The counterintuitive effect of summer-to-fall mixed layer deepening on eukaryotic new production in the Sargasso Sea

Sarah E. Fawcett, Michael W. Lomas, Bess B. Ward, and Daniel M. Sigman

1. Introduction

In much of the modern ocean, phytoplankton growth is limited by the availability of “fixed” nitrogen (N) [Dugdale and Goering, 1967; McCarthy and Carpenter, 1983; Ryther and Dunstan, 1971]. “New production” is the fraction of total phytoplankton growth in the sunlit surface ocean (the “euphotic zone”) that is fueled by externally supplied nutrients (“new” N; nitrate mixed up from depth, augmented by in situ N₂ fixation), whereas “regenerated production” refers to phytoplankton growth supported by “recycled” N (ammonium, simple organic N forms, and nitrite and nitrate produced in the euphotic zone) [Dugdale and Goering, 1967; Bronk et al., 1994]. On an annual basis, new production is balanced by the export of sinking organic matter from surface waters (“export production”), which maintains the sequestration of carbon in the ocean interior (i.e., the “biological pump”).

The Bermuda Atlantic Time-series Study (BATS) site is located at the northwestern margin of the North Atlantic subtropical gyre (the “Sargasso Sea”), an oligotrophic region characterized by high reliance on recycled N to fuel phytoplankton growth [Menzel and Ryther, 1960; Steinberg et al., 2001]. The hydrography and biogeochemistry of the upper water column at BATS are driven primarily by seasonal changes in heat flux and wind stress: in the winter and spring, cool temperatures and high winds drive a short period of deep vertical mixing (usually down to 200–250 m) that entrains nutrients (including subsurface nitrate) into the euphotic zone (upper ~100 m), where these nutrients support a short period of high primary productivity (the “spring bloom”). By contrast, the summer is characterized by strong thermal stratification of the upper water column; the mixed layer shoals (to <20 m at times), and there is strengthening of the seasonal pycnocline that defines the base of the mixed layer, which is presumed to impede the upward mixing of subsurface nitrate, such that the summer is considered the most oligotrophic period at BATS [Menzel and Ryther, 1960; Lohrenz et al., 1992; Michaels et al., 1994; Steinberg et al., 2001]. Surface nitrate concentrations are typically below detection at this time [Lipschultz, 2001], and recycled forms of N are thought to support most phytoplankton growth [Menzel and Ryther, 1960; Lipschultz et al., 2002]. As the surface ocean cools into the fall,
the mixed layer begins to deepen. The resultant weaker surface-to-deep density stratification should, according to the view outlined above, correspond with an increased supply of nutrients to phytoplankton. Relative to the spring bloom and its subsequent transition to summertime conditions at BATS, the biogeochemical variability of the upper ocean between the stratified summer period (July and August) and deep mixing in the spring (February through April) has received less attention. Lipschultz (2001) considers June to November the “oligotrophic period” but does not differentiate between the summer and fall. Yet despite the absence of measureable euphotic zone nutrients during this oligotrophic period, occasional incidents of high productivity have been observed [Steinberg et al., 2001]. In addition, geochemically derived estimates of new production at BATS imply that less than half of the Sargasso Sea’s annual nutrient requirement is met by deep-water entrainment during winter and spring mixing, such that alternate mechanisms of new N supply during the remainder of the year (i.e., the summer and fall) are required to support the estimated global rate of new production [Jenkins and Goldman, 1985; Spitzer and Jenkins, 1989; Sarmiento et al., 1993]. Furthermore, measurements of the annual cycle of dissolved inorganic carbon (DIC) at BATS reveal a biologically facilitated summertime drawdown of DIC in the mixed layer that occurs in the absence of measurable nitrate and phosphate [Michaels et al., 1994; Bates et al., 1996; Gruber et al., 1998]. This DIC drawdown exacerbates the need for additional sources of new N to the euphotic zone, and the mixed layer in particular, during the stratified period. Mesoscale features such as eddies have been recognized as important for transporting subsurface nitrate into the euphotic zone and thus driving export production [e.g., Falkowski et al., 1991; Jenkins and Wallace, 1992; McGillicuddy et al., 1998]; however, this nutrient supply would import a near-stoichiometric quantity of regenerated DIC into surface waters, such that it cannot explain the summertime DIC drawdown. N2 fixation and atmospheric N are plausible N sources for both the geochemically inferred summer-to-fall export production and the summertime mixed layer DIC drawdown at BATS because they represent sources of new N that are not stoichiometrically tied to carbon [Michaels et al., 1994; Gruber et al., 1998]. However, fluxes from both sources appear too small [Altabet, 1988; Knapp et al., 2005, 2008; Knap et al., 1986; Michaels et al., 1993]. Biological transport has also been suggested as a mechanism of nitrate supply [Villareal et al., 1999; Katiya and Dabiri, 2009; Johnson et al., 2010]; however, this process operating alone would appear to require small phytoplankton to migrate 75–100 m to transport nitrate from below the euphotic zone into the upper ~20 m of the water column. We recently reported δ15N measurements of the biomass of the prokaryotic cyanobacterial genera, Prochlorococcus and Synechococcus, and of small eukaryotic phytoplankton (<35 μm), which had been sorted by flow cytometry from bulk particulate N (PN) collected from the euphotic zone at BATS during the summer (δ15N, in permil versus atmospheric N2, = ([15N/14N]sample/[15N/14N]atm) – 1) × 1000. We found that the prokaryotes and eukaryotes had distinct N isotope signatures, indicating that Prochlorococcus and Synechococcus rely on recycled N, while small eukaryotic phytoplankton are uniquely responsible for consuming the nitrate supplied from below and thus dominate new production at BATS during the summer [Fawcett et al., 2011]. These eukaryotes appear to rely on subsurface nitrate for >50% of their N, despite the extremely low euphotic zone nitrate concentrations typical of the summer [Lipschultz, 2001]. This work provided direct evidence of nitrate assimilation throughout the euphotic zone (including the surface mixed layer) during the period at BATS generally considered most oligotrophic. However, the mechanism remains unknown by which nitrate is supplied to the euphotic zone and its shallow mixed layer in the face of what is considered intense upper ocean stratification. One important implication of the higher new production estimates derived from geochemical tracers at BATS [Jenkins, 1988, 1998; Spitzer and Jenkins, 1989] is that the contribution of the subtropics to organic carbon export appears significantly higher than previously assumed, rivaling that of the temperate and polar open ocean, including sites that are not limited by the major nutrients, nitrate and phosphate (Table 1). Furthermore, a comparison of these export flux estimates with 14C-derived measurements of primary productivity at BATS (12.7 mol C m–2 yr–1 [Michaels and Knap, 1996]) and Station P (17.9 mol C m2 yr–1 [Varela and Harrison, 1999]) suggest that the fraction of total primary production that is exported from surface waters (i.e., the “f-ratio” [Eppley and Peterson, 1979]) in the subtropics (0.28) is higher than that of the high-latitude North Pacific (0.11) and similar to that estimated for the mixed layer of the Pacific Subantarctic (0.27 [Reuer et al., 2007]). These comparisons are problematic for the view of the subtropical gyres as zones of oligotrophy induced by stratification-driven limitation of the supply of major nutrients, and they raise the question of how the nutrient supply can sustain such high rates of export production (i.e., net community production) in the strongly stratified tropics and subtropics.
In this study, we again apply our flow cytometry/N isotope analysis approach to investigate the extent and sensitivities of nitrate-supported production throughout the Sargasso Sea’s oligotrophic period. We collected particles throughout the euphotic zone at BATS in July, October, and December; separated the major phytoplankton groups using flow cytometry; and analyzed the $\delta^{15}$N of their biomass N. Samples were also collected from three stations along a transect into the southern, tropical sector of the gyre, which has been described as permanently stratified and oligotrophic throughout the year [Michaels and Knap, 1996; Lipschultz et al., 2002]. By comparing changes in phytoplankton biomass $\delta^{15}$N with seasonal and shorter-term changes in the hydrography and biogeochemistry of the Sargasso Sea, we can begin to characterize the mechanism(s) of nitrate supply to the euphotic zone and mixed layer. Furthermore, separating the different autotrophic populations from each other and from the bulk PN allows us to determine the relative importance of different phytoplankton groups for driving new and export production in the subtropical ocean.

### Table 1. Geochemically Derived Estimates of Export Production in Different Regions of the Ocean

<table>
<thead>
<tr>
<th>Ocean Region</th>
<th>Method Source</th>
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<tbody>
<tr>
<td>Subtropical North Atlantic (BATS)</td>
<td>Sediment traps [Steinberg et al., 2001; Lomas et al., 2013]</td>
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<tr>
<td>Subtropical North Atlantic (BATS)</td>
<td>$O_2/Ar$ [Spitzer and Jenkins, 1989]</td>
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<tr>
<td>Subtropical North Pacific (HOT)</td>
<td>$O_2/Ar$ [Emerson et al., 1997]</td>
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<td>$O_2/Ar$ [Hamme and Emerson, 2006]</td>
</tr>
<tr>
<td>Subarctic North Pacific (Station P)</td>
<td>$O_2/Ar$ [Emerson et al., 1991]</td>
</tr>
<tr>
<td>Western Equatorial Pacific</td>
<td>$O_2/Ar$ [Stanley et al., 2010]</td>
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<tr>
<td>Subarctic Zone (South of Australia)</td>
<td>N isotope model [D’Fiore et al., 2006]</td>
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<tr>
<td>Subarctic Zone (South of Australia)</td>
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### Table 2. Sampling Locations and Months Sampled

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<tr>
<th>Station</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Month Sampled</th>
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<tbody>
<tr>
<td>BATS</td>
<td>31°40’N</td>
<td>64°10’W</td>
<td>July, October, December 2009</td>
</tr>
<tr>
<td>PITSa</td>
<td>31°35’N</td>
<td>64°10’W</td>
<td>July 2009</td>
</tr>
<tr>
<td>Hydro Station Sa</td>
<td>32°10’N</td>
<td>64°34’W</td>
<td>July 2008</td>
</tr>
<tr>
<td>27°N</td>
<td>27°28.90’N</td>
<td>63°47.33’W</td>
<td>October 2009</td>
</tr>
<tr>
<td>23°N</td>
<td>23°40’N</td>
<td>65°22’W</td>
<td>October 2009</td>
</tr>
<tr>
<td>SS #12</td>
<td>31°12.40’N</td>
<td>63°57.67’W</td>
<td>December 2009</td>
</tr>
</tbody>
</table>

aStations sampled previously [Fawcett et al., 2011].
2. Materials and Methods

2.1. Field Collections

Samples were collected aboard the R/V *Atlantic Explorer* on BATS cruises B248, BV44 (validation cruise), and B253 in July, October, and December 2009. Samples were collected from the BATS site on all cruises, as well as from spatial station 12 (hereafter “SS #12”) in December. In October, we sampled two stations along the World Ocean Circulation Experiment A22 transect line (hereafter “27°N” and “23°N”, respectively) as well as the BATS site (Table 2 and Figures 1a and 1b).

For all stations, mixed layer depth (MLD) was determined from depth profiles of sigma-theta ($\sigma_\theta$) calculated from temperature and salinity measured in situ during the conductivity-temperature-depth (CTD) casts. MLD was defined as the depth of maximum water column stability ($E$), approximated by the stability equation for the upper 1000 m, is $-1/\alpha_\theta \times (\partial \alpha_\theta / \partial z)$ [Sverdrup et al., 1942]. The deep chlorophyll maximum was taken to be the depth of maximum fluorescence measured in situ during the CTD casts.

On ship, seawater was collected for analysis of suspended PN, nitrate ($NO_3^-$), and ammonium ($NH_4^+$) at various depths throughout the euphotic zone, selected based on CTD hydrographic and fluorescence data from previous casts (see Figures 2a and 2b for sample depths). Four 12 L Niskin bottles were tripped at each PN sample depth in order to collect sufficient material for N isotope analysis. In addition, Niskin bottles were tripped at regular intervals from the euphotic zone down to 1200 m to collect samples for later measurement of nitrate concentration ($[NO_3^-]$) and $NO_3^- \delta^{15}N$.

For PN collections, seawater was transferred into 4 L Thermo Scientific Nalgene low-density polyethylene carboys with 1/4 inch tygon tubing that was attached to 47 mm inline polycarbonate filter holders containing 0.4 $\mu$m polycarbonate filters. Seawater (4 L) was passed through each filter under gentle vacuum (<100 mm Hg). Filters were removed from the holders and transferred to 5 mL cryovials along with approximately 4 mL of 0.2 $\mu$m filtered seawater and 200 $\mu$L of 10% formaldehyde solution (PFA; ~0.5% final concentration). Cryovials were agitated to dislodge cells from the filters and encourage "fixation" by the PFA. Samples were held at 4°C for 1–4 h and then frozen at –80°C. Samples collected for bulk PN isotope analysis were treated in the same manner as PN samples intended for flow cytometry.

2.2. Laboratory Methods

2.2.1. Flow Cytometric Analysis

Cryovials were thawed at room temperature in the dark and agitated to remove fixed cells from the filters. Cells were then filtered through a 35 $\mu$m nylon mesh in order to remove the occasional large particle and/or any chains of cells that could clog the sorting tip. A high-speed jet-in-air Influx Cell Sorter (Cytopeia, Inc., Seattle, WA) was used for all sorts. Samples were sorted for the prokaryotic cyanobacteria, *Prochlorococcus* and *Synechococcus*, and for eukaryotic phytoplankton according to the sorting criteria and conditions.
Outlined in Fawcett et al. [2011], adapted from Casey et al. [2009]. Briefly, autotrophs were identified unstained by red (650 nm long pass) autofluorescence. Synechococcus was discriminated from Prochlorococcus by FSC-H and yellow/orange (580/30 nm) fluorescence. Autotrophs not included in the cyanobacterial gates were assumed to be eukaryotes. In some cases, Prochlorococcus and Synechococcus were grouped together and sorted as a single population ("total cyanobacteria"). Software abort rates were <1%, regular analysis of sorted populations demonstrated that sort purity was >95%, and mean recovery was 98.1 ± 1.1%.

2.2.2. Persulfate Oxidation of Organic N to NO₃⁻/CO₃²⁻
Sorted populations were gently vacuum filtered onto rinsed 25 mm, 0.2 μm polycarbonate filters and washed copiously with ultrahigh-purity deionized water (DIW). Both sorted and bulk PN sample filters were placed in combusted 4 mL Wheaton vials, and 1–2 mL of DIW was added. Vials were gently vortexed and then sonicated for 60 min to remove cells from filters and suspend them in the DIW.

Figure 2. Depth profiles of (a) nitrate concentration (open symbols) and (b) corresponding nitrate δ¹⁵N (shaded symbols), (c) upper water column density (sigma-theta, in kg/m³, calculated from temperature and salinity (conductivity) data from CTD casts at the time of particle sampling [http://bats.bios.edu]), with the MLD indicated on each profile by a short horizontal line, and shallow (0–200 m) (d) nitrate and (e) ammonium concentrations for all stations sampled in July (squares), October (circles), and December (triangles). Note that in Figure 2d, the 0–0.5 μM nitrate concentration range has been expanded (left of the dashed grey vertical line). For all profiles, the depth of the euphotic zone was ~100 m. For nitrate, error bars indicate ± 1 standard error of all measurements, including samples from duplicate Niskin bottles, duplicate samples from the same Niskin bottle, and replicate analyses of the same samples. For ammonium, error bars indicate ± 1 standard deviation of at least two samples from each depth, collected, stored, and analyzed independently. Data from PITS in July 2009 ("July PITS") and Hydro Station S in July 2008 ("July Hydro S") are from Fawcett et al. [2011].
Resuspended cells were oxidized to $\text{NO}_3^-$ using the persulfate oxidation method of Knapp et al. (2005). Briefly, 1 mL of potassium persulfate oxidizing reagent (POR) was added to sorted and bulk samples and triplicate vials containing two L-glutamic acid standards, U.S. Geological Survey (USGS)-40 and USGS-41 [Qi et al., 2003]. The POR was made by dissolving 1–2.5 g of sodium hydroxide and 1–2.5 g of four times recrystallized potassium persulfate [Knapp et al., 2005; after Koroleff, 1983] in 100 mL of DIW. Sample vials were capped tightly after POR addition, and autoclaved at 121°C for 55 min on a slow-vent setting.

After oxidation, sample pH was lowered to 5–8 using 12 N HCl in order to prevent the high pH of the POR killing the denitrifying bacteria (see below), after which the concentration and $\delta ^{15}$N of the resultant $\text{NO}_3^-$ were measured via chemiluminescent analysis [Braman and Hendrix, 1989] and the “denitrifier method” [Sigman et al., 2001; Casciotti et al., 2002]. Final N content and $\delta ^{15}$N of the oxidized samples were corrected for the POR-associated N blank, and N content was converted to N concentration ([N]) using the volume filtered for each sample, corrected for volume lost during flow cytometry. For PN $\delta ^{15}$N, error is reported for replicate samples, collected, sorted, and analyzed independently, rather than for replicate measurements of the same sample, in order to characterize the precision of the full protocol.

### 2.2.3. Water Column Nitrate Concentration and $\delta ^{15}$N

[NO$_3^-$] was determined by injecting 100–1000 μL of sample into a 95°C acidic solution of vanadium (V(III)) to reduce $\text{NO}_3^-$ to nitric oxide, followed by nitric oxide analysis via a chemiluminescent detector (Teledyne model #200 EU) [Braman and Hendrix; 1989; Garside; 1982], with a detection limit of ~0.02 μM.

The $\delta ^{15}$N of $\text{NO}_3^-$ was measured using the denitrifier method [Sigman et al., 2001], which involves quantitative bacterial conversion of nitrate to nitrous oxide for samples with $[\text{NO}_3^-] \geq 0.3$ μM. The isotopic composition of the nitrous oxide was measured by gas chromatography-isotope ratio mass spectrometry using a modified ThermoFinnigan GasBench II and DeltaPlus [Casciotti et al., 2002] or a Thermo MAT 253. NO$_3^-$ $\delta ^{15}$N measurements are reported as average values ± 1 standard error, as multiple samples were taken from each depth, and each sample was analyzed at least three times.

Samples with $[\text{NO}_3^-]<0.3$ μM were concentrated 10× via a parallel vortex evaporation technique (BUCHI Laborteknik) prior to N isotope analysis. Sample (20 mL) was aliquotted into combusted borosilicate centrifuge tubes that were positioned in a BUCHI Multivapor and rotated under increasing vacuum in a 70°C water bath until all seawater had evaporated. Two milliliters of DIW was then added, and the solution was centrifuged to separate precipitated salts from the liquid containing the dissolved $\text{NO}_3^-$. This liquid was transferred into combusted 4 mL Wheaton vials, with care being taken to leave the salt behind, and $[\text{NO}_3^-]$ was measured as described above to ensure >90% yield (yield was generally >95%). NO$_3^-$ $\delta ^{15}$N was then analyzed as described above. Low $[\text{NO}_3^-]$ standards and a few samples (with $[\text{NO}_3^-]$ of 0.35–1.42 μM) that had been analyzed previously without being concentrated were also run to control for any isotopic change associated with the evaporation technique; this was found to be minor (supporting information, Table S1).

### 2.2.4. Water Column Ammonium Concentrations

The ammonium concentration ([NH$_4^+$]) of surface samples (0–200 m) that had been stored at −20°C was measured according to the method of Holmes et al. (1999). From each depth, at least two separately collected samples were analyzed. Standards were made in NH$_4^+$-free seawater collected from 1000 m in the Sargasso Sea. After addition of working reagent (orthophthalaldialdehyde, sodium sulfite, and sodium borate), standards and samples were left in the dark at room temperature for 3 h before fluorescence was measured using a Trilogy fluorometer (Turner Designs model #7200-000, with colored dissolved organic matter/NH$_4$ filter model #7200-041). NH$_4^+$ standards were measured before and after the samples to account for any change in fluorescence, which was insignificant. [NH$_4^+$] was also measured for samples collected from Hydro Station S in July 2008 and Particle Interceptor Traps Site (PITS) in July 2009, for which nitrate and PN concentration and $\delta ^{15}$N data have already been published [Fawcett et al., 2011].

### 3. Results

#### 3.1. Upper Water Column Hydrography

In July, the mixed layer depth (MLD) at BATS was 27 m (Figure 2c). In October, MLD was 88 m at BATS, shoaling to 80 m at 27°N, and 45 m at 23°N. In December at BATS, MLD was 98 m, whereas at SS #12, a well-defined, low-density mixed layer (0–35 m) was superimposed on an ~100 m thick layer of almost constant density.
Figure 3
3.2. Nitrate and Ammonium Concentrations and $\delta^{15}$N

At all stations, $[\text{NO}_3^-]$ was low throughout the euphotic zone, although there was some variability with season (Figures 2a and 2d). At BATS in July and October, low-level nitrate (0.01–0.50 μM) was detectable in the euphotic zone below the mixed layer. By contrast, $[\text{NO}_3^-]$ was below detection throughout the euphotic zone at 27°N and 23°N in October, and at both stations in December.

For all seasons, the vertical structure of $\text{NO}_3^-\delta^{15}$N below 200 m was similar to previous studies from this region (Figure 2b) [Knapp et al., 2005, 2008; Fawcett et al., 2011]. The average $\text{NO}_3^-\delta^{15}$N below 1000 m was $\sim$5.2‰, and it decreased to a minimum of $\sim$2 to 2.6‰ in the thermocline (at 150–250 m). This bolus of low-$\delta^{15}$N $\text{NO}_3^-$ is thought to derive primarily from the oxidation of newly fixed low-$\delta^{15}$N PN throughout the tropical and subtropical Atlantic [Knapp et al., 2008]. $\text{NO}_3^-\delta^{15}$N increased into the euphotic zone as a result of isotope fractionation during nitrate assimilation by phytoplankton [Sigman et al., 1999], reaching values as high as 4.9‰ in July and 8.1‰ in October at BATS. Due to a lack of samples between 150 m and the base of the euphotic zone, we did not capture the full extent of the vertical $\text{NO}_3^-\delta^{15}$N profile for this month.

$[\text{NH}_4^+]$ was generally extremely low throughout the upper 200 m (<20 nM; Figure 2e), and there appeared to be no trend with depth or season, although the lowest concentrations were consistently found near the surface. The error bars in Figure 2e indicate one standard deviation from the mean $[\text{NH}_4^+]$ of two samples from the same depth, collected, stored, and analyzed separately. The relatively small magnitude of these error bars indicates that the considerable variability in $[\text{NH}_4^+]$ measurements with depth is representative of ammonium in the water column at the time of sampling and is not the result of analytical error or storage artifact (see also supporting information, Text S1, section S1).

3.3. Bulk and Sorted Autotrophic PN

3.3.1. Bulk Suspended PN

The concentration ([N]) of bulk suspended PN in the euphotic zone varied little with depth, month, or station (ranging from 0.2–0.3 μM; Figures S2a–S2f). The $\delta^{15}$N of bulk PN was generally low throughout the euphotic zone (seasonal average $\delta^{15}$N of $\sim$0.7 ± 0.8‰; Figure 3a–3f), as observed previously in the Sargasso Sea and elsewhere [Altabret, 1988; Casciotti et al., 2008; Fawcett et al., 2011; Montoya et al., 2002]. However, at BATS and 27°N in October, as well as at BATS in December, bulk PN $\delta^{15}$N was higher than the $\delta^{15}$N of all autotrophic populations (Figures 3b, 3c, and 3e). This $\delta^{15}$N difference results from isotope fractionation during remineralization, and will be interpreted in detail elsewhere [Fawcett, 2012; Fawcett et al., manuscript in preparation].

3.3.2. Sorted Autotrophic PN

Regardless of month or station location, the sorted fractions of Prochlorococcus, Synechococcus, and eukaryotic phytoplankton each comprised roughly a third of the euphotic zone autotrophic biomass N (Figures S2a–S2f), which is consistent with previous observations from BATS [DuRand et al., 2001; Fawcett et al., 2011].

In July at BATS, the $\delta^{15}$N of Prochlorococcus and Synechococcus was uniformly low, whereas the eukaryotes were always higher in $\delta^{15}$N (by $\sim$2‰) than both the prokaryotes and bulk PN (Figure 3a). An offset of this magnitude is consistent with previous summertime observations [Fawcett et al., 2011], although the $\delta^{15}$N of all particulate pools was generally lower than in previously reported July profiles, suggesting that recycled N had a lower $\delta^{15}$N at this site. At this station, our shallowest sample (30 m) was collected within the seasonal pycnocline, and the 2‰ offset between prokaryotes and eukaryotes is evident at this depth as well.

In October at BATS and 27°N, the $\delta^{15}$N of all phytoplankton was low and remarkably similar (Figures 3b and 3c). This was also true for Prochlorococcus and Synechococcus throughout the euphotic zone at 23°N (Figure 3d). At this station, eukaryotes at 30 m were also low in $\delta^{15}$N, whereas eukaryotes below the mixed layer had a high $\delta^{15}$N (2 to 3.9‰).

Figure 3. $\delta^{15}$N of bulk and flow cytometrically sorted components of the PN from the Sargasso Sea in (a) July at BATS, (b) October at BATS, (c) October at 27°N, (d) October at 23°N, (e) December at BATS, and (f) December at SS #12. For (N) of sorted components, see Figures S2a–S2f. The mixed layer depth at the time of sampling is indicated on the plots (MLD; dashed grey line). “Total cyanos” (purple triangles) represents a combined population of Prochlorococcus plus Synechococcus, sorted and analyzed independently of the individual genera. Note the different $\delta^{15}$N scale for Figure 3d (October 23°N) as compared to Figures 3b (BATS) and 3c (27°N). For all panels, error bars indicate the full range of values measured for replicate samples, commonly duplicates, collected, sorted, and analyzed independently.
In December, the δ15N of Prochlorococcus, Synechococcus, and the eukaryotes at BATS was similar and low (Figure 3e). At SS #12, the average δ15N of Prochlorococcus and Synechococcus was also low, whereas eukaryotic δ15N was once again high and increased toward the surface. At this station, we observed the highest eukaryote δ15N at 30 m (3.2‰), which was within the surface mixed layer (Figure 3f).

The number of occurrences of a particular δ15N for each of the sorted autotrophic populations is summarized in Figure 4. The δ15N of Prochlorococcus and Synechococcus was uniformly low (with no value > 0‰), regardless of season or depth (Figures 4a and 4b), whereas eukaryotic phytoplankton were far more variable and often higher in δ15N (14 of 26 observations > 0‰; Figure 4c).

### 4. Discussion

#### 4.1. δ15N of Sorted Autotrophic Populations

For all stations and seasons, the average δ15N of Prochlorococcus and Synechococcus was low, indicating that these prokaryotic phytoplankton use recycled N (NH₄⁺ and/or simple organic N forms such as amino acids) as their dominant N source throughout the summer and fall. This observation is consistent with previous summertime findings [Fawcett et al., 2011], and in the case of Prochlorococcus is supported by our current...
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P = assimilated by δ by Synechococcus the period of spring mixing [Synechococcus Indeed, October and BATS in December, eukaryotes had a lower all N pools at BATS suggests that the entire ecosystem had undergone an event of high-
ments from two stations near BATS in July (average eukaryotic
The difference in
metric of the extent to which eukaryotes consume subsurface nitrate, with greater reliance on this nitrate
understanding of their N metabolism: Prochlorococcus prefers reduced N sources, and some strains do not have the genetic capability to assimilate oxidized N forms [Moore et al., 2002, 2007; Dufresne et al., 2003; Zubkov et al., 2003; Zubkov and Tarran, 2005].
Marine Synechococcus strains have been reported to utilize all common reduced and oxidized forms of fixed N [Gilbert et al., 1986; Pael, 1991; Lindell et al., 1998; Collier et al., 1999; Moore et al., 2002], although their preference in the environment is thought to be for reduced N [Gilbert and Ray, 1990; Lindell and Post, 2001], likely because its assimilation is far less energetically expensive than that of nitrate [Dortch, 1990]. Our N isotope data are consistent with this preference. For instance, in October at BATS, the low δ15N of Synechococcus indicates assimilation of only recycled N despite detectable nitrate as shallow as 60 m (although it is also possible that the nitrate was recently introduced and thus its utilization is not yet apparent in phytoplankton δ15N). We cannot rule out the possibility of nitrate assimilation by Synechococcus under conditions of high availability (e.g., after spring mixing events).
Indeed, Synechococcus generally reaches its maximum abundance at BATS from March to May, directly after the period of spring mixing [DuRand et al., 2001; Olson et al., 1990], perhaps suggesting a growth response by Synechococcus to the increased nitrate supply that accompanies mixing events. Some amount of the N assimilated by Synechococcus and Prochlorococcus may derive from N2 fixation, although only via the recycled N pool, as neither of these taxa appears to fix N2 in the ocean [Partensky et al., 1999; Moore et al., 2002].
The δ15N of euphotic phytoplankton was variable, both with season and station. In July, eukaryote δ15N was consistently higher than the δ15N of the prokaryotes. We previously reported high eukaryote δ15N measurements from two stations near BATS in July (average euparotic δ15N of 3.0‰ at Hydro Station S in July 2008 and 5.0‰ at BATS in July 2009, with observations for individual populations as high as 12.7‰). We interpreted this high eukaryote biomass δ15N to indicate consumption of subsurface nitrate by the eukaryotes [Fawcett et al., 2011]. Here, while the average euparotic δ15N at BATS (0‰) was lower than those previous summertime measurements, the δ15N of the prokaryotes was also lower. As a result, the eukaryotes were consistently higher in δ15N than the prokaryotes by ~2‰, similar to other July profiles, suggesting significant nitrate consumption by euphotoic phytoplankton at this station as well. The observation of a shift to lower δ15N by both eukaryotes and prokaryotes at BATS implies that the δ15N of recycled N was lower at BATS than at PITS and Hydro Station S. Bulk PN at BATS was also almost 2‰ lower in δ15N than bulk PN at the other summer stations. The low δ15N for all N pools at BATS suggests that the entire ecosystem had undergone an event of high-δ15N N loss, such as from an export event that preferentially removed euphotoic biomass (e.g., in zooplankton fecal pellets), resulting in a lower-than-normal δ15N for the ammonium available for assimilation.
At 23°N in October and SS #12 in December, eukaryote δ15N resembled previous July profiles, indicating assimilation of subsurface nitrate by eukaryotes at these stations as well. By contrast, at BATS and 27°N in October and BATS in December, eukaryotes had a lower δ15N than that similar to the Δδ15N of the prokaryotes.
We interpret this euphotoic convergence on the δ15N of the prokaryotes as indicating a shift toward more complete reliance on recycled N (predominantly ammonium) by euphotoic phytoplankton.
The difference in δ15N between euphotoic phytoplankton and Prochlorococcus (Δδ15N = δ15N_euk − δ15N_pro), the latter assumed to assimilate only recycled N [Fawcett et al., 2011], provides a simple metric of the extent to which eukaryotes consume subsurface nitrate, with greater reliance on this nitrate

Figure 5. Cross plot showing the difference between the concentration-weighted euphotic zone average δ15N of euphotoic phytoplankton and Prochlorococcus (Δδ15N = δ15N_euk − δ15N_pro) relative to mixed layer depth (MLD) for all stations sampled in July (squares), October (circles), and December (triangles). Data from PITS in July 2009 ("July PITS") and Hydro Station S in July 2008 ("July Hydro S") are from Fawcett et al. (2011).
yielding higher values of $\Delta \delta^{15}N_{e-P}$ (Figures 5 and S3; see supporting information, Text S1, section S2.1). The caveats in this generalization should be recognized; for example, variations in nitrate $\delta^{15}N$ in the euphotic zone could affect $\Delta \delta^{15}N_{e-P}$, particularly in the case of individual profiles. However, this effect should cancel over the long term. In any case, in the eight profiles shown here, $\Delta \delta^{15}N_{e-P}$ increases as MLD decreases.

4.2. Controls on the Nitrate Supply to the Euphotic Zone

4.2.1. The Role of Summer-to-Winter Mixed Layer Evolution

While $\Delta \delta^{15}N_{e-P}$ is reasonably well correlated with mixed layer depth (MLD; Figure 5), the sense of this correlation is surprising. The shallow mixed layers of July are typically taken as emblematic of a time of high stratification and thus reduced nutrient input from below [Steinberg et al., 2001; Lipschultz, 2001]. Indeed, among the stations located at a similar latitude, the shallowest mixed layers are generally associated with the strongest surface-to-deep density difference (Figure 2c) [see also Steinberg et al., 2001]. Yet $\Delta \delta^{15}N_{e-P}$ is observed to be highest during these times when mixed layers are shallowest (i.e., July), implying the greatest reliance of eukaryotes on nitrate under such conditions. Given that it is less energetically expensive to assimilate reduced N forms [Cochlan and Harrison, 1991; Dortch, 1990; Harrison et al., 1996], one might suspect a connection between $\Delta \delta^{15}N_{e-P}$ and ammonium availability, which in turn might be related to mixed layer depth. However, our data show no compelling correlation between $\Delta \delta^{15}N_{e-P}$ and water column ammonium concentration (see supporting information, Text S1, section S2.2).

These observations have led us to reconsider the relationship between seasonal stratification changes and the barriers to nitrate supply to the euphotic zone from the underlying ocean (Figure 6). During early and
middle summer, largely due to high heat fluxes and low wind stresses [Steinberg et al., 2001], a very shallow, low-density “mixed layer” develops at the surface of the euphotic zone. July is typically considered a time of high water column stability brought on by the intense thermal stratification that is characterized by such a shoaling of the mixed layer. However, the depth of this mixed layer is generally ~20 m, with the pycnocline extending no deeper than 40 m (Figure 6a), so it does not contribute to stratification at the base of the euphotic zone. There is thus relatively little buoyancy impediment to low levels of nitrate being supplied from the subsurface, across the base of the euphotic zone and into the photosynthetically active lower euphotic zone.

The reduced buoyancy impediment to subsurface nitrate supply to the base of the summer euphotic zone can be thought of as a decoupling of the pycnocline from the nutricline. The depth of the pycnocline, which varies considerably during the summer-to-fall transition at BATS, from 20–40 m in July to 90–120 m in December, determines the shallowest depth to which subsurface water can be mixed up with relatively little energy input. By contrast, the depth of the nutricline remains much more constant throughout the oligotrophic period (~80–100 m), largely because of nitrate uptake by phytoplankton throughout the euphotic zone. The substantial vertical decoupling between the pycnocline and nutricline in July (i.e., when mixed layers are shallowest; Figure 6a) increases the potential for the upward flux of subsurface nitrate and thus perhaps speaks to the observed nitrate assimilation by eukaryotes in the July euphotic zone. Conversely, the increased vertical coupling of the pycnocline to the nutricline into the fall and early winter (Figure 6b) may underlie the apparent decrease in euphotic zone nitrate supply at these times of year. This effect reaches its maximum when the pycnocline depth overlaps with the base of the euphotic zone, as in December at BATS. By the early spring, the base of the mixed layer deepens below the base of the euphotic zone, mixing nutrients into the euphotic zone in the process [Steinberg et al., 2001].

However, while the seasonal change in pycnocline/nutricline coupling may be important to the summer-to-fall change in nitrate supply, it cannot in itself explain the nitrate supply. The lack of density gradient across the base of the summer euphotic zone translates to a minimal energetic impediment to vertical water exchange, but a mechanism is still required to drive exchange across this boundary. In this regard, the data remain mysterious. Biological nitrate transport may be responsible [Johnson et al., 2010]; however, there is no evidence that the small eukaryotes that are consuming nitrate in the summer are capable of this process. Vertical phytoplankton migration or biologically induced turbulent mixing appear more plausible as mechanisms of nitrate import into the mixed layer, across the sharp (and thus thin) summertime pycnocline [Villareal et al., 1999; Katija and Dabiri 2009; Johnson et al., 2010]. It is also possible that weather events or submesoscale processes (e.g., “frontogenesis” [Calil and Richards, 2010; Thomas et al., 2008]) drive such transport. An apparent example from SS#12 in December, which was sampled specifically because of its location adjacent to an eddy, is described in section 4.2.3. However, it is unclear why such events and processes would lead to nitrate supply to the euphotic zone during each of our summer samplings but few of our fall/winter samplings (Figure 4c), absent the seasonal change in mixed layer depth (Figure 5). Clearly, additional samplings will test the current evidence for seasonality, helping to distinguish among the mechanisms of nitrate supply.

4.2.2. The Meridional Gradient in Mixed Layer Depth

The same counterintuitive linkage between mixed layer depth and nitrate supply appears to apply to the differences in eukaryote \( ^{15}\text{N} \) with latitude in the three October depth profiles (Figures 3b–3d). The two northern stations, BATS and 27°N, were characterized by a thick, low-density surface mixed layer, with the depth of its base similar to the depth of the euphotic zone, and the \( ^{15}\text{N} \) of all phytoplankton was low. At the southernmost station (23°N), the pycnocline at the base of the surface mixed layer was shallower, which, according to our interpretation (i.e., Figure 6a), should reduce the energetic barrier to nitrate supply across the base of the euphotic zone, consistent with the higher average \( ^{15}\text{N} \) of the eukaryotes in this profile. Remarkably, the surface-to-deep density gradient at 23°N was the strongest of all our profiles, yet this did not seem to prevent nitrate penetration into the lower euphotic zone. In addition to the effect of pycnocline-nutricline decoupling proposed above, nitrate supply to the 23°N station may have been increased by the fact that the nutricline is shallower at 23°N than it is further to the north [Palter et al., 2005; Knapp et al., 2008]. The low \( ^{15}\text{N} \) of eukaryotes and correspondingly low \( \Delta^{15}\text{N}_{\text{e,p}} \) in the mixed layer at this station suggests that the strong pycnocline at the base of the mixed layer isolated it from the deeper euphotic zone, preventing any nitrate supply into the mixed layer. However, despite the “permanently oligotrophic” nature of this region [Lipschultz et al., 2002], the high eukaryotic \( ^{15}\text{N} \) at 23°N indicates that nitrate was by some mechanism
available to support new production in the sub–mixed layer euphotic zone. Interestingly, the associated productivity would be largely undetected by satellite ocean color observations.

4.2.3. Mesoscale Drivers of Nitrate Supply
Mesoscale eddies are ubiquitous features in the subtropical ocean and are important for surface ocean ecology and biogeochemistry because they affect vertical nutrient transport and light availability [Robinson, 1983; Falkowski et al., 1991; McGillicuddy et al., 1998; Siegel et al., 2011]. “Submesoscale pumping” is one mechanism by which eddies influence primary production, causing elevated chlorophyll concentrations in the high velocity regions surrounding the eddy [Calli and Richards, 2010; Siegel et al., 2011]. It has been hypothesized that such increases in chlorophyll result from nutrient injections at the outer frontal regions of eddies due to instabilities induced by high velocity currents [Calli and Richards, 2010].

Within our data set, there is tentative evidence of submesoscale pumping driving seasonally anomalous eukaryote δ15N through an increase in the nitrate supply to the euphotic zone. The unusually shallow, low-density surface layer that we observed at SS #12 in December (Figure 2c) likely resulted from the passage of an anticyclonic eddy to the southwest of BATS (Figure S5a; see also supporting information, Text S1, section S2.3) that generated a strong, southeastward flowing jet directly over SS #12 (Figure S5b). Indeed, SS #12 was specifically sampled because of its location adjacent to this eddy. The high eukaryote δ15N at SS #12, as well as an increase in 60–100 m fluorescence at this station (Figure S5c), are likely a response to nitrate supplied by submesoscale pumping at the front between the eddies, which is apparent in the acoustic Doppler current profiler velocity on 9 and 10 December (Figure S5b).

The similarity between average eukaryote δ15N (2.2‰) and the δ15N of subsurface nitrate (2.2‰) at SS #12 in December is consistent with an episodic nitrate supply event to the euphotic zone that has been rapidly, and completely, drawn down by eukaryotic phytoplankton. The observed pattern of increasing eukaryote δ15N from the base of the euphotic zone up toward the base of the mixed layer can be explained by isotope discrimination during nitrate assimilation as the consumption of newly supplied nitrate progresses upward [Sigman et al., 1999]. It should be noted that the eddy interaction driving the nitrate flux coincided with a shoaling of the mixed layer, leading to consistency of the December interstation δ15N differences with the broader mixed layer/nitrate assimilation correlation described above (see Figure 6a). This may not be coincidental: the eddy-induced input of dense subsurface water that supplied nitrate to the euphotic zone can also lead to a shoaling of the base of the mixed layer [McGillicuddy et al., 1998]. Eukaryotes collected from within the mixed layer had the highest δ15N of all depths sampled at this station, suggesting that the strong pycnocline did not prevent the upward transport of nitrate, via physical or biological means, into the mixed layer.

5. Conclusions and Implications

The data reported in this study support and extend the findings of our earlier work [Fawcett et al., 2011], confirming that eukaryotic phytoplankton in the Sargasso Sea specialize in the assimilation of nitrate supplied from depth, thereby dominating new production in this region. Our seasonal N isotope data imply that if nitrate is available, it will be consumed exclusively by eukaryotic phytoplankton. While this does not rule out the possibility of nitrate assimilation by the prokaryotes, which may occur during times of prolonged nitrate availability (e.g., following spring mixing), in the summer and fall, Prochlorococcus and Synechococcus rely predominantly on N recycled within the euphotic zone, thus contributing little to new production.

Given the discrepancy between direct measurements [Steinberg et al., 2001] and geochemically derived estimates [e.g., Jenkins and Goldman, 1985] of new and export production in the Sargasso Sea, evidence for the direct supply of nitrate to the euphotic zone in the face of strong upper ocean stratification has long been sought [Jenkins, 1988; Michaels et al., 1994; Bates et al., 1996; Lipschultz et al., 2002], as has the mechanism for this supply [Villareal et al., 1999; Katija and Dabiri, 2009; Johnson et al., 2010]. Our summertime data provided direct evidence of nitrate assimilation in both the euphotic zone and mixed layer at BATS [Fawcett et al., 2011], indicating the existence of nitrate supply mechanisms other than the deep mixing events characteristic of winter and spring. With the extension of our approach to different seasons and latitudes, we observe that nitrate assimilation by eukaryotes coincides with very shallow mixed layers, a condition typically taken as indicative of strong upper ocean stratification. However, the shallow July mixed layers at BATS are also associated with a weaker density contrast between the sub–mixed layer euphotic zone and the deeper...
waters below. That is, the sharp and shallow pycnocline that results from strong surface warming in July actually protects the euphotic zone below it from the sources of buoyancy at the surface, reducing the energetic (i.e., buoyancy) barrier to the so far unknown mechanism that is responsible for the import of subsurface nitrate into the sub–mixed layer euphotic zone. While the mechanism remains mysterious, the observed nitrate transport into the euphotic zone may help to explain the surprisingly high estimates of export production in the subtropical ocean [Jenkins, 1988; Spitzer and Jenkins, 1989]. Further, the evidence for nitrate uptake in (and thus nitrate transport into) the summer mixed layer may also help to explain the substantial and mysterious summertime DIC drawdown at BATS [Michaels et al., 1994; Bates et al., 1996; Gruber et al., 1998], especially if the mechanism for nitrate transport is related to vertical migration of planktonic organisms [Villereal et al., 1999; Katija and Dabiri, 2009; Johnson et al., 2010].

The nitrate demand suggested by eukaryotic $\delta^{15}$N in the euphotic zone during the growing season can be compared with the estimates of export production that geochemical approaches require over this period. Eukaryotes constitute approximately 0.05 $\mu$M of suspended PN during the oligotrophic period, and we estimate that they rely on subsurface nitrate for $\sim$45% of their N at this time. Assigning a growth rate of 0.5 day$^{-1}$ to the eukaryotes [Goericke and Welschmeyer, 1998; Cuvelier et al., 2010], integrating over the whole euphotic zone (0–100 m), and assuming that the oligotrophic period extends from June until December, we calculate a eukaryotic nitrate demand of $\sim$0.25 mol N m$^{-2}$. This nitrate would support the removal of approximately 1.8 mol C m$^{-2}$, which is about 50% of the annual estimate derived from measurements of the upper ocean oxygen mass balance [Spitzer and Jenkins, 1989]. Geochemical estimates of new production suggest that approximately half of the Sargasso Sea’s annual N requirement (0.50 $\pm$ 0.14 mol N m$^{-2}$ yr$^{-1}$ [Jenkins, 1988; Spitzer and Jenkins, 1989; McGillicuddy et al., 1998]) is met by deep-water entrainment during spring mixing, such that $\sim$0.25 mol N m$^{-2}$ must be supplied during the oligotrophic period. Our N isotope measurements imply that all of this N goes to eukaryotic new production during the summer and fall.

We also have direct evidence for nitrate consumption in the southern, tropical sector of the Sargasso Sea, which, due to permanently strong surface-to-deep stratification, is considered oligotrophic throughout the year [Lipschutz et al., 2002]. At 23$^\circ$N in October, however, eukaryotic phytoplankton assimilated nitrate in the euphotic zone below the mixed layer, driving significant new production at this site. The relatively high contribution of the tropics and subtropics to carbon export as implied by geochemical tracers [e.g., Spitzer and Jenkins, 1989; Emerson et al., 1997] appears to be predominantly attributable to eukaryotic phytoplankton and fueled by nitrate supplied to the lower euphotic zone below the surface mixed layer. In short, a strong pycnocline at the base of the mixed layer does little to inhibit the nitrate supply to the lower euphotic zone if the mixed layer is much shallower than the depth of the euphotic zone.

The $\sim$25 $\mu$M DIC drawdown in the mixed layer at BATS over the course of the growing season (April to October) [Marchal et al., 1996; Bates et al., 1996; Gruber et al., 1998] occurs in the apparent absence of nitrate and phosphate, and apparently isolated from nutrient-rich deep water by the low-nutrient, sub–mixed layer euphotic zone [Bates et al., 1996]. In order to explain such a drawdown, any transport of nutrients into the mixed layer must occur without simultaneous transport of DIC, such that nitrate supplied to the mixed layer via high winds or storms (as suggested above) cannot account for any net removal of DIC. However, vertical transport by organisms can. At the Hawaii Ocean Time-series station in the oligotrophic North Pacific, Johnson et al. [2010] observed a nitrate deficit between 100 and 250 m that corresponds with excess DIC consumption and oxygen production in surface waters. These observations can be explained if phytoplankton take up nitrate below the euphotic zone and then transport it upward from the subsurface into the euphotic zone and mixed layer. In the North Pacific, such episodic transport events are estimated to supply sufficient nitrate to support the removal of all mixed layer DIC remaining after N$_2$ fixation, diffusive transport, and vertical entrainment are taken into account [Johnson et al., 2010].

At BATS, our data suggest that throughout much of the growing season, low levels of nitrate are regularly supplied to the sub–mixed layer euphotic zone from the higher nutrient waters below, by either physical or biological means. Under these conditions, migrating phytoplankton that grow in the mixed layer might be able to exploit whatever mechanism imports nitrate into the euphotic zone below the mixed layer, if that mechanism is not also biological. Any mixed layer carbon fixation supported by such biologically supplied nitrate would drive a net removal of DIC from the mixed layer and thus help to explain the summertime DIC drawdown.
During every month of our study, there was insufficient resident nitrate in the euphotic zone to fuel the observed extent of euphotic zone nitrate utilization, or to support mixed layer DIC removal in the hypothetical case that biological transport imports all sub-mixed layer euphotic zone nitrate (but no DIC) into the mixed layer. This reinforces the importance of continuous exchange between the sub-mixed layer euphotic zone and the underlying dark ocean, which would allow many times more nitrate to cross the base of the euphotic zone than is observed in the nitrate inventory of the oligotrophic period.

The data reported here support previous work indicating the importance of eukaryotic nitrate assimilation in the euphotic zone at BATS during seasons previously thought to be dominated by recycled production. In contrast, while the evidence for nitrate assimilation in the summertime mixed layer is robust for the times it was observed [Fawcett et al., 2011], its ubiquity requires further testing. Further measurements of the summer mixed layer will also be important for understanding the relationship between nitrate assimilation and the still-mysterious summertime DIC drawdown in the mixed layer near Bermuda.

Acknowledgments
We thank Katye Altieri, Julie Granger, and Lija Treibergs for sampling help; Sergey Oleynik and M. Alexandra Weigand for laboratory support; John Casey and Stacey Goldberg for flow cytometric analyses and expertise; and D. Gwyn Evans for ancillary data and discussion regarding the anticyclonic eddy at SS #12 in December. We are grateful to BATS, the captain and crew of the R/V Atlantic Explorer, the faculty and staff of the Bermuda Institute of Ocean Sciences, particularly Doug Bell, Steve Bell, Rod Johnson, and Debra Lomas. This paper benefited from discussions with Michael Bender and from reviews by Ken Johnson and an anonymous reviewer. This work was supported by the Charrack Foundation, the Siebel Energy Grand Challenge of Princeton University, and the US NSF through grants OCE-0752356 (BATS), OCE-1136345, and OCE-1060947.

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D. Gwyn Evans for ancillary data and discussion regarding the anticyclonic eddy at SS #12 in December. We are grateful to BATS, the captain and crew of the R/V Atlantic Explorer, and the faculty and staff of the Bermuda Institute of Ocean Sciences, particularly Doug Bell, Steve Bell, Rod Johnson, and Debra Lomas. This paper benefited from discussions with Michael Bender and from reviews by Ken Johnson and an anonymous reviewer. This work was supported by the Charrack Foundation, the Siebel Energy Grand Challenge of Princeton University, and the US NSF through grants OCE-0752356 (BATS), OCE-1136345, and OCE-1060947.

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