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The role of *Synechococcus* in vertical flux in the Costa Rica upwelling dome

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ABSTRACT

Despite evidence that picophytoplankton contribute to export from marine pelagic ecosystems to some extent, few field studies have experimentally evaluated the quantitative importance of that flux or specifically assessed the relative strengths of alternate ecological pathways in transporting picophytoplankton carbon to depth. In experimental studies in the Costa Rica Dome (CRD), we used a combination of methods – flow cytometry (FCM), microscopy, pigments, dilution assays, mesozooplanton gut contents and sediment traps – to follow production, grazing and export fates of the dominant picophytoplankter, *Synechococcus* spp. (*Syn*), relative to the total phytoplankton community. *Syn* accounted for an average of 25% (range 9–50%) of total phytoplankton production during four 4-day drifter experiments at CRD sites. During the same experiments, sediment trap deployments at the base of the euphotic zone measured total organic carbon export ranging from 50 to 72 mg C m^{-2} d^{-1}. Flow cytometry measurements of the trap samples showed that only 0.11% of this carbon was recognizable as ungrazed sinking *Syn*. Phycoerythrin (PE) measurements on the same samples, which we attributed mostly to transport of intact cells in mesozooplankton fecal pellets, gave export contributions of unassimilated *Syn* eight-times higher than ungrazed sinking cells, though still <1% of total carbon. Grazing of mesozooplankton on *Syn* was confirmed by PE measurements of mesozooplankton guts and the visual presence of *Syn* cells in fecal pellets. Microzooplankton grazing estimates from dilution experiments, combined with degradation rates of mesozooplankton fecal material in the water column, allowed us to estimate indirectly the additional flux of carbon transferred through protozoan grazers before being exported as mesozooplankton fecal pellets. Assuming one to three protozoan trophic steps, this *Syn* pathway contributed on average an additional 0.5–5.7% of organic carbon flux. A similar budget for total phytoplankton, based on chlorophyll *a* and phaeopigments was consistent with fecal pellets as the dominant mechanism of sinking carbon. Therefore, while *Syn* sinking as ungrazed cells or aggregates were minor components of export, the indirect trophic pathway involving mesozooplankton predation on protozoan consumers of *Syn* comprised the major mode of bulk carbon export for *Syn*-generated primary production.

1. Introduction

Phototrophic cells in the picoplankton size class (<2 μm) are known to be major contributors to phytoplankton biomass and production in the open ocean (Li et al., 1983; Brown et al., 1999; Poulton et al., 2006). Their role in vertical carbon flux, however, is both poorly quantified and heavily debated. Generally, it is believed that picophytoplankton are too small to sink individually or to be grazed efficiently by most fecal pellet-producing mesozooplankton, making them less likely to contribute significantly to carbon export compared to larger taxa (Michaels and Silver, 1988). Inverse modeling studies initially questioned this interpretation, inferring the need for large fluxes of ungrazed picophytoplankton to balance their estimates of system-level rates (e.g. Richardson and Jackson, 2007). Although that inference has itself been criticized (Stukel and Landry, 2010), such models were important in highlighting the potential importance of aggregation mechanisms to enhance gravitational sinking of the ungrazed picophytoplankton production. Among direct observations, distinguishable picophytoplankton cells are often present in flow cytometric and microscopic examinations of sediment trap contents, though at concentrations that account for only a small fraction of total carbon flux (Silver and Gowing, 1991; Rodier and Le Borgne, 1997; Waite et al., 2000). Diagnostic pigments that might be indicative of much larger concentrations of partially degraded picophytoplankton have also been detected in sediment trap material (Lamborg et al., 2008) and mesopelagic regions of the water column (Lomas and Moran, 2011). In addition, genetic sequencing, at least at one

Abbreviations: CRD, Costa Rica Dome; ETP, eastern tropical Pacific; FCM, flow cytometry; PE, phycoerythrin; Phaeo, phaeopigments; *Syn*, *Synechococcus*; CE, conversion efficiency; EE, egestion efficiency; GGE, gross growth efficiency.

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site, has shown picoeukaryotes to be overrepresented in sediment trap material relative to diatoms when compared to the overlying water column (Amacher et al., 2009).

Despite mounting evidence that picophytoplankton contribute to export to some extent, few field studies have experimentally evaluated the quantitative importance of that flux, and, perhaps more importantly, none have specifically assessed the relative strengths of alternate ecological pathways in transporting picophytoplankton carbon to depth. Three distinct pathways exist for concentrating and facilitating the export of picophytoplankton carbon, each with its own efficiency of transport and implication for plankton ecology. Picophytoplankton may be incorporated into sinking aggregates (Waite et al., 2000; Richardson and Jackson, 2007). They may be grazed by mesozooplankton, either individually (Pfannkuche and Lochte, 1993; Gorsky et al., 1999) or as a by-product of feeding on aggregates (Wilson and Steinberg, 2010), and exported as unassimilated material within fecal pellets (Pfannkuche and Lochte, 1993; Waite et al., 2000). They may also be grazed by protozoans in the microbial portion of the food web, with these in turn being consumed and incorporated into fecal pellets by mesozooplankton. This latter mechanism constitutes an indirect transport pathway of picophytoplankton carbon to depth (Stukel and Landry, 2010) that may retain little or no indication (pigments or DNA) of its origins as picophytoplankton production.

The Costa Rica Dome (CRD) is a unique region of open-ocean upwelling and shoaling of isopycnals in the eastern tropical Pacific (ETP), centered at 9°N, 90°W (Fiedler, 2002). One property of the region is its remarkably large populations of the picocyanobacteria, *Synechococcus* spp. (*Syn*), with concentrations typically exceeding 10^5 cells mL^{-1} and often $>10^6$ cells mL^{-1} (Li et al., 1983; Saito et al., 2005). The CRD thus offers a unique opportunity to study the export flux role of a dominant picophytoplankton that is readily distinguished and quantified by microscopy, flow cytometry and characteristic pigments. Here we measure phytoplankton (total and *Syn*) standing stocks and growth rates, grazing by micro- and mesozooplankton, and vertical fluxes in four water parcels across the CRD. Based on analyses of *Syn* by flow cytometry and the diagnostic pigment phycoerythrin (PE), and in comparison to a pigment-carbon budget for total phytoplankton, we show (1) that *Syn* sinking as ungrazed cells or aggregates is a minor component of export, (2) that mesozooplankton grazing/fecal pellet transport provides the main export mechanism for distinguishable *Syn* cells from the euphotic zone, and (3) that the indirect trophic pathway of mesozooplankton predation on protozoan primary consumers of *Syn* comprises the major mode of bulk carbon export for *Syn*-generated primary production.

2. Methods

2.1. Experimental design and sampling

Using a semi-Lagrangian experimental design similar to that in Landry et al. (2009), we conducted four experimental studies involving water-column sampling and rate measurements of phytoplankton production and growth, grazing losses to micro- and mesozooplankton, and export fluxes into sediment traps. These were done on the CRD FLUZiE (Flux and Zinc Experiments) cruise aboard R/V Melville in July 2010. For each 4-day study, which we called an experimental "cycle", we followed a marked water parcel with a satellite-tracked drift array with a holey-sock drogue (3×1-m) centered at 15-m depth. The drifter served both as the moving frame of reference for our sampling and experimental measurements and as an in situ incubator for daily bottle experiments for rate determinations that were attached in coarse net bags to a tether line beneath the surface float. We also deployed for the duration of each 4-day cycle a second drogued drift array with sediment traps at two depths to quantify particulate fluxes from the euphotic zone. Seawater samples were collected from Niskin bottles mounted on a CTD-equipped rosette, typically within 100 m of the drift array. Early morning samples (0200 local time) from the Niskin bottles were used both for daily assessments of standing stocks and to set up dilution experiments. Oblique net tows through the full euphotic zone were taken to measure mesozooplankton biomass and gut pigment contents.

We used a combination of flow cytometry (FCM), microscopy and pigment analyses to follow the production and fate of *Syn* and the total phytoplankton community through various processes (Table 1). Production and microzooplankton (protozoan) grazing rates were determined from daily dilution incubations at 8 depths in the euphotic zone. Grazing by mesozooplankton was assessed from gut content analyses of PE and phaeopigments and followed to its fate as unassimilated *Syn* and total phytoplankton in fecal pellets collected in the sediment traps. Finally, we used protozoan grazing measurements, pigment degradation rates, and gross growth efficiency assumptions to constrain estimates of the amount of *Syn* and total phytoplankton transported to depth in fecal pellets by indirect trophic transfer.

2.2. Phytoplankton biomass assessments

We used various FCM, pigment and microscopy methods to estimate water-column standing stocks and cell:pigment or C:pigment ratios for both *Syn* and the total phytoplankton community. Stock estimates for *Syn* came from direct FCM cell counts, assuming a cell carbon content of 101 fg C $cell^{-1}$ (Garrison et al., 2000), and from measurements of the cyanobacteria marker pigment phycoerythrin (PE). For total phytoplankton, we used Chl *a* as the pigment indicator and combined FCM (photosynthetic bacteria) and epifluorescence microscopy (eukaryotes) to assess total carbon biomass.

FCM analyses were done with live samples onboard ship and with frozen preserved samples in the laboratory. Different instruments were used but with very similar results for *Synechococcus* (lab cells $mL^{-1} = -1771 + 0.974 * \text{ship cells } mL^{-1}$; $r = 0.99$, $n = 160$ paired samples taken from the same Niskin bottles). Results from the ship instrument (a Beckman-Coulter XL with a 15-mW 488-nm argon ion laser) are used in the present study for all water-column stock and rate assessments for *Syn* and for sediment trap

Table 1
Assessment of export pathways. Carbon export (stemming from either *Syn* or total phytoplankton) was determined for three distinct pathways: Sinking of ungrazed cells and aggregates, sinking of unassimilated cells within mesozooplankton fecal pellets, and trophic transfer through protozoans to mesozooplankton. PrGr is protozoan grazing, GGE is protozoan gross growth efficiency, TL is the number of protozoan trophic levels separating *Syn* from mesozooplankton, PigDeg is the pigment degradation rate prior to export (includes degradation within mesozooplankton guts and remineralization of fecal pellets in the euphotic zone and is calculated as the ratio of sediment trap pigment flux to mesozooplankton pigment ingestion).

Pathway	Sinking cells/aggregates	Herbiv. fecal pellets	Trophic transfer
Assessment	Direct	Direct	Indirect
Measurements	Flow cytometry	Pigments	Protozoan grazing, pigment degradation
Calculation	*Syn* flux × Carbon:*Syn*	PE flux × Carbon:PE	$PrGr \times GGE^{TL} \times PigDeg$