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# Concentrations, turnover rates and fluxes of polyamines in coastal waters of the South Atlantic Bight

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**Abstract** Polyamines are short-chain aliphatic compounds containing multiple amine groups. They are important components of the cytosol of eukaryotes and are present at mmol $L^{-1}$ concentrations inside phytoplankton cells, while complex polyamines play a role in biosilica deposition. Concentrations of polyamines measured in seawater are typically in the sub-nmol $L^{-1}$ range, implying rapid and efficient uptake by osmotrophs, likely bacterioplankton. We measured turnover rates of three polyamines (putrescine, spermidine and spermine) using $^{3}$H-labeled compounds and determined their concentrations by HPLC to estimate polyamine contributions to dissolved organic matter and bacterioplankton carbon and nitrogen demand. These measurements were made on transects from the inner shelf to the Gulf Stream across the South Atlantic Bight (SAB) during April and October of 2011 and in salt marsh estuaries on the Georgia coast during August of 2011 and April of 2012. We found that turnover rates of polyamines were similar to those of amino acids (arginine and glutamic acid) measured in the same samples; however, fluxes of polyamines into bacterioplankton were much lower than amino acid fluxes as a result of low ambient concentrations. Turnover rates and fluxes of polyamines decreased from near-shore waters to the shelf-break, following the pattern of chlorophyll *a* concentration. Polyamine uptake accounted for less than 10 % of bacterial N demand and 5 % of bacterial C demand on average, with a large variation among water masses.

**Keywords** Polyamines · Dissolved organic nitrogen · Bacterioplankton · Spermidine · Spermine · Putrescine · DFAA · South Atlantic Bight

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## Introduction

Dissolved organic matter (DOM) released directly from phytoplankton or as a result of grazing or viral lysis is the main source of carbon and nitrogen for heterotrophic bacteria in the sea (Ducklow et al. 2001; Agustí and Duarte 2013), fueling the "microbial loop" (Azam et al. 1983). Heterotrophic bacteria assimilate and catabolize organic compounds from DOM and simultaneously release inorganic C, N and P produced through cellular

respiration. DOM is a complex mixture of compounds of varying reactivities. Within this mixture, dissolved free amino acids (DFAA) constitute a small (~1 %) but very labile pool of compounds with rapid turnover rates, contributing significantly to bacterial C and N requirements (Fuhrman 1987, 1990; Suttle et al. 1991). Taurine, glycine betaine, dimethylsulfoniopropionate and polyamines have molecular weights and intracellular concentrations similar to amino acids. These compounds have various metabolic functions in cells, ranging from osmolytes to DNA stabilization. Metabolic pathways for their degradation have been identified in bacterioplankton and they can produce TCA cycle intermediates, indicating their potential to contribute to bacterial biomass production (Mou et al. 2010; Denger et al. 2006; Moran et al. 2007); however, their concentration and turnover in the environment have received less attention than amino acids.

Polyamines are short-chain aliphatic organic compounds containing multiple amine groups. Putrescine (PUT), spermidine (SPD) and spermine (SPM) are typically the dominant polyamines found in living cells and in seawater (Tabor and Tabor 1985; Incharoensakdi et al. 2010; Kusano et al. 2007; Lu and Hwang 2002; Marián et al. 2000; Nishibori et al. 2001a, b). Polyamines are involved in a wide variety of biological reactions, including the synthesis of DNA, RNA and proteins. They contribute to membrane stability and stress responses in higher plants, fungi and bacteria (Tabor and Tabor 1985; Incharoensakdi et al. 2010; Jantaro et al. 2003; Alcázar et al. 2006). In seawater, polyamines are found mostly in algae and bacteria (Hamana and Matsuzaki 1985, 1992; Nishibori et al. 2006; Nishibori and Nishijima 2007; Incharoensakdi et al. 2010). Polyamine pools are maintained in cells by *de novo* biosynthesis and by uptake of exogenous compounds. Biosynthesis starts with the formation of PUT either from ornithine via ornithine decarboxylase (ODC) or arginine via arginine decarboxylase and agmatine ureohydrolase (ADC). PUT can then be used as a substrate to form SPD, followed by synthesis of SPM (Tabor et al. 1958; Tabor and Tabor 1966, 1985; Fuell et al. 2010). Putrescine (PotFGHI) and spermidine-preferential uptake systems (PotABCD) have been found in *E. coli*, yeast, cyanobacteria and most of marine bacterial groups (Incharoensakdi et al. 2010; Igarashi and Kashiwagi 1999; Moran et al. 2007; Mou et al. 2010, 2011), indicating that these organisms can use exogenous polyamines.

Polyamines measured in seawater could come directly from living or dead organisms or be produced from protein and amino acid degradation (Höfle 1984). Polyamine concentrations are reported to reach 30 nmol $L^{-1}$ in coastal seawater during algal blooms and have been reported to be as high as 250 nmol $L^{-1}$ in a coastal salt pond (Nishibori et al. 2001a; Lee and Jørgensen 1995). The relative distribution of polyamines between organisms and seawater is similar to that of DFAA, suggesting similarities in their geochemical processing. As with DFAA, heterotrophic bacteria may catabolize polyamines for carbon and nitrogen or as sources of energy, in addition to using them directly in other metabolic processes as mentioned above (Lee 1992; Höfle 1984).

Previous studies of polyamine cycling in seawater are few in number and were mainly conducted in nearshore coastal waters or semi-enclosed marine basins (Lee 1992; Lee and Jørgensen 1995). Measuring turnover rates of polyamines in a broader range of water masses will help in elucidating their role in marine C and N cycling. Based on their turnover rates, polyamine fluxes into bacterioplankton and their contributions to bacterial C and N demand can also be estimated for comparison with other compounds. In this study, we determined turnover rates, concentrations and uptake of polyamines along an inshore to offshore transect and compared them to similar measurements made with select amino acids. We used our data to estimate the potential contribution of polyamines to bacterial C and N demand to obtain a general idea of the contribution of polyamines to bacterial production. We correlated these parameters with biological and environmental conditions across our study areas and used these analyses to identify factors potentially driving spatial variability of polyamine cycling.

## Materials and methods

### Sampling locations

Samples were collected on two cruises of the R/V Savannah in the South Atlantic Bight (SAB) on 18–22 April and 2–6 October 2011. A total of 13 stations were occupied along three inshore–offshore transects

of the SAB between Savannah, Georgia and Jacksonville, Florida (Fig. S1a). Sample sites covered the near-shore, middle shelf and the shelf-break at the edge of the Gulf Stream along the SAB. Water properties at near-shore stations were influenced by river inflow and tidal exchange with salt marshes and estuaries. In contrast, offshore stations, especially those along the shelf-break, were oceanic and influenced by the Gulf Stream. Station locations and sampling depths are given in Supplementary Table 1. Seawater samples were collected using 12 L Go-Flo bottles mounted on a CTD rosette. Because the SAB shelf is shallow, samples were only collected at the surface and near the bottom except at shelf-break stations where mid-depth samples were also collected. Profiles of environmental variables (temperature, salinity, dissolved oxygen, relative fluorescence and photosynthetically active radiation—PAR) were taken by sensors mounted on the CTD during hydrocasts to collect water samples.

Inshore samples were collected during 13–16 August 2011 and 17–20 April 2012 at Georgia Coastal Ecosystems-Long Term Ecological Research (GCE-LTER) sampling sites, located on the Georgia, USA coast adjacent to the SAB (http://gce-lter.marsci.uga.edu/ and Fig. S1b). We collected 2 L of near-bottom water during high and low tide from Sapelo Sound (SP, GCE stations 1, 2 and 3) and Altamaha Sound (AL, GCE stations 7, 8 and 9). We also collected surface water samples from a floating dock at Marsh Landing (ML) on the Duplin River every 3 h over 24-h periods. The water passing the dock on flood tide comes from Doboy Sound, which receives input from both the Atlantic Ocean and the Altamaha River, while the Duplin River drains a salt marsh with limited input of groundwater from Sapelo Island on ebb tide. The Sapelo Island National Estuarine Research Reserve (SINERR) maintains an observatory at Marsh Landing that collects meteorological, water quality and nutrient data (available at http://cdmo.baruch.sc.edu, station

**Table 1** Turnover rates, summed concentrations and uptake rates of polyamines (PUT, SPD and SPM) and amino acids (ARG and GLU) in different regions of the SAB and at GCE-LTER sites

| Time/location | Turnover rate ($day^{-1}$) | | Concentration (nmol $L^{-1}$) | | Uptake rate (nmol $L^{-1}$ $day^{-1}$) | |
|---|---|---|---|---|---|---|
| | PA | AA | PA | DFAA | PA | AA |
| SAB (Apr 2011) | | | | | | |
| Near-shore | 0.75 ± 0.62‡ | 2.3 ± 1.4‡ | 0.70 ± 0.36 | 75 ± 67 | 12.3 ± 4.7‡ | 38.4 ± 15.5‡ |
| Mid-shelf | 0.05 ± 0.03 | 0.28 ± 0.15 | 0.77 ± 0.64 | 34 ± 8.9 | 0.65 ± 0.37 | 4.7 ± 3.0 |
| Shelf-break | 0.01 ± 0.01 | | 2.2 ± 1.9 | 30 ± 37 | 0.14 ± 0.14 | – |
| SAB (Oct 2011) | | | | | | |
| Near-shore | 0.43 ± 0.47‡ | 0.68 ± 0.27‡ | 1.3 ± 1.8 | 214 ± 100 | 5.7 ± 1.9‡ | 18.4 ± 3.1‡ |
| Mid-shelf | 0.04 ± 0.04 | 0.19 ± 0.08 | 1.4 ± 2.5 | 479 ± 778 | 0.74 ± 0.35 | 6.0 ± 5.5 |
| Shelf-break | 0.02 ± 0.01 | 0.05 ± 0.04 | 0.53 ± 0.69 | 153 ± 57 | 0.23 ± 0.11 | 1.1 ± 1.3 |
| GCE-LTER (Apr 2012) | | | | | | |
| SP | 1.2 ± 0.8 | 2.4 ± 1.1 | 1.9 ± 2.2 | 160 ± 45 | 18.8 ± 7.8 | 47.0 ± 25.0 |
| ML | 1.3 ± 0.9 | 1.8 ± 1.1 | 0.9 ± 0.5 | 278 ± 141 | 28.8 ± 9.0 | 65.7 ± 25.1 |
| AL | 1.9 ± 0.8 | 1.1 ± 0.5 | 0.4 ± 0.2 | 101 ± 61 | 22.7 ± 8.0 | 17.1 ± 5.4‡ |
| GCE-LTER (Aug 2011) | | | | | | |
| SP | 0.7 ± 0.5 | 1.4 ± 0.9 | – | – | – | – |
| ML | 0.3 ± 0.2 | 0.8 ± 0.5 | – | – | – | – |
| AL | 1.2 ± 1.3 | 1.1 ± 0.7 | – | – | – | – |

The results are presented as mean ± SD of data from samples collected at different stations in the same region. DFAA concentration was the total of 20 dissolved amino acid species, not just ARG plus GLU. Uptake rate was calculated as the measured turnover rate times the sum of measured the in situ substrate concentration ($S_n$) plus 5 nM added labeled substrate ($S_a$)

*PA* polyamines, *AA* amino acids, *SP* Sapelo Sound, *ML* Marsh Landing, *AL* Altamaha Sound, – indicates missing data

‡ Indicates the statistical significance (ANOVA and pairwise *t* test, $p < 0.05$) of turnover rates, concentrations and uptake rates of polyamines and amino acids, respectively. The comparison was conducted among different regions of the SAB or at GCE-LTER sites

LD). Profiles of temperature and salinity were measured at other stations using a CTD and discrete samples were taken to measure nutrients, chlorophyll and other water quality variables (http://gce-lter.marsci.uga.edu/).

## Turnover rate, concentration and uptake rate of polyamines and amino acids

Seawater samples were drawn directly from Go-Flo bottles into clean, acid-washed 500 mL polypropylene bottles. A series of 10 mL subsamples were quickly transferred to 15 mL snap-cap, disposable test tubes. Radiolabeled polyamines [2,3-$^{3}$H(N)]-putrescine (80 Ci mmol$^{-1}$, American Radiolabeled Chemicals (ARC)); [terminal methylene-$^{3}$H]-spermine (50 Ci mmol$^{-1}$, ARC); [terminal methylene-$^{3}$H (N)]-spermidine (39 Ci mmol$^{-1}$, PerkinElmer) and two amino acids L-[2,3,4-$^{3}$H]-arginine (54.6 Ci mmol$^{-1}$, PerkinElmer); and L-[3,4-$^{3}$H]-glutamic acid (51.1 Ci mmol$^{-1}$, PerkinElmer) were added to five replicate subsamples to final concentrations of 5 nmol L$^{-1}$ ($\sim$1 μCi per tube). Arginine (ARG) and glutamic acid (GLU) were chosen as representatives of DFAA in seawater because they are readily processed by microbial communities (Crawford et al. 1974; Hollibaugh 1978) and have distinct functional groups. In addition, Ferguson and Sunda (1984) showed that the turnover rate of GLU in samples from the Gulf of Mexico approximated that of the turnover rate of an amino acid mixture based on regression analysis (slope = 1.04, intercept = 0.076, r = 0.94). Note that measured polyamine concentrations were generally <5 nmol L$^{-1}$ (see below), so that the amendments we used are not tracer additions, especially for polyamines. We used these amendments to obtain reliable counts in small volume, short duration incubations and because we had no information, in advance, on polyamine concentrations in our samples. The turnover rates we measured are thus approximations of turnover rates that would have been measured in a true tracer experiment: they may be elevated if uptake in situ was limited by substrate concentration, or they may be depressed because the substrate pool is larger than in situ. As a consequence, our estimates of polyamine fluxes and the contributions of polyamines to bacterial C and N demand are also likely biased; however, we assume that the spatial and temporal patterns of activity we observed reflect underlying ecosystem processes.

Duplicate control samples were filtered immediately after the addition of the substrate. The remaining triplicates of each substrate were incubated in the dark for $\sim$1 h at the in situ temperature. Incubations were terminated by filtering samples through 0.45 μm pore size Millipore filters (type HA) held on a model 1225 Sampling Manifold (Millipore, Massachusetts). Each filter was rinsed with 10 mL of unlabeled seawater to wash out unincorporated substrate, and then placed in a 7 mL scintillation vial. Ethyl acetate was added immediately to stop biological activity and to dissolve the filters, then scintillation cocktail was added prior to counting on a Beckman Coulter LS 6500. CPM were converted to DPM using counting efficiencies determined by the instrument using an external standard, then turnover rates were calculated by Eq. (1).

$$\text{Turnover rate} = (\text{dpm}_{\text{experimental}} - \text{dpm}_{\text{control}})/(dpm_{added} \times incubation\ time) \quad (1)$$

A second water sample was collected for determining concentrations of polyamines and DFAA. The sample was filtered through 3 μm and then 0.2 μm pore size membrane filters at low vacuum ($\sim$1 cm Hg) and the filtrate was frozen at −80 °C until analysis. Concentrations of polyamines and DFAA were determined by high-pressure liquid chromatography (HPLC) following derivatization with *o*-phthaldialdehyde/ethanethiol (OPA/ET) and 9-fluorenylmethyl chloroformate (FMOC) reagents (Lu et al. 2014; Körös et al. 2008). The limits of detection for PUT, SPD and SPM were 0.05, 0.01 and 0.1 nmol L$^{-1}$, respectively, and 0.01 – 0.1 nmol L$^{-1}$ for DFAA species. Uptake rates were calculated by multiplying turnover rates (d$^{-1}$) from Eq. 1 by concentrations (nmol L$^{-1}$) we measured in the same sample ($S_n$) plus the 5 nM added as tracer ($S_a$).

## Fate of polyamine carbon

We quantified respiration of polyamine C using putrescine as a model compound. [2, 3-$^{14}$C]-putrescine (100 mCi mmol$^{-1}$, ARC) and [1,4-$^{14}$C]-putrescine (110 mCi mmol$^{-1}$, ARC) were mixed in equimolar proportions and added to 10 mL seawater samples to a final concentration of 50 nmol L$^{-1}$ ($\sim$0.1 μCi per tube). After incubation in the dark at the in situ temperature for $\sim$ 0.5 h, samples were filtered through 0.45 μm pore size Millipore filters

(type HA) and the radioactivity of $^{14}C$ collected on the filter was determined by liquid scintillation counting as above. The $^{14}C$ retained by the filter was considered to be incorporated carbon. One milliliter of 1 N $Na_2CO_3$ was added to the filtrate which was then frozen and held at −80 °C until analysis. In the laboratory, dissolved inorganic $^{14}C$ was stripped from the filtrate and trapped on a phenethylamine-saturated filter following Hobbie and Crawford (1969). The trapped $^{14}C$ was quantified by liquid scintillation counting. Phenethylamine-captured $^{14}C$ was confirmed to be respired carbon from control experiments with labeled substrate added to filtered sterilized seawater. The percentages of C incorporated and respired were calculated by dividing the radioactivity from filter and filtrate, respectively, by their sum.

## Bacterial production and abundance

Bacterial production (BP) was determined from $^3H$-L-leucine incorporation into bacterial protein (Kirchman et al. 1985; Simon and Azam 1989). L-[4,5-$^3H$(N)]-leucine (60 Ci mmol$^{-1}$, ARC) was added to 4 replicate 5 mL water samples to a final concentration of 20 nmol L$^{-1}$ (~5 μCi/tube). Two control tubes were filtered through 0.45 μm-pore size Millipore filters immediately after adding $^3H$-L-leucine. Duplicate experimental samples were incubated for ~1 h in the dark at the in situ temperature, and then filtered as above. Samples were extracted with cold 5 % trichloroacetic acid (TCA) and 80 % ethanol after filtration (Hollibaugh and Wong 1992) and filters were radioassayed by liquid scintillation counting as above. L-Leucine incorporation rate was converted to bacterial protein production (BPP) using a factor of 3,565 g protein (mole leucine incorporated)$^{-1}$ (Simon and Azam 1989). The BPP measurement can be converted to the rate of dry weight production by multiplying by 1.6, and into bacterial carbon demand (BCD) by multiplying the rate of dry weight production by 0.54 (Simon and Azam 1989). Bacterial nitrogen demand (BND) was estimated from BCD assuming that the C: N ratio of bacterial cells is 5:1 (Lee and Jørgensen 1995).

Bacterial abundance was determined by quantitative PCR of 16S rRNA genes using an iCycler iQ5 qPCR System (BioRad) and 2× SYBR® Green I Dye (BioRad) following previously described protocols (Buchan et al. 2009). Samples (~1 L) for DNA extraction were collected at each station and depth on both cruises. They were pressure filtered (at ~60 kPa) through 0.22 μm Durapore filters (Millipore) and frozen in 2 mL of lysis buffer (0.75 mol L$^{-1}$ sucrose, 40 mmol L$^{-1}$ EDTA, 50 mmol L$^{-1}$ Tris, pH 8.3). DNA was extracted and purified by phenol–chloroform extraction as described previously (Tolar et al. 2013; Bano and Hollibaugh 2000). Triplicate 25 μL reactions containing 2 μL of template DNA were run with gene-specific primers (BACT1369F: 5′-CGGT GAATACGTTCYCGG-3′ and PROK1492R: 5′-GG WTACCTTGTTACGACTT-3′) for each sample (Suzuki et al. 2000). Samples were run alongside standards (ranging from 10 to $10^7$ copies μL$^{-1}$) and checked for PCR inhibition by comparing tenfold dilutions of DNA run on the same plate. Specificity of each reaction was confirmed with a melt curve. The detection limit was $9.12 \times 10^4$ copies L$^{-1}$ with qPCR efficiency ranging from 95.6 to 106 %. Gene abundance was converted to copies L$^{-1}$ of sample filtered by assuming 100 % extraction efficiency and calculated as in Tolar et al. (2013).

## Chemical analyses

The concentration of biogenic silica (BSi) was measured as an estimate of the abundance of diatoms in SAB samples (Nelson et al. 1995; Brzezinski et al. 1997). BSi was analyzed following the protocol described in Brzezinski and Nelson (1989), modified from Strickland and Parsons (1977) and Paasche (1973). Briefly, a total volume of 0.6–1 L of water was filtered through a 0.6 μm pore size polycarbonate membrane filter, which was then frozen at −80 °C until analysis. Filters were placed in 4 mL of 0.2 N NaOH, heated to 95 °C for 40 min, then the solution was neutralized by adding 1 mL of 1 N HCl. The sample was centrifuged for 10 min at 1,000x*g*, and then the supernatant was used to quantify silicic acid following Brzezinski and Nelson (1989). Extracts from coastal seawater samples were diluted tenfold for BSi measurement. Samples for nutrient determination were filtered through 0.22 μm Durapore filters (Millipore) immediately after collection and stored frozen at −80 °C until analysis. Ammonium ($NH_4^+$) was measured using a fluorometric method described in Holmes et al. (1999). Nitrate plus nitrite ($NO_x^-$) was analyzed by reducing nitrate to nitrite with cadmium

(Jones 1984), and nitrite ($NO_2^-$) was determined following Strickland and Parsons (1972).

### Statistical analyses

Principal components analysis (PCA) of biological (bacterial production and abundance) and environmental data (relative fluorescence, dissolved oxygen, attenuation coefficient, temperature and salinity) was used to group SAB stations for better understanding of the patterns of polyamine cycling. The average PAR attenuation coefficient (K) at each station was calculated from PAR profiles collected with other environmental data during CTD casts (Lin et al. 2009). Redundancy analysis (RDA) was applied to identify factors that might influence turnover rates, concentrations and fluxes of polyamines and amino acids. RDA is a canonical analysis that combines regression and ordination in a multivariate model. Linear relationships are assumed between response and explanatory variables in RDA (Legendre and Legendre 2012). The log-transformed nutrient data ($NH_4^+$, $NO_2^-$, $NO_x^-$), biological data (BSi concentrations, relative fluorescence bacterial production and abundances) and environmental variables (temperature and salinity) measured during the two cruises were analyzed with 999 Monte-Carlo permutations using the software package CANOCO (4.5; ter Braak and Smilauer 2002). Individual *t* tests run in R (http://www.r-project.org/) were used to test the statistical significance of spatial differences in turnover rates, concentrations and uptake rates of polyamines and amino acids using log-transformed data.

## Results

### Water column characteristics

The temperature and salinity of surface water encountered on the April cruise ranged from 19 °C and 29 psu at near-shore stations to 27 °C and 36.7 psu at shelf-break stations, respectively. Bottom water (~450 m) temperature was 7.9 °C at shelf-break stations. In October, the salinity was uniformly ~36 psu across the SAB except at station 1 (surface: 23.5 psu, bottom: 27.6 psu). Surface water temperature ranged from 24.2 °C at near-shore stations to 29.2 °C at the shelf-break and decreased to 7.3 °C at 450 m. The water column was well mixed at near-shore and mid-shelf stations (<50 m) during both cruises, while the water column at the shelf-break was weakly stratified during April and well stratified in October with a thermocline at ~50 m. Temperature was uniform at inshore GCE-LTER sites (April 23 ± 0.5 °C; August 31 ± 0.6 °C); however, salinity varied significantly between sites (SP 31 ± 1 psu, ML 30.3 ± 0.1 psu and AL 8.9 ± 9.9 psu in April; SP: 35.6 ± 0.2 psu, ML: 32.2 ± 0.4 psu and AL: 14.7 ± 11.2 psu in August) due to tidal mixing and variable riverine freshwater input, especially at AL stations 7–9.

Relative fluorescence (RF) and BSi, representing chlorophyll *a* (Chl *a*) and the abundance of diatoms, respectively, decreased from near-shore to offshore waters during both cruises (Fig. S2a and S2c). Relative fluorescence was highest at mid-depth at shelf-break stations, comparable to or even higher than those measured at the mid-shelf stations. L-Leucine incorporation rates and the abundance of bacterial 16S rRNA genes also decreased from inshore to offshore (Fig. S2b, d). L-Leucine incorporation rates and bacteria 16S rRNA gene abundance covaried in April but not in October; for example, bacterial abundance was low at Gray's Reef (GR) in October, yet bacterial production was extremely high.

We used PCA of biological and environmental variables to group similar stations in the SAB (Fig. 1). The first two PCA axes explained 88.0 % of the variation between stations in April and 94.8 % of the variation between stations in October. The contribution of each variable to principal components axes is given in Table S2. Overall, the analysis supported separating the stations into three groups: near-shore, mid-shelf and shelf-break. Environmental conditions at GR varied between cruises so GR was grouped with mid-shelf stations in April and near-shore stations in October.

### Turnover rates of polyamines and amino acids

Turnover rates of polyamines (PUT, SPD and SPM) and amino acids (ARG and GLU) were faster at near-shore stations and decreased rapidly offshore in the SAB during both cruises (Fig. 2; Table 1). Polyamine turnover varied similarly to that of amino acids but the difference between near-shore and offshore waters was greater. Polyamine turnover rates at mid-shelf and shelf-break stations in April were 6.7 % and 1.3 %, respectively, of those at near-shore stations. Turnover

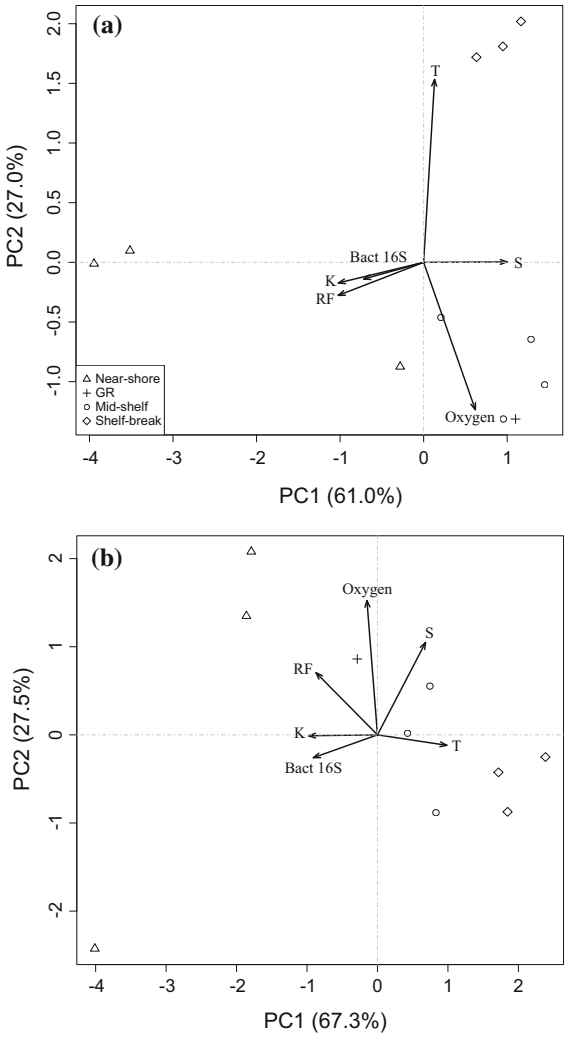


**Fig. 1** PCA of environmental characteristics of SAB samples collected during April (**a**) and October (**b**) cruises. *RF* relative fluorescence (Chl *a* fluorescence), *K* attenuation coefficient, *T* temperature, *S* Salinity, *Oxygen* dissolved oxygen, *Bact 16S* bacterial 16S rRNA gene abundance. *Symbols* represent samples from different water regions on the SAB; *triangles* near-shore; *cross* Gray's Reef (GR); cycles, mid-shelf; *diamonds* shelf-break

rates of polyamines measured on the October cruise were similar to those measured on the April cruise, while amino acids turned over slower than April (*t* test, $p = 0.005$; Fig. 2; Table 1). On both cruises, turnover rates of SPD and SPM were faster than those of PUT at nearly all stations (*t* test, $p < 0.05$; Fig. 2).

The turnover rates of polyamines and amino acids in samples collected from inshore GCE-LTER sites also varied spatially (Fig. 3; Table 1). Polyamine turnover rates were relatively faster in samples from the riverine estuary Altamaha Sound (AL) than other areas, especially PUT turnover rates. As with samples collected on the cruises, the turnover rates of SPD and SPM were greater than the turnover of PUT in April samples (*t* test, $p < 0.05$), but in August PUT turnover rate was similar to the other two species (*t* test, $p > 0.05$), with relatively faster turnover rates in some samples collected at AL (Fig. 3). Polyamine turnover rates were significantly faster (*t* test, $p = 0.002$) at inshore GCE sites in April when compared to turnover rates measured at near-shore SAB stations (Table 1).

## Concentrations of polyamines and DFAA

Polyamine concentrations were very low compared to total DFAA concentrations measured in the same samples (Fig. 4; Table 1). Concentrations of individual polyamines were typically <1 nmol $L^{-1}$ (Fig. 4). On the April cruise, the summed concentrations of polyamines were similar in near-shore and mid-shelf samples but increased ~threefold at shelf-break stations. In contrast, higher concentrations of DFAA were found at near-shore stations. DFAA pools were 1–2 orders of magnitude greater than polyamine pools in SAB samples (Fig. 4a, b; Table 1). PUT and SPD were detected in all samples, but SPM was only detected in samples from the shelf-break. SPD was the most abundant polyamine encountered on the October cruise. Relatively high concentrations of SPD were found in surface samples from near-shore stations 3 ($3.3 \pm 0.2$ nmol $L^{-1}$, mean ± SE) and GR ($5.3 \pm 4.2$ nmol $L^{-1}$), as well as in one mid-shelf sample (Stn 8; 7.0 nmol $L^{-1}$; Fig. 4c). DFAA were uniformly distributed with concentrations <400 nmol $L^{-1}$ (Fig. 4d), except for one extremely high concentration found in surface water of Stn 8 ($2{,}210 \pm 493$ nmol $L^{-1}$) where polyamine concentrations were also elevated. DFAA concentrations were significantly greater in October versus April (*t* test, $p = 0.0006$) and the DFAA pool was more than 2 orders of magnitude larger than the polyamine pool, which did not change significantly between the two cruises (*t* test, $p > 0.05$).

Concentrations of polyamines and DFAA were only measured at inshore GCE-LTER sites in April of 2012 (Fig. 4e, f) and SPD was the most abundant polyamine species detected in those samples. PUT was only detected in samples collected during low tide at GCE 2 in SP but the concentration in this sample was >4 nmol $L^{-1}$. On average, the concentrations of polyamines at inshore sites

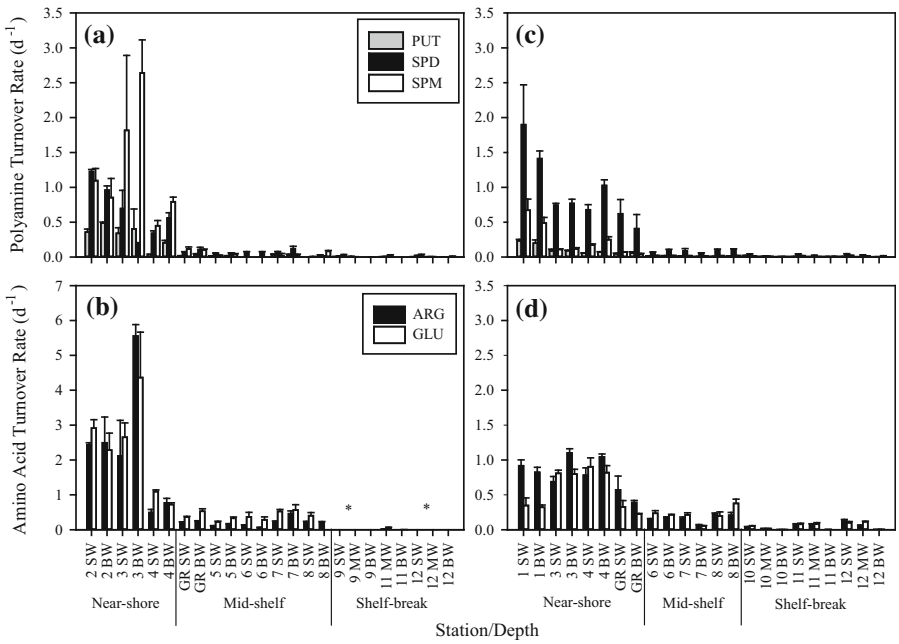


**Fig. 2** Turnover rates of polyamines and amino acids in samples collected from the SAB in April 2011 (**a**, **b**) and October 2011 (**c**, **d**). Numbers on the abscissa identify the station as per Fig. S1a. *SW* surface water, *MW* mid-water, *BW* bottom water. Stations are allocated to regions of the shelf (near-shore, mid-shelf and shelf-break) based on water quality properties analyzed by PCA. *Error bars* indicate the standard error of triplicate measurements of one sample and *asterisks* indicate lost data

were similar to those measured at near-shore and mid-shelf stations of the SAB on the April cruise, with concentrations of individual polyamines consistently $<1$ nmol $L^{-1}$. DFAA concentrations were significantly higher at inshore GCE sites than at near-shore SAB stations (*t* test, $p < 0.05$).

## Uptake rates of polyamines and amino acids

Uptake rates of polyamines (Fig. 5a, c) and amino acids (Fig. 5b, d) were highest at near-shore stations and decreased at mid-shelf and shelf-break stations on both cruises, a distribution similar to turnover rates. Uptake rates of polyamines and amino acids at near-shore stations were twofold higher in April than October, but were similar at mid-shelf and shelf-break stations on both cruises (Table 1). The summed uptake rates of ARG and GLU were approximately threefold, eightfold and fivefold greater than polyamine uptake rates at near-shore, mid-shelf and shelf-break stations, respectively (Table 1). On average, uptake rates of all substrates were higher at inshore GCE-LTER stations than at near-shore SAB stations in April (*t* test, $p < 0.05$; Fig. 5e, f; Table 1).

## Fate of putrescine carbon

We found that $26 \pm 17$ % (mean $\pm$ SD) of PUT carbon processed (uptake plus respiration) was released as $^{14}CO_2$ in samples collected during the April cruise. A larger fraction ($51 \pm 8.8$ %) of PUT carbon was respired in samples collected in October, and elevated respiration of PUT carbon was observed primarily at near-shore and mid-shelf stations. Compared to the low respiration of PUT C detected across the SAB on the April cruise, a large fraction of the PUT carbon was respired by samples collected in April at inshore GCE sites (Table 2); however, the respiration of PUT carbon decreased in samples collected at Marsh Landing during August (Table 2).

## Contributions to bacterial demand

The contributions of polyamines and DFAA to BCD and BND were compared using fluxes calculated as the product of measured turnover rates and measured in situ substrate concentrations ($S_n$). Our analysis indicated that C and N supplied by polyamines accounted for only a small proportion of the total

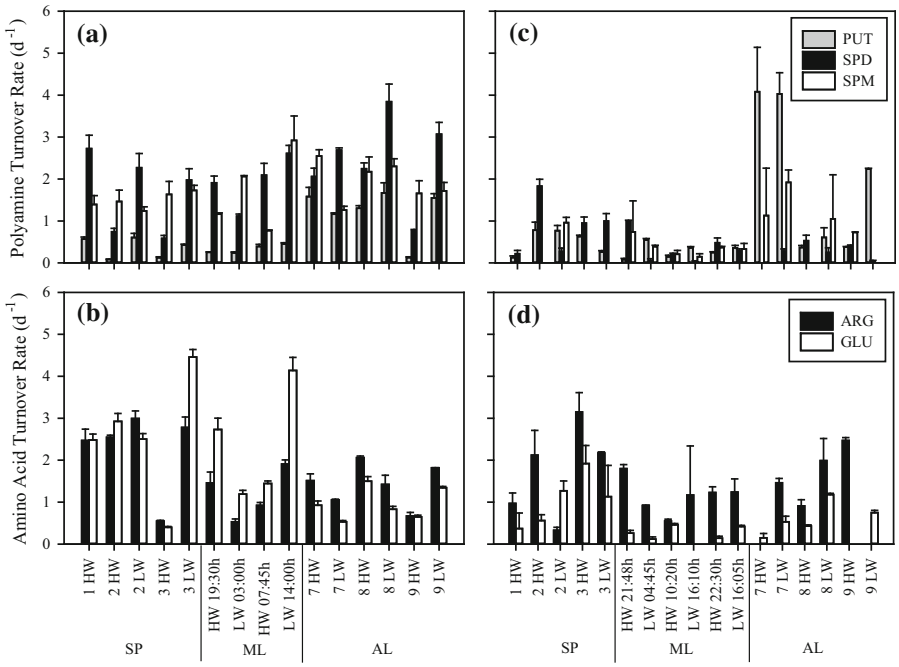


**Fig. 3** Turnover rates of polyamines and amino acids in samples collected from GCE-LTER sites located in Sapelo Sound (SP-1, 2 and 3), Marsh Landing (ML) and Altamaha Sound (AL-7, 8 and 9) in April of 2012 (**a**, **b**) and August of 2011 (**c**, **d**). *Numbers* on the abscissa identify the station as per Fig. S1a. Samples from ML were collected over a 24-h period. *HW* high tide water sample, *LW* low tide water sample. *Error bars* indicate the standard error of triplicate measurements of one sample

bacterial demand for C and N in these samples, in contrast to a much larger contribution supplied by ARG plus GLU (Table 3). Polyamines contributed <4 % of BCD and 8 % of BND in samples collected in the SAB. Maximum contributions were at shelf-break stations on the April cruise and at near-shore stations on the October cruise (Table 3). Polyamine contributions to BND were highest at inshore GCE-LTER sites, especially at Marsh Landing (ML) where 14 % of BND could be supplied by polyamines. In contrast, the contribution of polyamine C to BCD was low (<3 %) at inshore stations and similar to those we measured in the SAB (Table 3).

## Environmental factors versus turnover rates, concentrations and uptake of polyamines and amino acids

RDA was only applied to samples collected in the SAB due to incomplete coverage of ancillary environmental variables at other stations. The RDA biplot (Fig. 6a) showed that 72 % of the variability in the turnover rate—environment relationship was explained by canonical axis 1 (RDA 1). Adding the second axis (RDA 2) increased the variance explained to 76 %. Bacterial 16S rRNA gene abundance was omitted from subsequent statistical analyses due to its low contribution to the turnover rate - environment model. Chlorophyll fluorescence (RF, $p = 0.001$) and salinity ($p = 0.003$) contributed significantly to RDA1 while nitrite concentration contributed significantly to RDA 2 ($p = 0.002$). Turnover rates of polyamines and amino acids were both positively correlated with chlorophyll fluorescence and negatively correlated with salinity on RDA1. SPD turnover rate was correlated with the concentrations of biogenic silica (BSi) and ammonium, while turnover rates of the other four substrates we measured (PUT, SPM, ARG and GLU) were more closely associated with L-leucine incorporation rates (LEU), a proxy for bacterial production.

The first two canonical axes only explained 25.5 % of the variability in the concentration-environment relationship (17.8 % for RDA1 and 7.7 % for RDA2; Fig. 6b). RDA 1 was not statistically significant ($F = 6.78$, $p = 0.37$); however, polyamine concentrations were shown to cluster closely with $NO_2^-$ concentrations

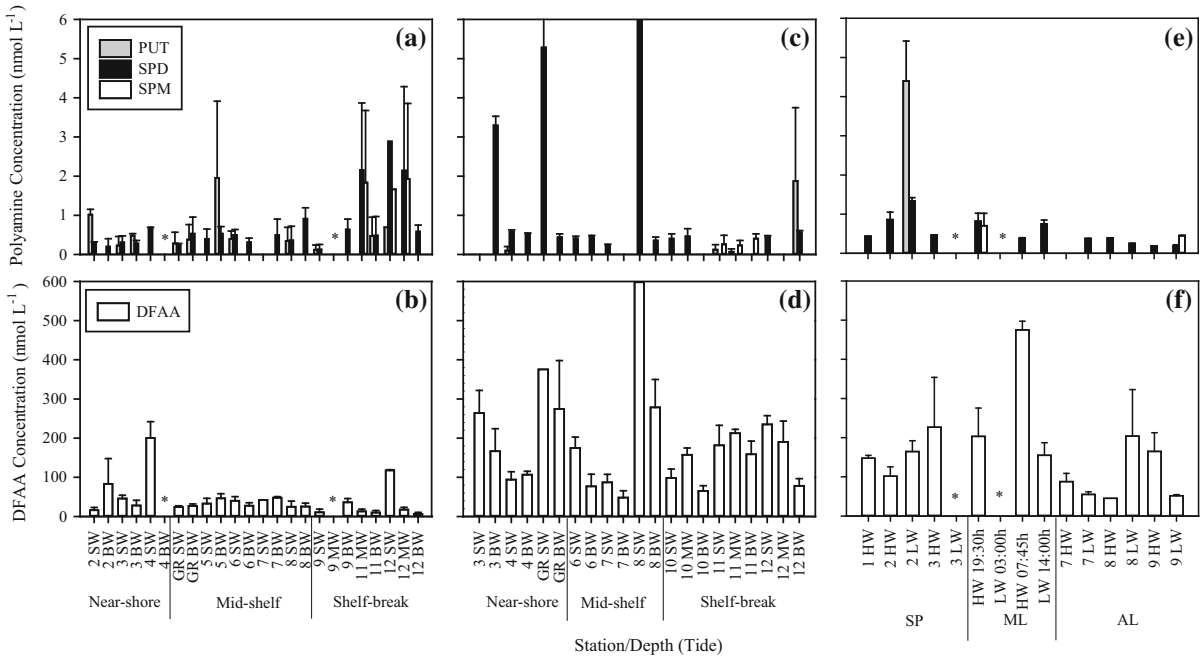


**Fig. 4** Concentrations of polyamines and DFAA in samples collected from the SAB in April of 2011 (**a**, **b**) and October of 2011 (**c**, **d**) as well as from GCE-LTER sites located in Sapelo Sound (SP-1, 2 and 3), Marsh Landing (ML) and Altamaha Sound (AL-7, 8 and 9) in April of 2012 (**e**, **f**). *Numbers* on the abscissa identify the station as per Fig. S1. *Error bars* indicate the standard error of triplicate measurements of one sample and *asterisks* indicate the lost data

and bacterial 16S rRNA gene abundance, while DFAA concentrations varied with temperature, bacterial production, salinity and $NO_x^-$ concentrations.

The uptake—environment relationship shown in Fig. 6c indicates that 82.3 % of the variability was explained by RDA1 and only 2.1 % of the variability was explained by RDA 2. Chlorophyll fluorescence ($p = 0.001$) and bacterial production ($p = 0.003$) contributed significantly to RDA 1; the former was more closely correlated with polyamine uptake while the latter was strongly associated with amino acid uptake. Although BSi was not a significant factor in the uptake-environment model, it had a stronger correlation with polyamine uptake than chlorophyll fluorescence. Weightings for all four canonical axes are given in Table S3.

## Discussion

### Polyamine pool

Polyamine concentrations were very low (N.D. to nmol $L^{-1}$) at our study sites, <1 % of the total DFAA concentrations in most samples. Our findings are similar to those reported for Japanese coastal waters (mostly <5 nmol $L^{-1}$; Nishibori et al. 2001a, b, 2003), but were much lower than the concentrations of PUT measured in an eutrophic salt pond (N.D. to 250 nmol $L^{-1}$; Lee and Jørgensen 1995); indicating significant spatial variability in polyamine concentrations. Instead of PUT, which is usually reported to dominate the polyamine pool in seawater (Nishibori et al. 2001a, b, 2003), SPD was the dominant polyamine in our samples and was widely distributed across our study area. PUT was detected frequently on the April cruise but was only detected occasionally on the October cruise or at GCE-LTER sites; however, when present it contributed significantly to total polyamine concentrations. The predominance of SPD and PUT was possibly due to their distribution in algal cells, especially diatoms (Hamana and Matsuzaki 1985), and variation in the species composition of phytoplankton biomass. SPD is also abundant in cyanobacteria, in some species of green algae and in members of the Raphidophyceae (Hamana and Matsuzaki 1982, 1992; Jantaro et al. 2003; Badini et al. 1994;

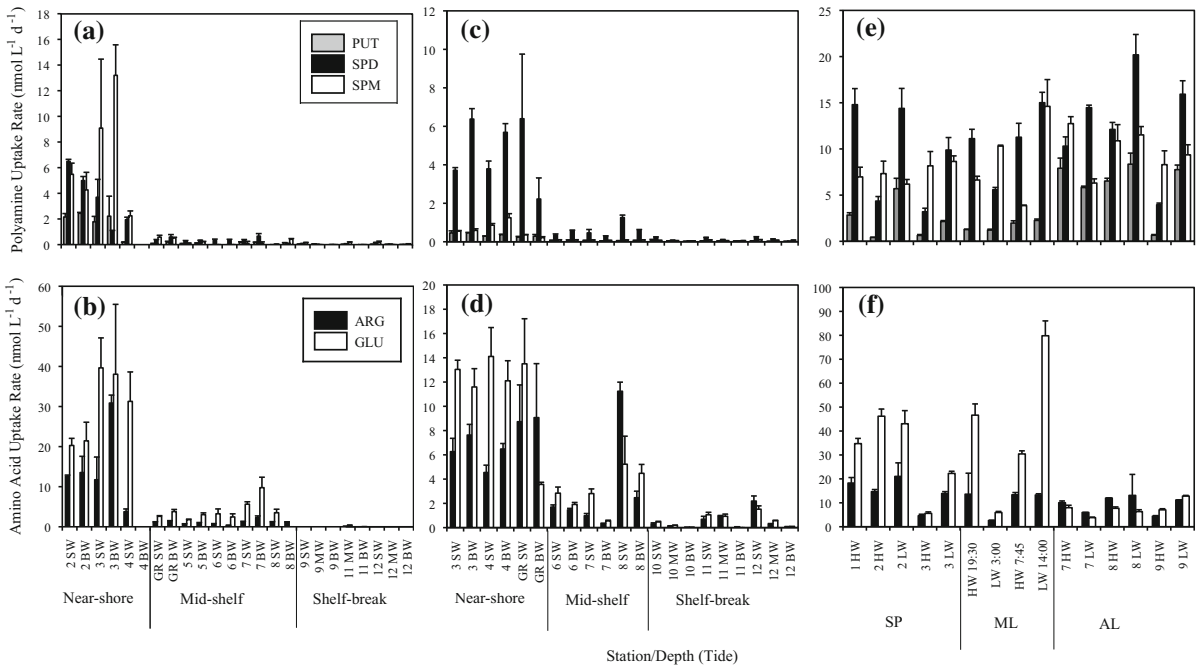


**Fig. 5** Uptake of polyamines and amino acids in samples collected from the SAB in April of 2011 (**a**, **b**) and October of 2011 (**c**, **d**) as well as from GCE-LTER sites located in Sapelo Sound (SP-1, 2 and 3), Marsh Landing (ML) and Altamaha Sound (AL-7, 8 and 9) in April of 2012 (**e**, **f**). *Numbers* on the abscissa identify the station as per Fig. S1. *Error bars* indicate the standard error of triplicate measurements of one sample

**Table 2** Respiration and assimilation of putrescine carbon (PUT C) in different regions of the SAB and at GCE-LTER sites

| Time/location | PUT C Respiration (%) | PUT C Assimilation (%) |
|---|---|---|
| SAB (Apr 2011) | | |
| Near-shore | 24 ± 15 | 76 ± 15 |
| Mid-shelf | 32 ± 23 | 68 ± 23 |
| Shelf-break | 22 ± 12 | 78 ± 12 |
| SAB (Oct 2011) | | |
| Near-shore | 56 ± 7.3 | 44 ± 7.3 |
| Mid-shelf | 60 ± 11 | 40 ± 11 |
| Shelf-break | 38 ± 14 | 62 ± 14 |
| GCE-LTER (Apr 2012) | | |
| SP | 75 ± 4.4 | 25 ± 4.4 |
| ML | 74 ± 11 | 26 ± 11 |
| AL | 60 ± 11 | 40 ± 11 |
| GCE-LTER (Aug 2011) | | |
| ML | 33 ± 7.3 | 67 ± 7.3 |

The results are presented as mean ± SD of data from samples collected at different stations in the same region

Nishibori et al. 2006). The occasional detection of SPM at low concentrations in our samples may reflect its distribution in specific eukaryotes, because the free SPM has only been found in yeast and a few eukaryotic algae (Hamana and Matsuzaki 1982, 1985). Thus, detection of different polyamine species in our samples could be as much due to the diversity of their sources as to removal by selective uptake (Nishibori et al. 2003).

## Polyamine turnover and uptake rates

The similarity in the distribution of polyamine and DFAA turnover rates in our samples suggest that a similar mechanism (bacterial uptake) controls their distribution and that polyamines are a biologically active fraction of DOM. However, as discussed above, the supply of polyamines may be as important as uptake in determining composition and distributions. Our RDA analysis showed that the turnover rates of both polyamines and amino acids correlated significantly with chlorophyll fluorescence and salinity. The close coupling between polyamine turnover and

**Table 3** Contributions of polyamines (sum of PUT, SPD and SPM) and amino acids (sum of ARG and GLU) to bacterial C and N demands (BCD and BND, respectively)

| Time/location | BCD (ng $L^{-1}$ $day^{-1}$) | PA C % | AA C % | BND (ng $L^{-1}$ $day^{-1}$) | PA N % | AA N % |
|---|---|---|---|---|---|---|
| SAB (Apr 2011) | | | | | | |
| Near-shore | 5,029 ± 3,114 | 0.47 ± 0.32 | 39 ± 27 | 1,006 ± 623 | 2.4 ± 1.6 | 105 ± 70 |
| Mid-shelf | 1,146 ± 1,013 | 0.38 ± 0.45 | 14 ± 14 | 229 ± 203 | 2.4 ± 2.3 | 39 ± 38 |
| Shelf-break | 170 ± 122 | 1.3 ± 1.2 | 0 | 34 ± 24 | 6.3 ± 5.9 | – |
| SAB (Oct 2011) | | | | | | |
| Near-shore | 4,106 ± 2,728 | 3.1 ± 2.5 | 24 ± 10 | 821 ± 546 | 7.8 ± 6.2 | 43 ± 16 |
| Mid-shelf | 594 ± 197 | 2.6 ± 4.4 | 46 ± 61 | 119 ± 39 | 6.5 ± 11 | 129 ± 222 |
| Shelf-break | 170 ± 165 | 0.7 ± 0.4 | 22 ± 22 | 25 ± 21 | 1.8 ± 1.1 | 60 ± 65 |
| GCE-LTER (Apr 2012) | | | | | | |
| SP | 4,703 ± 1,830 | 1.5 ± 1.9 | 9.6 ± 5.8 | 941 ± 366 | 8.9 ± 11.3 | 56 ± 34 |
| ML | 4,375 ± 1,592 | 2.3 ± 1.8 | 23 ± 8.5 | 875 ± 318 | 14 ± 10 | 137 ± 49 |
| AL | 6,232 ± 2,107 | 0.7 ± 0.4 | 2.8 ± 2.6 | 1246 ± 421 | 4.1 ± 2.4 | 16 ± 15 |

The results are presented as mean ± SD of samples collected from different stations in the same region. BCD and BND represent bacterial C and N production, respectively, calculated from L-leucine incorporation rates following Simon and Azam (1983). PA C % and AA C % represent percentages of BCD from polyamines and amino acids, respectively. PA N % and AA N % represent percentages of BND from polyamines and amino acids, respectively

– Indicates missing data

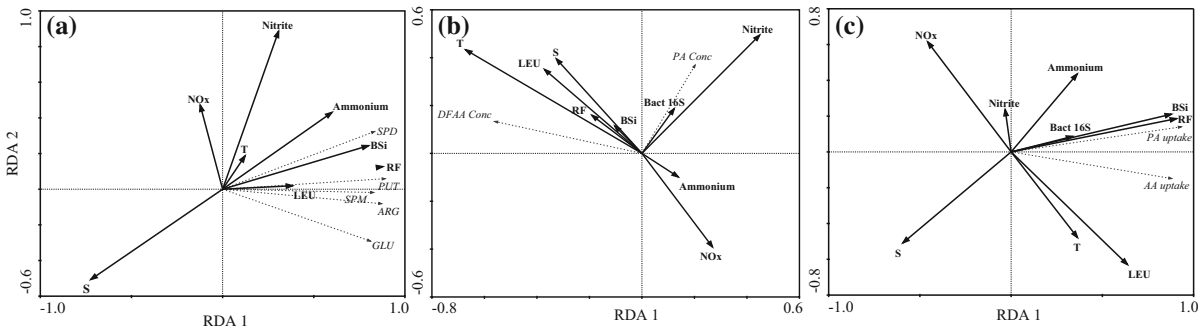


**Fig. 6** Redundancy analysis (RDA) ordination plots of turnover rates (**a**), concentrations (**b**) and uptake rates (**c**) versus environmental conditions for polyamines and amino acids. The length and angle of *arrows* show the contribution of each variable to RDA axes. The projected length of turnover rates, concentrations and uptake rates on a particular environmental variable indicates the correlation between two variables. *PA Conc* summed polyamine concentrations, *DFAA Conc* total concentrations of 20 dissolved free amino acids. *PA uptake* summed polyamine uptake, *AA uptake*, summed uptake of ARG and GLU, *LEU* L-leucine incorporation rates, *RF* relative fluorescence, equivalents of Chl *a* fluorescence, *T* temperature, *S* salinity, *Bact 16S* bacterial 16S rRNA gene abundance, *BSi* concentrations of biogenic silica

chlorophyll fluorescence implies a link to phytoplankton, presumably as a potential source of polyamines (Lee and Jørgensen 1995), in the biogeochemical cycling of polyamines in seawater. Higher phytoplankton biomass could result in more polyamines released to seawater, stimulating polyamine uptake by bacterioplankton, while the composition or physiological condition of the phytoplankton crop may affect the composition of the polyamine pool. We did not collect samples for chlorophyll measurements at inshore sites. GCE-LTER data indicates that chlorophyll concentrations are higher at freshwater stations in Altamaha Sound (AL, GCE Stn 7–9) than in the more saline water of Sapelo Sound (SP, GCE 1–3) during April and August, consistent with the pattern of polyamine turnover rates we found. However, elevated chlorophyll concentrations and low salinity tend to co-occur in near- and in-shore waters; thus, it was

not surprising that turnover rates correlated with both variables.

We measured BSi (Fig. S2a, c) to estimate the abundance of diatoms and found a strong correlation between SPD turnover rates and BSi, suggesting a link between diatoms and the supply of SPD. Long-chain polyamines (LCPAs) have been detected in diatom frustules as a component of silafins, a silica-precipitating protein. In LCPAs, putrescine, spermidine and spermine moieties are attached to N-methylated derivatives of polypropyleneimine units (Kröger et al. 2000; Bridoux and Ingalls 2010). However, whether the simple, short-chain polyamines in seawater originate from these long-chain polyamines, and the possible biochemical processes linking these two polyamine pools, have not been studied.

As we expected, the patterns of polyamine and amino acid turnover rates across the SAB were similar to the pattern of bacterioplankton heterotrophic activity measured as L-leucine incorporation rate (LEU). Previous studies focused on the cycling of PUT, partly as a consequence of the availability of labeled substrates. The turnover rates of PUT we measured are comparable to those reported previously (Table 4; Lee and Jørgensen 1995; Lee 1992), which were faster in more productive water ($> 0.1$ $d^{-1}$) and slower in stratified or less productive waters (0.001–0.1 $day^{-1}$). Pools of the other two polyamine species we measured (SPD and SPM) turned over more rapidly than PUT in most of our samples. The $K_m$ of SPD uptake by the energy-dependent polyamine uptake system in *E.coli* is about tenfold less than that of PUT, indicating more effective uptake of SPD at low substrate concentrations (Tabor and Tabor 1966). Although SPM was undetectable in samples from most stations, we measured rapid turnover of SPM in SAB samples suggesting that heterotrophic bacteria may use it preferentially. Previous studies with *E.coli* found that SPM was taken up more rapidly than other polyamines at the pH of seawater (Tabor and Tabor 1966, 1985), consistent with results from our study.

The turnover rates and uptake rates we calculated are underestimates because we did not correct them for respiration. However, our studies showed that a larger fraction of PUT carbon was incorporated than respired, comparable to the results of a previous study (Lee and Jørgensen 1995) showing that 40–100 % of PUT carbon was incorporated into biomass by bacteria from a salt pond. Our incubations were of <1 h duration and Höfle (1984) suggested that a longer incubation period (>6 h) was required before PUT carbon was respired significantly and concluded that the uptake of radiolabeled polyamines can be regarded as a reasonable estimate of bacterial polyamine utilization, as long as short incubations are used to make the measurements (Lee 1992).

**Table 4** Comparisons of concentrations and turnover rates of putrescine in natural waters from early studies with results from this study

| Environment | Putrescine | | Reference |
|---|---|---|---|
| | Concentration (nmol $L^{-1}$) | Turnover rate ($day^{-1}$) | |
| Eutrophic stratified pool | 0–250 | 2.4–16.8 | Lee and Jørgensen (1995) |
| Oxic layer of a eutrophic salt pond | 10 | 2 | Lee (1992) |
| Oxic layer of a stratified trench with low production | 5 | 0.032 | Lee (1992) |
| Anoxic layer of a stratified trench with low production | 15 | 0.006 | Lee (1992) |
| SAB near-shore (Apr/Oct) | 0.34/0.02 | 0.24/0.11 | This Study |
| SAB mid-shelf (as above) | 0.34/0 | 0.03/0.01 | |
| SAB shelf-break (as above) | 0.18/0.26 | 0.005/0.007 | |
| GCE-LTER inshore (Apr/Aug) | 4.4[a]/– | 0.7/0.94 | |

– Samples were not collected

[a] The concentration was only detected in one sample

Variability in polyamine uptake was determined primarily by variability in turnover rates, rather than variability in concentration. Polyamine concentrations were generally low and uniform in our samples, thus polyamine concentrations in incubations to determine turnover rates were determined primarily by the concentration of added radiolabeled substrate (5 nmol $L^{-1}$). Because we were unable to add truly tracer concentrations of radiolabeled substrates to our samples, to some extent these measurements may approach "potential" rates rather than "in situ" uptake rates. We assumed that the spatial and temporal distribution of the uptake rates we measured approximate the spatial and temporal distribution of true in situ rates. These uptake rates were then used in statistical analyses in an effort to elucidate the patterns of polyamine utilization. As a conservative measure, we used the polyamine concentrations we measured, rather than the sum of the measured plus the added tracer ($S_n + S_a$ in the parlance of Hobbie et al. 1968), in our calculations of BCD and BND. Using the total pool size ($S_n + S_a$) would increase calculated fluxes into bacterioplankton by orders of magnitude in some cases (Table 1).

The similarity of the patterns of polyamine and amino acid uptake in the study area suggest similar pathways of biogeochemical cycling. The primary source of both classes of compounds in the environment is likely phytoplankton biomass. It is anomalous that, given the similarity in concentrations of these classes of compounds in phytoplankton cytosol, implying similar fluxes of polyamines and DFAA into the environment as a consequence of viral lysis (Suttle et al. 1990; Cottrell and Suttle 1995) or "sloppy feeding" by grazers (Conover 1966; Lampert 1978; Hasegawa et al. 2001), in situ concentrations are so different. Amino acids may "leak" from phytoplankton more readily than polyamines, or release of DFAA by hydrolysis of detrital proteins may account for the apparent differential in sources. Alternatively, if polyamine fluxes into the plankton are comparable to those of DFAA, the results imply the existence of a highly efficient removal mechanism for polyamines.

Statistical analysis of our data suggests that the composition and concentration of the polyamine pool is tightly coupled to phytoplankton production, especially by diatoms, while DFAA concentration was controlled by bacterial uptake, as reported previously (Lee and Jørgensen 1995). The strong correlation between polyamine uptake and phytoplankton biomass re-emphasizes that phytoplankton are the predominate source of polyamines in the SAB. Due to low in situ concentrations, polyamine uptake contributes less to BCD and BND than DFAA uptake, as shown by the weaker correlation between polyamine uptake and bacterial production. Bacterial uptake of polyamines thus appears to be controlled mainly by release from phytoplankton. In contrast, the relatively high concentrations of DFAA are closer to their $K_t$ in seawater (Crawford et al. 1974), resulting in the actual uptake of DFAA approaching $V_{max}$; hence the uptake of DFAA is controlled by the ability of bacteria to transport these compounds. The low in situ concentrations of polyamines also suggests rather significant differences in the affinities of bacterial transport systems for polyamines versus amino acids or, alternatively, that there additional sinks for polyamines competing with uptake by bacteria.

## Polyamine contribution to bacterial C and N production

We used the turnover rates and in situ concentrations we measured to estimate the potential contribution of polyamines to BCD and BND. Low fluxes of polyamines into bacterioplankton suggest that they are less important to bacterial production than amino acids. Our calculations supported this prediction, showing that polyamines contributed a very small proportion to bacterial C and N demand compared to amino acids. Due to low C:N ratios (PUT 4:2, SPD 7:3 and SPM 10:4), the contributions of polyamines to bacterial N demand were more significant, indicating the potential of polyamines to serve as a source of nitrogen for bacterial production. However, as discussed above, our estimates of the contribution of polyamines to bacterial production could be underestimated because we did not correct for respiration and regeneration, while the elevated substrate concentrations we used to measure turnover rates is another potential source of bias.

Unlike C, the fate of assimilated polyamine N has not been fully characterized. We assume that the amine groups of respired polyamines would be remineralized by heterotrophic bacteria and released as ammonia, as shown previously for amino acids (Hollibaugh 1978; Goldman et al. 1987; Goldman and Dennett 1991), or used to produce amino acids from α-

ketoacids by a transaminase, followed by incorporation into protein (Bagni et al. 1978; Rastogi and Davies 1990). The correlation of polyamine uptake with ammonium concentration and with bacterial 16S rRNA gene abundance suggests regeneration of polyamine N is important; however, additional studies are required to test this hypothesis.

## Conclusions

Polyamine turnover rates in the SAB are rapid and comparable to those of amino acids, indicating that they are available to bacterioplankton despite their low concentrations (sub-nmol $L^{-1}$). The extremely low polyamine concentrations limit polyamine uptake and their potential to be significant sources of C and N to bacteria as compared to amino acids. The cycling of polyamines and DFAA varied spatially and were both strongly influenced by phytoplankton abundance and microbial activity; however, polyamine uptake was more closely correlated with phytoplankton biomass while DFAA uptake was more closely related to bacterial production. More polyamine carbon was assimilated than respired in our experiments. The fate of N in polyamines has not been studied; however, the close association between polyamine uptake and ammonium concentration suggests heterotrophic remineralization of amine groups in polyamine compounds.

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