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Decoupling between bacterial production and primary production over multiple time scales in the North Pacific Subtropical Gyre

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ABSTRACT

We measured rates of ^{3}H-leucine (^{3}H-Leu) incorporation, as a proxy for bacterial production, at Station ALOHA (22°45′N, 158°W) in the oligotrophic North Pacific Subtropical Gyre (NPSG). We report measurements conducted between January 2011 and April 2013, examining variability in ^{3}H-Leu incorporation over diel, daily, and monthly time scales. Rates of ^{3}H-Leu were evaluated in the context of contemporaneous ^{14}C-based primary productivity (^{14}C-PP) to identify potential temporal coupling between these measures of productivity. Throughout the upper ocean (0–125 m), rates of ^{3}H-Leu incorporation measured in the light (^{3}H-Leu$_{Light}$) were stimulated (1.5-fold, on average) relative to measurements in the dark (^{3}H-Leu$_{Dark}$). At monthly scales, rates of ^{3}H-Leu$_{Light}$ and ^{3}H-Leu$_{Dark}$ varied 4.9-fold and 3.8-fold, respectively, while rates of ^{14}C-PP varied 1.7-fold. Rates of ^{14}C-PP were often elevated during summer months (May through August) when incident light flux was greatest, while rates of both ^{3}H-Leu$_{Light}$ and ^{3}H-Leu$_{Dark}$ often peaked in early fall (August through October) when seawater temperatures were maximal. Near-daily measurements of ^{3}H-Leu incorporation and ^{14}C-PP conducted over a 62-day period in the summer of 2012 revealed that rates of ^{3}H-Leu$_{Light}$ and ^{3}H-Leu$_{Dark}$ varied ~2.5 and 2.0-fold, respectively, similar to ~1.8-fold daily variability observed in rates of ^{14}C-PP. Over diel time scales, rates of ^{3}H-Leu$_{Light}$ and ^{3}H-Leu$_{Dark}$ demonstrated different patterns, with rates of ^{3}H-Leu$_{Light}$ elevated at mid-day and rates of ^{3}H-Leu$_{Dark}$ greatest in the early evening. Together, these results suggest that in this oligotrophic ecosystem, photosynthetic production of organic matter and bacterial production can be temporally uncoupled across daily to seasonal scales.

1. Introduction

The ocean supports nearly half of global net primary productivity (PP), with much of that production occurring in the oligotrophic gyres of the open ocean (Behrenfeld and Falkowski, 1997; Field et al., 1998). A significant fraction of this photosynthetically-fixed carbon supports the growth and metabolic activities of bacterioplankton (del Giorgio et al., 1997; Duarte and Cebrian, 1996; Ducklow and Carlson, 1992). Bacterial production (BP) of biomass is a central component of aquatic food webs (Azam et al., 1983; Pomeroy, 1974), estimated to account for 10–15% of net PP in open ocean ecosystems (Ducklow, 1999; Ducklow and Carlson, 1992), with bacterial demand for organic matter (inclusive of requirements for biomass production and respiration) accounting for > 70% of contemporaneous rates of primary production (Church, 2008; Kirchman, 2004). Hence, estimates of the magnitude and variability associated with bacterial growth in the sea are critical to understanding ocean carbon cycling.

Examining spatiotemporal coupling between BP and PP provides insight into the relative dependence of bacterial growth on contemporaneous phytoplankton production. The nature of the coupling between BP and PP is generally quantified based on correlative or regression analyses (Ducklow and Carlson, 1992; Joint and Pomroy, 1987). Such analyses provide insight into the strength of coupling between photosynthetic production of organic matter and its consumption by heterotrophic bacteria (Billen et al., 1990; Ducklow and Carlson, 1992). The nature of such coupling can be regulated by numerous processes, including availability of inorganic and organic nutrients, grazing pressure, and temperature (Alonso-Sáez et al., 2008; Billen et al., 1990; Carlson et al., 1996; Cotner and Biddanda, 2002; Fouilland et al., 2014; Shiah and Ducklow, 1994). To date there are few studies examining temporal coupling between BP and PP in open ocean ecosystems (although see Carlson et al., 1996; Ducklow and Carlson, 1992; Ducklow et al., 2012; Steinberg et al., 2001; Van Wambeke et al., 2008); this coupling has also been examined in coastal, estuarine, and

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freshwater systems (*e.g.* Fuhrman et al., 1985; Pace and Cole, 1994; Shiah and Ducklow, 1994). Some of these studies describe significant positive correlations between BP and PP; however, the nature of these relationships can be complicated by lagged responses in BP relative to PP (*e.g.* Steinberg et al., 2001). For example, at the Bermuda Atlantic Time-series Study (BATS) in the Sargasso Sea, rates of BP demonstrate weak to moderate seasonality, generally increasing 2–3-fold during mid-summer and declining into fall and winter (Carlson et al., 1996; Steinberg et al., 2001). In contrast, rates of ^{14}C-primary production (^{14}C-PP) demonstrate relatively strong seasonality, varying up to 5-fold over the year, peaking in early spring (Michaels et al., 1994; Steinberg et al., 2001). The seasonal-scale decoupling of PP and BP in this ecosystem coincides with patterns in the production and removal of dissolved organic carbon (DOC), resulting in concentrations of DOC increasing throughout the spring and summer (Carlson et al., 1994; Hansell and Carlson, 1998). Such seasonal-scale decoupling in ^{14}C-PP and BP in this ecosystem results in net production of DOC, which is available for subsequent export (*via* convective mixing) during the winter (Carlson et al., 1994). Hence, the coupling between contemporaneous ^{14}C-PP and BP plays potentially important roles in carbon export and cycling.

Since 1988, the Hawaii Ocean Time-series (HOT) program has conducted near-monthly measurements of ocean biogeochemistry and hydrography at the open ocean field site Station ALOHA (22°45′N, 158°00′W) in the North Pacific Subtropical Gyre (NPSG). This ecosystem is characterized by a deep euphotic zone (the depth to which 1% of the surface light flux penetrates at Station ALOHA averages 105 ± 10 m), persistently low concentrations of inorganic nutrients, and picoplankton (< 2 μm) comprising a large fraction of upper ocean biomass (Campbell et al., 1994; Rii et al., 2016). Moreover, rates of PP in this ecosystem appear sustained in large part by intensive recycling of nutrients through the metabolic activities of planktonic microorganisms (Karl, 1999). HOT program measurements of ^{14}C-PP reveal significant seasonality, with rates increasing 2–3-fold in summer, coincident with increased insolation (Church et al., 2013; Karl et al., 2012; Letelier et al., 2004). Despite these seasonal-scale increases in ^{14}C-PP, rates of photosynthetic production of DOC (as measured by production of extracellular ^{14}C-DOC) do not demonstrate significant seasonality or apparent relationships to rates of ^{14}C-PP (Viviani et al., 2015). To date however, less is known about temporal variability in BP, or possible relationships between rates of BP and PP in this ecosystem. Previous work at ALOHA has demonstrated that sunlight stimulates rates of ^{3}H-leucine (^{3}H-Leu) incorporation (Church et al., 2006, 2004), a proxy for BP, an effect that appears largely driven by incorporation of ^{3}H-Leu by the unicellular cyanobacterium *Prochlorococcus* (Björkman et al., 2015; Church et al., 2006, 2004).

In this study, we measured rates of ^{3}H-Leu incorporation and ^{14}C-PP over diel to near-monthly time scales at Station ALOHA. Doing so allowed us to evaluate potential coupling between BP and PP over a range of time scales and provided insight into factors controlling these processes in this persistently oligotrophic habitat.

2. Methods

2.1. ^{14}C-primary production and ^{3}H-leucine incorporation measurements

Samples were collected at or in the vicinity of Station ALOHA on 24 different cruises between January 2011 and April 2013. On each cruise, rates of ^{3}H-Leu incorporation into protein were measured (Kirchman et al., 1985; Simon and Azam, 1989). Coincident measurements of plankton assimilation of ^{14}C-bicarbonate were utilized as a proxy for net PP (Marra, 2009; Steemann Nielsen, 1952). Seawater samples for both measurements of production were collected from pre-dawn vertical hydrocasts at 6 discrete depths (5, 25, 45, 75, 100, 125 m) using polyvinyl chloride sampling bottles affixed to a rosette sampler equipped with Sea-Bird 911+ conductivity, temperature, and depth (CTD) sensors (Karl and Lukas, 1996). Measurements of ^{14}C-PP were conducted following HOT program protocols (Letelier et al., 1996). Briefly, seawater was subsampled under subdued light from the rosette sampling bottles into triplicate acid-cleaned 500 ml polycarbonate bottles. Samples were inoculated with ^{14}C-bicarbonate to a final activity of ~1.85 MBq, affixed to a free-drifting array, and incubated *in situ* over the full photoperiod (ranging 11–13 h). During the summer of 2012, rates of ^{14}C-PP were measured daily as part of the Center for Microbial Oceanography: Research and Education (C-MORE) HOE-DYLAN cruises. For these near-daily scale measurements, seawater was collected at 25 m from a predawn CTD rosette cast; triplicate 500 ml polycarbonate bottles were filled from the CTD rosette bottles, inoculated with ~1.85 MBq ^{14}C-bicarbonate, and placed for the duration of the photoperiod in a seawater-cooled, deckboard incubator, shaded with blue Plexiglass to ~30% of surface irradiance.

At the end of the photoperiod, bottles were retrieved from the incubator or the *in situ* array, and 250 μl was subsampled from each sample bottle into a 20 ml glass scintillation vial containing 500 μl of β–phenethylamine for subsequent determination of the total activity of ^{14}C added to each sample. The remaining sample volume was gently vacuum filtered onto 25 mm diameter glass fiber filters (Whatman GF/F). Filters were placed in glass 20 ml scintillation vials and stored frozen until analysis. At the shore-based laboratory, filters were thawed and 1 ml of 2 mol L^{-1} hydrochloric acid was added to each filter; filters were allowed to passively vent in a fume hood overnight. After venting, 10 ml of Ultima Gold (Perkin Elmer) liquid scintillation cocktail was added to each filter and to the total activity vials; vials were placed in a liquid scintillation counter for determination of the resulting ^{14}C activities. Samples were stored in the dark and recounted after a month; the values from the second counts were used to calculate rates of ^{14}C-PP (Karl et al., 1998).

Seawater for measurements of ^{3}H-Leu incorporation was collected from the same CTD hydrocasts and depths as coincident ^{14}C-PP measurements. Polyethylene amber bottles (125 ml capacity) were subsampled from the CTD rosette bottles, and duplicate acid-cleaned 12 ml polycarbonate centrifuge tubes (Nalgene Oak Ridge) were filled from each depth. Each polycarbonate tube was inoculated with 20 nmol L^{-1} (final concentration) 3,4,5-^{3}H-leucine (Perkin Elmer; stock specific activities ranged from 108 to 144 Ci/mmol). An additional 1.5 ml per depth was subsampled into 2 ml microcentrifuge tubes (Axygen; Pace et al., 2004) containing 100 μl of 100% (w/v) ice-cold trichloroacetic acid (TCA) to serve as a killed blank. The polycarbonate sample tubes were capped and incubated *in situ* over the photoperiod to measure rates of ^{3}H-Leu incorporation in both dark (through use of black cloth bags; hereafter ^{3}H-Leu_{Dark}) and light (hereafter ^{3}H-Leu_{Light}) on the same free-drifting array utilized for the ^{14}C-PP measurements. At the end of the photoperiod, triplicate 1.5 ml subsamples were removed from each tube and added to 2 ml microcentrifuge tubes (Axygen) containing 100 μl of 100% ice-cold TCA; these tubes were stored frozen until analysis. Samples were processed following a modified method of the microcentrifuge method (Smith and Azam, 1992). Microcentrifuge tubes were spun at ~23,900*g* for 15 min at 4 °C in a refrigerated microcentrifuge; supernatants were decanted, 1 ml ice-cold 5% TCA was added to each microcentrifuge tube and samples were spun for an additional 5 min at ~23,900*g* at 4 °C. Supernatants were decanted and 1 ml of 80% ethanol was added to each sample, and tubes were spun for an additional 5 min at ~23,900*g* at 4 °C. After decanting supernatants, samples were left uncapped for 12–16 h in a fume hood to evaporate any residual ethanol from the microcentrifuge tubes. When samples had completely dried, 1 ml of Ultima Gold LLT scintillation cocktail was added to each tube, the tubes were vortexed, placed into 7 ml polyethylene scintillation vials (serving as a carrier vials) and counted on a liquid scintillation counter.

During the summer of 2012, rates of ^{3}H-Leu incorporation were measured at near-daily time scales during a series of cruises to Station