COMPARISON OF THE FATE OF DISSOLVED ORGANIC MATTER IN TWO COASTAL SYSTEMS: HOG ISLAND BAY, VA (USA) AND PLUM ISLAND SOUND, MA (USA)

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APPROVAL SHEET

This thesis is submitted in partial fulfillment of

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ABSTRACT

Coastal systems such as the Hog Island Bay (HIB) lagoon on the ocean-side of Virginia's eastern shore and the Plum Island Sound (PIS) estuary in Massachusetts may play important roles in transforming dissolved inorganic and organic nutrients during their transport to the coastal ocean. Although the dissolved inorganic nitrogen (DIN) in HIB is derived from agriculture and enters the system via groundwater, the dissolved organic matter (DOM) is autochthonous. The predominant nitrogen source in PIS is allochthonous: dissolved organic nitrogen (DON) is derived from forests and DIN enters the system from suburban areas. We hypothesized that the lability of the DOM sampled would be greater: (1) in HIB than in PIS, and (2) in HIB after the macroalgal population crashed mid-summer than in other seasons. We also hypothesized that the rates of gross mineralization would be significantly higher than rates of net mineralization, indicating rapid consumption of the ammonium produced. Nitrification was expected to be the primary fate of ammonium, and immobilization into bacterial biomass was expected to be secondary. In order to test these hypotheses, the DOM was characterized using synchronous fluorescence spectroscopy. Then, net mineralization was determined using bioassays bimonthly from February to October in HIB and from May to September in PIS. Gross nitrogen mineralization and nitrification were measured using the isotope pool dilution technique with $^{15}NH_4^+$ and $^{15}NO_3^-$ additions, respectively. Synchronous fluorescence characterization indicated that the DOM in PIS was predominantly terrestrially-derived humic material, whereas that in HIB was mostly proteinaceous and likely algal-derived. The results of the net mineralization incubations suggested that the DOM in HIB was more labile than that in PIS: 27% of the initial DOC and 9% of the initial DON was utilized within three weeks at HIB compared to 7% of DOC and 6% of DON in PIS. In addition, the DOM sampled in HIB in August was highest in concentration (582 μM in August compared to an average of 212 μM for all other months) and was more labile (54% of initial DON was utilized in August compared to 0-27% in other months) than DOM sampled in other seasons. Average gross mineralization rates were 3-6 times greater than net mineralization rates, suggesting that 16% to 33% of the ammonium produced by mineralization was immediately consumed. Nitrification rates were highly variable and ranged from 11% to 500% of gross mineralization, suggesting that nitrification was a significant fate for ammonium in the systems, but the level of importance varied with season and sampling location. Immobilization into bacterial biomass was not a permanent fate of ammonium in our study, but ammonium was likely processed through particulate nitrogen transiently and re-released as DON via viral lysis, grazing, or exudation by bacterial cells. Our results indicate that HIB has the potential to alter the bioavailability of DIN and DOM more significantly than PIS due to the longer residence times, increased importance of labile autochthonous DOM, and higher significance of benthic-pelagic coupling in HIB.

INTRODUCTION

Population growth with accompanying land-use changes and increased use of fertilizer in the coastal areas of the United States during the past several decades have changed the quantity and quality of inorganic and organic inputs to the coastal ocean (Meybeck 1982, Hamilton and Helsel 1995, Hopkinson and Vallino 1995, Nixon 1995, Hopkinson et al. 1998). There has been an increase in the percentage of land area used for agriculture and urban/suburban areas, and a concurrent decrease in wetland and forested areas. Aquatic systems such as estuaries and coastal embayments are often viewed as potential buffer zones between the land and the ocean, protecting the ocean from anthropogenic influences on land. Although many studies in the past decade have examined the role of these systems as traps or sinks of inorganic nutrients and organic matter (Nowicki and Oviatt 1990, Morell and Corredor 1993, Nielson et al. 1995, Anderson et alia. *in press*), and an average of 70% of total dissolved nitrogen (TDN) in rivers is dissolved organic nitrogen (DON; Meybeck 1982), not much is known regarding the fate of DON and its lability. Bioavailability of DON is known to vary spatially and temporally with different sources (Seitzinger et al. 2002), but the variability is poorly understood. Little work has been done on coastal lagoons compared to estuaries; yet coastal lagoons are especially important along the east and Gulf coasts of the United States.

Estuaries are defined ecologically as aquatic systems where fresh water from streams and rivers mix with ocean water. Coastal lagoons are embayments along the coast with predominantly marine input. They are typically shallow, well mixed, and

receive limited freshwater input (Boynton et al. 1996). Both estuaries and lagoons receive some freshwater input on their landward edge and dissolved constituents are transformed during transport through the system toward the coastal ocean. Estuaries and lagoons can act as filters, removing and transforming nutrients and organic matter in the water as it is transported, therefore playing a role in regulating eutrophication of the coastal ocean. Nixon (1995) defined eutrophication as "an increase in the rate of supply of organic matter to an ecosystem." The potential direct and indirect impacts of increased organic matter input include increased primary and secondary production (possibly including harmful algal blooms) and decreased oxygen concentrations, which in severe cases cause fish kills (Paerl et al. 1998).

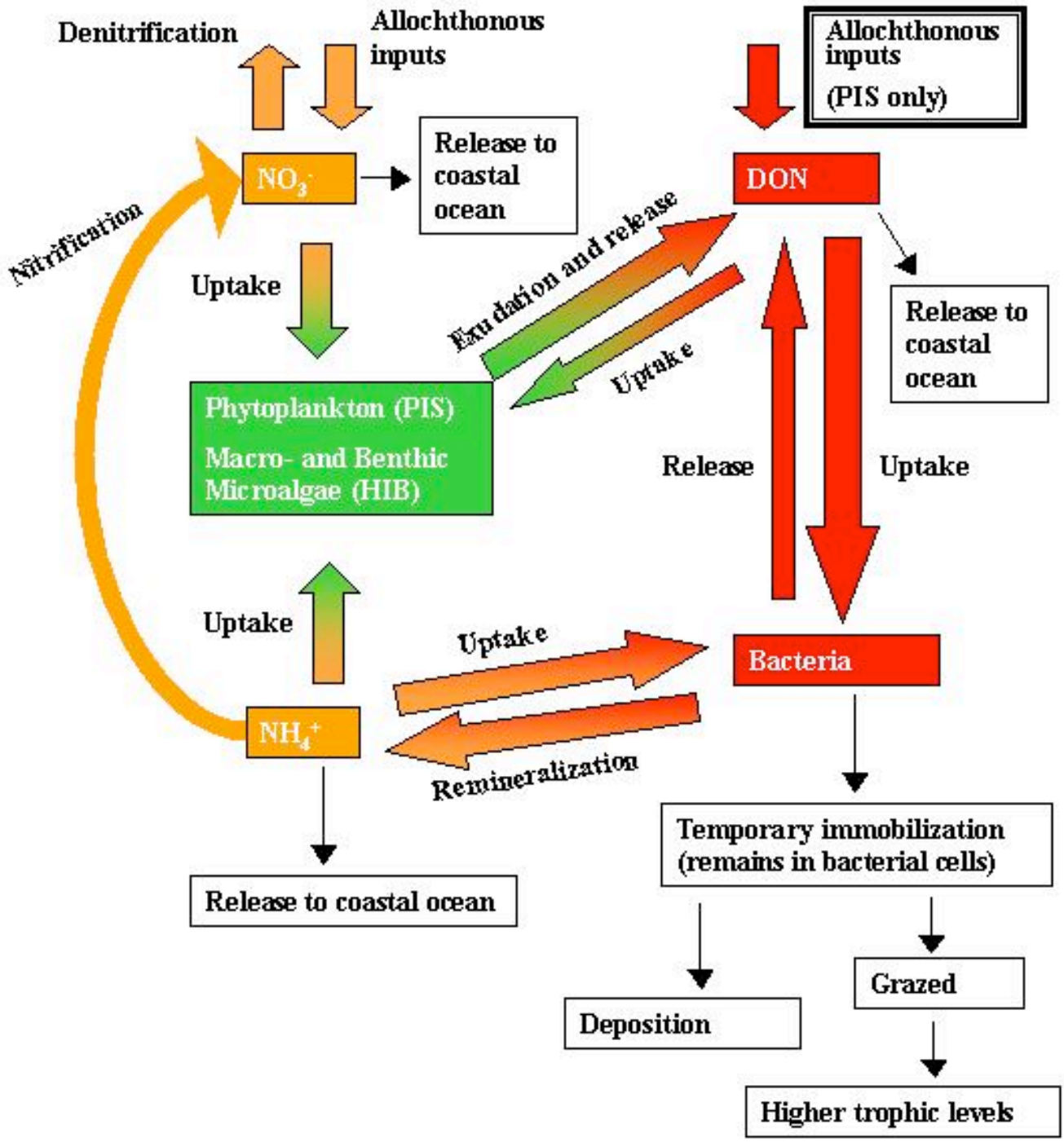
Nowicki and Oviatt (1990) used mesocosms in Narragansett Bay to estimate rates of nitrogen and phosphorus trapping over an annual cycle. They found that most nutrients that entered the system were exported, regardless of treatment level or season. However, much of the inorganic nitrogen and phosphorus was transformed to dissolved and particulate organic matter. This transformation may reduce the ability of the nutrients to initiate either primary or secondary production. Other studies have shown that coastal lagoons and estuaries do retain, at least temporarily, or remove a significant amount of the nitrogen they receive (Morell and Corredor 1993, Nielson et al. 1995, Anderson et alia. *in press*). In these studies, a significant portion of the incoming nutrient pool was removed by uptake into benthic microalgae and macroalgae, denitrification, or by sorbing to particles and settling to the sediments. Benthic-pelagic coupling is likely to have a strong effect on nutrient cycles in lagoons, because they are shallow and light

penetrates the water column to the sediments (McGlathery et alia. 2001, Anderson et alia. *in press*).

There are many processes within the water column of aquatic systems that affect the concentration and form of dissolved constituents (figure 1). Within the inorganic pool, ammonium can be transformed to nitrate via nitrification and nitrate can be removed from the system via denitrification or converted back to ammonium by dissimilatory nitrate reduction. Inorganic nutrients taken up by primary producers and heterotrophic bacteria are transformed into particulate organic matter. The primary producers (phytoplankton, benthic microalgae, and macroalgae) release organic matter by passive release, death and cell lysis, and when grazed (Bronk and Glibert 1993). Release by phytoplankton is a significant source of DON to the water column. In laboratory studies, 25-41% of the DIN taken up by phytoplankton was re-released as DON (Bronk and Glibert 1993, Bronk et alia. 1994). Macroalgae similarly have been shown to release significant amounts of DON during growth and decomposition (Tyler et alia. 2001). Microbial communities release dissolved organic matter (DOM) to the water column as a result of grazing, viral lysis, and secretion of exoenzymes (Middelboe et al. 1995, McCarthy et al. 1998). In addition, allochthonous inputs of DOM are significant in some aquatic systems with sources including marshes, surface water run-off, point-source pollution, and groundwater (Valiela et al. 1997a, Valiela et al. 1997b, Hopkinson et al. 1998, Hopkinson et al. 1999).

DOM in the water column of a lagoon or estuary has four possible fates: export to the coastal ocean, adsorption to particles and deposition to the sediments, uptake by primary producers, and uptake by bacteria (figure 1). Some DOM may remain in the

Figure 1. Conceptual model for nitrogen cycling in Plum Island Sound and Hog Island Bay.


Denitrification
Allochthonous inputs
Allochthonous inputs (PIS only)
NO_3^-
Release to coastal ocean
DON
Nitrification
Uptake
Exudation and release
Uptake
Release to coastal ocean
Phytoplankton (PIS)
Macro- and Benthic Microalgae (HIB)
Release
Uptake
Uptake
Uptake
Bacteria
NH_4^+
Remineralization
Release to coastal ocean
Temporary immobilization (remains in bacterial cells)
Deposition
Grazed
Higher trophic levels

water column and be exported to the ocean by tides and currents. DOM may sorb onto mineral and organic particles and be deposited on the bottom of the basin, where it enters the benthic metabolic cycle, is humified, or is temporarily or permanently buried as sediment organic matter. Primary producers may take up DON to support production of new biomass or for respiration (Palenik and Morel 1990, Antia et al. 1991). The primary fate of labile DOM, however, is uptake by heterotrophic bacteria for respiration or incorporation into biomass. Cole and colleagues (1988), in a review of bacterial production in many aquatic systems, reported that approximately 60% of primary production in the water column is metabolized by bacteria. Another review found an average of 17% of water column dissolved organic carbon (DOC) was utilized by bacteria within one to two weeks (Søndergaard and Middelboe 1995). A study of the Delaware and Hudson rivers found that 40-72% of DON was utilized within fifteen days, with most incorporated into bacterial biomass and a small amount remineralized to DIN (Seitzinger and Sanders 1997). Incorporation versus mineralization is determined by bacterial growth efficiency; if the incorporation rate is greater than the mineralization rate, there will be net immobilization (Buchsbaum et al. 1991).

Closing the nitrogen cycle requires regeneration of inorganic nitrogen from DON by the microbial community. However, whether DIN is released or consumed by bacteria during decomposition depends on the lability of the DOM being utilized, its C:N ratio, and the growth efficiency of the bacterial community. Net ammonium regeneration decreases and C:N of bacterial biomass increases as organic substrate C:N increases (Goldman et al. 1987, Hopkinson et al. 1989, Goldman et al. 2000). Heterotrophic bacteria may preferentially utilize DIN over DON as a nitrogen source to support growth

(Zweifel et al. 1993, Middelboe et al. 1995), and ammonium uptake can account for 20-60% of total bacterial nitrogen uptake (Wheeler and Kirchman 1986). Bacteria outcompete phytoplankton for ammonium at low concentrations due to the small size and high surface area to volume ratios of bacteria, and the uptake of ammonium decreases the efficiency of remineralization (Zweifel et al. 1993). One study found that microbial ammonium uptake was higher in oligotrophic than in eutrophic waters (contributing up to 50% of total nitrogen uptake), possibly due to limiting labile DON in the oligotrophic systems (Hoch and Kirchman 1995). Goldman and Dennett's (2000) findings demonstrated that uptake of ammonium was not inhibited by the presence of amino acids. The above studies demonstrate the complexity of DON utilization in natural systems and the relationship between DON and DIN uptake and remineralization. Ammonium regeneration can potentially be predicted based on bacterial growth efficiency and the C:N ratio of the substrate and of the bacterial cells; however, little is known about the C:N ratio of the substrate being utilized by bacteria in natural waters (Kroer 1993, Kirchman 1994).

The rates of the above-described processes and the extent to which they alter the pools of dissolved constituents in the water column vary spatially and temporally. Søndergaard and Middelboe (1995) speculated that microbial populations in eutrophic systems have a higher affinity for DOC than those in oligotrophic systems, explaining a gradient in the percentage of labile DOC observed across systems. Seitzinger and colleagues (2002) found significant differences in bioavailability between different sources of DON and seasons in New Jersey watersheds, with utilization ranging from 0-73%. The differences in response were not consistent between sites, which indicated that

a combination of factors affected the bioavailability of the DON and plankton community composition. Bacterial processes are also strongly affected by temperature (Hopkinson et al. 1989, Hoch and Kirchman 1993, Shiah and Ducklow 1995). In addition, inputs of allochthonous nutrients and composition of organic matter vary with season and the adjacent landscape. The mesohaline Chesapeake Bay varies from being net autotrophic during the late spring through early fall (during which times allochthonous inputs of inorganic nitrogen support phytoplankton production) to being net heterotrophic in the late fall when much autochthonous DIN is being produced by microbial remineralization (Bronk et al. 1998).

My research examined microbial water column processes and their potential to transform nutrients and organic matter during transport to the coastal ocean in two coastal systems with differing sources of nutrients and DOM. Water column nitrogen cycling was examined in view of: (1) the role of nitrogen as a potential limiting nutrient for the growth of aquatic primary producers (Carpenter and Capone 1983); (2) the spatial and temporal variability of DON lability in these 2 systems; and (3) the multiple processes that affect transport and fate of DIN and DON within a given system. A comparison of a coastal lagoon and an estuary was performed: Hog Island Bay (HIB) on the ocean side of Virginia's Delmarva Peninsula and Plum Island Sound (PIS) in Massachusetts. Both systems are Long Term Ecological Research (LTER) sites with extensive sets of available biological, chemical, and physical data. The two systems receive significantly different forms of nitrogen from a variety of sources. HIB receives mostly nitrate from agricultural sources via groundwater (Reay et al. 1992). The nitrate supports production of macroalgae and benthic microalgae, which release DON and DIN to the water column

(McGlathery et alia. 2001, Tyler et alia. 2001). At the freshwater end, PIS receives DON primarily from forests and urban/suburban areas (Hopkinson et. al. 1998).

OBJECTIVES AND HYPOTHESES

The objective of this study was to determine the fate of DOM in two coastal embayments. Net mineralization of DOM, gross mineralization of DON, and nitrification were measured in order to determine the lability and turnover times of nitrogen compounds and to assess the relative importance of microbial mineralization versus immobilization in these systems. Measurements were made bimonthly because sources of DON were expected to vary seasonally (Bronk et al. 1998). In addition, samples were taken along a transect from land to sea in order to examine the spatial variability of DON lability and the potential for removal of DOM and DIN within the systems.

Specific hypotheses were:

1. DOM collected following decomposition of macroalgae blooms in HIB will be more labile than DOM sampled during other seasons.
2. Autochthonously produced DOM in HIB will be more labile than the DOM in PIS, which is predominantly allochthonous in origin.
3. Rates of gross mineralization in incubations will be significantly higher than rates of net mineralization from both systems indicating rapid bacterial consumption of the ammonium produced by mineralization.
4. The primary mechanism for consumption of ammonium during incubations will be nitrification. A secondary mechanism for removal of ammonium will be bacterial immobilization.

MATERIALS AND METHODS

Study Sites and Characteristics

Plum Island Sound, Massachusetts (USA): PIS is a 24-km long estuarine system receiving freshwater from three rivers (figure 2). The Parker River watershed has a 155-km^2 basin that is 50% forested (mostly conservation land), 25% urban, 13% agriculture, and 12% wetland (Hopkinson et al. 1998). The Rowley River watershed is much smaller (26-km^2 basin) and is composed mostly of forest and salt and tidal freshwater marshes, although there is some residential development in the upper watershed. The Ipswich River has a 404-km^2 drainage basin that is predominantly suburban-residential, including suburbs of Boston (Vallino and Hopkinson 1998). The PIS watershed in its entirety is 37% forest and 35% urban/suburban (PIE LTER Site Review 2001; table 1). Previous work in these three rivers has shown that they retain 80-90% of the nitrate they receive, and that DON is the major form of nitrogen exported to the estuary. Also, 90% of the total nitrogen derived from the forest is DON, whereas the urban and suburban inputs are mostly NO_3^-. The annual average concentration of TDN in the Parker River where it enters the sound is 39 □M, 53-70% of which is DON (Hopkinson et al. 1998 and 1999). Approximately 7% of the PIS watershed is agricultural land (table 1), and the agricultural runoff contains both DON and DIN with relative amounts varying seasonally. The residence time of water parcels in PIS has been found to range from 34 days in the upper estuary to 0.5 days in the lower estuary, depending on river flow. The system has semi-diurnal tides with an average tidal range of 2.9 meters (Vallino and Hopkinson 1998).

Figure 2. Map of study sites. a) East Coast of the United States for reference. b) Plum Island Sound; stations are designated by red dots and are Middle Bridge, Newbury, and Plum Island, from left to right. c) The black square is Hog Island Bay; stations are Creek, Shoal, and Hog from left to right.

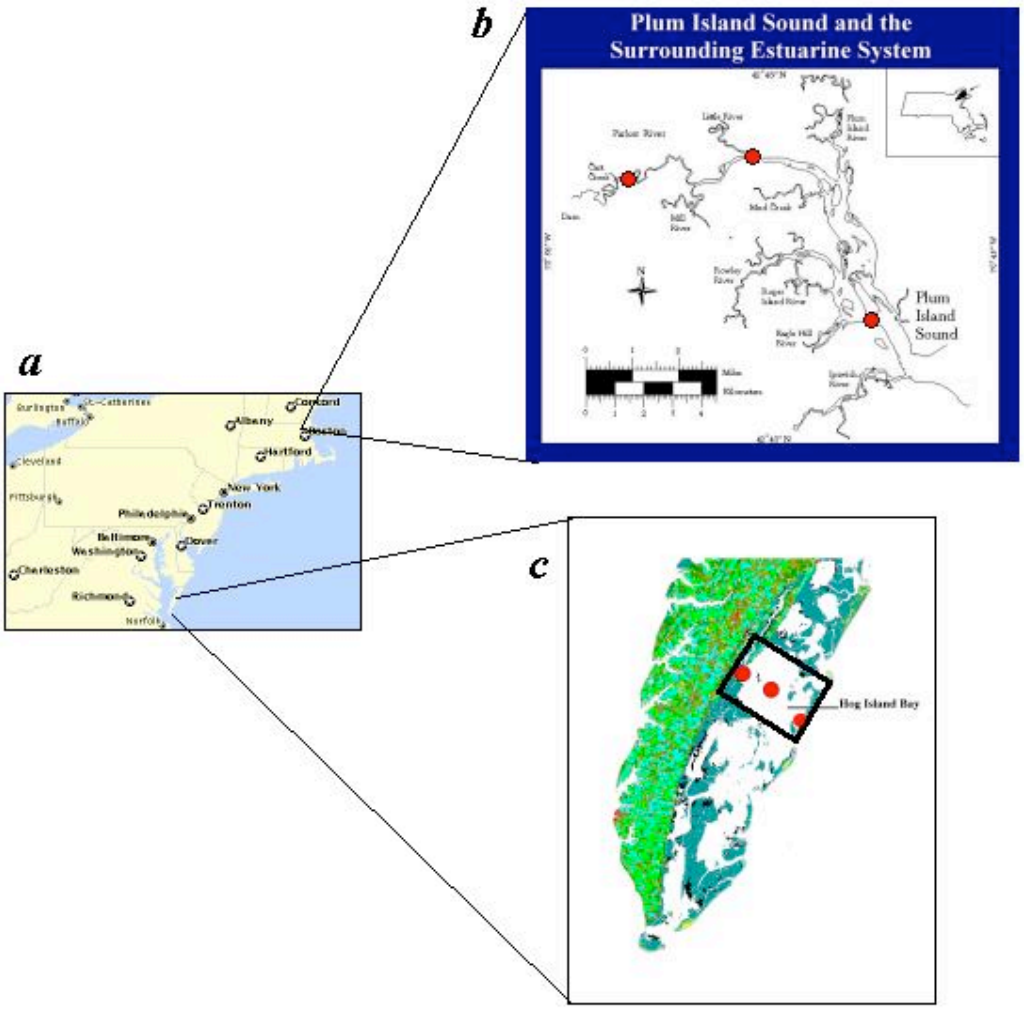

a
b
Plum Island Sound and the Surrounding Estuarine System
Plum Island Sound
c
Hog Island Bay

Table 1. Land use in Plum Island Sound Watershed in 1971, 1991, and 2001. From Plum Island Ecosystem LTER Site Review (2001): http://ecosystems.mbl.edu/pie/3yrSiteReview.pdf

Land Use	1971	1991	2001
Agriculture	7%	7%	7%
Forest	58%	46%	37%
Wetland & water	10%	15%	21%
Urban/Suburban	25%	32%	35%

The stations used in this study include two within the Parker River (a freshwater station and a mesohaline station) and one within the main stem of the Sound below the entrance of the Rowley River. The freshwater station is Middle Bridge, which has a salinity close to zero psu during ebb tide. It is surrounded by freshwater marsh with *Typha* as the predominant flora. The mesohaline station, Newbury, is also surrounded by marsh (*Typha* and *Spartina alterniflora* dominated); however, it is located in a residential area. Plum Island, the polyhaline station, has a salinity of 25 to 30 psu and is located in a small yacht club adjacent to the open sound.

Hog Island Bay, Virginia (USA): HIB is a coastal lagoon on the ocean side of Virginia's Delmarva Peninsula, located in the Virginia Coast Reserve (managed by the Nature Conservancy) and is a Long Term Ecological Research site (figure 2). The Virginia Coast Reserve contains barrier islands, deep channels, shallow shoals, marshes, mud flats, and tidal creeks. It is shallow (average depth is one meter at mean low water), well mixed, and receives little freshwater input. Residence time estimates for the lagoon range from four days near the barrier island to over 30 days in the shoals and near the land margin (Fugate *unpublished data*). The system has semi-diurnal tides with a 1.2 to 1.5 meter range. The main source of nutrients and organic matter to the lagoon is believed to be a shallow, unconfined aquifer on the mainland Delmarva Peninsula, which is strongly impacted by agriculture (Reay et al. 1992). The watershed has a 442-km^2 basin, 55% of which is agricultural (Hamilton and Helsel 1995). Most of the inputs are dissolved inorganic nitrogen (DIN; Wu *unpublished data*) and the primary producers create organic matter using the allochthonous nutrients. DON comprises 52-98% of TDN

within the water column in HIB (Tyler et alia. 2001). Seagrasses have been absent from HIB since the 1930s, and phytoplankton do not appear to play a significant role in the system, as water column chlorophyll *a* was low (<3 μg l^{-1}) during all months of this study except during August, following the crash of the macroalgal populations and the significant release of DIN and DON to the water column. In late summer, chlorophyll *a* values of 15 □g l^{-1} have been observed (McGlathery et alia. 2001). The major primary producers in HIB are benthic microalgae and macroalgae, with dominant macroalgal genera *Ulva, Gracilaria,* and *Cladaphora* (McGlathery et alia. 2001). The autochthonous DON produced by the macroalgae, especially following a bloom, has been shown to be significantly higher than background levels of DON (Tyler et alia. 2001). Also, it has been hypothesized that the macroalgal DON is more labile than that from allochthonous sources (McGlathery et alia. 2001, Tyler et alia. 2001); this thesis examined this hypothesis.

The stations in HIB are Creek, Shoal, and Hog. The salinity at all three stations was approximately 32 psu during most seasons. Creek is located near the mainland in a small tidal creek (approximately 5 meters across) and is surrounded by tidal salt marsh dominated by *Spartina*. Shoal is adjacent to a remnant oyster reef located in the middle of the Lagoon approximately 200 meters from the deep-water channel. Hog is located on the bay side of a barrier island that occupies the margin between the lagoon and the Atlantic Ocean.

Sampling and Incubation Methods

Samples were taken for incubations bimonthly at the three HIB stations described above (Creek, Shoal, and Hog) starting in February 2000 and ending in October 2000 (five sampling events). PIS samples were taken at Middle Bridge, Newbury, and Plum Island in May, July, and September 2000. Three replicate surface water samples were collected at each station during ebb tide in acid-washed polycarbonate bottles. Subsamples were taken from each of these bottles for DOC, DON, chlorophyll *a*, inorganic nutrients (NO_3^-, NO_2^-, NH_4^+, and PO_4^+), and bacterial abundance. Samples were then filtered using a pre-combusted (500ºC, 5 hours) 142-mm Gelman A/E glass fiber 1.0 µm pore-size filter in the laboratory using a low-pressure peristaltic pump to remove detritus, phytoplankton, and most grazers.

The filtrate from each replicate was partitioned into three subsamples for determinations of net mineralization, gross mineralization, and nitrification.

1. Net mineralization: Incubations were performed in acid-washed polycarbonate bottles in a dark incubator at *in situ* temperature for 21 days. Subsamples were taken from all bottles at 0, 3, 5, 7, 14, and 21 days and analyzed for DOC, DON, inorganic nutrients, and bacterial abundance. In addition, during the summer sampling at each site and station, subsamples were taken for characterization of the DOM by synchronous fluorescence.

2. Gross mineralization: Determinations were made using the isotope pool dilution method. $(^{15}NH_4)_2SO_4$ was added to a final concentration of 5 μM and an enrichment of 40-atom% ^{15}N. Incubations were performed in acid-washed polycarbonate bottles in the dark for 7 days. Subsamples were taken from incubation bottles at 0, 3, 5, and 7 days and stored frozen until analyzed. $^{15}NH_4^+$ was removed by diffusion (Holmes et al. 1998). Isotope dilution is a procedure in which both the concentration and enrichment of the product pool, NH_4^+ in the case of mineralization, are measured over time. As bacteria remineralize organic matter to ammonium, the ammonium pool is diluted with more and more ^{14}N. The equations of Wessel and Tietema (1992; page 48 of this thesis) were used to calculate rates of mineralization and NH_4^+ consumption based on the ^{15}N: ^{14}N ratios and ammonium concentrations measured over time.

3. Nitrification: Determinations were made using the isotope pool dilution technique with $^{15}NO_3^-$ additions followed by a seven-day dark incubation with subsamples collected at 0, 3, 5, and 7 days. This procedure is similar to that of gross mineralization; however changes in enrichment and concentration of the nitrate pool are measured to determine the amount that has been created due to bacterial nitrification (conversion of ammonium to nitrate) and consumption due to denitrification, dissimilatory reduction to ammonium, or immobilization. Prior to removal of NH_4^+ by diffusion, NO_3^- was reduced by the addition of Devarda's alloy (Sigman et al. 1997).

Chemical analyses

DOC samples were stored frozen in pre-combusted (500ºC, 5 hours) glass vials until analyzed using a Shimadzu TOC-5000A. Samples were acidified with 1M phosphoric acid, inorganic carbon was purged by bubbling, and DOC was analyzed by the Pt-catalyzed high-temperature combustion method.

DON samples were analyzed by persulfate oxidation in sealed 10-milliliter ampoules (Grasshoff et al. 1983). The oxidizing reagent was made fresh daily by diluting 7.5 grams of NaOH to 500 milliliters with deionized water and then adding 25 grams of double re-crystalized $K_2S_2O_8$ (J.T. Baker, Instra-analyzed reagent grade) and 15 grams of H_3BO_3. Re-crystalization of the $K_2S_2O_8$ was performed by dissolving $K_2S_2O_8$ in warmed (approximately 50-60ºC) Nanopure water (super-saturated solution, approximately one liter of water for 150 g $K_2S_2O_8$). The mixture was refrigerated in a sealed glass flask for 1-2 days and the water was then decanted off and discarded. The $K_2S_2O_8$ crystals were re-dissolved as described above, and after decanting the second time, the $K_2S_2O_8$ was dried at 28ºC for three days. Five milliliters of sample and one milliliter of oxidizing reagent were autoclaved (121ºC, 15 psi) in a sealed pre-combusted (500ºC, 5 hours) glass ampoule for 40 minutes. This process converted all organic nitrogen to nitrate, and the nitrate produced was determined within three days using an Alpkem autoanalyzer. DON was calculated as TDN minus DIN (NO_3^-, NO_2^-, and NH_4^+). The accuracy of the method was verified using 12.5 and 25.0 μM L-leucine standards.

Dissolved inorganic components were analyzed as follows. All NO_3^- and NO_2^- samples were analyzed using an Alpkem autoanalyzer. NH_4^+ samples were analyzed using the phenol hypochlorite method (Solorzano 1969). PO_4^+ was analyzed by the

molybdate method (Parsons 1984). NH_4^+ and PO_4^+ concentrations were determined using a Shimadzu UV-1601 spectrophotometer.

DOM samples were characterized at Florida International University by synchronous fluorescence spectroscopy (De Souza Sierra et al. 1994). Spectra were obtained using a Perkin Elmer LS50B spectrofluorometer with a 150-watt Xenon arc lamp by scanning at a constant offset value of 30 nm between the excitation and emission wavelengths; the slit width used was 10 nm. Two categories of DOM can be identified using the synchronous fluorescence technique: a high molecular weight, humic fraction, can be distinguished from a low molecular weight, labile fraction (De Souza Sierra et al. 1994, Coble 1996).

Samples for analysis of bacterial abundance were fixed with glutaraldehyde (final concentration of 2%) and refrigerated for no more than 3 days. Samples (3 ml) were filtered with 120 µl of acridine orange onto 0.22 µm black polycarbonate filters, mounted on slides, and frozen. Bacterial counts were performed via epifluorescence microscopy. Ten fields of view were counted per slide, with a minimum of 30 cells counted per field of view.

Gross mineralization samples were analyzed using the ammonium diffusion method (Holmes et al. 1998). First, diffusion packets were created daily using one pre-combusted (500ºC, 5 hours) glass fiber GF/D filter (Whatman, 1.0 cm diameter) and two Teflon membranes (Millipore, 10.0 µm pore size, 25 mm diameter) that were previously rinsed with 10% HCl and deionized water. The GF/D filter was acidified with 25 µl of 2.5M $KHSO_4$ and sealed between the two Teflon membranes by pushing down firmly with a scintillation vial. Ammonium concentrations were determined from subsamples,

and the appropriate volume of sample to diffuse was calculated to collect approximately 30-60 μg of nitrogen. The samples were thawed and measured into acid washed polycarbonate bottles for analysis. Pre-combusted (500ºC, 5 hours) KCl was added to each sample to a final concentration of 1M to increase the salinity of the sample and increase the efficiency of NH_4^+ diffusion. Pre-combusted (500ºC, 5 hours) MgO (Mallinckrodt USP Food Grade powder) was then added (3.0 g per liter of sample) to raise the pH to approximately 9.7 and convert all NH_4^+ to NH_3 gas and allow it to be trapped on the acidified GF/D filter in the Teflon packet. Samples were incubated on a shaker table at 40°C for 14 days, and then the filter packet was removed, rinsed in 10% HCl and deionized water, and dried in a dessicator with silica gel and over concentrated sulfuric acid for one to two days.

Nitrification samples were prepared by a modification of the ammonium diffusion method (Sigman et al. 1997). First, samples were thawed and measured into acid washed glass beakers. KCl was added to a final concentration of 1M and MgO was added (3.0 grams/liter of sample). The samples were then boiled on hot plates in a fume hood to a final volume of approximately 100 milliliters. This step reduced the volume to increase diffusion efficiency and removed the ammonium and labile DON from the water sample, leaving only nitrate. Each 100-milliliter sample was poured into an acid washed polycarbonate bottle; 0.5 grams of MgO, 0.3 grams of Devarda's alloy (Fluka puriss. powder), and a diffusion packet as described above were added. Samples were incubated at room temperature on a shaker table for 7 days. The filter packet was then removed, rinsed in 10% HCl and deionized water, and dried in a dessicator with silica gel and over concentrated sulfuric acid for 1-2 days.

The glass fiber filters from the diffusion experiments were shipped to the University of California, Davis, USA, for analysis of ^{15}N enrichment using a Europa isotope ratio mass spectrometer linked to an elemental analyzer.

Statistical Analysis

The effects of site, station, and season were determined using 3 separate analysis of variance (ANOVA) models (Underwood 1997). The models were used to examine the following six responses: DOC and DON utilization rates, percent DOC and DON utilized, gross mineralization, and nitrification. The DOC and DON utilization rates were calculated as the slope of a linear regression of the time course data for concentrations of DOC and DON, respectively. The three replicate slopes for each site were compared using a difference of two means t-test (Zar 1996). Replicates that were not statistically different were pooled and the regressions re-run to determine the station utilization rate. Percent DOC and DON utilized were calculated from the initial and final concentrations. Gross mineralization and nitrification were calculated following the equations of Wessel and Tietema (1992).

The overall experiment was designed to test the following three effects: site (Plum Island Sound vs. Hog Island Bay), station along a transect (landward, middle, and seaward), and season (sampling months from February to October). The full three-factor model including site, station, and season as crossed factors was unbalanced (Underwood 1997), due to the absence of winter sampling at PIS. The three-factor model was analyzed using only data from spring, summer, and autumn (figure 3c). The two-factor

model testing the effects of station and season was analyzed separately for each of the two sites (PIS and HIB; figures 3a and 3b).

Averages are presented in the text as mean ± standard error. When seasons are compared, all three stations within a system are averaged. When stations are compared, all seasons are averaged. A significance level of 0.05 was used for all statistical analyses. The Tukey multiple comparisons test was used to conduct pairwise comparisons between factor levels in main effects with greater than 2 levels when p-values were less than 0.05 (Underwood 1997). Comparisons between measured parameters, such as utilization rates and DOM C:N ratios, were performed using a model 2 regression function (Sokal and Rohlf 1981). All statistical analyses except the model 2 regressions were performed using the Minitab software package (www.minitab.com).

Figure 3. Analysis of variance models used for all six responses.

A. Two-factor model for Plum Island Sound.

Station	May	July	September
	Middle Bridge	Middle Bridge	Middle Bridge
	Newbury	Newbury	Newbury
	Plum Island	Plum Island	Plum Island

Month

B. Two-factor model for Hog Island Bay.

Station	February	April	June	August	October
	Creek	Creek	Creek	Creek	Creek
	Shoal	Shoal	Shoal	Shoal	Shoal
	Hog	Hog	Hog	Hog	Hog

Month

C. Three-factor model including site, station, and season.

Site	Station	Spring	Summer	Autumn
PIS		Landward	Landward	Landward
		Middle	Middle	Middle
		Seaward	Seaward	Seaward
HIB		Landward	Landward	Landward
		Middle	Middle	Middle
		Seaward	Seaward	Seaward

Season

RESULTS

Site characterizations

Plum Island Sound: Salinities at Middle Bridge, Newbury, and Plum Island were approximately 0, 20, and 30 psu, respectively. Temperatures were 13 ± 1ºC, 20 ± 1ºC, and 17 ± 1ºC for May, July, and September samplings, respectively. Initial concentrations of DOC and DON averaged over all three seasons were highest at Middle Bridge (freshwater station; 703.9 ± 12.9 μM and 32.5 ± 1.0 μM, respectively) and lowest at Plum Island (polyhaline station; 242.9 ± 20.6 μM and 13.0 ± 0.8 μM; table 2). This is consistent with data collected during the same sampling seasons along the entire Parker River, which show a decrease in DOC and DON concentrations from the headwaters to the mouth of the estuary (PIE LTER Site Review 2001). There was a positive curvature to the mixing curves for DOC and DON concentrations (figure 4). Overall, DON was 87 ± 3% of TDN, and Newbury had the highest DIN concentration with DON contributing 77 ± 3% of TDN at that station.

Carbon to nitrogen (C:N) ratios of the DOM also varied spatially and temporally. In May, C:N increased along the estuary from 23.7 ± 1.6 at Middle Bridge to 31.2 ± 1.8 at Plum Island; whereas in July and September, the C:N decreased from 20.9 ± 0.4 to 13.8 ± 2.5 and 21.6 ± 0.2 to 16.2 ± 0.5, respectively (table 2). The overall C:N averages for the three stations from landward to seaward were not significantly different and the average for all sites and sampling times in PIS was 21.6 ± 2.4. However, C:N ratios measured during the three sampling months (May, July, and September) were significantly different ($p = 0.009$).

Table 2. Average initial concentrations and standard error of DOC, DON, and DIN in Plum Island Sound for all sampling events.

Station	Sampling month	Initial DOC Concentration (□M)	Initial DON Concentration (□M)	Initial C:N of DOM	Initial DIN Concentration (□M)
Middle Bridge	May	689 ± 34	29 ± 0.1	23.7 ± 1.6	4.06 ± 0.08
	July	733 ± 13	35 ± 0.4	20.9± 0.4	1.80 ± 0.36
	September	690 ± 5	32 ± 0.1	21.6 ± 0.2	2.60 ± 0.09
Newbury	May	705 ± 8	24 ± 0.1	29.4 ± 0.3	5.11 ± 0.10
	July	546 ± 6	34 ± 0.3	15.9 ± 0.2	9.59 ± 0.27
	September	497 ± 4	30 ± 0.3	16.4 ± 0.3	12.88 ± 0.28
Plum Island	May	322 ± 11	10 ± 0.1	31.2 ± 1.8	0.60 ± 0.06
	July	194 ± 9	15 ± 1.9	13.8 ± 2.5	1.57 ± 0.07
	September	212 ± 6	13 ± 0.1	13.6 ± 0.5	2.32 ± 0.08

Figure 4. Initial concentrations of DOC, DON, NH_4^+, and NO_3^- in PIS.

DOM C:N in May was significantly higher than in both July and September, which were not different from each other (29.9 ± 2.4, 16.7 ± 2.2, and 18.1 ± 1.7, respectively).

Synchronous fluorescence spectroscopy of samples taken from Middle Bridge (landward, freshwater station) had significant peaks at 360 and 400 nm (figure 5), indicative of terrestrially derived humic substances (De Souza Sierra et al. 1994, Coble 1996). As the water was transported down the estuary, some humic substances remained at Newbury, but humics were much less prevalent than in samples from Middle Bridge. The maximum peak at Newbury occurred at 300 nm. Samples from Plum Island did not indicate the presence of humic substances; the peak occurred at 280-300 nm, suggesting fresh, labile DOM such as proteins (De Souza Sierra et al. 1994, Coble 1996).

The concentrations of DIN were uniformly highest at Newbury, 9.19 ± 0.06 □M, with concentrations at both endmembers lower and similar to each other (table 2; figure 4). Nitrate concentrations in May did not follow this pattern; concentrations at Middle Bridge in May were much higher than those found in July or September (3.48, 0.53, and 0.71 □M, respectively). The lowest overall chlorophyll *a* concentrations were found in May, with an average of 10 □g l^{-1}, compared to July and September when chlorophyll *a* concentrations were 57 and 48 □g l^{-1}, respectively (figure 5). The higher nitrate concentrations found in May likely resulted from both high winter/spring flow rates and low nitrate uptake by phytoplankton. Along the transect, chlorophyll *a* concentrations were determined to be lowest at the Plum Island site and highest at the Middle Bridge site. Concentrations were consistently low at Plum Island (3-6 □g l^{-1}) most likely due to short residence times (PIE LTER Site Review 2001).

Figure 5. Synchronous fluorescence spectroscopy analysis of DOM from Middle Bridge in Plum Island Sound. Green bars represent the range of emission peaks from algal-derived proteinaceous material. Red bars represent the range of peaks from humic substances. Ranges from De Souza Sierra et al. 1994.

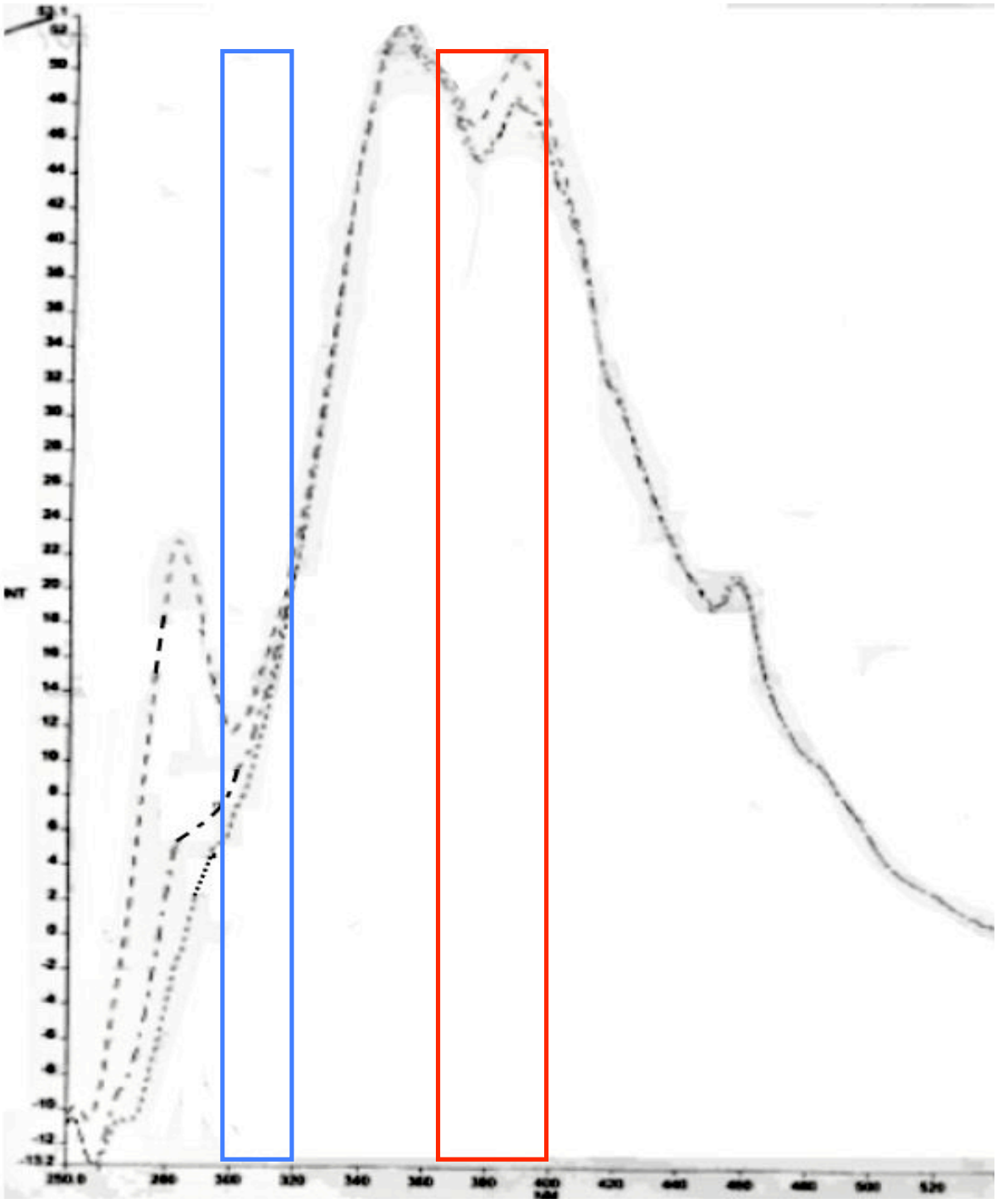
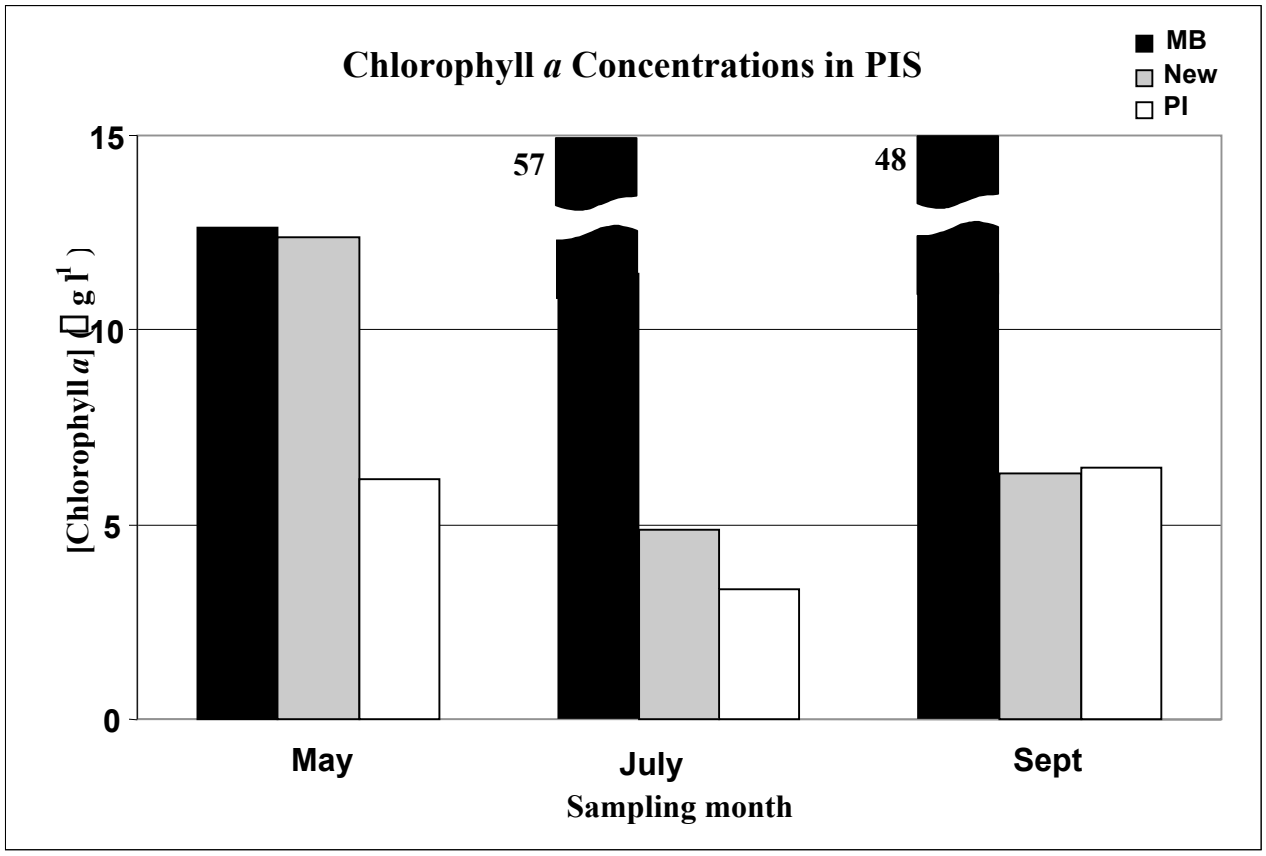

NT

Figure 6. Chlorophyll *a* concentrations at Plum Island Sound stations at time of sampling.


Chlorophyll a Concentrations in PIS
MB
New
PI
15
10
5
0
57
48
[Chlorophyll a] (□g l-1)
May
July
Sept
Sampling month

Hog Island Bay: Salinities at Creek, Shoal, and Hog stations were not significantly different and averaged 32 ± 1 psu. Temperatures were highest in June and August (average 27 ± 0.5ºC) and lower in the spring and autumn (average 16 ± 1ºC). Initial concentrations of DOC for all stations and seasons ranged from 136.0 μM to 590.9 μM with a mean of 265.9 ± 22.9 μM (table 3; figure 7). Highest concentrations were found in August (all three stations averaged, 561.0 ± 34.0 μM) and were consistently found at Creek (average for all seasons, 291.46 ± 35.4 μM). DON concentrations ranged from 9.3 μM to 24.2 μM (mean 13.1 ± 0.6 μM) and highest concentrations were again found in August (17.6 ± 1.7 μM) and at Creek (15.7 ± 1.3 μM). DON comprised 92 ± 1% of TDN, with no significant differences between seasons or stations.

DOM C:N was significantly higher in August than in other months (35.2 ± 3.4; p=0.001). Also during August, the C:N increased along the transect from landward Creek (24.4 ± 0.1) to seaward Hog (39.6 ± 5.1), whereas in February and October, C:N decreased along the transect (23.8 ± 2.6 to 18.3 ± 1.6 and 17.6 ± 0.9 to 14.5 ± 0.3, respectively). There was no station trend in April or June. Major peaks in synchronous fluorescence spectroscopy occurred at 283 nm (figure 8), indicative of labile protein-like material (De Souza Sierra et al. 1994, Coble 1996).

DIN concentrations ranged from 0.13 μM in February to 3.11 μM in August (table 3; figure 7). Average chlorophyll *a* concentrations were 3.3 μg l^{-1}, with the highest concentrations found in August at an average of 6.0 μg l^{-1} (figure 9).

Table 3. Average initial concentrations and standard error of DOC, DON, and DIN in Hog Island Bay for all sampling events.

Station	Sampling month	Initial DOC Concentration (□M)	Initial DON Concentration (□M)	Initial C:N of DOM	Initial DIN Concentration (□M)
Creek	February	240 ± 2	10 ± 1.0	23.8 ± 2.6	0
	April	193 ± 6	13 ± 0.2	14.5 ± 0.6	0.95 ± 0.19
	June	298 ± 9	17 ± 0.1	17.1 ± 0.6	3.26 ± 0.04
	August	591 ± 4	24 ± 0.2	24.4 ± 0.1	3.15 ± 0.19
	October	235 ± 12	13 ± 0.1	17.6 ± 0.9	1.32 ± 0.09
Shoal	February	182 ± 5	9 ± 0.2	19.6 ± 0.8	0.31 ± 0.16
	April	136 ± 1	10 ± 0.1	13.7 ± 0.3	0.85 ± 0.14
	June	209 ± 12	12 ± 0.1	17.3 ± 1.0	1.06 ± 0.05
	August	549 ± 69	15 ± 0.4	37.9 ± 5.7	4.20 ± 0.40
	October	190 ± 4	12 ± 0.1	15.4 ± 0.3	0.46 ± 0.01
Hog	February	174 ± 8	9 ± 0.1	18.3 ± 1.6	0.07 ± 0.07
	April	146 ± 9	10 ± 0.1	14.4 ± 0.8	0.64 ± 0.12
	June	238 ± 4	14 ± 0.1	17.0 ± 0.3	0.12 ± 0.11
	August	553 ± 75	14 ± 0.2	39.6 ± 5.1	1.99 ± 0.18
	October	163 ± 2	11 ± 0.1	14.5 ± 0.3	1.19 ± 0.10

Figure 7. Initial concentrations of DOC, DON, and DIN in Hog Island Bay at sampling.

Figure 8. Synchronous fluorescence spectroscopy analysis of DOM from Creek in Hog Island Bay. Green bars represent the range of emission peaks from algal-derived proteinaceous material. Red bars represent the range of peaks from humic substances. Ranges from De Souza Sierra et al. 1994.

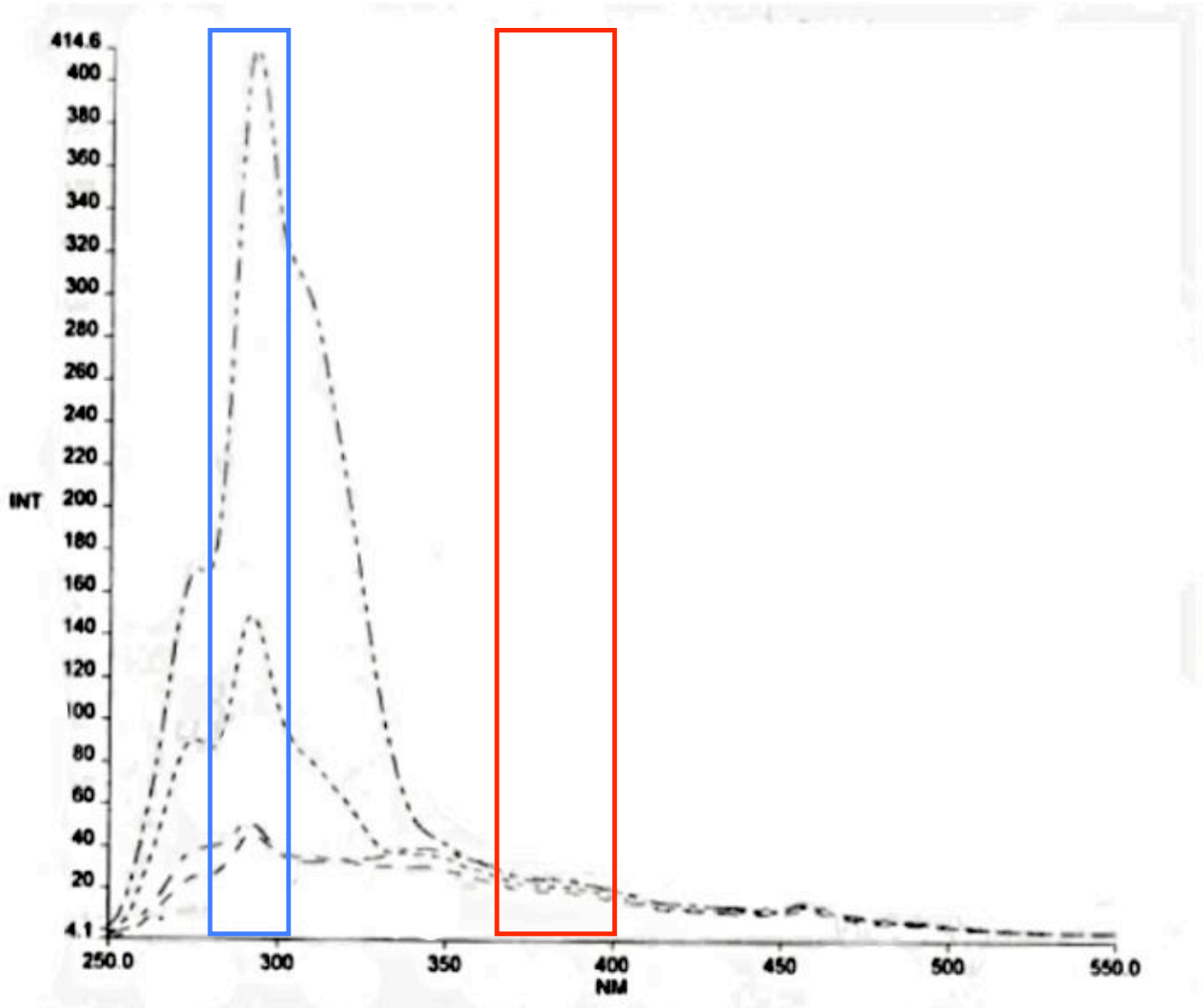
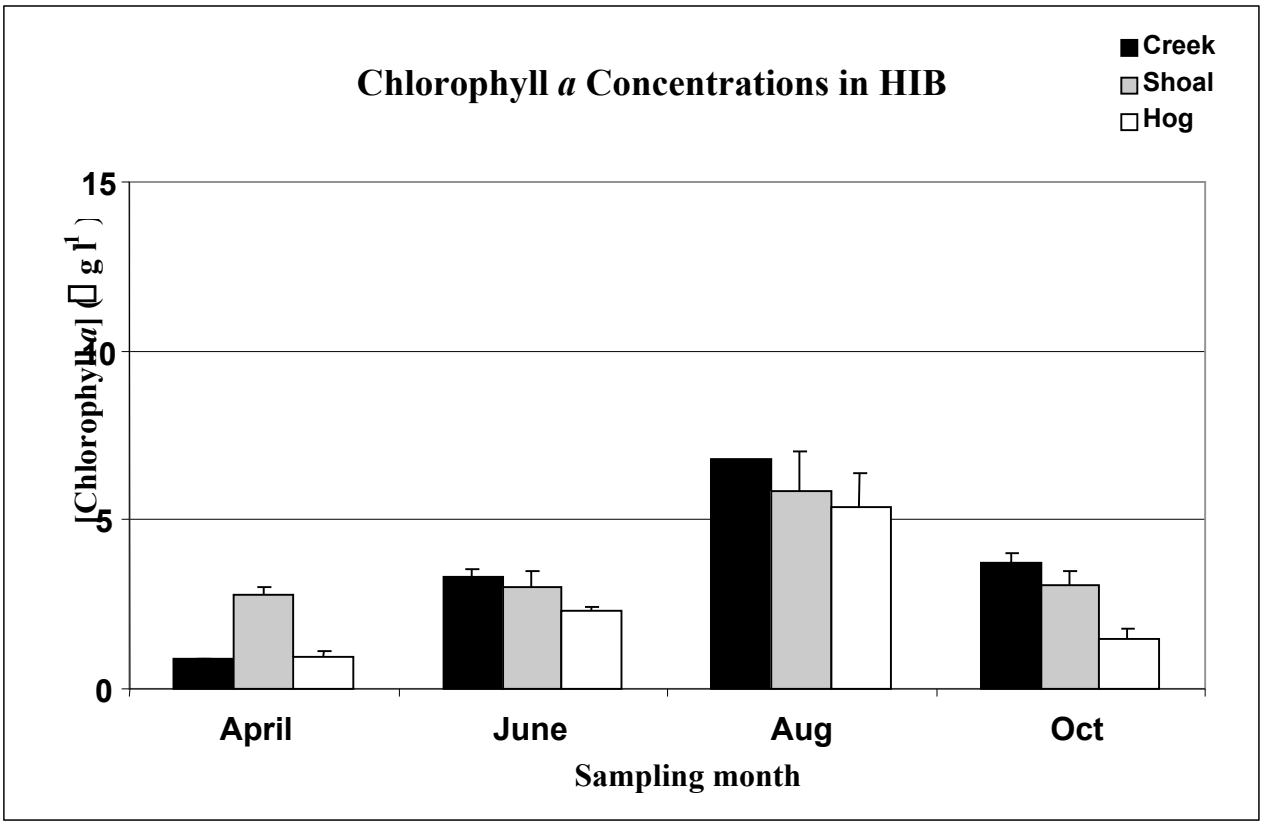

414.6
400
380
360
340
320
300
280
260
240
220
INT
200
180
160
140
120
100
80
60
40
20
4.1
250.0
300
350
400
450
500
550.0
NM

Figure 9. Chlorophyll *a* concentrations at Hog Island Bay stations at time of sampling.


Chlorophyll a Concentrations in HIB
Creek
Shoal
Hog
15
10
5
0
April
June
Aug
Oct
Sampling month

Method verifications

Precision and accuracy of the DON method was verified using standards of 12.5 μM and 25.0 μM L-leucine for all analyses; mean concentration of the 12.5 μM standards was 12.42 ± 0.35 μM (n=13; CV= 0.03), and that of the 25.0 μM standard was 25.18 ± 1.0 μM (n=26; CV=0.04).

Ammonium and nitrate recoveries for gross mineralization and nitrification samples were calculated using 5 μM, 10 μM, and 20 μM standards, and by measuring the amount of ammonium or nitrate in the sample compared to that recovered by diffusion and analyzed in the elemental analyzer at University of California, Davis. Recovery efficiency of ammonium for gross mineralization standards averaged 64.5 ± 4%, and varied with concentration, indicating decreased efficiency at higher concentrations: recovery of 5 μM standards was 74 ± 4%, 10 μM was 78 ± 3 %, and 20 μM was 50 + 5%. The isotope signal for the 30 atom % ^{15}N standards was 35.7 ± 3 atom %. Ammonium recovery from all gross mineralization samples (sample ammonium concentration measured by the elemental analyzer compared to that measured in our lab) averaged 126 ± 3%.

Recovery of the nitrate in the nitrification standards was 103 ± 3%. There were no significant differences between different standard concentrations. The isotope signal for the 30 atom % ^{15}N standards was 24.3 ± 0.6 atom %. Nitrate recovery from samples ranged from 14% to 175% and averaged 59 ± 3%.

Net mineralization time courses

DOC and DON utilization rates were calculated as slopes of linear regression lines in the time course data ([DOC] or [DON] vs. time). Negative numbers indicated removal from the water column, or microbial utilization of DOC or DON. Replicates were analyzed using a difference of two means t-test. Only one replicate (August, Creek, Replicate #1) was found to be significantly different than the other two replicates in the set It is indicated in bold (data tables in Appendix A) and was not included in the pooled data set. Data in figures are the average of the pooled replicates with error bars showing standard error between replicates. Figures of utilization rates show the absolute values of the rates, so that utilization of organic matter is shown as a positive number.

Plum Island Sound: No significant differences were detected in DON utilization between stations or seasons. The average rate of DON utilization was 0.065 ± 0.018 mmol-N m^{-3} d^{-1} (figure 10), and the percent of initial DON utilized after 3 weeks was 5.7 ± 2.0 % (figure 11). DON utilization did not correlate with C:N of the organic matter.

DOC utilization at PIS did correlate with DOM C:N and there were significant differences between stations and seasons. DOC utilization rate was highest at Newbury (mesohaline; $p < 0.0001$), with an average rate over all seasons of 4.003 ± 0.782 mmol-C m^{-3} d^{-1}, compared to Middle Bridge (1.613 ± 0.634 mmol-C m^{-3} d^{-1}) and Plum Island (2.048 ± 0.333 mmol-C m^{-3} d^{-1}; figure 12). Seasonally, the highest rates were in July (average of three stations, 3.428 ± 0.641 mmol-C m^{-3} d^{-1}; $p<0.0001$). There was no utilization of DOC in May at any station, and September DOC was utilized at a rate of

Figure 10. Plum Island Sound DON utilization rates. Rates are presented as absolute values, so that a positive number indicates utilization of DON.

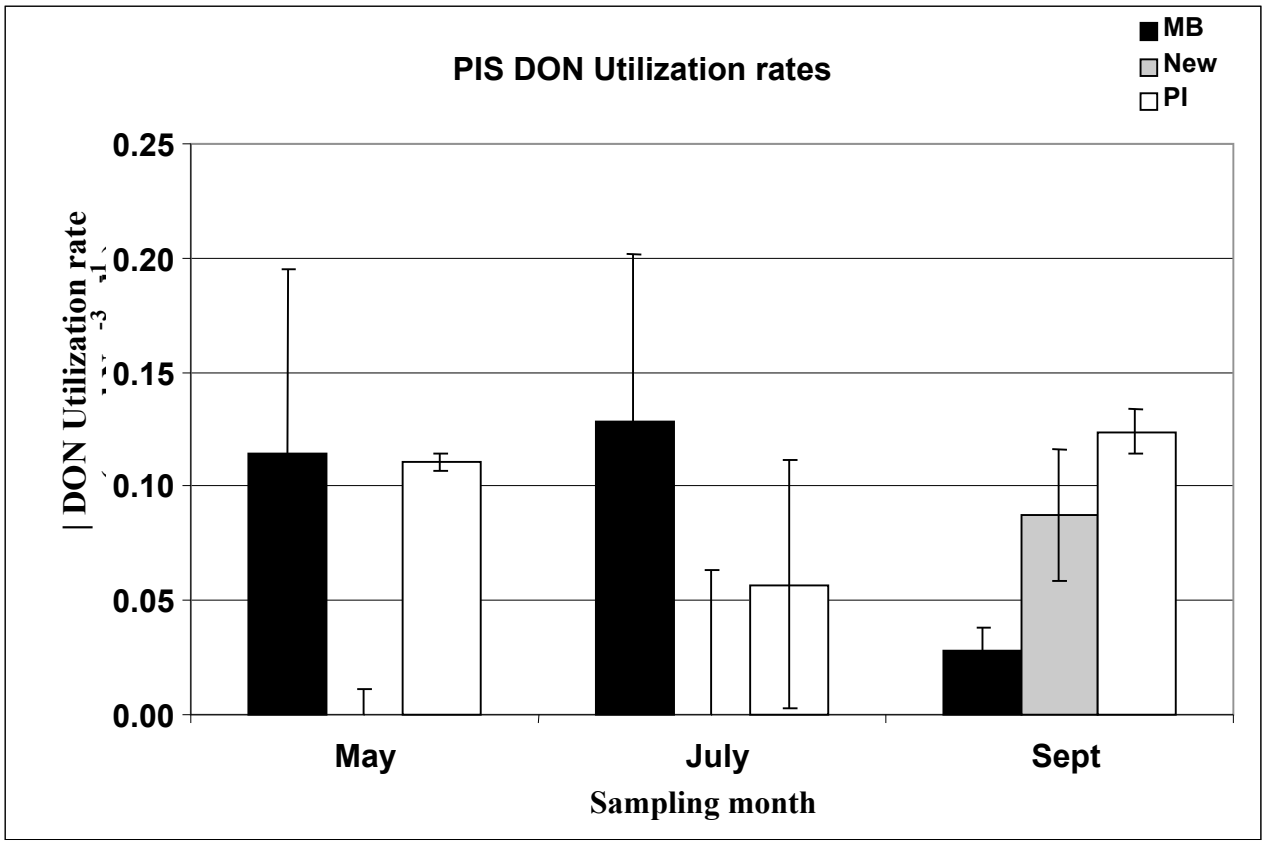

PIS DON Utilization rates
MB
New
PI
0.25
0.20
0.15
0.10
0.05
0.00
May
July
Sept
Sampling month

Figure 11. Plum Island Sound percent of initial DON utilized in three weeks.

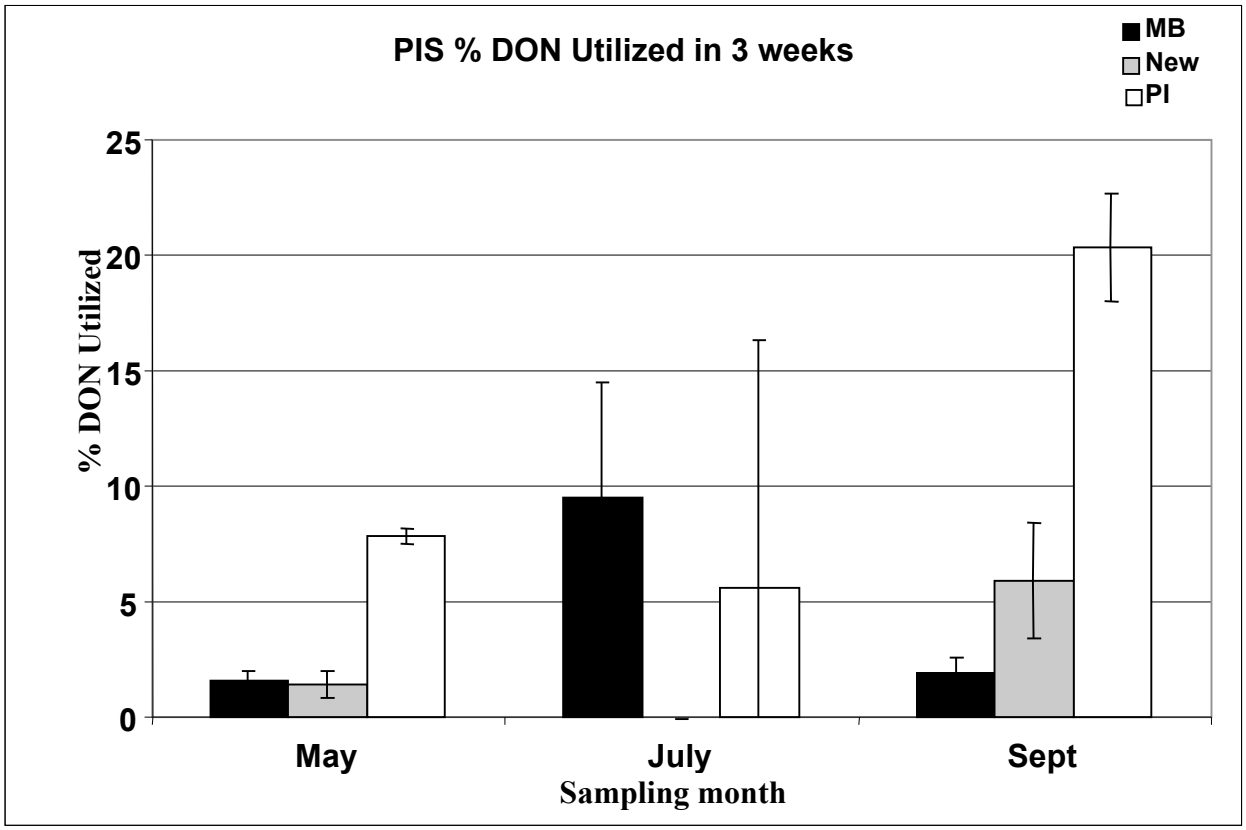
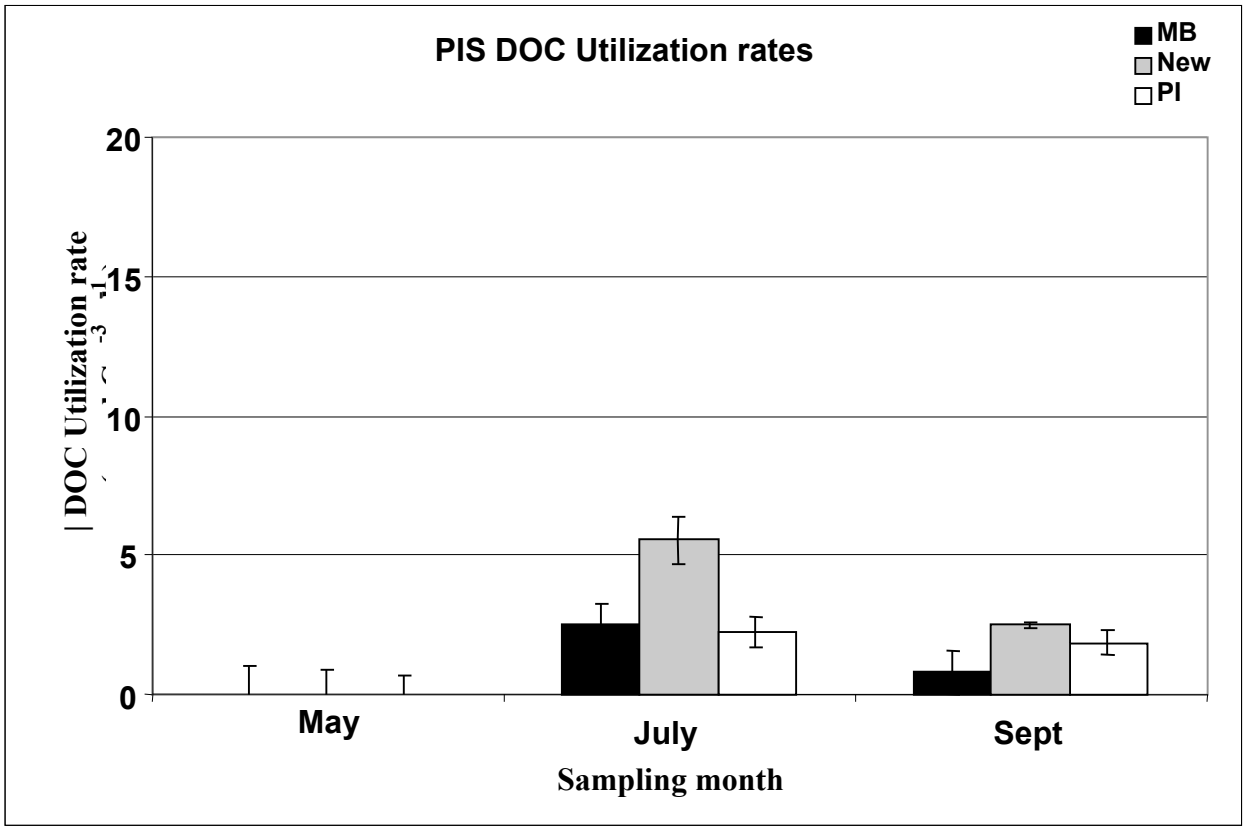

PIS % DON Utilized in 3 weeks
MB
New
PI
25
20
15
10
5
0
% DON Utilized
May
July
Sept
Sampling month

Figure 12. Plum Island Sound DOC utilization rates. Rates are presented as absolute values, so that a positive number indicates utilization of DOC.


PIS DOC Utilization rates
MB
New
PI
20
15
10
5
0
May
July
Sept
Sampling month

1.682 ± 0.370 mmol-C m^{-3} d^{-1}.

Percent of initial DOC utilized in 3 weeks followed a slightly different pattern. Both the highest rate of DOC utilization and the percent of DOC utilized were measured in July (18.2 ± 2.8% compared to 12.8 ± 3.3% in September); however, the highest percent of DOC used was at Plum Island (23.3 ± 2.9%) compared to Middle Bridge (6.7 ± 2.5%) and Newbury (16.4 ± 2.6%; figure 13). Percent of DOC utilized and rates of utilization correlated with the initial DOM C:N (model 2 regression; Sokal and Rohlf 1981). As C:N increased, percent of initial DOC utilized and utilization rate both decreased, indicating a decrease in lability with increasing C:N (figure 14).

Hog Island Bay: Significant differences in DON utilization rates were detected between seasons ($p<0.0001$) and stations ($p=0.026$). There was also a significant interaction effect ($p=0.008$). The highest utilization rates averaged for all stations were measured in August: 0.098 ± 0.026 mmol-N $m^{-3}d^{-1}$. Rates in April (0.039 ± 0.005 mmol-N $m^{-3}d^{-1}$), June (0.061 ± 0.007 mmol-N $m^{-3}d^{-1}$), and October (0.045 ± 0.004 mmol-N $m^{-3}d^{-1}$) were not significantly different from each other, but were all higher than February, which was not significantly different than zero. Along the gradient from land to sea, the highest average rates of utilization were at Creek (0.065 ± 0.019 mmol-N $m^{-3}d^{-1}$), but they were not significantly different from those at Hog (0.050 ± 0.008 mmol-N $m^{-3}d^{-1}$). The interaction effect was caused by the high utilization rates in August at Creek.(I'm a little worried about attributing the high utilization in August to the Macroalgal crash since it only shows up at Creek; who knows, it could have been runoff from the uplands or DON from the organic rich benthic sediments at Creek). The utilization rate at Creek in

August (0.173 ± 0.054 mmol-N $m^{-3}d^{-1}$) was much higher than the overall average (0.050 ± 0.007 mmol-N $m^{-3}d^{-1}$; figure 15).

Figure 13. Plum Island Sound percent of initial DOC utilized in three weeks.

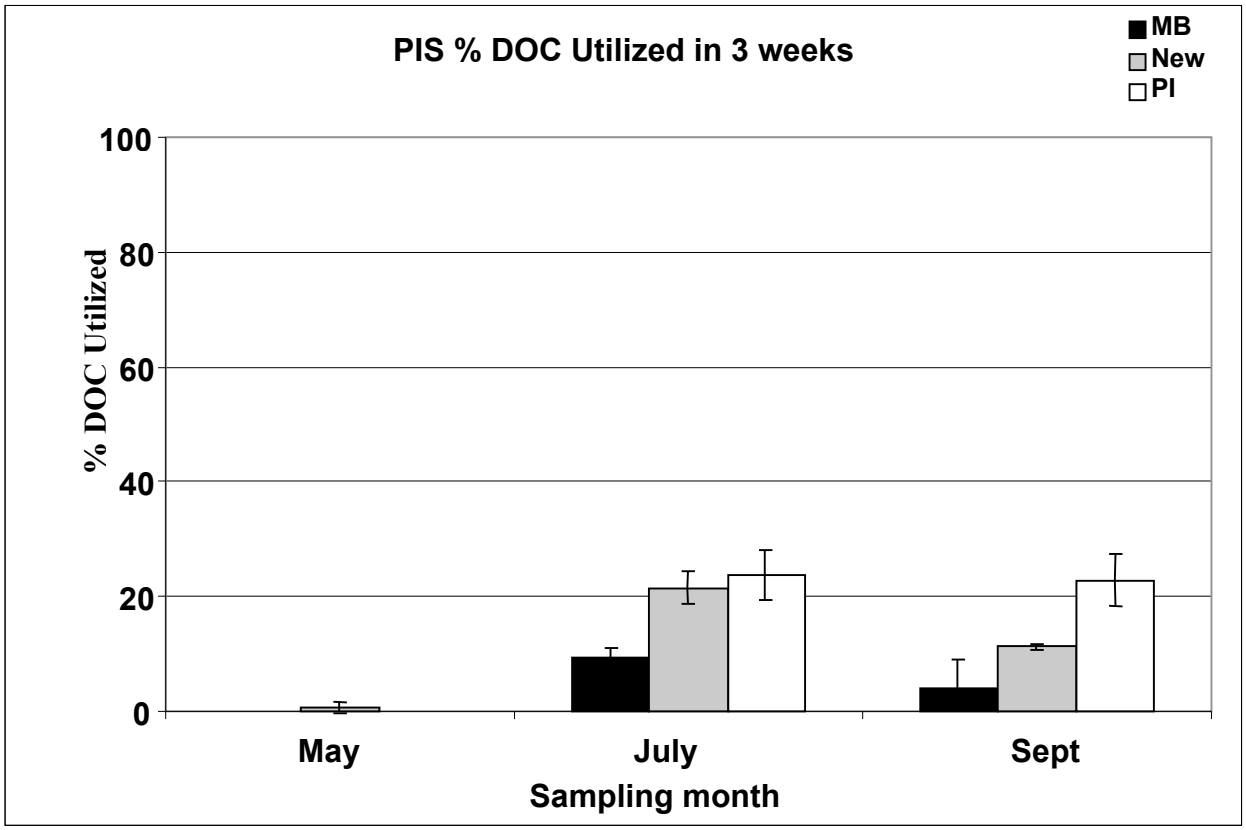
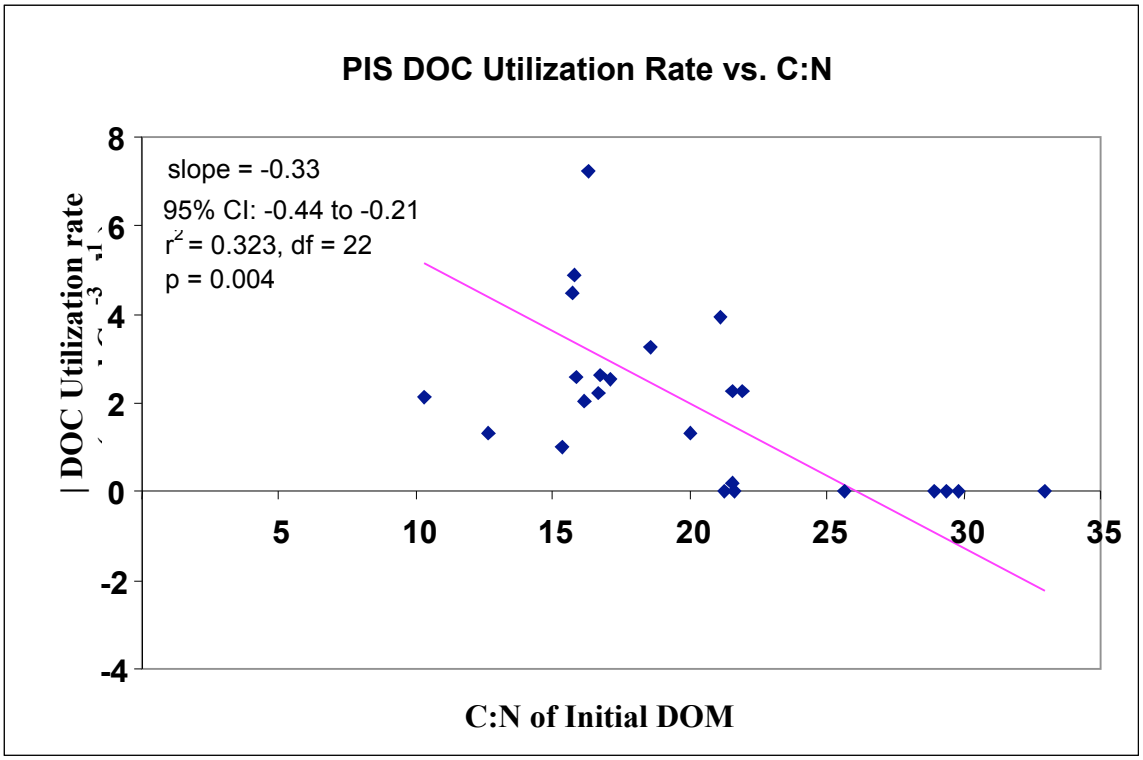

PIS % DOC Utilized in 3 weeks
MB
New
PI
100
80
60
40
20
0
% DOC Utilized
May
July
Sept
Sampling month

Figure 14. Plum Island Sound DOC utilization compared to initial C:N of dissolved organic matter.


PIS DOC Utilization Rate vs. C:N
slope = -0.33
95% CI: -0.44 to -0.21
r2 = 0.323, df = 22
p = 0.004
| DOC Utilization rate
8
6
4
2
0
-2
-4
5
10
15
20
25
30
35
C:N of Initial DOM

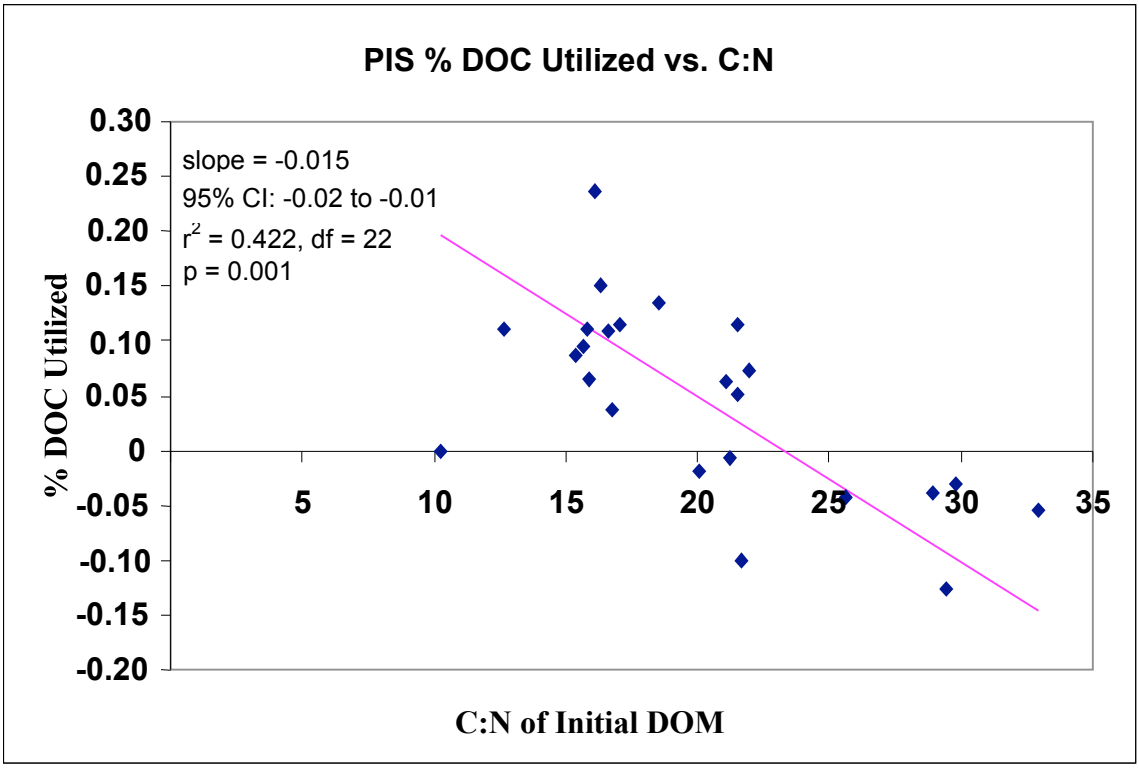
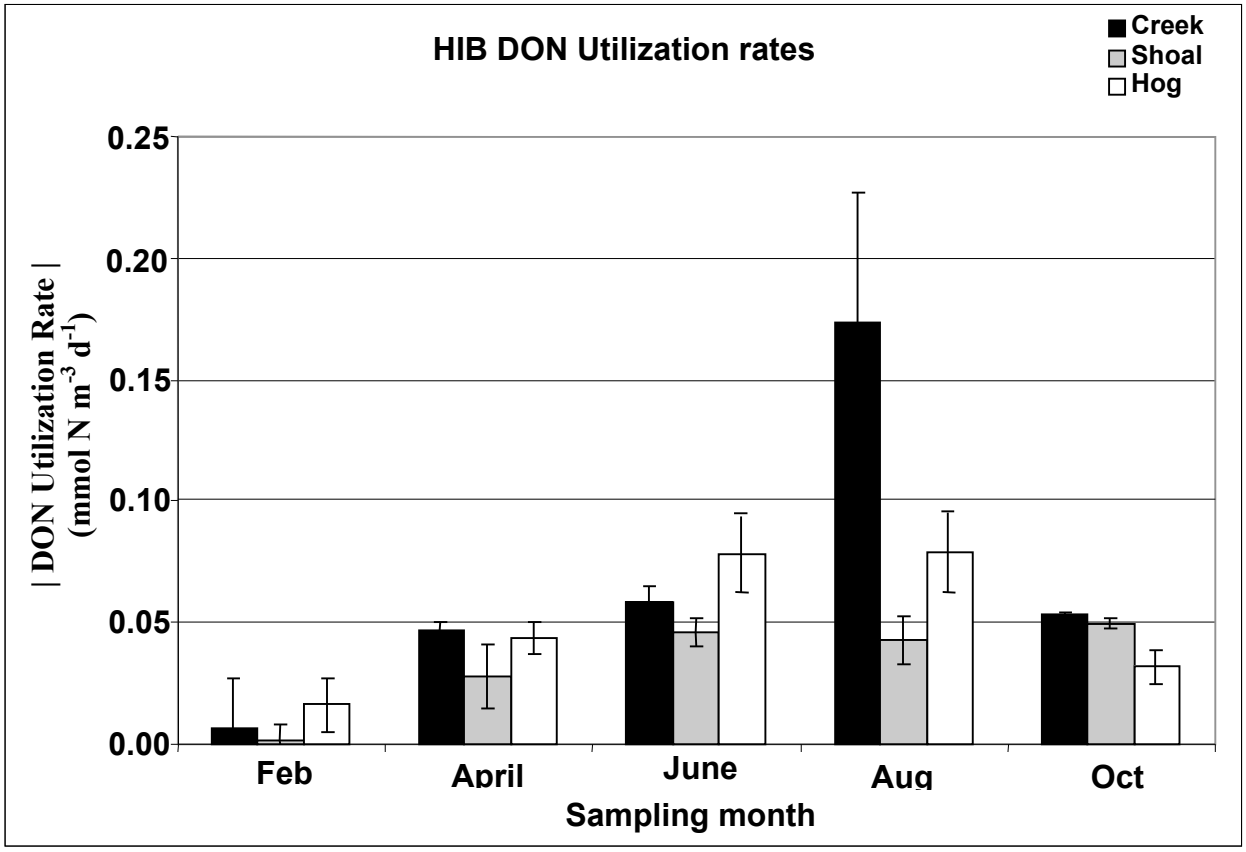

PIS % DOC Utilized vs. C:N
slope = -0.015
95% CI: -0.02 to -0.01
r2 = 0.422, df = 22
p = 0.001
% DOC Utilized
0.30
0.25
0.20
0.15
0.10
0.05
0
-0.05
-0.10
-0.15
-0.20
5
10
15
20
25
30
35
C:N of Initial DOM

Figure 15. Hog Island Bay DON utilization rates. Rates are presented as absolute values, so that a positive number indicates utilization of DON.


HIB DON Utilization rates
Creek
Shoal
Hog
| DON Utilization Rate |
(mmol N m-3 d-1)
0.25
0.20
0.15
0.10
0.05
0.00
Feb
April
June
Aug
Oct
Sampling month

There were significant differences in the percent of initial DON utilized after three weeks between seasons ($p<0.0001$) but no station or interaction affects were observed. In February there was no measurable utilization of DON (figure 16). The average percent of initial DON utilized was 8.5 ± 0.8% for all months other than February.

Utilization of DOC in HIB followed similar trends as DON. Significant differences in DOC utilization rates were detected only between seasons ($p<0.0001$; figure 17). There was no measurable utilization of DOC in April, and low utilization was measured in October (0.312 ± 0.186 mmol-C m^{-3} d^{-1}). DOC utilization in February (2.159 ± 0.244 mmol-C m^{-3} d^{-1}) and June (3.646 ± 0.279 mmol-C m^{-3} d^{-1}) were not significantly different from each other, and rates were highest in August (9.763 ± 2.237 mmol-C m^{-3} d^{-1}).

Percent of initial DOC utilized after 3 weeks showed significant differences between seasons ($p<0.0001$) and stations ($p=0.04$) with no interaction effects observed. April is not included in this comparison because the DOC samples for the last sampling period were lost; however, all time points between zero and 21 days indicated no DOC utilization. Percent of initial DOC utilized was highest in August (54.0 ± 3.9%), as was observed with the utilization rates. Percents utilized in February (24.1 ± 2.1%) and June (27.1 ± 1.9%) were not significantly different from each other, and lowest percent utilized was observed in October (4.4 ± 2.4%; figure 18). Comparing stations across the lagoon transect, Shoal (mid-lagoon) had the highest percent of DOC utilized (30.7 ± 4.7%) compared to Creek (landward; 20.5 ± 4.2%); Hog (seaward) was not significantly different from either Shoal or Creek (28.2 ± 5.7%).

Figure 16. Hog Island Bay percent of initial DON utilized.

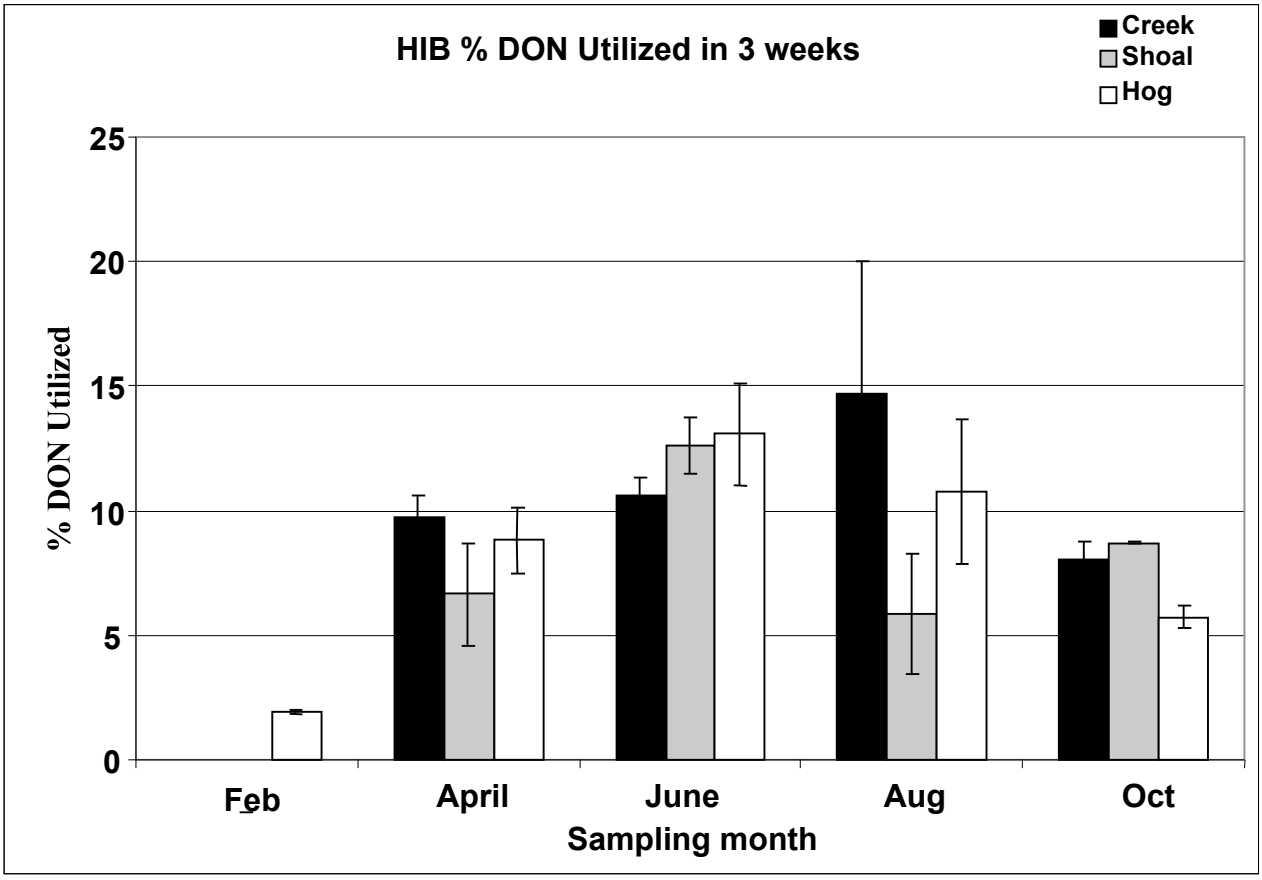

HIB % DON Utilized in 3 weeks
Creek
Shoal
Hog
% DON Utilized
25
20
15
10
5
0
Feb
April
June
Aug
Oct
Sampling month

Figure 17. Hog Island Bay DOC utilization rates. Rates are presented as absolute values, so that a positive number indicates utilization of DOC.

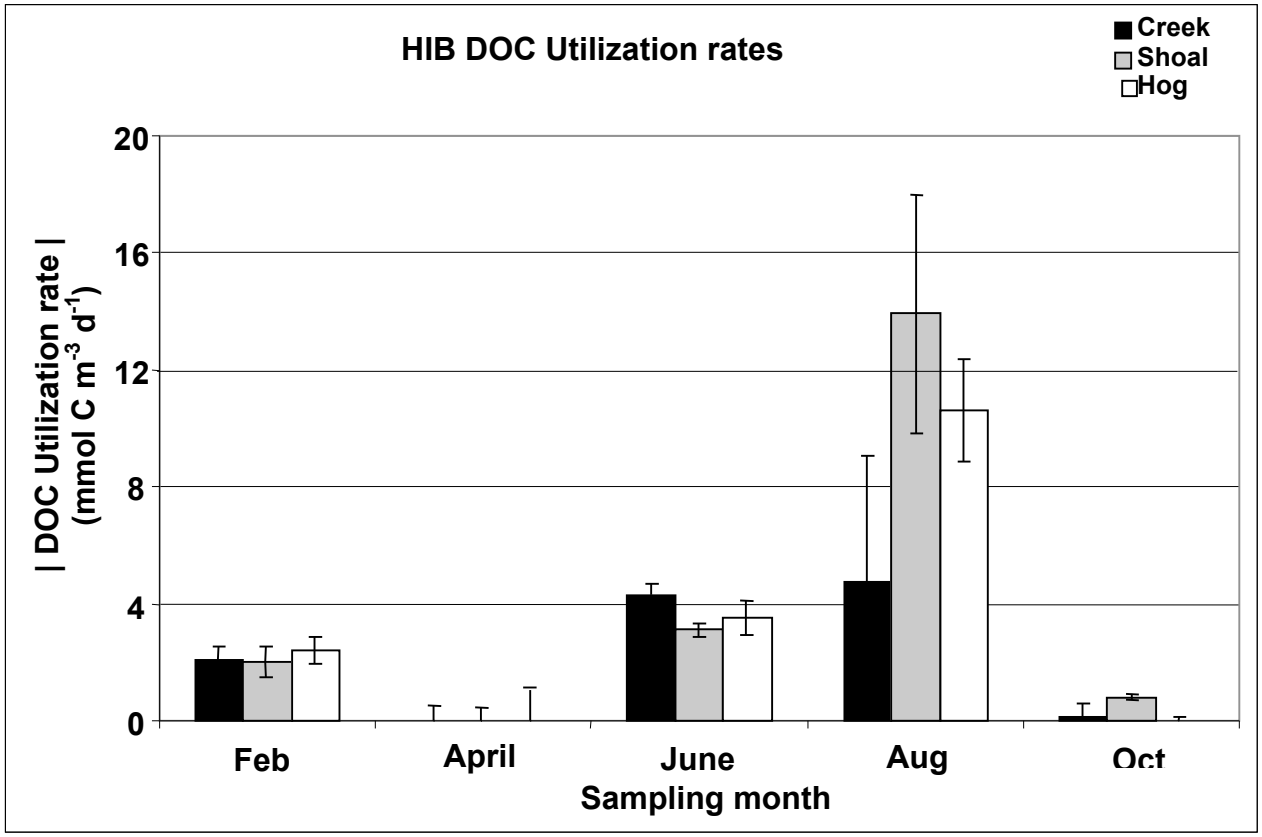
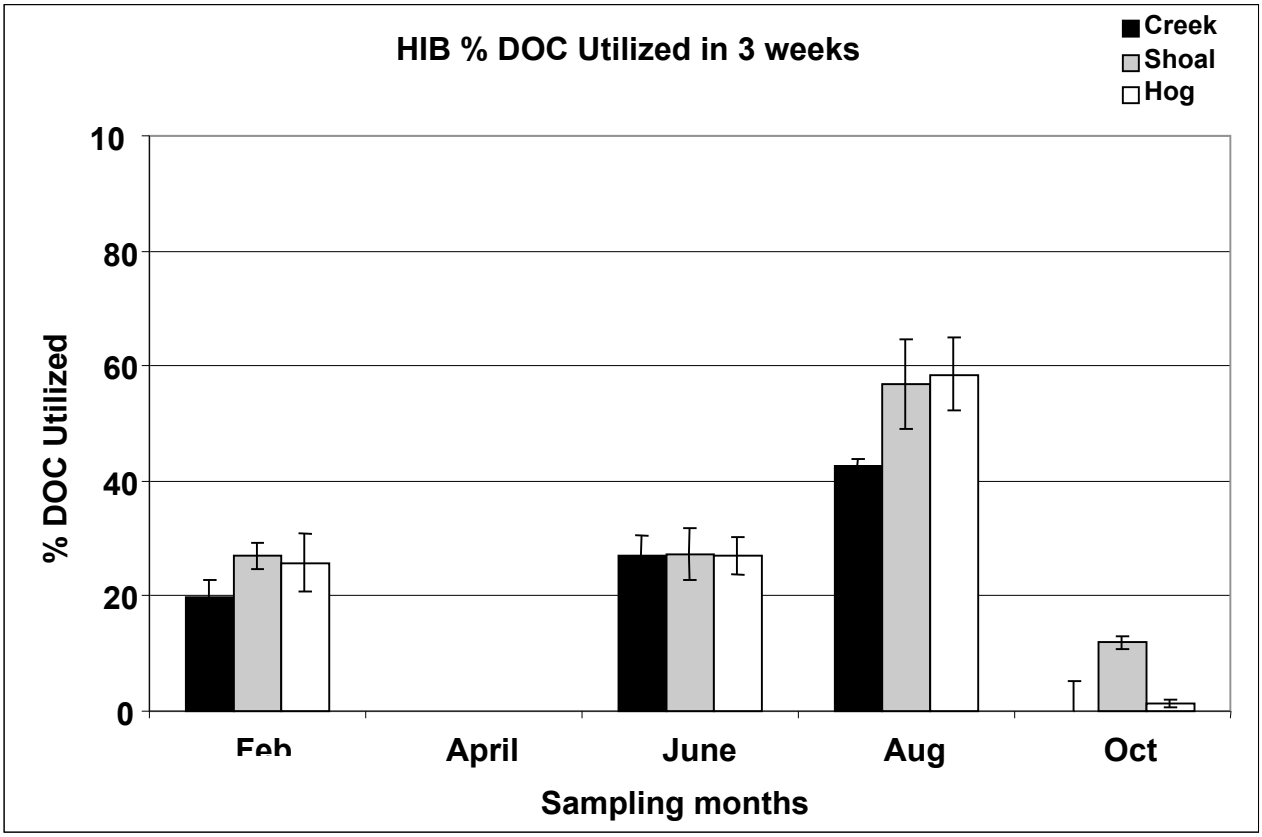

HIB DOC Utilization rates
Creek
Shoal
Hog
| DOC Utilization rate |
(mmol C m-3 d-1)
20
16
12
8
4
0
Feb
April
June
Aug
Oct
Sampling month

Figure 18. Hog Island Bay percent of initial DOC utilized in three weeks.


HIB % DOC Utilized in 3 weeks
Creek
Shoal
Hog
% DOC Utilized
10
80
60
40
20
0
Feb
April
June
Aug
Oct
Sampling months

Site comparison of PIS vs. HIB: Comparisons between the two sites were done using a 3-factor ANOVA, with the following factors: site (PIS and HIB), station (landward, middle, and seaward), and season (spring, summer, and autumn; figure 3). Spring included April sampling in HIB and May sampling in PIS, summer included June and July, and autumn included August and September. There was no difference detected between the two sites for DON utilization rate averaged over stations and seasons. The average DON utilization rates for PIS and HIB were 0.065 ± 0.018 and 0.050 ± 0.007 mmol-N $m^{-3}d^{-1}$, respectively (table 4). The rates varied significantly only between stations ($p=0.004$), indicating that the landward (0.075 ± 0.017 mmol-N $m^{-3}d^{-1}$) and seaward (0.068 ± 0.010 mmol-N $m^{-3}d^{-1}$) stations were not significantly different from each other, but both were higher than the middle-estuary or middle-lagoon station (0.023 ± 0.012 mmol-N $m^{-3}d^{-1}$).

The percent of initial DON utilized after 3 weeks did show a significant difference between sites ($p=0.012$) in addition to the difference between stations ($p=0.025$). This parameter indicated that, in general, a greater percentage of DON was metabolized in HIB (8.5 ± 1.0%) than in PIS (5.7 ± 2.0%; table 4). At both sites the percent of DON utilized was highest at the most seaward station (9.7 ± 1.7%), lower at the landward station (not significantly different; 6.4 ± 1.8%), and lowest at the middle station (4.7 ± 1.3%).

DOC utilization rates were significantly higher at HIB than at PIS (2.543 ± 0.789 and 0.912 ± 0.378 mmol-C $m^{-3}d^{-1}$, respectively; $p=0.002$; table 4). There were also season and station effects. In general, rates of DOC utilization in summer (3.567 ± 0.340 mmol-C $m^{-3}d^{-1}$) and autumn (5.723 ± 1.473 mmol-C $m^{-3}d^{-1}$) were not significantly

Table 4. Summary of results for Plum Island Sound and Hog Island Bay. Numbers represent the overall averages over stations and seasons in each site for each parameter calculated.

	DON utilization rate (mmol-N $m^{-3}d^{-1}$)	% of Initial DON Utilized	DOC utilization rate (mmol-C $m^{-3}d^{-1}$)	% of Initial DOC Utilized	Gross mineralization—NH_4^+ production (mmol-N $m^{-3}d^{-1}$)
Plum Island Sound	0.065 ± 0.018	5.7 ± 2.0	0.912 ± 0.578	7.0 ± 3.0	0.248 ± 0.015
Hog Island Bay	0.050 ± 0.007	8.5 ± 1.0	2.543 ± 0.789	26.7 ± 2.8	0.237 ± 0.082

different from each other, but both were greater than spring, when there was no measurable DOC utilization at either site ($p<0.0001$). In contrast to DON, DOC utilization was highest at the middle stations (3.152 ± 1.064 mmol-C $m^{-3}d^{-1}$; $p=0.019$); the landward (1.231 ± 0.725 mmol-C $m^{-3}d^{-1}$) and seaward (1.411 ± 0.993 mmol-C $m^{-3}d^{-1}$) stations were not significantly different from each other. There was a site/season interaction ($p<0.0001$) because the landward station behaved very differently during different seasons.

Intersite comparison of the percent of initial DOC utilized after three weeks incubation could not be performed due to missing DOC data in the HIB April samples. However, analysis of data collected after 1-week incubation indicated that percent utilization followed a similar trend as the DOC utilization rate. In general, percent of DOC utilized was greater at HIB than at PIS (13.7 ± 4.1% and 3.3 ± 1.8%, respectively; $p<0.0001$). Percent utilized after three weeks was 26.7 ± 2.8% in HIB and 7.0 ± 3.0% in PIS (table 4). There was a significant interaction effect between site and season ($p<0.0001$), due to the fact that in spring PIS had greater DOC utilization, but the difference between the two sites in the spring was small. The seasonal comparison showed that DOC metabolism in autumn (31.8 ± 6.4%) was greater than in summer (13.4 ± 1.9%), and spring rates were not significantly different from zero ($p<0.0001$). Percent of initial DOC utilized was greater at the middle (22.4 ± 3.7%) and seaward (20.0 ± 4.8%) stations than at the landward station (11.6 ± 3.5%), but there was also a significant season/station interaction effect ($p=0.035$).

Gross mineralization and nitrification

Gross mineralization of DON to ammonium (turnover of the ammonium pool) and nitrification of ammonium to nitrate were measured using the isotope pool dilution method. Production and consumption of the ammonium (or nitrate) were calculated from changes in total ammonium (or nitrate) concentrations and changes in ^{15}N enrichment. The following equations were used:

$$\text{Production} = \frac{\ln \frac{(\text{atom\% } t_f - k)}{(\text{atom\% } t_0 - k)}}{\ln \frac{[NH_4^+ t_f]}{[NH_4^+ t_0]}} * \frac{[NH_4^+ t_0] - [NH_4^+ t_f]}{\text{time}}$$

$$\text{Consumption} = \left[1 + \frac{\ln \frac{(\text{atom\% } t_f - k)}{(\text{atom\% } t_0 - k)}}{\ln \frac{[NH_4^+ t_f]}{[NH_4^+ t_0]}} \right] * \frac{[NH_4^+ t_0] - [NH_4^+ t_f]}{\text{time}}$$

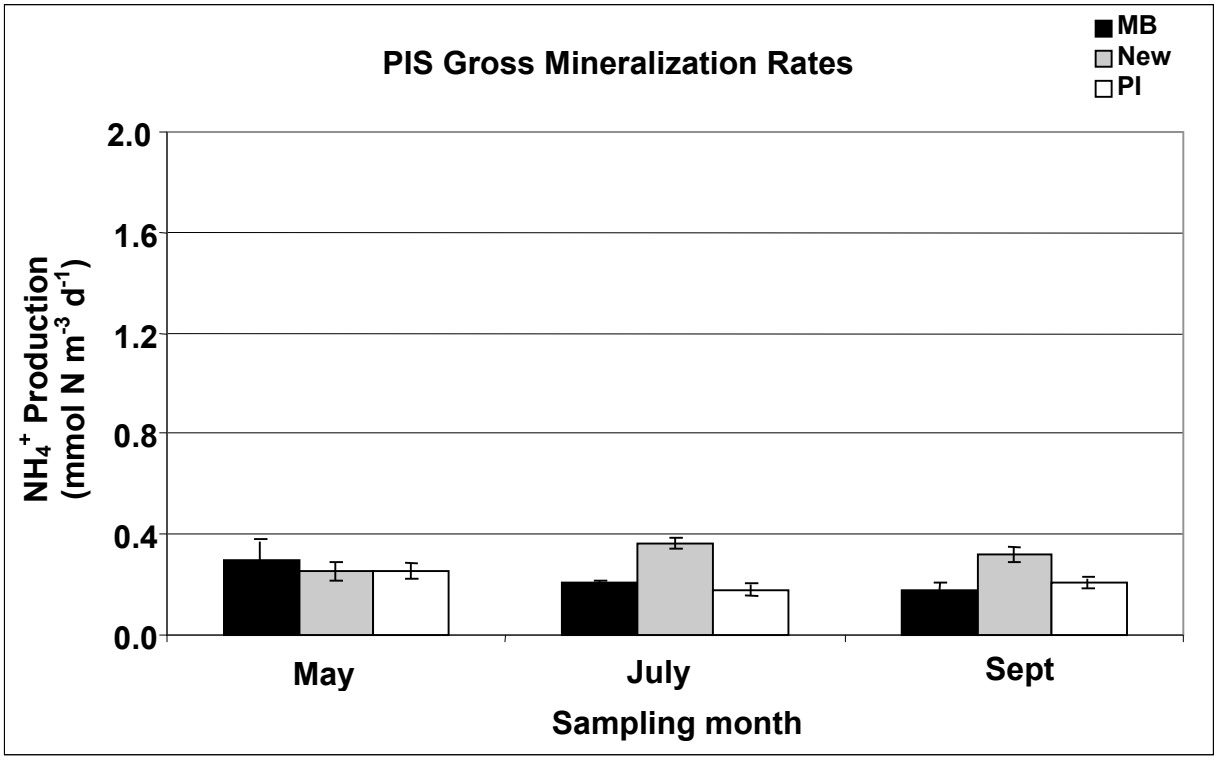
where "k" is the natural abundance of ^{15}N, 0.3663 atom%; "t_f" represents the final time of the incubation; "t_0" is the starting time; and "time" refers to the duration of the incubation. Assumptions for the model used are: (1) mineralizable DOM is not limiting; (2) no dissimilatory nitrate reduction is occurring (Wessel and Tietema 1992). Both assumptions were met, as the concentrations of DOC and DON were never depleted and incubation bottles were opened and remained oxic.

Plum Island Sound: Production of ammonium did not vary over seasons, average of all stations and seasons was 0.248 ± 0.015 mmol-N $m^{-3}d^{-1}$ (figure 19). Along the estuarine gradient, ammonium production was higher at Newbury, the middle station (0.341 ± 0.020 mmol-N $m^{-3}d^{-1}$), than at both endmembers (0.193 ± 0.015 and 0.192 ± 0.014 mmol-N $m^{-3}d^{-1}$), which were not significantly different from each other (p=.002). Nitrification rates were positive only at Newbury (0.261 ± 0.107 mmol-N $m^{-3}d^{-1}$); nitrification at Middle Bridge and Plum Island was not measurable (figure 20).

Hog Island Bay: Production of ammonium only occurred during April, June, and August; production was highest in April (0.872 ± 0.258 mmol-N $m^{-3}d^{-1}$; p=0.042; figure 19). Rates in June and August were not significantly different from each other (0.246 ± 0.029 and 0.293 ± 0.028 mmol-N $m^{-3}d^{-1}$, respectively). On average, no station differences were detected; however, in April gross mineralization was not observed at Creek (landward station), whereas at Shoal (middle) and Hog (seaward) ammonium was produced: 1.250 ± 0.010 and 1.097 ± 0.478 mmol-N $m^{-3}d^{-1}$, respectively. Nitrification rates were only significantly greater than zero at Shoal (0.388 ± 0.256 mmol-N $m^{-3}d^{-1}$). Creek and Hog nitrification rates were 0.066 ± 0.143 and 0.066 ± 0.063 mmol-N $m^{-3}d^{-1}$, respectively (figure 20).

Site comparison of PIS vs. HIB: Significant differences in gross mineralization were detected between sites (p=0.044) and seasons (p=0.027). Rates in spring (averaged for all stations in both sites, 0.589 ± 0.149 mmol-N $m^{-3}d^{-1}$) were greater than in summer (0.248 ± 0.021 mmol-N $m^{-3}d^{-1}$) and in autumn (0.264 ± 0.020 mmol-N $m^{-3}d^{-1}$), which

Figure 19. Plum Island Sound and Hog Island Bay gross mineralization ammonium production.


PIS Gross Mineralization Rates
MB
New
PI
2.0
1.6
1.2
0.8
0.4
0.0
NH_4^+ Production (mmol N m^{-3} d^{-1})
May
July
Sept
Sampling month

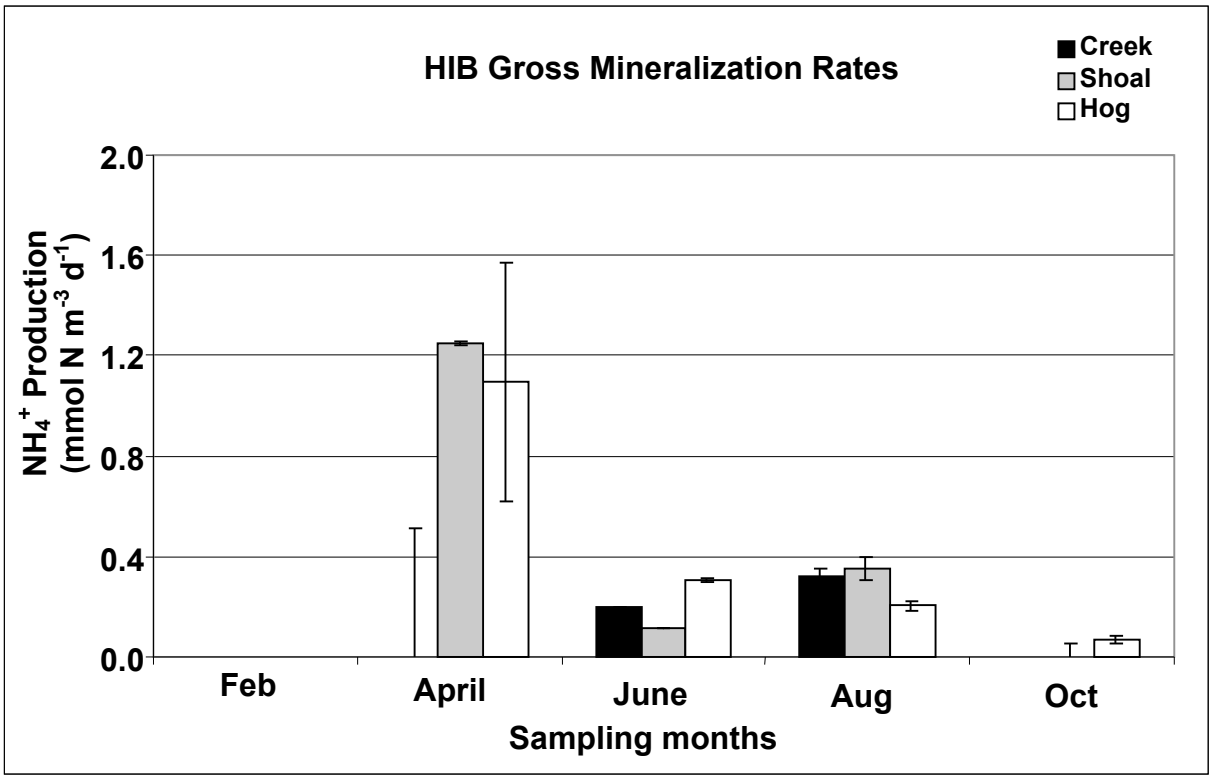
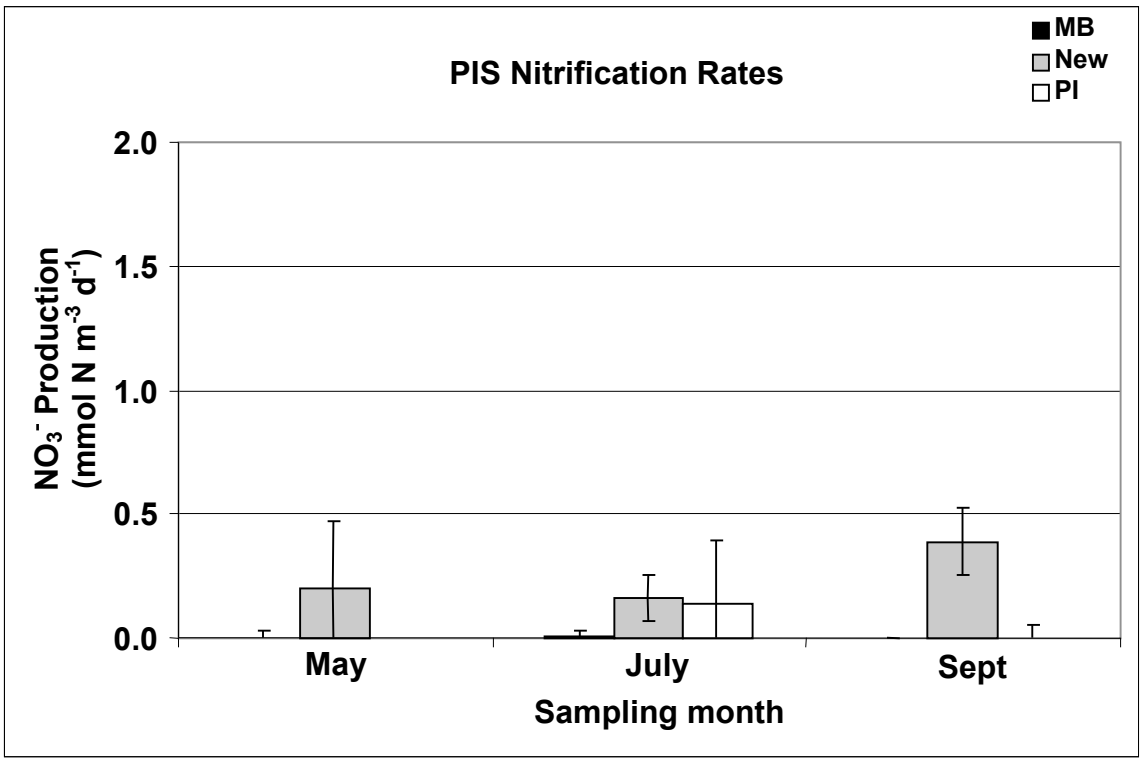
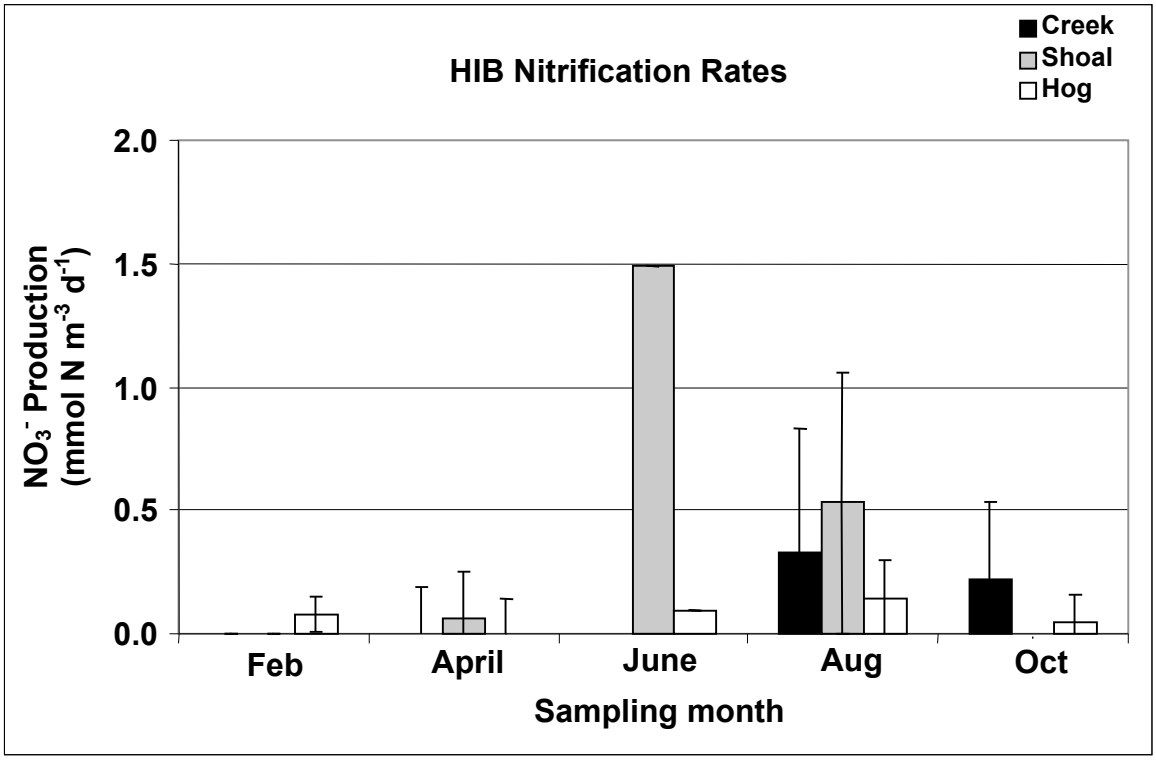

HIB Gross Mineralization Rates
Creek
Shoal
Hog
2.0
1.6
1.2
0.8
0.4
0.0
NH_4^+ Production (mmol N m^{-3} d^{-1})
Feb
April
June
Aug
Oct
Sampling months

Figure 20. Plum Island Sound and Hog Island Bay gross nitrification rates.


PIS Nitrification Rates
MB
New
PI
2.0
1.5
1.0
0.5
0.0
NO_3^- Production (mmol N m^{-3} d^{-1})
May
July
Sept
Sampling month


HIB Nitrification Rates
Creek
Shoal
Hog
2.0
1.5
1.0
0.5
0.0
NO_3^- Production (mmol N m^{-3} d^{-1})
Feb
April
June
Aug
Oct
Sampling month

were not significantly different from each other. High ammonium production was measured in HIB in April (0.872 ± 0.258 mmol-N $m^{-3}d^{-1}$); this rate was significantly higher than the average of all data collected (0.242 ± 0.073 mmol-N $m^{-3}d^{-1}$). Overall, the averages of gross ammonium production in PIS (0.248 ± 0.015 mmol-N $m^{-3}d^{-1}$) and HIB (0.237 ± 0.082 mmol-N $m^{-3}d^{-1}$) were similar (table 4).

Methodological problems encountered

Gross mineralization and nitrification: Recovery efficiencies of ammonium standards by diffusion were low (64.5 ± 4.0%) compared to experimental samples (126 ± 3%). The recovery efficiency of experimental samples indicated that more ammonium was recovered after the ammonium diffusion procedure than was measured prior to treatment. The additional ammonium, most likely derived from abiotic breakdown of DON at the high pH (>9.7) required for diffusion, diluted the $^{15}N\text{-}NH_4^+$ pool, thereby causing us to overestimate gross mineralization.

The recovery efficiency of nitrification nitrate standards was 103 ± 3%, but sample recoveries ranged from 14-175%, average 59 ± 3%. The comparison of standards and samples indicated that there were methodological errors in sample recovery. It is likely that the salinities of the samples affected recovery efficiency. Middle Bridge in PIS was the only freshwater station and the recovery efficiencies for MB samples were 126 ± 7%. At Newbury (mesohaline PIS station), Plum Island (polyhaline PIS station) and HIB sample recovery efficiencies were 48 ± 5%, 39 ± 5%, and 52 ± 3%, respectively.

Therefore, the excess KCl added to the samples may have interfered with sample recovery (C. Tobias, USGS, Reston, VA., *pers. comm.*).

In addition to the low recovery efficiencies in the gross mineralization and nitrification samples, many samples were lost during analysis. Diffusion packets came apart in 9% of the incubations, saturating the acidified filter with water. Those samples were not analyzed.

Presence of grazers in incubations: In each incubation, bacterial abundance decreased in the first seven days and then stabilized for the remaining 14 days. Although filtration to 1.0 μm should have removed most grazers, that was obviously not the case. In a few bacterial abundance slides some microheterotrophs were visible, but quantification was not possible based on the low abundances observed. Presence of grazers has been observed in many other studies (Sanders et al 1992; Søndergaard and Middelboe 1995; Seitzinger and Sanders 1997). To avoid this problem in future work, water column bacteria samples should be isolated, sonicated to remove microheterotrophs as described in Seitzinger and Sanders (1997), and added to 0.2 μm filtered DOM.

DISCUSSION

The objective of this study was to compare the fate of DOM in two coastal systems with different DOM sources. PIS is a river-fed estuary with significant terrestrial organic inputs (Hopkinson et al. 1999), whereas HIB is a coastal lagoon with limited freshwater input delivered primarily as base flow (Reay et al 1992). The nitrogen in base flow is primarily in the form of DIN (J. Stanhope, VIMS, *pers. comm.*) As water is transported through an estuary or coastal lagoon, dissolved constituents are transformed and may be removed from the water column. Such transformations may be important in reducing anthropogenic impacts, such as eutrophication, on the coastal ocean. This study focused on nitrogen because of its role as a potential limiting nutrient for primary producers in the coastal ocean (Carpenter and Capone 1983). Many studies have focused on the fate of DIN within coastal systems, but DON has historically been overlooked. In addition, bioavailability of DON and DOC vary through space and time (tables 5 and 6), and the variability is poorly understood.

Plum Island Sound

The dominant source of DOM to Plum Island Sound was terrestrial in origin; DOM entered the estuary at Parker Dam (freshwater head of estuary). Three major pieces of evidence suggest that DOM was terrestrial in origin. First, major synchronous fluorescence peaks from samples at MB (freshwater station) occurred at 400 nm (figure 5). Humic substances characteristically have peaks between 360-400 nm, whereas more labile proteins peak at 280-310 nm (De Souza Sierra et al. 1994, Coble et al. 1996).

Table 5. Comparison of percent of initial DOC utilized in various systems.

System	% Initial DOC utilized	Incubation time	Source
Hog Island Bay	27 ± 3	21 days	This study
Plum Island Sound	7 ± 3	21 days	This study
Cross- system review	17	5 – 7 days	Søndergaard and Middelboe 1995
Bothnian Sea	1 – 7	4 days	Zweifel et al. 1993
Sargasso Sea	6 – 9	4 – 9 days	Carlson and Ducklow 1996
Southeastern USA rivers	2 – 18	35 – 58 days	Moran et al. 1999
Agricultural run-off, New Brunswick, NJ	9 – 14	10 days	Wiegner and Seitzinger 2001
Forest run-off, Stanton, NJ	6 ± 3	10 days	Wiegner and Seitzinger 2001

Table 6. Comparison of net and gross percent of initial DON utilized in various systems.

System	Net % Initial DON utilized	Gross % Initial DON utilized	Incubation time	Source
Hog Island Bay	9 ± 1	19 – 31*	21 days	This study
Plum Island Sound	6 ± 2	14 – 23*	21 days	This study
Delaware River		40 – 72	15 days	Seitzinger and Sanders 1997
Hudson River		40	15 days	Seitzinger and Sanders 1997
South Sweden Wetlands		2 – 16	9 days	Stepanauskas et al. 1999
Lilliån & Stridbacken Streams, Sweden		19 – 28	14 days	Stepanauskas et al. 2000
Lilliån & Stridbacken Streams, Sweden after spring flood		45 – 55	14 days	Stepanauskas et al. 2000
Forest watershed, New Jersey		24 ± 17	12 days	Seitzinger et al. 2002
Urban/suburban watershed, New Brunswick, NJ		59 ± 11	12 days	Seitzinger et al. 2002
Agricultural pastures, New Brunswick, NJ		30 ± 14	12 days	Seitzinger et al. 2002
Agricultural and forest run-off, NJ		25 ± 13	10 days	Wiegner and Seitzinger 2001

* Lower numbers in the range represent the DON gross utilization corrected for recovery efficiencies of $^{15}NH_4^+$ standards (35% recovery loss) and overestimation based on DON breakdown (26%; described within "Methodological problems encountered"). This represents the maximum possible overestimation of gross mineralization. The upper number represents uncorrected numbers.

Fresh DOM released from aquatic primary producers, such as phytoplankton, would not create a large humic signal such as the one found at MB. Samples from MB contained organic matter that was relatively refractory compared to that in other systems and possibly leached from forests or originating from soil microorganisms. Previous work has demonstrated that forested uplands are an important source of DON to the PIS watershed (Hopkinson et al. 1999). Bacterial processing of DON within the watershed is likely to produce peptidoglycans, components of bacterial cell walls, which are refractory and thus remain in the water column longer than unprocessed DOM (McCarthy et al. 1998). All of this evidence suggests that the humic substances found in MB samples were largely refractory and likely derived from soils in the surrounding watersheds. Other work in PIS has also indicated the importance of allochthonous inputs to the estuary. A study using carbon isotopes determined that the primary source of DOM to PIS at the Parker Dam was modern (within the last 50 years) and derived from terrestrial primary production; very little of the DOM sampled was autochthonously produced (Raymond and Bauer 2001). In addition, because the system is net heterotrophic it requires an allochthonous input of DOM (Alderman et al. 1995, Balsis et al. 1995).

DOC lability averaged over three season (indicated by percent DOC utilized) was lower at MB than at Newbury (New; mesohaline station) or Plum Island (PI; polyhaline station): 6.7 ± 2.5, 16.4 ± 2.6, and 23.3 ± 2.9%, respectively. These percentages of labile DOC are on the high end relative to what has been reported in other studies (table 5). Gross nitrogen mineralization rates were also lower at MB than at New (0.193 ± 0.015 mmol-N m^{-3} d^{-1} and 0.341 ± 0.020 mmol-N m^{-3} d^{-1}, respectively), indicating that

heterotrophic bacteria were not remineralizing DON to ammonium as rapidly, most likely because the DON was less labile.

In July and September, phytoplankton biomass was high at MB (figure 6) and corresponded with low standing stocks of nitrate in the water column (1.5 and 2.3 μM, respectively). Nitrate concentrations were higher in May (4.1 μM) when phytoplankton biomass was lowest (10.4 $\mu g\ l^{-1}$; figure 6). This is consistent with long term data indicating that depleted nitrate concentrations are often found in the upper estuary when residence times are longer (i.e. summer) and diatom blooms occur (PIE LTER Site Review 2001). Phytoplankton primary production in July and September provided an autochthonous source of DOM above the background of allochthonously-derived DOM. This source was indicated in the DOC and DON mixing curves by a positive curvature compared to a theoretical linear decrease caused by mixing alone (figure 4). In addition, the input of autochthonous DOM produced by phytoplankton in July and September lowered the overall C:N of DOM in the estuary. DOM C:N ratios in PIS were lower in July and September than in May (16.7 ± 2.2, 18.1 ± 1.7, and 29.9 ± 2.4, respectively). These data suggest an increased importance of phytoplankton DOM in July and September, because phytoplankton C:N tends to be near the Redfield ratio of 6.7:1 (Redfield 1958), whereas terrestrial primary producer C:N ratios are 4-10 times higher (Vitousek et al. 1988).

The amount of autochthonous DOM at New can be calculated using the measured DOM concentrations (figure 21a) and the predicted losses due to dilution and bacterial metabolism (figure 21b). The decrease in DOM during transport downstream (μM / psu) was calculated from the slope of the [DOC] or [DON] versus salinity curve. The slope

was multiplied by the salinity difference between New and MB to find the potential dilution loss during transport from MB to New.

$$\text{Dilution loss} = \frac{[\text{DOC}]\text{ at MB} - [\text{DOC}]\text{ at PI}}{\text{Salinity at PI} - \text{Salinity MB}} * (\text{Salinity at New} - \text{Salinity MB})$$

Next, the maximum possible loss due to bacterial metabolism was calculated using the highest net DOC and DON utilization rates measured (whichever was higher, MB or New). Transport time from MB to New was estimated at five days (Vallino and Hopkinson 1998), and utilization rates (mmol-N m^{-3} d^{-1}) were multiplied by five days to obtain the amount of potential metabolic loss during transport.

Predicted concentrations (P_{DOC}) were calculated based on both the losses due to dilution and bacterial metabolism. Measured concentrations at MB were used as the initial values, and the calculated dilution and utilization losses were subtracted from these initial concentrations:

P_{DOC} = MB [DOC] – dilution loss – metabolic utilization loss.

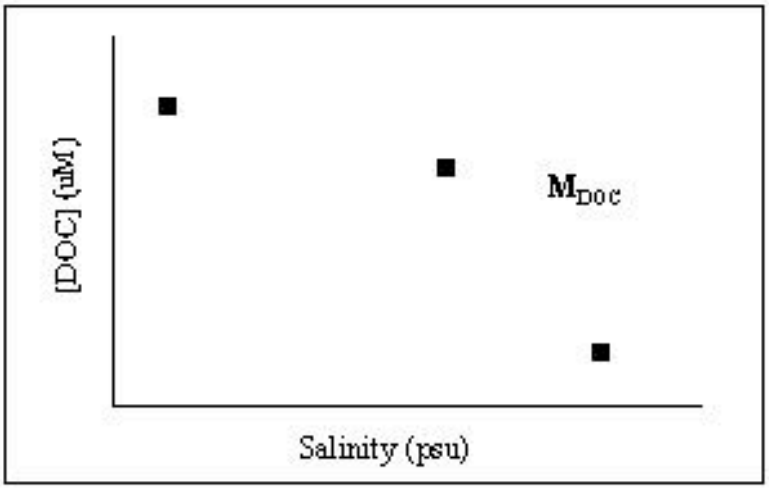
Calculated in this way, the predicted mixing curve would be concave (figure 21b). Autochthonous production was estimated by subtracting the predicted concentration at New from measured concentrations (figure 21c). Therefore, the overall equation for calculating autochthonous DOM inputs (AP) was:

$AP = M_{DOC} - P_{DOC}$

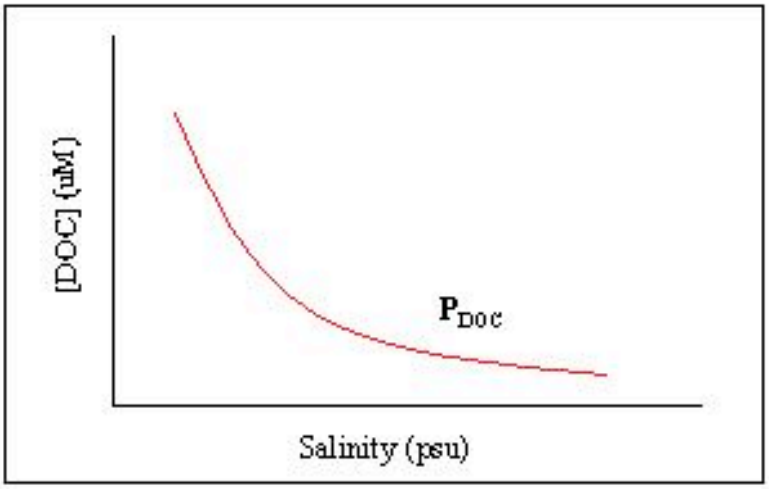
where M_{DOC} was [DOC] measured at New and P_{DOC} was [DOC] predicted at New.

Figure 21. Conceptual diagram of autochthonous DOM calculations. a) M_{DOC} = measured [DOC] vs. salinity. b) P_{DOC} = calculated mixing curve based on dilution and metabolism losses. c) AP = Autochthonous Production, calculated as the difference between measured and predicted values.

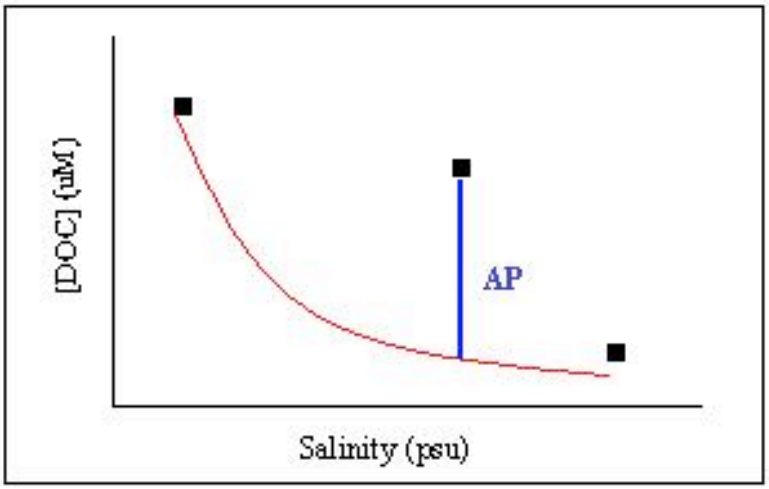
a


[DOC] (uM)
M_{Doc}
Salinity (psu)

b


[DOC] (uM)
P_{Doc}
Salinity (psu)

c


[DOC] (uM)
AP
Salinity (psu)

Autochthonous DOM concentrations calculated in this way could be overestimates because the maximum net microbial utilization rates were used for this calculation; however, they are more likely underestimates as losses due to particle sorption, uptake by benthic communities, or uptake by primary producers were not included in the calculations.

Based on the above calculations, we determined that one third of the total DOM at New was autochthonous (table 6). Highest autochthonous DOM inputs at New occurred in May (278 μM), but the C:N ratio of this material was much higher in May than in July and September (35.8 versus 13.7 and 11.8, respectively; table 6). High C:N and low chlorophyll *a* concentrations in May suggest that the source of this DOM was likely release from the sediments or surrounding marshes. The autochthonous inputs in July and September had lower C:N ratios and the chlorophyll *a* concentrations at MB were higher than in May (57 and 48 μg l^{-1} for July and September, respectively). Therefore, the autochthonous inputs in July and September were more likely from phytoplankton exudation and were likely to be more labile to microbial metabolism. The highest rates and percentages of DOC utilized were measured in July (3.248 ± 0.641 mmol-N $m^{-3}d^{-1}$ and 18.2 ± 2.8%; figures 12 and 13), when the overall C:N and the C:N of autochthonous DOM were lowest and temperatures were highest.

Organic matter from allochthonous and autochthonous sources was mineralized during transport along the estuary, indicated by the trend of decreasing DOM concentrations along the transect from land to sea (figure 4). Based on synchronous fluorescence analysis, DOM at New contained much less humic material than did DOM from MB, and a larger peak of fresh, labile DOM was observed at 283 nm. Other

Table 7. Calculated maximum quantities of autochthonous DOC and DON production at Newbury in PIS.

Sampling Month	Estimated autochthonous DOC production (□M)	% of total DOC	Estimated autochthonous DON production (□M)	% of total DON	C:N of autochthonous DOM
May	278.33	37%	7.77	33%	35.82
July	165.04	30%	12.08	35%	13.66
September	127.79	26%	10.83	36%	11.80

work has shown that mid-estuary DOM in PIS consists of a combination of material derived from allochthonous and autochthonous sources (Hopkinson et al. 1998). DOC utilization was highest at New (figure 12) indicating that labile DOC was a larger component of total DOM than at the other sites. This corresponds to results showing that bacterial production was higher mid-estuary than at freshwater or polyhaline endmembers (PIE LTER Site Review 2001).

Higher concentrations of DIN at New indicated an input of inorganic nutrients mid-estuary (table 2; figure 4). Some of the DIN was likely remineralized DON; however, based on net mineralization rates calculated for MB and New and the estimated transport time between the two stations of five days, a maximum of 5-10% of the difference in DIN concentrations could be accounted for by remineralization of DON in the water column. Other potential sources of DIN are sediment remineralization or external sources from surrounding uplands. Newbury is a small town along Route 1A, and has more paved areas and houses surrounding it than the other two stations. Therefore, local surface water run-off and groundwater seepage are likely DIN sources.

Concentrations of DOM, DIN, and chlorophyll *a* at PI were low due to rapid flushing. Synchronous fluorescence DOM peaks occurred at 283 nm, indicating a labile, protein-like pool of DOM (data not shown). Percent of initial DOC utilized was highest at PI (23.3 ± 2.9%; figure 13), indicating higher DOC lability than at New or MB. DOC utilization was inversely related to DOM C:N (figure 14), indicating that DOM with a higher C:N was less labile than DOM with a C:N closer to that of bacterial biomass. This result correlates to relationships found in other studies between different DOM sources and utilization (Goldman and Dennett 1987, Goldman and Dennett 2000, Hunt et al.

2000). In addition, the salinity change along the transect of the estuary could alter the bioavailability of the DOM by altering the microbial community composition or the chemical structure of DOM by releasing ammonium due to cation exchange (Stepanauskas et al. 1999).

Hog Island Bay

The origin of a majority of the DOM in HIB is autochthonous. Allochthonous inputs are derived from an aquifer highly impacted by agriculture (Reay et al. 1992) and DON constitutes only 6% of TDN entering the system (J. Stanhope, VIMS, *pers. comm.*). However, within the lagoon DON is an important component of the nitrogen pool. In all seasons and stations in this study, 91 ± 1% of the TDN was DON, compared to a range of 52-98% reported by Tyler et alia. (2001) for this system. The potential sources of autochthonous DOM were phytoplankton, benthic microalgae, macroalgae, and sediment flux. Phytoplankton biomass was low (<6 □g l^{-1} chlorophyll *a*) throughout the year in HIB (figure 9). In August, when chlorophyll *a* concentrations were highest, the DOM C:N was highest (35.2 ± 2.8), which suggests that neither phytoplankton (C:N of 6.7; Redfield 1958) nor benthic microalgae (C:N of 9; Sundback et al. 2000) were the primary source of DON. Although the sediments may be an important source of DOM to the water column, the major source is likely the macroalgal population with predominant taxa *Ulva lactuca* and *Gracilaria tikvahiae* (McGlathery et alia. 2001). Macroalgae tend to dominate littoral zone systems such as HIB that have relatively short residence times

that discourage phytoplankton blooms (Valiela et al. 1997b). Growth occurs in annual boom-bust cycles, with maximum growth rates occurring in the late spring during highest nutrient influx followed by a population crash mid-summer (Viaroli et al. 1993, Valiela et al. 1997b, McGlathery et alia. 2001). The crash is most likely due to high summer temperatures and self-shading within the mat (Valiela et al. 1997b, Tyler et alia. 2001). DON is released by macroalgae into the water column both during growth and as a result of decomposition following crash of the bloom (Buchsbaum et al. 1991, Tyler et alia. 2001). The excess DOM released following a crash may result in anoxic events as has been observed in the lagoon of Venice and on occasion at some mid-lagoon sites in HIB (Sfriso et al. 1987, Viaroli et al. 1993).

In the present study, concentrations and highest utilization of DOC and DON in HIB occurred in August when temperatures were highest (27 ºC). Temperature plays an important role in bacterial processes (Hopkinson et al. 1989, Hoch and Kirchman 1993, Shiah and Ducklow 1995), and is a confounding factor in this study as highest temperatures occurred simultaneously with the decline of the macroalgal population. DOM C:N ratios were also highest in August (35.2 ± 2.8), as one might expect if macroalgae were the source, because macroalgae have high C:N values relative to other aquatic primary producers (Enriquez et al. 1993) with a range of 10:1 to 45:1 in HIB (McGlathery et alia. 2001). During early July 1998 more than 38 mmol-N $m^{-2}d^{-1}$ of DON were released into the water column following a crash of a macroalgal bloom (Tyler et alia. 2001). Given the ambient DON concentrations typically measured prior to a crash of the bloom (11 □M in April 2000), the influx of 38 mmol-N $m^{-2}d^{-1}$ of organic matter with high C:N ratios would likely affect the overall composition of the DOM pool;

however, the degree of impact would depend upon the distribution and abundance of macroalgae throughout the lagoon. In general, a direct relationship between DOM utilization and DOM C:N was observed. This is somewhat counterintuitive as most studies show that DOM with lower C:N tends to be more labile (Goldman and Dennett 1987, Goldman and Dennett 2000, Hunt et al. 2000). However, the observed relationship in this study was driven by the very high DOM decomposition rates in August, at a time when DOM C:N was higher than usual.

Rates of DOC utilization in August in HIB were two orders of magnitude greater than those of DON (figures 15 and 17), and percent of initial DOC utilized was four times greater than that of DON (figures 16 and 18). Rapid utilization of DOC resulted in significantly decreased ambient DOC concentrations in the water column between August and October (561 ± 34 □M and 196 ± 11 □M, respectively; table 3). DON concentrations did not decrease proportionately (17.6 ± 1.7 □M to 12.3 ± 0.3 □M), contributing to the decrease in DOM C:N ratio from August to October (32 to 16).

Much of the DOM in HIB in August was not remineralized by the microbial community within the water column. The estimated residence times within HIB range from four days near the barrier islands to 30+ days inland and in shoal areas (D. Fugate, VIMS, *pers. comm.*). Assuming a 30-day residence time and using the utilization rates calculated above, only 52% of the DOC and 17% of the DON would be utilized within the water column in a 30-day period. Therefore, some of the DOM in August could have entered the coastal ocean and contributed to eutrophication there. However, in a shallow, well-mixed system such as HIB it is likely that the benthic community mineralized a significant amount of the remaining DOM because benthic gross mineralization rates are

much greater than those in the water column in this system (0.93 - 6.53 mmol-N $m^{-2}d^{-1}$; Anderson et alia. *in press*). The DOM remaining after 30 days of microbial processing within the lagoon was likely to be recalcitrant and not readily utilizable by bacteria in the coastal ocean. Thus, even in the summer, when DOM concentrations were highest, the lagoon functioned to protect the coastal ocean by removing much of the labile DOM.

Immobilization of DIN

One might have expected increased DIN concentrations during the incubations of samples in this study concomitant with measured gross mineralization rates; however, there were much lower changes in standing stocks of DIN than predicted in HIB or PIS incubations. Possible fates of mineralized ammonium include bacterial immobilization and nitrification. When C:N is high, as was observed in PIS DOM and in HIB DOM sampled in August, bacteria are more likely to use ammonium to build biomass (Kirchman 1994, Hoch and Kirchman 1995, Middelboe et al. 1995, Gardner et al. 1996). In fact, ammonium has been found to supply 10-65% of nitrogen needs of bacteria (Wheeler and Kirchman 1986, Kiel and Kirchman 1991, Hoch and Kirchman 1995, Middelboe et al. 1995, Middleburg and Nieuwenhuize 2000). In this study, DOM C:N ratios ranged from 13:1 to 40:1; however, water column DOM C:N does not generally reflect the C:N utilized by the microbial community (Kroer 1993). Therefore, to estimate the C:N ratio of the substrate utilized by the bacterial population in these incubations, DOC utilized was divided by the DON utilized. The results showed that C:N of the

substrate utilized (26.0 ± 4.2 in PIS and 76.9 ± 34.1 in HIB) was much higher than the C:N of typical bacterial biomass. Thus, in order to maintain a low C:N in bacterial biomass, the cells utilized inorganic nitrogen in the form of recycled ammonium. The utilization of recycled ammonium is reflected in the excess gross mineralization over net mineralization rates; however, immobilization into bacterial biomass was likely not a permanent fate of the ammonium (discussed below).

System comparison

We hypothesized that the DOM in HIB would be more labile than in PIS. Indeed we did observe that the DOM sampled in HIB was primarily autochthonous and more labile than the DOM in PIS, which was predominantly allochthonous. There were no significant differences between DON utilization rates in PIS and HIB; however, the percent of initial DON utilized was significantly higher in HIB (8.5 ± 1.0%) than in PIS (5.7 ± 2.0%). DOC utilization was almost three times faster in HIB than in PIS (2.543 ± 0.789 and 0.912 ± 0.378 mmol-C $m^{-3}d^{-1}$, respectively) and percent utilized was almost four times higher (26.7 ± 2.8% and 7.0 ± 3.0%, respectively). Characterization of the DOM by synchronous fluorescence suggested that DOM in HIB was more protein-like, whereas DOM in PIS it contained more refractory humic-like substances.

The percent of initial DOC utilized at PIS (7.0 ± 3.0%) was well within the range of those reported for other systems (table 5). Utilization of DOC was reported to vary from 2-18% in various rivers in the southeastern U.S. (Moran et al. 1999), from 1-9% in

open sea and ocean samples (Zweifel et al. 1993, Carlson and Ducklow 1996), and from 6-14% in surface water run-off collected in New Jersey watersheds (Weigner and Seitzinger 2001; table 5). Utilization in HIB (27 ± 3%) was higher than those discussed above, most likely due to the importance of autochthonous DOM in the system.

Depending on the method of calculation, the percent of DON mineralized ranged from 6% to 23% in PIS and from 9% to 31% in HIB. There are errors inherent in each method. Net mineralization rates (the lower percentage in each range) assume that immobilization into particulate nitrogen (PN) is not an important fate of ammonium. Seitzinger and Sanders (1997) found that immobilization into bacterial PN was significant (73% of DON utilization). Their study used diluted initial bacterial abundances to maximize growth, and they observed significant increases in bacterial abundance over time. In addition, using their data, we calculated bacterial biovolumes (1.13 μm^3) that are much higher than reported elsewhere (Bratbak 1985, Bjornsen 1986, Nagata 1986, Lee and Fuhrman 1987, Nagata and Watanabe 1990). Our incubations included ambient bacterial abundances at the initial time point, and abundances decreased over time in every replicate due to the presence of grazers. Therefore, there was no increase in bacterial PN during the incubations, and immobilization into PN was most likely not a permanent fate of DON. However, we were unable to enumerate grazers, and it is possible that grazer populations increased and some nitrogen was immobilized into microheterotroph biomass. Given the average final bacterial abundance in this study of 1.8×10^9 cells $liter^{-1}$ and using a carbon conversion factor of 20 fg-C $cell^{-1}$ and a bacterial C:N of 4 (Lee and Fuhrman 1987), 0.65 μM-N (5% of the initial DON concentration) was stored in bacterial biomass. This compares to results reported by Seitzinger and

Sanders (1997) of 62 μM-C and 13 μM-N in bacterial biomass, which corresponds to 248 fg-C $cell^{-1}$ and 182 fg-N $cell^{-1}$ based on their final bacterial abundance of 3 x 10^9 cells $liter^{-1}$. The carbon conversion factor we used above, although canonical, has been described as an overestimate for typical bacterial cells (Joint and Pomroy 1987) and is on the upper end of conversion factors detailed in a review by Ducklow (2000). Immobilization into PN was not a permanent fate of ammonium in our study, but ammonium could have been processed through PN transiently and re-released as DON via viral lysis, grazing, or exudation by bacterial cells similar to what has been described for phytoplankton cells in Ward and Bronk (2001).

DON utilization rates based upon gross mineralization (the higher number in each range above) assume that all of the ammonium mineralized mixes homogeneously with the pool of labeled ammonium prior to either immobilization or nitrification. In addition, measurement of gross mineralization suffers from some operational problems. In order to trap ammonium for isotopic analysis, the pH is adjusted to >9.7. In this process DON may be abiotically broken down to ammonium, diluting the ^{15}N pool and causing an overestimation of mineralization. We estimated from measurements made before and after alkalization that abiotic breakdown of DON accounted for approximately 26% of the calculated gross mineralization rate. In addition, the $^{15}NH_4^+$ standards had low recovery efficiencies (65%). If corrected for these errors, the gross percent of DON utilized could be overestimated by a maximum of 61%, giving us DON utilizations of 13% in PIS and 19% in HIB, which are within the range reported in other studies (table 6).

CONCLUSIONS

Hypotheses and conclusions

1. DOM derived from decomposition of macroalgae blooms in HIB will be more labile than that sampled during other seasons.

The DOC in HIB was more labile in August than in other months. Although there was no large macroalgal population bloom and crash in 2000 as there was in 1998 (Tyler et alia. 2001), the population declined in July, and highest rates of utilization (figures 15 and 17) and highest percents of initial DOM utilized (figures 16 and 18) were measured in August.

2. DOM will be more labile in HIB than in PIS.

DOC and DON were more labile in HIB than that in PIS. Synchronous fluorescence analysis of the DOM pool indicated that the DOM in PIS was more humic-like; whereas in HIB the DOM was more protein-like. In addition, DOC utilization rates and percent of initial DOC and DON utilized were significantly higher in HIB than in PIS.

3. Rates of gross mineralization will be significantly higher than rates of net mineralization in incubations from both systems.

Rates of gross mineralization were on average 8 times higher than rates of net mineralization in incubations from both systems indicating rapid bacterial consumption

of the ammonium produced by mineralization for nitrification or immobilization into biomass. Although immobilization was not a permanent fate of ammonium, it is likely that ammonium was taken up by bacterial cells, made into biomass, and re-released as DON due to viral lysis, grazing, or exudation.

4. The primary mechanism for consumption of ammonium during incubations will be nitrification. A secondary mechanism for removal of ammonium will be bacterial immobilization.

Bacterial immobilization and nitrification were both potential sinks of ammonium produced by mineralization. Further quantification of the rates or distinctions of importance were not clear due to methodological errors.

Riverine and lagoonal systems serve an important ecological function as nutrient and organic matter filters for the coastal ocean. Microbial communities in both PIS and HIB altered the lability and composition of the DOM. Our results indicate that Hog Island Bay has the potential to alter the bioavailability of DIN and DOM more significantly than Plum Island Sound due to increased importance of labile autochthonous DOM, and higher significance of benthic-pelagic coupling in HIB.

APPENDIX A

Data Tables

Table 8. Rates of Plum Island Sound DOC utilization, DON utilization, and DIN remineralization; calculated as slopes of a linear regression line.
An asterisk indicates $p < 0.05$.

Site	Month	Rep #	DOC utilization rate (mmol-C $m^{-3}d^{-1}$)	DON utilization rate (mmol-N $m^{-3}d^{-1}$)	DIN remineralization rate (mmol-N $m^{-3}d^{-1}$)
MB	May	1	3.856	-0.0413 *	0.041 *
		2	1.349	-0.0271	0.027
		3	4.747	-0.275	0.050 *
	July	1	-3.938 *	0.0169	-0.0214
		2	-2.256	-0.191	0.227 *
		3	-1.335	-0.211	0.222 *
	September	1	-2.263	-0.020	0.022
		2	0.332	-0.048 *	0.050 *
		3	-0.216	-0.017	0.019
New	May	1	1.132	-0.015	0.015
		2	1.065	0.012	-0.012
		3	-1.608	0.021	0.006
	July	1	-4.502 *	-0.005	0.046
		2	-4.866	0.189	-0.203
		3	-7.212	0.006	0.051
	September	1	-2.621 *	-0.030	0.033
		2	-2.226	-0.123 *	0.130 *
		3	-2.594	-0.109 *	0.113 *
PI	May	1	4.979	-0.117 *	0.117 *
		2	2.732	-0.103 *	0.103 *
		3	3.111	-0.111 *	0.112 *
	July	1	-1.336	-0.021	0.090 *
		2	-2.154	-0.164	0.075
		3	-3.252 *	0.014	0.052
	September	1	-0.987	-0.110 *	0.114 *
		2	-2.027	-0.143 *	0.149 *
		3	-2.535	-0.119 *	0.124 *

Table 9. Rates of HIB DOC utilization, DON utilization, and DIN remineralization; calculated as slopes of a linear regression line. An asterisk indicates $p < 0.05$.

HIB Site	Month	Rep #	DOC utilization rate (mmol-C $m^{-3}d^{-1}$)	DON utilization rate (mmol-N $m^{-3}d^{-1}$)	DIN mineralization rate (mmol-N $m^{-3}d^{-1}$)
Creek	Feb	1	-1.913	-0.019	0.017
		2	-2.948 *	0.046	0.042 *
		3	-1.384	-0.006	0.0056 *
	April	1	0.943	-0.044	0.048
		2	0.678	-0.042	0.048
		3	2.349	-0.054	0.061
	June	1	-4.058	-0.047	0.046
		2	-3.867 *	-0.067	0.067
		3	-5.014 *	-0.063	0.062
	August	1	**2.603**	**-0.281 ***	0.281 *
		2	-4.389	-0.127 *	0.127 *
		3	-12.428	-0.112 *	0.112 *
	Oct	1	-0.581	-0.054 *	0.059
		2	-0.597	-0.052 *	0.052 *
		3	0.782	-0.054 *	0.054 *
Shoal	Feb	1	-2.888 *	0.011	-0.008
		2	-2.002 *	-0.007	0.014
		3	-1.139	-0.009	0.007
	April	1	3.202	-0.047	0.047
		2	2.521	-0.002	0.001
		3	1.547	-0.035	0.034
	June	1	-3.415 *	-0.045	0.045
		2	-2.646	-0.036	0.036
		3	-3.254 *	-0.056	0.056
	August	1	-6.4	-0.057	0.057
		2	-20.298	-0.025	0.025
		3	-15.054	-0.047	0.047
	Oct	1	-0.779	-0.048 *	0.048 *
		2	-0.950	-0.054 *	0.054 *
		3	-0.667	-0.047 *	0.047 *
Hog	Feb	1	-3.264 *	-0.009	0.010
		2	-2.167	-0.038	0.019
		3	-1.723	-0.002	0.002
	April	1	6.810	-0.031	0.051 *
		2	6.783	-0.054 *	0.055 *
		3	3.644	-0.046	0.047
	June	1	-4.650 *	-0.049	0.049
		2	-3.368 *	-0.104 *	0.104 *
		3	-2.541	-0.080 *	0.081 *
	August	1	-9.791	-0.088 *	0.088 *
		2	-14.030	-0.103 *	0.102 *
		3	-8.084	-0.047 *	0.047 *
	Oct	1	0.152	-0.019	0.019
		2	-0.283	-0.035 *	0.035 *
		3	0.117	-0.042 *	0.042 *

Table 10. Pooled rates of HIB and PIS DOC utilization, DON utilization, and DIN remineralization; calculated as averages of slopes of linear regression lines. Only replicates that were not significantly different from one another were included in the pooled data set (t-test, $p > 0.05$).

Site	Month	DOC utilization rate (mmol-C $m^{-3}d^{-1}$)	DON utilization rate (mmol-N $m^{-3}d^{-1}$)	DIN mineralization rate (mmol-N $m^{-3}d^{-1}$)
HIB Creek	Feb	-2.082 *	0.010	0.021 *
	April	1.323	-0.047 *	0.052 *
	June	-4.313 *	-0.059 *	0.059 *
	August	-5.954	**-0.119** *	0.173 *
	Oct	-0.213 *	-0.054	0.055 *
HIB Shoal	Feb	-2.010 *	-0.002	0.004
	April	2.423 *	-0.028	0.028
	June	-3.105 *	-0.046	0.046
	August	-13.917 *	-0.043 *	0.043 *
	Oct	-0.799 *	-0.050 *	0.050 *
HIB Hog	Feb	-2.385 *	-0.011	0.011 *
	April	5.746 *	-0.044 *	0.050 *
	June	-3.520 *	-0.078 *	0.078 *
	August	-10.636 *	-0.079 *	0.079 *
	Oct	-0.005	-0.032 *	0.032 *
PIS Middle Bridge	May	3.317	-0.075	0.039 *
	July	-2.510 *	-0.128	0.142 *
	Sept	-0.716	-0.028	0.030
PIS Newbury	May	0.196	0.002	0.003
	July	-5.527 *	0.063	-0.046
	Sept	-2.480 *	-0.087 *	0.092 *
PIS Plum Island	May	3.607 *	-0.110 *	0.111 *
	July	-2.247 *	-0.057	0.072 *
	Sept	-1.850 *	-0.124 *	0.129 *

APPENDIX B

Preliminary Study on Filter Pore Size (October 1999)

A study was done to determine the optimum filter pore size for retention of the least number of bacteria and removal of phytoplankton and grazers. Filters examined were: 0.2 μm Supor, 0.7 μm Whatman GF/F (glass fiber), 1.0 μm Gelman A/E (glass fiber), and 1.2 μm Whatman GF/C (glass fiber). Whole water samples from the Creek and Shoal sites in HIB were passed through a filter of each pore size and bacterial abundance measurements were taken before and after filtration. The 0.7-μm pore size Whatman GF/F filters that had been used in preliminary studies were found to remove a significant portion of the bacterial population (59.4 and 34.1% for the two sites, Creek and Shoal, figure 8). All slides were also carefully checked for heterotrophic flagellates (grazers), phytoplankton, and cyanobacteria (data not shown). No significant difference in the abundances of flagellates was found between the 1.0 μm and 0.7 μm samples. Phytoplankton were successfully removed in both the 1.0 and 0.7 μm samples, as indicated by a lack of chlorophyll *a* measured after filtration. We determined that preferential removal of the larger size class of bacteria could bias the study. Also, the glass fiber filters were found to cause the least amount of lysis and increased nutrient levels in the samples (Gasol and Moran 1999). We therefore used 1.0 μm glass fiber filters in this thesis work.

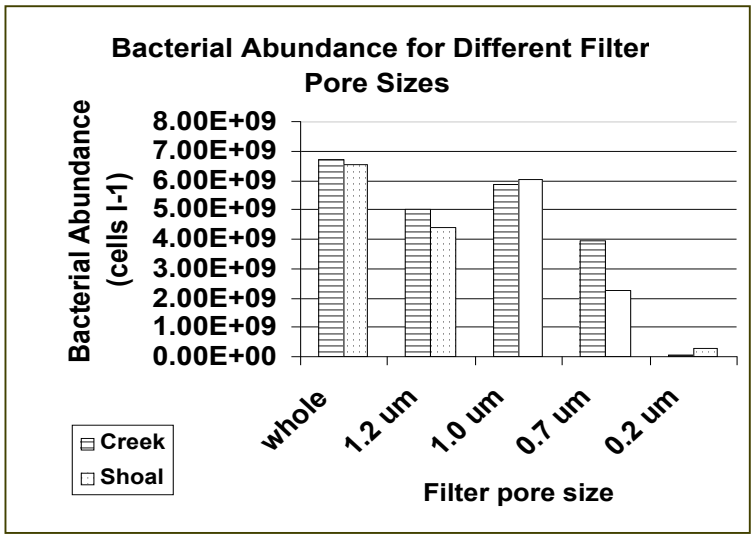


Figure 20. Bacterial abundance measured as a function of pre-filtration pore size for two HIB sites.

Pore Size	Creek	Shoal
Whole water	100%	100%
1.2 um	75.1%	66.9%
1.0 um	87.7%	92.2%
0.7 um	59.4%	34.1%
0.2 um	1.1%	4.6%

Table 11. Bacterial abundances as a percentage of whole water for different filter pore sizes.

APPENDIX C

Control samples

A composite filtered control incubation was attempted for each site, using one-third liter from each of the three replicate samples. The aim was to ensure that all organisms were removed, and that only abiotic processes that occurred within the incubation bottles were measured. The first attempt to make controls involved killing the bacteria within the samples using zinc chloride. The chemical clouded the water and interfered with spectrophotometric analysis of nutrients.

Next, a filtered control was attempted. Controls were filtered using a 0.2-µm pore-size Supor membrane and then a 0.02-µm pore-size Whatman Anodisc membrane. After 3-5 days incubation, bacterial abundance samples revealed similar or greater amounts of bacteria than in the unfiltered samples.

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