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, mesozooplankton 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. Mesozooplankton 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
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 (Plankhuche and Lochte, 1993; Waite et al., 2000). They may also be grazed by protozoans in the microzooplankton fraction 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 10^7 cells mL^{-1} and often ×10^7 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 FLUIZE (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 tow 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 pigments 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 *Syn* (lab cells mL^{-1} = 1771 ± 0.974 ± 0.160, 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>
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<tr>
<th>Pathway</th>
<th>Sinking cells/aggregates</th>
<th>Herbiv. fecal pellets</th>
<th>Trophic transfer</th>
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<tbody>
<tr>
<td>Assessment</td>
<td>Direct</td>
<td>Direct</td>
<td>Indirect</td>
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<tr>
<td>Measurements</td>
<td>Flow cytometry</td>
<td>Pigments</td>
<td>Protozoan grazing, pigment degradation</td>
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<tr>
<td>Calculation</td>
<td>Syn flux × Carbon:Syn</td>
<td>PE flux × Carbon:PE</td>
<td>PrGr × GGETL × PigDeg</td>
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</tbody>
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analyses (below). Results from the lab cytometer (Beckman-Coulter Altra with 200-mW UV and 1-W 488-nm argon ion lasers) are used only for total phytoplankton carbon biomass estimates because that instrument gave more reliable counts for Prochlorococcus. For the shipboard cytometer, an Orion syringe pump delivered 2.2-ml samples at a rate of 0.44 ml min⁻¹ (Selph et al., 2001). Syn were distinguished from light scatter (forward and 90° side scatter), chlorophyll and PE fluorescence, normalized to 6-μm fluorescent calibration beads. For the lab analyses, 2-ml samples were preserved (0.5% paraformaldehyde, final concentration), flash frozen in liquid nitrogen, and later stained with Hoechst 33342 at 1 μg ml⁻¹ final concentration before analysis (Monger and Landry, 1993). Fluorescence signals were normalized to 0.5 and 1.0-μm yellow-green (YG) polystyrene beads (Polysciences Inc., Warrington, PA). Listmode data files (FCS 2.0 format) of cell fluorescence and light-scatter properties were acquired with Expo32 software (Beckman-Coulter) and used with FlowJo software (Tree Star, Inc., www.flowjo.com). For biomass estimates, we used carbon contents of 32 and 101 fg C cell⁻¹ for Prochlorococcus and Synechococcus, respectively (Garrison et al., 2000).

Samples (250 ml) taken for fluorometric analyses of Chl a were immediately filtered onto GF/F filters, and the Chl a extracted with 90% acetone in a dark freezer for 24 h. Extracted samples were shaken, warmed in the dark to room temperature, settled and quantified on a calibrated Turner Designs model 10 fluorometer (Strickland and Parsons, 1972). PE was measured using the glycerol uncoupling method of Wyman (1992). Water-column samples were vacuum-filtered sequentially through a 20-μm filter to remove larger particles and a 0.6-μm polycarbonate filter to retain small cyanobacteria. The filters were then transferred to 20-ml glass scintillation vials with a saline 50% glycerol solution (35 g L⁻¹ NaCl, final concentration), briefly shaken on a vortex mixer, and refrigerated for 2–24 h to allow the cells to become suspended in solution. Fluorescence was measured on a Turner Designs TD-700 Fluorometer with a PE filter set (excitation 544 nm, emission 577 nm). The fluorometer was calibrated with a commercial R-phycocerythrin standard (Wyman, 1992; Dore et al., 2002). Epifluorescence microscopy was done for a subset of sampling depths (n = 120) sufficient to determine the mean C:Chl a ratio for the phytoplankton assemblage. Details of the methods are the same as Taylor et al. (2012). Seawater samples (500 mL) were preserved and cleared according to a modified protocol from Sherr and Sherr (1984), with sequential additions of 250 mL of buffered Lugol’s solution, 10 mL of buffered Formalin and 500 μL of sodium thiosulfate, followed by staining with 1 mL of proflavin (0.33% w/v) and 1 mL of DAPI (0.01 mg mL⁻¹). Aliquots of 50 mL were filtered onto 25-μm, 0.8-μm pore-size black polycarbonate filters to determine concentrations of nanophytoplankton, and the remaining 450-ml samples were filtered onto black 8.0-μm polycarbonate filters to determine concentrations of larger cells (microplankton). Filters were mounted onto glass slides and digitally imaged in Z-stack mode at 630X (nanoplankton) and 200X (microplankton). Filters were imaged for each slide, with each Z-stack level and position consisting of four fluorescent channels: Chl a, DAPI, FITC and phycoerythrin. The separate images were combined to produce one composite best-in-focus 24-bit RGB image for each position, and these were processed and analyzed using ImagePro software. Phytoplankton biovolumes (BV; μm³) were calculated from the length (L) and width (W) measurements of each cell using the geometric formula of a prolate sphere (BV = 0.524 LWH), where unmeasured height (H) was estimated as W for diatoms and 0.5 W for flagellates (Taylor et al., 2012). Biomass was calculated as carbon (C; pg cell⁻¹) using the equations of Menden-Deuer and Lessard (2000): C = 0.288 BV⁰.⁸¹¹ for diatoms and C = 0.216 BV⁰.⁹³⁹ for non-diatoms.

2.3. Dilution experiments
Rates of growth, production and protozoan grazing were estimated for Syn and for the total phytoplankton community from the results of 2-treatment dilution experiments (Landry et al., 1984, 2008) incubated in situ at 9 depths on the drift array, 4 times per cycle. Following the procedures described in Landry et al. (2009), we prepared one diluted treatment and one control bottle (2.7-L polycarbonate) per depth, respectively, with 33% whole seawater (diluted with 0.1-μm Suporcap filtered seawater) and 100% seawater collected from Niskin bottles from the CTD-rosette. Initial FCM samples were taken from each bottle prior to deployment, and initial samples for Chl a were taken from the same Niskin bottle as the incubation water. The experiments were incubated for 24 h (deployment/recovery ~0430 local time). Upon recovery, the incubation bottles were subsampled for FCM and Chl a. Instantaneous grazing rates (m) on Chl a or Syn cells were determined from net measured growth rates in treatment (kt) and control bottles (k) as m = (kt − k)/1.0–33. Instantaneous growth rates were determined as m = k_1. Biomass production (PP) and grazing (PG) estimates were computed as PP = μ + B and PG = m − B, where B is the initial (and in situ) phytoplankton biomass. For these, we used carbon biomass conversions of 101 pg C cell⁻¹ for Syn and C:Chl = 73 for total phytoplankton (see results).

2.4. Mesozooplankton grazing
Mesozooplankton were sampled daily with paired day-night oblique tows to 150-m depth with a ring net (0.71 m², 202-μm mesh size), for a total of 4 paired measurements per experimental cycle. A General Oceanics flowmeter was attached across the net mouth to record volume filtered, and a Vyper Suunto dive computer was fastened to the net frame to record tow depth and duration. Immediately upon recovery, organisms were anesthetized with carbonated water (Kleppel and Pieper, 1984). The samples were then split with a Folsom splitter for biomass and gut pigment determinations. Typically, 1/8 sample splits were filtered onto 200-µm Nitex filters for gut pigment analyses for phaeopigments (Phaeo) and PE and flash frozen in liquid N₂. Phaeo samples were thawed and extracted with a tissue homogenizer in 90% acetone, and the homogenate was centrifuged for 5 min at 3000 rev min⁻¹ to remove particulates. Concentrations of Phaeo were then measured using a Turner 10AU fluorometer (Strickland and Parsons, 1972). PE samples (only one pair of samples per cycle) were later washed from filters with glycerol saline solution, ground with a tissue homogenizer to release gut contents, and centrifuged to separate the supernatant from pellets containing crushed mesozooplankton carcasses. After refrigeration for 2–24 h, fluorescence was measured on a Turner Designs TD-700 Fluorometer with a PE filter set.

For each analysis, we computed the depth-integrated concentration of gut pigment (Phaeo, PE) in the euphotic zone as:

\[
GPC = \frac{pg \cdot f \cdot D}{vol}
\]

where GPC is gut pigment content (mg m⁻²), pg is the measured pigment value (mg), f is fraction of sample analyzed, D is depth of tow (m) and vol is the volume of water filtered (m³) (Décima et al., 2011). To compute grazing rates from gut content measurements, we utilized the gut turnover rate of 2.1 h⁻¹ measured for zooplankton in the Equatorial Pacific (Zhang et al., 1995), where temperature conditions were similar to the CRD.
2.5. Sediment traps

We deployed VERTEX-style sediment traps (8:1 aspect ratio, 7-cm diameter, with a baffle of 14 smaller tubes tapered at the top) on a satellite-tracked drifter array at two depths (base of the euphotic zone and 150 m) at the beginning of each 4-day experimental cycle, and recovered them at the end. At each depth, 8–12 replicate tubes were held vertically in place on a PVC cross piece (Knauer et al., 1979). All tubes were filled before deployment with a slurry of 0.1-μm filtered seawater amended with 50 g L\(^{-1}\) NaCl and 1% final concentration formalin. Upon recovery, water above the density interface in each tube was gently removed by peristaltic pump, and the samples were filtered through a 200-μm Nitex filter. Filters were examined under a dissecting microscope to remove zooplankton swimmers, and the remaining material was rinsed off the filter and returned to the sample. We do not believe that substantial numbers of mesozooplankton passed through the 200-μm Nitex mesh, because a few exploratory net tows with a 100-μm mesh suggested that 100–200-μm sized organisms comprised an insignificant portion of the total mesozooplankton biomass and small mesozooplankton are typically weak vertical migrators.

For analyses, 1/8 to 1/4 of the tube contents was filtered onto a 0.6-μm filter for PE measurements and 1/4 of the contents was filtered onto a pre-combusted GF/F filter for measurement of particulate organic carbon (POC). Smaller subsamples (50 mL) of the well-mixed trap contents were also removed for Chl a and phaeopigment (Phaeo) determinations, for FCM enumeration of Syn cells (20 mL), and for epifluorescence microscopy (EPI) of larger intact cells (50 mL). Chl a and Phaeo concentrations were measured, as above, by the fluorometric acidification method. POC analyses were done with a Costech 4010 Elemental combustion analyzer, following acidification to remove inorganic carbon. PE samples were ground with a tissue homogenizer prior to addition of glycero salin solution and refrigerated for 2–24 h. Fluorescence was measured on a Turner Designs TD-700 fluorometer with PE filter set.

FCM subsamples were stored on ice in the dark until analyzed within 1–6 h of collection with the shipboard cytometer (Beckman-Coulter XL). Just prior to analysis, the samples were briefly vortexed and pre-filtered through 200-μm Nitex mesh to disrupt delicate aggregates while keeping fecal pellets largely intact (frag-
roughly 24% degraded before reaching 150-m depth. Chl a was not detected in three of the eight traps and only registered as a significant amount of the pigment flux on Cycle 5, where the Chl a: Phaeo export ratio was still <0.1 at 90 m. The export pattern for PE was similar to Phaeo, with a regression of PE on Phaeo explaining 84% of the variability in PE flux (Fig. 4). The small intercept suggests that the two pigments are transported to depth in relatively constant proportion (geometric mean = 0.04 μg PE/μg Phaeo), implying a common mechanism of export.

Based on our mean conversion estimate of 6.4 fg PE per Syn cell, the PE fluxes into sediment traps are equivalent to total export of 3.3 \times 10^{10} to 9.8 \times 10^{10} Syn cells m^{-2} d^{-1} (Phyco, Table 2). Since vertically integrated standing stocks ranged from 9.0 \times 10^{11} to 7.4 \times 10^{12} m^{-2} (with peak cycle concentrations of 5.0 \times 10^{12} to 2.8 \times 10^{13} L^{-1}), total Syn export marked by a pigment tracer signal ranged from 0.04% to 1.06% of standing stock per day. From FCM measurements, estimates of Syn export as individually recognizable cells were almost an order of magnitude lower (Table 2), suggesting that most Syn export tracked by the PE pigment arrived in fecal pellets or aggregates that were not easily disrupted by the vortexing and 200-μm pre-screening that was done prior to FCM analysis of the trap material.

Export of recognizable diatom cells in sediment trap material was uniformly low, never exceeding 0.5 mg C m^{-2} d^{-1}, and typically far less than that. The dominant diatoms in the sediment trap were small pennates with linear dimensions of roughly 20 μm \times 3 μm, although larger pennate diatoms were responsible for most of the diatom flux in Cycle 5. Export of other microplankton (which were not identified to taxa but comprised mostly of a mixed community of flagellates) varied from 0.65 to 1.81 mg C m^{-2} d^{-1} at the base of the euphotic zone. Overall, the flux of microscopically identifiable microplankton was of the same magnitude as the phytoplankton mass flux inferred from Chl a.

3.4. Grazing rate measurements

Despite the common perception that picoplankton cells are too small to be grazed by typical mesozooplankton, we found significant PE concentrations in our measurements of mesozooplankton gut contents (Table 3). Grazing rates varied from 0.23 to 0.36 mg PE m^{-2} d^{-1}, equivalent to 3.7 \times 10^{10} to 5.8 \times 10^{10} Syn cells m^{-2} d^{-1}. Mesozooplankton fecal pellets thus can easily account for the average of 0.04 mg PE m^{-2} d^{-1} exported out of the euphotic zone. Intact Syn cells were also observed individually and in clumps within the fecal pellets of live mesozooplankton incubated in shipboard grazing experiments (Fig. 5), consistent with previous reports that they may not be significantly digested in passing through the guts of metazoan consumers (Silver and Bruland, 1981; Johnson et al., 1982; Gorsky et al., 1999). In calculations below, we assumed that all direct consumption of Syn cells by mesozooplankton passed intact (i.e. without pigment degradation) through their digestive systems and into their fecal pellets (e.g. Gorsky et al., 1999).

Phaeo concentrations (indicative of grazing on Chl a), were high in the mesozooplankton gut analyses (2.08–7.53 mg Chl a equiv. m^{-2} d^{-1}; Table 3), suggesting substantial grazing pressure of 12–44% of Chl a standing stock d^{-1} by the mesozooplankton community. When compared to Phaeo fluxes measured in traps at the base of the euphotic zone (Table 2), 74–84% of the Chl a consumed by mesozooplankton disappeared between ingestion and export from the euphotic zone (either degraded in the guts of mesozooplankton or lost to disintegration/recycling of fecal pellets during sinking). A similar comparison of PE grazing to PE vertical flux indicates comparable loss rates of 75–92% of PE consumed. The similarity in these estimates, coupled with the fact that sediment trap PE appeared to be largely associated with transport of intact Syn cells in pellets, suggests that physical disintegration and/or recycling of pellet contents during euphotic zone transit...
Table 2
Export fluxes of carbon, nitrogen, pigments and cells into sediment traps. All data are integrated over the depth interval shown (depth, m) in Column 1. Shown are: Chlorophyll a (Chl a, μg m⁻² d⁻¹), Phaeo (phaeopigments, μg Chl a equiv. m⁻² d⁻¹), PE (phycoerythrin, μg m⁻² d⁻¹), PE: Syn abundance as calculated by multiplying PE flux by Syn:PE ratio, cells m⁻³ d⁻¹), SYN (Syn abundance as measured by FCM (flow cytometry), cells m⁻³ d⁻¹), phytoplankton biomass (mg C m⁻² d⁻¹) measured by epifluorescence microscopy (diatoms, other micro), and Mass Flux of Carbon and Nitrogen (mg C or mg N m⁻² d⁻¹) measured by combustion analysis. Values are mean ± standard error.

<table>
<thead>
<tr>
<th>Cycle, depth, date (2010)</th>
<th>Pigments</th>
<th>FCM</th>
<th>EPI MICRO</th>
<th>Mass flux</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chl a</td>
<td>Phaeo</td>
<td>PE</td>
<td>Syn</td>
</tr>
<tr>
<td>C2, 90, July 4-8</td>
<td>0 ± 1.2</td>
<td>377 ± 40</td>
<td>20.4 ± 0.5</td>
<td>3.19 × 10⁷</td>
</tr>
<tr>
<td>C2, 150</td>
<td>1.2 ± 2.7</td>
<td>270 ± 23</td>
<td>21.4 ± 17.0</td>
<td>3.35 × 10⁹</td>
</tr>
<tr>
<td>C3, 90, July 9-13</td>
<td>21.2 ± 8.7</td>
<td>1219 ± 55</td>
<td>48.8 ± 15.3</td>
<td>7.63 × 10⁸</td>
</tr>
<tr>
<td>C3, 150</td>
<td>2.7 ± 5.3</td>
<td>1024 ± 27</td>
<td>32.6 ± 7.0</td>
<td>5.11 × 10⁹</td>
</tr>
<tr>
<td>C4, 90, July 15-19</td>
<td>0 ± 19.7</td>
<td>846 ± 105</td>
<td>25.5 ± 5.9</td>
<td>3.99 × 10¹⁰</td>
</tr>
<tr>
<td>C4, 150</td>
<td>0 ± 0</td>
<td>529 ± 55</td>
<td>19.2 ± 9.4</td>
<td>3.01 × 10¹⁰</td>
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<tr>
<td>C5, 90, July 20-23</td>
<td>122.6 ± 11.3</td>
<td>1368 ± 96</td>
<td>60.7 ± 7.6</td>
<td>9.50 × 10⁹</td>
</tr>
<tr>
<td>C5, 150</td>
<td>51.6 ± 6.4</td>
<td>1166 ± 86</td>
<td>48.7 ± 7.8</td>
<td>7.62 × 10⁸</td>
</tr>
</tbody>
</table>

was the most likely explanation for the low fraction of mesozooplankton pellets sinking into the traps. Vertically integrated estimates of protozoan grazing on the total phytoplankton community from dilution experiments were roughly equivalent to mesozooplankton grazing for Cycles 3 and 5, and close to double mesozooplankton grazing estimates for Cycles 2 and 4 (Table 3). Protozoan grazing rates on Chl a averaged 54% of growth rates, based on the geometric mean of the dilution results. When only grazing on Syn cells was considered, however, the results were strikingly different. Protozoan grazing pressure on Syn was roughly 50 times higher than the direct mesozooplankton grazing pressure on Syn suggested by PE gut content measurements. Protozoan grazing alone was able to account for the average loss of 127% of Syn production estimates in our experimental incubations (Fig. 6).

4. Discussion

4.1. Ecosystem pathways of vertical flux

Our experiments were designed to generate comparable contemporaneous estimates of export fluxes for Syn and for total phytoplankton (including picoplankton) by three distinct pathways: direct sinking of ungrazed cells, sinking of unassimilated Syn and phytoplankton carbon in mesozooplankton fecal pellets, and indirect trophic transfer of Syn and total phytoplankton production via protozoan grazers to mesozooplankton. Interpretations of results hinge on several assumptions about carbon:pigment ratios and the resistance of pigments to conversion to non-fluorescent material that are discussed below. However, by making consistent assumptions for PE and the Chl a:Phaeo pigment pair (see bottom line of Table 1), we can create from the measured rates a robust budget for sinking material.

The contribution of ungrazed Syn to vertical flux is determined by multiplying FCM-derived cell flux by the Syn cellular carbon content (101 fg C; Garrison et al., 2000).

\[ \text{Export}_{\text{ungrazed}} = \text{sedTrapSyn}_{\text{FCM}} \times 101 \text{ fg C cell}^{-1} \]  

(2)

This varies from 0.03 to 0.11 mg C m⁻² d⁻¹ at the base of the euphotic zone, accounting for approximately 0.11% of total carbon flux (Table 4). The contribution of total unassimilated Syn can likewise be calculated by dividing vertical PE flux by our measured PE:Syn ratio (6.4 fg PE cell⁻¹) and multiplying by the Syn cellular carbon content:

\[ \text{Export}_{\text{unassimilated}} = \text{sedTrapPE} \times \frac{\text{cells}_{\text{PE}}}{\text{PE}} \times 101 \text{ fg C cell}^{-1} \]  

(3)

This flux, which we attribute primarily to Syn carbon transported as intact cells in fecal pellets, varies from 0.32 to 0.95 mg C m⁻². If we subtract the contribution of Syn carbon (and PE) known to be from ungrazed cells, the direct transport of Syn carbon in fecal pellets averages 0.84% of total flux.

To estimate the amount of vertical flux supported by protozoan grazing on Syn, we first compute the total protozoan production.
Table 3

Mesozooplankton and protozoan grazing rates. Mesozooplankton grazing rates are shown as Chl a (phaeopigments, Phaeo) or phycoerythrin (PE) in units of mg m$^{-2}$ d$^{-1}$. PE$^*$ denotes the PE grazing converted to a cell-based Syn grazing (cells m$^{-2}$ d$^{-1}$) using the regression in Fig. 3. Protozoan grazing rates are derived from vertically integrated microzooplankton dilution experiment results, for the entire community as Chl a (mg Chl a m$^{-2}$ d$^{-1}$) and for Syn (cells m$^{-2}$ d$^{-1}$). The final two columns are the equivalent phytoplankton production rates measured by the dilution technique for the entire community (Chl a, mg m$^{-2}$ d$^{-1}$) and for Syn (cells m$^{-2}$ d$^{-1}$). Values are mean ± standard error.

<table>
<thead>
<tr>
<th>Cycle</th>
<th>Mesozooplankton</th>
<th>Protozoan</th>
<th>Production</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Phaeo</td>
<td>PE</td>
<td>PE*</td>
</tr>
<tr>
<td>2</td>
<td>2.08 ± 0.33</td>
<td>0.27 ± 0.08</td>
<td>4.24 × 10$^{10}$</td>
</tr>
<tr>
<td>3</td>
<td>7.53 ± 1.55</td>
<td>0.36 ± 0.07</td>
<td>5.67 × 10$^{10}$</td>
</tr>
<tr>
<td>4</td>
<td>3.2 ± 0.42</td>
<td>0.23 ± 0.10</td>
<td>3.55 × 10$^{10}$</td>
</tr>
<tr>
<td>5</td>
<td>7.29 ± 0.43</td>
<td>0.24 ± 0.04</td>
<td>3.76 × 10$^{10}$</td>
</tr>
</tbody>
</table>

Fig. 5. Epifluorescence image of a euphausiid fecal pellet produced during feeding experiments at sea (data not shown, from separate experiments conducted by M. Decima on this cruise). The sample was stained with proflavin (protein stain) and DAPI (DNA stain), then mounted on a black filter and excited with blue (450–490 nm) light and UV (340–380 nm) light. With this preparation, proflavin staining imparts a green fluorescence to proteins, allowing the edge of the fecal pellet to be visualized, and orange fluorescence is from naturally-fluorescing phycoerythrin in Synechococcus cells. Scale bar is 50 μm.

Fig. 6. Syn production (x-axis) and protozoan grazing on Syn (y-axis) as measured by the microzooplankton dilution technique. Solid line is the regression of grazing against growth (grazing = 1.28 × growth + 0.02, R$^2$ = 0.84).

Based on grazing of Syn that is available to mesozooplankton as the product of protozoan grazing on Syn (Table 3) and an assumed protozoan gross growth efficiency of 30% (Straile, 1997):

Production$_{protozoa}$ ≤ Gf$_{protozoa-Syn}$ × GGE$_{protozoa}$  
(4)

(Note that the above equation is an inequality, as it assumes only one protozoan trophic step between Syn and mesozooplankton. Additional trophic steps within the protozoa—grazing of protozoans on protozoans—would decrease the total production available to mesozooplankton, see Section 4.2.) We then estimate the fraction of this carbon that is lost by pellet remineralization before sinking out of the base of the euphotic zone by comparing the pigments consumed by mesozooplankton (Phaeo and PE, Table 3) to the pigment fluxes measured in the sediment traps (Table 2):

Remineralization = 1 − (Pigment$_{mesozoo}$/CE)  
(5)

where CE is the conversion efficiency of pigment to non-fluorescent molecules within mesozooplankton guts. For the Chl a/phaeopigment pair, Durbin and Campbell (2007) have shown that the mesozooplankton gut evacuation calculations take into account the loss of pigments to non-fluorescent phaeophorbides, thus the quantity Pigment$_{mesozoo}$/CE is equivalent to the mesozooplankton grazing rate found in Table 3. For PE, CE = 1 for Syn cells that pass through mesozooplankton guts undigested. From these calculations we thus calculate remineralization for Phaeo as 74–84%, and 75–92% for PE. Assuming for each cycle that the carbon in sinking fecal pellets is lost proportionally to the mean loss rates of the two pigments and that only one trophic step separates Syn from mesozooplankton, we calculate an upper estimate of the proportion of vertical flux transported by Syn through mesozooplankton grazing on protozoans from:

Export$_{multiv}$ ≤ Production$_{protozoa}$ × EE$_{mesozoo}$ × (1 – Remineralization)  
(6)

where EE$_{mesozoo}$ is the egestion efficiency of mesozooplankton (30%; Conover, 1966).

Across our four experimental cycles, this upper estimate for the multivorous export pathway averaged 5.6% of total flux. Upper limits for the total contributions of Syn to vertical flux at the base of the euphotic zone can thus be determined as 9.2%, 4.9%, 8.3% and 4.0%, respectively, for Cycles 2–5. Contributions of Syn to total primary production in the same water parcels are 50.2%, 13.6%, 28.2% and 8.7% (Table 4).

The only difference between the equations for Chl a and PE is that while we assume that Syn passes undigested through mesozooplankton guts, most of the total organic carbon
ingested by mesozooplankton is subject to digestion and absorption during gut passage. Thus, Eq. (3) is rewritten as:

\[
\text{Export}_{\text{unassimilated}} = \text{SEDTrapPhaeo} \times C: \text{Chl}_{\text{phyto}} \times \left( \frac{\text{EE}_{\text{mesozoo}}}{\text{CE}} \right)
\]  

(7)

where as before EE_{mesozoo} is the egestion efficiency of the mesozooplankton and CE is the conversion efficiency of Chl a to phaeo (instead of non-fluorescent molecules). We again assume that EE_{mesozoo} = 0.3, and further make the conservative assumption that CE = 1 (but see Section 4.2). These calculations indicate that 2.2% of total carbon flux from the euphotic zone.

However, it is worth noting that this may be due to our conservative estimate of the flux of herbivorous fecal pellets. Nevertheless, it is clear that fecal pellets of suspension feeding mesozooplankton constitute a dominant mechanism of export flux from the euphotic zone.

4.2. Sensitivity to assumptions

As Fig. 3b and d makes clear, pigment:carbon ratios are not constant in the ocean, but typically increase with depth. Our decision to use average pigment:carbon ratios from a linear regression of biomass on pigment assumes that the depth strata with maximum biomass contribute most to mass fluxes (especially grazing). The bead-normalized PE fluorescence per Syn cell determined from FCM analyses of sediment trap samples was, in fact, similar to that measured in water-column samples from ~50 m, suggesting that our estimate of 6.4 fg PE cell^{-1} for sinking Syn was reasonable.

While our pigment:carbon ratios in the water column seem robust, another site of decoupling of the pigment to carbon ratio is within mesozooplankton guts. Syn cells likely pass through mesozooplankton guts intact and even if they are degraded, PE (a protein) is likely digested at a similar rate to total Syn. However, the equivalence of ingested and egested pigment:carbon ratio is unlikely to be true for total phytoplankton. It is important to note that our assumption of CE = 1 (the conversion efficiency of Chl a to phaeo) in Eq. (7) may lead to a large underestimate of the herbivorous fecal pellet pathway (but not the herbivorous pathway for Syn, where we assumed that CE = EE, Eq. (3)). While early studies assumed that Chl a was quantitatively converted to phaeopigments (Downs and Lorenzen, 1985), it has since been shown that the fraction of Chl a converted to non-fluorescent particles can be highly variable (Conover et al., 1986; Dagg and Walser, 1987; Penny and Frost, 1991). Goericke et al. (2000) measured the conversion of Chl a to non-fluorescent phaeophorbides (1-CE, in our Eq. (7)), and found it to range from 40% to 70%. For a more accurate estimate of the C:Phaeo ratio within fecal pellets, we could, rather than assuming that the C: pigment ratio remains constant as material passes through the gut, take the midpoint of the Goericke et al. (2000) measurements (CE = 45%) while still assuming 70% assimilation efficiency of carbon. In this case, the C:Phaeo ratio of herbivorous fecal pellets, and hence their contribution to flux, would increase by a factor of (1/CE) = 2.2. While this affects the herbivorous pathway for total phytoplankton, altered estimates of CE will not alter export estimates of the trophic transfer pathway, since the true (but unknown) CE is taken into account in typical mesozooplankton gut evacuation experiments (Durbin and Campbell, 2007). Under the aforementioned assumption of CE = 0.45, the percent total contribution of Syn production to export would remain 9.2%, 4.9%, 8.3% and 4.0% for Cycles 2–5, respectively, but the percent contribution of total phytoplankton production to export would be 38.2%, 101.1%, 97.5%, and 107.8%, which is remarkably good agreement for three of our four water parcels.

That export was predominantly supported by mesozooplankton fecal pellet production is further supported by the measured balance of growth and grazing in our experiments. According to the Chl-based rate estimates in Table 3, the mean (±s.d.) difference between total phytoplankton community production and consumption by protozoans is 5.4 ± 1.8 mg Chl equiv. m^{-2} d^{-1}. The fact that this is statistically indistinguishable from the estimated rate of mesozooplankton grazing (5.0 ± 2.8 mg Chl equiv. m^{-2} d^{-1}) indicates that phytoplankton production was balanced by total grazing pressure, leaving little excess production available for direct export. Using these same methods and assumptions, similar production-grazing balances have also been described for the HNLC equatorial region from a large data set of many stations (Landry et al., 2011).

The assumption of greatest importance to our calculation of the total contribution of Syn to export is that made to calculate the magnitude of indirect trophic transfer from protozoan grazing, since this is, according to our results, the pathway that transports the most Syn-generated production to depth. The most conservative guess is that a single protozoan trophic step separates Syn from mesozooplankton. While a wide range of grazers is known to feed on Syn (Caron et al., 1991; Jeong et al., 2005; Frias-Lopez et al., 2009), the dominant grazers of picoplankton in both

Table 4
Proportion of total and export production. First column shows the proportion of total primary production attributable to Syn as assessed by dividing the microzooplankton egestion-derived production rates of Syn by Chl a based production rates. Next four columns show the fraction of total export supported by total phytoplankton due to sinking of ungrazed cells, herbivorous fecal pellets (with the conservative assumption that CE = 1, see Eq. (7)), herbivorous fecal pellets (with the more realistic assumption that CE = 0.45, see Section 4.2), and a potential upper estimate of mesozooplankton fecal pellets produced from grazing on protozoans. Final three columns show the equivalent proportion of export supported by Syn production through each of those three pathways. For more details see Section 4.1.
oligotrophic and upwelling regions of the open ocean are <5-μm protozoans (Calbet and Landry, 1999; Christaki et al., 2001; Sherr and Sherr, 2002). Considering that much larger cells like diatoms are also subject to high rates of protozoan grazing in the open ocean (Landry et al., 2011), it could well be that the nanoflagellate predators of Syn are in turn the prey of other protozoans. Assuming 30% gross growth efficiencies for each trophic step separating Syn from mesozooplankton decreases the contribution of the indirect trophic transfer pathway to flux by 70%. Two protozoan trophic steps would result in total Syn contributions to export ranging from 2.1% to 3.1%, while three steps would reduce their composite contributions to only 1.3–1.6% of vertical carbon flux. It is thus clear that the assumption of one protozoan trophic step gives an upper limit for Syn contribution to export flux. The minimum estimate (~1%) is the amount directly measured by PE flux into sediment traps.

4.3. The role of picophytoplankton in vertical flux

Based on inverse model analysis, Richardson and Jackson (2007) argued that picophytoplankton dominated vertical carbon export in the open ocean and that the mechanism was primarily through aggregation and sinking of ungrazed cells. However, if picophytoplankton production is almost entirely consumed by protozoan grazers as in the present experiments and many others (Latasa et al., 1997; Selph et al., 2011), ungrazed cells seem unlikely to be the major export mechanism. Indeed, further results of inverse analyses with better constraints on size-structured production and grazing rates, have indicated significant, though not dominant, contributions of picophytoplankton-derived production to export, but mainly through indirect trophic transfers after initial grazing (Stukel and Landry, 2010).

Our present field results indicate that direct flux of ungrazed picophytoplankton (as measured flow cytometrically) was a negligible component of both total carbon export and picophytoplankton loss from the euphotic zone. While it is possible that the greater Syn export estimates suggested by PE concentrations in the sediment traps may have originated from robust aggregates that resisted our disruption approach (vortexing), the strong similarity between the percentages of mesozooplankton-ingested Phaeo and PE reaching the sediment traps (Fig. 4) suggests that most PE was transported in fecal pellets. While direct grazing of mesozooplankton on Syn cells was a small loss term for picophytoplankton in our experiments, its impact on export of Syn (Table 3) was two orders of magnitude higher than direct cell sinking (Table 2). The primary role of aggregation may be to make picophytoplankton available to mesozooplankton (Wilson and Steinberg, 2010), rather than to catalyze sinking directly. Aggregation may still play a role in export, however, if pellets are transported in association with aggregates, or if pellets degrade to amorphous aggregates.

Appendicularians, salps and large pyrosome colonies, all capable of feeding directly on small particles, were abundant at various times during our experiments in the CRD, so metazoan grazing cannot be ruled out as an important mechanism for cell concentration and aggregation, such as the uningested cells in discarded appendicularian houses. Our observations – the presence of abundant intact Syn cells in fecal pellets and evidence that most pellets never leave the euphotic zone – also raise intriguing possibilities that Syn can benefit in some ways from being in fecal pellets (e.g. enhanced nutrient environment and protection from protozoan grazers) and that the cells in disintegrating pellets may be repeatedly cycled by mesozooplankton. We note that even in the early days of microbial loop discoveries, Johnson et al. (1982) envisioned viable intact Syn cells being transported to great ocean depth in the fecal pellets of large grazers. While our results stop at 150-m deep sediment traps, they are consistent with this mechanism of picophytoplankton delivery to greater ocean depths.

While this is only a single study, our conclusions are in agreement with data from other sites. Picophytoplankton and their diagnostic pigments are frequently found in sediment traps (Rodier and Le Borgne, 1997; Waite et al., 2000; Lamborg et al., 2008), as suspended particulates in the mesopelagic zone (Lomas and Moran, 2011; Sohrin et al., 2011), and even in deep-sea sediments (Lochte and Turley, 1988; Pfannkuche and Lochtke, 1993). Nevertheless, when the carbon transported by these cells has been directly measured and compared to concurrent total POC flux, it has typically accounted for a minor (on the order of 0.1%) proportion of sediment trap flux (Turley and Mackie, 1995; Rodier and Le Borgne, 1997; Waite et al., 2000). Using water-column pigments and 234Th measurements, Lomas and Moran (2011) estimated that the cyanobacteria contribution to export was an order of magnitude less than their contribution to biomass, although still a significant 5% each for Syn and Prochlorococcus. Their study assumed, however, that the contribution of pigments to sinking flux was equal to their contribution to standing stocks in the mesopelagic. The growing evidence that picocyanobacteria production is balanced by protozoan grazing in the open ocean (Liu et al., 2002; Hirase et al., 2008; Selph et al., 2011) also suggests that little picophytoplankton production remains for direct export. In contrast, the low export efficiency (e-ratio = export/14C-PP was ~5%) in the picophytoplankton-dominated CRD is consistent with other studies that find high ratios associated with communities dominated by diatoms and large phytoplankton (Buesseler, 1998; Buesseler et al., 2008). Nevertheless, we find that picophytoplankton may contribute significantly to export through protozoan grazing pathways that link them to mesozooplankton. Constraining the number of trophic transfers within the protozoan grazer chain therefore remains an important step toward accurately linking the production of plankton size classes to biogeochemical fluxes.

5. Conclusions

Despite variability in the contribution of Syn to biomass and primary production, several robust patterns emerged from four water parcels studied in the CRD. While Syn contributed greatly to total primary production (25% of PP, varying from 9% to 50%), its direct contribution to export flux was negligible. PE measurements suggested that unassimilated Syn did not exceed 1.25% of total POC export into sediment traps at the base of the euphotic zone, and based on a comparison to FCM-derived Syn flux estimates (an order of magnitude lower) and high PE concentrations in mesozooplankton guts, we attribute most of this direct flux to transport of undigested Syn in mesozooplankton fecal pellets. In contrast to the direct flux of unassimilated Syn carbon, the export supported indirectly by Syn may be considerably higher. Protozoa consumed most, close to all, of Syn production. Depending on assumptions about the gross growth efficiency of protozoa and the mean number of protozoan trophic steps, the trophic pathway from Syn through protozoa to mesozooplankton fecal pellets contributed 0.5–6.6% of total POC flux. Given the order-of-magnitude uncertainty in this multivorous transport pathway, determining the efficiency of the protozoan link is thus critical for assessing the total contribution of Syn to export. Despite this uncertainty, comparison of the PE and Chl a budgets clearly shows that most export in the CRD is associated with herbivorous mesozooplankton fecal pellets.

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References


