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Seasonal cycling of putrescine and amino acids in relation to biological production in a stratified coastal salt pond

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Abstract. Seasonal cycles of concentrations and microbial uptake of dissolved free amino acids and the polyamine, putrescine, were followed during summer stratification of a coastal salt pond. Stratification began in May and was clearly seen in profiles of temperature, salinity, pH and alkalinity. Primary production exhibited a mid-August maximum and the O_2-H_2S interface shoaled at that time. POC and phytopigments roughly followed the pattern of primary production. Cycling of putresince, like the amino acids, was strongly influenced by primary production and microbial decomposition. Putrescine concentration profiles appeared to follow the pattern of primary production more closely, while amino acids appeared to follow the pattern of microbial production. The absence of production of putrescine during the decomposition of dissolved ornithine and the correlation of putrescine concentration with primary production suggest a direct source from algae in the water column.

Microbial uptake of amino acids and putrescine together accounted for 60–90% of the bacterial C production measured in oxic waters and almost 300% of that measured in the anoxic bottom layer. Since other organic carbon and nitrogen compounds are also being taken up, these data suggest that tracer uptake methods as we used them may overestimate the true microbial uptake rates, or release of other organic compounds by microbes occurs at the same time. Further work on carbon and nitrogen budgets is needed to resolve the apparent imbalance between organic C and N incorporation and bacterial production.

Introduction

Heterotrophic bacteria require organic compounds for growth. The uptake and removal of these compounds from seawater by marine bacteria is thought to be important in controlling the distribution of organic carbon in the sea (Williams et al. 1976; Billen et al. 1980; Larsson & Hagström 1982). By selectively removing organic compounds produced by planktonic organisms, bacteria can change the relative as well as absolute concentrations of labile organic compounds. Studies on bacterial uptake have frequently centered on organic nitrogen compounds, usually amino acids. Little is known about the bacterial cycling or organic nitrogen compounds other than amino acids since

measurement of these compounds in seawater has been very difficult due to their low concentration.

In this study, we measured heterotrophic uptake of dissolved free amino acids (DFAA) and putrescine in coastal seawater from spring to fall. The diamine putrescine (1,4-diaminobutane) is found in all living organisms and plays a regulatory role in protein and nucleic acid synthesis (Cohen 1971; Bachrach 1973). In cells, putrescine bonds electrostatically to nucleic acids, stabilizing the double helix of DNA, and can also bind to membrane phospholipids, thus protecting the membrane from lysis (Galston 1983). In addition to its role in cellular structure and metabolism, putrescine is also produced during the catabolic decarboxylation of arginine and ornithine during protein decomposition (Lehninger 1970; Metzler 1977).

Likely sources of both putrescine and amino acids in seawater are production during the decomposition of protein and other organic compounds and release from the intracellular pool of organisms, where amino acid concentrations, for example, are usually 10^3–10^6 times higher than in seawater (Simon & Azam 1989; Martin-Jézéquel et al. 1992). In addition, amino acids are known to be excretory products of zooplankton and flagellates (Andersson et al. 1985; Riemann et al. 1986; Nagata & Kirchman 1991). In spite of these likely sources, the concentration of these compounds in seawater is relatively low. For amino acids, bacterial uptake is mainly responsible for keeping amino acid concentrations low (Azam & Hodson 1977; Fuhrman 1987). Previous experiments have shown that low concentrations of putrescine are also at least partially due to bacterial uptake (Höfle 1984; Lee 1992). Here we present results of heterotrophic uptake experiments with natural populations under oxic and anoxic conditions in a small marine pond. To do this, we measured natural concentrations of amino acids and putrescine as well as incorporation and respiration of radiolabeled compounds over short periods of time. We also measured primary and bacterial production during these experiments. We continued to measure amino acid concentrations and putrescine turnover times in the pond throughout a summer season and correlated these with changes in biological activity in the pond.

Method

Sampling location and methods

Salt Pond is a small (29 ha), shallow (<6 m), eutrophic, semi-enclosed marine glacial basin located on Cape Cod, Massachusetts, USA. Kim & Emery (1971) describe the pond as highly stratified with an oxygenated, nutrient-depleted epilimnion and an anoxic, more saline hypolimnion. At our sampling location,

hydrogen sulfide concentrations in the anoxic waters are high (5 mM), and the depth below which H_2S is present varies seasonally from about 3 m in the summer to 5 m in the winter (Wakeham et al. 1984).

Water samples were taken from Salt Pond during August, 1983, and every other week beginning in May 1985 (referred to as SP1) and ending in October 1985 (referred to as SP13). The sampling site, in the deepest part of the pond (5.5 m), was located using two anchored moorings. A rowboat was tied off to these two moorings during sampling in order to maintain a uniform position from week to week. Water from depths of 1, 2, 3, 4, and 5 m was obtained using a peristaltic pump and silicone tubing lowered using PVC weights. The approximate flow rate was 1.25 l/min and the tubing was allowed to flush for about 2 minutes between sampling depths.

During the 1985 summer, the pond was sampled three times a day, at approximately 10 AM, 3 PM and 4 AM for ^{14}C-putrescine uptake, ^{3}H-putrescine uptake, dissolved free amino acid (DFAA) concentrations, sulfide presence, and temperature. (SP1 was sampled at 10 AM, noon, and 10 PM.) In addition, the following parameters were measured at 10 AM: Chl-*a* and phaeopigment concentrations, particulate organic carbon and nitrogen, pH, alkalinity, and salinity. ^{14}C-primary productivity measurements were begun at 10 AM and continued for 5–6 h. During two sampling periods in May, bacterial production, DFAA assimilation, and putrescine concentrations were also measured. Water for Chl-*a*, particulate organic carbon and nitrogen, and label uptake experiments was pumped into 2-1 polycarbonate bottles and transported back to the laboratory (~20 min) for processing. Samples for amino acid and putrescine concentrations and alkalinity were obtained in separate polyethylene bottles and placed on ice for transport back to the lab. Water samples for ^{14}C-productivity were taken individually, radioisotope was added, and the bottles were hung on an incubation line attached to the permanent mooring within 10 minutes of collection.

Temperature and sulfide presence were measured at the sampling site. Water for these samples was pumped into 50-ml flasks. For the sulfide measurement, 5 ml of *p*-phenylenediamine HCl reagent and 5 ml of ferric chloride reagent, prepared according to Strickland & Parsons (1972), were added to the flask and the presence of sulfide was determined visually by the formation of Lauth's violet (or the appearance of a blue-violet color). Salinity samples were pumped into 250 ml glass bottles and tightly capped. Salinity was calculated from measurements of sample conductivity and comparison to standard seawater on an Autosal salinometer. Carbonate alkalinity was measured according to the method of Strickland & Parsons (1972). The pH of water samples was measured on an Orion Model 399A pH meter.

Chlorophyll a

Water samples (10–250 ml) were filtered in duplicate through Whatman 2.4 cm GF/C filters. The filters were placed in darkened scintillation vials and 5–10 ml of methanol were added. The samples were refrigerated in the dark overnight, then homogenized with a vortex mixer and refiltered. Chl-*a* and phaeopigment concentrations in the methanol extract were determined on a Turner fluorometer and calibrated using a Chl-*a* standard (Sigma) (Parsons et al. 1984). Duplicate analyses agreed to within ±5%.

Particulate organic carbon and nitrogen

Samples for particulate organic carbon and nitrogen were obtained by filtering pond water onto precombusted (450 °C, 16 hrs.) Whatman 2.1 cm GF/C filters. The filters were dried approximately 6 hours in a 60 °C oven. Particulate carbon and nitrogen on the filters were measured on a Perkin-Elmer CHN Analyzer. Duplicate analyses agreed to within ±10%.

Amino acid and putrescine concentrations

Water samples used for the determination of DFAA concentrations were filtered through a 0.2 μm Nuclepore filter. The filtrate was collected and frozen for later analysis. The amino acid concentrations in these samples were determined by gradient HPLC using either Beckman 110A or Varian Model 5000 solvent pumps equipped with a 25-cm Beckman Ultrasphere ODS 5-μm column and either a Kratos FS 950 or FS 970 fluorometer with a Shimadzu C-R3A integrator. The sample was derivatized before injection with *o*-phthaldialdehyde (OPA) and analyzed according to Jones et al. (1981) and Lindroth & Mopper (1979). Concentrations were determined by comparison to authentic amino acid standards. Duplicate analyses agreed to within ±20%. In a number of the samples, it was necessary to remove the ammonium which overwhelmed any signal from some of the later-eluting amino acids. Ammonium was removed by mixing 1 ml of the water sample with 10 μl of pH 10.5 borate buffer. This mixture was then evaporated to dryness in a centrifugal evaporator. Ammonium-free double-distilled water (1 ml) was used to reconstitute the sample which was then derivatized and analyzed as usual. Eliminating the interference from ammonium using this technique appeared to proceed without loss of amino acids or contamination as judged by comparing concentrations of the acidic (early eluting) amino acids in treated and untreated water samples.

Ambient putrescine concentrations were measured in Salt Pond using reversed-phase HPLC of OPA derivatives. Using an HPLC system similar

to that for the amino acids, putrescine in the 1985 samples was eluted using 85% methanol/15% 0.05 M sodium acetate (pH 5.9) buffer isocratically at 1 ml/min. The amino acids elute before 15 minutes, while putrescine elutes at approximately 18–19 minutes and another polyamine, cadaverine (1,5-diaminopentane), can be seen occasionally in samples at about 22 minutes. The 1983 samples were run under the conditions reported in Lee (1992). Only samples from 1983 and SP1, SP2, and SP6 were analyzed because the remaining samples were lost in a freezer accident. Duplicate analyses agreed to within ±20%.

Primary productivity measurements

Primary production rates in Salt Pond were measured at one meter intervals from the surface of the pond to a depth of 5 m. At each depth, two light and one dark bottle were used for measuring the incorporation of ^{14}C-sodium bicarbonate at each depth. The final activity of $H^{14}CO_3^-$ added to each bottle was approximately 0.01 μCi/ml. Samples were pumped into glass incubation bottles, the radioisotope was added and the bottles were immediately placed back in the water on a moored incubation line. Incubations were usually begun at 10 AM and lasted 5–6 h. At the end of the incubation period, bottles were retrieved from depth, placed in a dark bucket and returned to the lab for processing. A 1-ml sample was withdrawn for counting the exact activity of ^{14}C added, and added to a scintillation vial containing 500 μl Protosol (Dupont). The remaining sample was used for duplicate measurement of the radioactivity incorporated into the particulate phase. The samples were filtered onto GF/C filters, the filters were then rinsed with prefiltered pond water and placed into a scintillation vial containing 1 ml 10% 12 N HCl. The filters were shaken in the acid and allowed to stand open overnight (~12 hours) in order to degas any unincorporated $H^{14}CO_3^-$. Radioactivity in both the total samples and filtered samples were determined by liquid scintillation counting (LSC) in a Packard Tricarb counter using Aquasol as the scintillation cocktail. Counting was to 1% error and quench corrections were made by the channels ratio method. Duplicate analyses usually agreed to within ±15%. Variability was higher in anoxic waters.

Heterotrophic assimilation

Heterotrophic uptake of DFAA and putrescine was measured in two diel experiments. Both respiration to CO_2 and incorporation into the particulate fraction were measured (Hobbie & Crawford 1969; Billen et al. 1980). For the amino acid experiments, either ^{14}C-ornithine or an equimolar mixture of ^{14}C-labeled glutamic acid, serine, glycine and alanine was added to 50-ml

triplicate water samples from each depth and to formalin-killed controls. Specific activities of the uniformly-labeled isotopes ranged from 58 (ornithine) to 285 mCi/mmol (glutamic acid). Addition of the tracers (0.005 μCi/50 ml) caused an increase in concentration of 1.7 nM for ornithine and 0.3 to 0.8 nM for the other amino acids.

The samples were incubated in sealed flasks at the *in-situ* pond water temperature for 20 to 30 minutes. During this period, a maximum of 10% of the tracers were taken up in most of the samples. The incubations were stopped by adding buffered formalin (2/3 37% formaldehyde, 1/3 saturated sodium borate) to a final concentration of 2%. Then, 30 ml were removed from the incubation flasks and filtered through 0.2 μm Metricel GA-8 membrane filters. Radioactivity on the filters was assayed by LSC. To determine heterotrophic respiration of the amino acids, the remaining 20 ml of incubation samples were acidified by injecting 0.5 ml 18 N H_2SO_4 into the sealed flasks. A filter-paper wick was soaked with 500 μl Protosol and suspended above the solution in each flask. The flasks were shaken on a rotary shaker for one hour to allow the $^{14}CO_2$ to degas and become trapped on the wick. The wick was then removed from the bottle and placed into a scintillation vial containing 200 μl 1 M TRIS-HCl, pH 7.0 to neutralize the Protosol and counted by LSC as above. Assimilation (gross uptake) of all individual amino acids was determined as incorporation (filter activity) plus respiration (activity on wicks). Assimilation of DFAA was calculated assuming that the amino acid mixture was representative of the rest of the DFAA pool. Replicate analyses agreed within $\pm$15%.

Putrescine uptake was measured in a similar fashion to amino acid uptake using the ^{14}C-labeled tracer. Methods used in 1983 are reported in Lee (1992). In the 1985 experiments, 50-ml water samples were placed in sealed flasks and labeled putrescine ([1,4-^{14}C]-putrescine; specific activity 104.6 mCi/mmol) was added, 0.57 nM in SP1-3 and 7.64 nM during SP4-13. The samples were incubated in the dark at room temperature (~24 °C) for 15 minutes (SP1-3) and 30 min and 1 hr (SP4-13). Total activity was counted in 500 μl of the sample. A 10–20 ml fraction was filtered through 0.2 μm membrane filters as above in order to measure heterotrophic incorporation. Respiration of putrescine was measured by collecting and counting $^{14}CO_2$ from a sample that was poisoned with 3.5 ml of borate-buffered formalin solution to a final formalin concentration of 1.2 %. Analyses of duplicate samples was not conducted, but respiration after 30 min and 1 hr in the individual samples generally agreed well ($\pm$15%) and were averaged.

Because we were concerned that the concentrations of ^{14}C-tracer added approached 10% of the ambient putrescine concentrations, and thus could not be considered a true 'tracer', we also conducted experiments with tritiated

putrescine during SP4-13. For these experiments, 50-ml water samples from 1 and 5 m depth were incubated in duplicate in glass bottles with 0.47 nM added [2,3-^{3}-H]-putrescine (specific activity 47.5 Ci/mmol). These samples were kept in the dark at room temperature for 30 minutes or one hour. Ten or 20 ml samples, depending on pond biomass, were filtered onto 0.2 μm membrane filters as above and counted by LSC. Samples (1 ml) for the measurement of total radioactivity were counted directly. Incorporation of label could then be directly calculated and compared with the ^{14}C results. Respiration (to ^3H_2O) was not measured. Neither the ^{14}C nor the ^3H-putrescine were uniformly labeled. We make the assumption here that degradation of both compounds went completely to CO_2 and H_2O.

To determine whether ornithine was a precursor of putrescine, incubation experiments with ^{14}C-ornithine were conducted just before SP1, after SP6, and during SP7 and SP8. Samples from 1, 3, and 5 m were spiked with 27 nCi of ^{14}C-ornithine, incubated for various times up to 24 h, and filtered through Whatman GF/F filters. Ornithine and putrescine in these time-course samples were separated by HPLC as OPA derivatives; the peaks were collected by fraction collector and the eluant mixed with Aquasol and counted by LSC.

Bacterial production

Production of bacteria in the pond was determined from ^3H-thymidine incorporation into bacterial DNA (Fuhrman & Azam 1980). ^3H-thymidine was added to 25-ml triplicate water samples (one a formalin control) to a final concentration of 5 nM. After 30 min, the incubations were stopped with formalin. The samples were treated with cold trichloroacetic acid, filtered through 0.22 μm membrane filters and radioassayed. Replicate analyses agreed within $\pm 10\%$. Incorporation of thymidine was converted to cell production using a factor of 2.1×10^{18} cells/mol (Smits & Riemann 1988). Carbon flux of the bacterial production was calculated using a biomass of 0.35 pg C/μm^3 (Bjørnsen 1986) and an estimated mean cell volume of 0.1 μm^3.

Results and discussion

Contribution of DFAA and putrescine assimilation to bacterial production

During the first two sampling dates in May, heterotrophic uptake rates of putrescine and an amino mix were measured. These are gross rates and include both respiration and incorporation. In early May, temperature stratification was just beginning in the pond although salinity stratification occurred

throughout the study period. Between the first (SP1) and second (SP2) sampling times, water temperature increased and temperature stratification became evident (Fig. 1). The chlorophyll concentration maximum near the bottom at those two times was most likely a remnant of an earlier spring phytoplankton bloom. Spring blooms in April have been observed previously in Salt Pond (Wakeham et al. 1984). Chl-*a* levels during SP1 reached 800 μg/l, the highest measured during this experiment. The primary productivity maximum at 5 m during SP1 moved up in the water column to 3 m at SP2. The bacterial production maximum remained near the bottom at both times even though sulfide become apparent at 5 m during SP2. Bacterial production exceeded primary production during SP1 (Table 1). The 2-fold higher bacterial production relative to primary production between 0 and 4 m suggests that bacteria were still using organic C from a previous spring bloom. By SP2 primary production in the upper waters was higher and bacterial production no longer exceeded primary production.

Concentrations, turnover times and uptake rates of putrescine and amino acids at 3 times each day during SP1 and SP2 are shown in Figs. 2 and 3. There was little difference in the general patterns observed in these parameters for either putrescine or the amino acids at the three times of day, although amino acid concentrations and uptake at 5 m were noticeably higher during the late afternoon. The general pattern in composition of individual amino acids also did not show any particular trend with time of day during these two sampling times. Dominant amino acids were glycine, serine, alanine and glutamic acid (data not shown). Concentrations and uptake rates of putrescine largely followed the general pattern of primary production where highest values were at the bottom during SP1 and at 3 m during SP2. Concentration and uptake of amino acids appeared to be more closely coupled with bacterial production, as the highest values were at the bottom during both sampling times.

A comparison of putrescine incorporation using both ^{14}C- and ^{3}H-putrescine shows that a similar pattern in turnover time was obtained from the two techniques (Fig. 4). The concentration of ^{3}H-putrescine used was 16 times less than that of the ^{14}C-compound. However, only the turnover due to incorporation of label could be compared since we did not measure respiration of the tritiated compound. Incorporation of ^{14}C-putrescine throughout the summer accounted for between 40–75% of the total label that was taken up in the upper 3 m and 60–100% in the anoxic waters (data not shown). Thus, a smaller fraction of the labeled putrescine taken up was respired than incorporated. Incorporation of amino acids during SP1 and SP2 accounted for between 50–65% of the total label taken up.

The contribution of DFAA and putrescine assimilation to the carbon and nitrogen budget of the pond during SP1 and SP2 was evaluated (Tables 1 and

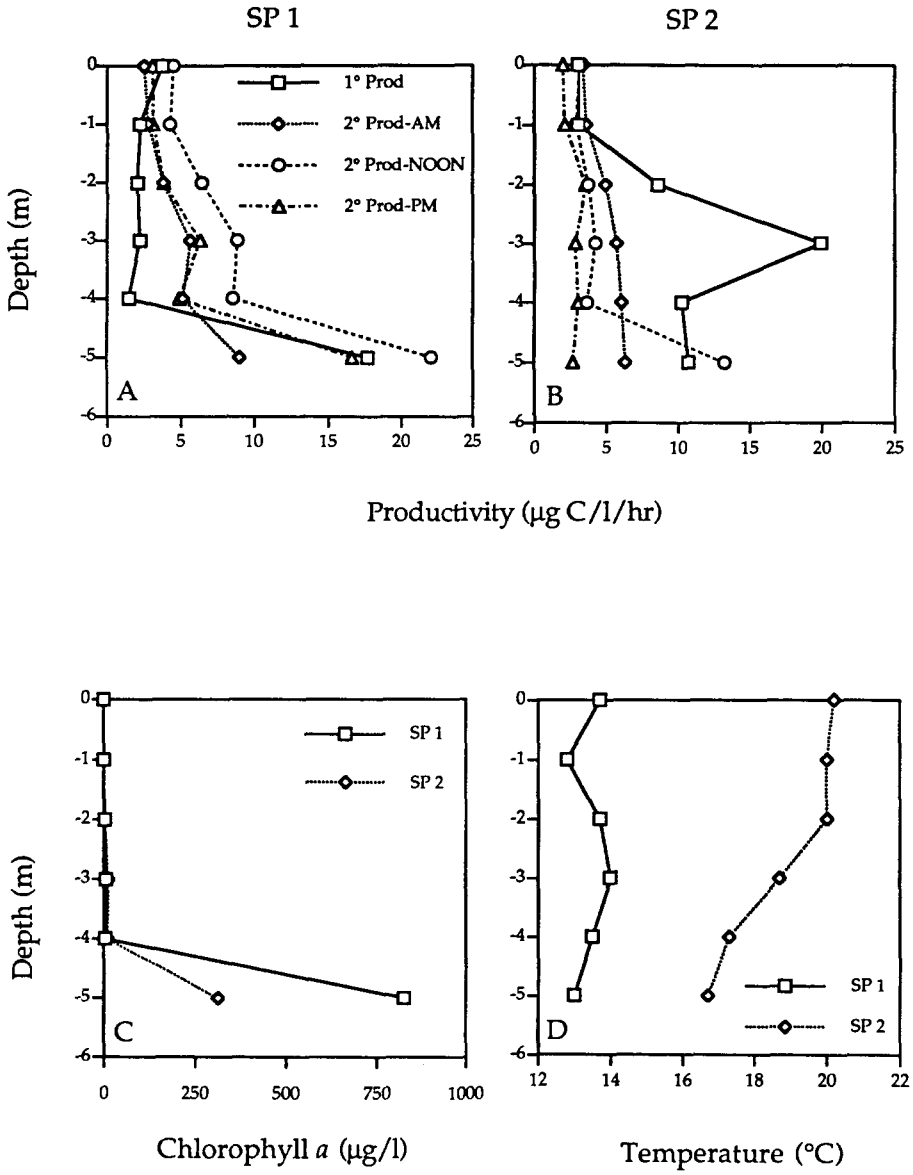

Fig. 1. Primary (1°) and bacterial (2°) production (A, B), Chl-*a* concentrations (C) and temperature (D) during the first two sampling periods.

2). During SP1, DFAA incorporation was about half of the total bacterial C production and about equal to bacterial N production. At 5 m during SP2, DFAA incorporation was an unrealistically high proportion of both bacterial

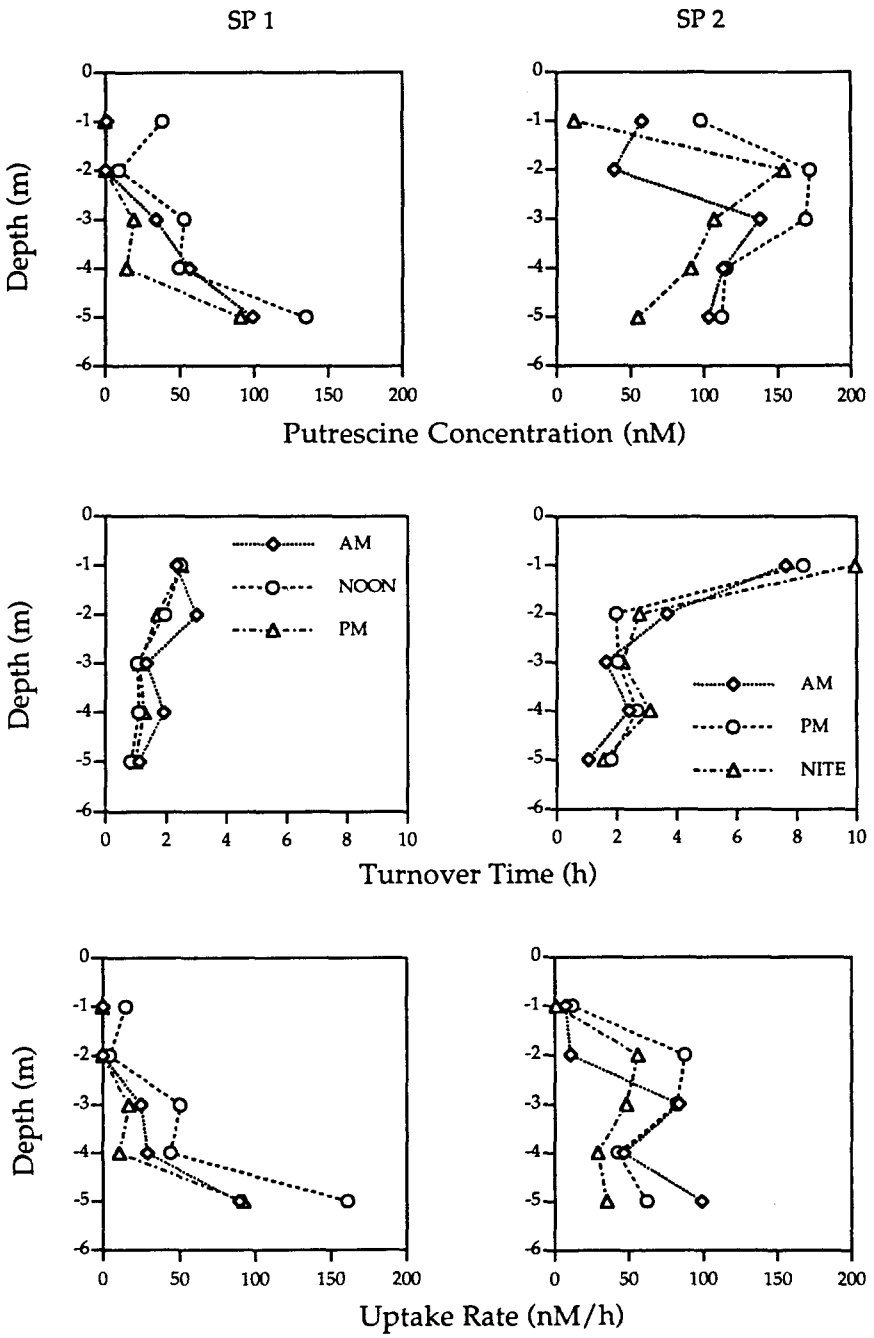

Fig. 2. Concentration, turnover time, and uptake rate of putrescine during the first two sampling periods.

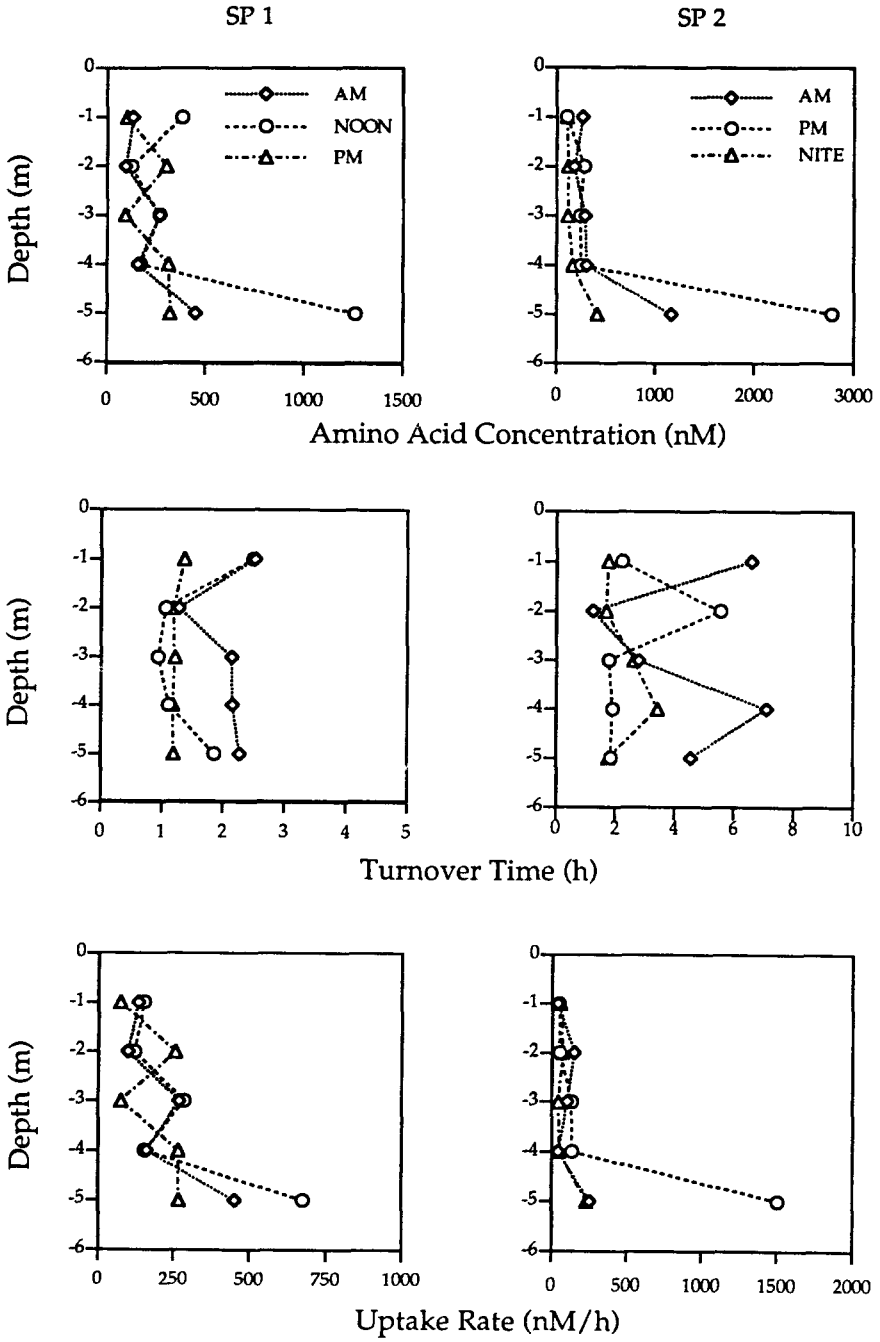

Fig. 3. Concentration, turnover time, and uptake rate of amino acids during the first two sampling periods. Uptake rate was calculated by dividing the total amino acid concentration by the average turnover time of the amino acid mixture.

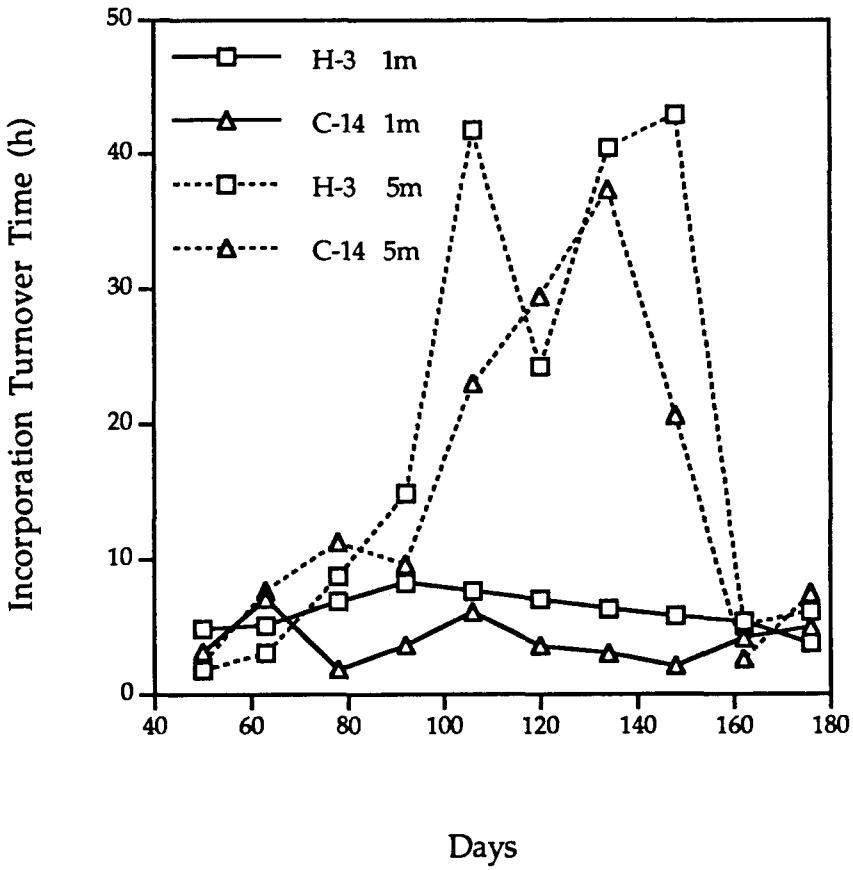

Fig. 4. Comparison of turnover time due only to incorporation of ^{14}C-putrescine and ^{3}H-putrescine at 2 depths during SP4-13. Time is days after May 1 (SP1).

C and N production. Sulfide was present at 5 m at this time and since very few anaerobic bacteria incorporate thymidine (Wellsbury et al. 1993), bacterial incorporation was probably underestimated. On the other hand, previous workers have also found C and N imbalances using similar approaches. Keil & Kirchman (1991) found that organic nitrogen incorporation was 14 to 246% of the bacterial N production measured. They suggested that this might be due to either incorrect bioconversion factors or to 'luxurious' nitrogen uptake.

In another coastal pond, Jørgensen et al. (1993) reported that 148% of the bacterial production was sustained by organic nitrogen incorporation. They suggested that the nitrogen incorporation rate measured did not represent *net* bacterial nitrogen incorporation, since other organic nitrogen compounds like methylamines or urea could have been released simultaneously. This view is

Table 1. Bacterial carbon incorporation in Salt Pond on May 1 (SP1) and May 8 (SP2)[a].

	May 1		May 8	
Depth	0–4 m	5 m	0–4 m	5 m
Bacterial production				
May 1 (μg C/l/12 h)	319	202		
May 8 (μg C/l/17 h)			291	144
Primary production				
May 1 (μg C/l/12 h)	142	213		
May 8 (μg C/l/17 h)			763	183
Bacterial production/				
Primary production (%)	225	95	38	79
DFAA incorporation[b]				
May 1 (μg C/l/12 h)	152	115		
May 8 (μg C/l/17 h)			106	389
Putrescine incorporation				
May 1 (μg C/l/12 h)	29	40		
May 8 (μg C/l/17 h)			111	37
DFAA incorporation/				
Bacterial production (%)	48	57	36	264
Putrescine incorporation/				
Bacterial production (%)	9	20	38	26

[a] Carbon incorporation rates indicate integrated values of 1 l of water from 0, 1, 2, 3 and 4 m depth, or 5 m depth, over the studied periods. Respiration rates are not included in these calculations.
[b] Assuming 3 atoms of C per mol amino acid.

supported by the putrescine N incorporation rates which when added to the amino acid incorporation are more than 100% of the bacterial production. The carbon to nitrogen imbalance in contribution of amino acids and putrescine suggests that compounds with a C:N of about 0.5 would be regularly excreted by bacteria. The common methylamines have a C:N in the range of 0.9–2.6; that of urea is 0.4. Obviously more work on carbon budgets using a variety of organic compounds is necessary to resolve this apparent imbalance between organic C and N incorporation and bacterial production. Other processes that decouple apparent C and N uptake rates must also be considered, such as extracellular oxidation of amino acids and amines by phytoplankton (Palenik & Morel 1990; Pantoja & Lee 1994).

Table 2. Bacterial nitrogen incorporation in Salt Pond on May 1 (SP1) and May 8 (SP2)[a].

Depth	May 1		May 8	
	0–4 m	5 m	0–4 m	5 m
Bacterial production[b]				
May 1 (μg N/l/12 h)	64	40		
May 8 (μg N/l/17 h)			58	29
DFAA incorporation[c]				
May 1 (μg N/l/12 h)	66	49		
May 8 (μg N/l/17 h)			46	166
Putrescine incorporation				
May 1 (μg N/l/12 h)	17	24		
May 8 (μg N/l/17 h)			64	21
DFAA incorporation/				
Bacterial production (%)	103	122	78	575
Putrescine incorporation/				
Bacterial production (%)	27	60	110	72

[a] Nitrogen incorporation rates indicate integrated values of 1 l of water from 0, 1, 2, 3 and 4 m depth, or 5 m depth, over the studied periods. Respiration was not included in these calculations.
[b] Assuming the carbon production values shown in Table 1 and a bacterial C:N of 5:1.
[c] Assuming 1.1 atoms of N per mol amino acid.

Sources of putrescine

The exact source of dissolved putrescine in Salt Pond is unknown. Putrescine is found in every living cell, but is also a decomposition product of the protein amino acid arginine, with the non-protein amino acid ornithine as an intermediate. Dissolved putrescine could result from decomposition of protein either within the water column, or in the sediments with subsequent diffusion into the water column. The maxima in uptake rates of putrescine at SP1, 2 and 6 coincided with the depths of maximum primary productivity (but not chlorophyll) suggesting a source from actively photosynthesizing algae (Fig. 5). To investigate whether ornithine decomposition is a source of putrescine in the water column, we measured ornithine uptake in the pond and conversion of ^{14}C-ornithine to ^{14}C-putrescine. Ornithine turnover times were longer (5–8 h for SP1 and 10–50 h for SP2; data not shown) than turnover times of the amino acid mix and putrescine shown in Figs. 2 and 3. They followed the same general pattern as putrescine with a surface (1 m) maximum for SP2 (Fig. 2). Ornithine was only detected in the 5 m samples

during SP1 and 2 so addition of label (1.7 nM) was not at tracer level in the 1–4 m samples.

During the labeled ornithine incubations, an attempt was made to measure the appearance of labeled putrescine by HPLC. At no time did we see production of ^{14}C-putrescine from ^{14}C-ornithine, although we incubated the samples until 95% of the ornithine disappeared in the oxic incubations (8 h) and 10% disappeared in the anoxic experiments (24 h). It is possible that any putrescine produced was taken up before we could measure it, since the turnover time for putrescine was shorter than that of ornithine. However, a transitory pool would have been expected due to isotope dilution.

The fact that ornithine was only present in bottom waters during SP1 and 2 (and was always highest at 5 m during the rest of the study) suggested a source of ornithine in anoxic waters or sediments, presumably from protein (arginine) decomposition. Putrescine concentrations were highest near the bottom on 4 of the 5 days for which we have data (Fig. 6). Although we have no sediment incubation data from Salt Pond, incubation of cores from Limfjord in Denmark showed an elevation in putrescine of 108 nM in 7 h, suggesting that diffusion of putrescine out of the sediment may occur.

Seasonal dynamics

Throughout the summer days after May 1, Salt Pond progressed through many of the changes expected due to seasonal increases in light and temperature. The Pond was stratified by the end of May and reached maximum temperature and stratification in mid-August (Day 106, Fig. 7A). During the end of August, cooler weather and heavy rains were reflected in the temperature profiles and even more strongly in the surface salinity values (Fig. 7B). To complete the seasonal picture, Hurricane Gloria passed through on September 27, reducing stratification in the Pond, although not completely mixing it. Water temperature was more uniform throughout the water column for a period of two weeks although the average temperature decreased during this time. Salinity, alkalinity, and pH all showed the effects of mixing by the hurricane, although complete mixing of these parameters did not occur at any time during this study (Fig. 7B and unpublished data).

The depth of maximum primary production moved up the water column as stratification increased through the summer (Fig. 8A). Primary production was highest at the bottom (5 m) at SP1, 3 m by SP2, 2 m by SP3, and the surface (0 m) by SP6 where it remained except for the rainy period before the hurricane and in the Fall (Oct. 23 – SP13) when temperatures were again at early May levels. Primary productivity in the surface waters (0 m) increased to a maximum in mid-August at the maximum water temperature (Fig. 7A). Peak values of about 80 μgC/l/h in surface waters agree well with those

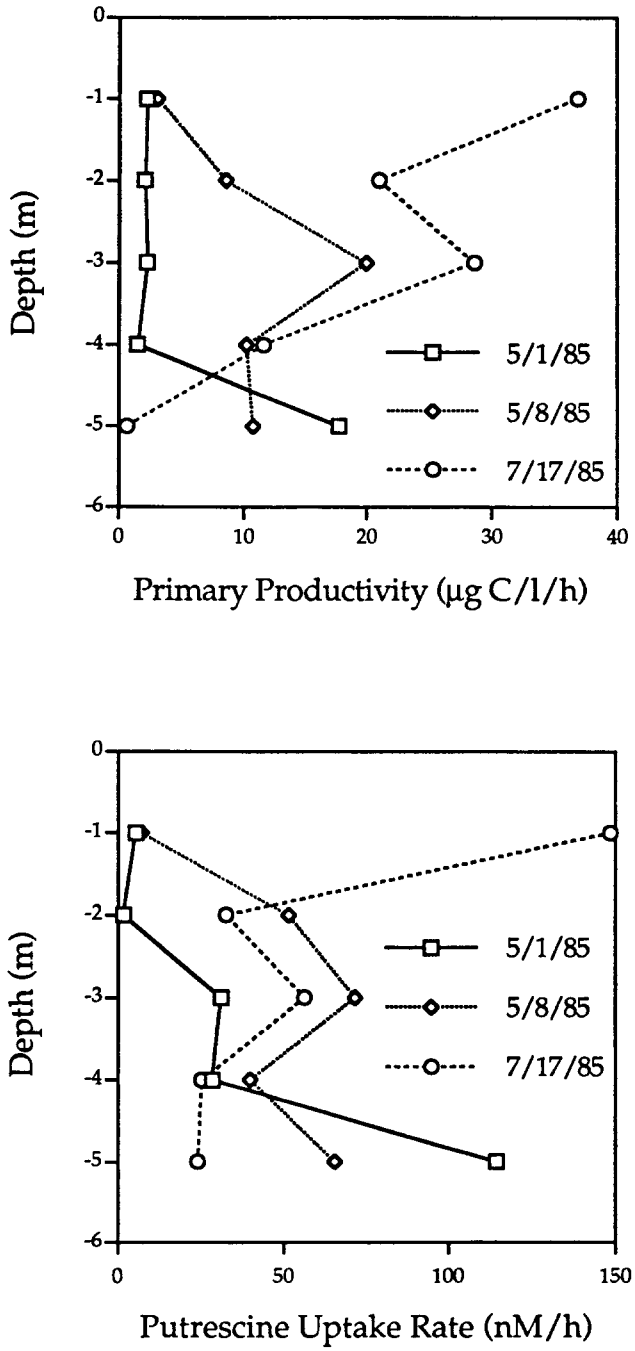

Fig. 5. Primary production and putrescine uptake rate with depth for SP1, 2 and 6. The uptake rates for SP1 and 2 were obtained by dividing putrescine concentrations by label turnover time at the 3 times sampled and then averaging the 3 profiles.

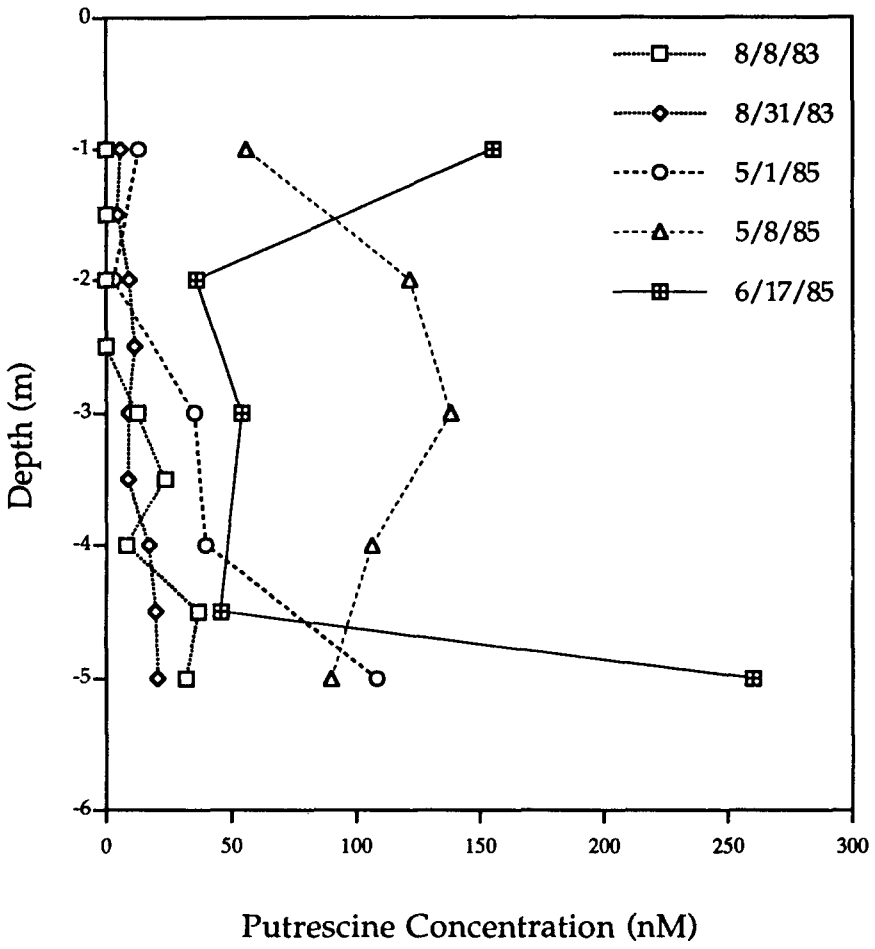

Fig. 6. Putrescine concentrations in 1983 and during SP1, 2 and 6. Concentrations for SP1 and 2 are averages of the 3 sampling times.

reported by Lohrenz et al. (1987) for 1983. During the cool, rainy period in late August and early September before the hurricane, primary production decreased dramatically. Fig. 7B clearly shows the effect of the rain water in decreasing the surface salinity during this time. The highest productivity measured in the Pond occurred in surface waters after the hurricane had passed through. Presumably the reduction in stratification caused by the hurricane resulted in a replenishment of nutrients from the deeper to the surface waters, stimulating productivity.

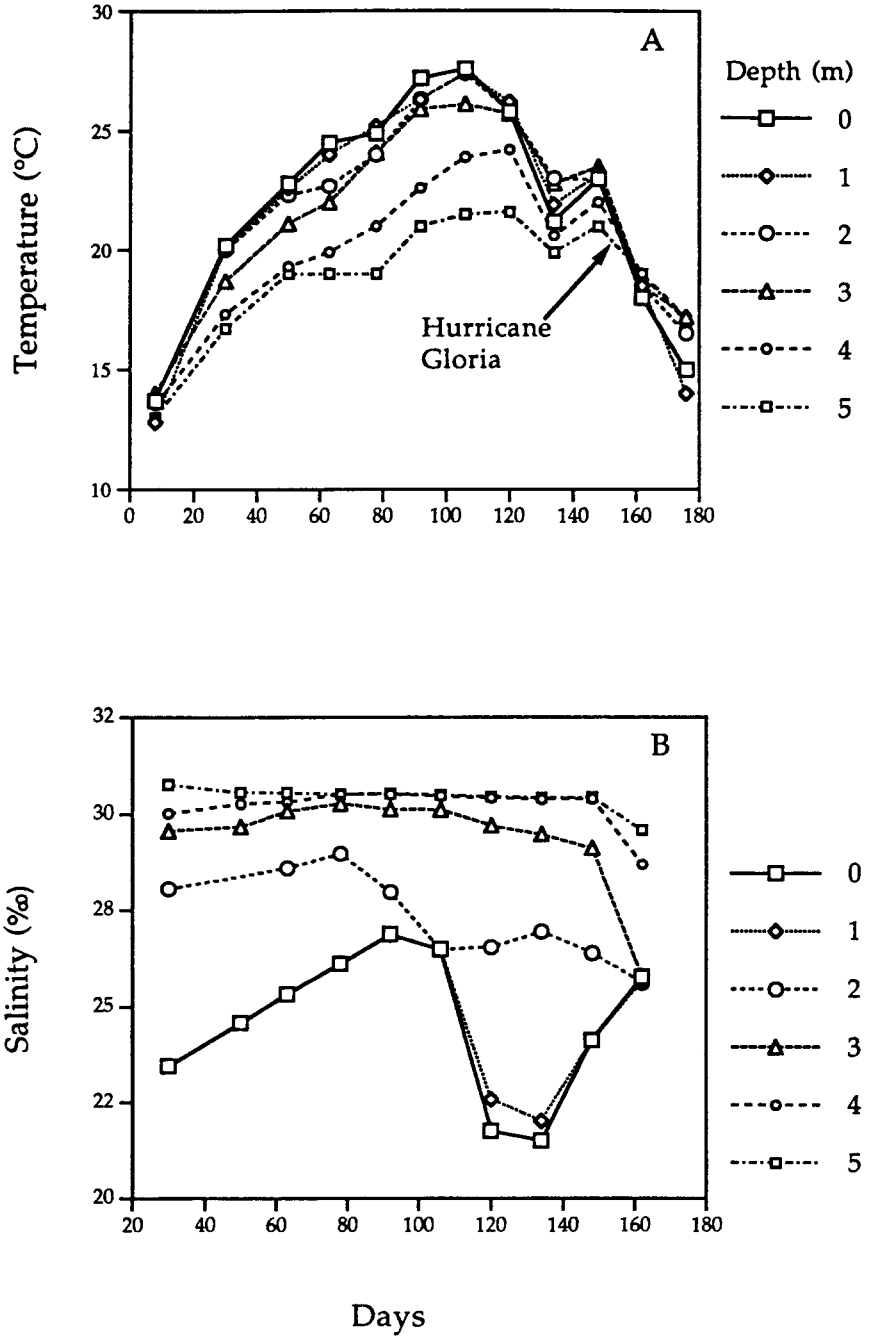

Fig. 7. Temperature (A) and salinity (B) at 6 depths taken at 12 sampling times (SP2-13) Time is days after May 1 (SP1).

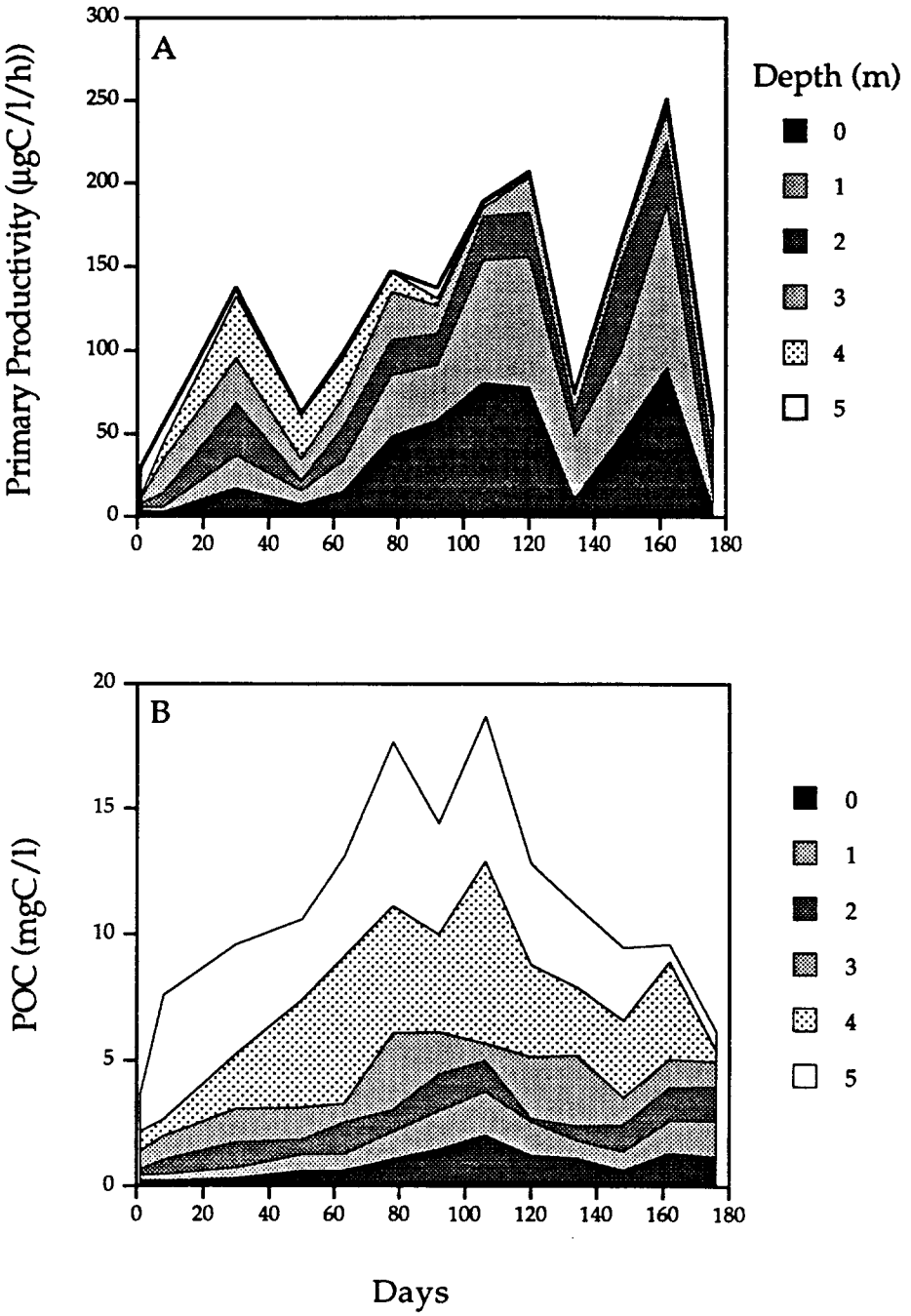

Fig. 8. Primary productivity (A) and particulate organic carbon (B) at 6 depths taken at 13 sampling times (SP1-13). Time is days after May 1 (SP1). Concentrations are additive for ease of comparison at different depths, but the total is not representative of any integrated value.

Changes in alkalinity and pH in the pond (data not shown) were compatible with the likely processes occurring there (Stumm & Morgan 1981; Morel 1983). Alkalinity in the upper 3 m ranged between 0.5 and 2 meq/l, and clearly increased during the early June, mid-August and early October primary productivity maxima, as would be expected from photosynthetic assimilation of NO_3^-. In the deeper waters, alkalinity was >3 meq/l, the upper detection limit of our analyses. Higher deep-water values would be caused by the decomposition of organic matter via denitrification and sulfate reduction. pH ranged between 6.6–7.6 below 3 m and 7.5–8 in the upper 2 m. A small increase in surface water pH values accompanied the increase in alkalinity during the productivity maxima due to removal of CO_2 from the water. Decreases in pH in the deeper water during the productivity maxima are reflective of CO_2 production during organic matter decomposition. Both pH and alkalinity were most strongly stratified in mid-August.

Surface (0 m) POC values correlate generally with primary productivity, peaking in mid-August at about 2 mgC/l (Fig. 8B). This pattern agrees with the findings of Lohrenz et al. (1987) for 1983, although POC values were about 8 times higher then. POC concentrations below 3 m were higher than in surface waters even in May (SP1-3). The high particle concentrations in deeper water could originate from resuspension of bottom sediments. However, the strong stratification of the Pond and the lack of correlation between high POC and mixing events (Hurricane Gloria) argue against this as a major process here. An alternative explanation is that POC in the bottom waters in May was a remnant of a spring bloom earlier in the year that had maximum productivity at 5 m. High POC values in deeper waters during mid-summer probably resulted from sinking material derived from surface production, such as sinking phytoplankton aggregates and zooplankton fecal pellets.

We have no information about the zooplankton in Salt Pond; however, the pigment data suggest that zooplankton grazing increased during the summer. Early in May (SP1) before temperature stratification was strong and H_2S was present in the water column, the peak in primary production was near the bottom of the pond (5 m, Figs. 1A & 8A). At that time the pigment data (Fig. 9A) show the highest level of chlorophyll measured in the pond at 5 m. Phaeopigments, a product of grazing organisms (Welschmeyer & Lorenzen 1985), were not as prevalent as later in the summer. Primary productivity in early May was not as high as later in the summer and the community of grazing organisms may not have been present to the extent necessary to remove the POC formed at that time.

The pigment data clearly shows the progression of the primary productivity maximum up the water column throughout the summer (Fig. 9). Until the beginning of July (Day 63), the Chl-*a* maximum was in the deeper waters.

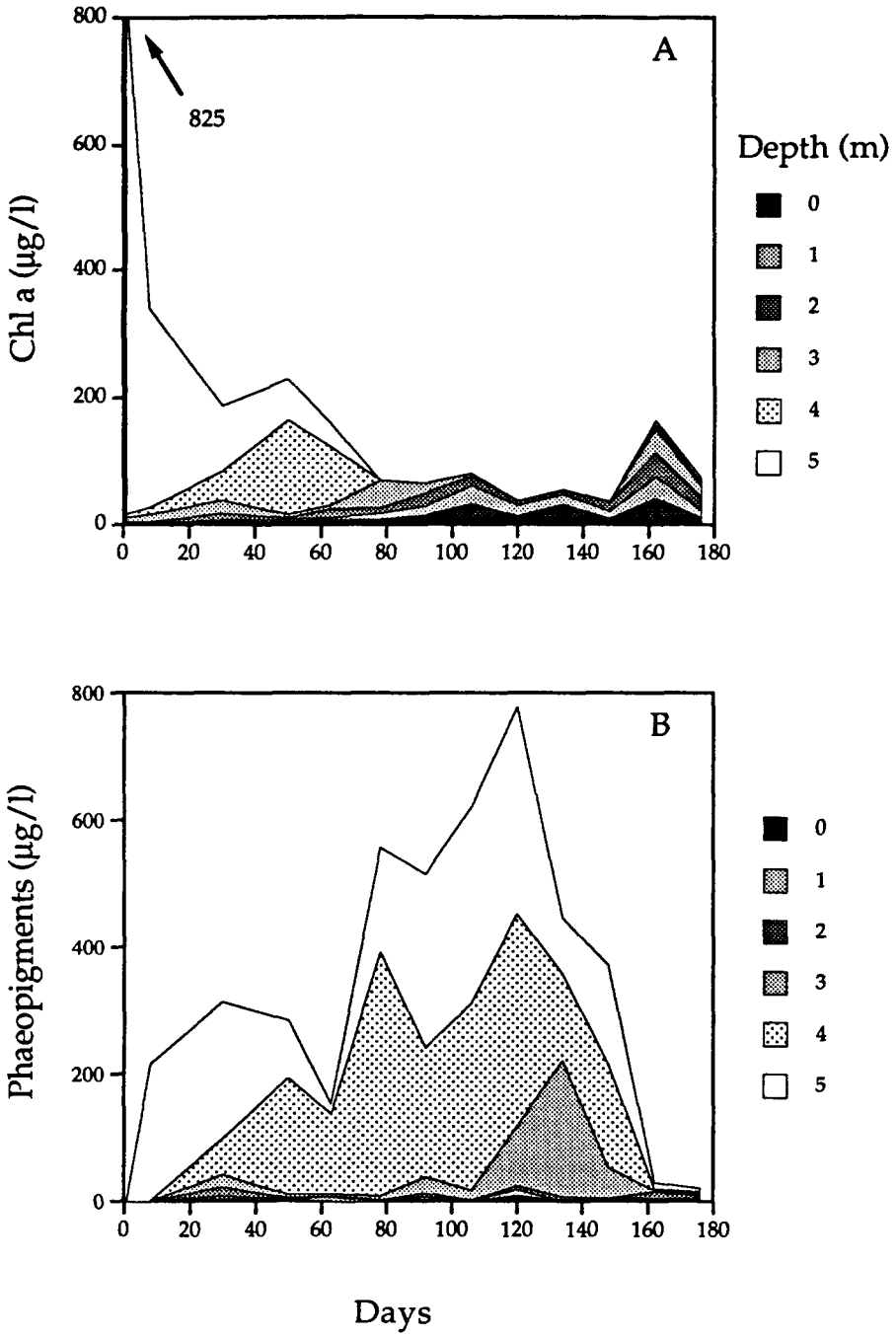

Fig. 9. Chlorophyll *a* (A) and phaeopigment (B) concentrations at 6 depths taken at 13 sampling times (SP1-13). Time is days after May 1 (SP1). Concentrations are additive for ease of comparison at different depths, but the total is not representative of any integrated value.

From July on, the productivity maximum was in the more shallow waters and is reflected by the higher Chl-*a* values there. Maximum phaeopigment concentrations were always in the deeper waters throughout the summer. The high productivity which occurred in the surface waters after Hurricane Gloria is reflected in the shallow pigment data but is surprisingly not seen in the POC or deeper pigment values. Clearly more information about zooplankton dynamics in the pond is necessary to explain the pigment distributions. The picture that emerges of Salt Pond during summer stratification is of a thick, cool bottom water with low oxygen, high alkalinity, POC and pigments (vichyssoise) overlain by a lens of warmer, fresher water higher in O_2 and lower in organic matter (consommé).

Seasonal changes in organic nitrogen cycling

Average total amino acid concentrations were highest in the bottom waters in early May (Fig. 10) when both primary and secondary productivity were higher there. Later in the summer, the DFAA maximum moved up the water column to 3–4 m in mid-August, always below the O_2-H_2O interface. This maximum was always more marked during the PM sampling period, and to a lesser extend in the NITE profiles, than in the AM samples (data not shown). AM profiles were usually fairly uniform with depth. Changes in the DFAA pools may have been correlated with changes in bacterial production, but we have no bacterial production data after SP2. Alternatively, DFAA concentration changes may have been influenced by activity of photosynthetic bacteria, e.g., by extracellular release. Wakeham et al. (1987) measured bacteriochlorophyll at the same site and time and found more present above 5 m in May, and maximum concentrations between 3–4 m on August 27. DFAA concentration maxima were also at these depths at these times, thus correlating more closely with biomass of photosynthetic bacteria than with primary production, which occurred higher in the water column. The individual amino acid composition of the DFAA also suggested a strong bacterial influence, but due to heterotrophic rather than phototrophic bacteria. High DFAA concentrations observed at 3–4 m during SP8 and 10 were characterized by an abnormally high proportion of β-alanine (data not shown), a bacterial decomposition product of aspartic acid and uracil (Lee & Cronin 1982). Ornithine, the microbial decomposition product of arginine, was present throughout the water column during SP9-12. Further study is necessary to elucidate the exact source of DFAA in Salt Pond.

Putrescine turnover times were low (<10 h) in the upper 3 m throughout the study period (Fig. 11). The turnover time at 4 and 5 m gradually increased as the depth at which sulfide was present became more shallow (Fig. 10) until a maximum of 63 h was reached at SP10. This high turnover

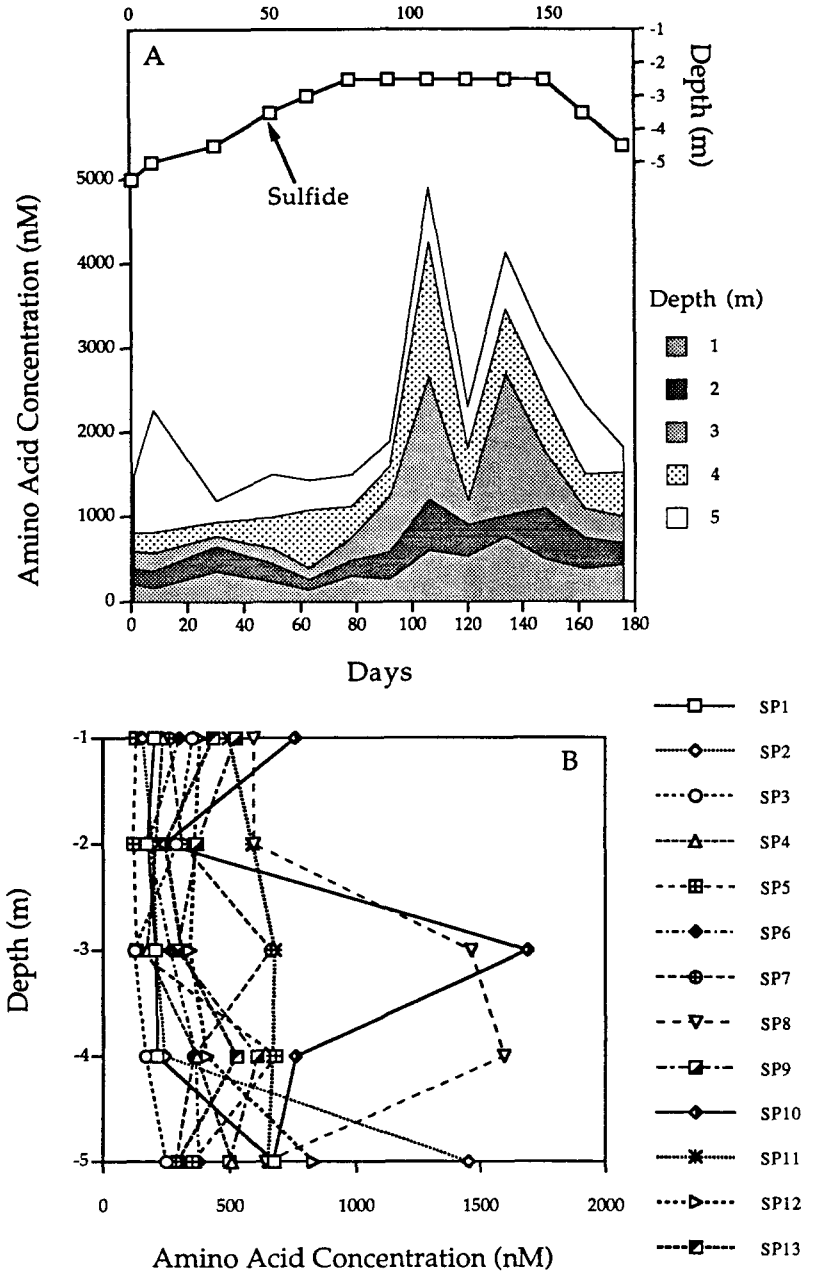

Fig. 10. (A) Total dissolved free amino acid concentrations at 5 depths taken at 13 sampling times (SP1-13). Time is days after May 1 (SP1). Concentrations are additive for ease of comparison at different depths, but the total is not representative of any integrated value. Depth at which sulfide is present is shown. (B) DFAA concentrations with depth at the various sampling times. Concentrations shown in both (A) and (B) are averages of three samples taken at different times of day.

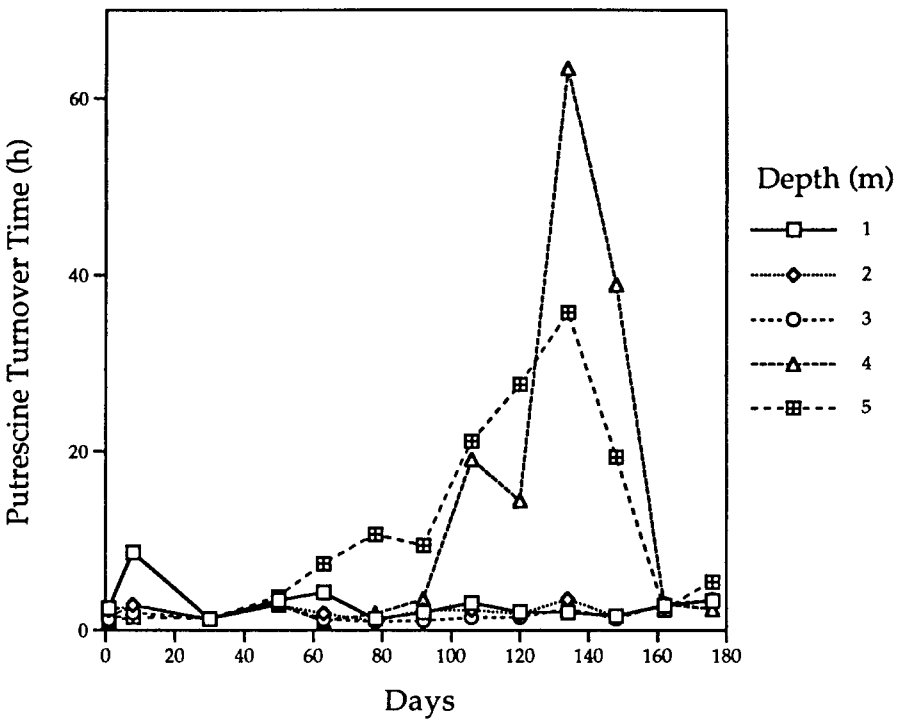

Fig. 11. Putrescine turnover time at 5 depths measured at 13 sampling times (SP1-13). Time is days after May 1 (SP1).

time dropped precipitously after the hurricane mixed (and oxygenated) the water column. We do not have concentration data for putrescine throughout the summer. However, if putrescine concentrations were to follow DFAA concentration patterns, increasing at the mid-depth waters in mid-August, the higher turnover times would not necessarily imply lower uptake rates since the higher concentrations would balance the higher turnover times as was the case at 1 m during SP1 (see Fig. 2). We observed this during 1983 for putrescine and also for other organic N compounds at other locations (Lee 1992).

Conclusions

During 1985, profiles of temperature, salinity, pH, alkalinity, POC and pigments in Salt Pond clearly showed the natural progression of stratification that is common in productive coastal ponds. Cycling of putrescine in Salt Pond, like the amino acids, was influenced by primary production and micro-

bial decomposition. Putrescine concentrations appeared to follow the pattern of primary production more closely, while amino acids appeared to follow the pattern of microbial production. Putrescine turnover times increased in deeper anoxic waters during the progressive stratification of the pond with time, either due to an increase in concentration in the deeper waters or to a decrease in anaerobic decomposition rate there.

Putrescine did not appear to be produced through decomposition of dissolved ornithine, an argument against protein decomposition as a major source in the water column. The correlation of putrescine concentration with primary production during the first two sampling periods instead suggests a direct source from algae in the water column. Diffusion out of the sediments into the overlying water is also possible.

Microbial uptake of amino acids and putrescine together accounted for 60–80% of the bacterial C production measured in shallower, oxic waters, and almost 300% of that measured in the anoxic bottom water. A large contribution to bacterial production from DFAA uptake might be expected, as more than half of the biomass of living organisms consists of protein, but a correspondingly high uptake of putrescine was not expected. Since other organic carbon and nitrogen compounds are probably also being taken up, these data suggest that tracer uptake methods as we used them may overestimate the true microbial uptake rates, or release of other organic compounds by microbes may occur at the same time. Turnover times measured using both ^{3}H- and ^{14}C-tracers in samples taken at the same time showed similar patterns over the summer. However, if there is a difference in chemical vs. biological behavior of the tracers, this difference would likely be identical with both tracers. Further work on carbon and nitrogen budgets is needed to resolve the apparent imbalance between organic C and N incorporation and bacterial production.

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