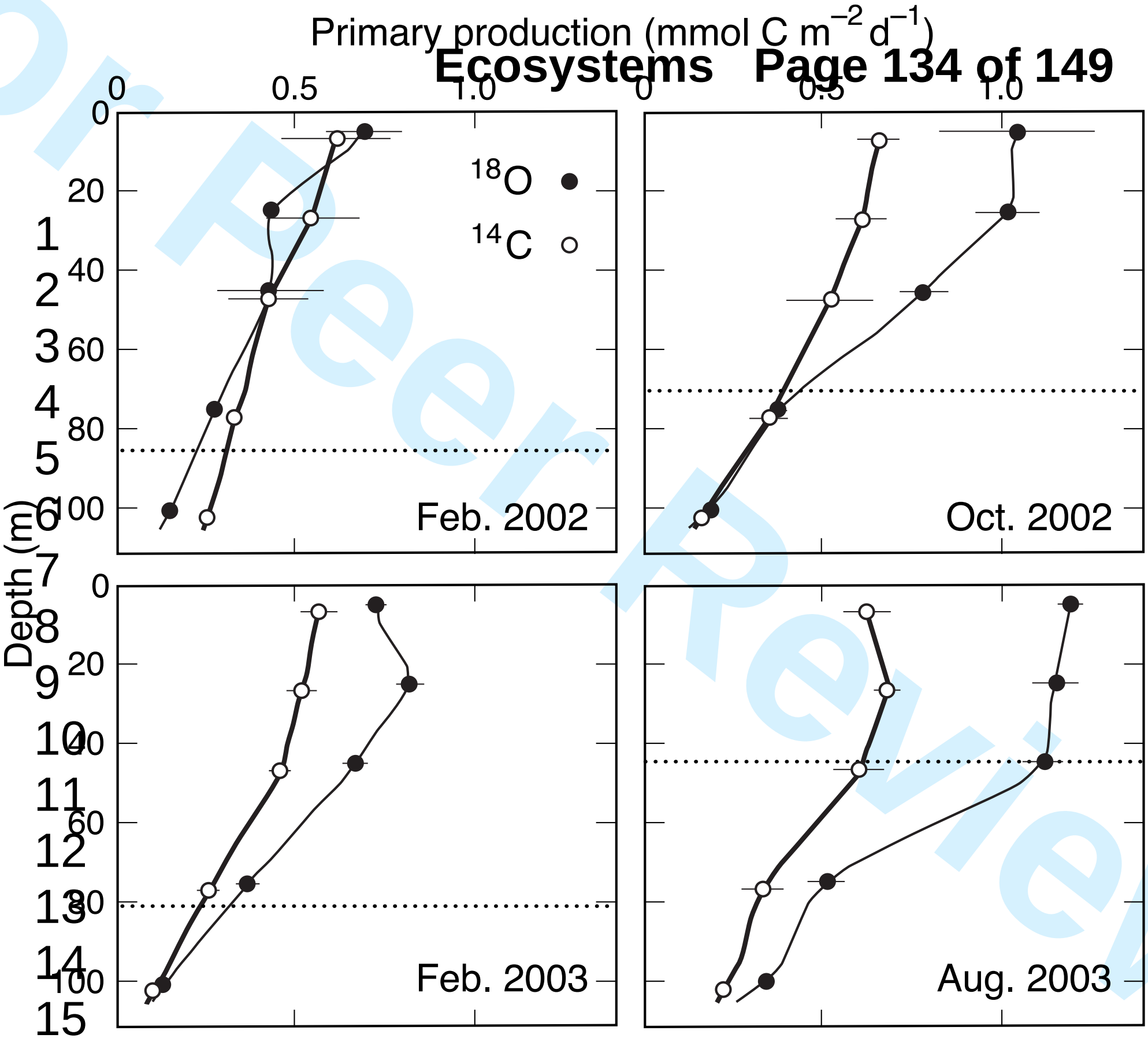
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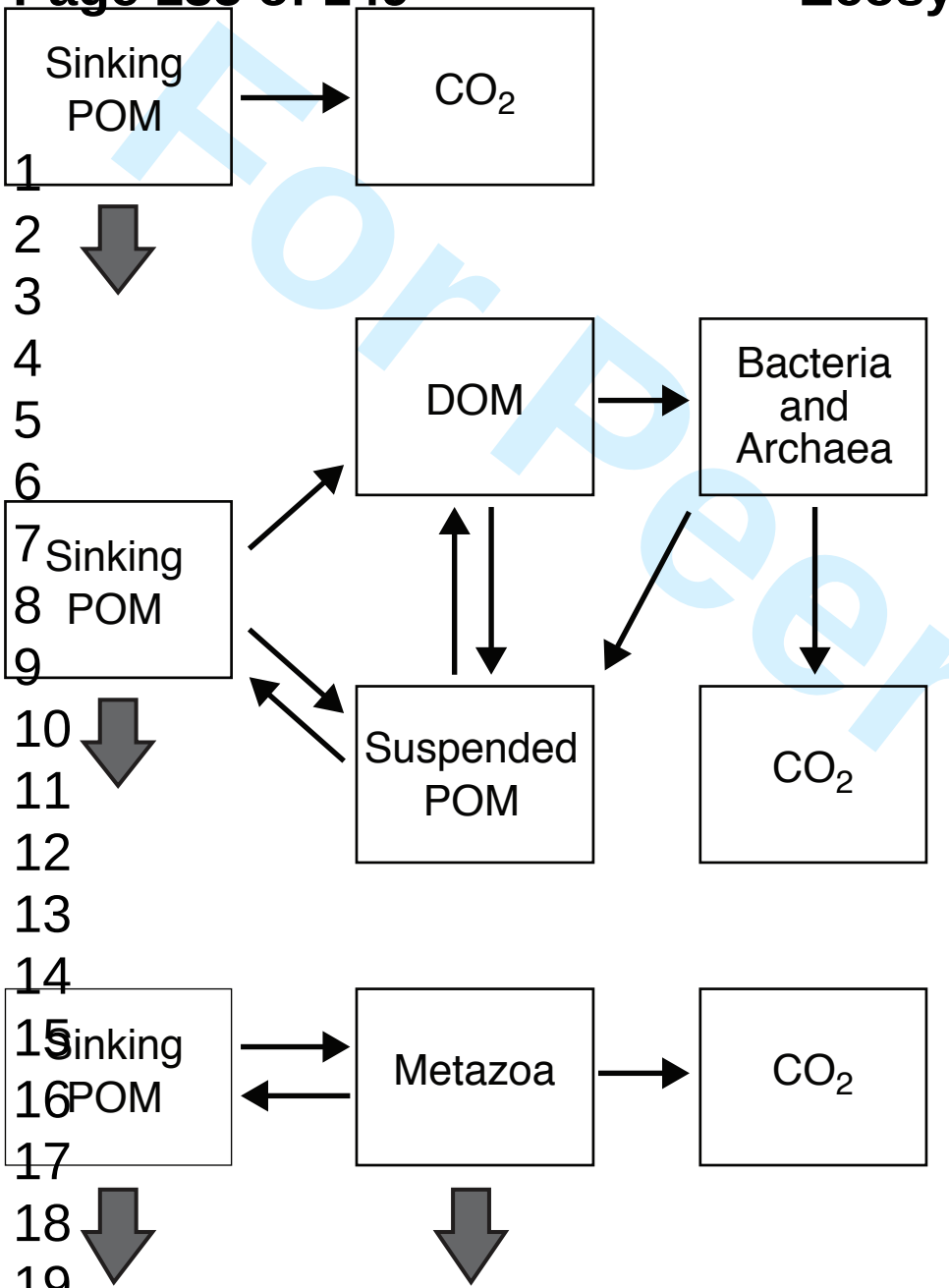
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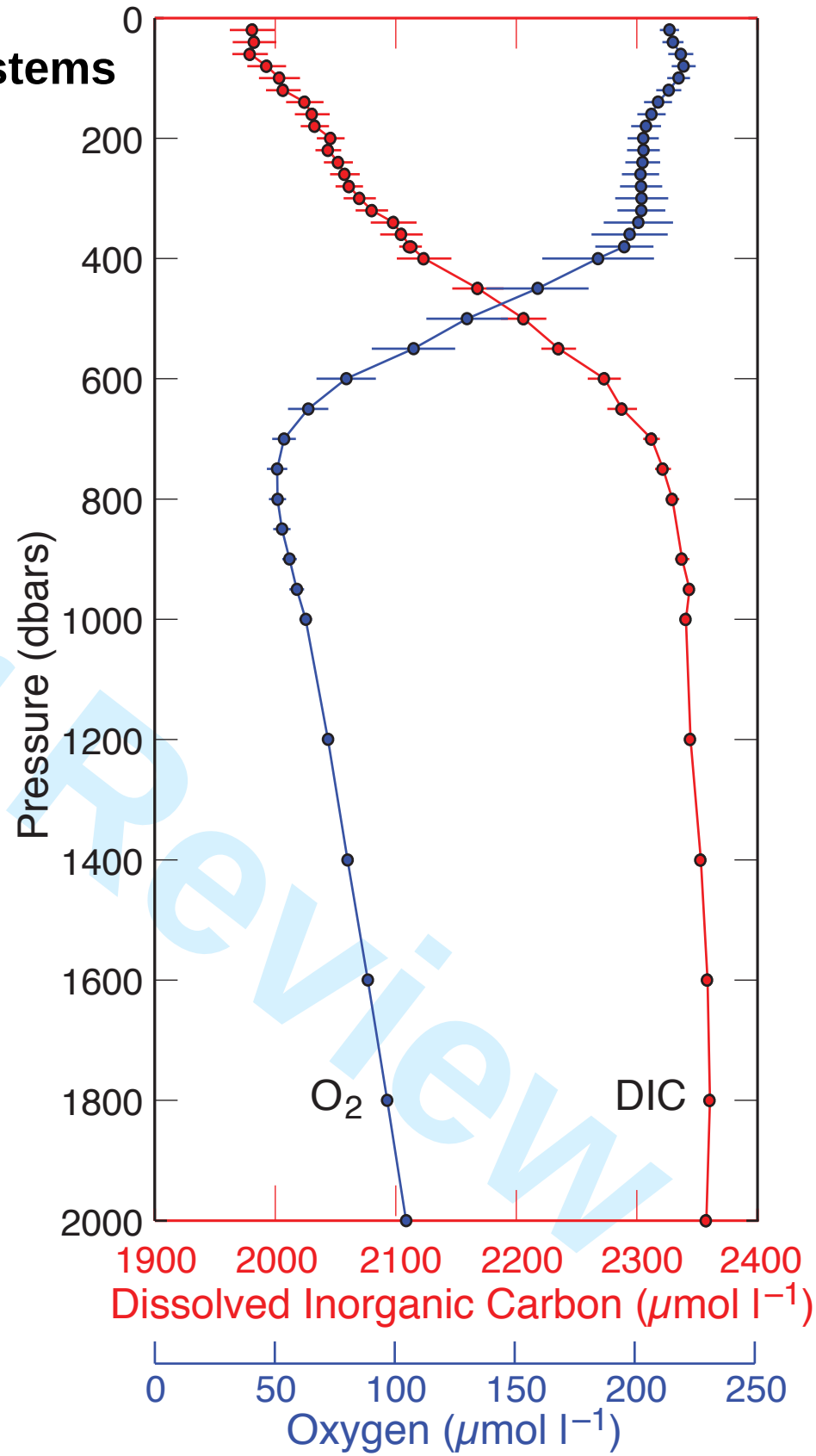

Primary production
(mg C m−3 d−1)
Chlorophyll a (mg m−3)
Assimilation No.
(g C g chl a−1 hr−1)
0
2
4
6
8
10
0.05
0.10
J F M A M J J A S O N D
J A S
Month


300
200
100
0
^{14}C assimilation (amol C cell^{-1} h^{-1})
0.20
0.15
0.10
0.05
0
^{3}H-Leu assimilation (amol cell^{-1} h^{-1})
HRF-PRO
LRF-PRO
NPB
0
100
200
300
400
500
600
Light flux (μmol photons m^{-2} s^{-1})

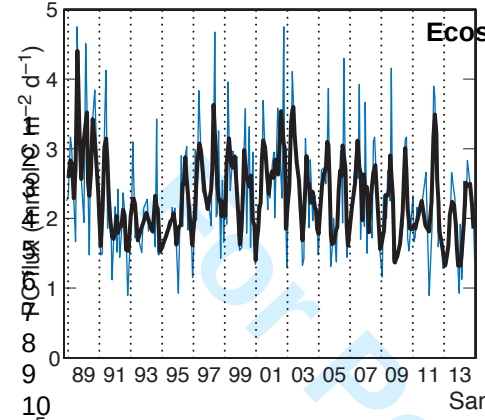


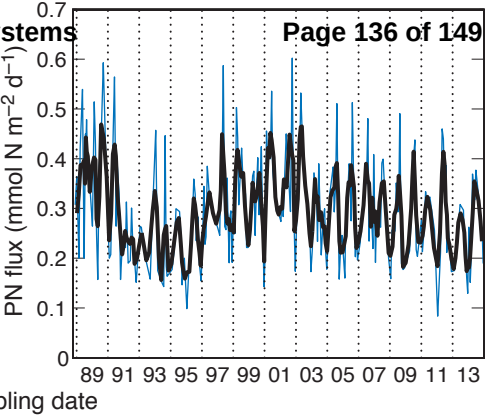
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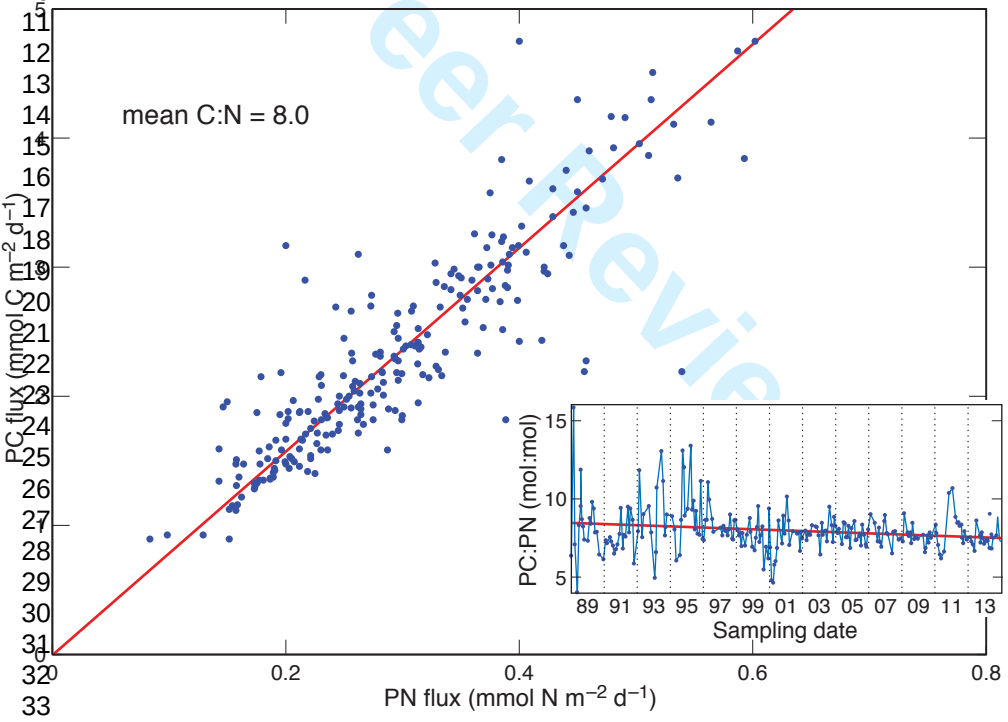




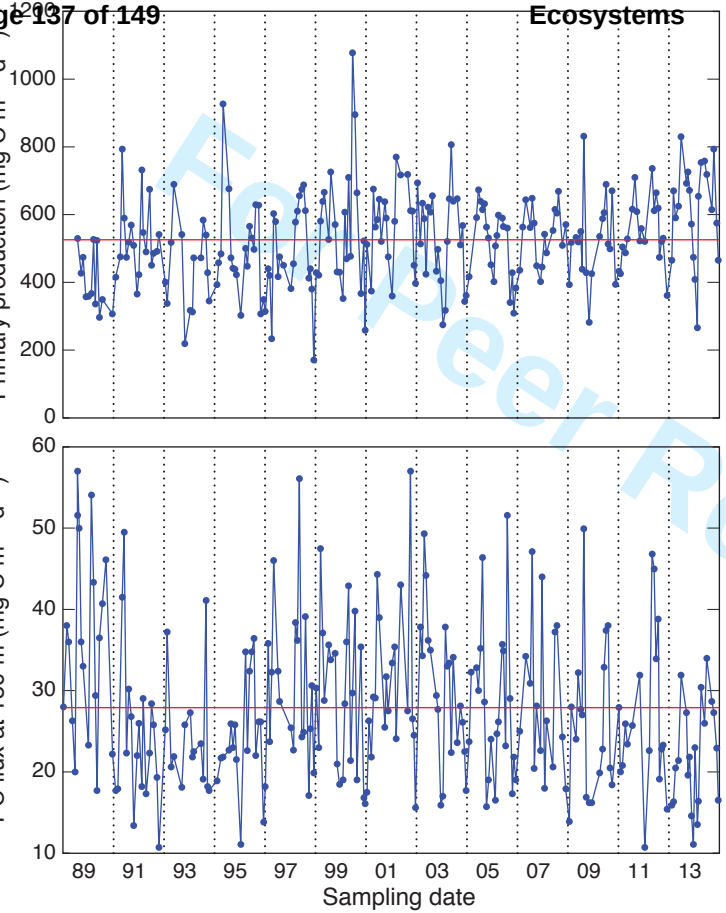
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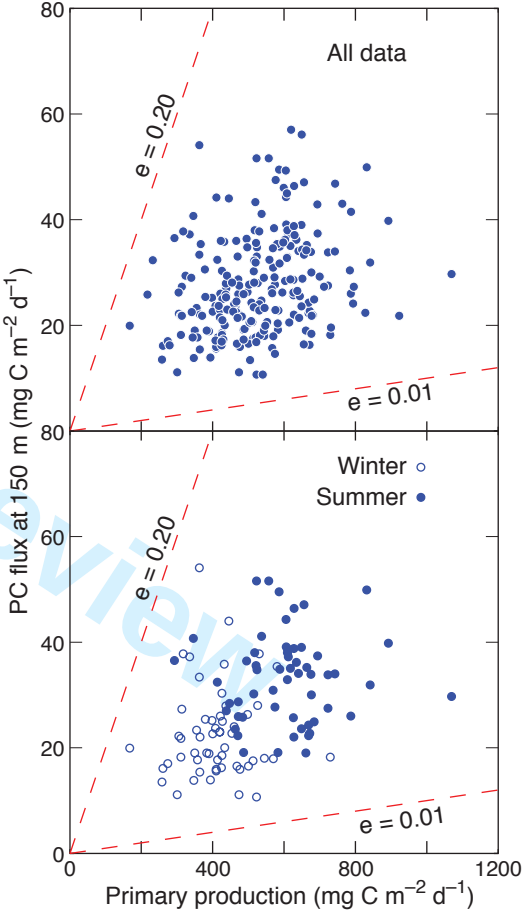




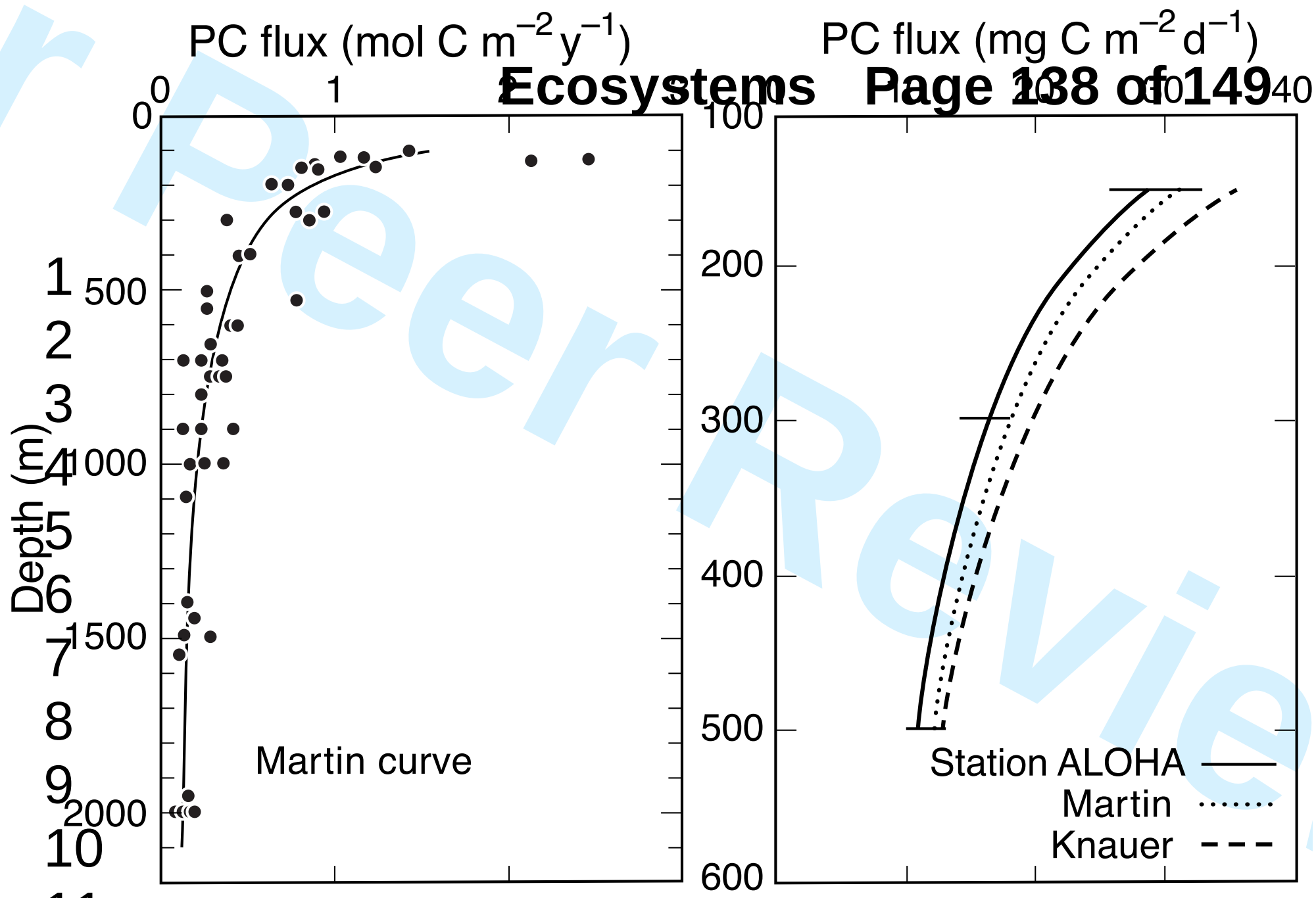


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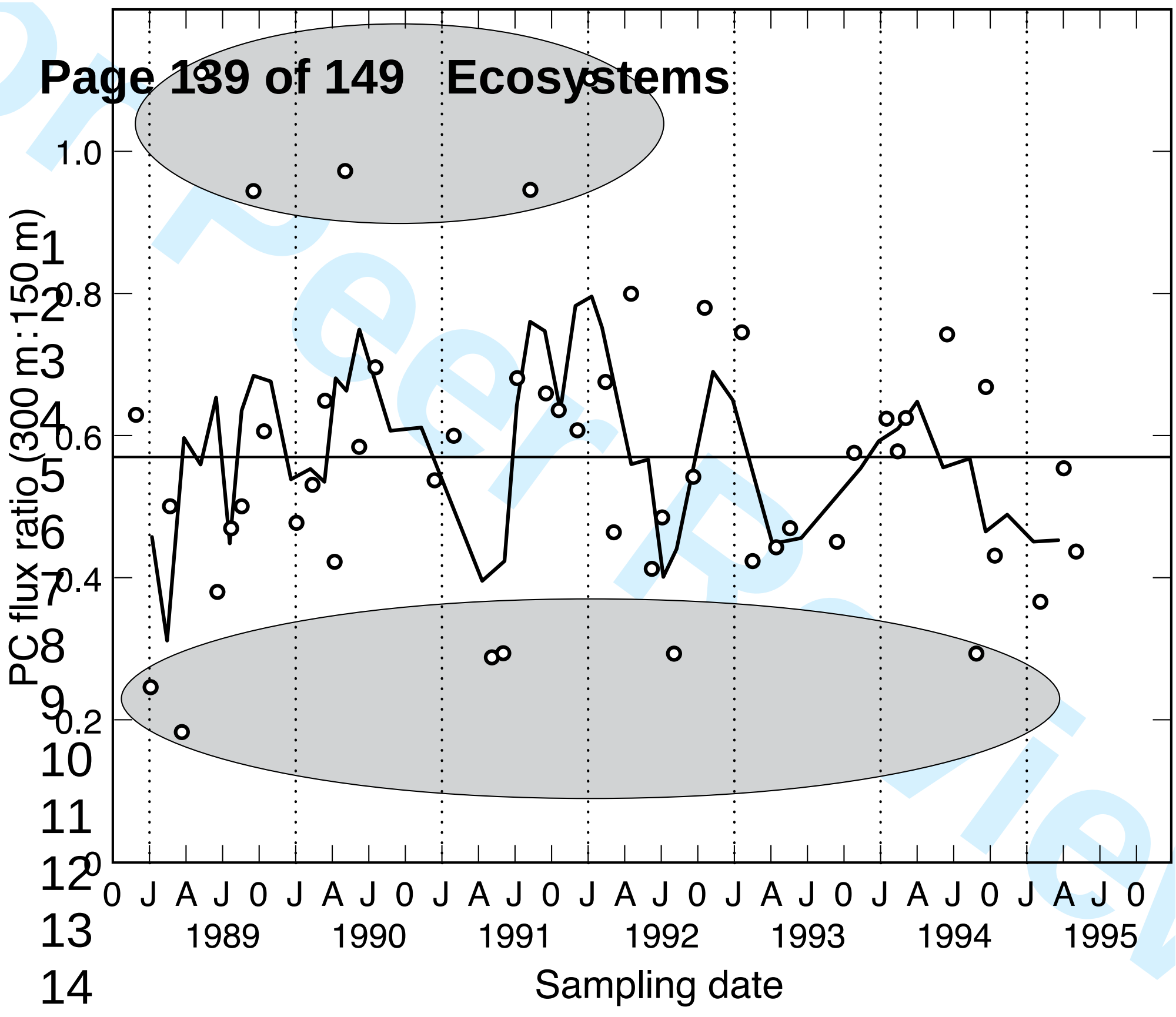


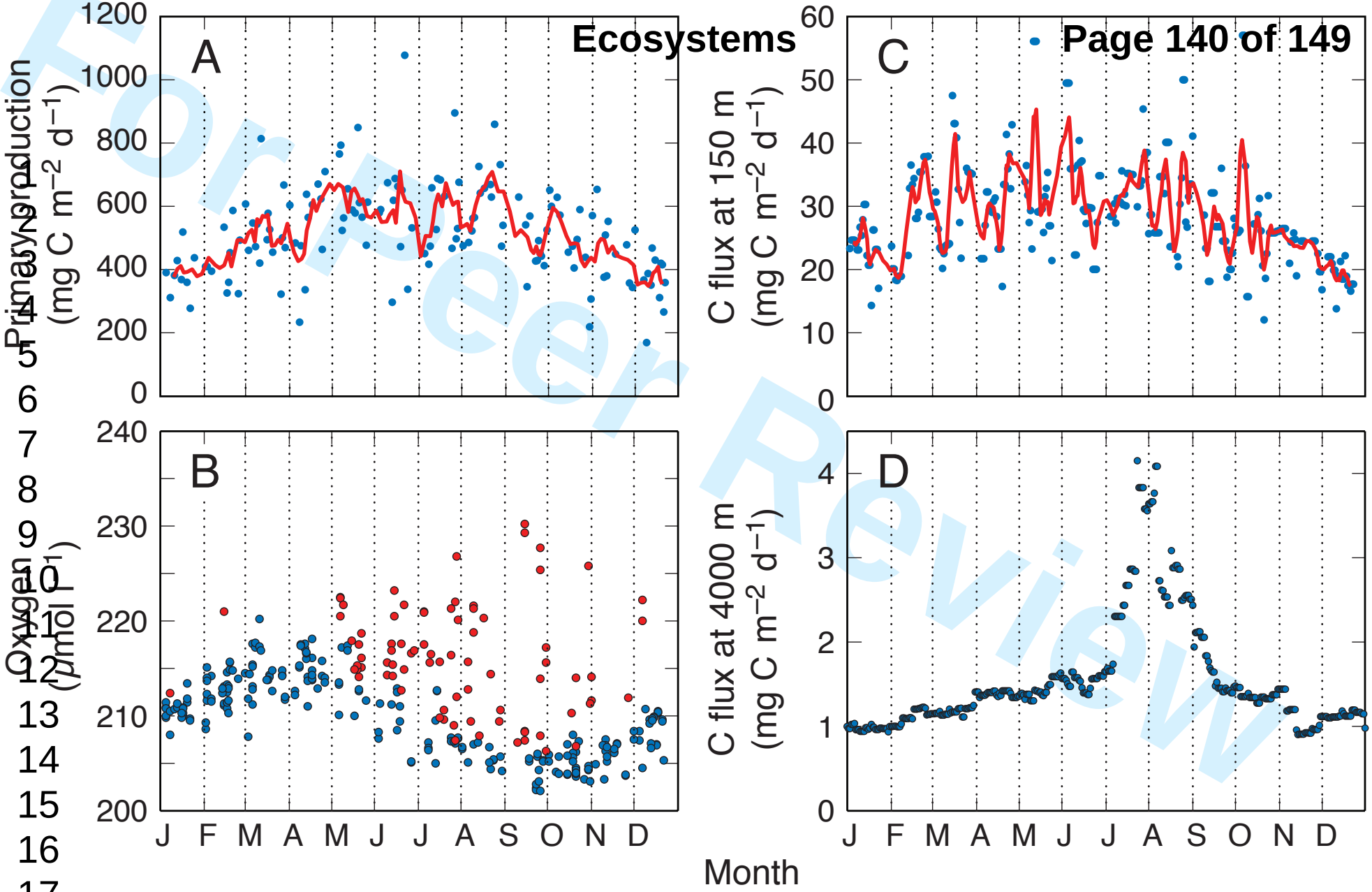


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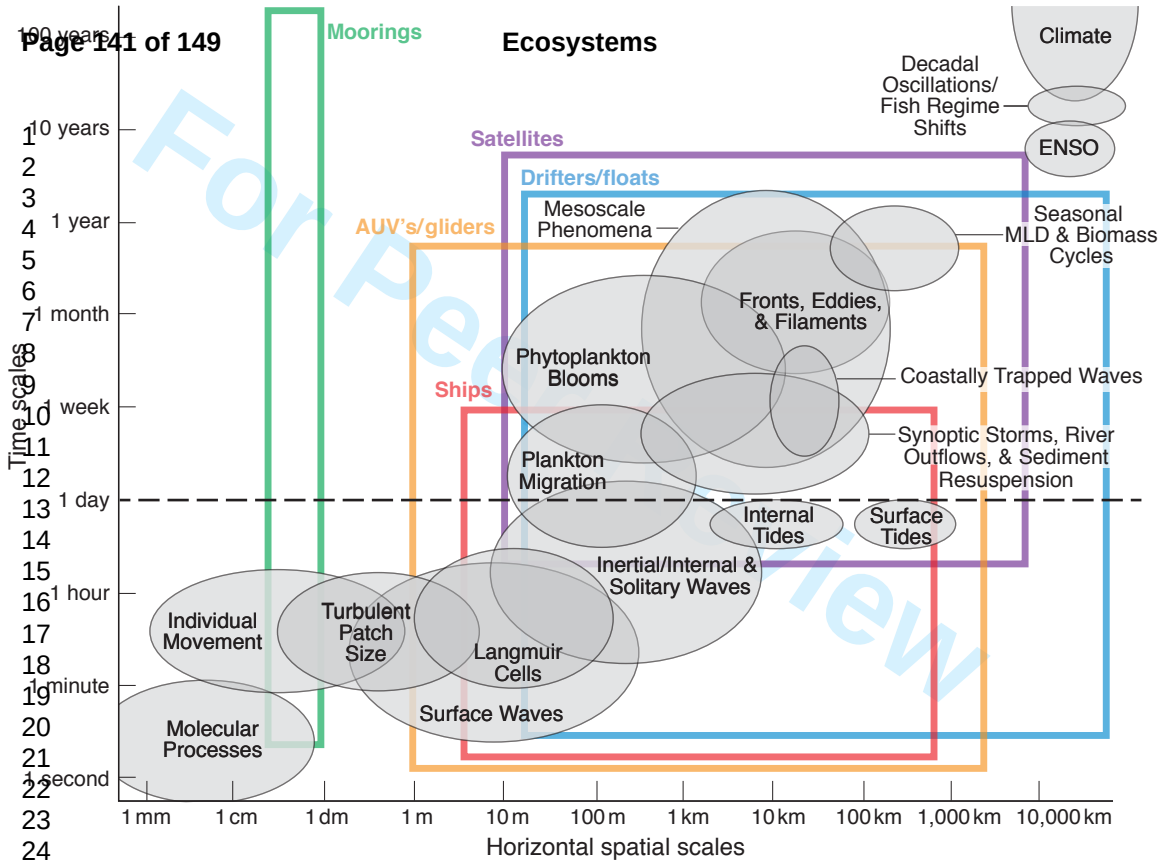


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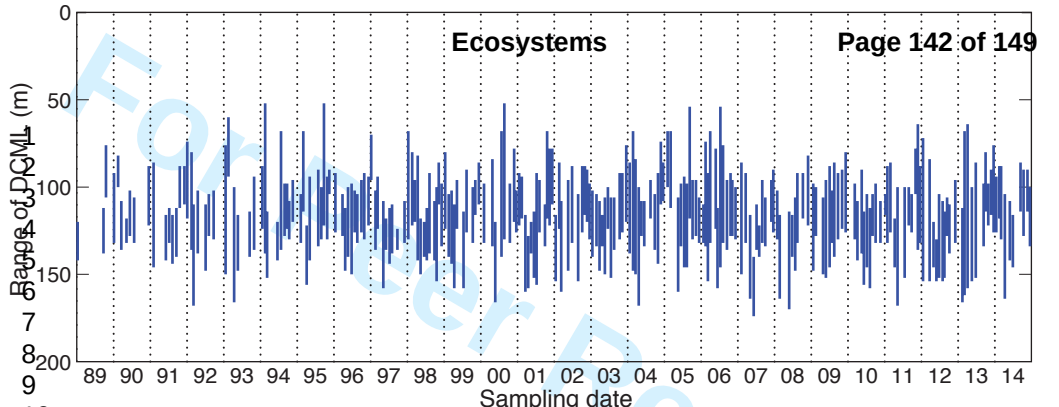

PC flux ratio (300 m:150 m)
1.0
0.8
0.6
0.4
0.2
0
J A J O J A J O J A J O J A J O J A J O J A J O J A J O J A J O
1989
1990
1991
1992
1993
1994
1995
Sampling date

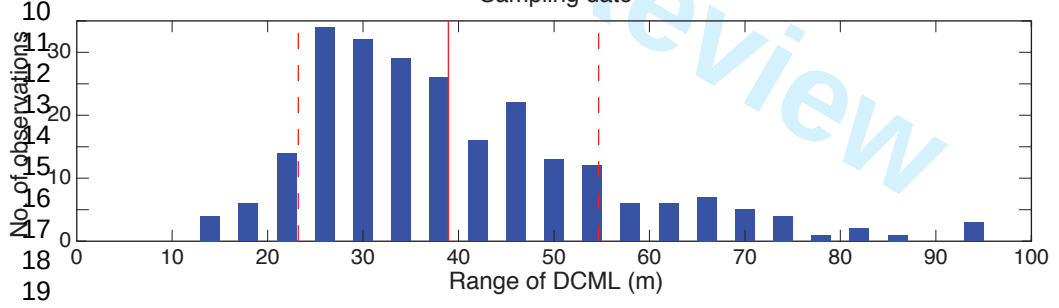


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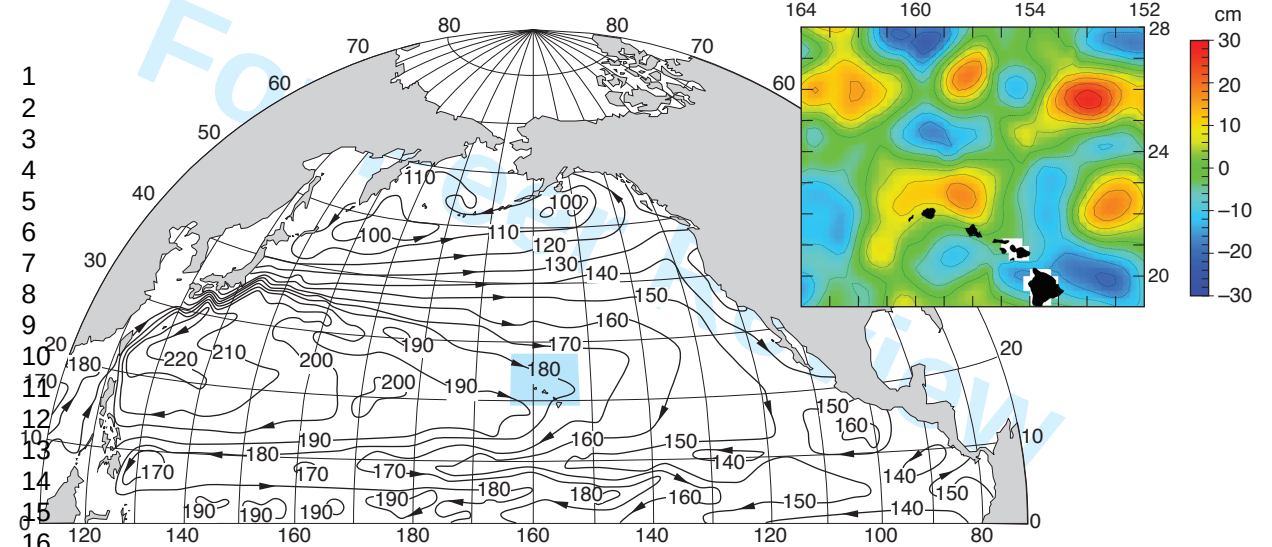


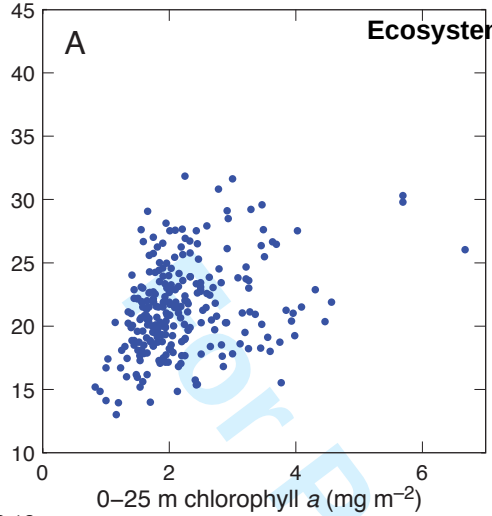
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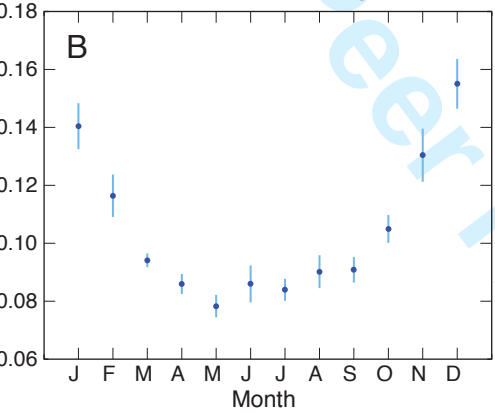


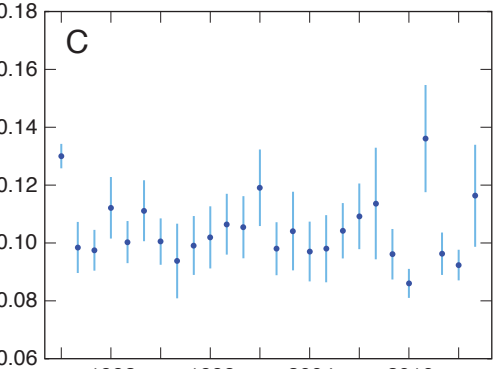


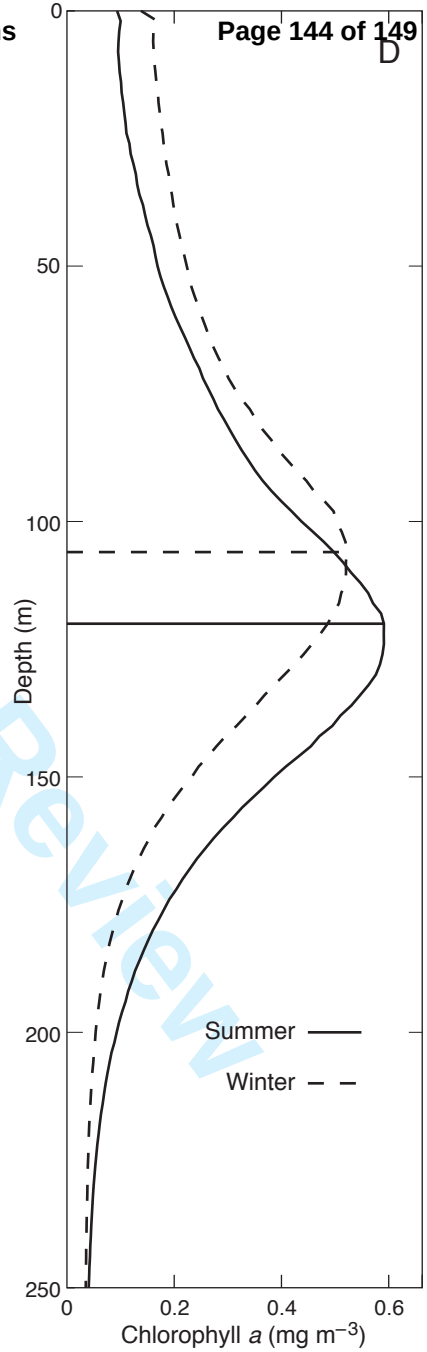
For Peer Review


164
160
154
152
28
24
20
cm
30
20
10
0
−10
−20
−30
80
70
60
50
40
30
20
10
0
120
140
160
180
100

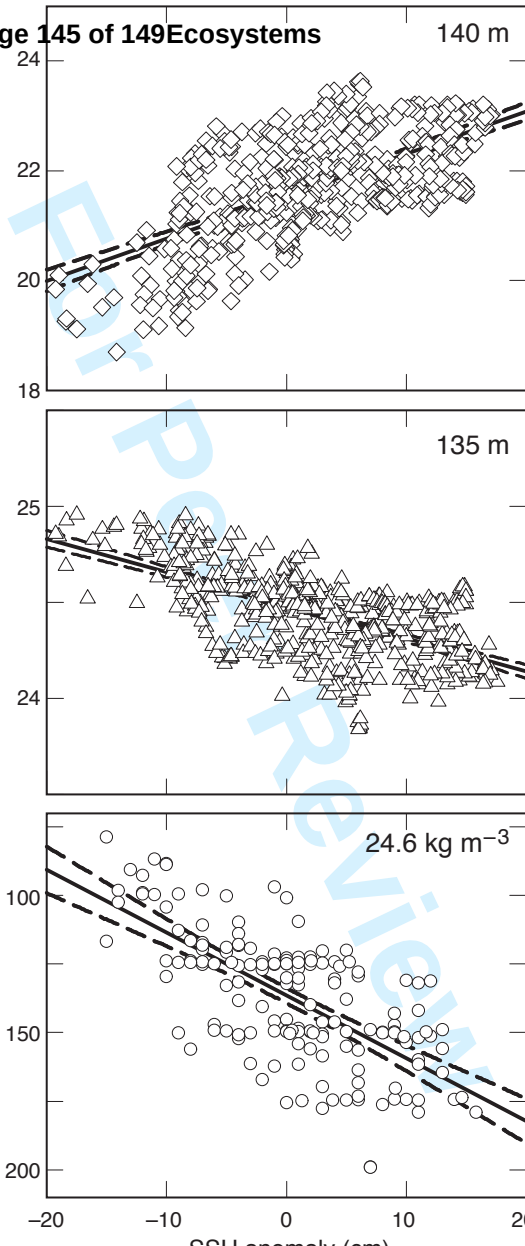




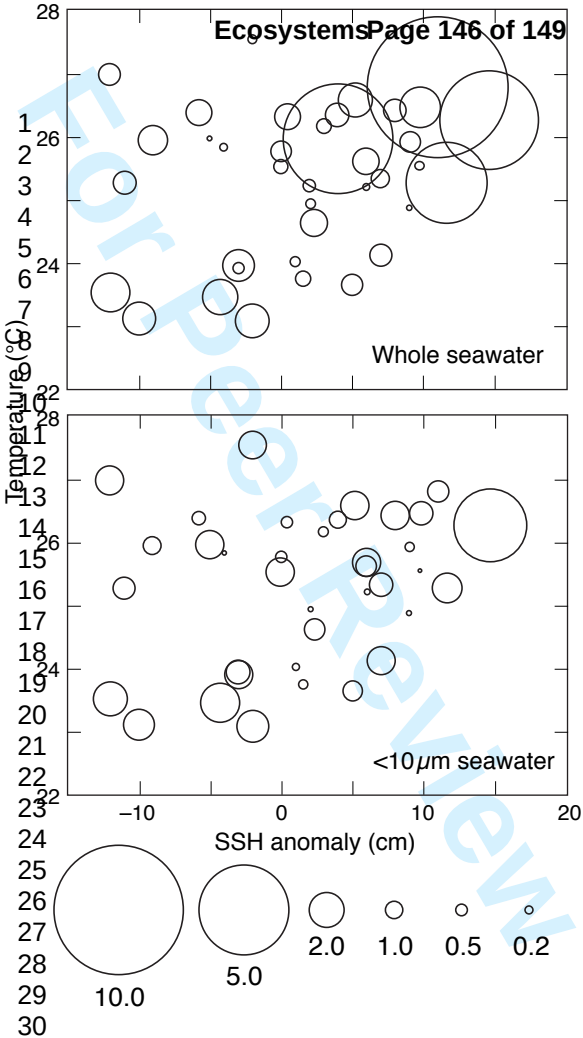




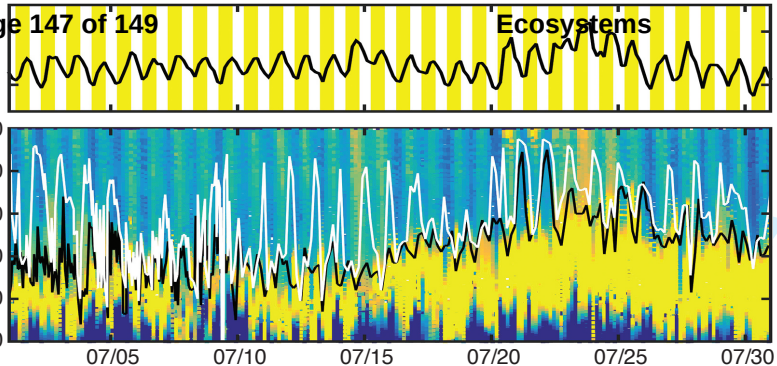
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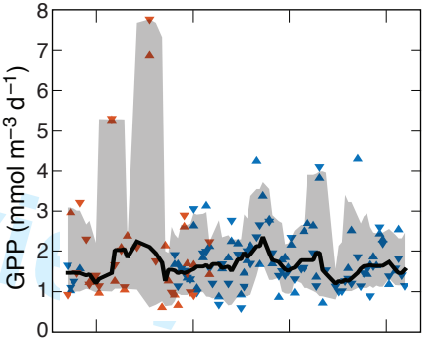


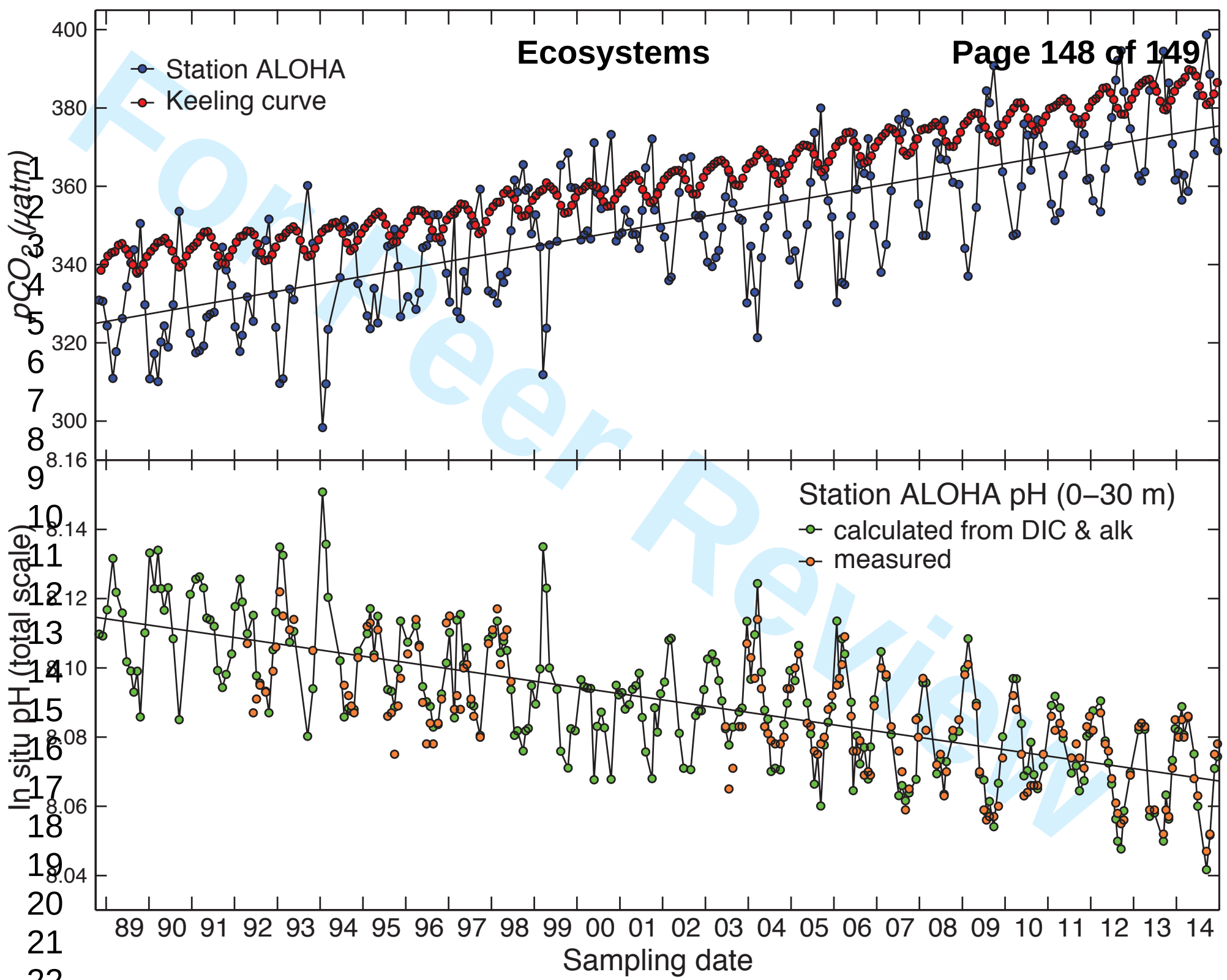
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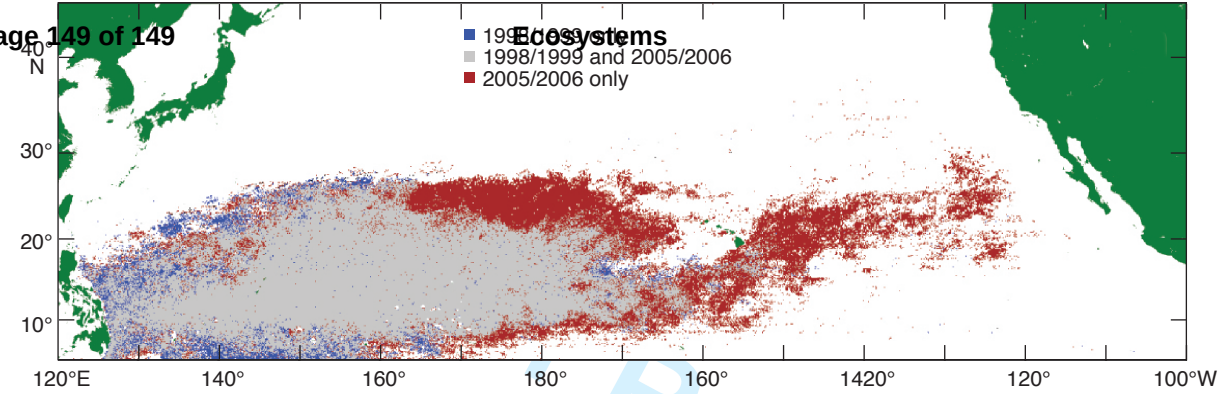
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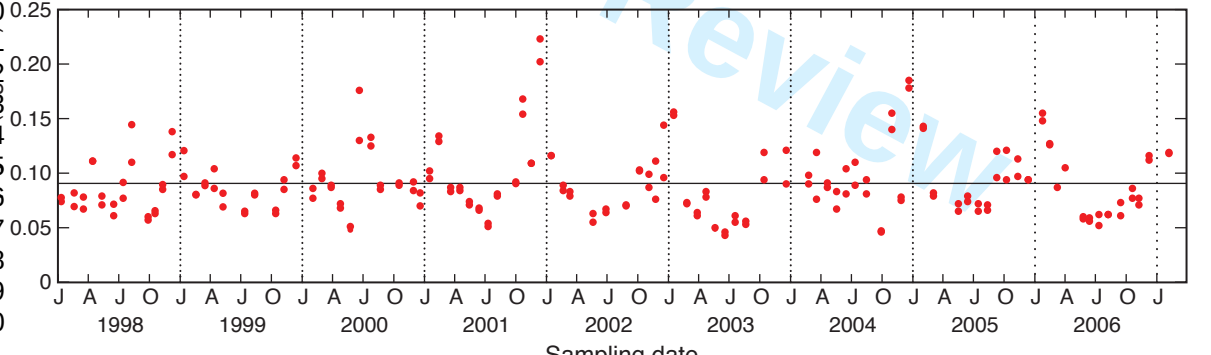

ΔO2 (%)
Depth (m)
07/05
07/10
07/15
07/20
07/25
07/30
0.5
1.0
1.5
2.0
2.5
ΔO2 (%)


GPP (mmol m−3 d−1)
Jun
Jun
Aug
Sep



For Peer Review


Ecosystems
1998/1999 only
1998/1999 and 2005/2006
2005/2006 only
40° N
30°
20°
10°
120°E
140°
160°
180°
160°
1420°
120°
100°W


0.25
0.20
0.15
0.10
0.05
0
J A J O J A J O J A J O J A J O J A J O J A J O J A J O J A J O J
1998
1999
2000
2001
2002
2003
2004
2005
2006
Sampling date

For Review