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DNA-based molecular fingerprinting of eukaryotic protists and cyanobacteria contributing to sinking particle flux at the Bermuda Atlantic time-series study

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ABSTRACT

We used denaturing gradient gel electrophoresis (DGGE) to examine the protist and cyanobacterial communities in the euphotic zone (0–120 m) and in corresponding 150 m particle interceptor traps at the Bermuda Atlantic Time-series Study (BATS) in a two-year monthly time-series from May 2008 to April 2010. Dinoflagellates were the most commonly detected taxa in both water column and trap samples throughout the time series. Diatom sequences were found only eight times in the water column, and only four times in trap material. Small-sized eukaryotic taxa, including the prasinophyte genera *Ostreococcus*, *Micromonas*, and *Bathycoccus*, were present in trap samples, as were the cyanobacteria *Prochlorococcus* and *Synechococcus*. *Synechococcus* was usually overrepresented in trap material, whereas *Prochlorococcus* was underrepresented compared to the water column. Both seasonal and temporal variability affected patterns of ribosomal DNA found in sediment traps. The two years of this study were quite different hydrographically, with higher storm activity and the passing of a cyclonic eddy causing unusually deep mixing in winter 2010. This was reflected in the DGGE fingerprints of the water column, which showed greater phylotype richness of eukaryotes and a lesser richness of cyanobacteria in winter of 2010 compared with the winter of 2009. Increases in eukaryotic richness could be traced to increased diversity of prasinophytes and prymnesiophytes. The decrease in cyanobacterial richness was in turn reflected in the trap composition, but the increase in eukaryotes was not, indicating a disproportionate contribution of certain taxa to sinking particle flux.

1. Introduction

Marine phytoplankton play a major role in the removal of carbon dioxide from the atmosphere and subsequent sequestration into the deep sea, a process known as the biological carbon pump (Volk and Hoffert, 1985). Rising global ocean temperatures, increased stratification, reduced nutrient input to the sea surface (Bopp et al., 2005; Falkowski and Oliver, 2007), and lowered pH (Hays et al., 2005) will have various effects on the plankton community, most likely selecting for small sized phytoplankton (Falkowski and Oliver, 2007). In several modeling studies, these changes have been projected to lead to a decrease in export flux (Bopp et al., 2001; Cox et al., 2000; Fung et al., 2005). However, in light of recent data showing the ability of small phytoplankton to sink through packaging mechanisms, this shift to smaller cells may not necessarily lead to a less efficient biological carbon pump.

The planktonic community in oligotrophic gyre regions is dominated by pico- and nanoplankton for most of the year (DuRand et al., 2001). Contrary to the traditional size-based tenet that only large taxa with heavy mineral tests dominate particle flux (Boyd and Newton, 1999; Michaels and Silver, 1988; Sarthou et al., 2005), we now have growing evidence that pico- and nanoplankton contribute to the flux out of the upper water column. In a modeling study, Richardson and Jackson (2007) hypothesized that picoplankton contribute to export production at a rate proportional to their primary production. In the western subtropical North Atlantic, Brew et al. (2009) observed a significant correlation between particle flux and picoplankton abundance and suggested that aggregation may be an important mechanism controlling export of pico- and nanoplankton. In a companion study by Lomas and Moran (2011), high performance liquid chromatography (HPLC)-based pigment analysis showed that the relative abundances of pico- and nanophytoplankton groups collected from within and below (down to 500 m) the

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euphotic zone were similar, and from this inferred that these groups were exported to depth. On a carbon-basis, their study found that nanoeukaryotes, specifically prymnesiophytes, contributed to particulate organic carbon (POC) flux in proportion to their carbon biomass, while cyanobacteria contributed one-tenth of their carbon biomass to POC flux. Similarly, in the eastern subtropical North Atlantic, Amacher et al. (2009) showed that in 18 S rRNA gene clone libraries at ESTOC (European Station for Time series in the Ocean), small plankton representation in subeuphotic zone sediment trap samples was roughly in proportion to their abundance in the water column. In addition, diatoms, while abundant in the water column were rare in corresponding trap samples. In subtropical gyre regions dominated by pico- and nanoplankton it is important to consider that small phytoplankton may play a major role in the flux of carbon out of the euphotic zone.

The Bermuda Atlantic Time-series Study (BATS), located in the western subtropical North Atlantic gyre, has been sampled on a monthly basis since 1988. This area stays stratified during most of the year (Steinberg et al., 2001). In late winter erosion of the thermocline, driven by increased wind stress and changes in heat flux, results in deep convective mixing (Cianca et al., 2007). This increases the availability of nutrients in the euphotic zone (average depth 100 m), causing a phytoplankton bloom as seen by elevated chlorophyll a (Chl-a) levels (Helmke et al., 2010). Most of what is known about the phytoplankton community in the euphotic zone at BATS comes from microscopy, flow cytometry, and HPLC data. The area is dominated by small pico- and nanoplankton during most of the year, with significant seasonal and interannual variability in phytoplankton community structure (Caron et al., 1999; DuRand et al., 2001; Steinberg et al., 2001). *Synechococcus* dominates the cyanobacterial community during the winter/spring bloom, while *Prochlorococcus* dominates during summer and fall (DuRand et al., 2001). The eukaryotic community is dominated by nanoplankton; abundant eukaryotes include prymnesiophytes and pelagophytes, with dinoflagellates and prasinophytes less abundant and diatom blooms occurring rarely, typically during winter/spring bloom periods (DuRand et al., 2001; Krause et al., 2009; Lessard and Murrell, 1996; Lomas and Bates, 2004; Steinberg et al., 2001). Particulate Organic Carbon (POC) flux at BATS has been found to be highest in winter and spring, lower in summer, and lowest in fall (Helmke et al., 2010).

Identification of taxa in particle traps proves to be difficult due to the packaging of cells into amorphous aggregates and fecal pellets and the degradation of pigmentation. Organisms with mineral tests, such as diatoms and coccolithophorids, can be identified, but this potentially overestimates the contributions of those phytoplankton taxa to flux while underestimating that of small and unarmored taxa. Analysis of 18 S rRNA sequences from community DNA (e.g., Amacher et al., 2009; Díez et al., 2001) allows us to determine which organisms contribute to the flux of carbon out of the euphotic zone; particularly useful for small and non-mineral ballasted organisms. While some phytoplankton may sink directly in aggregates or detritus, a considerable proportion will be transported in the form of fecal pellets ($45 \pm 16\%$ of POC flux, Steinberg et al., 2012). There may be some degradation of DNA in zooplankton guts. Several studies of copepod diets (Martin et al., 2006; Nejstgaard et al., 2003; Nejstgaard et al., 2008; Troedsson et al., 2009) have shown that digestion and degradation of DNA passing through zooplankton guts may only be partial. Additionally, Martin et al. (2006) found that prey species contained in fecal pellets reflected the actual diet, suggesting no differential degradation of DNA. Intact and even culturable cells have been found in aggregates and fecal pellets of salps (filter feeding planktonic tunicates) collected in particle traps and sediments (Fischer et al., 1996; Pfannkuche and Lochte, 1993; Turley and Mackie, 1995), in fecal pellets (Jansen and Bathmann, 2007; Martin et al., 2006), and in the hindguts of mesozooplankton (Wilson and Steinberg, 2010). Furthermore, because DNA is a chemically stable molecule more resistant to breakdown than other tracer compounds, such as pigments and proteins, cells need not be living or even intact in order to obtain DNA sequences that can be used for molecular analysis (Nejstgaard et al., 2008).

In this study we used denaturing gradient gel electrophoresis (DGGE) to obtain community "fingerprints"(i.e. Díez et al., 2001; Moon-van der Staay et al., 2001) in order to examine eukaryotic protist and cyanobacterial communities in the upper water column and taxa collected in 150 m particle trap material. Regular monthly sampling at the BATS station over a two year time period allowed us to observe the sinking patterns of different taxa in response to seasonality as well episodic events. Additionally, we determined the efficiency of DNA recovery in order to accurately quantify the DNA present in water column samples and to determine the flux of DNA out of the euphotic zone. These data give us a better understanding of the dynamics of plankton communities and their contribution to flux.

2. Methods

2.1. Field sampling

Sampling was conducted on a monthly basis (twice monthly during the winter bloom) from May 2008–April 2010 at BATS. Samples for DNA analysis were collected in addition to the core parameters as part of the BATS program (including temperature, salinity, nutrients, chlorophyll, primary production, flow cytometry, HPLC, and deployment of PITs, surface-tethered particle interceptor traps, http://bats.bios.edu/). In this study we will be including several of these core parameters in our analysis.

From the upper water column, samples were collected in Niskin bottles attached to a rosette with conductivity, temperature, and depth (CTD) sensors from four depths, generally at 10 m, at the deep chlorophyll maximum (DCM) and two additional depths above or below the DCM, based on the temperature and fluorescence profile (See Figs. 6 and 7). For molecular analysis, a 2 L sample was prefiltered through a 100 μm mesh (to exclude larger zooplankton) and filtered onto GF/F glass fiber filters, placed in 1 mL lysis buffer (40 mM EDTA, pH 8, 100 mM Tris–HCl, pH 8, 100 mM NaCl, 1% sodium dodecyl sulfate, and water; (Countway et al., 2005), immediately frozen in liquid nitrogen for shipping, and ultimately stored at $-80\ ^{\circ}C$ at ASU until extraction.

In addition to the standard (poisoned brine, 50 g l-1 above ambient seawater and 0.74% formalin) BATS three-day PITs deployed at 150, 200 and 300 m, a separate non-poisoned brine particle trap array was deployed for 24 h at 150 m for this project. In order to identify specific taxa contributing to particle flux, the 150 m poisoned and 24-h non-poisoned trap samples were subjected to DNA-based molecular analysis. After retrieval, particles in each PIT collection tube were drained through a 0.8 μm polycarbonate filter at the base of the tube. The filters were then frozen in liquid nitrogen, thawed briefly in the lab to pick out zooplankton swimmers using a dissecting microscope, placed in 1 mL lysis buffer, frozen again in liquid nitrogen for shipping, and then stored at $-80\ ^{\circ}C$ at ASU until extraction. Filters from two tubes of the 24 h non-poisoned trap were combined in the first step of extraction for a higher yield of DNA. POC flux was determined from the three day fixed traps as part of the BATS core program.