Impact of Benthic Algae on Dissolved Organic Nitrogen

in a Temperate, Coastal Lagoon

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Abstract

Coastal lagoons are a common land-margin feature world-wide. The shallow nature of lagoons leads to substantial benthic-pelagic coupling and dominance by benthic autotrophs. Increased inputs of nitrogen (N) from coastal watersheds may cause nuisance macroalgal blooms. However, little is known about the dynamics of dissolved organic nitrogen (DON), an important component (50- 95%) of the total dissolved N (TDN) pool. The objectives of this dissertation were to: (1) quantify benthic-pelagic fluxes of specific dissolved organic and inorganic N (DIN) compounds along an environmental gradient in Hog Island Bay, an algal-dominated lagoon at the Virginia Coast Reserve LTER site, (2) determine how uptake and release by benthic macro- and microalgae impacts DON cycling and (3) estimate the turnover and retention of N by macroalgae.

Sediment-water column DON fluxes were highly variable but comparable to DIN fluxes; fluxes of individual compounds (urea and dissolved free and combined amino acids [DFAA, DCAA]) often proceeded concomitantly in different directions. Where sediment metabolism was net autotrophic due to microalgal activity, TDN fluxes, mostly comprised of DIN, urea and DFAA, were directed into the sediments. Heterotrophic sediments, particularly beneath macroalgal mats, were a net source of TDN, mostly as DIN. Isolated crashes of dense macroalgal mats resulted in an order of magnitude increase in DIN and DON release. When present, living macroalgae controlled benthic-pelagic coupling by intercepting DIN, urea and DFAA fluxes and releasing DON, mostly as DCAA. Separate estimates from ^{15}N isotope dilution field experiments showed that macroalgae release ~50% of total N uptake. *Ulva lactuca* took up DIN, urea and DFAA

throughout the lagoon, but DON uptake was only important where DIN was low. In the laboratory, urea and AA uptake rates were consistently higher for *U. lactuca* than for *Gracilaria tikvahiae*. Uptake and ^{15}N and ^{13}C assimilation rates varied for individual amino acids, suggesting different uptake mechanisms. Overall, macroalgae act as a conduit whereby both organic and inorganic N are taken up, transformed, and re-released to the water column on short time scales (minutes-hours). Benthic algae thus clearly influence benthic-pelagic coupling and the retention of N moving across the land-sea interface.

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Exultation is the going of an inland soul to sea …
--Emily Dickinson

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Chapter 1
Introduction: Impact of benthic algae on dissolved organic nitrogen dynamics in temperate, coastal lagoons

Background:

Coastal lagoons, benthic algae and dissolved organic nitrogen

Shallow lagoonal estuaries are an important land margin feature worldwide, making up approximately 13% of the world's coastline (Cromwell, 1973; Kjerfve, 1989). Behind the chain of barrier islands that lines nearly half of the Atlantic and Gulf coasts of the United States is a series of back-barrier lagoons (Hayden and Dolan, 1979). Increasing human populations and land use changes in the watershed have resulted in increased nutrient inputs to coastal ecosystems (NRC, 1993). Shallow estuaries, in particular, may function as an important filter for nutrients, specifically nitrogen (N), moving from the land-margin to the coastal ocean (Boynton *et al*., 1996). In spite of the importance of shallow lagoons, little work has been done to investigate the fate of inorganic or organic N in shallow estuaries (Boynton *et al*., 1996). Among the various processes responsible for the transformation, retention, or removal of nitrogen in coastal lagoons are uptake and release and mineralization by primary producers and bacteria, burial, denitrification and transport to the coastal ocean (Figure 1-1). The research presented here describes the control of nitrogen cycling, particularly dissolved organic nitrogen, by benthic macro- and microalgae in a shallow, back-barrier lagoon on the Virginia coast.

Ecosystem functions in shallow lagoons, which have a much higher ratio of surface area to volume than deeper, riverine estuaries, are often dominated by benthic

processes (Nixon, 1981; Martens, 1982; Nowicki and Nixon, 1985; Sand-Jensen and Borum, 1991). The mineralization of metabolizable organic compounds often drives the biogeochemical processing in the sediments and water column of shallow lagoons (Martens, 1982; Anderson *et al*., in press). In addition, because the majority of the sediment surface lies within the photic zone, benthic autotrophs are often the dominant autotrophs and benthic primary production is often more important than pelagic production (Sand-Jensen and Borum, 1991; McGlathery *et al*., 2001).

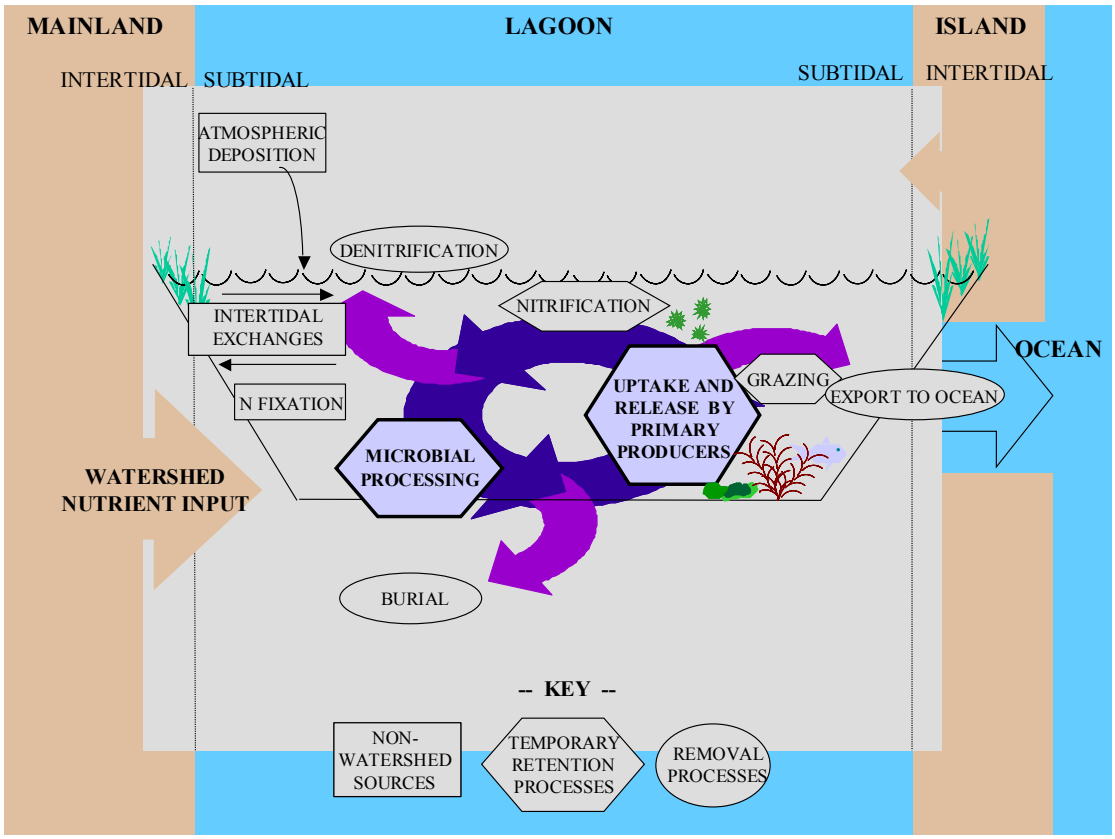

Figure 1-1 Schematic diagram illustrating the potential fate and transformation of reactive nitrogen as it moves across the land margin interface, through the lagoon, and out to the coastal ocean. Processes highlighted in bold text were the focus of the research presented here.

Seagrasses, macroalgae and benthic microalgae are the most common types of benthic primary producers in shallow systems (Sand-Jensen and Borum, 1991). Both macroalgae and microalgae potentially play a very important role in nitrogen (N) cycling. Benthic-pelagic nutrient coupling may be altered by the presence of these algae at the sediment surface; nitrogen efflux from the sediment may be intercepted by algal uptake, and diel photosynthetic oxygen release can have an effect on redox-dependent biogeochemical processes (Sundback and Graneli, 1988; Rizzo, 1990; Sundback *et al*., 1990; Lavery and McComb, 1991a; Sundback *et al*., 1991; Sfriso *et al*., 1992; D'Avanzo and Kremer, 1994; Rysgaard *et al*., 1995; Krause-Jensen *et al*., 1996; Rysgaard *et al*., 1996; McGlathery *et al*., 1997; Krause-Jensen *et al*., 1999; Sundback *et al*., 2000). In some cases, macroalgal interception of nutrients regenerated within the sediments may limit phytoplankton growth in the overlying waters (Smith and Horne, 1988; Valiela, 1992c; Thybo-Christesen *et al*., 1993; McGlathery *et al*., 1997). In addition, macroalgae have the ability to rapidly assimilate and store N in excess of growth demands (Bird *et al*., 1982; McGlathery *et al*., 1996), serve as integrators of N availability in coastal waters and are often better indicators of water quality than standing stock nutrient concentration (Bjornsater and Wheeler, 1990; Fong *et al*., 1994a; Jones *et al*., 1996). While the influence of both micro- and macroalgae on benthic-pelagic coupling of DIN has been well studied, the effect of these primary producers on dissolved organic nitrogen is much less understood.

It is generally accepted that N limits algal growth in temperate estuaries (Howarth, 1988). Loading rates of nutrients are orders of magnitude higher than

historical values, and the effect of these inputs on estuarine ecosystems is considerable (NRC, 1993). While larger estuaries have high phytoplankton production in response to increased N loading, shallow lagoons typically have increased macroalgal growth (Lee and Olsen, 1985; Sand-Jensen and Borum, 1991; Sfriso *et al*., 1992; Valiela, 1992c; Fong *et al*., 1994b; Duarte, 1995; Valiela *et al*., 1997; Kinney and Roman, 1998). Fast growing, opportunistic species of macroalgae have 'boom and bust' life cycles, little structural material, and rapid decomposition following senescence (Buchsbaum *et al*., 1991; Duarte, 1995; Bourgues *et al*., 1996) so that nutrient cycling in macroalgal-dominated systems is often accelerated relative to seagrass-dominated systems (Valiela, 1992c; Duarte, 1995; Viaroli *et al*., 1995). A sufficient increase in macroalgal biomass and subsequent eutrophication (increased supply of organic matter, sensu Nixon, 1995) may lead to a change in net ecosystem metabolism, a shift from net autotrophy to net heterotrophy, and a shift from a grazer-dominated food chain to a decomposer-dominated food chain (Ferrari *et al*., 1993). In some highly eutrophic European lagoons, macroalgal blooms and subsequent crashes regulate overall nitrogen cycling and dystrophic crises frequently occur (Sfriso *et al*., 1992; Ferrari *et al*., 1993; Viaroli *et al*., 1993; Viaroli *et al*., 1995; Rysgaard *et al*., 1996). However, much of the work on coastal eutrophication has been in deep estuaries; less is known about the effects of eutrophication in shallow lagoons.

Dissolved organic nitrogen (DON) makes up a large fraction of the total nitrogen (N) in marine systems (Sharp, 1983). Coastal systems receive inputs of DON (and DIN) from allochthonous sources (Meybeck, 1982; Hopkinson, 1998), including atmospheric

deposition (Paerl *et al*., 1990; Cornell *et al*., 1995; Paerl, 1995), and autochthonous production. While the inputs of DON are often high, DON is currently not a part of budgets that relate nutrient loading to processing in and export from estuaries. Previous studies in shallow estuaries indicate that the magnitude of DON fluxes between the sediments and the water column can vary widely (e.g. Hopkinson, 1987; Teague *et al*., 1988; Dollar *et al*., 1991; Lomstein *et al*., 1998;), but are in some cases a large proportion of the total dissolved nitrogen (TDN) flux (Boynton *et al*., 1980; Teague *et al*., 1988; Lomstein *et al*., 1998). However, 'DON' is a collective term for a variety of compounds that can differ substantially in molecular weight and bioavailability: from small, highly bioavailable, low C:N compounds like amino acids and urea to very large, refractory, high C:N polyphenolic compounds. In order to understand the reactivity of the DON pool, it is necessary to resolve the relative proportions of the different constituent compounds. By lumping together all organic N containing compounds, a great deal of information may be lost (Boynton *et al*., 1980; Burdige and Zheng, 1998).

Bioavailable compounds, such as amino acids and urea can make up significant portion of the DON pool, and contribute to the benthic flux of DON (e.g. Boucher and Boucher-Rodoni, 1988; Lomstein *et al*., 1989; Burdige and Zheng, 1998). In addition, these small, labile organic compounds may represent an important source of N for both heterotrophic and autotrophic microorganisms, as well as for benthic plants (e.g. Jorgensen, 1982; Hanisak, 1983; Admiraal *et al*., 1984; Jorgensen, 1984; Flynn and Butler, 1986; Lomstein *et al*., 1989; Palenik and Morel, 1990a; Tupas and Koike, 1990; Antia *et al*., 1991; Keil and Kirchman, 1993; Chisholm *et al*., 1996; Nilsson and

Sundback, 1996; Rondell *et al*., 2000). The importance of DIN in temperate ecosystems may be overestimated due to anthropogenically enhanced inputs from the watershed and the atmosphere (VanBreeman, 2002). In using organic N directly, rather than inorganic N, the organism is effectively bypassing the mineralization of organic N to inorganic N. This capability may provide a competitive advantage to these organisms, especially at low ambient DIN:DON, and suggests that utilization of organic nitrogen may be much more important than previously recognized.

Because DON may be an important component of the benthic N flux, uptake by benthic algae may significantly influence DON fluxes, as has been shown for fluxes of DIN. Benthic microalgae capable of DFAA uptake are often opportunistic, 'blooming species' (Nilsson and Sundback, 1996) and high DON may stimulate the growth of brown tide forming phytoplankton (Berg *et al*., 1997b). Less is known about DON utilization or uptake kinetics in macroalgae, although uptake has been demonstrated for a variety of species (Nasr *et al*., 1968; Schmitz and Riffarth, 1979; Bird *et al*., 1980). While some species of phytoplankton use cell-surface oxidases to cleave the amino group from amino acid prior to uptake as ammonium (Palenik and Morel, 1990a; Palenik and Morel, 1990b), other species of phytoplankton may actually use DFAA carbon at the end of blooms when light, but not N, is limiting (Lewitus and Koepfler, 1997). Direct uptake and assimilation of organic compounds provides fixed C under light-limiting conditions, and would be advantageous for macroalgae growing in turbid environments. Heterotrophic growth in the dark has been demonstrated in both red and green algae (Markager and Sand-Jensen, 1990; Robaina *et al*., 1995). Heterotrophic uptake of

organic N by macroalgae has not been included in nutrient cycling models, and may provide an important link between N and C cycles in estuarine ecosystems.

A substantial fraction of the total N pool is contained in macroalgal biomass, especially during blooms. In addition to the release of DON during senescence and decay, macroalgae, like phytoplankton, (Bronk *et al*., 1994) may "leak" DON and DIN into the water during active growth. Up to 41% of the DIN taken up by phytoplankton is released as DON (Bronk *et al*., 1994). Over relatively short time scales, algae act as a conduit whereby DIN (and DON) is taken up, transformed, and subsequently re-released to the water column. Thus, estimates of N turnover in the macroalgal pool based solely on uptake rates and growth are likely to greatly underestimate the actual quantity of N passing through the macroalgal pool. Despite the significance of DON, and the potential importance of benthic algae in regulating N cycling, we know relatively little about the role of primary producers in regulating DON standing stock concentrations, fluxes or transformations in shallow estuaries. The work presented here represents an effort to learn more about the ways that macroalgae, and to some extent microalgae, control estuarine DON dynamics by uptake, transformation and release.

Site description: Hog Island Bay and the Virginia Coast Reserve LTER

Hog Island Bay is a shallow coastal lagoon and part of the Virginia Coast Reserve Long Term Ecological Research project (VCR-LTER). The VCR is comprised of 14 barrier islands, shallow shoals and deep channels, mudflats, marsh islands, fringing marshes, and tidal creeks extending westward from the Delmarva Peninsula. The

semidiurnal tidal range is approximately 1.5 m at the mainland and 1.2 m at Hog Island (Santos, 1996). Of the total benthic surface area of the lagoon (15,085 ha), 37% is intertidal marshes and flats and 46% is less than 2 m deep at mean low water (Oertel, 2001). The lagoon has an overall hydraulic turnover time of approximately 1 day (Oertel, 2001); however, water residence times are spatially highly variable (Fugate *et al*., 2002).

The primary sources of N to Hog Island Bay are seepage of nutrient enriched groundwater (Lee and Olsen, 1985; Reay *et al*., 1992) and atmospheric deposition (Paerl *et al*., 1990). Macroalgae and benthic microalgae are the dominant primary producers with *Gracilaria tikvahiae*, *Ulva lactuca*, and *Bryopsis plumosa* being the most abundant macroalgae; phytoplankton chlorophyll *a* (chl *a*) production is generally low throughout the year and seagrasses have been locally extinct since the 1930s. A transect along an environmental gradient of nutrient and organic matter inputs has been established across Hog Island Bay with 4 representative sites: a mainland harbor, a mainland tidal creek, a mid-lagoon shoal, and a back-barrier island embayment (Figure 1-2). This transect provides an ideal situation for measuring the influence of macroalgae on N dynamics under a variety of environmental conditions. A more detailed site description follows in subsequent chapters.

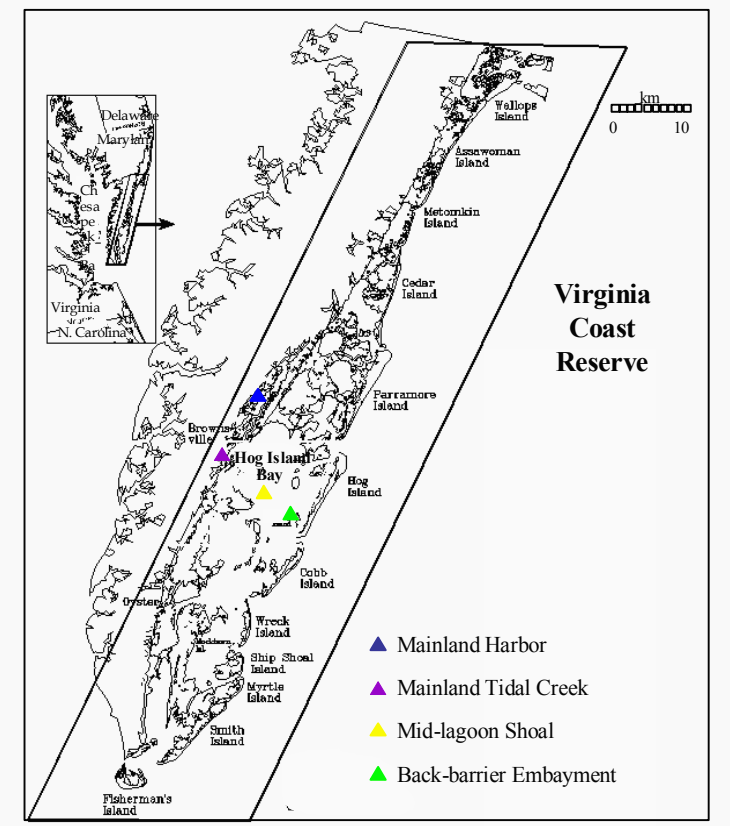

Figure 1-2 Map of the Virginia Coast Reserve showing the location of the four primary sites used in this study.

Objectives

The overall objective of the work presented here was to determine the patterns of DON distribution and transformation across a gradient of organic matter inputs in Hog Island Bay and to characterize the role of the dominant benthic primary producers, macro- and microalgae, in regulating these transformations.

The specific objectives of my dissertation were to:

- quantify the temporal and spatial patterns of DON relative to DIN in the water

column, and in sediment - water column fluxes and to partition the N pools into a variety of bioavailable components (e.g. NO_3^-, NH_4^+, urea, dissolved free amino acids (DFAA) and total amino acids (TAA)) compounds;

- quantify the role that benthic algae play in regulating water column standing stock and sediment-water column fluxes of DON through uptake and release of specific organic compounds;
- estimate the turnover rates of macroalgal tissue N pools and relate this to N availability and algal productivity.

A guide to the dissertation

The body of this dissertation is divided up into 4 chapters. The first two chapters represent an initial (Chapter 2) and a more in-depth (Chapter 3) investigation of the spatial and temporal variation in dissolved inorganic and organic nitrogen in Hog Island Bay. Specifically, the experiments presented within these chapters were designed to look at sediment fluxes of nitrogen across an environmental gradient, and to quantify the impact that macroalgae and microalgae have on these fluxes. Chapter 2 was published in 2001 in the journal Estuarine, Coastal and Shelf Science, and is included here as the final draft of this manuscript. In Chapters 3-6, this work is referred to as "Tyler et al. 2001". Chapter 4 details a series of field and laboratory experiments that investigate the uptake and release of nitrogen by the common macroalga *Gracilaria tikvahiae*. This experiment represents an attempt to quantify the total amount of nitrogen that passes through the

macroalgal pool on a daily basis. Finally, Chapter 5 summarizes a series of laboratory experiments where I measured the uptake of a variety of labile organic nitrogen compounds (urea and amino acids) by the two most common macroalgae in Hog Island Bay, *G. tikvahiae* and *Ulva lactuca*. In the final chapter (6), I have attempted to tie together the main results of these four chapters, to draw some final conclusions and to indicate areas where future research is needed.

Chapter 2
Macroalgal mediation of dissolved organic nitrogen fluxes in a temperate coastal lagoon

Introduction

Dissolved organic nitrogen (DON) makes up a large fraction of the total nitrogen (N) in marine systems (Sharp, 1983). Coastal systems receive inputs of DON from allochthonous sources (Meybeck, 1982; Hopkinson, 1998) including atmospheric deposition (Paerl *et al*., 1990; Cornell *et al*., 1995; Paerl, 1995) and autochthonous production. In lagoonal systems with little riverine input the majority of new DON most likely comes from autochthonous production or from enriched groundwater. Because they are usually shallow, lagoons are for the most part littoral zone systems. As such, shallow lagoons tend to be dominated by benthic primary producers, such as seagrasses, macroalgae and benthic microalgae rather than by phytoplankton. Fast-growing species of macroalgae are at a competitive advantage over slow-growing seagrasses and perennial macroalgae under conditions of increased nutrient loading (Duarte, 1995) so that anthropogenically impacted lagoons typically display increased growth of opportunistic macroalgae (Lee and Olsen, 1985; Sfriso *et al*., 1992; Valiela, 1992a; Valiela *et al*., 1997; Kinney and Roman, 1998). Thus in anthropogenically impacted lagoonal systems the remineralization of macroalgae contributes significant amounts of DON.

In deeper estuaries, some studies have shown DON to be an important component of the overall N flux (Lomstein *et al*., 1989; Enoksson, 1993; Blackburn *et al*., 1996; Cowan and Boynton, 1996), while others have shown that DON fluxes were either insignificant (Nixon, 1981; Burdige and Zheng, 1998) or only seasonally important

(Boynton *et al*., 1980). Few studies have examined sediment fluxes of DON in shallow systems, even though the high sediment surface area to water volume ratio of lagoons increases the relative importance of sediment-water column interactions (Nowicki and Nixon, 1985; Sand-Jensen and Borum, 1991). The magnitude of sediment nutrient fluxes is generally related to the magnitude of primary production in the system, as primary production is the source of organic matter to the sediments (Nixon, 1981). Duarte (1995) described that in macroalgal-dominated systems nutrient cycling may be accelerated in comparison to systems dominated by vascular plants because macroalgae, which have little structural material and decompose rapidly (Buchsbaum *et al*., 1991), have the potential to contribute significantly and rapidly to sediment fluxes following senescence. For example, the addition of dead algal material to sediment cores has been shown to significantly increase fluxes of ammonium (NH_4^+) and DON to the water column (Hansen and Blackburn, 1992; Enoksson, 1993). In extreme cases, such as in the Sacca di Goro, Italy, massive blooms of *Ulva rigida* periodically crash, releasing significant amounts of dissolved and particulate organic matter and leading to severe dystrophic crises (Sfriso *et al*., 1992; Viaroli *et al*., 1993; Viaroli *et al*., 1995).

Nutrients regenerated in the sediments and released to the water column are thought to support a significant portion of primary production in coastal ecosystems (e.g. Nixon, 1981; Fisher *et al*., 1982; Koop *et al*., 1990; Cowan and Boynton, 1996). Macroalgal uptake of dissolved inorganic nitrogen (DIN) influences the flux of DIN between the sediment and the overlying water column, and may limit phytoplankton growth (Valiela, 1992c; Thybo-Christesen *et al*., 1993; McGlathery *et al*., 1997).

Because some species of macroalgae can take up specific DON compounds in addition to DIN (Nasr *et al*., 1968; Hanisak, 1983; Chisholm *et al*., 1996), they may influence water column concentrations and sediment fluxes of DON as well. However, the impact of living macroalgae on sediment fluxes of DON has not been investigated.

Dissolved organic nitrogen is a collective term for a variety of compounds that can differ substantially in molecular weight and bioavailability, from small, highly bioavailable, low carbon (C):N compounds like amino acids and urea to very large, refractory, high C:N polyphenolic compounds. Previous studies have indicated that a substantial fraction of the sediment DON flux is comprised of low carbon (C):N compounds, such as urea and amine compounds (Boucher and Boucher-Rodoni, 1988; Lomstein *et al*., 1989; Burdige and Zheng, 1998), which generally are more labile and available to water column organisms. Knowledge of the concentration of biologically important components of the DON pool, such as urea, is important in understanding overall system dynamics.

We investigated the role of macroalgae in regulating DON fluxes and transformations in Hog Island Bay, a shallow macroalgal-dominated lagoon located on the Virginia Coast. The specific objectives of this study were to characterize the importance of DON in the water column total dissolved nitrogen (TDN) pool and to determine the influence of the macroalga *Ulva lactuca* on water column concentrations and sediment-water column fluxes of DON, urea and DIN during active growth and following senescence. The study presented here represents one of the first attempts to characterize DON fluxes in temperate lagoons.

Methods

Site description

Hog Island Bay (Figure 2-1), a shallow coastal lagoon situated off of the Delmarva Peninsula, is part of the Virginia Coast Reserve (VCR) Long Term Ecological Research site (LTER). The VCR is comprised of 13 barrier islands, and numerous shallow shoals, deep channels, mudflats, marsh islands, fringing marshes, and tidal creeks extending westward from the Peninsula. Due to the small catchment area and lack of fluvial inputs, the primary sources of allochthonous N to Hog Island Bay are most likely seepage of nutrient enriched groundwater (Lee and Olsen, 1985; Reay *et al*., 1992), and atmospheric deposition (Paerl *et al*., 1990). There is a gradient of organic matter and nutrient inputs across Hog Island Bay from the mainland to the islands, with the highest concentrations of dissolved N and sediment organic matter found closest to the mainland (McGlathery *et al*. unpublished data). Within Hog Island Bay, N is transformed by algal and bacterial uptake, remineralization, nitrification and denitrification. Seagrasses here have been locally extinct since the 1930s and macroalgae are the dominant primary producers. Dominant macroalgal species include *U. lactuca, Gracilaria tikvahiae* and *Cladophora* sp. Benthic microalgae also may be important primary producers but phytoplankton production is low throughout the year.

We established a transect across Hog Island Bay with sites representing the 3 subtidal habitat types: a mainland tidal creek (Creek), two mid-lagoon shoals (Shoal 1 and Shoal 2), and a back-barrier island embayment (Hog; Figure 2-1). The water depth at

all sites is <1 m at mean low water and the tidal range is approximately 1.2 m at Hog Island and 1.5 m at the mainland (Santos, 1996). Atlantic Ocean water enters the lagoon through Machipongo Inlet at the southern tip of Hog Island. The Creek site is a small tidal creek (approx 5 m across) bordered by *Spartina alterniflora* marsh. The sediments are fine-grained and silty and often coated with a well-developed microalgal turf. Macroalgal biomass is generally low, (<20 g dw m-2, McGlathery *et al*., 2001) and often partially buried in the sediment, although large floating mats of *U. lactuca* and *G. tikvahiae* were observed ephemerally during the summer of 1998. The Shoal sites in the mid-lagoon border remnant oyster reefs and the sediments are fine-grained sands. Substantial macroalgal mats develop at specific locations in the Shoal region of the mid-lagoon, with the peak biomass occurring in June/July (>450 g dw m^{-2} at Shoal 1 and >650 g dw m^{-2} at Shoal 2, McGlathery *et al*., 2001). In early July 1998, the algal populations at Shoal 2 crashed, probably as a result of high temperatures and self-shading within the algal mat. No crash was observed at Shoal 1. The Hog Island site is a shallow embayment with coarser-grained sandy sediments. Algal biomass here is typically low (5-15 g dw m^{-2}, McGlathery *et al*., 2001) and relatively constant throughout the year.

N Flux measurements

Sediment - water column nitrogen fluxes were measured on October 30, 1997 and May 4, July 8, and August 18, 1998. Six sediment cores (8 cm I.D., 12 cm sediment, 18 cm water column), water and *U. lactuca* were collected at each of the sites. The cores were returned to the laboratory and held overnight in outdoor flowing seawater tables to

maintain ambient field temperatures. Experimental treatments were: sediment only and sediment + algae. *U. lactuca* and water from each site were used with the respective sediment. Parallel water blanks were used to correct for concentration changes in the overlying water column. In July 1998 additional cores from Shoal 2 were collected in order to measure fluxes immediately following the crash of the macroalgal bloom.

Prior to initiation of the experiment, the overlying water was drained using a siphon and carefully replaced, without disturbing the sediment surface. *U. lactuca* was added to the sediment + algae cores, large air bubbles were removed and the cores were sealed with a rubber stopper. *U. lactuca* density (equivalent to 100 - 200 g dw m^{-2}) simulated moderately dense patches in the lagoon. The overlying water was stirred with a magnetic stir bar (approximately 60 rpm) throughout the experiment to prevent build-up of concentration gradients that may interfere with diffusion across the sediment-water and thallus-water interfaces. Fluxes were measured over a 12 hour period (6 hr light, 6 hr dark). Surface irradiance during the light treatment ranged from 700 to 1200 μmol photons m^{-2} s^{-1} and was similar to that measured in *situ* (McGlathery *et al*., unpublished data). The water depth in the cores (17-20 cm) approximates low tide water levels in the field so that light reaching the water-sediment or water-algae interface within the cores was likely similar to low tide conditions. Samples for ammonium (NH_4^+) and nitrate + nitrite ($NO_3^- + NO_2^-$) were collected at 2 hr intervals; samples for urea and total dissolved nitrogen (TDN) were collected at 6 hr intervals. All samples were filtered immediately (Gelman Supor, 0.45 μm) and frozen, with the exception of NH_4^+ samples, which were analyzed within 3 hr of collection.

Ammonium was measured using the phenol-hypochlorite method (Solorzano, 1969). Nitrate + nitrite was measured using an Alpkem "Flow Solution" Autoanalyzer (Perstorp, 1992). Urea was measured using a modification of the methods described by Mulvenna & Savidge (1992) and Goeyens *et al*. (1998). TDN was measured as NO_3^- following alkaline persulfate digestion in pre-combusted sealed ampoules (modified from Koroleff, 1983), and DON was calculated by difference between TDN and DIN (NH_4^+ + NO_3^- + NO_2^-). Several additional organic nitrogen standards were used to examine the recovery efficiency of the method (Table 2-1). The percent recovery was 95-100%, with the exception of ATP (85%). The limits of detection for the TDN method were 0.6 μM (calculated as 2 times the standard deviation of the blanks, n=10, as defined by Willason and Johnson 1986).

Flux calculations

Fluxes were estimated based on the slope of the change in concentration over time using the equation:

$$J = \frac{dC}{dt} \bullet \frac{V}{A}$$

where J is the flux rate in μmol m^{-2} h^{-1}, A is the core area, V is the water volume, C is the concentration and t is time. Sediment and sediment + algae fluxes were corrected for changes in the water blanks. Uptake and release by *U. lactuca* were calculated by subtracting the mean fluxes measured in sediment only chambers for that site from the sediment + algae chambers, and is expressed per gram dry weight of algae. Daily

sediment flux and algal uptake rates were obtained by multiplying the measured hourly rate by the actual number of daylight or dark hours for that date.

Elemental analysis

Following each experiment, the algae used in each core was rinsed with deionized water, patted dry and frozen. They were later freeze-dried and ground to homogeneity with a mortar and pestle. Small sediment cores (4 cm I.D.) were collected from each site at the time of the experiment, sectioned (0-2, 2-5, 5-10 cm), freeze-dried and ground with a mortar and pestle. Sediment samples were acidified with 20% HCl to remove carbonates. C and N content of sediment and algae were measured on a Carlo Erba NA 2500 Elemental Analyzer.

Results

Site characteristics

Water column TDN ranged from 16 - 33 μM and was, in general, highest at the Creek site and lowest at the Hog site, with intermediate levels at the Shoal site throughout the seasons (Figure 2-2). DON represented 52-98% of the TDN pool, and ranged from 11-30 μM at Creek, 9-22 μM at Shoal 1, and 12 - 20 μM at Hog. Highest values occurred in July. At all sites, DIN was highest and made up a greater proportion of

TDN in October than in other months. Urea was generally low in the October and May (<1 μM) and highest in the July and August at all sites (up to 2 μM) when it made up a maximum of 9% of the TDN pool at Shoal 1 in August.

As with water column N, sediment %N and %C were highest at Creek, intermediate at Shoal 1, and lowest at Hog (Table 2-2). At Hog, the %N was consistently around 0.01%. The C:N in these sandy sediments was approximately 9 in May and July, declined in August, and rose in October, due mainly to changes in C content. Although the overall %N was higher, a somewhat similar pattern was seen in the C:N at Shoal 1 where there was a rise in October due to decreased %N. Following the crash of the macroalgal bloom in early July at Shoal 2, the sediment %N was 0.4% and %C was 2.5%, yielding a C:N of 7.2. Three weeks later the sediment N and C had dropped back down to values approaching those of Shoal 1, and the C:N had increased to 11.3. The Creek site had the highest C:N in the summer months of July and August, and lowest in May and October.

Mean macroalgal tissue N content was 2.5 ± 0.4, 2.0 ± 0.3 and 1.1 ± 0.2 % for Creek, Shoal 1 and Hog macroalgae, respectively (Figure 2-3). Variations in tissue N content indicate a gradient of high to low N availability from the mainland seaward, echoing the patterns seen in the water column N and sediment N. There was a weak relationship ($p = 0.07$; SPSS for Windows ver. 8.0, linear regression function) between macroalgal N and water column DIN. At all sites the highest values occurred in October.

Fluxes

The concentration of DIN in all cores incubated with algae declined to a constant concentration close to zero within a few hours after the initiation of the experiment due to rapid DIN uptake by *U. lactuca*. This results in light flux rates that represent only the initial surge uptake and dark flux rates of zero. Since only the light values for macroalgal DIN uptake are interpretable, and even these are not representative of the potential uptake rate, the DIN fluxes for the sediment + algae cores are not discussed at length.

The DIN sediment flux rates at the Creek were not significantly different from zero (Figure 2-4) except during October when there was substantial uptake of DIN, most likely as a result of high initial concentrations. The mean urea flux to the water column (188 μmol m^{-2} d^{-1}) was 60 fold greater than the mean urea-free DON flux (not shown, 3 μmol m^{-2} d^{-1}) at the Creek site (Table 2-3). The highest overall urea flux was measured in August (Figure 2-4). Fluxes for the rest of the DON pool were not significantly different from zero, with the exception of August, when the net flux was negative at this site. In contrast, *U. lactuca* in these cores released DON into the water in all months with the exception of August (Figure 2-5). The urea released from the sediments was taken up immediately by the algae, except for July when the uptake rate was not significantly different from zero.

The Shoal sites exhibited the most dynamic fluxes of both DON and DIN (Figure 2-4). In May, fluxes of all dissolved N compounds were not different from zero at Shoal 1. Even though the macroalgae did not exhibit an episodic crash at Shoal 1, the summer DON fluxes were two fold higher than those measured at Hog or Creek at any time. At

Shoal 2 in July, the 5-10 cm layer of decomposing macroalgae at the sediment surface resulted in a large efflux of DON and DIN. The net TDN flux, >70 mmol m^{-2} d^{-1}, was an order of magnitude greater than at any other site. The mean urea flux at Shoal 1 was negative, in contrast to positive mean fluxes at the other sites (Table 2-3). Shoal macroalgae took up urea only in October and DON only in August (Figure 2-5). DON was released in all other months, with highest rates in July.

In May, the Hog Island sediments demonstrated the only positive flux of DIN aside from post-crash Shoal 2 (Figure 2-4). In all other months DIN was either taken up by these sediments (August and October) or showed an insignificant flux (July). The sediments released urea in all months and DON in August, but took up DON in October. Macroalgal uptake rates for urea were significant in May, July and October (Figure 2-5). The DON release rates by macroalgae, although positive in all months except May, were so variable that we were unable to discern any patterns.

Overall, there was an inverse relationship between sediment C:N and net sediment TDN fluxes ($p=0.06$; SPSS for Windows ver. 8.0, linear regression function). Macroalgal DON release rates did not appear to be proportional to tissue N content ($p=0.62$; SPSS for Windows ver. 8.0, linear regression function). Macroalgal release of DON appeared to occur primarily in the light, although light-dark differences were not significantly different due to high variability (Table 2-4). No significant differences in algal uptake or sediment release were seen for urea in light versus dark comparisons. Sediment release of DON occurred significantly more in the light than in the dark (Table 2-4). DIN uptake by sediments was significantly higher in the light than in the dark.

Discussion

DON is an important component of the total dissolved nitrogen pool in Hog Island Bay, comprising 58 - 95% of water column TDN. While river-dominated estuaries often have substantial allochthonous inputs of DON (Meybeck, 1982; Hopkinson, 1998), the DON in this system appears to come from autochthonous macroalgal production within the lagoon in addition to enriched groundwater. In spite of the variation in measured fluxes across the different habitat-types in the lagoon, the relative proportions of DON, DIN and urea remain relatively constant between sites, suggesting that the lagoon is very well mixed. Benthic macroalgae also appear to act as a conduit whereby DIN and some DON compounds (urea) are taken up during production, and subsequently released to the water column as different DON compounds during active growth. In addition, particulate organic nitrogen (PON) is provided to the sediments following senescence. The input of DON to the water column from decomposing organic matter in the sediments and from living macroalgae on the sediment surface has important consequences for the metabolism of heterotrophs and autotrophs capable of DON uptake. The uptake of bioavailable dissolved N compounds and conversion to PON and DON by macroalgae adds an additional step to the processing of N as it moves across the land-sea interface and may prolong its retention within the lagoon.

A proportion of the nutrient demand for estuarine primary production is often supported by nutrients recycled within the sediments and released to the water column (e.g. Nixon, 1981; Fisher *et al*., 1982; Koop *et al*., 1990; Cowan and Boynton, 1996).

Indeed, in other coastal ecosystems, it has been shown that macroalgal uptake intercepts N released from the sediments (Valiela, 1992b; Bierzychudek *et al*., 1993; McGlathery *et al*., 1997). However, the sediments of Hog Island Bay were not a source of DIN to the water column, with the exception of Shoal 2 following the crash of the algal bloom and Hog in May. As such, it appears that the macroalgae instead may prevent the downward diffusion of water column DIN to the sediments, especially because *U. lactuca* takes up N more rapidly than the sediments in the lagoon.

While sedimentary fluxes of DIN do not appear to be a significant component of macroalgal nutrition in Hog Island Bay, sediment-derived urea may be important. In using organic N directly, the algae effectively bypass the complete mineralization of organic N to inorganic N. Not all species of macroalgae are capable of urea uptake and under conditions of low DIN:urea availability, those species that can are at a competitive advantage. Recent evidence suggests that urea may be a more important source of N for primary producers than originally thought. For example, Cho *et al* (1996) demonstrated that bacterial production of urea-N in the water column of the Southern California Bight was sufficient to supply 35-91% of the daily phytoplankton N demand. While sediment release of urea is likely to be closely coupled to macroalgal productivity in Hog Island Bay, at this point we do not know enough about the N demand of the macroalgal population to determine the relative importance of urea. Other, as yet unidentified, DON compounds, such as amino acids, may also play an important role in meeting algal N demand. Because macroalgae may serve as an indicator of an integrated measure of N availability, the high tissue N near the mainland suggests that terrestrial and groundwater

N, entering at the land-water interface, are important to the overall N budget. This also lends support to the idea that the lagoon is well mixed, because higher concentrations of N in the water column are not consistently observed close to the mainland. Overall mean macroalgal uptake rates for DIN and urea calculated from our flux incubations were 1.2 ± 0.5 and 0.6 ± 0.2 μm g dw^{-1} d^{-1}. Maximum uptake rates (V_{max}) of 138 ± 78 μmol g dw^{-1} h^{-1} for ammonium (Fujita, 1985) and 11.7 ± 0.6 μmol g dw^{-1} h^{-1} for urea (Tyler, unpublished data.) have been measured under non-limiting conditions, which are significantly higher than those reported here. However, N availability was relatively low at our sites and the rates obtained in this study may be a more realistic estimate of the impact that algae have on DIN and urea from water column and sediment sources in the lagoon.

In addition to the uptake of urea and DIN from the sediments and water column, respectively, living macroalgae also have a substantial impact on the net DON release from the benthos to the water column. The mean total DON flux in cores with algae was 331 μmol m^{-2} d^{-1} higher than the fluxes in the sediment only cores (Table 2-3; using urea-free DON flux the difference is 450 μmol m^{-2} d^{-1}). Like phytoplankton (Bronk *et al*., 1994), the macroalga *U. lactuca*, appears to "leak" DON into the water during active growth. Over relatively short time scales, DIN, urea, and possibly other small DON compounds are taken up, transformed and subsequently released to the water column as DON. Release was higher in the light than the dark, indicating a possible association with photosynthesis.

In dense patches, living macroalgae may be a more important source of DON for the water column than the sediments. Given the mean release rate of urea free DON by *U. lactuca* (3.5 ± 0.9 μm g dw^{-1} d^{-1}) and the range of macroalgal biomass found at the sites used in this study (0 to > 650 g dw m^{-2}) there is the potential for the release of more than 2 mmol N m^{-2} d^{-1} from actively growing algae to the surrounding water (assuming all species have similar release rates). DON release by macroalgae has not been reported in the literature, however DOC release has been documented previously, with a wide range in the values reported (e.g. Khailov and Burlakava, 1969; Moebus and Johnson, 1974; Brylinsky, 1977). While the bioavailability of this released organic matter is as yet unknown, these inputs are likely to fuel heterotrophic metabolism in the water column (Brylinsky, 1977; Valiela *et al*., 1997).

Our measurements of macroalgal uptake and release of dissolved N are based on an estimate of the net change between cores incubated with and without algae. However, the macroalgae may have important indirect effects on nitrogen dynamics that were masked by our methods. For example, microalgal chlorophyll *a* is generally low across the lagoon (1-8 μg cm^{-2}; McGlathery *et al*. 2001), however the light-dark difference in DIN uptake by sediments suggests that benthic microalgae were responsible for DIN uptake in the light. Uptake of water column NH_4^+ by benthic microalgae has been shown to limit coupled nitrification-denitrification in the sediments (Rysgaard *et al*., 1995), which would in turn prolong the retention of N within the lagoon by slowing the loss as N_2. Where macroalgae are present in sufficient biomass, they will outcompete the microalgae for water column nutrients and, by shading the sediment surface, for light.

Further, over the diurnal cycle, macroalgal production and consumption of O_2 may alter the redox status at the sediment surface (Lavery and McComb, 1991a), which may in turn affect sediment fluxes of DIN and DON (Kristensen and Blackburn, 1987; Hansen and Blackburn, 1991; Miller-Way *et al*., 1994) and may affect coupled nitrification-denitrification (Krause-Jensen *et al*., 1999).

Delivery of organic matter to the sediment surface from decomposing macroalgal tissue is an additional source of DON to the sediments and water column. Our data show clearly that in a macroalgal-dominated lagoon such as Hog Island Bay, DON was a major component of the TDN flux from the sediment to the overlying water column. This is consistent with the conclusion of Bartoli *et al*. (1996) who inferred high DON fluxes from sediment porewater profiles in the Lagoon of Venice. The mean total DON and urea sediment fluxes measured at the individual sites in this study fall within the range of reported values for a variety of coastal ecosystems (Table 2-5). In Hog Island Bay, macroalgal biomass peaks in mid-summer and rapidly declines (McGlathery *et al*., 2001). In spite of this decline, the benthos (including macroalgae) remained net autotrophic during the study period (McGlathery *et al*., 2001), with the exception of Shoal 2. The net uptake of DIN and release of DON observed at Hog, Shoal 1 and Creek are consistent with a net autotrophic metabolism.

The flux of nutrients from sediments is generally thought to be proportional to the amount of organic matter delivered to the sediment surface (Nixon, 1981). Kelly & Nixon (1984) and Kelly *et al*. (1985) demonstrated a positive relationship between sediment nutrient regeneration and primary production in experimental mesocosms and

many others have reported increases in sediment DIN, DON and/or urea fluxes with the addition of organic material to the sediment surface both *in situ* (Jensen *et al*., 1990) and experimentally (Enoksson, 1993; Sloth *et al*., 1995; Therkildsen *et al*., 1996). The Shoal sites in Hog Island Bay had the highest N flux rates throughout the year, suggesting that sediment N release was related to macroalgal biomass. The ratio of carbon to nitrogen in sediments, often an indicator of the lability or age of the sediment organic matter, also may be related to sediment N fluxes (e.g. Kristensen and Blackburn, 1987; Caffrey, 1995; Hall *et al*., 1996). Low C:N values in the summer at both Shoal sites were most likely indicative of the input of fresh organic matter from macroalgal senescence, and were linked to large fluxes of DON. This is concordant with the observations of Hansen & Blackburn (1992) who observed both an increase in the magnitude of the DON flux as well as an increase in the proportion of the TDN flux made up by DON following the simulated deposition of an algal bloom. The magnitude of the summertime DON fluxes following the macroalgal decline at Shoal 2 rivals the highest values reported for coastal systems (Table 2-5). At both the Creek and Hog sites, where inputs of macroalgal detritus were low, the sediments were not a net source of N to the water column, even though urea release was high. The Creek site, closest to the mainland and with muddy sediments, had the highest sediment N content and also the highest C:N, probably reflecting the salt marsh sources of refractory organic material with a relatively high C:N. In contrast, the sandy, low-N sediments at Hog had insignificant fluxes in May and July followed by a large flux of DON when the sediment C:N dropped in August. This release, which

suggests a greater relative availability of high N organic material, coincided with a period of macroalgal decline at this site (McGlathery *et al*., 2001).

Fast growing, opportunistic species of macroalgae have 'boom and bust' life cycles, little structural material, and rapid decomposition following senescence relative to vascular plants (Buchsbaum *et al*., 1991; Duarte, 1995; Bourgues *et al*., 1996). The labile fractions of macroalgal-derived organic matter disappear within days to weeks (Buchsbaum *et al*., 1991; Enriquez *et al*., 1993). The pulse of organic material at both Shoal 1 and Shoal 2 appeared to be utilized by October, when the C:N value more than doubled and flux rates decreased. The increase in C:N also indicates that N was metabolized and removed from the sediments more quickly than C. Given the biomass measured at Shoal 2 in June of 1998 and the measured flux rates, all of the N contained in the previously living macroalgae would have been released to the water column within approximately 13 days (this estimate is based on the N content of all algal species found at the site and ignores losses by denitrification). This is in agreement with the increased C:N measured 3 weeks after the crash. The release of nutrients following the macroalgal decline was thus an ephemeral occurrence that accelerated nutrient cycling, as has been shown in other systems (Buchsbaum *et al*., 1991; Duarte, 1995; Viaroli *et al*., 1996). A sudden increase in oxygen demand is also typically associated with such events (Valiela, 1992c; Duarte, 1995; Viaroli *et al*., 1995).

In contrast to the substantial net DON flux at the Shoal site, the urea component of this flux was negligible. However, at the Creek and Hog sites, urea made up the majority of the N flux to the water column. This may indicate a highly developed

infaunal community at the Creek and Hog sites in comparison to the Shoal site (Lomstein *et al*., 1989) and is consistent with Sundback *et al*. (1990) who demonstrated an impoverished infaunal community below macroalgal mats. Across all the sites, urea was a very important component of the N flux, comprising 32% of N released from the sediments. This is consistent with other studies that also have shown urea to be an important component of the sediment N flux: 36-70% in northern Bering Shelf sediments (of urea + NH_4^+ + NO_3^- + NO_2^-, Lomstein *et al*., 1989) and ~30% in the Bay of Pampoul, France (of urea + NH_4^+, Boucher and Boucher-Rodoni, 1988). Likewise, Burdige & Zheng (1998) suggested that the low C:N of benthic DOM fluxes in Chesapeake Bay intimates the potential importance of labile compounds such as urea and amino acids. Our data suggest that while net sediment-water column DON fluxes are related to macroalgal biomass, flux rates of specific DON compounds may be controlled by a more complex set of factors.

Short-term (hours) flux measurements such as ours tend to be more variable than those from longer-term incubations (days-weeks) (Nixon, 1981; Teague *et al*., 1988; Cowan and Boynton, 1996; Burdige and Zheng, 1998). However, in longer term experiments, DON released from the sediments may be mineralized in the water column to CO_2 and DIN, causing an underestimate of DON fluxes and an overestimate of DIN fluxes. In some cases, we observed large differences between replicates which meant that making corrections for water column and sediment activity occasionally required subtracting a highly variable number from another highly variable number. For example, the August macroalgae+sediment DON flux measurement for the Hog site yielded a

mean of 5.6 ± 4.8 μmol m^{-2} d^{-1}; the mean sediment flux was used to calculate this. If the range of sediment flux values had been used, we would have calculated algal DON release/uptake rates ranging from -4.4 to +19.7 μmol m^{-2} d^{-1}. This variability sometimes made trends in the data less significant. However, the release of DON from mid-lagoon shoal sediments in July was significant in spite of the variability, and was likely to have had an effect on overall system metabolism in the lagoon. We measured the highest water column DON concentrations across the lagoon in July, perhaps as a result of the massive release of DON from the decomposing algal mat. Further, water column chlorophyll *a* more than doubled following the crash at Shoal 2, suggesting the stimulation of phytoplankton production by the release of nutrients (McGlathery *et al*., 2001). In this study, we were only able to address diffusive fluxes from the sediments. Advection by tidal forcing and the upwelling of N-rich groundwater may add substantially to the amount of dissolved N crossing the sediment-water interface.

Conclusions and future research directions

Our results clearly show that macroalgae play an important role in the uptake of DIN from the water column and urea from the sediments, and that other DON compounds are released to the water column during active growth as well as following senescence. Where decaying macroalgal biomass was deposited on the sediment surface, as at Shoal 1 and Shoal 2, it appears that mineralization was incomplete, as most of the N flux from the sediment occurred as DON rather than DIN (or N_2). In the absence of advection of DON directly from the system, mineralization to DIN in the water column

adds an additional step to the processing of N as it moves across Hog Island Bay to the coastal ocean, prolonging the retention within the lagoon (Anderson *et al*., in press). In lumping together the individual compounds into 'DON', the dynamics of individual compounds are masked. When comparing the urea fluxes with the DON fluxes measured in this study, for example, it is evident that in many cases urea is behaving differently than the remainder of the DON pool. Discrepancies such as this underscore the importance of measuring fluxes of specific DON compounds, especially highly bioavailable compounds such as urea and amino acids. Future studies in this system will address DON fluxes on a compound specific basis in all seasons and will relate this to macroalgal biomass and productivity throughout the lagoon.

Figures & Tables for Chapter 2: Macroalgal mediation of DON fluxes

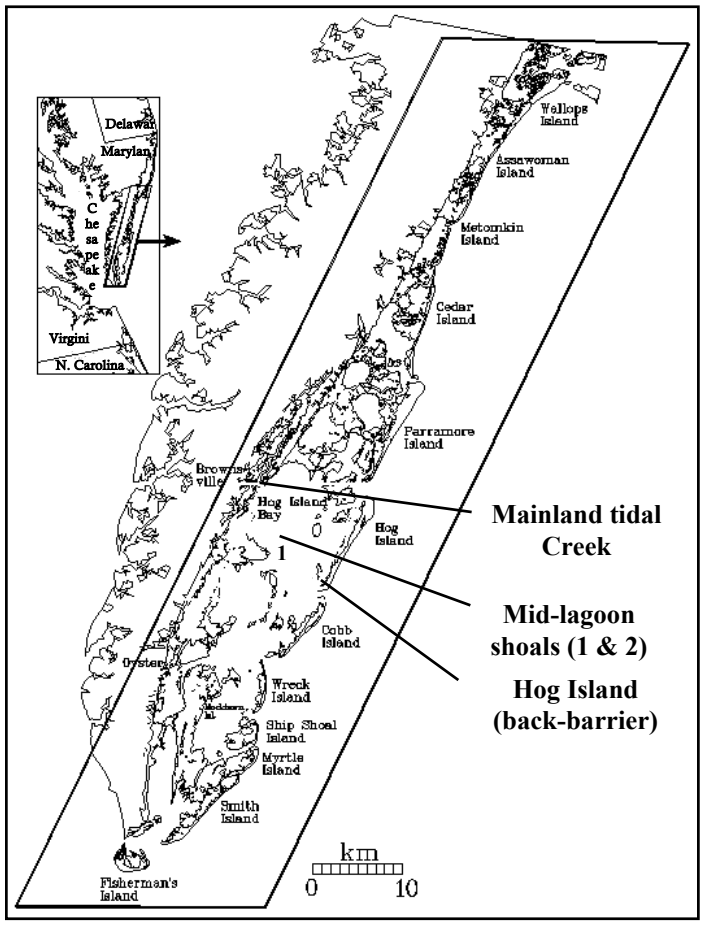

Figure 2-1 Site diagram of the Virginia Coast Reserve LTER site showing the 3 sites used in this study.

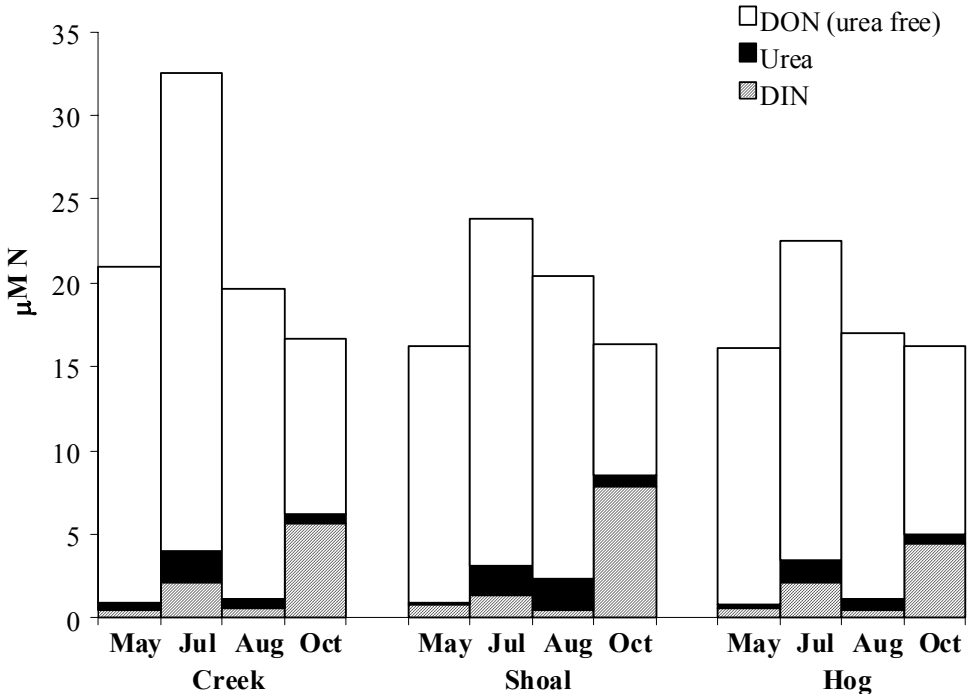

Figure 2-2 Water column concentrations of DON (urea free), urea and DIN (NO_3^- + NO_2^- + NH_4^+) from May, July and August 1998 and October 1997.

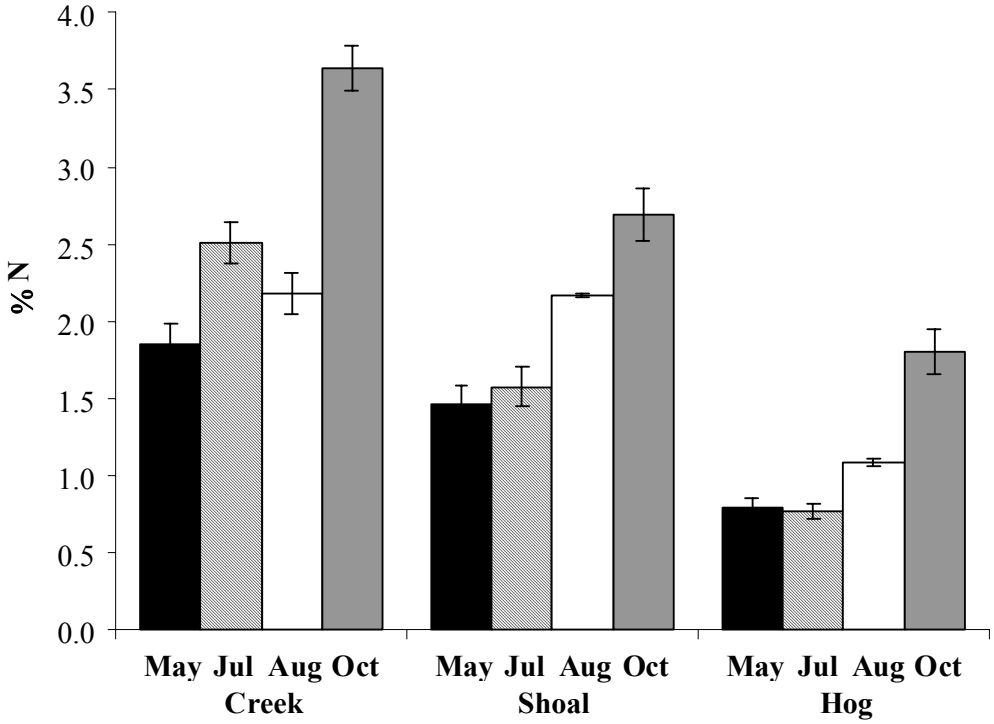

Figure 2-3 Nitrogen content of *Ulva lactuca* used in sediment flux incubations from May, July and August 1998 and October 1997.

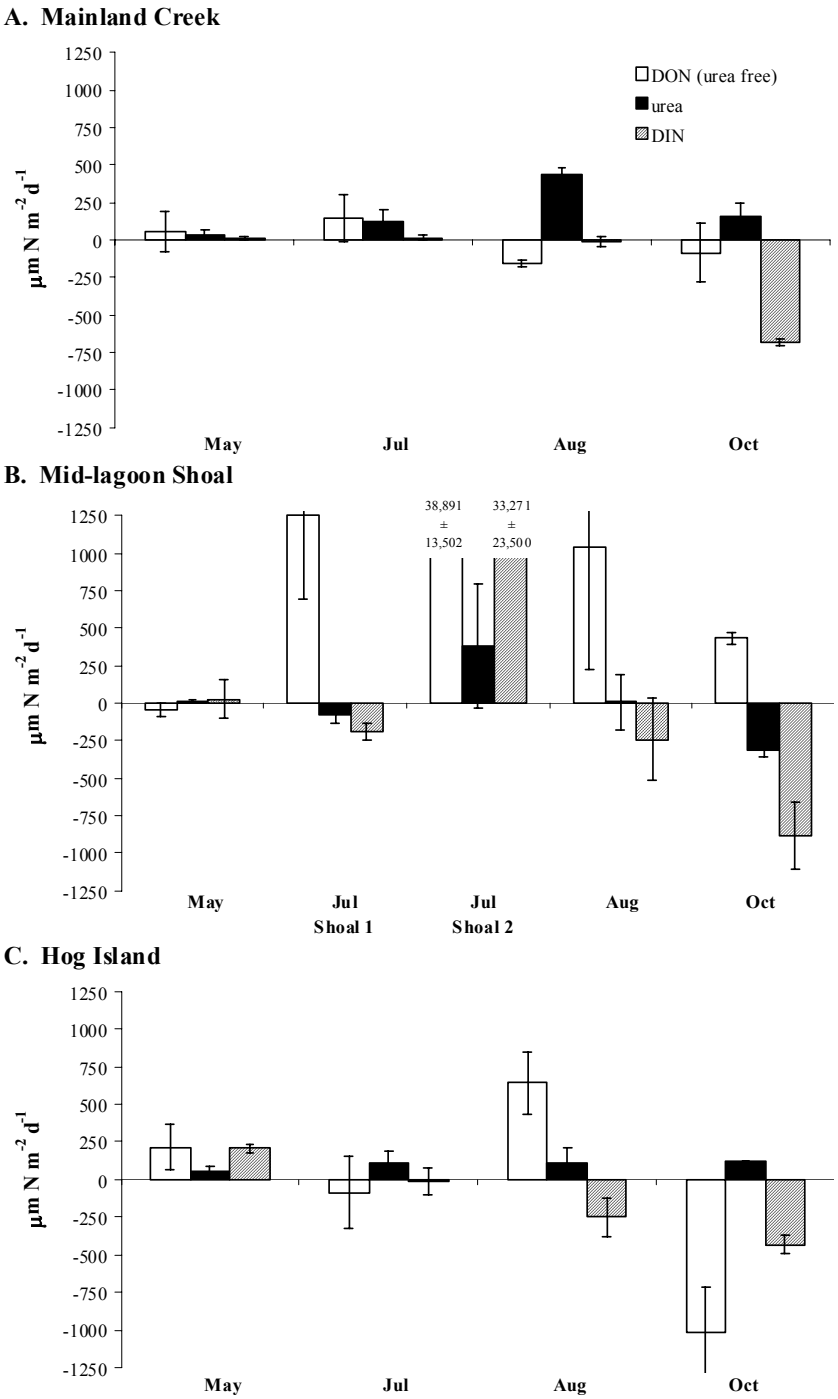

Figure 2-4 Sediment - water column fluxes of DON (urea free), urea and DIN at the (A) mainland creek, (B) mid-lagoon shoal(s) and (C) Hog Island sites from May, July and August 1998 and October 1997. Positive numbers indicate release from the sediment to the water column and negative indicate uptake from the water column. The May, August and October fluxes are from Shoal 1 only.

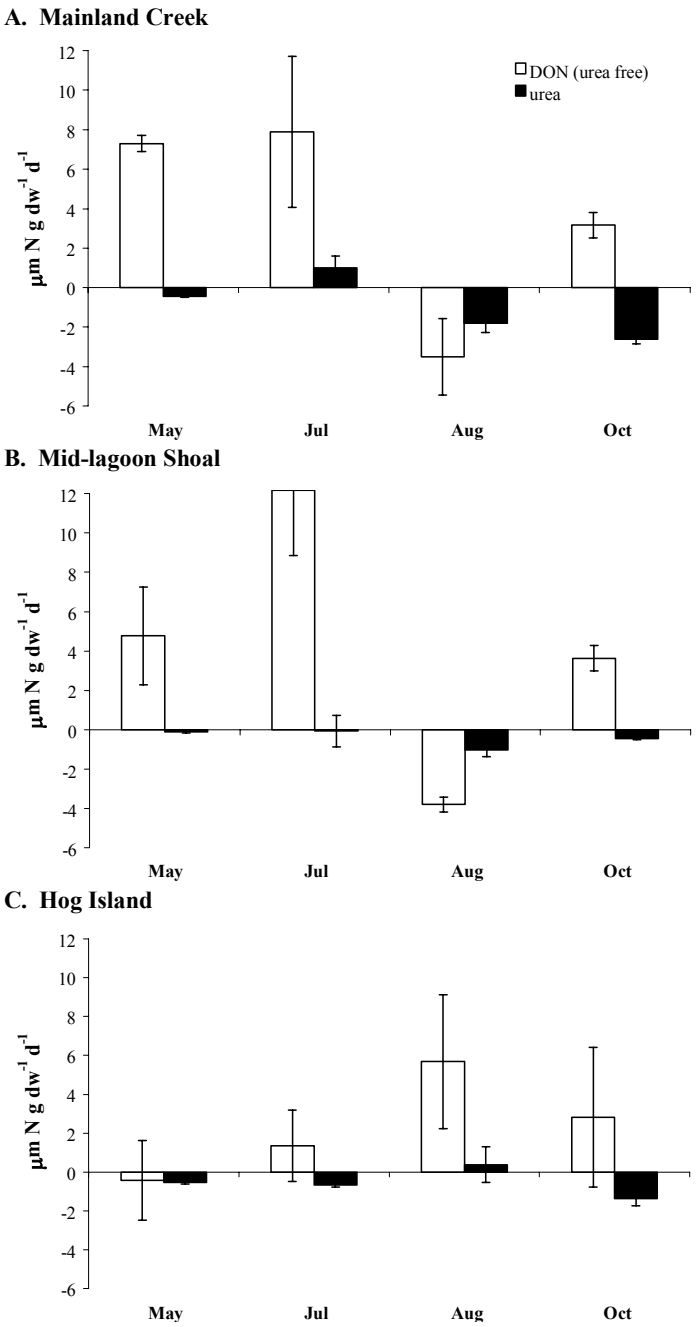

Figure 2-5 Macroalgal uptake/release of DON (urea free) and urea at the (A) mainland creek, (B) mid-lagoon shoal(s) and (C) Hog Island sites from May, July and August 1998 and October 1997. Positive numbers indicate release to the water column and negative indicate uptake from the water column. Shoal 2 July 1998 values were not included.

LEU	HUM	GLY	NIC	UREA	ATP	EDTA
97.9 ± 0.3	95.8 ± 0.2	98.1 ± 0.5	100.7 ± 0.5	100.5 ± 0.2	84.8 ± 0.1	95.2 ± 0.3

Table 2-1 Recovery of 20 μM organic nitrogen standards. Abbreviations are: LEU, leucine; HUM, humic acid; GLY, glycine; NIC, nicotinic acid; UREA, urea; ATP, adenosine triphosphate; EDTA, ethylenediaminetriacetic acid. Percent recovery noted in italics above bars. N = 2 for HUM & EDTA; N = 4 for all others.

		%N	%C	C:N
Creek	May 4	0.12 ± 0.01	1.07 ± 0.09	10.7 ± 0.1
	July 30	0.14 ± 0.02	1.93 ± 0.33	16.1 ± 1.0
	Aug 18	0.09 ± 0.00	1.24 ± 0.14	15.5 ± 1.8
	Oct 30	0.11 ± 0.00	1.05 ± 0.05	11.4 ± 0.9
Shoal1	May 4	0.04 ± 0.00	0.35 ± 0.03	11.8 ± 0.2
	July 30	0.02 ± 0.00	0.17 ± 0.02	9.4 ± 0.5
	Aug 18	0.04 ± 0.01	0.26 ± 0.11	7.9 ± 1.3
	Oct 30	0.02 ± 0.01	0.26 ± 0.04	19.0 ± 4.9
Shoal2	July 9	0.40 ± 0.11	2.47 ± 0.64	7.2 ± 0.2
	July 30	0.09 ± 0.00	0.87 ± 0.01	11.3 ± 0.3
Hog	May 4	0.01 ± 0.00	0.07 ± 0.01	8.9 ± 0.4
	July 30	0.01 ± 0.00	0.10 ± 0.01	9.3 ± 0.4
	Aug 18	0.01 ± 0.00	0.03 ± 0.01	3.4 ± 0.5
	Oct 30	0.01 ± 0.00	0.10 ± 0.00	14.3 ± 4.1

Table 2-2 Seasonal surface sediment %N, %C (carbonate-free) and C:N. Values are from top 2 cm section of each sediment core. Errors are the standard error of the mean.

	treatment	TDON	DON (urea free)	urea	DIN
Creek	sediment	72.9 ± 82.0	3.0 ± 75.3	188.1 ± 53.2	-166.1 ± 90.2
	sediment + algae	367.8 ± 174.6	417.6 ± 201.2	67.7 ± 67.5	-304.4 ± 165.2
Shoal 1	sediment	583.0 ± 289.2	690.9 ± 284.0	-91.9 ± 59.1	-322.4 ± 130.9
	sediment + algae	1066.9 ± 268.9	1325.3 ± 323.4	-146.6 ± 51.6	-596.8 ± 235.3
Hog Island	sediment	-9.2 ± 199.2	-61.5 ± 208.1	101.5 ± 28.1	-110.9 ± 85.8
	sediment + algae	184.4 ± 228.1	214.4 ± 224.5	16.4 ± 36.5	-299.9 ± 195.5
Overall mean	sediment	208.9 ± 124.8	202.8 ± 130.5	65.9 ± 33.7	-202.3 ± 61.0
	sediment + algae	539.7 ± 143.7	652.4 ± 166.3	-20.9 ± 33.7	-397.5 ± 113.9

Table 2-3 Mean daily sediment fluxes of total DON (including urea), urea free DON, urea and DIN from all experiments. A positive number indicates a flux from the sediment to the water column. Shoal 2 fluxes are not included in the overall mean. Units are μmol N m^{-2} d^{-1}, errors are the standard error of the mean, n=12.

	light	dark	p
Ulva lactuca			
DON (urea free)	0.23 ± 0.10	0.06 ± 0.10	0.206
urea	0.00 ± 0.03	-0.06 ± 0.03	0.148
DIN	-0.13 ± 0.04	0.02 ± 0.01	0.003
sediment			
DON (urea free)	21.84 ± 12.43	-11.58 ± 9.11	0.034
urea	4.35 ± 2.53	1.35 ± 2.89	0.437
DIN	-13.79 ± 4.36	-3.91 ± 1.49	0.038

Table 2-4 Light and dark fluxes of DON (urea free), urea and DIN. A positive number indicates release from the sediment or *U. lactuca* to the water column. Numbers represent pooled data from all experiments (n=36), except for Shoal 2 in July 1998. Errors are the standard error of the mean. Significance based on t-test between light and dark, equal variances not assumed. Units are μm gdw^{-1} hr^{-1} for macroalgal uptake/release and μm m^{-2} hr^{-1} for sediment fluxes.

Table 2-5 (following page) Comparison of sediment-water column DON fluxes from selected coastal systems. Comparison was limited to studies that directly measured fluxes, rather than estimations based on pore-water profiles. All values are in mmol m^{-2} d^{-1}.

System	DON flux			urea flux			Source
	range	mean		range	mean		
Narragansett Bay, RI, USA	0.2 - 0.7	0.3					range: Nixon et al. 1976, mean: Nixon & Pilson 1984
Patuxent River Estuary, MD, USA	-0.7 - 0.4						Boynton et al. 1980
Georgia Bight, GA, USA	~-1.9 - ~5.9	0.20					Hopkinson 1987
Bay of Pampoul, France					2.1		Boucher & Boucher-Rodoni 1988
Atchafalaya - Fourleague Bay, LA, USA	-116 - 107	-17	upper bay				Teague et al. 1988
		7.3	lower bay				
Northern Bering Shelf					0.7		Lomstein et al. 1989
Tomales Bay, CA, USA	-3.5 - -0.1	-0.3					Dollar et al. 1991
Aarhus Bight, Denmark		0.85	oxic				Hansen et al. 1991
		2.2	anoxic				
Svalbard, Norway	0.3 - 2.1	0.93		-0.05 - 0.11	0.01		Blackburn et al. 1996
Chesapeake Bay	-6.0 - 13.2						Cowan & Boynton 1996
Chesapeake Bay	-0.01 - 0.42	0.18	middle bay				Burdige & Zheng 1998
		0.11	south bay				
Young Sound, Greenland					0.03		Rysgaard et al. 1998
Hog Island Bay, VA, USA	-0.01 - 1.07	0.21		-0.15 - 0.19	0.07		this study
	38.89		post-macroalgal crash				

Chapter 3
Control of sediment–water column fluxes of inorganic and organic nitrogen by benthic micro- and macroalgae in a temperate lagoon: a compound specific approach

Introduction

Shallow lagoons are an important land margin feature world-wide, making up approximately 13% of the world's coastline (Cromwell, 1973). Behind the chain of barrier islands that lines nearly half of the Atlantic and Gulf coasts of the United States is a series of back-barrier lagoons (Hayden and Dolan, 1979). Owing to the shallow nature of these systems, the ratio of surface area to water volume is often high and the majority of the sediment surface falls within the photic zone. As a result, benthic processes are an important component of overall ecosystem function. Indeed, benthic primary production is often more important than pelagic production, and sediment mineralization of nutrients may drive overall biogeochemical cycling (Nixon, 1981; Martens, 1982; Sand-Jensen and Borum, 1991). In addition, coastal systems are often important features in the processing of land-derived nutrients, particularly nitrogen. In spite of the importance of shallow lagoons, little work has been done to investigate the fate of inorganic or organic nitrogen in these shallow systems (Boynton *et al*., 1996). In the work presented here, we describe the most recent results of a continuing investigation into the fate of nitrogen in Hog Island Bay, a shallow lagoon on the Virginia, USA coast. Specifically, we have investigated the role of the two dominant groups of benthic primary producers, macroalgae and microalgae, in regulating sediment-water column exchanges of dissolved inorganic and organic N.

DON makes up a high proportion of the dissolved N in seawater (Sharp, 1983). It is often in greater concentrations than inorganic nitrogen and is important for

heterotrophic metabolism. In addition, a large proportion of the dissolved nitrogen entering coastal systems by atmospheric deposition may be organic (Paerl *et al*., 1990; Cornell *et al*., 1995; Paerl, 1995; Cornell *et al*., 1998). Previous studies in Hog Island Bay and other, similarly shallow estuaries indicate that the magnitude of DON fluxes can vary widely (e.g. Boynton *et al*., 1980; Hopkinson, 1987; Teague *et al*., 1988; Dollar *et al*., 1991; Lomstein *et al*., 1998; Tyler *et al*., 2001). However "DON" is a collective term for a very large variety of compounds ranging widely in molecular weight and bioavailability. By lumping together all organic N containing compounds, a great deal of information may be lost (Boynton *et al*., 1980; Burdige and Zheng, 1998).

Bioavailable compounds, such as amino acids and urea may make up significant portion of the DON pool, and contribute to the benthic flux of DON (e.g. Boucher and Boucher-Rodoni, 1988; Lomstein *et al*., 1989; Burdige and Zheng, 1998). In addition, these small, labile organic compounds may represent an important source of N for both heterotrophic and autotrophic microorganisms, as well as for benthic plants (e.g. Jorgensen, 1982; Admiraal *et al*., 1984; Jorgensen, 1984; Flynn and Butler, 1986; Lomstein *et al*., 1989; Palenik and Morel, 1990a; Tupas and Koike, 1990; Keil and Kirchman, 1993; Cho *et al*., 1996; Nilsson and Sundback, 1996; Rondell *et al*., 2000). Under conditions of low nutrient availability, organic nitrogen may contribute more to primary production than previously recognized (VanBreeman, 2002). In the present study we sought to partition bulk water column DON and sediment-water column fluxes of DON into specific component compounds (bulk DON, dissolved free and combined amino acids [DFAA, DCAA], urea).

The dominant benthic primary producers in shallow systems are seagrasses, macroalgae and benthic microalgae (Sand-Jensen and Borum, 1991). As human inputs of N to shallow coastal systems increases, there is often a shift in the dominant group of producers, from seagrasses to macroalgae, and perhaps eventually to phytoplankton where loading is high enough and residence time sufficiently long (Valiela *et al*., 1997). In the unvegetated sediments of shallow waters, benthic microalgae are often important components of the microbial mats at the sediment surface. Both microalgae and macroalgae are capable of rapid nutrient uptake, particularly in comparison to seagrasses (Duarte, 1995), so that their presence at the sediment surface is likely important in determining the movement of dissolved nutrients across the sediment – water interface (Valiela *et al*., 1997). While the influence of both micro- and macroalgae on benthic-pelagic coupling has been well studied (e.g. Sundback and Graneli, 1988; Rizzo, 1990; Sundback *et al*., 1990; Lavery and McComb, 1991a; Sundback *et al*., 1991; Sfriso *et al*., 1992; D'Avanzo and Kremer, 1994; Rysgaard *et al*., 1995; Krause-Jensen *et al*., 1996; Rysgaard *et al*., 1996; McGlathery *et al*., 1997; Krause-Jensen *et al*., 1999; Sundback *et al*., 2000), the effect of primary producers on dissolved organic nitrogen fluxes is much less understood. By investigating sediment fluxes at four very different sites across Hog Island Bay, in the light and dark, and in the presence and absence of macroalgae, we hope to show the importance of different N compounds in the N nutrition of primary producers, and the overall impact of the primary producers on benthic processing of inorganic and organic nitrogen.

Methods

Site description

Hog Island Bay, located within the Virginia Coast Reserve LTER site, is a typical back-barrier lagoonal estuary extending westward from the Delmarva Peninsula, VA. The primary deep channel, Great Machipongo, runs from the town of Willis Wharf on the Peninsula, southeast across the bay, and meets the Atlantic Ocean at the southern end of Hog Island. The semidiurnal tidal range is approximately 1.5 m at the mainland and 1.2 m at Hog Island (Santos, 1996). Of the total benthic surface area of the lagoon (15,085 ha), 37% is intertidal marshes and flats and 46% is less than 2 m deep at mean low water (Oertel, 2001). The lagoon has an overall hydraulic turnover time of <2 tidal cycles; however, water residence times of individual water parcels are spatially highly variable (Fugate *et al*., 2002). The small, agricultural watershed is drained by several small creeks, but there is no major riverine input. These creeks may contain high concentrations of dissolved N resulting from overland flow following rain events, but the greatest sources of N to the system are likely nutrient enriched groundwater (Lee and Olsen, 1985; Reay *et al*., 1992; Neikirk, 1996) and atmospheric deposition (Paerl *et al*., 1990; Paerl and Fogel, 1994). There is a gradient of nutrient inputs and sediment organic matter across Hog Island Bay from the mainland to the islands, with the highest concentrations of dissolved N and sediment organic matter found closest to the mainland (McGlathery *et al*. 2001; Tyler *et al*. 2001; Anderson *et al*. in press).

Seagrasses have been locally extinct since the 1930s, so that macroalgae, microalgae and phytoplankton are the dominant primary producers. The dominance of each of these functional groups of primary producers varies across the lagoon and shifts throughout the year (McGlathery *et al*. 2001). Macroalgal biomass, which is dominated by *Gracilaria tikvahiae, Bryopsis plumosa* and *Ulva lactuca,* peaks in July. Following the decline of the macroalgae and the associated release of N to the water column, phytoplankton may exhibit a peak in productivity (McGlathery *et al*. 2001). Benthic microalgal productivity ranges from 4-99% of total benthic productivity, with highest rates in the late summer (McGlathery *et al*. 2001).

Four shallow (<1 m at MLW) subtidal sites that represent the range of environmental conditions within Hog Island Bay were selected. Closest to the mainland, the Willis Wharf ("WW") site was located near the head of Parting Creek, a small tributary of Machipongo Channel. Historically, shellfish processing plants were located here and more recently, aquaculture facilities discharge water into the creek. The "Creek" site was located on the margin of a small secondary tidal creek (approx 5 m across) flowing through well-developed *Spartina alterniflora* marsh. Macroalgal biomass was generally low at both of these sites (<10 g dw m^{-2}, McGlathery et al. 2001; Table 3-1), and often partially buried in the fine-grained, muddy sediments at Creek. Green-gold mats of microalgae often coat the surface at both sites; however they were not as common during this experiment at Creek as in previous years (pers. obs.). In the mid-lagoon, a third site ("Shoal") was established in close proximity to a series of relict oyster reefs. The remaining oyster shells provide an attachment site for many species of

macroalgae and the reef itself serves as a barrier, trapping floating macroalgal mats. All sampling took place in the fine-grained sandy sediments just to the east of the reefs. Macroalgal biomass at Shoal is characteristically an order of magnitude higher than at the other sites, with patchy mats >650 g m^{-2} (McGlathery et al. 2001; Table 3-1). Finally, at the eastern edge of the lagoon, a back-barrier site was chosen at the southern end of Hog Island ("Hog"). This site, characterized by coarse-grained sands and low organic content sediments, has biomass of the same order of magnitude as Creek but microalgal chl *a* that is often 2x higher than elsewhere (McGlathery et al. 2001; Table 3-1).

N Flux measurements

Sediment-water column fluxes of dissolved nitrogen were measured in small flux chambers (8 cm i.d., 12 cm sediment, 18 cm water column) in October 1998 and January, March, May, June and August 1999. In July of 1999, an additional experiment was conducted at Shoal only, in an attempt to capture the high fluxes previously observed following the crash of the macroalgal mats. The macroalgae did not exhibit the massive die-off as in previous years, however, and biomass declined more slowly (Table 3-1). Measurements from this month are included in figures, but were not included in statistical analyses. Sediment cores, water and *U. lactuca* were collected from each site by hand. After collection, the cores were carefully transported to the laboratory in Charlottesville, VA and held overnight in a Conviron® environmental growth chamber at ambient temperatures. Stoppers were removed from the cores overnight to allow gas exchange with the air.

At the initiation of the experiment, the over-lying water was siphoned from each core and carefully replaced with fresh water taken from each site. Experimental treatments (sediment only, sediment + algae, and water "blanks") were run in triplicate. *U. lactuca* biomass in the cores, equivalent to 50 - 85 g m^{-2}, approximated the mean monthly biomass in the lagoon (42.9 ± 82.1 g m^{-2}, McGlathery *et al*. unpub. data). Macroalgal thalli were rinsed in seawater prior to insertion in the cores to avoid release of DOM as a result of wounding. After refilling the cores, and addition of algae, a small magnetic stir bar, suspended from a flexible metal holster was inserted into each core and the core was capped with an acrylic top. All remaining air bubbles were released through a small hole in the top and a rubber stopper was inserted to seal the chamber. Cores were placed in random sequence into filled aquaria in the environmental chamber. The water column of each core was gently stirred (~ 60 rpm) throughout the experiment to prevent the build-up of concentration gradients at the sediment-water column interface. Fluxes were measured over a 12 hour period (6 hr light, 6 hr dark). Dissolved oxygen was measured and samples for ammonium (NH_4^+) and nitrate + nitrite ($NO_3^- + NO_2^-$) were collected at 3 hr intervals; samples for urea, amino acids and total dissolved nitrogen (TDN) were collected at 6 hr intervals. Dissolved oxygen was measured using an Orion Model 842 meter with a self stirring probe. All nutrient samples were immediately filtered (Gelman Supor, 0.45 μm) and frozen, with the exception of NH_4^+ and urea samples, which were analyzed within 3 hr of collection. Samples for amino acid analysis (20 ml) were filtered through mixed cellulose ester filters using gentle vacuum pressure (<5 cm Hg Fuhrman and Bell, 1985) and immediately frozen.

Nutrient Analyses

Ammonium was measured using the phenol-hypochlorite method (Solorzano, 1969). Nitrate + nitrite was measured using an Alpkem "Flow Solution" Autoanalyzer (Perstorp, 1992). Urea was measured using a modification of the methods described by Mulvenna & Savidge (1992) and Goeyens et al. (1998). TDN was measured as NO_3^- following alkaline persulfate digestion in pre-combusted sealed ampoules (modified from Koroleff, 1983), as discussed in Tyler et al. (2001).

DFAA concentrations were determined by pre-column derivatization with *o*-phthaldialdehyde, separation by HPLC using a two eluent gradient (eluent 1: 80% NaAc buffer, 19% HPLC grade methanol, 1% tetrahydrofuran; eluent 2: 80% HPLC grade methanol, 20% NaAc buffer; Gilson 231 Autosampler and 401 Dilutor; Dionex 4000 Gradient Pump; Alltech Guard Column and Adsorbosphere OPA HR Separator Column), and detection by fluorescence (St. John's Associates Fluorescence detector; Jones *et al*., 1981; Gorzelska and Galloway, 1990). All glassware was rinsed with deionized water, soaked overnight in a 15% HCl solution, rinsed with deionized water 3×, air dried, rinsed with HPLC grade acetone, rinsed 5× with nanopure water and shaken to remove excess water droplets. Total dissolved amino acids were measured after hydrolysis of 1 ml water samples in pre-ashed ampoules. One ml 12N HCl was added, the ampoule was sealed and heated to 100^{o}C for 24 hr (Pedersen *et al*., 1999). The ampoules were then opened and dried in a vacuum dessicator. Following re-dissolution in 2 ml nanopure water, samples were analyzed as described above. Dissolved combined amino acids

(DCAA) were calculated from the difference between total and free amino acids. Nanopure water blanks were run through the entire filtration, storage and analysis procedure for both DFAA and DCAA to ensure that contamination had not occurred. Amino acids measured were: aspartic acid, glutamic acid, asparagine, serine, glutamine, histidine, glycine, threonine, arginine, alanine, tyrosine, γ-amino butyric acid (GABA), methionine, phenylalanine, isoleucine and leucine. Standard abbreviations are used for all amino acids, except as noted. Proline is not detected using this method and due to co-elution with an unidentified compound, valine was not able to be satisfactorily resolved. ASN and GLN are converted to ASP and GLU, respectively, by the hydrolysis procedure and are reported together. Individual amino acid concentrations were changed to moles N L^{-1} and all results are expressed this way.

Flux calculations

Fluxes were estimated based on the change in water column concentration over time, as described in Tyler et al. (2001). Water blanks were used to correct sediment and sediment + algae treatments. Likewise, *U. lactuca* uptake and release was calculated by subtracting out the corresponding sediment flux from each site and dividing by the biomass of macroalgae in each core. Daily fluxes were calculated using the number of hours of light or dark on the day of the experiment. Annual sediment fluxes were calculated by multiplying each individual replicate by the number of days in the "season" that it represents. One randomly selected replicate from each "season" was chosen and these six estimates were summed, yielding an annual flux rate. This was repeated for the

2 remaining replicates, and the resulting 3 annual estimates were averaged to give a single annual flux rate and error estimate for each site. This method assumes that the variance across sampling times was equal, which may not be true. It does, however, enable a calculation of the potential variability in our annual estimates. For sites and times where the macroalgal biomass was less than that used in the experimental treatments, annual macroalgal uptake was calculated in a manner similar to that for the sediment flux; each seasonal estimate (as mmol g dw^{-1} $time^{-1}$) was multiplied by the corresponding local biomass (g dw m^{-2}; Table 3-1) and by the number of days in that season, and summed. For cases where the field biomass was greater than that used in the cores (Shoal site, Jan 99 through Aug 99), we also performed an additional calculation because even at the low biomass used in these experiments, all sediment-derived N was consumed. In a dense mat, only macroalgae at the bottom will have access to sediment N; algae at the top of the mat will likely derive N primarily from the water column. Thus, multiplying our measured uptake (mostly sediment-derived N) by total biomass may overestimate available N. Therefore, we calculated water column N use as the difference between total uptake and the sediment flux measured in sediment only cores (total uptake – sediment flux = water column N uptake). The uptake of water column N was multiplied by the standing algal biomass and this value then added to the areal sediment flux to obtain the total areal uptake of N. Our closed experimental system may underestimate the water column N availability found in the field where tides continually bring in new nutrients. However, these adjusted calculations likely provide a more accurate estimate of the net macroalgal impact. This correction was made only for

components where the net uptake in macroalgae + sediment cores was greater in magnitude than the net sediment efflux in sediment only cores (NO_3^-, NH_4^+ and urea).

Elemental analysis

The macroalgae from each sediment + algae core was removed following the experiment, rinsed briefly with deionized water to remove salts and sediment, patted dry and frozen. Samples were later lyophilized and ground to homogeneity with a coffee mill. C and N content was measured using a Carlo Erba NA 2500 Elemental Analyzer.

Data Analysis

The influence of macroalgae on daily sediment fluxes was analyzed across all sites and dates using a one-way ANOVA. Light-dark differences in hourly flux rates and hourly uptake rates were similarly analyzed. Differences between sites and dates were analyzed using a two-way ANOVA (3 sites x 6 dates), and significant differences between sites or dates were distinguished using Tukey's HSD post-hoc test. Pearson correlation analysis was used to identify significant relationships between sediment flux rates and water temperature, macroalgal biomass, benthic chlorophyll *a* and sediment N and C:N content (Table 3-1). Relationships between algal uptake rates and water temperature and algal tissue N were also analyzed using this method.

Results

Site Characteristics

The water column concentrations of dissolved N, listed in Table 3-2, clearly show the pattern of decreasing N across the lagoon. Highest concentrations were observed at WW and lowest at Hog, although the only significant difference between sites was between WW and Hog (DON $F = 4.5$, $p = 0.014$; DCAA $F = 5.0$, $p = 0.012$). TDN was highest in late summer and lowest in winter and spring. Overall, DON was 55 – 97% of the N pool, and was proportionately greater at Hog and Shoal than at Creek and WW. DCAA were comparable to NH_4^+ and NO_3^-, with urea slightly lower and DFAA very low relative to the other components. The average mole % (as mol N) for individual DFAA are shown in Figure 3-1A. The most common DFAA (> 4 mole %) in the water column were SER (11%), HIS (20%), GLY (14%), ARG (13%), ASP (7%), GLU (6%) and ALA (4%). Because these percentages are based on concentrations of amino acid N, the relative importance of N rich amino acids, such as HIS and ARG, increases. The most abundant DCAA (Figure 3-1B) were GLY (26%), ALA (14%), HIS (12%), THR (9%), ASP (9%), SER (8%), GLU (7%) and ARG (5%). For the most part, *U. lactuca* tissue N reflected the gradient in water column nutrient concentrations; WW and Creek had the highest %N and Hog the lowest (Figure 3-2). There was a uniform decrease in %N at all sites during the spring, followed by an increase above 2% in June. From June to August, the N content continued to increase at all sites except Hog.

Sediment Fluxes

On an annual basis, sediments at Hog and WW were net autotrophic (3.4 and 4.3 mol m^{-2} y^{-1}, respectively), Creek was approximately neutral, and Shoal was net heterotrophic (-1.3 mol m^{-2} y^{-1}). WW and Hog sediments released O_2 during all seasons and Creek and Shoal consumed O_2 from March to June. Shoal continued to be net heterotrophic in July and August whereas Creek was net autotrophic in August. All sites were autotrophic in October (Table 3-3; Figure 3-3). Maximum net heterotrophy at the Shoal coincided with maximum macroalgal biomass.

Average daily TDN fluxes (all sampling periods weighted equally) varied significantly across Hog Island Bay (Table 3-3); Creek and Shoal produced a net efflux of TDN (220 and 276 μmol m^{-2} d^{-1}, respectively) and WW and Hog a net influx (-816 and -243 μmol m^{-2} d^{-1}, respectively; Table 3-3). Overall fluxes were highest during the summer months (Figure 3-4; Figure 3-5). Annual fluxes (sampling periods time-weighted) were similar: TDN efflux at Creek and Shoal (91 and 98 mmol m^{-2} y^{-1}, respectively) and influx at WW and Hog (-303 and -69 mmol m^{-2} y^{-1}, respectively; Figure 3-6). The individual components of the flux often behaved differently from the net TDN flux, with uptake and release of different compounds occurring simultaneously. On an annual basis, the NH_4^+-N released from the Creek sediments was 94% of the total efflux (153 mmol m^{-2} y^{-1}). Similarly, at the Shoal, NH_4^+ (65%) was also the primary component of the mean efflux (247 mmol m^{-2} y^{-1}), with urea (32%) and DCAA (2%) exhibiting temporal importance. There was net uptake of DON at these sites, with only a small

percentage of the compounds identified (4% urea, 7% DFAA, 11% DCAA at Creek; 1% DFAA at Shoal) and the remainder unknown. At WW and Hog, the sediment efflux of TDN was small relative to the influx (efflux = 4 and 34 mmol m^{-2} y^{-1}, respectively), and was made up of urea (100% at WW; 39% at Hog) and DCAA (61% at Hog). DIN dominated the TDN influx at these sites (NH_4^+ = 39%, NO_3^- = 12% at WW; NH_4^+ = 25, NO_3^- = 31% at Hog); unknown DON compounds were also important (49% and 43% at WW and Hog, respectively), but DFAA were not (<1%).

There were substantial seasonal differences at all sites, resulting in high variance of the mean daily fluxes (Figure 3-4; Figure 3-5; Table 3-3). The highest NH_4^+ effluxes were in summer at Creek and Shoal, while there was still a net influx of both NH_4^+ and NO_3^- at WW and Hog. Total DON fluxes were generally directed into the sediments during the warmer months at all sites except WW in June. Significant DON release occurred in October and January at Hog and in October at Shoal when the release was predominantly made up of urea. Overall, no significant seasonal trends in sediment urea uptake or release were observed and it was only a substantial component of the flux at Hog and Shoal. DFAA fluxes were generally small relative to the total DON flux and did not vary between sites. DFAA were generally directed into the sediments, with the exception of relatively large releases measured at Shoal and Hog in January. On average, all measured amino acids were taken up by the sediments except for ARG, TYR, GABA (Figure 3-1A). DCAA fluxes exhibited high variability across the lagoon, and differences between sites were seen only between WW and Hog; there was no interpretable pattern of DCAA release relative to season. Overall, only GABA and ILE

were taken up as DCAA by the sediments (mean DCAA uptake = 1.9 μmol m^{-2} d^{-1}) and all other amino acids were released (mean DCAA release = 27.3 μmol m^{-2} d^{-1}; Figure 3-1B).

Sediment NH_4^+ release and DFAA uptake were much greater in the dark (Table 3-4). Individual DFAA also showed distinct light-dark differences, with significantly greater uptake in the dark for ASP, GLU, SER, HIS, THR, ALA, MET, ILE, LEU. Correlations between sediment fluxes and the predictor variables (macroalgal biomass, benthic chl *a*, sediment N and C:N and temperature) varied among the measured flux components. The DO flux correlated strongly with the other predictor variables macroalgal biomass ($r = -0.35$, $p = 0.002$), chl *a* ($r = 0.31$, $p = 0.009$) and temperature ($r = 0.28$, $p = 0.023$) and was the most consistent predictor for DIN, total DON and TDN fluxes (NH_4^+ $r = -0.52$, $p = 0.000$; NO_3^- $r = -0.42$, $p = 0.000$; DON $r = -0.30$, $p = 0.013$; TDN $r = -0.58$, $p = 0.000$). The NH_4^+ flux was also strongly correlated with macroalgal biomass ($r = 0.47$, $p = 0.000$) and chl *a* ($r = -0.33$, $p = 0.005$). The release of urea and DON were both proportional to the C:N of the sediments (urea $r = 0.32$, $p = 0.014$; DON $r = 0.33$, $p = 0.014$).

Influence of Macroalgae on Sediment Fluxes

Where macroalgal biomass was high the annual benthic (sediments + macroalgae) TDN fluxes are dictated by macroalgal uptake and release of N (Figure 3-6). At WW, Creek and Hog, macroalgae have a small impact the net benthic TDN flux (additional uptake = 17, 19, and 8 mmol m^{-2} yr^{-1}, respectively) but at Shoal, net TDN fluxes

decreased by 112 - 619 mmol m^{-2} y^{-1} (depending on macroalgal uptake calculations) because of macroalgal uptake. On an annual basis, the benthos, including macroalgae, at Shoal imported DIN, urea and DFAA and exported DCAA. The same trend was seen in the daily measurements, as shown in Figure 3-7. In the presence of macroalgae, DIN and urea fluxes to the water column were prevented; benthic uptake of DFAA and release of DCAA were greater than in sediment only cores. Averaged over all sites and dates, the sediment + macroalgae treatment resulted in a >500 μmol m^{-2} d^{-1} change in the NH_4^+ flux and >100 μmol m^{-2} d^{-1} change in the urea flux (Table 3-5). The flux of NO_3^- and DFAA from the water column was 2-3 fold greater than the flux to the sediments alone (Table 3-5). All DFAA were taken up by *U. lactuca,* except HIS. Total benthic DON uptake was less in sediment + algae cores, but not significantly so due to high variability. However, the DCAA flux, which is insignificant in sediment only cores, increased to 172 μmol m^{-2} d^{-1} in cores with macroalgae.

Macroalgal uptake and release, corrected for the sediment fluxes, also varied between dark and light; uptake of NH_4^+ and NO_3^- were higher in the light, while uptake of urea and DFAA were higher in the dark (Table 3-4). The uptake of all individual amino acids was greater in the dark, but only significantly so for GLU, ASN, THR, ARG, TYR, GABA and PHE. DCAA were released only in the light, with all amino acids released; GLY (18%), GLU (14%), ALA (11%), HIS (11%), and SER (5%) were the most abundant (Figure 3-1). Mean macroalgal uptake and release rates are shown in Figure 3-8. The average daily macroalgal uptake (as DIN, urea and DFAA) at each site varied significantly across the lagoon, from 24.6 μmol g dw^{-1} d^{-1} at Creek, to 15.8 and

13.4 at WW and Shoal, and 3.2 at Hog. NH_4^+, NO_3^- and urea uptake were all correlated with the N content of algae, indicating that the uptake rate in the field has led to the greater accumulation of N in the macroalgal tissue (NH_4^+ r = -0.60, p = 0.000; NO_3^- r = -0.30, p = 0.044; urea r = -0.28, p = 0.018). The relative importance of DON increased as DIN availability decreased. At WW and Creek, DIN was the majority of the uptake (NH_4^+ = 80%; NO_3^- = 9%), with urea (10%) and DFAA (2%) contributing a smaller proportion. At Shoal, DIN was still dominant (NH_4^+ = 73%; NO_3^- = 7%), but urea became more important at 17%. At Hog, where water column DIN was lowest, NH_4^+ made up only 12%, NO_3^- was 29% and organic nitrogen contributed the remainder (urea = 39%; DFAA = 20%). There was a general trend of increasing DCAA release from the macroalgae as the N content of the algae decreased (r = -0.41, p = 0.013). Averaged across all sites, this release was equivalent to 22% of the total uptake of N by the macroalgae. NH_4^+ uptake was greater during the warmer months (r = -0.59, p = 0.000) and DFAA uptake was greater during the colder months (r = 0.35, p = 0.026).

Discussion

The pattern of nitrogen uptake and release by the benthos in Hog Island Bay indicates clearly that benthic processes were strongly influenced by the primary producers. Where sediments were net autotrophic (WW and Hog) the sediments were a TDN sink; where sediments were net heterotrophic (Creek and Shoal) the sediments were a TDN source. This suggests that microalgae were important in controlling the TDN flux

at WW and Hog. At Shoal, where macroalgal biomass was 1-2 orders of magnitude greater than elsewhere in the lagoon, the macroalgae shaded the sediment surface and caused the total benthos (sediments + macroalgae) to be a TDN sink. Thus, where benthic primary producers were important, the benthic community as a whole removed dissolved N from the water column. While the TDN flux gives an indication of the overall N balance, the individual components that make up this net flux are highly variable, both in space and time, and a closer examination reveals further differences between the sites.

DIN Fluxes

Like many other systems, we observed high between- and within-site variability in sediment DIN fluxes, with order of magnitude differences between sites and seasons (e.g. Fisher *et al*., 1982; Nowicki and Nixon, 1985; Reay *et al*., 1995; Berelson *et al*., 1998). The daily sediment NH_4^+ and NO_3^- flux values in Hog Island Bay were low (range = -1.2 – 2.0 mmol NH_4^+ m^{-2} d^{-1} and -0.4 – 0.4 mmol NO_3^- m^{-2} d^{-1}), but fell within the range observed in similar shallow estuaries (-8.1 – 15.6 mmol NH_4^+ m^{-2} d^{-1}, 0 – 0.1 mmol NO_3^- m^{-2} d^{-1}; Fisher *et al*., 1982; Nowicki and Nixon, 1985; Rizzo, 1990; Reay *et al*., 1995; Rysgaard *et al*., 1996; Tyler *et al*., 2001; Anderson *et al*., in press). Often greater DIN fluxes are found in muddy compared to sandy sediments (Fisher *et al*., 1982; Nowicki and Nixon, 1985; Sundback *et al*., 1991; Reay *et al*., 1995) and in sediments with higher organic content (Jensen *et al*., 1990; Enoksson, 1993; Caffrey, 1995; Sloth *et al*., 1995; Therkildsen *et al*., 1996). In contrast, in Hog Island Bay, the relationship

between sediment type or organic content and DIN fluxes was less clear, probably due to the strong influence of the primary producers.

Consistent with other studies, light DIN fluxes were significantly lower than dark fluxes at all 4 sites due to benthic microalgal activity (Nowicki and Nixon, 1985; Sundback and Graneli, 1988; Rizzo, 1990; Sundback *et al*., 1991; Reay *et al*., 1995). Microalgal control of DIN fluxes is further demonstrated by the negative correlation between NH_4^+ and NO_3^- fluxes and both DO production and benthic chl *a* (NH_4^+ only). Where DO production was consistently high (Hog and WW) both NH_4^+ and NO_3^- were removed from the water column. Net heterotrophy at Creek and Shoal suggests a less active microalgal community at these sites, but the daytime NH_4^+ fluxes were still reduced by 50% and 23%, respectively, over the nighttime fluxes. The light-dark differences in DIN efflux that we observed were equal to and higher than those measured in a shallow Rhode Island lagoon (25% reduction, Nowicki and Nixon, 1985). It is likely that while microalgae take up NO_3^- directly from the water column, NH_4^+ fluxes are prevented not because of direct uptake by the microalgae, but rather because of the redox "filter" created by photosynthetic O_2 production (Sundback and Graneli, 1988).

Nitrogen mineralization at Creek and Shoal may have been high enough to swamp both microalgal demand and the redox "filter" effect, and thus resulted in an efflux of NH_4^+ from the sediments. The consistent release of mineralized N by Creek sediments from March through August suggests a constant supply of OM. The potential sources of this organic material include: small amounts of macroalgae buried in the mud; POM from nearby *Spartina* marshes; and possibly seepage of nutrient- and organic

matter-enriched groundwater entering through the creekbank. At Shoal, the correlation between macroalgal biomass and NH_4^+ fluxes indicates that decomposing macroalgae are the source of organic matter. High summertime flux rates are common in temperate estuaries (e.g. Boynton *et al*., 1980; Callender and Hammond, 1982; Fisher *et al*., 1982; Rizzo, 1990) and are associated with high phytoplankton production in the overlying water column in deeper estuaries (Kelly and Nixon, 1984; Kelly *et al*., 1985; Nixon, 1981). During the rest of the year at Creek and Shoal, and at all times of the year at WW and Hog, DIN fluxes were low. This is consistent with previous work in Hog Island Bay (Anderson *et al*., in press) which demonstrated that DIN fluxes from the sediments were consistently negligible because bacterial immobilization and microalgal uptake were capable of removing all mineralized N, in spite of high mineralization rates in the sediments. In the previous study, because sediment fluxes were low, phytoplankton in the water column had a greater effect on water column nutrients than the benthos (Anderson *et al*., in press). We may attribute the difference between the high fluxes observed at Creek and Shoal during the summer of 1999 (sediments heterotrophic) and the low fluxes of 1998 (sediments autotrophic McGlathery *et al*., 2001) to high interannual variability associated with a decline in microalgal biomass at Creek and increased macroalgal biomass at Shoal (McGlathery *et al*., in prep.). In addition to supplying organic matter for mineralization within the sediments, macroalgal mats can decrease light availability at the sediment surface (>90%, Krause-Jensen *et al*., 1996; Astill and Lavery, 2001). This shading may inhibit microalgal growth and explain the

inverse relationship between macroalgal biomass and both sediment chl *a* and sediment DO production.

Macroalgae appear to have been transported to the mid-lagoon shoals by wind and tides and remained entrained behind the oyster reefs until biomass declined in mid- to late summer. Decomposition of algal tissue at the bottom of macroalgal mats leads to high nutrient concentrations within the mat (Sundback *et al*., 1990; Lavery and McComb, 1991a; Bierzychudek *et al*., 1993; Thybo-Christesen and Blackburn, 1993; McGlathery *et al*., 1997; Trimmer *et al*., 2000; Astill and Lavery, 2001). The sustained efflux of NH_4^+ from sediments beneath macroalgal mats indicates that there was also some incorporation of labile N into the sediments and that this N may be released for some time following senescence. In addition, the redox status at the sediment surface influences both DIN and DON fluxes (Kristensen and Blackburn, 1987; Hansen and Blackburn, 1991; Miller-Way *et al*., 1994); macroalgal oxygen production and consumption may thus exert a significant indirect effect on nutrient cycling (Valiela *et al*., 1997). Further, if sediments beneath the mats were anoxic during the summer, nitrification may thereby be prevented and the NH_4^+ flux would increase. At Shoal, macroalgae were capable of intercepting all of the DIN (and urea) released by the sediments and little sediment-derived N was likely to reach the water column. Macroalgae may thereby uncouple sediment-water column interactions and outcompete phytoplankton for sediment-derived N (Valiela *et al*., 1997).

DON Fluxes

The range of daily DON fluxes in Hog Island Bay was large (-1.6 – 1.7 mmol m^{-2} d^{-1}) and without a consistent pattern based on site or date. However, extrapolated annually, we found a net uptake of DON by the sediments at all sites (Figure 3-6). This is consistent with past studies of sediment DON fluxes in moderately shallow estuaries (range = -17.0 – -0.1 mmol m^{-2} d^{-1}; Boynton *et al*., 1980; Teague *et al*., 1988; Dollar *et al*., 1991). However, others have found DON fluxes directed consistently out of the sediments (range = 0.2 – 107 mmol m^{-2} d^{-1}; Hopkinson, 1987; Teague *et al*., 1988; Lomstein *et al*., 1998). The same variability has been found in deeper estuaries, where in some cases DON is an important component of the TDN efflux (Lomstein *et al*., 1989; Enoksson, 1993; Blackburn *et al*., 1996; Cowan and Boynton, 1996) and in others it is small or insignificant (Nixon, 1981; Burdige and Zheng, 1998). On an annual basis, the total DON flux was approximately the same order of magnitude as the DIN flux, but proceeded in the same direction (influx of both) at WW and Hog and in opposite directions (influx of DON only) at Creek and Shoal. The greatest sediment uptake, at WW, cannot be explained by the influx of any of the individual compounds that we measured in this study. However, the water column DON at this site was higher than elsewhere in the lagoon, and it is possible that it consisted of other, equally labile compounds.

The lack of a net flux of DON from the sediments during most seasons suggests that porewater DON was either mineralized within the sediments, taken up by sediment microorganisms, or buried. This is consistent with Anderson *et al*. (in press) who

suggested that rapid mineralization and immobilization in the sediments of Hog Island Bay prevented the efflux of N to the water column. The correlation between O_2 efflux and DON influx indicates uptake by the benthic microalgae, consistent with Rondell et al. (2000) who showed that microbial mat communities can use small DON compounds as their primary N source. At Shoal, the maximum uptake of DON occurred simultaneously with maximum DIN efflux, O_2 uptake, and macroalgal biomass, suggesting that the heterotrophic community in the sediment was highly active at that point and capable of quickly utilizing all sediment and water column derived DON. This is consistent with Trimmer et al. (2000) who showed enhanced rates of mineralization beneath macroalgal mats. In a previous study, we observed huge releases of DON and DIN following the crash of the macroalgal bloom (Tyler *et al*., 2001). There was not an episodic crash during the summer of 1999; rather, it appears that macroalgal N was more slowly released to the sediments and water column during late summer senescence, where it was mineralized to DIN or urea, or denitrified.

Even though the mean DON flux at all sites was negative, the high C:N of sediment OM at Hog and Shoal in the fall coincided with a contrasting net DON efflux. This relationship has been observed previously (Blackburn *et al*., 1996; Lomstein *et al*., 1998). Lomstein *et al*. (1998) suggested that when sediment organic C:N is high, mineralized N is rapidly immobilized by sediment bacteria resulting in low DIN fluxes. Anderson *et al*. (in press) observed high rates of gross mineralization, but low rates of net mineralization at these same sites in 1998. However, hydrolysis of detritus at the sediment surface may still lead to a positive flux of DON. The high flux at Shoal in

October was comprised of urea and DCAA (65% of total flux; Figure 3-4; Figure 3-5) and overall urea was a more important here than at the other sites. This is consistent with Burdige and Zheng (1998), who suggested that DOM effluxes from (deep) Chesapeake Bay sediments may consist of small, labile compounds such as amino acids and urea.

In a previous study, we documented a large DON release by living macroalgae; in the presence of macroalgae, benthic DON release was >250% higher (Tyler et al. 2001). In the current study, the variability in total DON fluxes was sufficiently high that there was not a significant effect of the macroalgae on total DON. However, the high DCAA release in sediment + algae cores in this study corroborates our previous work. In general, DON fluxes are quite difficult to interpret because a single value represents the net flux of hundreds of compounds. At best we have identified 10-40 % of the DON pool as urea and amino acids. While this leaves the bulk of the pool to be identified, a closer examination of the individual compounds may provide more information.

DON Fluxes: Specific Compounds

The range of urea fluxes measured in this study (-0.2 – 1.3 mmol m^{-2} d^{-1}; mean = 0.05 mmol m^{-2} d^{-1}) was similar to values previously observed in Hog Island Bay (mean = 0.07 mmol m^{-2} d^{-1}, Tyler *et al*. 2001). These values were also comparable with the few other studies of urea fluxes that exist for both shallow (2.1 mmol m^{-2} d^{-1}; Boucher and Boucher-Rodoni, 1988) and deeper systems (range = 0.01 – 0.7 mmol m^{-2} d^{-1}; Lomstein *et al*., 1989; Blackburn *et al*., 1996; Rysgaard *et al*., 1998). In each of the cases cited above, and at Shoal in Hog Island Bay, urea made up a substantial fraction of the TDN

flux (33% of TDN at Shoal). Microalgae can survive with only urea as an N source (Rondell *et al*., 2000) and in Hog Island Bay microalgal uptake may intercept the flux to the water column. The DO flux was lower during the colder months so that the higher flux rates of urea during the months of October, January or March may be related to lower microalgal uptake. However, there was often a great deal of variability associated with a positive urea flux among individual cores (Figure 3-5). Lomstein *et al*. (1989) found high rates of urea production associated with benthic infaunal activity and while we did not specifically look at infaunal densities, the irregular high rates that we observed may be due to the occasional presence of bioturbating infauna. Our results contrast with Boucher and Boucher-Rodoni (1988) who saw high urea fluxes in the summertime. Macroalgae further decrease the release of urea (and DIN) to the water column.

Even though DFAA were only a small proportion of water column TDN in Hog Island Bay, highly labile amino acids have such rapid turnover that low concentrations may not indicate relative importance (e.g. Hagstrom *et al*., 1984). Sediment fluxes of DFAA were also low (range -112 – 139 μmol m^{-2} d^{-1}; mean = -18 μmol m^{-2} d^{-1}), and were a very small percentage of the total influx of DON to the sediments (0-5%). Again, however, rapid consumption in the sediments or water column may mask fluxes at the time scale of these experiments (6 hr). Our rates were much lower than in the shallow Kysing Fjord, Denmark (1300 μmol m^{-2} d^{-1}; Jorgensen, 1982) or somewhat deeper Cape Lookout Bight, North Carolina, USA (52 - 257 μmol m^{-2} d^{-1}; Burdige and Martens, 1990). However, relative to Hog Island Bay, the organic content of the sediments in

Cape Lookout Bight was much higher (3-5% organic C, 0.5%N; Burdige and Martens, 1988), as were the water column nutrients in Kysing Fjord (Jorgensen, 1982) which may contribute to the higher DFAA flux rates. Our measurements may also be somewhat lower because we did not measure lysine, valine, proline or the non-protein amino acids β-aminoglutaric acid, ornithine or taurine, some of which may be important in sediment porewater or fluxes (e.g. Henrichs and Farrington, 1979; Jorgensen, 1982; Burdige and Martens, 1990). Moreover, while anoxic conditions may prevent DFAA consumption within the sediments and foster an efflux (Henrichs *et al*., 1984), the sediments in Hog Island Bay appeared oxic, for the most part (except beneath macroalgal mats at Shoal and at Creek in the summer). Thus, we may anticipate the observed influx and indeed, the greatest DFAA consumption occurred where O_2 production was always positive and the sediment surface thereby oxidized (WW).

The lack of DFAA efflux suggests that all AA produced as intermediates in the breakdown of organic matter were rapidly mineralized to NH_4^+ (Lomstein *et al*., 1998) or were otherwise consumed by sediment bacteria (Stanley *et al*., 1987) or microalgae, which are capable of both light and dark DFAA uptake (e.g. Jorgensen, 1982; Admiraal *et al*., 1984; Jorgensen, 1984; Flynn and Butler, 1986; Nilsson and Sundback, 1996). Dark uptake of DFAA may provide a competitive advantage to buried microalgae (Nilsson and Sundback, 1996) and DFAA uptake by phytoplankton is likely to occur in the dark, particularly under N limiting conditions (Flynn and Butler, 1986). Consistent with this prediction, we observed a significantly greater dark influx of both DFAA. Cyanobacteria, diatoms and autotrophic flagellates are all capable of some DFAA

utilization (Nilsson and Sundback, 1996) and Admiraal *et al*. (1984) found that diatoms were capable of taking up all amino acids tested, with more rapid uptake at low DIN. The uptake of HIS, GLY and ALA was much greater than the relative concentration of these three amino acids in the water column, suggesting some preferential uptake. HIS, which has the highest uptake based on the mole % of N, contains 4 N atoms, making it a valuable N source, even at low concentrations. GLY and ALA are aliphatic neutral amino acids, with small side chains, possibly making them easier to assimilate than some of the larger amino acids. DFAA were also temporally important in macroalgal N nutrition.

DCAA fluxes (-375 - 426 μmol m^{-2} d^{-1}) were approximately 3-fold greater than the DFAA fluxes, but were far more erratic and on an annual basis insignificant at all sites except Hog (Figure 3-5, Figure 3-6). Total, hydrolyzable AA may make up a high percentage of sediment porewater TDN in some cases, (20-70% Henrichs *et al*., 1984; 30-40% Burdige and Martens, 1988), but it does not seem that these AA were released to the water column with any regularity in Hog Island Bay. Overall, sediment DCAA fluxes were not correlated with any other fluxes or predictor variables, and do not appear to be influenced by microalgae or by the input of macroalgal OM at the Shoal. In the presence of macroalgae, however, mean benthic fluxes of DCAA increased nearly 8-fold. The higher release in the light suggests that this release is a photosynthetically driven process, although Harlin and Craigie (1975) found no difference in light-dark DOC release rates for a brown macroalgae.

Phytoplankton may release 25 - 41% of DIN uptake as DON on short time scales (Bronk *et al*., 1994) and much of this release may be DFAA and DCAA (Flynn and Berry, 1999). Jorgensen (1982) found increased water column DFAA in the presence of *U. lactuca*, but based on the DFAA composition concluded that the macroalga was only indirectly responsible and that the DFAA were exudates from bacteria stimulated by algal DOM release. The algal DOM release may have been DCAA, which were not measured. In the current study, macroalgal DCAA release was 22% of the total N uptake, indicating a substantial loss of N to the water column. While in some cases, the DCAA in estuarine waters may not be available for bacterial utilization (Keil and Kirchman, 1993), others have suggested that DCAA are an important substrate for bacterial growth (Hollibaugh and Azam, 1983; Hagstrom *et al*., 1984; Tupas and Koike, 1990). If the released DCAA are bioavailable, these exudates will fuel heterotrophic activity in the waters surrounding a macroalgal mat (Brylinsky, 1977; Johnsen and Lein, 1989; Valiela *et al*., 1997), potentially increasing the oxygen demand. This rapid release of N suggests that macroalgal N turns over at two different rates following uptake: a rapid release as DCAA (and other compounds) and a slower release during senescence. The rapid uptake and release indicates that actual uptake is greatly underestimated if based solely on tissue N.

Macroalgal uptake of dissolved N

The N uptake rates reported here (range for NH_4^+ = 0 – 5 μmol g dw^{-1} h^{-1}) are much lower than the maximum uptake rates for an opportunistic green macroalga such as

U. lactuca (e.g. V_{max} for NH_4^+ = 138 μmol g dw^{-1} h^{-1}; Fujita, 1985), but probably represent true field uptake. Macroalgal N demand was met by several different forms of dissolved N, with DON playing an increasingly important role as DIN availability decreased. When DIN (generally as NH_4^+) was readily available, it constituted the majority of uptake. Nonetheless, urea made up only 12% of the mean uptake, but overall was more important than NO_3^- (9%) and was seasonally more important than either NH_4^+ or NO_3^-. Likewise, DFAA uptake was also generally very low (3% of total), but also temporally important. Urea (Bronk and Glibert, 1993; Cho *et al*., 1996) and DFAA (Lewitus and Koepfler, 1997) may be temporally important N sources for phytoplankton as well.

At the low-nutrient Hog site, DFAA and urea were nearly 90% of the total annual uptake of known compounds (-3.6 mmol m^{-2} y^{-1} as DIN, urea and DFAA). If we include uptake of "unknown" DON compounds (-8.4 mmol m^{-2} y^{-1}), it is evident that DON provides nearly all of the N demand. Recently, the importance of DON in nutrient poor ecosystems has received greater attention, and it has been suggested that plants growing in these depauperate environments may be better adapted to use DON, rather than DIN (VanBreeman, 2002). If this is true, then the Hog macroalgae may be better acclimated to, or have induced uptake mechanisms for, DON uptake.

U. lactuca appeared to assimilate all measured DFAA (except HIS), but GLY and SER, in particular, were selectively taken up relative to their water column concentrations (Figure 3-1). The higher uptake rates of DFAA and urea in the dark are difficult to explain, particularly because water column release is not greater (data not

shown). However, as proposed for microalgae, dark uptake may provide a competitive advantage during N limitation or turbidity events. Heterotrophic uptake of glucose and acetate has been shown for *U. lactuca* (Markager and Sand-Jensen, 1990) and may also occur with urea or DFAA.

In semi-shallow, phytoplankton-dominated estuaries, sediments may contribute 28-35% of the N to support new primary production (Fisher *et al*., 1982). In this study, the efflux of DIN and urea is sufficient to meet 27 - 75% of the macroalgal uptake (depending on calculation method). Some additional N is likely supplied by recycling within the macroalgal mat (Lavery and McComb, 1991a; Thybo-Christesen and Blackburn, 1993; McGlathery *et al*., 1997; Trimmer *et al*., 2000). The high tissue %N of *U. lactuca* at Creek and WW indicates high N availability (Bjornsater and Wheeler, 1990; Fong *et al*., 1994a; Horrocks *et al*., 1995). Hog and Shoal had virtually identical tissue N content between January and June, when both water column and sediment fluxes were low. In August and October, however, sediment N effluxes and macroalgal decomposition at Shoal likely led to increased tissue N at this site. The strong correlation between NH_4^+, NO_3^- and urea uptake with %N was likely due to this seasonal release from the sediments.

Conclusions

While DIN, primarily as NH_4^+, was the dominant and most predictable component of the sediment – water column N flux, DON was also important to TDN standing stocks and fluxes. DIN fluxes were greatest in the summertime, providing a

large proportion of the benthic algal N demand. When DIN standing stocks and fluxes were low, both urea and DFAA were temporally important as an N source for autotrophs. Urea fluxes, in particular, often followed a different trend than bulk DON fluxes. The information gained by investigating the individual DON compounds underscores the importance of measuring more than just bulk DON.

We have shown that the relative role of the benthos can vary dramatically, even over short distances within a small estuary. This variability complicates intercomparisons, even between similar types of estuaries. Where macroalgal biomass is high, dense mats control benthic-pelagic coupling by intercepting sediment release of available N, by removing N from the water column, and by subsequently re-releasing DON and DIN to the water column. This release occurs over short and long time scales, with rapid release of DCAA and other compounds by living macroalgae, and by the release of DON, DIN (and PON) by senescing macroalgae at the end of the growing season. The release of bioavailable DON has clear implications for water column heterotrophic activity. However macroalgal biomass is often patchy and the effects of high biomass thus localized. Where macroalgal biomass is low, and microalgal productivity creates net autotrophy within the sediments, the benthos may be a significant net sink for TDN. Both micro- and macroalgae can influence coupled nitrification-denitrification (Henriksen and Kemp, 1988; Lavery and McComb, 1991a; Rysgaard *et al*., 1996; Krause-Jensen *et al*., 1999) and may thereby dictate N_2 removal from the system. Overall, in a shallow estuary such as Hog Island Bay, the primary producers

clearly control N cycling and are important in determining the retention and removal of N passing from the land through the lagoon and out to the coastal ocean.

Figures & Tables for Chapter 3:
Influence of algae on N fluxes: compound specific approach

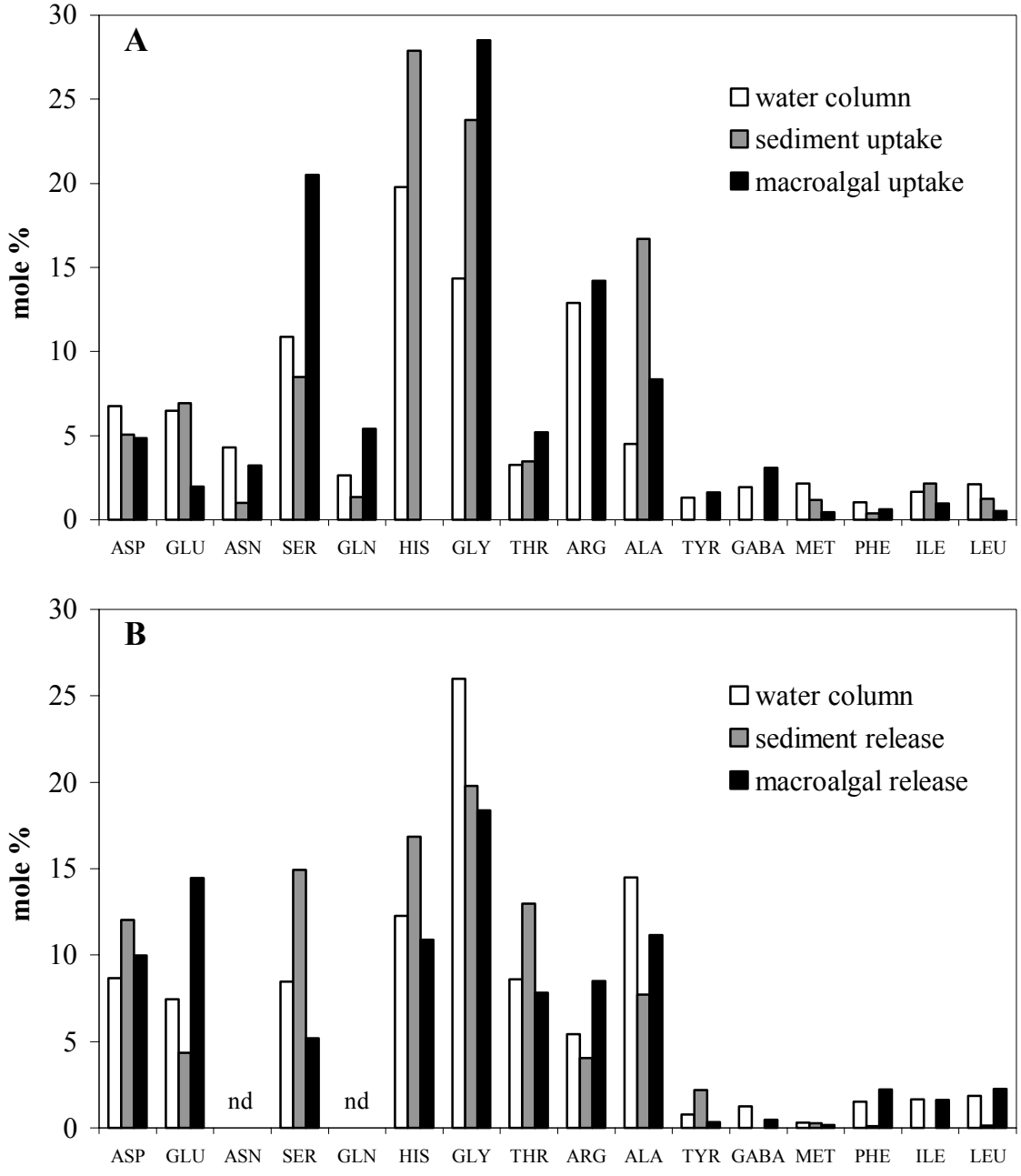

Figure 3-1 Mole % N for water column standing stock, sediment uptake and release and macroalgal uptake and release for (A) DFAA and (B) DCAA. A zero value indicates that no release or uptake of this amino acid was measured except where noted as "nd"; these amino acids are not recovered after hydrolysis as described in the methods.

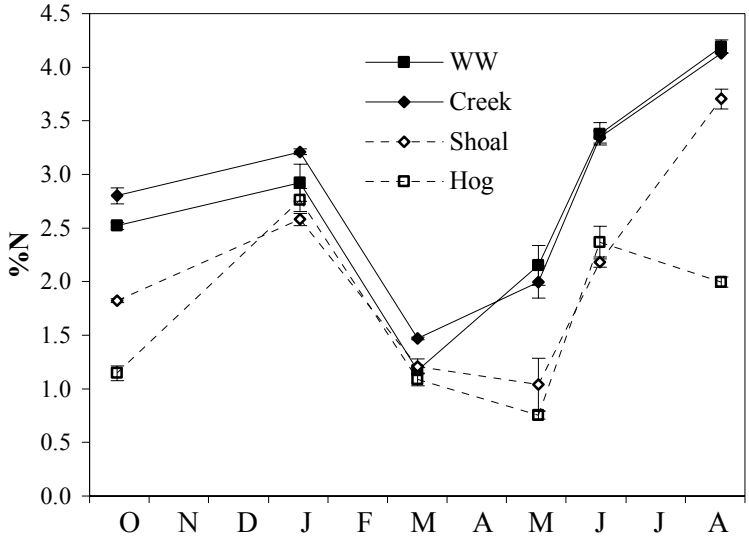

Figure 3-2 Tissue N content of *U. lactuca* used in experiments. Error bars represent the standard error of the mean.

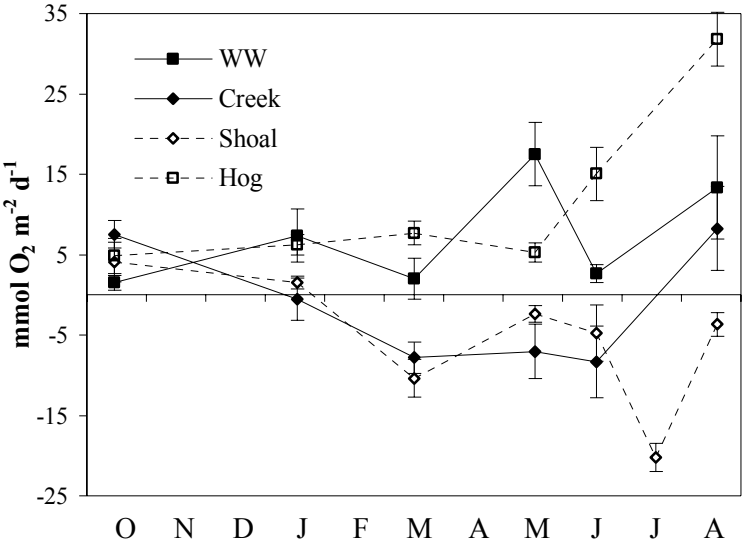

Figure 3-3 Daily fluxes of dissolved oxygen (mean and standard error) from the sediments. A positive value indicates a flux out of the sediments; a negative value indicates a flux into the sediments.

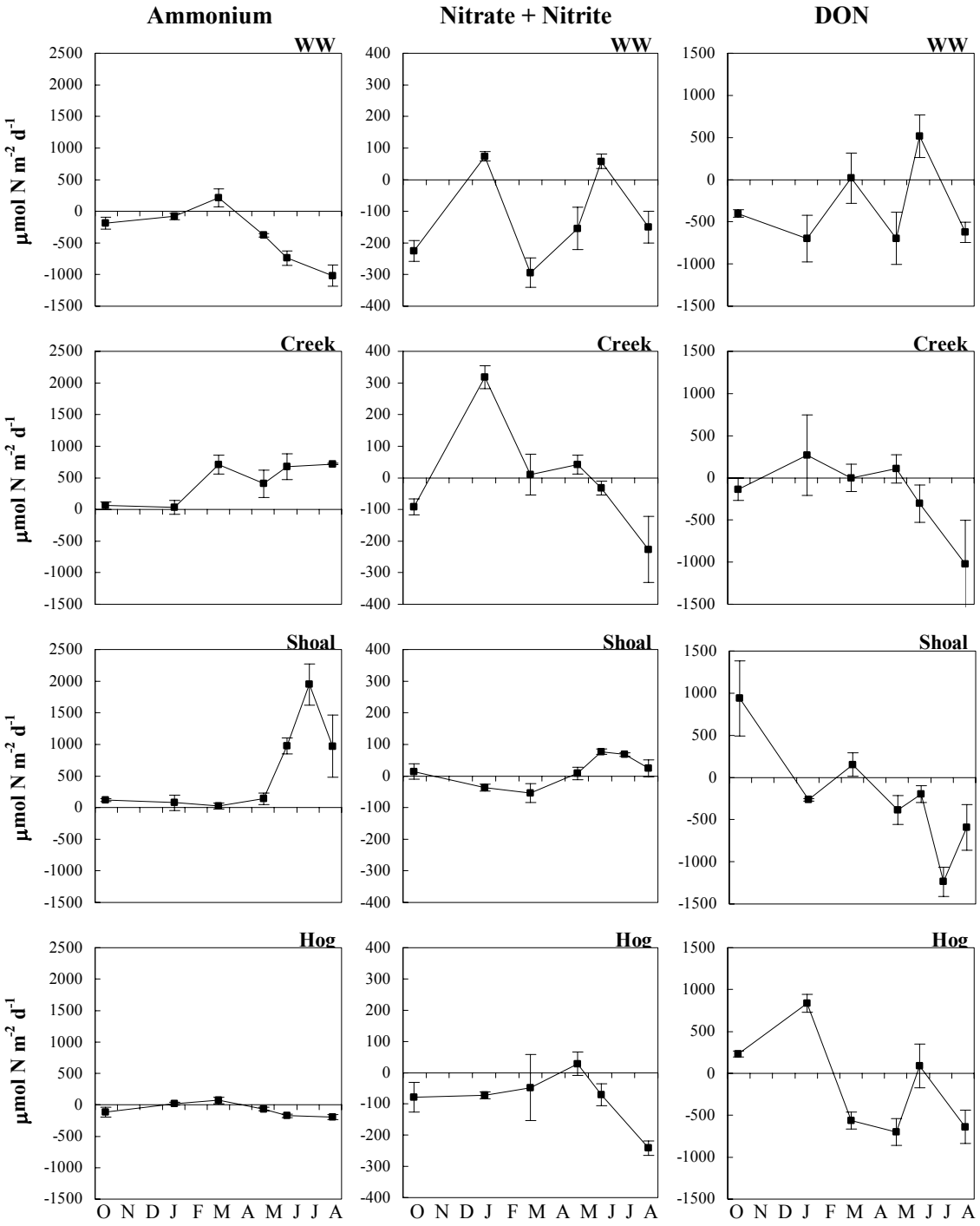

Figure 3-4 Daily sediment fluxes (mean and standard error) of ammonium, nitrate + nitrite and DON across Hog Island Bay. Positive values indicate a flux from the benthos to the water column; negative values indicate a flux from the water column to the benthos.

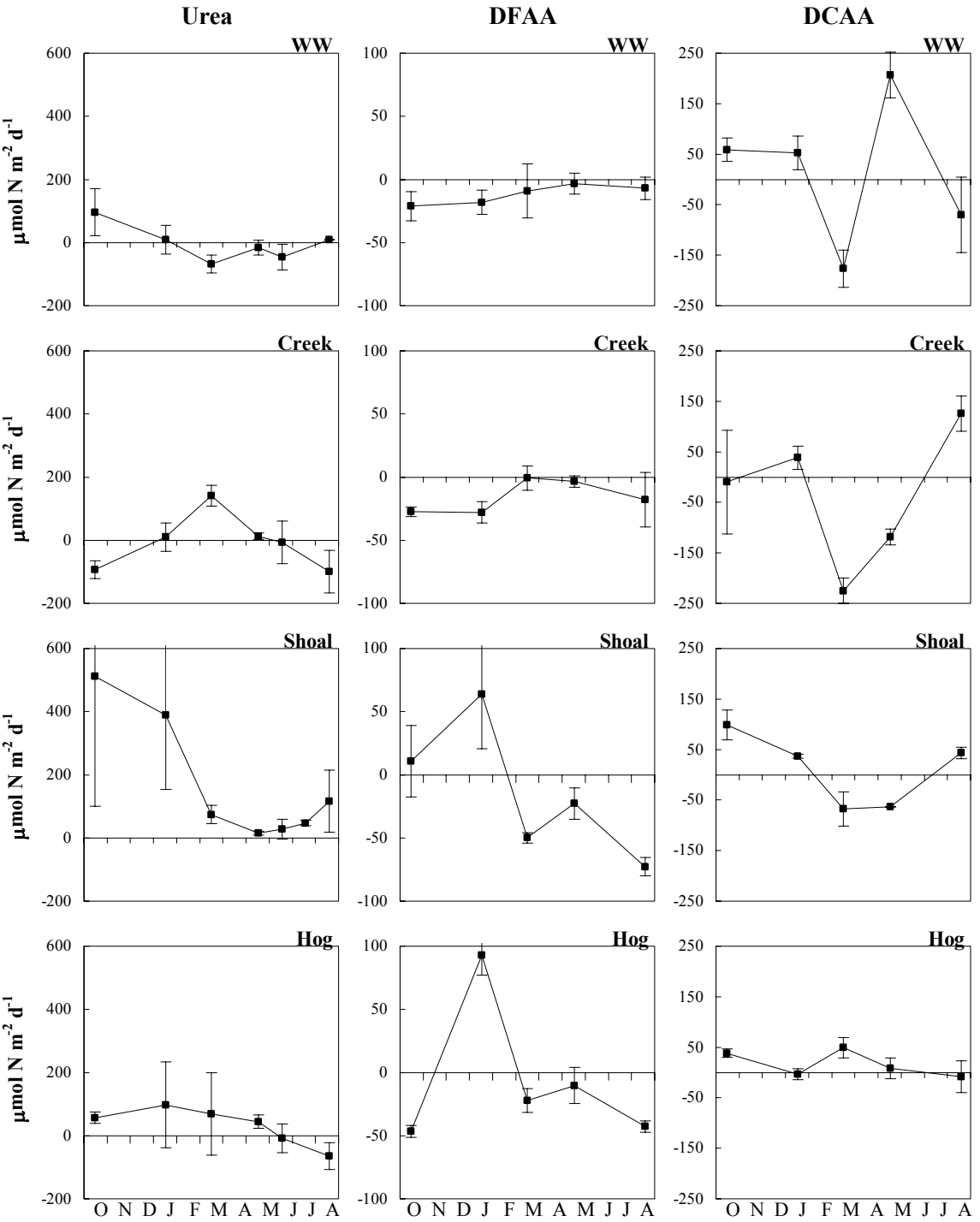

Figure 3-5 Daily sediment fluxes (mean and standard error) of urea, dissolved free and dissolved combined amino acids across Hog Island Bay. Positive values indicate a flux from the benthos to the water column; negative values indicate a flux from the water column to the benthos.

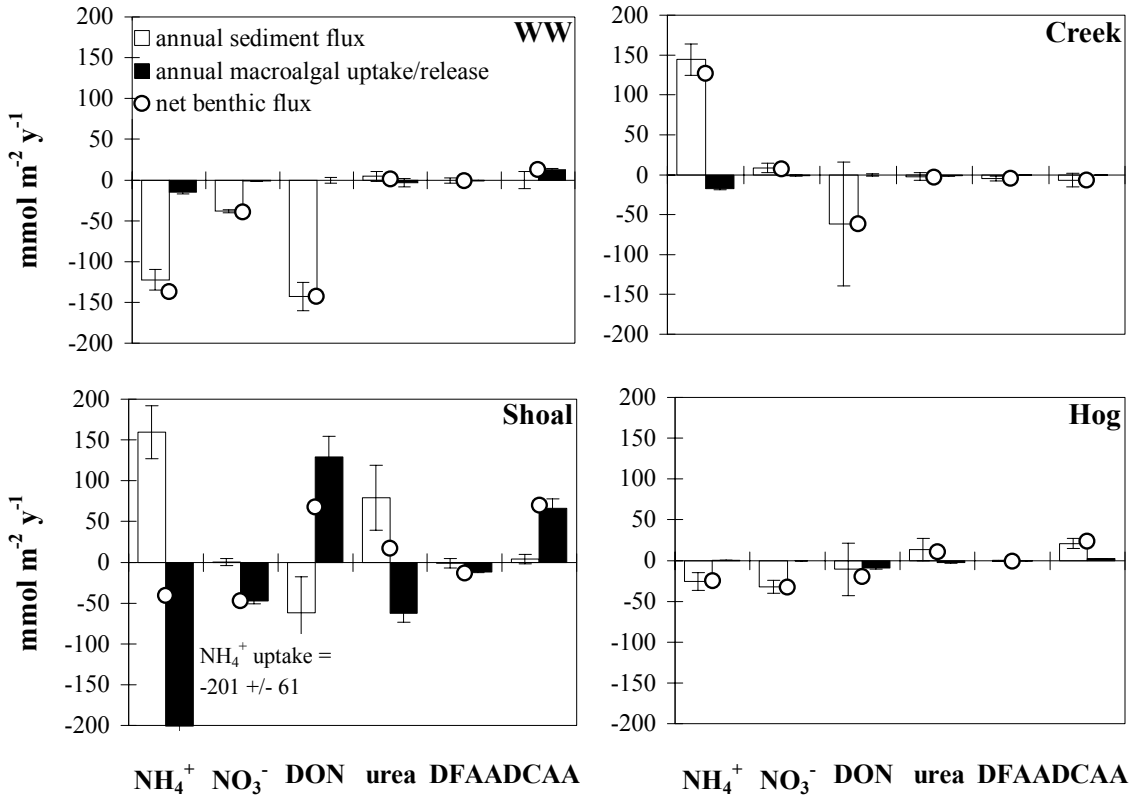

Figure 3-6 Calculated annual sediment-water column fluxes, macroalgal uptake/release based on local biomass and corrected for high biomass as described in the text, and net benthic flux (sum sediment flux + macroalgal uptake/release) across Hog Island Bay. Positive numbers represent a release from the benthos to the water column. All values are in mmol N m^{-2} y^{-1}. Errors bars approximate the standard error of the mean.

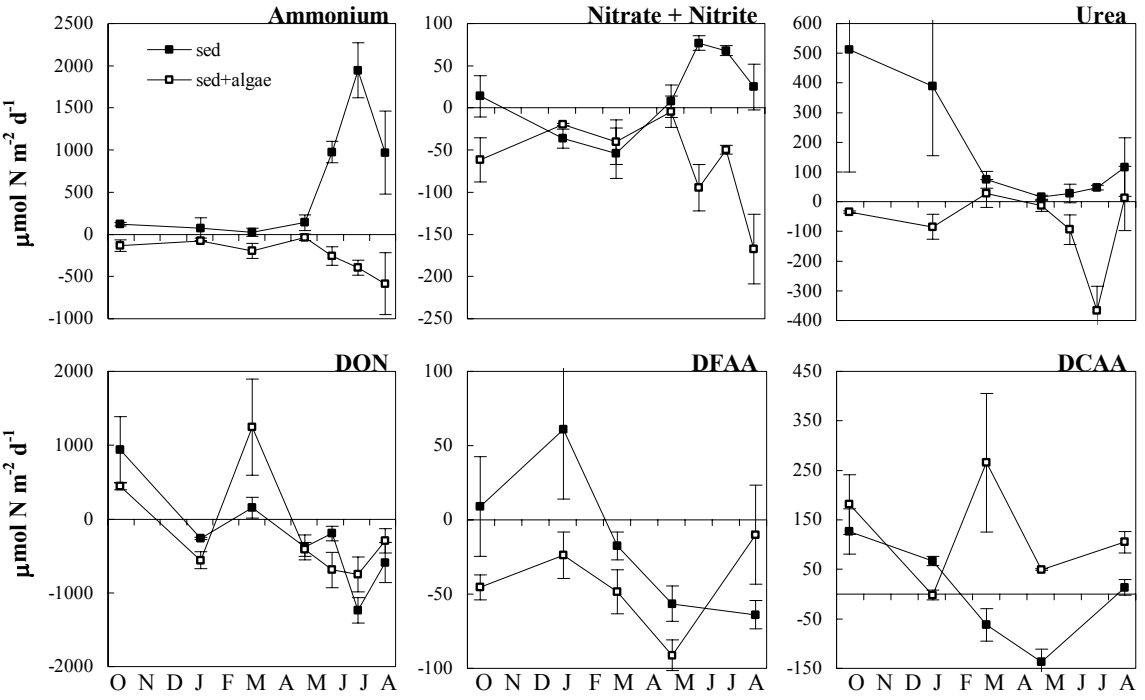

Figure 3-7 Benthic fluxes of dissolved N in sediment only and sediment + algae cores from Shoal. Sediment fluxes are the same as those shown in Figure 3-4 and Figure 3-5. The general patterns are representative of the differences between the two treatments at all sites. Positive values indicate a flux from the benthos to the water column; negative values indicate a flux from the water column to the benthos. Error bars are the standard error of the mean.

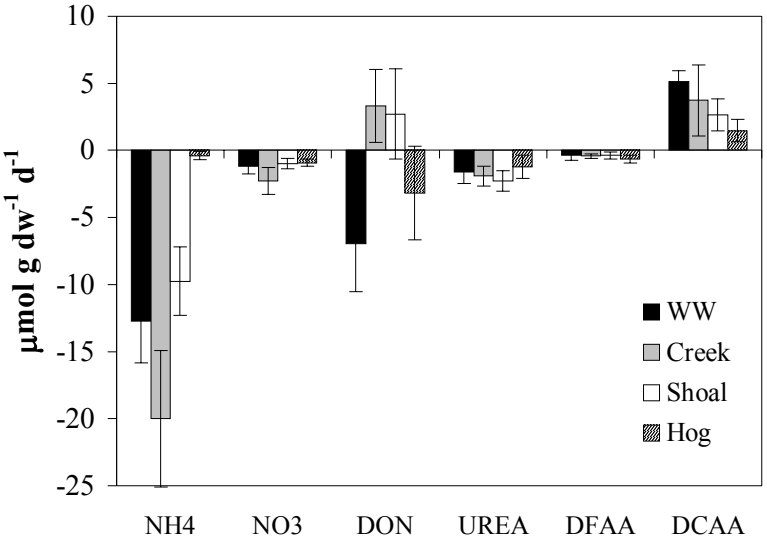

Figure 3-8 Average daily uptake and release of dissolved N by *U. lactuca* during incubations for each site. Positive values indicate a release from the macroalgae to the water; negative numbers indicate uptake by the macroalgae from the water and/or sediment. All values are in µmol N g dw^{-1} d^{-1}. Error bars are the standard error of the mean.

		Oct 98	Jan 99	Mar 99	May 99	Jun 99	Aug 99
	WW	0	11	10	1	7	0
biomass	Creek	0	3	0	0	6	3
(g dw m^{-2})	Shoal	28	76	86	170	193	243
	Hog	9	5	3	4	4	3
	WW	14	18	45	35	20	63
Chl *a*	Creek	27	40	29	26	27	12
(mg m^{-2})	Shoal	36	33	38	40	22	13
	Hog	89	37	64	101	49	58
	WW	0.11	0.12	0.12	0.13	0.12	0.10
%N	Creek	0.11	0.11	0.11	0.09	0.12	0.15
	Shoal	0.02	0.05	0.00	0.08	0.06	0.06
	Hog	0.01	0.00	0.00	0.00	0.01	0.00
	WW	12.9	11.8	11.9	10.8	9.2	11.7
C:N	Creek	11.4	11.1	11.9	10.5	8.8	10.6
	Shoal	19.0	9.3	--	13.5	7.5	11.0
	Hog	14.3	--	--	--	11.3	12.4

Table 3-1 Site characteristics used as predictor variables for fluxes and in calculations of the net impact of macroalgae on sediment-water column fluxes. The units are: g dry weight m^{-2} for macroalgal biomass; mg m^{-2} for benthic chlorophyll *a*; g g^{-2} for sediment %N; mol:mol for C:N. Sediment values are the top 2 cm of sediment cores, sectioned from 0-1 cm and 1-2 cm and averaged. Data from McGlathery *et al*. in prep.

		Willis Wharf		Creek		Shoal		Hog	
		mean	range	mean	range	mean	range	mean	range
TDN	**µM**	22.2	12.7 - 45.7	15.7	9.1 - 30.0	10.7	7.0 - 14.2	9.0	4.8 - 13.8
NH_4^+	**µM**	4.3	0.5 - 14.4	2.7	0.5 - 8.6	0.8	0.0 - 2.5	0.7	0.0 - 2.4
	% TDN	15	3 - 31	14	4 - 29	7	0 - 17	7	0 - 17
NO_3^-	**µM**	2.5	1.1 - 5.7	1.7	0.4 - 5.0	0.6	0.0 - 1.1	0.7	0.0 - 1.5
	% TDN	11	7 - 15	10	3 - 17	6	0 - 13	8	0 - 14
DON	**µM**	15.4	10.0 - 25.7	11.3	7.5 - 16.4	9.3	6.2 - 13.0	7.6	4.5 - 10.8
	% TDN	73	56 - 83	76	55 - 89	87	75 - 97	85	73 - 97
urea	**µM**	1.0	0.4 - 2.3	1.0	0.3 - 2.0	0.4	0.2 - 0.7	0.4	0.2 - 0.8
	% TDN	4	3 - 6	6	2 - 10	4	2 - 8	6	2 - 8
DFAA	**nM**	216	79 - 425	59.2	24 - 106	157	39 - 156	123	56 - 218
	% TDN	1	0 - 5	0	0 - 1	2	0 - 3	1	1 - 2
DCAA	**nM**	2178	1346 - 2986	1277	407 - 2013	1265	736 - 1200	1013	705 - 1217
	% TDN	12	5 - 21	10	3 - 25	12	8 - 18	12	8 - 17

Table 3-2 Dissolved N concentrations in the water column at the 4 sites across Hog Island Bay and percent of the total dissolved nitrogen in the water column (TDN). All values are in µM or nM N. Values are the annual mean and range calculated from water samples taken at the time of collection of cores and algae for flux experiments.

		F	p		mean	subset
DO	site	33.3	0.000	WW	7 ± 2	a
	date	10.8	0.000	Creek	-1 ± 2	b
	site×date	5.6	0.000	Shoal	-3 ± 1	b
				Hog	12 ± 2	a
NH_4^+	site	39.7	0.000	WW	-327 ± 104	a
	date	2.3	0.056	Creek	418 ± 90	b
	site×date	7.0	0.000	Shoal	384 ± 126	b
				Hog	-78 ± 29	c
NO_3^-	site	10.5	0.000	WW	-115 ± 36	a
	date	12.6	0.000	Creek	9 ± 47	b
	site×date	6.3	0.000	Shoal	6 ± 13	b
				Hog	-80 ± 27	a
DON	site	1.3	0.301	WW	-361 ± 141	a
	date	7.0	0.000	Creek	-122 ± 146	a
	site×date	3.5	0.001	Shoal	-45 ± 154	a
				Hog	-137 ± 152	a
UREA	site	3.6	0.019	WW	-4 ± 22	a
	date	1.2	0.312	Creek	-1 ± 26	a
	site×date	0.9	0.552	Shoal	189 ± 82	b
				Hog	32 ± 31	ab
DFAA	site	1.5	0.225	WW	-36.3 ± 14.8	a
	date	4.4	0.006	Creek	-13.8 ± 14.4	a
	site×date	2.4	0.023	Shoal	-13.6 ± 16.0	a
				Hog	-11.8 ± 16.2	a
DCAA	site	3.1	0.040	WW	-48.6 ± 46.3	a
	date	1.5	0.220	Creek	49.9 ± 51.4	ab
	site×date	5.3	0.000	Shoal	19.1 ± 28.0	ab
				Hog	74.9 ± 34.3	b
TDN	site	19.1	0.000	WW	-816 ± 187	a
	date	8.2	0.000	Creek	220 ± 177	b
	site×date	3.6	0.000	Shoal	276 ± 166	b
				Hog	-243 ± 156	c

Table 3-3 ANOVA results for daily sediment fluxes, in μmol N m^{-2} d^{-1}. Mean and standard error are for each site, across all dates. A positive number denotes a flux out of the sediment; a negative number indicates a flux into the sediment. N = 15 for AA and 18 for all other measurements. Significantly different subsets for the site comparison are denoted by different letters.

	light	dark	F	p value
Sediment fluxes				
NH_4^+	-1.6 ± 3.3	11.2 ± 3.2	7.6	0.007
NO_3^-	-2.7 ± 1.2	-1.0 ± 0.9	1.3	0.254
DON	-5.7 ± 5.2	-9.1 ± 6.1	0.2	0.669
Urea	0.9 ± 0.8	1.3 ± 1.4	0.1	0.820
DFAA	678 ± 671	-1753 ± 892	4.8	0.030
DCAA	180 ± 2120	1705 ± 2705	0.2	0.658
***Ulva lactuca* uptake/release**				
NH_4^+	-0.47 ± 0.11	-0.20 ± 0.05	4.8	0.030
NO_3^-	-0.08 ± 0.02	-0.01 ± 0.02	5.2	0.024
DON	-0.10 ± 0.13	0.25 ± 0.13	3.4	0.066
Urea	0.03 ± 0.02	-0.18 ± 0.03	34.0	0.000
DFAA	33 ± 27	-59 ± 27	6.0	0.016
DCAA	258 ± 56	18 ± 43	11.1	0.001

Table 3-4 ANOVA results for comparison between light and dark sediment fluxes and macroalgal uptake/release of dissolved nitrogen. Sediment values are in μmol N m^{-2} h^{-1} (AA in nmol); macroalgal values are in μmol N g dw^{-1} h^{-1}. Error bars represent the standard error of the mean and n = 72 each for light and dark for all measurements except AA where n = 60. A positive value indicates a flux from the benthos to the water column; a negative value indicates a flux into the benthos from the water column.

	Sediment		Sediment + Algae			
	mean	**range**	**mean**	**range**	**F**	**p value**
NH_4^+	101 ± 60	-1226 - 1948	-438 ± 93	-3532 - 386	23.7	0.000
NO_3^-	-46 ± 17	-370 - 370	-119 ± 15	-427 - 237	9.9	0.002
DON	-164 ± 74	-1665 - 1777	-136 ± 108	-1665 - 2811	0.0	0.837
Urea	57 ± 26	-184 - 1327	-65 ± 16	-374 - 350	16.1	0.000
DFAA	-19 ± 8	-112 - 139	-39 ± 7	-239 - 38	4.2	0.043
DCAA	24 ± 22	-375 - 427	191 ± 36	-273 - 1415	16.8	0.000

Table 3-5 ANOVA table for comparison between sediment and sediment + algae treatments. All units are in μmol N m^{-2} d^{-1}. Error is the standard error of the mean. N = 72 for each treatment for all components except amino acids, where n = 60. A positive value indicates a flux from the benthos to the water column; a negative value indicates a flux into the benthos from the water column.

Chapter 4
Uptake and release of nitrogen by the macroalgae *Gracilaria tikvahiae* (Rhodophyta) across a nutrient gradient in a coastal lagoon: estimates based on ^{15}N isotope dilution

Introduction

Shallow lagoons are an important land-margin feature (Cromwell, 1973; Hayden and Dolan, 1979; Kjerfve, 1989). The shallow nature of these estuaries means that the benthos is in the photic zone, and thus seagrasses, macro- and microalgae are the dominant primary producers. In response to increased nutrient loading, there can be a shift in dominance of primary producers, from seagrasses to macroalgae and eventually to phytoplankton where loading is sufficiently high and residence time sufficiently long (Valiela *et al*., 1997). Many species of macroalgae are capable of rapid nutrient uptake, storage and growth (e.g. Hanisak, 1983; Fujita, 1985; Pedersen and Borum, 1997) and therefore are able to outcompete phytoplankton and mat-forming benthic microalgae (Sundback *et al*., 1990; Sand-Jensen and Borum, 1991; Fong *et al*., 1993; Duarte, 1995; Valiela *et al*., 1997), as well as seagrasses (Hauxwell *et al*., 2001) under moderately high levels of nutrient input.

Dense macroalgal mats can have a significant impact on the benthos, as well as on the coupling between the benthos and the water column (Sundback *et al*., 1990; Ferrari *et al*., 1993; Valiela *et al*., 1997). The uptake and storage of nutrients (and carbon) by macroalgae can represent a significant sink where biomass is high (Smith, 1981; Valiela *et al*., 1997). Most macroalgae have little structural tissue, and thus decomposition takes place rapidly following senescence (Buchsbaum *et al*., 1991; Enriquez *et al*., 1993). This release upon senescence can have significant ecosystem impacts, including high

concentrations of organic matter and inorganic nutrients, an increase in heterotrophy in the water column, and a concomitant increase in oxygen consumption. Often, in macroalgal dominated systems, there is the potential for dystrophy following the crash of a bloom and hence the system may be unstable (Sfriso *et al*., 1992; Viaroli *et al*., 1995; Rysgaard *et al*., 1996).

The release of dissolved organic matter by living macroalgae has been acknowledged in the literature for decades, with estimates of dissolved organic carbon (DOC) release ranging from 0.5 up to 40% of total carbon fixed by photosynthesis (Khailov and Burlakava, 1969; Harlin and Craigie, 1975; Brylinsky, 1977; Penhale and Capone, 1981; Carlson and Carlson, 1984). Macroalgae are also capable of releasing a variety of compounds for defense against herbivores and other 'fouling' organisms (Hay and Fenical, 1988). However, the concomitant release of nitrogen, as dissolved organic nitrogen (DON), has largely been ignored. Macroalgae may also release some nitrogen as dissolved inorganic nitrogen (Naldi and Wheeler, 2002). Since N is generally thought to limit primary production in temperate estuaries (Howarth, 1988), an accurate estimate of uptake and release of N, by living and senescent macroalgae, may be important in determining their overall impact on ecosystem production and metabolism.

In phytoplankton, 25 – 41% of DIN uptake may be rapidly released back to the water column as DON (Bronk *et al*., 1994). This release may cause an underestimate of the total uptake of N estimated by ^{15}N uptake experiments (Bronk *et al*., 1994). By releasing DON during active growth, macroalgae may increase the total flux of DON from the benthos to the water column substantially (Tyler *et al*., 2001); much of this

release may be comprised of combined amino acids (Chapter 3), as has also been shown for phytoplankton (Flynn and Berry, 1999). This release indicates that estimates of macroalgal N demand based solely on biomass and tissue N content will underestimate significantly the actual quantity of N passing through the macroalgal N pool. In the work presented here, we have used ^{15}N isotope dilution in laboratory and field experiments to estimate the total uptake of N, as the sum of assimilated and 'leaked' N, by the red macroalga *Gracilaria tikvahiae* along a nutrient gradient in a shallow lagoon on the Virginia coast. The work presented here may provide an improved estimate of the overall impact of macroalgae on N retention in shallow, macroalgal dominated estuaries.

Methods

Site description

G. tikvahiae, for both the laboratory and field experiments, was collected from Hog Island Bay, a shallow, back-barrier lagoon located within the Virginia Coast Reserve LTER site. The lagoon (150 km^2) extends eastward from the southern portion of the Delmarva Peninsula, VA and meets the Atlantic Ocean at the southern end of Hog Island. The lagoon is very shallow, with more than 80% of the total area <2m deep at mean low water (Oertel, 2001). Water residence time varies substantially (Fugate *et al*., 2002), but portions are well flushed (Oertel, 2001). The watershed, which is predominantly agricultural, is drained by a few small creeks but no large riverine input enters the

lagoon. Much of the input of nutrients from land is likely through groundwater (Lee and Olsen, 1985; Neikirk, 1996; Reay *et al*., 1992), however atmospheric deposition (Paerl and Fogel, 1994; Paerl *et al*., 1990) and runoff after storm events may also contribute to the total N loading. There is a gradient of nutrient inputs and thereby water column N across the lagoon, with the highest concentrations closest to the mainland (McGlathery *et al*. 2001; Tyler *et al*. 2001).

Macroalgae, microalgae and phytoplankton are the dominant primary producers in Hog Island Bay as seagrasses have been absent from the lagoon since the wasting disease of the 1930's. *G. tikvahiae* is the dominant macroalgae, but *Ulva lactuca* and *Bryopsis plumosa* occasionally demonstrate temporal importance (McGlathery *et al*. unpub. data). Biomass varies greatly across the lagoon and generally peaks in the mid-summer. For this experiment, we chose three sites, representing the range of environmental conditions in the lagoon. Closest to the mainland, the "Creek" site is a small, secondary tidal creek surrounded by *Spartina alterniflora* marsh. The water column dissolved N (DIN 1 – 7 μM; DON 10 – 17 μM) were higher here than at the other 2 sites (McGlathery *et al*. unpub. data). Maxima for both DIN and DON were in the late summer/early fall; minima are in the winter. Macroalgal biomass here was relatively low (monthly mean: 1 – 22 g dw m^{-2}), peaking in the fall (McGlathery *et al*. unpub. data). Moving eastward, the second site is in the mid-lagoon adjacent to a series of relict oyster reefs ("Shoal"). The reefs act as a barrier, trapping floating macroalgae and also providing attachments points so that macroalgal biomass was highest here throughout the year (monthly mean: 15 – 300 g dw m^{-2}). During the period of maximum

biomass, in the mid-summer, dense mixed-species mats form and in patches biomass can be >650 g dw m^{-2}. During the summers of 1999 and 2000, biomass declined gradually as temperatures became warmer; in other years there have been isolated "crashes" of the mats that led to the release of large quantities of dissolved N (Tyler *et al*. 2001). The third site, at the southern end of Hog Island ("Hog") had sandy sediments and biomass comparable to the Creek site (monthly mean: 1 – 25 g dw m^{-2}), also peaking in the mid-summer. DIN at both Shoal and Hog was always <2 μM and peaked in October; DON (7 – 13 umol) peaked in the late summer (McGlathery *et al*. unpub. data).

Dissolved Oxygen Production

Macroalgae (*G. tikvahiae*) used for DO measurements in field were collected 10 d prior to field production measurements and held in the laboratory as described in the section "estimation of leakage and assimilation". The day before the field DO incubations, water was collected from each site and filtered through 0.2 μm Nuclepore filters to remove bacteria, phytoplankton and other microorganisms. Thalli were cut the evening before the incubation and kept in seawater over night to minimize the potential impact of a wound response. Wet weights of the individual thalli were approximately 0.3 g. Incubations were performed in 300 ml glass BOD bottles. Bottles were filled with the filtered water and initial DO concentration and temperature was measured. The bottles were kept at in *situ* temperatures in a cooler until placed in the field. The bottles, 3 light and 3 dark (foil-wrapped) were placed at random in a rack which was secured on top of a cinder block to keep the bottles raised just slightly above the sediment surface.

Macroalgae were placed into the bottles immediately prior to placing the rack at the site. The racks were retrieved from each site 2 – 4 hours later, and the macroalgae were immediately retrieved from the bottles and placed in individual bags to keep each thallus separate. The bottles were kept in a cooler in the dark, until returned to the laboratory where the final oxygen and temperature were recorded. Measurements were generally made within 2 hr of collection, and it is unlikely that changes associated with residual microbial activity altered the residual DO concentrations in any substantial way relative to production or consumption by the algae. Macroalgae were patted dry and the wet weight was recorded. They were later freeze-dried and the final dry weight was recorded. Net daily oxygen production was calculated based on the numbers of hours of light and dark at each sampling time. All measurements were made at or near low tide on sunny, clear days, and we acknowledge that this may cause an overestimate of the actual daily production. However, during most months, light intensity at the bottom (usually > 100 $\mu E\ m^{-2}\ s^{-1}$) was likely sufficient for maximum photosynthesis (Lobban and Harrison, 1997).

Light extinction

Water column light extinction was measured concurrent with the DO production measurements using a Li-Cor model 193SA meter with a 4π spherical quantum sensor. Duplicate profiles were measured at each site, with point measurements at the surface and at 10, 25, 50, 75, 100, 125 and 150 cm from the surface. The light extinction coefficient was calculated from each profile.

Estimation of N assimilation and leakage

The assimilation and leakage of N by *G. tikvahiae* was measured using an isotope dilution technique during October 1999 and February, April and July 2000. Macroalgal thalli, labeled in the laboratory with ^{15}N, were placed in the field at each of the three sites for 10 – 16 d. At periodic intervals (approximately 1, 3, 6, 10 and 16 d) 3 – 5 thalli were collected from each site and the change in biomass, total N content (%N) and atom % ^{15}N was measured. Leakage and assimilation were then calculated, as described below, from the changes in these values over time.

Labeling

Approximately 10 days before the initiation of the experiment, *G. tikvahiae* was collected from Hog Island Bay. The algae were grown in a Conviron™ environmental growth chamber in Charlottesville, VA at ambient field temperatures and a light-dark cycle appropriate for each season. "Daytime" light intensity was approximately 550 $\mu E\ m^{-2}\ s^{-1}$. Small mats were maintained in plastic tubs containing 6 L low nutrient seawater collected from Machipongo Inlet at the southern end of Hog Island. The tubs were bubbled continuously to maintain aeration and motion of the water. Macroalgae were fertilized daily with a 10:1 solution of NH_4Cl:KH_2PO_4. We used 10:1 N:P rather than the Redfield ratio of 16:1 to ensure that neither N nor P would limit growth of fertilized. Tubs were given sufficient N to sustain tissue N at 3% of dry weight at a growth rate of 10% d^{-1} during October, April and July; in February we assumed 2.5% N

and 5% d^{-1} growth due to colder field conditions and lower tissue N content in the winter months. Actual growth rates were generally slightly lower, and final tissue N concentrations were 3.5 - 4% N. Tubs containing macroalgae intended for the DO production measurements described above were fertilized with $^{14}NH_4Cl$; tubs with algae intended for the field assimilation and leakage experiments were fertilized with a solution of $^{14}NH_4Cl$ and $^{15}NH_4Cl$ (98 atom % ^{15}N, Sigma Chemical Co.) at ~2 atom % ^{15}N in October, February and April and ~50 atom % ^{15}N in July. Macroalgae were grown for 10 days and were then transferred to the LTER laboratory on the Eastern Shore of Virginia where they were prepared for field incubation.

Field incubations

The labeled macroalgal mats were cut into small, equal mass (1.0 ± 0.05 g wet weight) thalli by breaking off the apical branches. Several thalli were retained for initial weight and N measurements and the remainder were placed in cages in the field. The cylindrical cages (30 cm long x 10 cm diameter) used for field incubations had clear plastic ends and 0.5 mm clear Nytex™ mesh on the sides to maintain water flow through the cages. Cages were attached to stakes driven into the sediment at each site and were suspended horizontally by cords approximately 20-30 cm above the sediment surface. Two cages were used at each site, and collections were made from both cages at each sampling. The mesh itself decreased light availability minimally, but some fouling of the mesh did occur. Epiphytes and other fouling organisms were scrubbed from the cages every 2 -3 days, and the remaining algal thalli were switched to new, clean cages each

week. Because of the high light intensities, growth was probably not impacted severely by the fouling. In addition, the site with the highest fouling and sediment accumulation (Creek) did not show appreciably different growth rates. At the initiation of each experiment, new *G. tikvahiae* samples were collected from the field to obtain baseline ^{15}N and tissue N content.

Thalli collected at each interval were quickly rinsed in deionized water to remove sediments and salt, gently patted dry, weighed and immediately frozen. These samples were later freeze-dried, re-weighed and ground to a fine powder using a coffee mill. All ^{15}N and %N analyses were performed by the University of California at Davis Stable Isotope Facility using a Europa Scientific Integra Isotope Ratio Mass Spectrometer.

Laboratory Experiment

Coincident with the February field experiment, we also conducted an additional experiment under controlled conditions in the environmental growth chamber in the laboratory. Labeling was conducted in the same manner as for the field experiment, and at the same time. Individual thalli were prepared as described above and kept in tubs containing 3 L of low nutrient seawater. Macroalgae were fertilized with a 10:1 $^{14}NH_4Cl$:KH_2PO_4 solution at a rate to sustain 5% growth and a tissue N content of 2.5%. The water was changed daily to prevent re-uptake of exuded ^{15}N; however, bacterial processing of DON may be sufficiently rapid that mineralized N was available for re-uptake (Flynn and Berry, 1999). Thalli were removed from the tubs at 4, 7, 13, 21 and 28 d and analyzed as described above.

Calculations

Relative macroalgal growth rates (μ_M) were calculated by fitting an exponential equation to the observed increase in biomass with time using least squares regression analysis. Likewise, where a decrease was observed, μ_N and μ_{15} were calculated for the change in %N and atom % ^{15}N, respectively. When an increase in %N occurred, a fit to a logistic equation was used, and the measured field N concentration was used as the upper bound. Leakage of N from macroalgal tissue was calculated based on the difference between the observed atom %N in the macroalgal tissue and the atom %N expected if all original plus all newly assimilated N were maintained in the tissue. All calculations were done using the fitted equations obtained from the changes in thallus mass, %N and atom % ^{15}N and were estimated on a one day time-step. Newly assimilated N was calculated as the difference between time steps for total thallus N (thallus weight x %N/100). We assumed that the ratio of ^{15}N:^{14}N for newly assimilated N should be equivalent to the ^{15}N:^{14}N of macroalgae found in the field ($\delta^{15}N$ ~10‰). The total expected ^{15}N and ^{14}N in the tissue was then calculated based on the new N plus N at the previous time-step. The difference between the expected ^{15}N and the measured ^{15}N was assumed to be "leakage" (Figure 4-1). Because *Gracilaria* sp. is exceptionally capable of storing N in excess of growth demands (e.g. Fujita, 1985; Lapointe, 1985; Naldi and Wheeler, 1999; Rosenberg and Ramus, 1982; Ryther *et al*., 1981; Smit *et al*., 1997), not all N is necessarily metabolically active and there is likely a difference in the susceptibility to leakage between stored N and the pool in active use. To estimate the ^{15}N:^{14}N ratio of the leaked N, and thus the total leaked N, we therefore partitioned the tissue N into 3 compartments,

"active", "structural" and "storage" (Figure 4-1; after Hanisak, 1983). The quantity of N in the combined active and structural compartments, nominally the critical N content below which the growth rate drops off (Nc, sensu Hanisak, 1983; Lavery and McComb, 1991b; Pedersen and Borum, 1996), was set at 2.3%, the level at which *G. gracilis* is N replete (Smit *et al*., 1997). We acknowledge that a given species of macroalgae may not have a single N_c that is applicable to all environmental conditions (Fujita *et al*., 1989; Lapointe and Duke, 1984). We are utilizing N_c as a cut-off point, above which storage of excess N is likely to occur. For *G. tikvahiae*, the structural component (0.8%) is relatively static (Hanisak, 1983) and along with the storage pool, which is primarily of proteins and pigments (Bird *et al*., 1982; Naldi and Wheeler, 1999; Rosenberg and Ramus, 1982), is likely not susceptible to leakage. Thus, in our model only the active compartment, set at a constant magnitude of 1.5% (= 2.3% - 0.8%) was subject to leakage, while the structural (0.8%) and storage compartments (N in excess of 2.3%) were not (Figure 4-1). At the onset of each experiment, we assumed that all 3 compartments had an equal ^{15}N:^{14}N. The ^{15}N:^{14}N in the active pool was diluted by uptake of N from the water column and was recalculated for each day based on the newly assimilated N. New N in excess of 2.3% was shunted to the structural (to maintain %0.8 N) and storage compartments, at an equivalent ^{15}N:^{14}N found in the total active compartment. Leaked N was also assumed to have a ratio of ^{15}N:^{14}N equivalent to the active compartment prior to storage. Under N limiting conditions, where the N stores were depleted, all remaining N >0.8% was subject to leakage. Daily leakage and assimilation values were calculated for each thallus as a whole. These values were then

divided by the thallus mass at each time step, and the resulting values averaged over the 14 day simulation. A detailed description of these calculations is provided in Appendix G. Errors for daily leakage and assimilation were calculated using a bootstrap procedure (Efron and Tibshirani, 1986). The mean and standard deviation for each measured parameter were assumed to be normally distributed and the combined results for leakage and assimilation were calculated using a Monte Carlo resampling (n = 1,000) of the different measured parameters. A standard deviation was calculated from the resulting data set for each combined result.

In April, thalli in the cages at Creek and Shoal became fragmented within a few days of the initiation of the field incubation, preventing an accurate assessment of the growth rate based on increase in weight. To estimate the growth at these two sites, we used the site-specific relationship between DO production and growth obtained from the other measurements. The relationship between DO and growth is relatively good for these two sites (Creek r = 0.88; Shoal r = 0.99), so while not ideal, this method is likely a close estimate of the actual growth rate.

The leakage and assimilation values obtained from the experiment were multiplied by field biomass data (McGlathery *et al*. unpub. data) in order to obtain a rough estimate of the areal impact of N uptake and release by macroalgae in the field. *G. tikvahiae* was consistently 80 – 95% of the total macroalgal biomass in the field, so assuming an equal uptake and leakage rate for all algae was likely satisfactory.

Results

Light

The mean light extinction coefficient (Figure 4-2) decreased moving across the lagoon, from 1.8 ± 0.2 at Creek, to 1.4 ± 0.2 at Shoal and 1.2 ± 0.1 at Hog. In spite of this trend, there was no significant difference overall between sites ($F = 3.6$, $p = 0.072$) due to the inter-seasonal variation. The highest rates at all sites were measured in February, with relatively constant rates across the other months.

DO

Daily dissolved oxygen production is shown in Figure 4-3. There were significant seasonal effects, with February < April and October < April and July ($F = 36.2$, $p < 0.001$), but no significant differences between sites.

Field N content

Macroalgal tissue N reflects clearly the gradient in N availability across the lagoon (Figure 4-4), with significantly higher ($F = 7.7$, $p < 0.001$) values closest to the mainland at the Creek (3.7 ± 0.2), intermediate at Shoal (2.6 ± 0.2) and lowest at Hog (2.0 ± 0.1). There were also significant differences between dates ($F = 1.7$, $p < 0.001$), but in post hoc tests only October was significantly higher than all other dates.

Growth, assimilation and leakage

The results of the laboratory incubation experiment are shown in Figure 4-5 and Figure 4-6. Thallus mass increased at approximately 5% d^{-1}, while the %N decreased at 2% d^{-1} and atom % ^{15}N decreased at 1% d^{-1}. Even though the total tissue N continued to increase over the course of the experiment, the assimilation rate declined nearly 50%; this is likely due to insufficient N supplied by fertilization. While the actual growth rate did approximate 5%, as assumed, the tissue N content decreased below the presumed 2.5% after ~20 days, likely due to leakage. The mean assimilation of N was 43.7 +/- 5.1 μmol N g dw^{-1} d^{-1}. The measured decline in tissue ^{15}N was consistently lower than that predicted based on the assumption that all N was retained (Figure 4-6); a similar pattern was observed for all field incubations (data not shown). The average daily leakage rate in the laboratory experiment was 17.2 +/- 1.9 μmol N g dw^{-1} d^{-1}. Leakage averaged 28% of total uptake (assimilation + leakage), but increased from 19% to 33% over the course of the experiment as the daily assimilation rate declined.

Growth rates, change in N content and change in atom % ^{15}N during the field experiments are shown in Table 4-1. The growth rates, which ranged from 1.4 – 4.4% d^{-1}, varied between site and date. Growth rates were lowest in February at all sites; July rates were highest at Creek and Shoal, but the maximum growth rate at Hog was measured in October. Shoal always had the lowest growth rates, and Hog the highest, with the exception of February when Creek was highest. The %N in the *G. tikvahiae* tissue decreased 0.8 – 2.9% d^{-1} throughout the course of the experiment at all sites and dates, except for the Creek site in October when we observed an increase in tissue N.

Overall, Creek had the smallest rate of %N decrease and Hog the highest. The %N in experimental thalli generally reached the %N of field algae from at each site within one week. The atom % ^{15}N decrease, which ranged from 0.6 – 3.7% d^{-1}, was the greatest at Hog during all seasons and lowest at Creek, with the exception of July 00 when Shoal had the smallest relative decrease in ^{15}N. Overall decreases were lowest in February and highest in July. The decrease in atom % ^{15}N was significantly linearly related to the rate of thallus growth, with higher rates of ^{15}N decrease at high growth rates ($r = 0.73$, $F = 10.5$, $p = 0.01$). There was not a significant relationship between the decrease in ^{15}N and the change in %N.

The mean average daily N assimilation was 31.3 ± 6.9 μmol N g dw^{-1} d^{-1}. N assimilation, averaged across the 14 days of the simulation, was generally highest at Creek and lowest at Shoal (Figure 4-7), except in February when Hog was the lowest. Assimilation varied seasonally, highest rates at all sites were measured in October and lowest in February. The average rate of N leakage across all sites and dates was 34.1 ± 5.5 μmol N g dw^{-1} d^{-1}. A sensitivity analysis for the constant N_c demonstrated that a 10% increase or decrease changed this mean value to 32.6 μmol N g dw^{-1} d^{-1} and 36.3 μmol N g dw^{-1} d^{-1}, respectively. The Creek always had the lowest leakage rates (20 ± 6 μmol N g dw^{-1} d^{-1}), Shoal the highest (45 ± 11 μmol N g dw^{-1} d^{-1}) and Hog intermediate (37 ± 8 μmol N g dw^{-1} d^{-1}), except in October when Hog was highest. Overall, leakage of N was 56% of total daily uptake (= leakage + assimilation), but varied substantially between sites and seasons. Leakage was 17 - 51% of total daily uptake at Creek, 51 - 86% at Shoal and 40 - 97% at Hog. There was an inverse relationship between field N content and

leakage ($R = 0.73$, $F = 11.3$, $p = 0.007$), indicating a higher leakage under low N conditions.

Discussion

Rapid uptake and release of dissolved N by macroalgae clearly has substantial impacts on N dynamics in Hog Island Bay. Both uptake and release vary markedly across the lagoon and are related to local environmental conditions as well as to the temporal variations associated with different seasons. During active growth, the average rate of leakage (34.1 μmol g dw^{-1} d^{-1}) was considerable relative to the average assimilation of new N (31.2 μmol g dw^{-1} d^{-1}). This intimates that a great deal more nitrogen passes through the macroalgal pool on a daily basis than previously thought, and that traditional estimates of the role of macroalgae in N retention based on biomass and N content may greatly underestimate total N uptake.

Comparison between field and laboratory measurements of N leakage

The mean release rate (all sites) in the field in February (13 ± 4 μmol N g dw^{-1} d^{-1}) was similar to the release measured in the laboratory at the same time of year (17 ± 2 μmol N g dw^{-1} d^{-1}). Even though the water was changed daily to prevent mineralization and re-uptake of ^{15}N during the laboratory experiment, bacterial processing of exuded ^{15}N may have been sufficiently rapid that mineralized N was available for re-uptake

(Flynn and Berry, 1999). If this was the case, the measured decline in ^{15}N would be lower and we would thereby calculate a smaller release rate. However, the laboratory and field measurements during this time period were very similar, suggesting that in this case, re-uptake of released N was minimal in the laboratory and that our field measurements can be closely replicated in the laboratory.

Patterns of assimilation, growth and leakage

The differences in the relative magnitude of assimilation, growth, and leakage between sites suggest spatially and temporally variable controls on these three parameters. Higher leakage at the lower N sites intimates that leakage may be a function of nutrient availability. The gradient in nitrogen inputs is reflected in tissue N values from field samples (Figure 4-4), in high N assimilation rates at Creek (Figure 4-7) and in the paler color of *G. tikvahiae* at Hog relative to Creek (pers. obs.), which is likely the result of a lack of stored phycoerythrin, a common N storage compound in the Rhodophyta (e.g. Ryther *et al*., 1981; Horrocks *et al*., 1995). The higher assimilation of new N at Hog relative to Shoal, in spite of lower standing stock concentrations, may be due to a greater overall nutrient flux because of faster water motion at Hog. Also, at Shoal, caged macroalgae were suspended above the macroalgal mat, so that only water column nutrients were available. Rapid recycling at the bottom of macroalgal mats can support new growth, even in the absence of new nutrient input (e.g. Lavery and McComb, 1991a; Trimmer *et al*., 2000; Astill and Lavery, 2001), and may contribute to the higher tissue N of field algae relative to the low measured assimilation of caged algae

at Shoal. Previous studies in Hog Island Bay have demonstrated that the release of N from Shoal sediments in the late summer, as biomass declined, was substantial and that macroalgal N content increased in the late summer here, likely as a result of this efflux (Tyler *et al*., 2001, and Chapter 3).

The low leakage at Creek relative to the other sites may be explained by several factors related to nutrient availability. In phytoplankton, the magnitude of N release can be related to the concentration gradient between the surrounding water and the cell interior, with higher diffusion out of the cell anticipated at lower water column concentrations (Flynn and Berry, 1999). If concentration-driven diffusion was a factor in this study, we would expect to find, as we did, the lowest release rates at the Creek, where water column nutrient concentrations were highest. This may also explain the difference between the high average leakage measured in this study, and the lower rates measured by Naldi and Wheeler (2002) for N replete macroalgae incubated at high nutrient concentrations (initial concentrations = 1 mM N). In addition, if re-uptake of exuded N occurred in the field, the probability of reabsorption would likely be related to the thickness of the boundary layer surrounding the thallus, which is in turn related to the local flow conditions. Current speeds are generally lowest at Creek (M. Thomsen, pers. comm.), again helping to explain the lower leakage measured here.

Further, if exudates included extracellular enzymes for the uptake of nutrients, such as alkaline phosphatase (Weich and Graneli, 1989), we would expect higher release associated with lower nutrient concentration. Standing stock phosphate concentrations were consistently higher at the Creek (~2 μM) than at Shoal or Hog (~1 μM, McGlathery

et al. unpub. data), perhaps contributing to greater release at the lower nutrient sites. Finally, our calculation of leakage assumed that leaked N had a ^{15}N:^{14}N equivalent to the total active pool ("old" N plus "new" N). If the leaked N was actually derived more from newly absorbed N (low ^{15}N), rather than from the total in the active pool (higher ^{15}N), then in using the higher ^{15}N:^{14}N in the active pool to calculate the ^{14}N leaked, we may have underestimated the ^{14}N leaked, and thereby underestimated the total N leaked. If this was the case, the underestimate would be greatest where N assimilation is highest - at Creek.

Variable macroalgal productivity across the lagoon may also be important in determining leakage. There are two somewhat opposing theories regarding the loss of DOC from phytoplankton. One states that the loss is passive and unavoidable (Bjornsen, 1988); the other describes an "income tax" concept, where loss is tightly coupled to photosynthesis (Zlotnik and Dubinsky, 1989). Leakage was highest during July, when DO production was also highest, suggesting some coupling between loss and temperature-driven photosynthesis. However, Shoal algae had the lowest overall growth rates and the highest leakage, indicating that the spatial variability of leakage at a single point in time was controlled by more than just relative productivity. Growth rates appear to be related to a number of factors, and different factors may be important at different sites. At Creek, nutrient availability was high, light availability wais low and growth rates were high; at Hog, light availability was high, nutrient availability was low and growth rates were high. Shoal, intermediate in both nutrients and light, had the lowest growth rate. High initial tissue N content may have supported the high growth at Hog

(Fujita, 1985; Lapointe and Duke, 1984; Lapointe and Ryther, 1979; Navarro-Angulo and Robledo, 1999). However, the same should be true at Shoal, but growth rates were low indicating that the high growth at Hog was determined by different factors. Overall, growth rates appear to be controlled by a combination of factors, including temperature, light and nutrient availability, and these factors in turn lead to the variability in leakage across the lagoon.

The storage of N for later use may give a competitive advantage to certain macroalgal species in systems with pulsed or seasonal N delivery (Asare and Harlin, 1983; Chapman and Craigie, 1977; Fujita, 1985; Lapointe and Duke, 1984; Rosenberg and Ramus, 1982; Trimmer *et al*., 2000). Generally, in temperate western Atlantic estuaries, storage follows late winter and early spring nutrient pulses (Asare and Harlin, 1983 Fujita, 1985; Rosenberg and Ramus, 1982), and enables high growth in summer, when temperature and light availability are more amenable, but nutrient concentrations are lower. In this experiment, N availability was highest in October and storage of N during the late summer and fall may have allowed the macroalgae to persist during the nutrient poor winter. However, during the winter at Shoal and Hog, growth rates were low (~1% d^{-1}), little new N was assimilated and at times the macroalgae were a net source of N to the water column. In a simulation model of productivity for the red macroalgae *Gelidium sesquipedale*, Duarte et al. (1997) found that the decline in biomass during colder months can be explained by mortality and frond breakage, as well as respiratory and "exudation" losses. This appears to be the case in Hog Island Bay as well, and during the winter almost all N taken up from the water column was immediately lost,

in contrast to other macroalgal-dominated systems where the macroalgae are a net sink for N during the winter.

What is it?

At this point, we can only speculate on the identity, bioavailability, and "purpose" of the material leaked from *G. tikvahiae*. However, there are a few potential "reasons" for the exudation of organic matter, including nutrient uptake and defense. Tyler et al. (Chapter 3) determined that the macroalga *Ulva lactuca* is capable of releasing 2 -5 μmol N g dw^{-1} d^{-1} as dissolved combined amino acids. Assuming an equivalent rate of release for *G. tikvahiae*, this suggests that up to 25% of release may be combined amino acids, as polypeptides or proteins. Macroalgae are known to excrete the proteinaceous enzyme alkaline phosphatase to aid in the uptake of phosphorus (Weich and Graneli, 1989). Penhale and Capone (1981) demonstrated that exuded organic matter by macroalgae may act to stimulate of N fixers living on the macroalgal thallus. N may be lost as a by-product of the exudation and stimulation of N fixers, but perhaps the loss is small relative to the net gain. Moreover, many species of macroalgae exude defensive chemicals (Hay and Fenical, 1988). However, *G. tikvahiae* is highly palatable (Hay *et al*., 1986) and prone to epiphytization by other algae and bryozoans (Schmitt *et al*., 1995) to the extent that settlement of propagules of *Ulva* and *Enteromorpha* is stimulated by exudates from *G. tikvahiae* (Santelices and Varela, 1993). The susceptibility of *G. tikvahiae* to epiphytization is the most evident during the summer months at the Hog site, where epiphytic algae (*Ceramium* sp. and *Polysiphonia* sp.) growing on *G. tikvahiae* can have

greater biomass than the *G. tikvahiae* itself (McGlathery et al. unpub data). This suggests that *G. tikvahiae* is not as heavily defended chemically as other species, and that leakage is likely not for defensive purposes. More research is necessary to clarify the identity and purpose of the leaked compounds, and it may be that overall leakage is driven simply by the diffusion gradient at the cell surface.

Comparison with other aquatic primary producers

The release of dissolved organic matter has been reported for all functional groups of aquatic primary producers. Seagrasses have been shown to release DOC to the water column from their leaves, but generally at rates less than 10% of C fixed (e.g. Penhale and Smith Jr, 1977; Wetzel and Penhale, 1979; Ziegler and Benner, 1999). Phytoplankton release is similar, with release rates up to 10-15% of recently fixed C (Giordano *et al*., 1994). Macroalgal release of DOC has also been reported, with large variation in the amount of photosynthate released. Releases up to 30-40% have been reported (Khailov and Burlakava, 1969), but the majority are less than 5% (Harlin and Craigie, 1975; Brylinsky, 1977; Penhale and Capone, 1981; Carlson and Carlson, 1984). The concomitant release of N has not been as fully investigated.

In estuarine phytoplankton, reported DON release rates ranged from 11 – 28 % of gross N uptake (see Table 2 in Bronk and Ward, 2000). Our values for N release by *G. tikvahiae*, 17 - 99%, represent a wider range. However, these rates encompass periods of little to no net assimilation of new N so that all N removed from the water column is subsequently leaked back out. A rough calculation of the C release yields a release of 7 -

41% of photosynthetically fixed C (assuming 1 mol O_2 : 1 mol C fixed and C:N of leaked material is equivalent to field tissue C:N). The highest proportion of release was during winter at Shoal and Hog, when DO production was low. Tyler et al. (2001) reported that the macroalga *Ulva lactuca* was capable of releasing 3.5 μmol N g dw^{-1} d^{-1} as urea-free DON. While this rate is lower than the values presented here, these measurements were based on the net difference in benthic fluxes observed in cores with and without macroalgae, and rapid uptake by water column heterotrophs may decrease the observed release.

Potential ecosystem impacts

The release of DIN and DON (and DOC) to the water column may have significant impacts on ecosystem processes. Tyler et al. (2001) demonstrated that where macroalgal biomass is high, release of DON to the water column by macroalgae could be much greater than the sediment DON efflux. Indeed, at the Shoal site in the summertime, the release of N from actively growing mats may be close to 6 mmol N m^{-2} d^{-1} (Figure 4-8). As has been shown following the senescence of seagrasses (Kemp *et al*., 1997), this increase in available organic matter is likely to fuel heterotrophic metabolism in the water column (Brylinsky, 1977; Valiela *et al*., 1997). Likewise, in Laguna Madre, Texas the release of DOC from seagrass beds supplied a large fraction of the bacterioplankton respiratory demand (Ziegler and Benner, 1999). Other related impacts include the promotion of N fixation on the fronds of *Laurencia* sp. and *Microdictyon* sp. (Penhale and Capone, 1981) and attraction of the toxic dinoflagellate *Prymnesium parvum* to mats

of *Cladophora* sp. (Johnsen and Lein, 1989). Further, exudates from *G. chilensis* were shown to stimulate the settlement of propagules of both *Ulva* sp. and *Enteromorpha* sp. (Santelices and Varela, 1993); this may explain why *G. tikvahiae* has a higher probability of being fouled by algae and bryozoans than other species of macroalgae (Schmitt *et al*., 1995).

Summary and conclusions

The release of nitrogen by benthic macroalgae can clearly be a substantial proportion of N recycling in shallow estuarine systems, particularly where biomass is high. It appears that, to some extent, this release may be related to low nutrient availability. It is also clear that if we estimated assimilation of new N for these algae, based on just growth rate and N content, in some cases we would underestimate the actual uptake of N by up to 100%. This is particularly true during conditions of low growth and N availability (winter), when there is no apparent removal of N from the water column by assimilation, but leakage still occurs. Several authors have suggested that the rapid release of DON to solution may cause an underestimate of total uptake in ^{15}N uptake experiments (e.g. Collos, 1992; Bronk *et al*., 1994). The results of this experiment, while on a longer time scale than most uptake experiments, indicate that this may be true. We suggest that when calculating the uptake of ^{15}N under experimental conditions that the leakage of N from tissue and subsequent dilution of tissue ^{15}N needs to be accounted for. In addition, it appears that macroalgae do not retain N for an entire growing season or even for the lifetime of an individual thallus; rather, uptake and

release is a continual, dynamic process that needs to be accounted for in estimates of the role of macroalgae in nutrient cycling.

Figures & Tables for Chapter 4: Macroalgal turnover of N

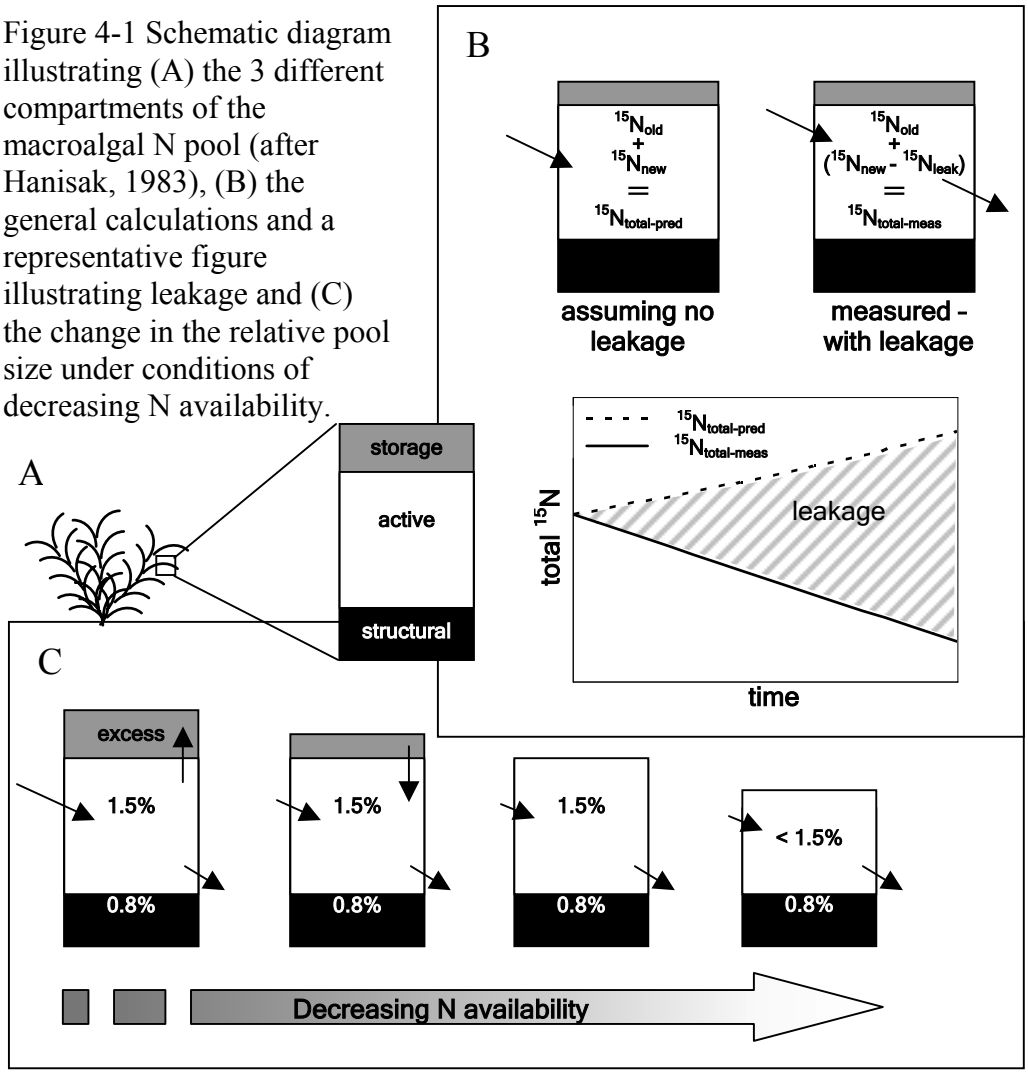

Figure 4-1 Schematic diagram illustrating (A) the 3 different compartments of the macroalgal N pool (after Hanisak, 1983), (B) the general calculations and a representative figure illustrating leakage and (C) the change in the relative pool size under conditions of decreasing N availability.

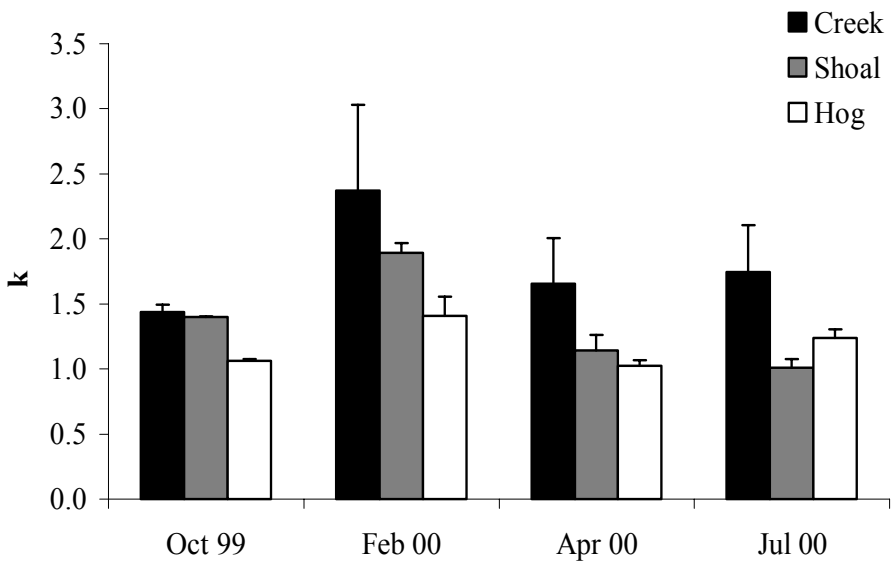

Figure 4-2 Light extinction coefficient (k) at each site estimated from measured light profiles. Error bars represent the standard error of the mean.

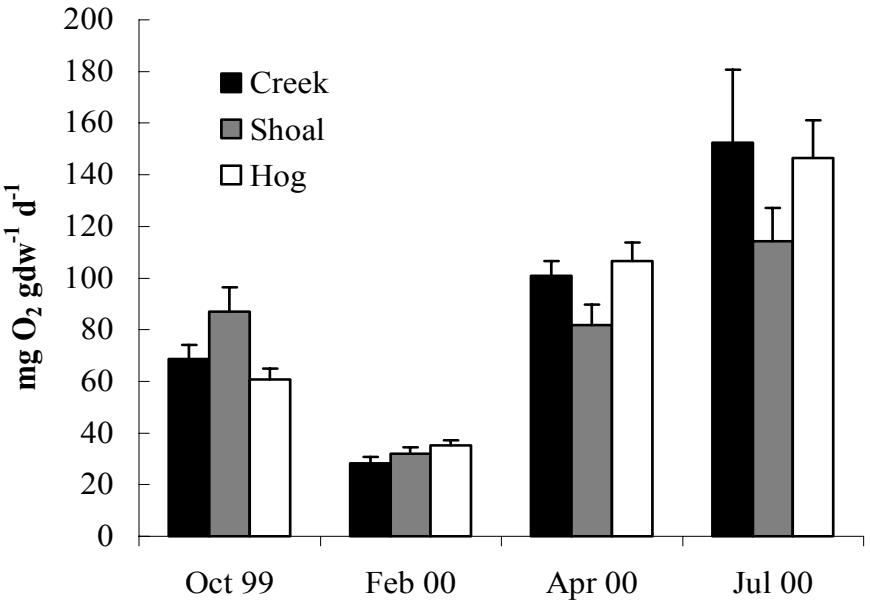

Figure 4-3 Daily dissolved oxygen production measured at the three sites. Error bars represent the combined standard error for light and dark measurements.

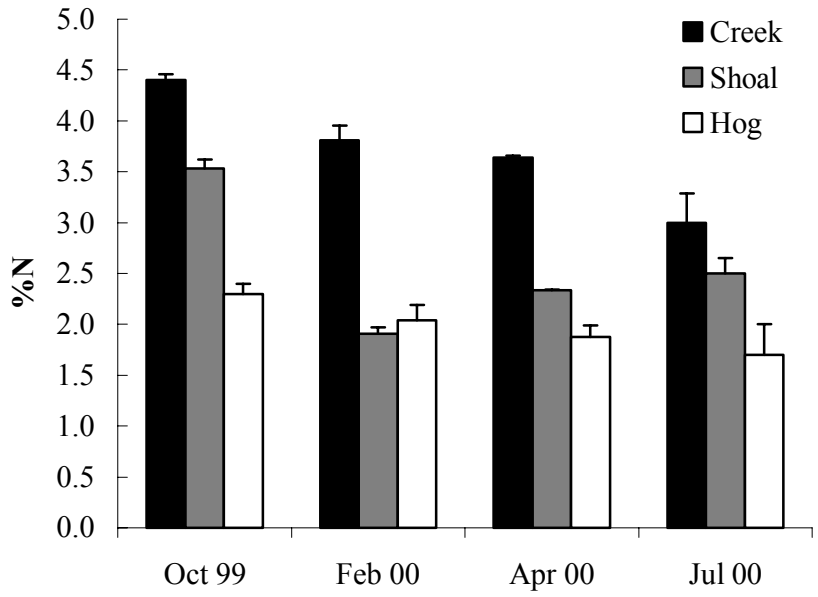

Figure 4-4 Percent nitrogen in *G. tikvahiae* tissue. Error bars represent the standard error of the mean.

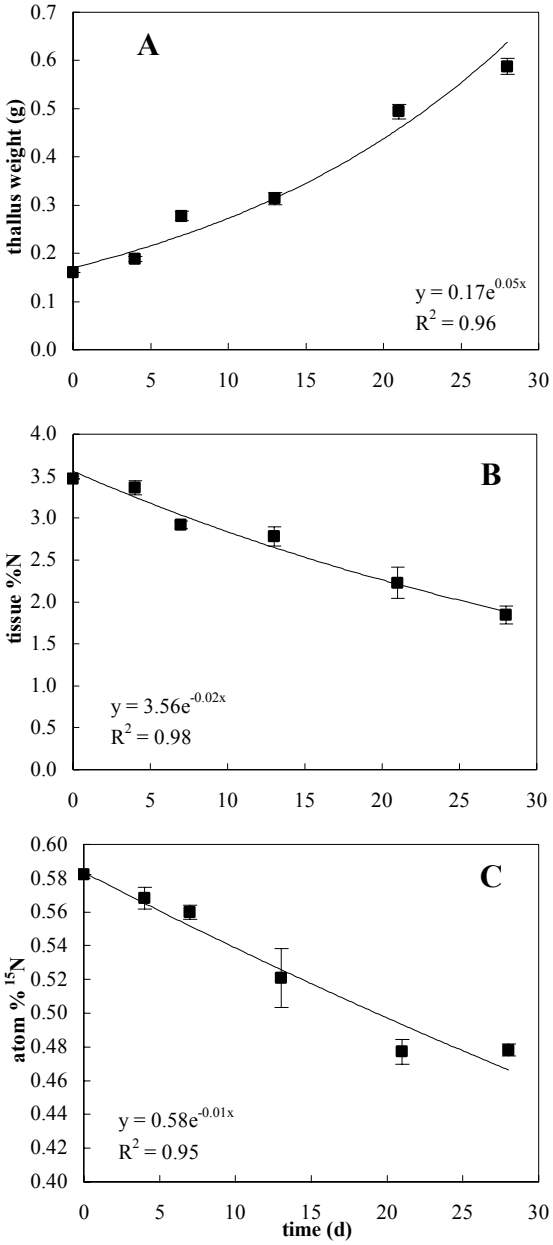

Figure 4-5 Results of the incubation of macroalgae measured in the laboratory for (A) thallus growth, (B) change in tissue %N and (C) tissue atom % ^{15}N. Best fit lines are shown with the associated equation and r^2 value. Error bars represent the standard error of the mean.

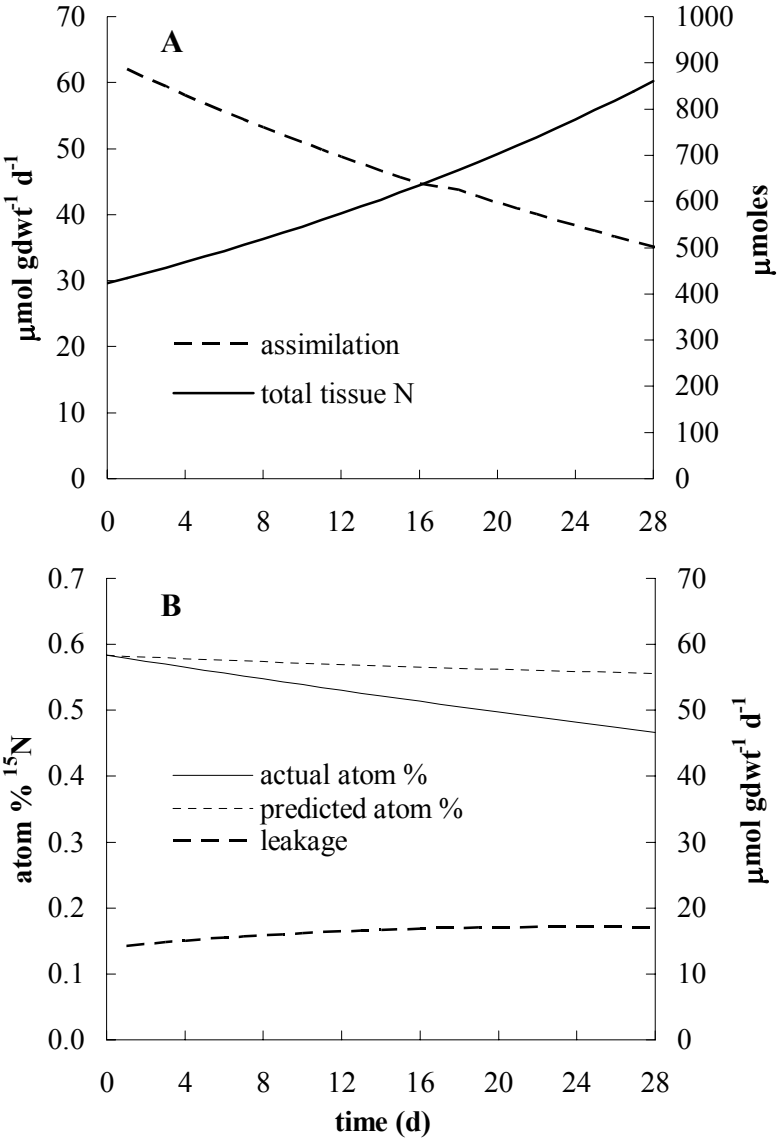

Figure 4-6 Estimated parameters for the calculation of leakage from the laboratory incubation. (A) Assimilation of new N g dw^{-1} and total thallus tissue N. (B) Actual atom % ^{15}N, predicted atom % ^{15}N based on uptake of new N and initial tissue values, and estimated leakage based on the difference between the predicted and actual atom % ^{15}N.

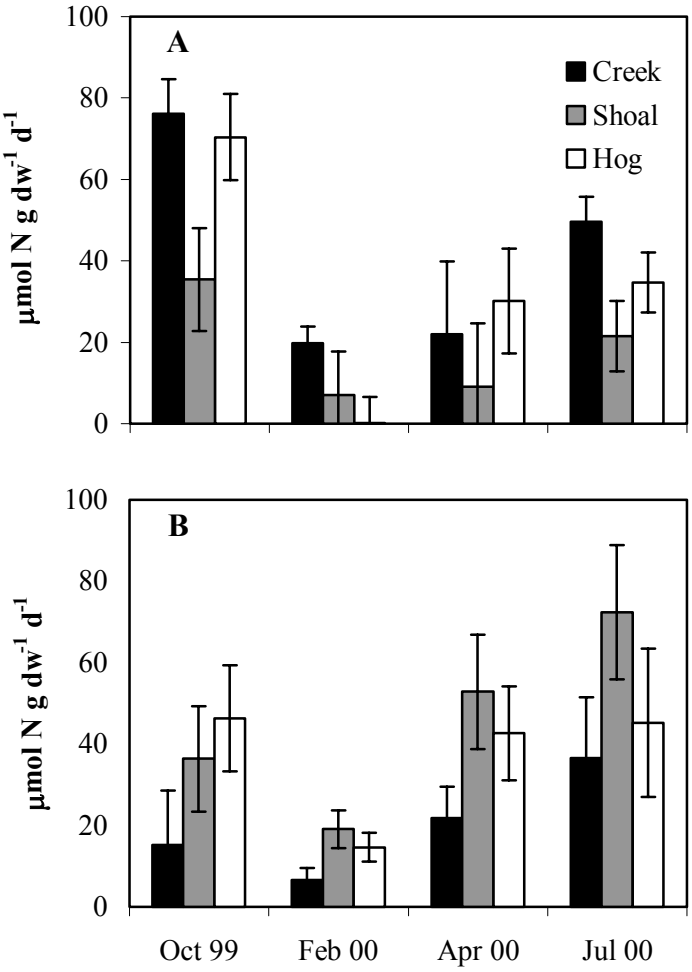

Figure 4-7 Daily assimilation and leakage rates for each site and season. Error bars represent the standard deviation of means calculated using the bootstrap procedure as described in the methods section.

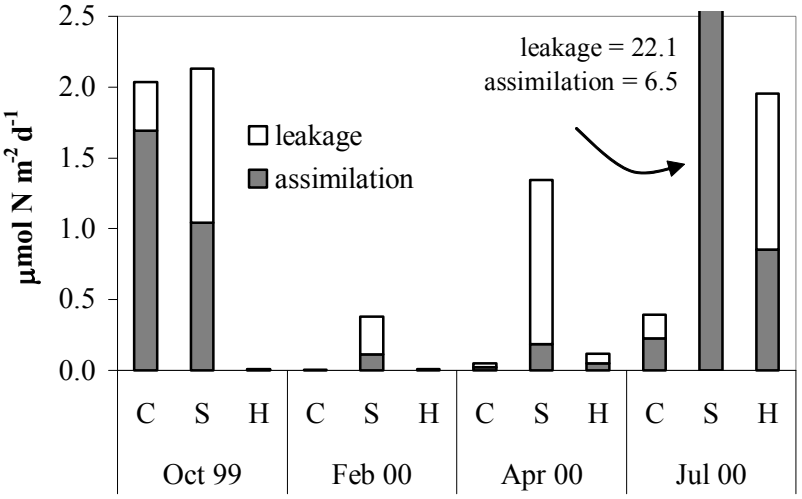

Figure 4-8 Areal assimilation and release of N for each season based on monthly mean biomass (from McGlathery *et al*. unpub. data) and calculated rates of assimilation and leakage. C = Creek, S = Shoal and H = Hog.

	thallus weight				%N				atom % ^{15}N			
	change (d^{-1})	F	p	r^2	change (d^{-1})	F	p	r^2	change (d^{-1})	F	p	r^2
Lab	4.7 ± 0.2	430	0.000	1.0	-2.2 ± 0.2	160	0.000	1.0	-0.8 ± 0.1	146	0.000	0.9
Oct 99												
Creek	2.4 ± 0.3	56	0.000	1.0	1.0 ± 0.7*	0	0.000	0.0	-1.2 ± 0.4	5	0.060	0.5
Shoal	2.3 ± 0.3	47	0.000	0.9	-1.0 ± 0.4	7	0.038	0.7	-1.5 ± 0.4	5	0.053	0.8
Hog	4.4 ± 0.3	194	0.005	1.0	-1.8 ± 0.4	21	0.002	0.9	-2.3 ± 0.4	27	0.002	0.8
Feb 00												
Creek	2.0 ± 0.2	101	0.000	0.9	-1.2 ± 0.0	820	0.000	1.0	-0.6 ± 0.1	24	0.001	0.9
Shoal	1.4 ± 0.5	8	0.018	0.6	-1.1 ± 0.1	74	0.001	0.9	-0.8 ± 0.1	120	0.058	1.0
Hog	1.5 ± 0.3	30	0.000	0.9	-1.5 ± 0.2	56	0.002	0.9	-0.6 ± 0.1	17	0.003	0.9
Apr 00												
Creek	2.6 ± ND†				-1.6 ± 0.9	3	0.104	0.8	-1.6 ± 0.6	7	0.038	0.5
Shoal	2.2 ± ND†				-1.8 ± 0.7	14	0.005	0.6	-2.2 ± 0.6	15	0.006	0.8
Hog	4.4 ± 0.6	51	0.000	0.9	-2.9 ± 0.4	44	0.000	0.9	-2.4 ± 0.5	25	0.000	0.9
Jul 00												
Creek	3.1 ± 0.2	192	0.000	0.9	-0.8 ± 0.2	16	0.001	0.6	-3.3 ± 0.5	52	0.000	1.0
Shoal	2.7 ± 0.4	57	0.000	0.9	-1.7 ± 0.3	36	0.000	0.8	-3.4 ± 0.4	61	0.000	0.9
Hog	4.0 ± 0.4	98	0.000	1.0	-2.3 ± 0.3	55	0.000	0.9	-3.7 ± 0.8	24	0.000	0.9

Table 4-1 Growth rates and change in %N and atom % ^{15}N during each experiment. All values are expressed as percent change d^{-1}. *Estimated using logistic equation, rather than exponential equation because of increase in N content over the course of the experiment. †Because fragmentation of thalli during the field incubation prevented an accurate estimate of growth, the site-specific relationship between DO production and growth rate was used instead.

Chapter 5
Uptake of urea and amino acids by *Ulva lactuca* (Chlorophyta) and *Gracilaria tikvahiae* (Rhodophyta)

Introduction

Primary productivity in temperate estuaries is often thought to be limited by nitrogen availability (Howarth, 1988). The majority of studies investigating nutrient uptake by and nutrient limitation of aquatic plants have focused on dissolved inorganic nitrogen (DIN), while organic nitrogen (DON), although acknowledged as an important component of the total dissolved nitrogen pool (Sharp, 1983), has largely been ignored as a potential nitrogen source. This may be due to the anthropogenically enhanced levels of inorganic nitrogen in well-studied temperate estuaries or perhaps to the ease of measurement of specific inorganic compounds (i.e. NH_4^+ and NO_3^-) relative to specific organic compounds (e.g. urea, amino acids). In shallow estuaries where sufficient light reaches the sediment surface, benthic macroalgae are important primary producers (Smith, 1981; Sand-Jensen and Borum, 1991; Valiela *et al*., 1997). While the uptake of inorganic compounds by various species of macroalgae has been quite well studied, relatively little is known about the uptake of organic nitrogen compounds (Lobban and Harrison, 1997). However, in recent years the importance of organic nitrogen in fulfilling the nitrogen demand, for both aquatic and terrestrial plants, has been more fully recognized (e.g. Antia *et al*., 1991; Chapin *et al*., 1993; Chisholm *et al*., 1996; Berg *et al*., 1997a; Turnbull *et al*., 1996; Mulholland *et al*., 1998; Nasholm *et al*., 1998).

Given the high turnover rate of urea in the water column, urea may be a substantial contributor to phytoplankton N demand (Cho *et al*., 1996), and may be important seasonally (Bronk and Glibert, 1993), or for specific bloom forming species

(e.g. Lomas *et al*., 1996; Glibert *et al*., 2001). Further, fluxes of urea from the sediments to the water column can be substantial (Boucher and Boucher-Rodoni, 1988; Lomstein *et al*., 1989; Lomstein *et al*., 1998; Tyler *et al*., 2001), thus urea may also be an important source of N for macroalgae situated at the sediment surface. Indeed, urea may provide the majority of the N demand for macroalgae when DIN availability is low (Chapter 3). Growth rates of macroalgae using urea-N can be equivalent to those using DIN (Probyn and Chapman, 1982; Thomas *et al*., 1985; Navarro-Angulo and Robledo, 1999). There have been, however, few studies of urea uptake kinetics in macroalgae (Probyn and Chapman, 1982).

While free amino acid concentrations are often quite low in seawater (Sharp, 1983), in low nutrient waters these compounds may represent a significant source of N to those organisms capable of uptake. Amino acid uptake has been demonstrated in both phytoplankton and benthic microalgae (Flynn and Butler, 1986; Antia *et al*., 1991; Nilsson and Sundback, 1996). Amino acid uptake in phytoplankton may occur by an extracellular mechanism, whereby free amino acids in the water column are split into NH_4^+, H_2O_2 and organic acids by cell-surface deaminating enzymes, and the NH_4^+ is subsequently taken into the cell (Palenik and Morel, 1990a; Palenik and Morel, 1990b; Pantoja and Lee, 1994). In low-nutrient waters, this mechanism may contribute up to 10% of the total NH_4^+ taken up by phytoplankton (Mulholland *et al*., 1998). The mechanism of amino acid uptake by macroalgae has not, to our knowledge, been investigated and few estimates of potential uptake and kinetic uptake parameters exist (Schmitz and Riffarth, 1979).

The objectives of the study presented here were to examine the uptake potential of various amino acids and urea by the two common estuarine macroalgal species *Gracilaria tikvahiae* (rhodophyta) and *Ulva lactuca* (chlorophyta). We have attempted to estimate the Michaelis-Menten kinetic uptake parameters for each compound under different environmental conditions (light, N availability, tissue N content), and have also attempted to gain some insight into the uptake mechanisms responsible for amino acid N assimilation. The results of these experiments may give some insight into the different adaptive strategies of these two species and show the potential ecological significance of organic nitrogen in macroalgal N nutrition.

Methods

Macroalgae used in the uptake experiments were collected from a mid-lagoon site in Hog Island Bay, VA during the summers of 1999 and 2000. Hog Island Bay, part of the Virginia Coast Reserve LTER site, is a small (150 km^2), shallow, back-barrier lagoon extending westward from the Delmarva Peninsula. The two species used, *Ulva lactuca* (chlorophyta) and *G. tikvahiae* (rhodophyta), together make up >80% of the total macroalgal biomass. Unless otherwise noted below, macroalgae were collected from the field, brought back to the laboratory in Charlottesville, VA and allowed to acclimate for 7 – 10 days in a Conviron™ Environmental Growth Chamber at a light intensity of 550 μE

m^{-2} s^{-1} (16 h light, 8 h dark) and approximately 20ºC. Macroalgae were maintained in shallow plastic tubs containing 6 L of low nutrient seawater collected from Machipongo Inlet, at the southern end of Hog Island, where the Atlantic Ocean enters Hog Island Bay. The water was continuously bubbled with air to ensure adequate water movement and aeration. Water was changed periodically, but no additional nutrients were added unless noted below. Uptake experiments were also conducted in this growth chamber.

For each experiment individual thalli (0.05 – 0.2 g dw) were incubated in 125 ml Erlenmeyer flasks containing 100 ml filtered (0.2 μm) seawater. Light intensity and temperature were maintained as above, unless otherwise noted. Flasks were shaken gently throughout the experiment to ensure mixing and prevent the build-up of a boundary layer at the macroalgal surface. In addition to filtration, we also used an antibiotic treatment for all uptake experiments (10 ppb ampicillin, 100 ppb erythromycin) in order to prevent bacterial mineralization of organic compounds and subsequent uptake of the mineralized N. The antibiotic decreased bacterial cells by >75% over untreated incubations ($p = 0.01$ based on acridine orange direct counts of bacteria, data not shown) and had no significant effect on macroalgal DO production or growth (DO production, $p = 0.2$; growth, $p = 0.2$, data not shown). At the conclusion of each experiment, thalli were removed from the flasks, rinsed 3x in fresh, filtered seawater and 1x in deionized water, patted dry, frozen and freeze dried. Dry weights were obtained, and where appropriate, samples were ground to homogeneity using a coffee mill for later carbon, nitrogen and stable isotope analysis.

Urea uptake experiments

Short term urea uptake experiment

To look closely at the initial (surge) uptake rates for *G. tikvahiae* and *U. lactuca*, we conducted a series of experiments with samplings at very short time intervals (0, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 15 and 20 min). The starting urea concentration (approximately 8 μM) was measured for each flask immediately before adding the macroalgae. We used low N macroalgae that received no additional nutrients during the laboratory assimilation period, and moderate N macroalgae that received a low level of N fertilization during the assimilation period. Moderate N algae received sufficient N to maintain a tissue content of 2.5% N at an estimated growth rate of 10% d^{-1}. This amount was satisfactory for *G. tikvahiae* (unfertilized = 2.02%, fertilized = 2.46%), but was insufficient for *U. lactuca* (unfertilized = 1.49%, fertilized = 1.72%), probably because of higher growth rates. Thus, for *U. lactuca*, the results are not necessarily indicative of uptake rates for N sufficient tissue.

Estimate of kinetic uptake parameters for urea

In order to estimate the kinetic uptake parameters defined by the Michaelis-Menten equation, uptake of urea by *U. lactuca* was measured over a 30 minute period. Several different initial concentrations of urea were used, ranging from 3 – 17 μmol, and samples were taken at 0, 2, 5, 10, 20 and 30 minutes. Rates were estimated for low-N and moderate-N *U. lactuca*, as described in the previous section. Kinetic uptake

parameters were estimated separately (calculations described below) for "surge" uptake (0 – 2 min) and "sustained" uptake (20-30 min).

Effect of light and ammonium on urea uptake

The effect of NH_4^+ and both conditioned and experimental light intensity on urea uptake by both *G. tikvahiae* and *U. lactuca* was examined in a 3 x 2 x 2 factorial design experiment, with three combinations of nutrient additions (Urea, Urea + NH_4^+ and NH_4^+ only), two levels of conditioned light (low = ~50 μE m^{-2} s^{-1}, ~550 high μE m^{-2} s^{-1}) and two levels of experimental light intensity (dark and light). Initial urea and ammonium concentrations were 10 μM and 15 μM, respectively. Samples were taken at 0, 10, 30, 60, 100 and 150 minutes after addition of macroalgae. A three-way ANOVA was performed separately on initial (0-10 min) and sustained (10 – 150 min) uptake rates for each species separately. A one-way ANOVA was performed on pooled data to compare overall rates between the two species.

Amino acid uptake

Uptake of common amino acids

The uptake of amino acids by *U. lactuca* and *G. tikvahiae* was measured using ^{15}N and ^{13}C labeled amino acids obtained from Isotec (Sigma Aldrich). Amino acid N assimilation was measured for glycine, alanine, serine, aspartic acid, glutamic acid and aminobutyrate (Figure 5-1). Experiments were conducted in 80 ml of water, as described

above, at initial amino acid concentrations of 0.2, 0.5, 1.5, 5 and 10 μM. Thalli were removed after 30 minutes and processed as described above.

Comparison between uptake methods

In order to estimate the validity of the ^{15}N uptake method, we did a comparison between uptake of ALA and NH_4^+, separately and in combination, using the ^{15}N method and using traditional calculations based on the disappearance of the nutrient from solution. Initial concentrations were 5 and 15 μM of alanine and NH_4^+, respectively. Alanine and NH_4^+ concentrations were measured prior to macroalgal addition (*G. tikvahiae* and *U. lactuca*), and at 1, 5, 10 15 and 30 minutes after addition. For comparison between the 2 methods, total uptake was calculated between 0 and 30 minutes, with an adjustment for the removal of each sample volume. The relationship between the two methods was analyzed using a linear least squares regression. This experiment also allowed us to determine potential "surge" uptake of amino acid during the first minute of exposure as well as the impact of NH_4^+ on alanine uptake.

Amino acid uptake in the light and dark

The effect of light and dark was measured for both species using glycine, at an initial concentration of 9.1 μM. After acclimation in the laboratory for 10 days, thalli were separated into smaller pieces (1 – 2 g ww) and held overnight under ambient light or in complete darkness. Individual thalli were incubated in separate flasks, with removal from solution at 0.5, 2 and 4 h.

Amino acid uptake following N fertilization

The effect of N status (N replete or N starved) on amino acid uptake rates was examined using macroalgae that had been held in the laboratory for 10 days in low nutrient seawater (as above), fertilized with NH_4Cl or fertilized with $NaNO_3$. The fertilization rates were sufficient to sustain an approximate growth rate of 5% d^{-1} and a tissue N content of 4%. The actual tissue %N values at the time of the experiment for the low N, NH_4^+ and NO_3^- treatments were 2.1 ± 0.1, 4.6 ± 0.1 and 4.1 ± 0.2 for *U. lactuca* and 2.5 ± 0.1, 4.9 ± 0.1 and 3.1 ± 0.1 for *G. tikvahiae*, respectively. Uptake rates for alanine and glycine were measured at an initial concentration of 5 μM; thalli were removed after 30 min and processed as described above. Statistical differences between uptake rates were identified using a one-way ANOVA with Tukey's HSD post-hoc test.

Amino acid carbon assimilation

Simultaneous assimilation of glycine and alanine carbon was estimated for both the C_1 (carboxyl carbon) and C_2 (central carbon) atoms (separately) using solutions containing 50% $^{15}N\ ^{12}C_{1\ and\ 2}$ and 50% $^{14}N\ ^{13}C_{1\ or\ 2}$ amino acid. C_2 uptake was measured at a variety of different initial concentrations; C_1 uptake was measured only at an initial concentration of 5 μM. Alanine and glycine were chosen because Palenik and Morel (1990a) demonstrated that alanine is active and glycine is inactive in the cell-surface amino acid oxidase system in phytoplankton. Thus, by examining the uptake of both N and C, we may gain insight into the mechanism whereby these amino acids are utilized

by macroalgae. The relationship between the uptake of ^{15}N and the uptake $^{13}C_2$ was analyzed using linear regression.

Nutrient Analyses

Ammonium was measured using the phenol-hypochlorite method (Solorzano, 1969). Urea was measured using a modification of the methods described by Mulvenna & Savidge (1992) and Goeyens et al. (1998). Alanine concentrations were determined by pre-column derivatization with *o*-phthaldialdehyde, separation by HPLC using a two eluent gradient (eluent 1: 80% NaAc buffer, 19% HPLC grade methanol, 1% tetrahydrofuran; eluent 2: 80% HPLC grade methanol, 20% NaAc buffer; Gilson 231 Autosampler and 401 Dilutor; Dionex 4000 Gradient Pump; Alltech Guard Column and Adsorbosphere OPA HR Separator Column), and detection by fluorescence (St. John's Associates Fluorescence detector Gorzelska and Galloway, 1990; Jones *et al*., 1981).

Calculations

Uptake rates (V (μmol g dw^{-1} h^{-1})) for urea, alanine and NH_4^+ based on the disappearance of the substrate in solution, were calculated for each time interval according to Equation 1:

$$V = \frac{\mu molN_i - \mu molN_f}{t \times B} \quad (1)$$

where μmol N_i and μmol N_f are the initial and final quantities of substrate in solution, t is time in hours and B is the dry weight of the algal thallus. In order to obtain the kinetic uptake parameters representing the maximum uptake rate (V_{max}) and the half saturation constant (K_m) for each nutrient, the Michaelis-Menten function was fitted to the relationship between V and the mean substrate concentration, S (in μM), for each time interval using non-linear, least squares regression according to Equation 2:

$$V = \frac{V_{\max} \times S}{K_m + S} \tag{2}$$

An estimate of the uptake affinity for each nutrient at low substrate concentrations was obtained based on the initial slope of the V versus S curve, defined as V_{max}/K_m (Healy, 1980). Uptake of ^{15}N labeled amino acids and ammonium was calculated according to Equation 3:

$$V = \frac{\left(N_f \times atom\%^{15}N_f\right) - \left(N_i \times atom\%^{15}N_i\right)}{atom\%^{15}N_{so\ln} \times t} \tag{3}$$

where N_f and N_i are the final and initial, respectively, tissue N content in μmol g dw^{-1}, atom % $^{15}N_f$ and atom % $^{15}N_i$ are the final and initial tissue atom percent, and atom % $^{15}N_{soln}$ is the ^{15}N content of the solution. N_f and N_i were calculated as the sum of the tissue $^{14}N_{i\ or\ f}$ and $^{15}N_{i\ or\ f}$, which were calculated as the product of the total tissue $N_{i\ or\ f}$ (g

N g dw^{-1}) and the atom $\%^{14}N_{i\ or\ f}$ or atom $\%^{15}N_{i\ or\ f}$, respectively. The average substrate concentration (in μM) over the time interval was calculated by averaging the initial substrate concentration and the initial concentration less the total uptake for each thallus (corrected for the volume of liquid in the flask).

Results

Urea uptake experiments

Short term urea uptake experiment

The uptake rate of urea as a function of time for the short term incubation experiments is shown in Figure 5-2. *U. lactuca* had a substantially higher rate of urea uptake than *G. tikvahiae* during the first 5 minutes, with rates >120 μmol g dw^{-1} h^{-1} in the first 30 seconds after exposure to urea. The maximum uptake rate measured for *G. tikvahiae* was approximately 40 μmol g dw^{-1} h^{-1}, during the interval between 1 and 2 min, but there was substantial variability in this measurement. For both species, the uptake rate decreased to a sustained rate between 5 and 15 min (~15 – 20 μmol g dw^{-1} h^{-1} for *U. lactuca*, 5-8 μmol g dw^{-1} $h^{-}1$ for *G. tikvahiae)* and trailed to 8 μmol g dw^{-1} h^{-1} for *U. lactuca* and 4 μmol g dw^{-1} h^{-1} for *G. tikvahiae* between 15 and 20 minutes. There were

no apparent differences between uptake rates of low or moderate tissue N macroalgae for either species.

Estimate of kinetic uptake parameters for urea

The Michaelis-Menten uptake curves for *U. lactuca* (V versus S) are distinctly different for surge uptake and sustained uptake (Figure 5-3). V_{max} and K_m were similar between the low N and moderate N macroalgae, and the affinity for urea as estimated by the initial slope (V_{max}/K_m) was likewise similar (4.3 – 4.5; Table 5-1). We were unable to estimate the kinetic uptake parameters for sustained uptake by the moderate tissue N macroalgae because the uptake rate did not level off, even at the highest concentrations. K_m was similar for surge and sustained uptake in the low N macroalgae (14 μM surge, 17 μM sustained), but the sustained V_{max} was 35% of the surge V_{max}, with a similarly decreased affinity (1.3).

Effect of light and ammonium on urea uptake

There was only a slightly higher initial uptake measured from 0 – 10 min in the light (17.8 light vs 15.6 dark; $F = 5.7$, $p = 0.02$) and higher sustained uptake rates of urea in the presence of ammonium (urea only – 4.6 ± 0.2 vs U + A 5.6 ± 0.3; $F = 6.25$, $p = 0.014$) for *U. lactuca*, but no other significant effects or interactions of treatments. The pooled results for each treatment type are shown in Table 5-2. There were highly significant differences between species for both the initial (all conditions U – 16.6 ± 0.5; G – 12.2 ± 0.7; $F = 28.4$, $p < 0.0001$) and sustained (all conditions U – 5.1 ± 0.2; G – 2.3 ± 0.1; $F = 149.1$, $p < 0.0001$) uptake rates.

Amino acid uptake experiments

Uptake of common amino acids

We observed high variability of Michaelis-Menten uptake curves and resulting kinetic uptake parameters among the different amino acids (Figure 5-4; Table 5-3). Where uptake rates were very low, the uptake parameters could not be reliably estimated using least squares non-linear regression; we have included the maximum measured uptake rate as an estimate of the potential V_{max} for these amino acids. Uptake rates for *U. lactuca* were consistently higher than rates for *G. tikvahiae*. Overall, V_{max} was highest for glycine for both species (*U. lactuca* 5.7 μmol g dw^{-1} h^{-1}, *G. tikvahiae* 1.8 μmol g dw^{-1} h^{-1}) and lowest for aminobutyric acid. However, the maximum observed uptake rates and the estimated affinities were consistently greatest for alanine.

Comparison between uptake methods

The concentration of alanine and NH_4^+ as a function of time for the disappearance from solution method demonstrated that both species were capable of alanine uptake, but *U. lactuca* was capable of much higher uptake rates (Figure 5-6;Table 5-4). There was no apparent impact of the presence of NH_4^+ on alanine uptake for either species, and vice versa (data not shown). Both species exhibited initially high surge uptake rates for ammonium, but not alanine. The estimated uptake rates of alanine and ammonium obtained for the two methods (Table 5-4) were consistent with one another and the statistical relationship was highly significant ($r^2 = 0.99$, $F = 1191$, $p < 0.0001$). The ^{15}N

method underestimated the uptake based on traditional disappearance methods by approximately 14% (Figure 5-7).

Amino acid uptake in the light and dark

There was no difference between the uptake rates of glycine in the light or dark for either species (Figure 5-5). The change in tissue ^{15}N was linear with respect to time for *G. tikvahiae* but exhibited a hyperbola shape for *U. lactuca*. Likewise, the uptake rate (1 – 1.6 μmol g dw^{-1} h^{-1}) for *G. tikvahiae* was relatively constant over the 4 h incubation, whereas the rate for *U. lactuca* was initially 3.3 μmol g dw^{-1} h^{-1} and leveled off to 1.4 μmol g dw^{-1} h^{-1} after 4 h.

Amino acid uptake following N fertilization

Nitrogen status had a significant effect on glycine and alanine uptake rates for *U. lactuca*, but not for *G. tikvahiae* (Table 5-5). All rates for *G. tikvahiae* were statistically similar, while glycine uptake by *U. lactuca* was significantly higher for low N and NH_4^+ fertilized thalli than for NO_3^- fertilized thalli. In contrast, alanine uptake by *U. lactuca* was greatest for the low N treatment than for either the NH_4^+ or NO_3^- fertilization treatments, which had similar uptake rates.

Amino acid carbon assimilation

For the simultaneous uptake of glycine and alanine ^{15}N and $^{13}C_2$, we would expect a ratio of ^{15}N:^{13}C of 0.52 and 0.37 for glycine and alanine, respectively, based on the initial atom % ^{15}N and ^{13}C of the solution. There was a significant relationship between

glycine ^{15}N and $^{13}C_2$ assimilation for *U. lactuca* ($r^2 = 0.41$, $F = 5.6$, $p = .04$), with a ratio of 0.62 (Figure 5-8). There was no significant relationship between ^{15}N and $^{13}C_2$ uptake for alanine in either species. For the simultaneous uptake of ^{15}N and $^{13}C_1$ we would expect a ratio of ^{15}N:^{13}C of 0.53 and 0.40 for glycine and alanine, respectively. The observed ratio for both amino acids and all species was approximately 0.1 and was not significant.

Discussion

It is clear from the experiments presented here that when concentrations of small, labile organic nitrogen compounds are sufficiently high, organic nitrogen has the potential to contribute a significant quantity of N to the overall N demand of estuarine macroalgae. The kinetic uptake parameters for urea and the various amino acids vary substantially between compounds, suggesting variable uptake mechanisms. The difference in uptake rates between species denotes that the importance of organic compounds may vary substantially between species of macroalgae, and may signify different adaptive strategies.

Urea uptake

Surge and sustained uptake

U. lactuca and *G. tikvahiae* clearly exhibit two distinct uptake rates for urea: a very high initial uptake rate and a much lower sustained, or assimilation rate. A similar phenomenon has been shown for ammonium uptake by a variety of macroalgae (e.g. Fujita, 1985; Harrison *et al*., 1989; McGlathery *et al*., 1996; Pedersen and Borum, 1997) and for urea by the brown macroalgae *Chordaria flagelliformis* (Probyn and Chapman, 1982). The change in these rates may be due to the rapid filling of internal pools, beyond which uptake proceeds more slowly due to feedback inhibition (Fujita *et al*., 1988; Harrison *et al*., 1989). The uptake by *U. lactuca* during the first 30 seconds of exposure (~120 μmol g dw^{-1} h^{-1}) is somewhat higher than the V_{max} observed during initial exposure for *C. flagelliformis* (V_{max} = 51-66 μmol g dw^{-1} h^{-1}; Probyn and Chapman, 1982), but this is probably because of the very short time interval over which we measured surge uptake. In a separate experiment with *U. lactuca*, (Table 5-1; Figure 5-3) we estimated a comparable V_{max} (65 – 76 μmol g dw^{-1} h^{-1}) over the first 2 min of the incubation, but a lower V_{max} during the first 10 min (Table 5-2). The differences between experiments were most likely due to the differences in initial substrate concentrations. *G. tikvahiae* also exhibited a slightly higher surge uptake rate, but this rate remained much lower than that measured for *U. lactuca*. Tissue N status did not appear to affect either sustained or surge uptake, although the differences between treatments (%N) were small. A greater difference between tissue N levels may have

yielded results similar to McGlathery *et al*. (1996), who showed that the maximum rate and duration of surge uptake is greater for N depleted macroalgae. Overall, the results of the short term experiment indicate that the intracellular urea pools of both species fill rapidly (within 15 min) upon exposure to urea, and that sustained uptake rates can be satisfactorily estimated after 15 minutes of incubation.

The sustained assimilation rates, which were much lower than the initial surge uptake, show a similar relative difference between species in all experiments (Table 5-2). The sustained V_{max} for *C. flagelliformis* was 13 μmol g dw^{-1} h^{-1} (Probyn and Chapman, 1982), which is somewhat higher. However, the results in Table 5-2 represent rates averaged over approximately 2 hours, with a similar initial urea concentration for all measurements, and are thus not intended to represent an estimate of V_{max}. Urea clearly exhibited saturatable uptake kinetics Figure 5-3, which indicates an active uptake process (Lobban and Harrison, 1997). While light history did not appear to be a factor for either species, incubation light intensity was a factor in uptake by *U. lactuca*. Lower uptake rates of urea after prolonged darkness (6 hr) have also been measured in long-term experiments with *C. flagelliformis* (Probyn and Chapman, 1982) as well as for the mat-forming cyanobacteria *Lyngbya gracialis* (Rondell *et al*., 2000). This further suggests that uptake and assimilation of urea is an active, energy demanding process. The greater uptake of urea in the presence of ammonium by *U. lactuca* is more difficult to explain. This result does indicate, however, that the uptake mechanisms for these two molecules are distinct, and that the presence of one does not inhibit uptake of the other.

Affinity at low concentrations

The affinity of *U. lactuca* for urea at low concentrations, as described by the initial slope of the uptake curve (4.5 for surge, 1.3 for sustained, Table 5-1), was low relative to the affinity of *U. lactuca* for ammonium (~12; Pedersen and Borum, 1997), but similar to the affinity for nitrate (~4.5; Pedersen and Borum, 1997), indicating that urea may represent an equally desirable N substrate under low NH_4^+ conditions. K_m for urea during surge and sustained uptake was similar (14 μM for surge, 17 μM for sustained, Table 5-1), and falls within the same range of K_m values measured for ammonium uptake by Pedersen (6 – 21 μM; 1997), but was less than that reported by Fujita (1985). This is consistent with *C. flagelliformis*, which also had a similar K_m for urea and ammonium (Probyn and Chapman, 1982). Although the affinity for urea at low concentrations was less than that for NH_4^+, it appears that urea has the potential to provide a significant quantity of N to both species examined here, when environmental concentrations are sufficiently high.

Amino acid uptake

Comparison between amino acid uptake methods

The lower (14%) uptake of alanine and NH_4^+ estimated by ^{15}N assimilation is consistent with other studies that suggest that uptake measured by ^{15}N assimilation routinely underestimates the actual N uptake because of the potential for adsorption onto the glass walls of the flask and the rapid turnover and re-release of N (e.g. Naldi and

Wheeler, 2002; Williams and Fisher, 1985). We utilized a short incubation time in the present study to eliminate potential re-release of the ^{15}N label, so re-release likely contributes only a small percentage of our underestimate. Schmitz and Riffarth (1979), in a study of ^{14}C labeled L-leucine uptake by the brown algae *Giffordia mitchellae*, demonstrated that repeated rinsing of macroalgal tissue after exposure to L-Leucine resulted in the release of 13% of the ^{14}C originally removed from solution. They presumed that this release was from L-leucine incorporated into the apoplast, but not yet assimilated into the cell proper. In the present study, a similar loss may have occurred during the rinsing of macroalgal thalli following removal from the incubation solution. This would result in a loss of the ^{15}N label from the tissue and hence a lower estimate of ^{15}N assimilation. However, the two methods agree quite well at both low (*G. tikvahiae*) and high (*U. lactuca*) uptake rates of alanine or NH_4^+. Yet, because of the potential for rapid loss of ^{15}N removed from solution, we caution that the rates reported here based on ^{15}N and ^{13}C uptake represent assimilation rates, rather than maximum uptake rates.

Uptake of different amino acids

Our data suggests that neutral amino acids may have the highest uptake rates in macroalgae, and that the potential for uptake is not necessarily proportional to the relative concentration of these amino acids in the environment (Chapter 3). The maximum uptake rate of amino acid N varies substantially between different types of amino acids, ranging from 0.1 to greater than 5 μmol g dw^{-1} h^{-1}. We measured the highest maximum uptake rates for the two aliphatic neutral amino acids glycine and

leucine; the lowest rate was also measured for an aliphatic neutral amino acid, aminobutyrate. Serine, an aliphatic hydroxy amino acid, was intermediate and the two acidic amino acids, aspartic and glutamic acids, had the lowest rates. As described further below, even among chemically similar molecules the mechanism for uptake appears to vary. Amino acid uptake rates for *U. lactuca* were higher than *G. tikvahiae* for all amino acids, as they were for urea. However, for the amino acids that we could reliably calculate K_m, the half-saturation constant was similar between the two species. The kinetic uptake parameters for L-leucine estimated by Schmitz and Riffarth (1979) for the brown algae *Giffordia mitchellae* were relatively high (V_{max} = 30-38 μmol g dw^{-1} h^{-1}, K_m = 3-14 μM); however, these rates were measured at very high (~1 mM amino acid-N) concentrations and may never be realized at the low concentrations found under field conditions. As such, the authors suggest that a more realistic estimate of amino acid uptake in the field is 0.3 μmol mol g dw^{-1} h^{-1}, if all amino acids have similar uptake parameters as L-leucine.

In Chapter 3, the results of the incubation of *U. lactuca* with sediments from Hog Island Bay were presented. In these experiments, we observed the highest uptake rates for glycine (124 nmol N g dw^{-1} d^{-1}), serine (89 nmol N g dw^{-1} d^{-1}), arginine (62 nmol N g dw^{-1} d^{-1}) and alanine (36 nmol N g dw^{-1} d^{-1}). Uptake rates for glutamic acid (9 nmol N g dw^{-1} d^{-1}), aminobutyric acid (13 nmol N g dw^{-1} d^{-1}) and aspartic acid (21 nmol N g dw^{-1} d^{-1}) were substantially lower, in spite of small differences in relative concentrations (Chapter 3). Glycine and serine were preferentially taken up, at rates higher than their relative concentrations to other amino acids in the water column. The results from direct

uptake experiments agree well with these flux results, with the exception of alanine. Alanine concentrations were generally low in Hog Island Bay, and this may explain the apparent discrepancy.

Mechanism of uptake

The lack of surge uptake of alanine for both species suggests that the mechanism of uptake, although clearly saturatable, differs from that for urea and NH_4^+. However, the uptake mechanism for alanine also may differ from that for glycine and other amino acids, and thus this result is not necessarily generalizable across all amino acids. Based on the low ratio of $^{15}N:^{13}C_1$ uptake, it seems that both amino acids are decarboxylated prior to uptake. However, based on the $^{15}N:^{13}C2_1$ uptake ratio, the remainder of the glycine molecule was clearly assimilated intact by *U. lactuca* (Figure 5-8). Glycine uptake was similar in the light and dark, suggesting that uptake may not be a terribly energy demanding process. Alanine, on the other hand, appears to be deaminated prior to uptake and only the amino group is assimilated. This is consistent with the results of Palenik and Morel (1990a) who found that the cell surface amino acid oxidase enzyme of phytoplankton is selective for specific L-amino acids. Of those measured in this study, they report that aspartic acid, glutamic acid, aminobutyrate and alanine are active; glycine and serine are inactive (Palenik and Morel, 1990a). The lack of a relationship in *G. tikvahiae* between N and C uptake may be due to the low overall uptake rate, or to the existence of a different uptake mechanism altogether.

The variability in uptake rates for fertilized versus unfertilized macroalgae also gives some insight into the mechanisms of amino acid uptake (Table 5-5). The low uptake rate of glycine by NO_3^- fertilized compared to N starved or NH_4^+ fertilized *U. lactuca* suggests that the glycine uptake mechanism was induced by both N starvation and NH_4^+ fertilization, but not by NO_3^- fertilization. Uptake of amino acids is also inhibited by NO_3^- in certain species of benthic microalgae; however, the effect is variable between different amino acids (Admiraal *et al*., 1984). In contrast, alanine uptake was high only after N-starvation. This may indicate that alanine uptake is induced only when necessary and that fertilization with either NO_3^- or NH_4^+ shuts off this potentially energy-demanding process. Further, we found no inhibition of alanine uptake by NH_4^+, in contrast to reported NH_4^+ inhibition of uptake in phytoplankton (Palenik and Morel, 1990b), which suggests that when N limited, *U. lactuca* is capable of simultaneous uptake of both amino acids and NH_4^+. These results confirm that *U. lactuca* assimilates glycine and alanine by different mechanisms.

The K_m values for alanine uptake in both *U. lactuca* and *G. tikvahiae* were similar to K_m values for phytoplankton (Palenik and Morel, 1990b), and were much lower than those measured in this study for urea. The affinity of both macroalgal species for alanine was likewise high. V_{max} for the amino acids examined here, estimated based on uptake kinetics or measured at 10 μM amino acid N, were all somewhat lower than the V_{max} for urea. These results suggest that the amino acid uptake mechanism in macroalgae is specific for low concentrations of amino acids, as are commonly found in shallow, estuarine environments.

Ecological significance

The observed variation in uptake kinetics between N-containing compounds suggests that there are distinctly different nutrient delivery mechanisms in the environment. Rapid uptake of nutrients and subsequent storage in macroalgal tissue allows for the temporal separation of nutrient uptake and growth, and may permit higher growth rates during periods of low nutrient availability (Fujita, 1985)**.** Both *Ulva* sp. and *Gracilaria* sp. are capable of storage of N in excess of growth demands (Ryther *et al*., 1981; Bird *et al*., 1982; Rosenberg and Ramus, 1982;Fujita, 1985). However the tissue turnover in *Ulva* is typically greater than in *Gracilaria*, which may allow *Gracilaria* to persist for a longer time under low nutrient conditions (Rosenberg and Ramus, 1982; Fujita, 1985). Both species exhibit similar "surge" uptake patterns for NH_4^+ uptake (Fujita, 1985), but *U. lactuca* clearly has an advantage over *G. tikvahiae* if urea is an important component of the pulsed N input to the system. The lack of a "surge" uptake for alanine suggests that the amino acid supply is not likely to come as "pulses", but rather may be a low, but consistent supply based on organic matter turnover in the water column or sediments. This would be consistent with the very rapid turnover of amino acids in seawater (Tupas and Koike, 1990). The high affinity and low K_m suggests that macroalgae, particularly *U. lactuca*, can take advantage of even very low amino acids concentrations. The ability to utilize amino acids may provide a competitive advantage to those species capable of uptake (Nilsson and Sundback, 1996).

In incubation experiments with sediments from Hog Island Bay, VA, uptake of both urea and amino acids by *U. lactuca* had a significant impact on both water column concentrations of urea and free amino acids and fluxes between the sediment and the water column. Total free amino acid concentrations ranged from 0.02 – 0.43 μM in Hog Island Bay (chapter 3). Overall free amino acid uptake, from both sediment and water column sources, was 0.43 μmol N g dw^{-1} d^{-1} (n = 50; chapter 3). Glycine, generally ~14% of the water column free amino acid pool, had concentrations ranging from 3 – 60 nM. At these concentrations, the uptake rate by *U. lactuca* would be 0.03 – 0.52 μmol g dw^{-1} h^{-1}, which indicates that *U. lactuca* has the potential to significantly impact water column glycine concentrations. This is true for all of the amino acids studied in this experiment. Likewise, the average concentration of urea in the waters of Hog Island Bay is low (0.7 uM), with a range from 0.2 – 2.3 μM and it constitutes only 2 – 10% of the standing stock TDN pool. Given the kinetic uptake parameters measured for *U. lactuca* in the present study, this would result in potential sustained uptake rates between 0.3 and 2.7 μmol g dw^{-1} h^{-1}. In addition, fluxes of urea from the sediments at times can be substantially greater than the concurrent dissolved inorganic nitrogen flux (range = -184 – 1327 μmol m^{-2} d^{-1}; Chapter 3). Where present at significantly high densities, macroalgae are capable of intercepting all urea effluxing from the sediments. This is particularly true because of the capacity for very rapid uptake of transient urea pulses by both *U. lactuca* and *G. tikvahiae*.

Overall, the importance of organic nitrogen to macroalgal N nutrition depends on the availability of both dissolved inorganic and organic nitrogen compounds. In Chapter

3, we demonstrated that where the inorganic N supply (from sediments and/or water column) was high, the total N uptake by macroalgae was high and amino acids and urea played a relatively small role in total N uptake (2-3% and 10-20%, respectively). Where dissolved inorganic nitrogen was low, either routinely or seasonally, total N uptake was also low, and both compounds played a much more important role (20% and 40%, respectively). However, uptake of the organic compounds measured in this study was not inhibited by ammonium, the major inorganic nitrogen component of both the water column and sediment effluxes in Hog Island Bay (Chapter 3), which suggests that the uptake of urea and amino acids is dependent only on the local availability.

Figures and Tables for Chapter 5: Uptake of dissolved organic nitrogen

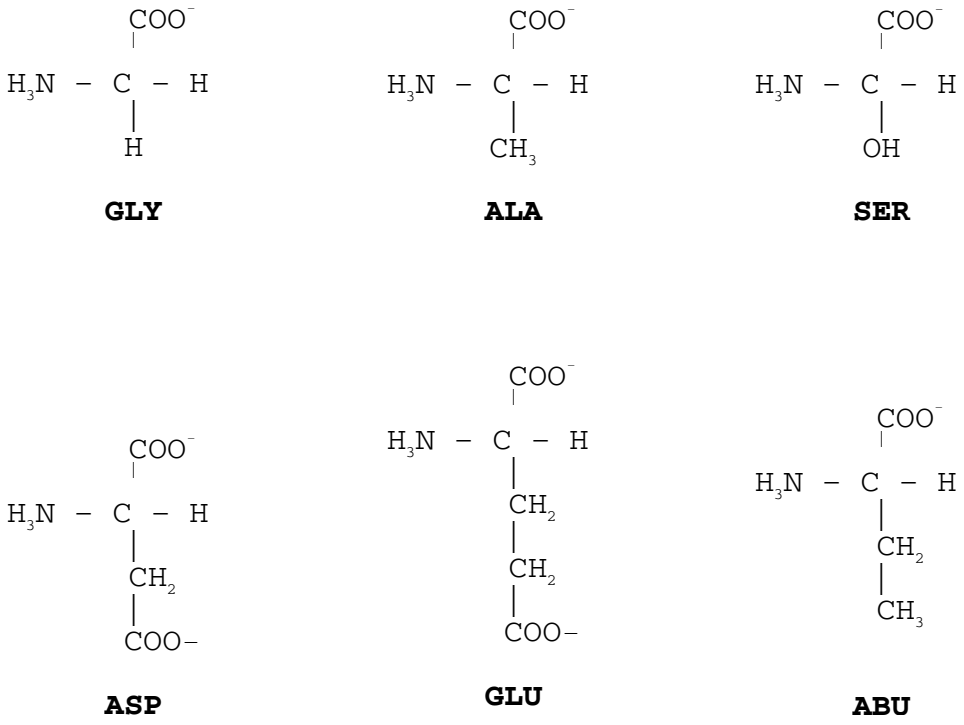

Figure 5-1 Chemical structures of amino acids used in the uptake experiments.

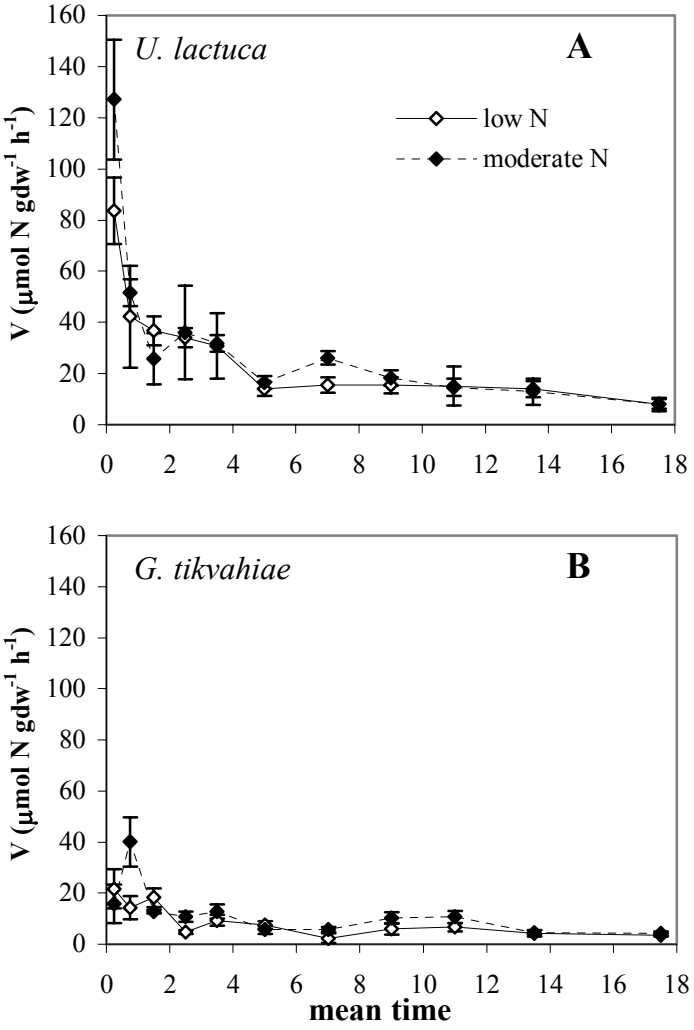

Figure 5-2 Results of the short term urea uptake experiments for (A) *U. lactuca* and (B) *G. tikvahiae* under pre-experimental conditions of low or moderate N availability. Starting N concentrations were measured for each replicate (~8 µM urea-N). The mean time is the halfway point between samplings. N = 5 for each treatment and error bars represent the standard error of the mean.

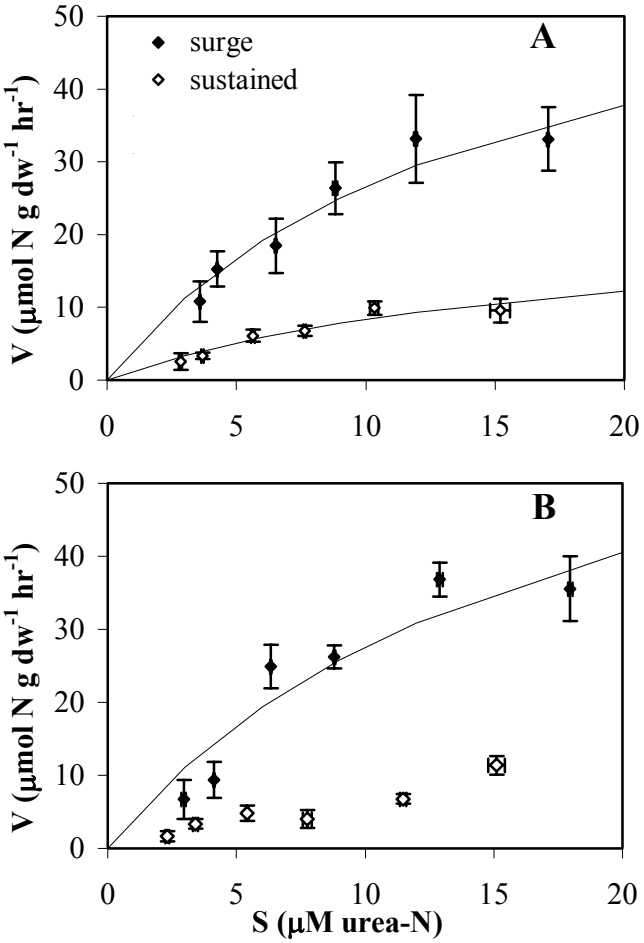

Figure 5-3 Surge (0-2 min) and sustained (after 30 minutes) uptake rates of urea by *U. lactuca* as a function of urea concentration (S) for (A) low tissue nitrogen and (B) moderate tissue nitrogen. Least squares regression lines, based on the kinetic uptake parameters shown in Table 5-1, are shown.

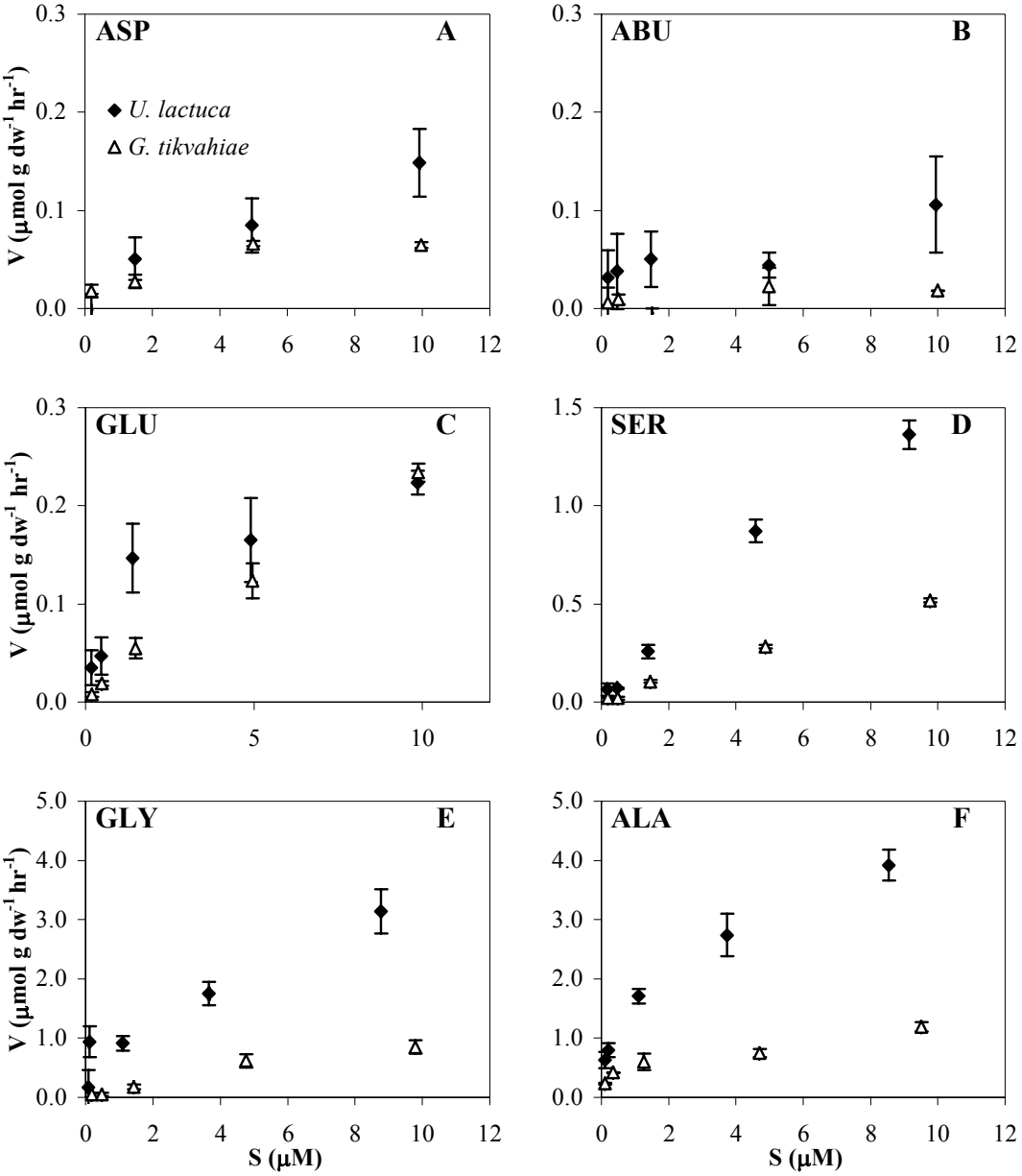

Figure 5-4 Amino acid uptake rate by *U. lactuca* and *G. tikvahiae* as a function of concentration for (A) aspartic acid, (B) aminobutyrate, (C) glutamic acid, (D) serine, (E) glycine and (F) alanine. Estimates for the kinetic parameters V_{max} and K_m based on these figures are given in Table 5-3. Errors are the standard error of the mean for n = 3.

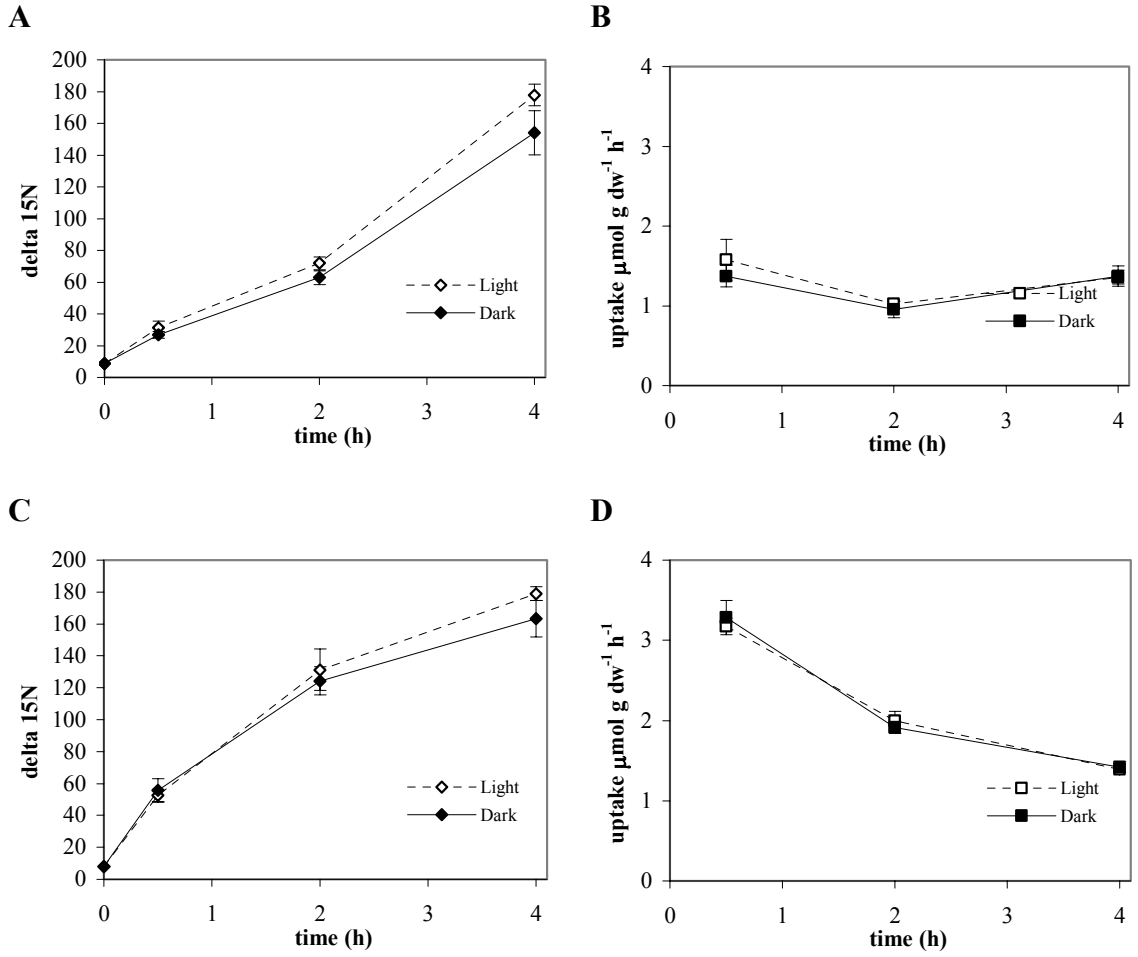

Figure 5-5 Change in δ15N (A & C) and uptake rates (B & D) of glycine over a 4 hour incubation for *G. tikvahiae* (A & B) and *U. lactuca* (C & D). The initial concentration of glycine was 9.1 µM. Error bars are the standard error of the mean for n = 3.

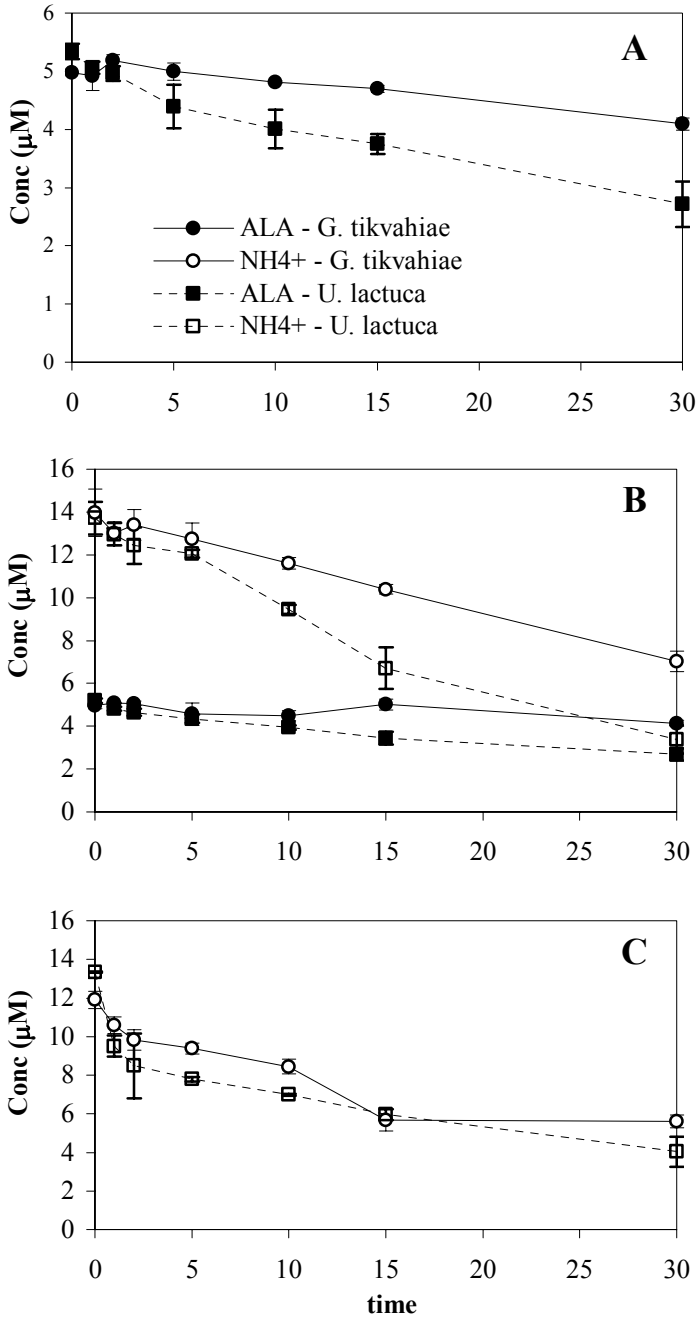

Figure 5-6 Change in alanine and ammonium concentration with time for (A) alanine only, (B) alanine + ammonium and (C) ammonium only for cultures of *U. lactuca* and *G. tikvahiae*. The uptake rates computed based on the initial and final concentrations were used for comparison with the ^{15}N uptake method.

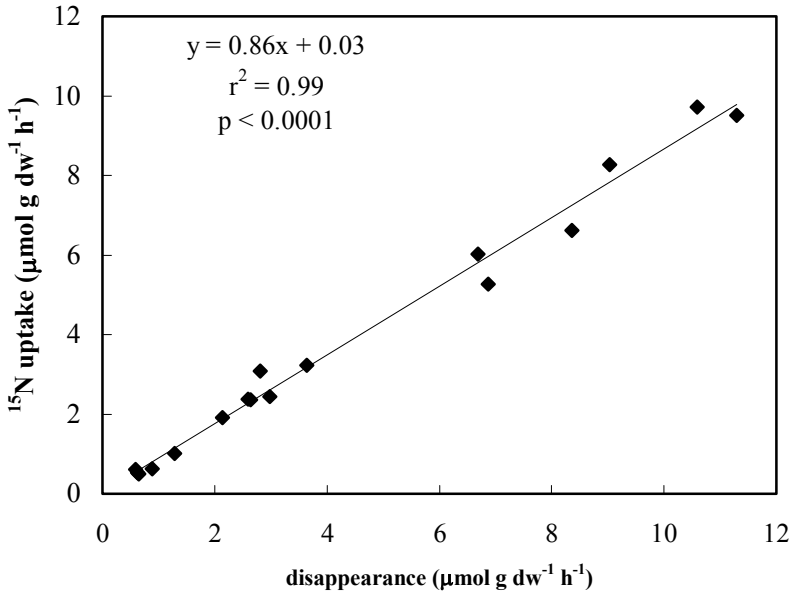

Figure 5-7 Comparison between uptake of alanine and ammonium estimated by the traditional disappearance from solution method and by ^{15}N uptake. The line approximates the least squares regression fit, and the equation, coefficient of determination and significance of the estimate are shown on the figure.

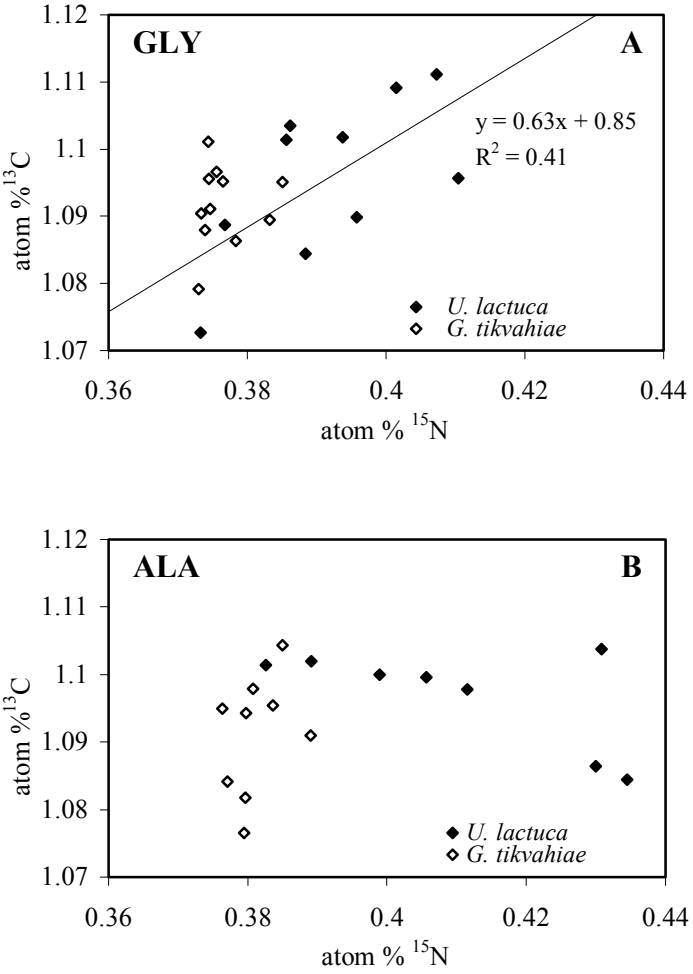

Figure 5-8 Comparison between change in atom % ^{15}N and ^{13}C for uptake of (A) glycine and (B) alanine by *U. lactuca* and *G. tikvahiae*. The ^{13}C label was on the C_2 carbon for each amino acid. The least squares regression line is shown for glycine uptake by *U. lactuca* and was the only significant relationship between ^{15}N and ^{13}C assimilation; the resulting equation and coefficient of determination are shown on Figure A.

	V_{max}	K_m	V_{max}/K_m	r^2
low N				
surge	65 ± 21	14 ± 8	4.5	0.53
sustained	23 ± 8	17 ± 10	1.3	0.64
moderate N				
surge	76 ± 24	18 ± 9	4.3	0.73
sustained	--	--	--	--

Table 5-1 Estimates of V_{max} (μmol N g dw^{-1} d^{-1}), K_m (μM-N), and the affinity for urea uptake at low concentrations (V_{max}/K_m; Healy 1980) for surge uptake (0 – 2 min after exposure) and sustained uptake (after 30 min exposure) for *U. lactuca*. Errors are the standard error of the estimate based on least squares regression.

		Initial uptake rate		Sustained uptake rate	
test	**treatment**	***G. tikvahiae***	***U. lactuca***	***G. tikvahiae***	***U. lactuca***
prior light					
	light	12.2 ± 0.9	16.3 ± 0.7	2.3 ± 0.1	5.4 ± 0.4
	dark	12.1 ± 1.0	17.0 ± 0.7	2.4 ± 0.2	4.8 ± 0.2
experimental light					
	light	11.0 ± 0.9	17.8 ± 0.8	2.5 ± 0.2	5.1 ± 0.3
	dark	13.2 ± 1.0	15.6 ± 0.5	2.2 ± 0.1	5.1 ± 0.2
nutrient					
	urea only	11.6 ± 0.9	16.1 ± 0.7	2.3 ± 0.1	4.6 ± 0.2
	urea + NH_4^+	12.7 ± 1.0	17.1 ± 0.8	2.4 ± 0.2	5.6 ± 0.3
overall mean		12.2 ± 0.7	16.6 ± 0.5	2.3 ± 0.1	5.1 ± 0.2

Table 5-2 Uptake rates (μmol N g dw^{-1} d^{-1}) for urea under different experimental conditions. "Initial" uptake was measured in the first 10 minutes of the experiment; "sustained" uptake was measured at several points between 10 - 150 minutes. Error bars represent the standard error of the mean. N = 20 for initial rates and n = 80 for sustained rates.

	V_{max}	K_m	V_{max}/K_m	r^2	V_{max} measured
U. lactuca					
ASP	0.37 ± 0.15	0.83 ± 0.57	0.4	0.90	0.15 ± 0.03
ABU	--	--	--	--	0.11 ± 0.05
GLU	0.24 ± 0.03	0.10 ± 0.05	2.3	0.71	0.22 ± 0.01
SER	3.90 ± 0.99	1.34 ± 0.49	2.9	0.98	1.36 ± 0.07
GLY	5.69 ± 2.53	0.60 ± 0.49	9.5	0.74	3.14 ± 0.37
ALA	4.80 ± 0.84	0.18 ± 0.10	26.4	0.82	3.92 ± 0.26
G. tikvahiae					
ASP	--	--	--	--	0.06 ± 0.00
ABU	--	--	--	--	0.02 ± 0.00
GLU	--	--	--	--	0.23 ± 0.01
SER	2.38 ± 0.61	2.83 ± 0.90	0.8	0.99	0.52 ± 0.01
GLY	1.79 ± 0.74	0.82 ± 0.56	2.2	0.89	0.85 ± 0.11
ALA	1.15 ± 0.13	0.07 ± 0.03	15.9	0.76	1.19 ± 0.08

Table 5-3 Kinetic uptake parameters for 6 different amino acids. V_{max} (μmol N g dw^{-1} d^{-1}) and K_m (μM-N) were estimated from uptake data shown in Figure 5-4 using least squares regression. The coefficient of determination (r^2) and standard error of the estimates are given. The affinity for amino acid uptake at low concentrations (V_{max}/K_m; Healy 1980) and the measured maximum uptake rate (starting concentration ~10 μM amino acid-N, n = 3, error is standard error of the mean) are also given. In some cases, where uptake rates were very low, the kinetic uptake parameters could not be reliably estimated and are not included.

	^{15}N method	**disappearance method**
U. lactuca		
^{15}ALA (only)	2.5 ± 0.4	2.8 ± 0.4
^{15}ALA (+ $^{14}NH_4^+$)	2.6 ± 0.2	2.8 ± 0.1
$^{15}NH_4^+$ (only)	9.2 ± 0.5	10.3 ± 0.7
G. tikvahiae		
^{15}ALA (only)	0.6 ± 0.0	0.8 ± 0.1
^{15}ALA (+ $^{14}NH_4^+$)	0.7 ± 0.1	0.8 ± 0.2
$^{15}NH_4^+$ (only)	6.0 ± 0.4	7.3 ± 0.5

Table 5-4 Comparison between ^{15}N uptake method and the traditional disappearance from solution method for uptake of ^{15}alanine only, ^{15}alanine in the presence of $^{14}NH_4^+$ and $^{15}NH_4^+$ only by *U. lactuca* and *G. tikvahiae*. Estimates are the mean μmol g dw^{-1} h^{-1} and standard error for n = 3.

	low N	NH_4^+	NO_3^-	p
Glycine				
U. lactuca	1.75 ± 0.20 [a]	1.87 ± 0.37 [a]	0.36 ± 0.22 [b]	0.016
G. tikvahiae	0.62 ± 0.11 [a]	0.66 ± 0.24 [a]	0.59 ± 0.05 [a]	0.950
Alanine				
U. lactuca	2.74 ± 0.36 [a]	0.64 ± 0.17 [b]	0.28 ± 0.11 [b]	0.002
G. tikvahiae	0.75 ± 0.07 [a]	0.22 ± 0.18 [a]	0.54 ± 0.02 [a]	0.088

Table 5-5 Uptake rates for glycine and alanine by *U. lactuca* and *G. tikvahiae* under different conditions of prior N fertilization. Treatments are: low N = no prior fertilization, NH_4^+ = prior fertilization with NH_4^+, NO_3^- = prior fertilization with NO_3^-. Significance differences between treatments for each combination of amino acid and species are noted by different letters; values with the same letter are not different from each other. Uptake rates are in μmol N g dw^{-1} h^{-1} with the standard error of the mean for n = 3.

Chapter 6

Conclusions

Dissolved organic nitrogen is clearly an important part of the nitrogen cycle in Hog Island Bay. Standing stocks and sediment fluxes are both high, and much of the DON turnover appears to be composed of small, labile compounds such as urea and free and combined amino acids. Nitrogen availability generally limits primary production in temperate estuaries (Howarth 1988), but at this point we do not know if the availability of nitrogen truly "limits" algal growth in Hog Island Bay. It is evident, however, that from the mainland to the barrier islands the availability of nitrogen decreases and that along this transition organic nitrogen begins to play a relatively more important role in fulfilling the algal nitrogen demand. This study has demonstrated that macroalgae are capable of utilizing many forms of dissolved nitrogen, even at low concentrations, but that there are distinct species-specific differences in organic nitrogen uptake kinetics. These differences may dictate the competitive dominance of individual species under conditions of variable inorganic and organic nitrogen supply.

The effect of benthic algae, both macro- and microalgae, on organic nitrogen standing stock and fluxes can be considerable. The dynamics of small, labile compounds such as urea and amino acids seem to be controlled by the primary producers. Both types of algae are capable of capping off benthic fluxes, thereby preventing the movement of dissolved compounds, inorganic and organic, between the sediment and the water column. However, the uptake of nitrogen by macroalgae is not entirely straightforward because of the rapid release of nitrogen from actively growing tissue. We believe, at this point, that approximately one quarter of this release is as dissolved combined amino acids. However, much remains to be learned about the nature of these exudates, and the

conditions under which release is stimulated. Moreover, this rapid turnover of nitrogen likely has a substantial impact on local production, both autotrophic and heterotrophic, in the water column. Further research into the impact of short-term N release on overall ecosystem metabolism is needed.

It is evident that macroalgae dominate ecosystem processes in the mid-lagoon shoal region of Hog Island Bay. During active growth, very substantial quantities of organic carbon and nitrogen are sequestered in macroalgal biomass trapped behind the oyster reefs. When these dense mats crashed in the late summers of 1998 and 2001, massive amounts of organic and inorganic nitrogen were released to the water column and a dystrophic crisis ensued. There is evidence of enhanced phytoplankton and bacterial metabolism following these crashes (McGlathery *et al*. 2001; Anderson *et al*. in press). However, these events are patchy and isolated. In many parts of the lagoon the macroalgal biomass is much lower and it appears that macroalgal biomass across the lagoon is controlled by a complex set of physical factors, including current and substrate, in addition to nutrients.

At this point we do not know enough about the distribution of macroalgae in other areas of lagoon to determine the relative contribution of these isolated events to overall system nitrogen transformations and retention. However, the body of work presented here demonstrates that where sufficiently dense, macroalgae are the primary driver of nutrient uptake, transformation and retention, and that organic nitrogen plays a very important role. In areas of the lagoon where benthic microalgae dominate the benthos,

they are likewise important drivers of nitrogen cycling and perform an equally critical role in mediating the transfer of nitrogen from the land margin to the coastal ocean.

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Appendix A: Standing stock water column dissolved N concentrations

The following table contains standing stock concentrations for all dissolved N compounds measured in the fill water at the start of each experiment. Ammonium, nitrate, urea and DON are in μM-N and amino acids are in nM-N.

Date	Site	NH_4^+	NO_3^-	urea	DON	DFAA	DCAA
Oct-98	WW	2.2	1.5	1.1	18.3	425	1574
Oct-98	Creek	2.8	0.4	0.4	11.9	36	477
Oct-98	Shoal1	2.5	0.6	0.4	11.2	45	1117
Oct-98	Hog	2.4	0.8	0.5	10.6	95	1372
Jan-99	WW	0.5	2.0	0.4	10.9	213	1346
Jan-99	Creek	0.5	0.9	1.2	11.3	38	826
Jan-99	Shoal1	0.2	0.2	0.2	6.6	161	967
Jan 99	Hog	0.2	0.2	0.4	4.5	84	834
Mar-99	WW	1.5	2.3	0.6	13.0	79	2986
Mar-99	Creek	0.6	1.1	0.7	7.5	30	2251
Mar-99	Shoal1	0.0	1.1	0.2	7.1	238	1458
Mar-99	Hog	0.0	0.6	0.4	6.5	111	997
May-99	WW	1.6	1.1	0.4	10.0	79	2665
May-99	Creek	0.8	0.8	0.3	11.0	116	1299
May-99	Shoal1	0.4	0.0	0.3	11.7	185	1443
May-99	Hog	0.4	0.0	0.2	10.8	56	968
Jun-99	WW	5.7	2.4	1.4	14.5	nd	nd
Jun-99	Creek	3.1	1.8	1.2	9.7	nd	nd
Jun-99	Shoal1	1.2	0.9	0.7	6.2	nd	nd
Jun-99	Hog	0.8	0.9	0.5	4.7	nd	nd
Aug-99	WW	14.4	5.7	2.3	25.7	284	2321
Aug-99	Creek	8.6	5.0	2.0	16.4	76	1535
Aug-99	Shoal1	0.4	0.8	0.7	13.0	154	1340
Aug-99	Hog	0.8	1.5	0.8	8.7	269	893

Appendix B: Standing stock water column amino acid concentrations

The following table contains standing stock concentrations of DFAA and DCAA in the fill water at the start of each experiment. Units are nM AA. SUM is the sum of all individual amino acids.

Standing stock water column concentrations - DFAA

Date	Site	ASP	GLU	ASN	SER	GLN	HIS	GLY	THR	ARG
Oct-98	WW	27	24	17	24	14	15	39	15	18
Oct-98	Creek	17	1	0	4	0	0	2	0	1
Oct-98	Shoal	21	5	0	2	0	0	0	2	2
Oct-98	Hog	5	4	3	1	0	8	6	0	2
Jan-99	WW	1	0	0	31	1	13	36	4	12
Jan-99	Creek	2	1	0	19	0	0	11	0	0
Jan-99	Shoal	0	4	21	44	1	2	13	18	2
Jan-99	Hog	2	4	0	14	0	5	14	6	4
Mar-99	WW	9	5	0	0	0	3	14	3	5
Mar-99	Creek	5	7	1	2	1	2	0	1	0
Mar-99	Shoal	11	7	0	28	1	26	34	6	9
Mar-99	Hog	3	9	0	5	0	7	7	5	9
May-99	WW	0	14	0	7	7	0	8	0	4
May-99	Creek	5	18	1	0	1	4	55	0	0
May-99	Shoal	9	22	4	12	0	18	36	0	1
May-99	Hog	14	18	0	20	0	0	2	0	0
Aug-99	WW	27	16	2	10	1	34	61	3	13
Aug-99	Creek	6	0	4	9	4	7	0	4	3
Aug-99	Shoal	9	3	0	17	4	17	9	14	4
Aug-99	Hog	14	18	6	50	2	21	50	8	0

		ALA	TYR	GABA	MET	PHE	ILE	LEU	SUM
Oct-98	WW	17	12	9	24	14	16	18	304
Oct-98	Creek	4	0	0	0	0	0	0	28
Oct-98	Shoal	0	0	0	5	0	0	0	37
Oct-98	Hog	9	0	4	0	2	12	9	65
Jan-99	WW	36	3	0	2	0	1	5	145
Jan-99	Creek	0	0	5	0	0	0	0	37
Jan-99	Shoal	4	1	2	2	2	1	0	117
Jan-99	Hog	1	0	0	2	1	1	0	55
Mar-99	WW	5	0	2	0	2	3	1	52
Mar-99	Creek	0	0	2	0	0	0	1	23
Mar-99	Shoal	8	3	3	5	2	5	5	154
Mar-99	Hog	2	3	8	0	1	4	2	65
May-99	WW	2	3	0	0	0	0	4	50
May-99	Creek	1	1	0	6	0	2	0	95
May-99	Shoal	1	4	14	11	4	0	0	135
May-99	Hog	1	0	0	0	0	0	0	55
Aug-99	WW	2	0	1	1	0	0	0	173
Aug-99	Creek	0	2	0	0	0	0	4	43
Aug-99	Shoal	2	1	3	0	0	0	4	88
Aug-99	Hog	29	4	0	2	0	2	5	211

Standing stock water column concentrations - DCAA

Date	Site	ASP	GLU	SER	HIS	GLY	THR	ARG	ALA
Oct-98	WW	136	109	145	123	351	139	5	233
Oct-98	Creek	19	45	52	13	125	28	14	59
Oct-98	Shoal	83	102	132	14	368	96	9	176
Oct-98	Hog	111	95	116	32	409	157	30	190
Jan-99	WW	112	135	89	50	343	131	35	144
Jan-99	Creek	54	64	61	50	167	60	16	107
Jan-99	Shoal	70	80	44	84	204	69	10	118
Jan-99	Hog	66	75	83	0	226	64	15	169
Mar-99	WW	343	302	256	53	930	261	23	399
Mar-99	Creek	201	182	203	43	708	210	43	278
Mar-99	Shoal	141	124	136	31	454	139	14	186
Mar-99	Hog	88	76	86	19	309	92	7	152
May-99	WW	240	119	359	77	767	252	26	439
May-99	Creek	132	59	153	16	306	103	21	279
May-99	Shoal	111	107	144	49	177	132	83	166
May-99	Hog	83	43	48	79	176	95	17	142
Aug-99	WW	200	173	154	147	557	190	0	410
Aug-99	Creek	94	80	79	100	322	83	19	194
Aug-99	Shoal	127	97	69	100	374	87	0	195
Aug-99	Hog	76	69	12	94	179	79	0	116

		TYR	GABA	MET	PHE	ILE	LEU	SUM
Oct-98	WW	5	2	0	18	15	25	1306
Oct-98	Creek	1	0	0	9	18	22	404
Oct-98	Shoal	4	0	7	20	13	23	1047
Oct-98	Hog	9	12	3	28	0	21	1213
Jan-99	WW	3	6	2	21	28	24	1124
Jan-99	Creek	9	13	0	14	20	19	655
Jan-99	Shoal	4	27	0	17	19	22	768
Jan-99	Hog	5	18	3	12	15	26	778
Mar-99	WW	16	56	14	26	34	58	2771
Mar-99	Creek	14	3	16	46	28	56	2033
Mar-99	Shoal	14	35	2	11	37	16	1342
Mar-99	Hog	4	19	10	9	28	10	910
May-99	WW	18	28	6	26	23	19	2401
May-99	Creek	8	56	8	17	14	4	1175
May-99	Shoal	19	12	0	23	28	44	1095
May-99	Hog	15	15	0	16	14	1	743
Aug-99	WW	7	5	2	38	57	51	1991
Aug-99	Creek	47	38	0	43	49	52	1200
Aug-99	Shoal	17	9	0	19	18	17	1128
Aug-99	Hog	1	7	15	19	16	16	700

Appendix C: Light-Dark Sediment and Sediment + Algae N Flux Rates

The following tables contain the hourly light and dark sediment fluxes of ammonium, nitrate, urea, DON, DFAA and DCAA measured during each flux experiment. Positive values indicate a flux from the sediment to the water column. The "core" column indicates the experimental treatment; S = sediment only, S+A = sediment plus *Ulva lactuca*. Units are μmol N m^{-2} h^{-1} and errors are the standard deviation for n = 3.

			NH_4^+		NO_3^-	
Date	**Site**	**Core**	**light**	**dark**	**light**	**dark**
Oct-98	WW	S	-24.5 ± 10.9	5.7 ± 2.2	-2.0 ± 6.4	-15.4 ± 7.7
Oct-98	WW	S+A	-3.2 ± 0.5	7.8 ± 0.0	-13.6 ± 1.8	-7.5 ± 1.9
Oct-98	Creek	S	3.4 ± 4.4	1.8 ± 1.1	-5.7 ± 1.8	-3.7 ± 1.5
Oct-98	Creek	S+A	2.4 ± 12.3	11.0 ± 6.1	-9.4 ± 4.7	2.3 ± 3.9
Oct-98	Shoal1	S	10.8 ± 3.2	0.3 ± 3.6	1.0 ± 1.6	0.2 ± 3.1
Oct-98	Shoal1	S+A	-13.5 ± 6.1	1.0 ± 1.7	-3.0 ± 1.2	-2.2 ± 1.4
Oct-98	Hog	S	-16.2 ± 9.3	4.4 ± 1.4	-3.2 ± 2.4	-3.4 ± 1.7
Oct-98	Hog	S+A	11.2 ± 9.5	-3.0 ± 2.8	-3.0 ± 1.3	1.6 ± 1.1
Jan-99	WW	S	-2.1 ± 3.8	-4.1 ± 1.3	-5.1 ± 3.7	8.8 ± 3.6
Jan-99	WW	S+A	-12.9 ± 2.6	-4.5 ± 5.0	-12.0 ± 4.0	10.5 ± 2.8
Jan-99	Creek	S	-1.4 ± 2.4	-4.5 ± 1.9	24.8 ± 2.2	5.2 ± 1.0
Jan-99	Creek	S+A	-2.4 ± 2.2	-15.1 ± 2.7	4.5 ± 3.1	-5.1 ± 3.1
Jan-99	Shoal1	S	-4.1 ± 5.3	8.2 ± 4.9	0.1 ± 0.4	-2.7 ± 0.7
Jan-99	Shoal1	S+A	-5.4 ± 3.4	-1.9 ± 2.4	1.3 ± 0.3	-2.3 ± 0.3
Jan-99	Hog	S	-6.3 ± 3.5	5.6 ± 0.7	-3.7 ± 0.9	-2.5 ± 1.4
Jan-99	Hog	S+A	4.0 ± 0.4	2.5 ± 1.2	-3.0 ± 1.3	-3.8 ± 0.2
Mar-99	WW	S	17.3 ± 6.3	0.1 ± 5.5	-24.1 ± 7.7	0.2 ± 5.7
Mar-99	WW	S+A	16.9 ± 1.2	-24.5 ± 8.2	-22.9 ± 2.4	5.2 ± 5.2
Mar-99	Creek	S	23.7 ± 7.4	35.5 ± 14.5	-2.9 ± 3.4	3.9 ± 8.8
Mar-99	Creek	S+A	-3.3 ± 11.0	-8.2 ± 4.5	-3.3 ± 4.2	-13.1 ± 8.0
Mar-99	Shoal1	S	-6.7 ± 2.7	9.2 ± 2.5	1.1 ± 0.9	-5.8 ± 3.3
Mar-99	Shoal1	S+A	-19.7 ± 6.6	4.1 ± 1.0	2.1 ± 2.0	-5.7 ± 1.5
Mar-99	Hog	S	11.7 ± 4.3	-6.3 ± 1.8	-0.5 ± 7.5	-3.6 ± 4.5
Mar-99	Hog	S+A	5.3 ± 2.8	-9.3 ± 5.1	-10.3 ± 2.6	2.7 ± 4.3

			NH_4^+		NO_3^-	
Date	**Site**	**Core**	**light**	**dark**	**light**	**dark**
May-99	WW	S	-25.3 ± 1.6	-2.3 ± 1.2	-9.5 ± 2.9	-2.0 ± 2.7
May-99	WW	S+A	-35.9 ± 4.2	-3.0 ± 0.7	-14.5 ± 2.2	4.5 ± 3.5
May-99	Creek	S	23.1 ± 0.0	30.2 ± 4.0	2.8 ± 0.8	0.2 ± 2.3
May-99	Creek	S+A	-23.5 ± 2.1	0.7 ± 1.4	-5.3 ± 3.7	-1.1 ± 1.7
May-99	Shoal1	S	5.8 ± 3.6	5.8 ± 4.1	0.3 ± 1.6	0.3 ± 1.3
May-99	Shoal1	S+A	-1.2 ± 2.3	-1.7 ± 1.8	0.0 ± 1.9	-0.4 ± 1.8
May-99	Hog	S	-5.3 ± 1.9	1.0 ± 1.3	0.9 ± 3.4	1.6 ± 4.7
May-99	Hog	S+A	-5.6 ± 1.5	-2.9 ± 0.4	-0.6 ± 0.6	1.3 ± 1.2
Jun-99	WW	S	-28.5 ± 5.8	-34.6 ± 5.9	4.0 ± 2.5	0.0 ± 2.4
Jun-99	WW	S+A	-102.8 ± 10.4	-53.1 ± 6.9	-7.7 ± 7.8	-4.7 ± 2.2
Jun-99	Creek	S	34.2 ± 8.5	18.8 ± 9.1	0.3 ± 2.9	-4.0 ± 2.3
Jun-99	Creek	S+A	-66.9 ± 15.1	-21.4 ± 7.1	-7.6 ± 4.2	-12.2 ± 1.8
Jun-99	Shoal1	S	13.3 ± 8.9	84.2 ± 6.3	3.4 ± 1.7	2.9 ± 2.0
Jun-99	Shoal1	S+A	-19.5 ± 6.2	3.3 ± 1.9	-9.3 ± 2.6	4.5 ± 5.1
Jun-99	Hog	S	-8.3 ± 2.2	-5.8 ± 0.5	-3.2 ± 1.6	-2.7 ± 1.3
Jun-99	Hog	S+A	-7.5 ± 0.8	-11.2 ± 3.2	-8.8 ± 1.9	-0.8 ± 1.7
Aug-99	WW	S	-99.7 ± 11.5	31.1 ± 34.4	-10.1 ± 2.6	-1.3 ± 4.2
Aug-99	WW	S+A	-240.3 ± 8.1	47.4 ± 25.1	-10.2 ± 3.4	-18.3 ± 7.5
Aug-99	Creek	S	-4.6 ± 3.9	74.5 ± 6.4	-16.7 ± 4.9	0.0 ± 7.9
Aug-99	Creek	S+A	-208.2 ± 7.5	85.0 ± 31.4	4.4 ± 23.8	-12.6 ± 1.0
Aug-99	Shoal1	S	45.9 ± 22.6	33.3 ± 17.9	-4.4 ± 1.3	8.0 ± 3.2
Aug-99	Shoal1	S+A	-22.0 ± 12.4	-27.3 ± 19.1	-14.2 ± 5.6	2.3 ± 3.4
Aug-99	Hog	S	-19.6 ± 2.8	6.2 ± 0.8	-11.9 ± 2.7	-7.7 ± 1.5
Aug-99	Hog	S+A	-19.5 ± 2.3	5.4 ± 0.0	-30.4 ± 0.9	2.9 ± 0.9

			Urea		DON	
Date	**Site**	**Core**	**light**	**dark**	**light**	**dark**
Oct-98	WW	S	-3.8 ± 2.3	10.4 ± 4.2	-20.0 ± 27.1	-14.1 ± 18.8
Oct-98	WW	S+A	-1.8 ± 0.4	-12.3 ± 2.0	-26.8 ± 12.5	53.4 ± 14.7
Oct-98	Creek	S	-1.2 ± 0.6	-5.7 ± 1.3	-13.2 ± 10.4	0.4 ± 1.5
Oct-98	Creek	S+A	3.9 ± 3.0	-3.3 ± 2.2	12.9 ± 2.6	127.5 ± 67.7
Oct-98	Shoal1	S	-0.4 ± 0.1	8.3 ± 8.1	-4.9 ± 7.3	75.1 ± 29.3
Oct-98	Shoal1	S+A	0.7 ± 1.3	-3.2 ± 1.3	42.8 ± 10.7	-1.4 ± 7.3
Oct-98	Hog	S	1.4 ± 0.6	3.2 ± 1.9	-2.2 ± 8.2	19.2 ± 7.3
Oct-98	Hog	S+A	4.0 ± 2.3	-1.6 ± 0.1	4.1 ± 6.4	16.8 ± 13.5
Jan-99	WW	S	-3.0 ± 1.0	2.7 ± 2.6	-49.0 ± 5.7	-15.1 ± 21.3
Jan-99	WW	S+A	-12.5 ± 6.6	0.8 ± 4.3	-46.9 ± 13.9	-7.6 ± 10.5
Jan-99	Creek	S	4.6 ± 8.7	-2.5 ± 3.6	-4.8 ± 16.0	22.4 ± 23.5
Jan-99	Creek	S+A	4.0 ± 7.3	-10.7 ± 6.3	19.7 ± 13.8	-2.4 ± 16.6
Jan-99	Shoal1	S	-1.5 ± 2.2	28.6 ± 16.0	62.1 ± 17.0	-133.6 ± 12.3
Jan-99	Shoal1	S+A	-7.8 ± 4.0	-0.5 ± 1.3	-22.9 ± 22.0	-58.0 ± 20.6
Jan-99	Hog	S	-4.6 ± 3.4	10.1 ± 10.1	17.5 ± 18.7	46.8 ± 11.5
Jan-99	Hog	S+A	-12.4 ± 3.9	-6.7 ± 0.5	-69.6 ± 39.1	-10.1 ± 11.3
Mar-99	WW	S	1.2 ± 1.4	-7.1 ± 1.6	74.8 ± 42.0	-77.3 ± 23.3
Mar-99	WW	S+A	4.2 ± 5.3	-11.5 ± 2.2	23.6 ± 24.8	-28.1 ± 5.4
Mar-99	Creek	S	10.7 ± 0.2	0.7 ± 3.0	-6.6 ± 6.8	6.8 ± 21.0
Mar-99	Creek	S+A	14.3 ± 8.9	-19.0 ± 6.1	46.3 ± 22.9	58.0 ± 20.8
Mar-99	Shoal1	S	1.1 ± 4.6	5.2 ± 3.8	17.9 ± 12.9	-5.8 ± 21.6
Mar-99	Shoal1	S+A	10.2 ± 11.6	-8.3 ± 9.4	138.8 ± 28.4	-39.8 ± 26.2
Mar-99	Hog	S	3.1 ± 1.9	2.7 ± 10.3	-30.2 ± 16.3	-16.4 ± 9.1
Mar-99	Hog	S+A	1.0 ± 1.4	-8.2 ± 4.3	20.0 ± 16.4	-47.4 ± 7.4

			Urea		DON	
Date	**Site**	**Core**	**light**	**dark**	**light**	**dark**
May-99	WW	S	9.4 ± 3.6	-15.1 ± 3.4	-39.3 ± 19.6	-14.3 ± 12.3
May-99	WW	S+A	28.5 ± 6.2	-40.0 ± 4.6	-9.3 ± 7.8	-75.6 ± 33.1
May-99	Creek	S	-0.6 ± 0.6	2.2 ± 1.5	19.7 ± 10.5	-17.4 ± 2.6
May-99	Creek	S+A	27.9 ± 16.5	-41.4 ± 7.3	34.4 ± 13.7	-38.6 ± 8.6
May-99	Shoal1	S	7.0 ± 1.2	-8.4 ± 1.2	1.1 ± 18.4	-40.4 ± 12.3
May-99	Shoal1	S+A	8.3 ± 1.7	-13.3 ± 0.7	-9.9 ± 3.4	-27.7 ± 9.4
May-99	Hog	S	6.3 ± 0.2	-4.6 ± 1.9	-56.0 ± 10.9	9.1 ± 9.1
May-99	Hog	S+A	14.1 ± 3.4	-13.6 ± 3.9	-39.2 ± 10.9	-11.4 ± 10.2
Jun-99	WW	S	-5.8 ± 4.0	4.3 ± 3.4	30.3 ± 17.3	-2.5 ± 10.7
Jun-99	WW	S+A	-6.0 ± 2.3	-8.3 ± 1.3	-105.1 ± 17.9	63.3 ± 36.9
Jun-99	Creek	S	-3.9 ± 1.3	5.5 ± 7.2	-8.3 ± 21.2	-19.6 ± 4.6
Jun-99	Creek	S+A	4.1 ± 8.1	-7.6 ± 4.8	-15.0 ± 30.2	-2.8 ± 66.5
Jun-99	Shoal1	S	0.7 ± 1.5	1.9 ± 1.3	4.2 ± 11.9	-27.8 ± 8.2
Jun-99	Shoal1	S+A	-5.0 ± 1.0	-2.1 ± 3.7	-48.2 ± 13.6	2.1 ± 8.3
Jun-99	Hog	S	1.7 ± 2.3	-3.6 ± 1.4	-31.6 ± 2.7	59.8 ± 30.2
Jun-99	Hog	S+A	0.6 ± 1.7	-3.3 ± 0.0	-27.0 ± 1.3	21.5 ± 3.4
Aug-99	WW	S	-4.3 ± 4.9	12.3 ± .	18.2 ± 27.7	-82.9 ± 50.0
Aug-99	WW	S+A	-3.7 ± 7.6	-8.7 ± 10.1	-101.1 ± 34.1	-7.4 ± 36.2
Aug-99	Creek	S	-3.4 ± 2.5	-5.1 ± 7.9	-71.2 ± 23.4	-6.2 ± 30.9
Aug-99	Creek	S+A	-6.8 ± 8.1	-24.5 ± 12.8	-58.6 ± 11.7	-49.1 ± 2.2
Aug-99	Shoal1	S	3.8 ± 4.1	6.2 ± 5.7	-63.2 ± 23.9	24.9 ± 31.5
Aug-99	Shoal1	S+A	2.9 ± 7.9	-2.6 ± 1.0	-72.5 ± 30.6	65.5 ± 23.7
Aug-99	Hog	S	3.7 ± 5.4	-10.9 ± 2.8	-5.5 ± 12.2	-53.7 ± 6.9
Aug-99	Hog	S+A	-1.9 ± 11.2	1.3 ± 3.0	5.4 ± 25.5	-23.2 ± 14.7

			DFAA		DCAA	
Date	**Site**	**Core**	**light**	**dark**	**light**	**dark**
Oct-98	WW	S	-0.4 ± 0.5	-1.3 ± 0.5	6.0 ± 0.1	-0.4 ± 1.8
Oct-98	WW	S+A	-0.7 ± 1.5	-1.2 ± 1.6	11.6 ± 1.5	8.1 ± 7.4
Oct-98	Creek	S	-0.8 ± 0.5	-1.4 ± 0.3	5.0 ± 2.9	-4.8 ± 6.2
Oct-98	Creek	S+A	2.5 ± 2.0	-1.3 ± nd	10.4 ± 2.9	6.6 ± nd
Oct-98	Shoal1	S	1.1 ± 0.5	-0.1 ± 2.0	0.0 ± 1.0	7.4 ± 1.4
Oct-98	Shoal1	S+A	1.0 ± 0.7	-3.1 ± 0.6	16.0 ± 3.2	-1.6 ± 1.3
Oct-98	Hog	S	-2.8 ± 0.7	-1.2 ± 0.8	3.7 ± 1.5	-0.2 ± 1.6
Oct-98	Hog	S+A	-3.2 ± 0.3	0.9 ± 0.9	6.7 ± 2.8	3.1 ± 1.4
Jan-99	WW	S	2.0 ± 0.8	-2.8 ± 0.1	-14.1 ± 1.8	13.6 ± 4.2
Jan-99	WW	S+A	3.2 ± 1.1	-3.6 ± 0.8	-2.8 ± 2.9	17.2 ± 3.8
Jan-99	Creek	S	-2.6 ± 1.2	0.6 ± 0.0	6.4 ± 0.9	-1.8 ± 1.2
Jan-99	Creek	S+A	-1.7 ± 2.7	-0.9 ± 1.8	4.1 ± 2.3	-0.8 ± 3.9
Jan-99	Shoal1	S	-0.4 ± 0.5	4.8 ± 3.3	-1.1 ± 0.6	3.4 ± 0.7
Jan-99	Shoal1	S+A	1.5 ± 1.5	-2.3 ± 0.6	6.4 ± 4.8	-0.6 ± 0.2
Jan-99	Hog	S	1.0 ± 0.6	5.5 ± 0.9	-2.1 ± 4.1	1.2 ± 1.9
Jan-99	Hog	S+A	1.6 ± 2.1	-3.9 ± 1.9	12.5 ± 3.0	-4.2 ± 2.9
Mar-99	WW	S	5.6 ± 1.4	-6.7 ± 0.9	-9.5 ± 1.3	-5.1 ± 4.4
Mar-99	WW	S+A	-0.4 ± 1.3	-4.1 ± 2.0	25.1 ± 12.1	-4.8 ± 7.3
Mar-99	Creek	S	-2.8 ± 0.5	2.9 ± 0.5	1.9 ± 5.5	-21.2 ± 3.8
Mar-99	Creek	S+A	-1.7 ± 2.5	-2.5 ± 1.9	57.3 ± 7.4	-5.3 ± 23.6
Mar-99	Shoal1	S	-1.7 ± 0.6	-2.4 ± 0.6	-0.9 ± 4.2	-4.8 ± 1.7
Mar-99	Shoal1	S+A	-3.9 ± 2.2	-2.0 ± 2.6	33.0 ± 10.5	-10.4 ± 5.8
Mar-99	Hog	S	3.1 ± 0.3	-5.2 ± 0.7	12.8 ± 10.1	-9.3 ± 11.4
Mar-99	Hog	S+A	-0.2 ± 0.5	-2.1 ± 3.0	19.5 ± 3.4	3.2 ± 7.4

			DFAA		DCAA	
Date	**Site**	**Core**	**light**	**dark**	**light**	**dark**
May-99	WW	S	8.9 ± 4.1	-13.0 ± 5.0	16.1 ± 7.4	-2.0 ± 8.2
May-99	WW	S+A	16.5 ± nd	-20.5 ± nd	52.3 ± 34.2	-22.0 ± 33.6
May-99	Creek	S	2.4 ± 1.1	-2.2 ± 0.4	-22.8 ± 2.0	20.6 ± 3.4
May-99	Creek	S+A	12.8 ± 5.6	-11.5 ± 4.8	-8.1 ± 0.8	16.0 ± 1.8
May-99	Shoal1	S	0.8 ± 1.1	-3.4 ± 0.5	0.6 ± nd	-7.3 ± nd
May-99	Shoal1	S+A	1.5 ± 2.3	-6.0 ± 2.3	4.3 ± 0.2	-1.4 ± 0.4
May-99	Hog	S	-1.3 ± 0.4	0.7 ± 0.7	2.3 ± 2.5	-2.4 ± 1.7
May-99	Hog	S+A	4.8 ± 1.6	-7.0 ± 1.6	5.3 ± 3.0	6.3 ± nd
Jun-99	WW	S	nd ±	nd ±	nd ±	nd ±
Jun-99	WW	S+A	nd ±	nd ±	nd ±	nd ±
Jun-99	Creek	S	nd ±	nd ±	nd ±	nd ±
Jun-99	Creek	S+A	nd ±	nd ±	nd ±	nd ±
Jun-99	Shoal1	S	nd ±	nd ±	nd ±	nd ±
Jun-99	Shoal1	S+A	nd ±	nd ±	nd ±	nd ±
Jun-99	Hog	S	nd ±	nd ±	nd ±	nd ±
Jun-99	Hog	S+A	nd ±	nd ±	nd ±	nd ±
Aug-99	WW	S	4.6 ± 0.7	-6.6 ± 0.1	13.3 ± 12.5	-23.8 ± 13.2
Aug-99	WW	S+A	-0.4 ± 0.8	0.3 ± 1.1	5.7 ± 1.3	0.7 ± 3.8
Aug-99	Creek	S	-3.8 ± 0.4	3.1 ± 1.6	-29.1 ± 1.4	49.4 ± 1.5
Aug-99	Creek	S+A	-4.3 ± 0.3	2.5 ± 0.9	-28.3 ± 1.1	42.1 ± 1.5
Aug-99	Shoal1	S	-3.7 ± 0.7	-2.2 ± 0.4	4.6 ± 1.2	-1.8 ± 2.4
Aug-99	Shoal1	S+A	-2.8 ± 0.7	-0.7 ± 2.1	9.7 ± 1.7	-0.1 ± 0.6
Aug-99	Hog	S	-0.9 ± 0.8	-2.9 ± 1.0	-7.8 ± 4.6	9.3 ± 8.9
Aug-99	Hog	S+A	-3.2 ± 0.8	-1.7 ± 0.7	-2.0 ± 5.2	9.4 ± 9.7

Appendix D: Light-Dark Macroalgal N Uptake and Release

The following tables contain light and dark hourly macroalgal release and uptake of ammonium, nitrate, urea, DON, DFAA and DCAA measured during each flux experiment. Positive values indicate a release from *U. lactuca*; negative values indicate uptake by U. lactuca. Units are μmol N g dw^{-1} h^{-1} ammonium, nitrate, urea, DON and nmol N g dw^{-1} h^{-1} for amino acids; errors are the standard error for n = 3.

		NH_4^+		NO_3^-	
Date	**Site**	**light**	**dark**	**light**	**dark**
Oct-98	WW	0.32 ± 0.02	0.03 ± 0.00	-0.18 ± 0.04	0.12 ± 0.03
Oct-98	Creek	-0.15 ± 0.38	0.19 ± 0.12	-0.05 ± 0.11	0.14 ± 0.08
Oct-98	Shoal1	-0.34 ± 0.13	0.01 ± 0.02	-0.05 ± 0.01	-0.03 ± 0.02
Oct-98	Hog	0.34 ± 0.11	-0.09 ± 0.04	0.00 ± 0.02	0.06 ± 0.01
Jan-99	WW	-0.33 ± 0.04	-0.04 ± 0.13	-0.26 ± 0.13	0.09 ± 0.09
Jan-99	Creek	-0.01 ± 0.03	-0.15 ± 0.04	-0.28 ± 0.04	-0.14 ± 0.04
Jan-99	Shoal1	-0.02 ± 0.06	-0.15 ± 0.04	0.02 ± 0.01	0.01 ± 0.00
Jan-99	Hog	0.15 ± 0.01	-0.04 ± 0.02	0.01 ± 0.02	-0.02 ± 0.00
Mar-99	WW	0.00 ± 0.03	-0.55 ± 0.24	0.04 ± 0.05	0.09 ± 0.11
Mar-99	Creek	-0.43 ± 0.17	-0.72 ± 0.07	0.00 ± 0.07	-0.29 ± 0.15
Mar-99	Shoal1	-0.24 ± 0.12	-0.10 ± 0.02	0.02 ± 0.04	0.00 ± 0.03
Mar-99	Hog	-0.10 ± 0.05	-0.04 ± 0.07	-0.14 ± 0.03	0.08 ± 0.06
May-99	WW	-0.19 ± 0.08	-0.01 ± 0.01	-0.09 ± 0.05	0.10 ± 0.04
May-99	Creek	-0.60 ± 0.04	-0.38 ± 0.04	-0.11 ± 0.05	-0.01 ± 0.02
May-99	Shoal1	-0.08 ± 0.02	-0.09 ± 0.03	-0.01 ± 0.03	0.00 ± 0.02
May-99	Hog	0.00 ± 0.02	-0.04 ± 0.00	-0.02 ± 0.01	0.00 ± 0.01
Jun-99	WW	-1.18 ± 0.17	-0.29 ± 0.11	-0.18 ± 0.11	-0.08 ± 0.04
Jun-99	Creek	-1.40 ± 0.21	-0.56 ± 0.10	-0.11 ± 0.06	-0.11 ± 0.03
Jun-99	Shoal1	-0.54 ± 0.11	-1.32 ± 0.08	-0.21 ± 0.05	0.04 ± 0.08
Jun-99	Hog	0.01 ± 0.01	-0.08 ± 0.05	-0.08 ± 0.02	0.02 ± 0.02
Aug-99	WW	-2.50 ± 0.10	0.32 ± 0.47	0.00 ± 0.06	-0.29 ± 0.13
Aug-99	Creek	-4.53 ± 0.16	0.19 ± 0.68	0.43 ± 0.50	-0.28 ± 0.00
Aug-99	Shoal1	-0.88 ± 0.21	-0.79 ± 0.29	-0.12 ± 0.07	-0.07 ± 0.04
Aug-99	Hog	0.00 ± 0.04	-0.01 ± 0.00	-0.33 ± 0.02	0.19 ± 0.01

		Urea		DON	
Date	**Site**	**light**	**dark**	**light**	**dark**
Oct-98	WW	0.03 ± 0.01	-0.34 ± 0.03	-0.09 ± 0.18	1.03 ± 0.28
Oct-98	Creek	0.10 ± 0.06	0.05 ± 0.04	0.65 ± 0.08	2.56 ± 1.43
Oct-98	Shoal1	0.01 ± 0.02	-0.15 ± 0.02	0.60 ± 0.08	-0.99 ± 0.03
Oct-98	Hog	0.04 ± 0.03	-0.06 ± 0.00	0.08 ± 0.08	-0.01 ± 0.17
Jan-99	WW	-0.25 ± 0.21	0.00 ± 0.17	0.29 ± 0.60	0.30 ± 0.29
Jan-99	Creek	-0.01 ± 0.10	-0.11 ± 0.09	0.34 ± 0.19	-0.34 ± 0.23
Jan-99	Shoal1	-0.10 ± 0.07	-0.43 ± 0.05	-1.25 ± 0.39	1.14 ± 0.39
Jan-99	Hog	-0.11 ± 0.05	-0.25 ± 0.01	-1.24 ± 0.53	-0.84 ± 0.19
Mar-99	WW	0.09 ± 0.13	-0.11 ± 0.06	-1.12 ± 0.65	1.08 ± 0.22
Mar-99	Creek	0.05 ± 0.14	-0.32 ± 0.09	0.83 ± 0.34	0.85 ± 0.35
Mar-99	Shoal1	0.17 ± 0.22	-0.25 ± 0.18	2.26 ± 0.51	-0.66 ± 0.51
Mar-99	Hog	-0.03 ± 0.02	-0.16 ± 0.07	0.68 ± 0.20	-0.44 ± 0.12
May-99	WW	0.33 ± 0.12	-0.44 ± 0.11	0.60 ± 0.21	-1.12 ± 0.52
May-99	Creek	0.36 ± 0.20	-0.56 ± 0.09	0.19 ± 0.17	-0.27 ± 0.11
May-99	Shoal1	0.02 ± 0.02	-0.06 ± 0.02	-0.14 ± 0.06	0.13 ± 0.08
May-99	Hog	0.08 ± 0.03	-0.10 ± 0.04	0.22 ± 0.14	-0.25 ± 0.12
Jun-99	WW	0.00 ± 0.04	-0.20 ± 0.03	-2.14 ± 0.22	1.03 ± 0.59
Jun-99	Creek	0.11 ± 0.11	-0.18 ± 0.07	-0.09 ± 0.42	0.23 ± 0.92
Jun-99	Shoal1	-0.09 ± 0.01	-0.06 ± 0.06	-0.87 ± 0.25	0.50 ± 0.16
Jun-99	Hog	-0.02 ± 0.03	0.01 ± 0.00	0.07 ± 0.03	-0.57 ± 0.11
Aug-99	WW	0.01 ± 0.13	-0.37 ± 0.17	-2.12 ± 0.60	1.33 ± 0.62
Aug-99	Creek	-0.06 ± 0.18	-0.45 ± 0.32	0.30 ± 0.28	-0.96 ± 0.12
Aug-99	Shoal1	0.00 ± 0.11	-0.11 ± 0.01	-0.15 ± 0.42	0.55 ± 0.35
Aug-99	Hog	-0.09 ± 0.20	0.22 ± 0.06	0.20 ± 0.47	0.55 ± 0.27

		DFAA		DCAA	
Date	**Site**	**light**	**dark**	**light**	**dark**
Oct-98	WW	-1.9 ± 23.3	1.4 ± 22.4	88.9 ± 29.2	137.8 ± 122.9
Oct-98	Creek	64.5 ± 44.4	3.2 ± nd	115.5 ± 56.4	412.4 ± nd
Oct-98	Shoal1	-1.3 ± 8.7	-42.2 ± 12.3	202.7 ± 23.3	-120.4 ± 22.0
Oct-98	Hog	-4.2 ± 3.5	23.9 ± 9.2	38.5 ± 36.2	42.0 ± 18.9
Jan-99	WW	28.7 ± 36.9	-17.6 ± 28.1	422.5 ± 174.3	77.3 ± 82.6
Jan-99	Creek	12.3 ± 36.7	-19.8 ± 24.4	-33.6 ± 32.2	15.2 ± 54.4
Jan-99	Shoal1	32.1 ± 23.0	-105.0 ± 17.1	122.6 ± 91.3	-52.9 ± 9.3
Jan-99	Hog	6.2 ± 28.9	-136.7 ± 23.2	211.6 ± 39.5	-77.2 ± 40.4
Mar-99	WW	-128.7 ± 31.5	50.0 ± 45.2	641.7 ± 67.4	43.7 ± 149.5
Mar-99	Creek	21.3 ± 43.1	-93.3 ± 37.4	907.5 ± 107.6	233.2 ± 370.4
Mar-99	Shoal1	-40.1 ± 40.9	6.7 ± 48.4	630.8 ± 189.0	-108.1 ± 107.6
Mar-99	Hog	-47.0 ± 2.6	40.4 ± 38.4	93.1 ± 38.5	170.3 ± 88.9
May-99	WW	133.5 ± nd	-129.6 ± .	635.0 ± 593.2	-322.7 ± 611.5
May-99	Creek	124.2 ± 69.0	-111.3 ± 57.8	189.0 ± 4.1	-62.6 ± 28.2
May-99	Shoal1	14.6 ± 32.9	-38.6 ± 36.7	50.3 ± 5.0	81.8 ± 17.2
May-99	Hog	70.3 ± 20.7	-86.8 ± 20.7	27.8 ± 27.8	124.3 ± nd
Jun-99	WW	nd ±	nd ±	nd ±	nd ±
Jun-99	Creek	nd ±	nd ±	nd ±	nd ±
Jun-99	Shoal1	nd ±	nd ±	nd ±	nd ±
Jun-99	Hog	nd ±	nd ±	nd ±	nd ±
Aug-99	WW	-89.5 ± 14.4	124.6 ± 24.6	-133.6 ± 20.0	432.3 ± 57.7
Aug-99	Creek	-10.8 ± 6.1	-13.0 ± 19.8	16.4 ± 23.3	-165.9 ± 44.5
Aug-99	Shoal1	12.1 ± 8.6	18.8 ± 26.5	69.7 ± 26.4	23.2 ± 8.8
Aug-99	Hog	-40.9 ± 14.6	22.1 ± 10.9	104.1 ± 95.1	-2.4 ± 176.8

Appendix E: Daily Amino Acid Flux Rates

The following tables contain the daily sediment fluxes of DFAA and DCAA measured during each flux experiment. Positive values indicate a flux from the sediment to the water column. The "core" column indicates the experimental treatment; S = sediment only, S+A = sediment plus *Ulva lactuca*. Units are nmol AA m^{-2} d^{-1} and errors are the standard deviation for n = 3.

Mean Daily Flux Rates - DFAA - 1

Date	Site	Core	ASP	GLU	ASN
10-98	WW	S	-862 ± 1581	-2809 ± 686	136 ± 895
10-98	Creek	S	-3308 ± 969	617 ± 782	-362 ± 83
10-98	Shoal1	S	5223 ± 8246	-1934 ± 1512	259 ± 741
10-98	Hog	S	-1769 ± 2327	-4340 ± 3324	-656 ± 832
10-98	WW	S+A	-4052 ± 2988	-3317 ± 987	17 ± 489
10-98	Creek	S+A	-2670 ± nd	871 ± nd	-409 ± nd
10-98	Shoal1	S+A	-1088 ± 2703	-2417 ± 1901	-1895 ± 88
10-98	Hog	S+A	-801 ± 3599	-3654 ± 671	-1790 ± 142
1-99	WW	S	-205 ± 1478	-106 ± 151	-14 ± 20
1-99	Creek	S	-27 ± 578	-1344 ± 110	388 ± 52
1-99	Shoal1	S	2398 ± 6546	2939 ± 2432	-464 ± 912
1-99	Hog	S	5594 ± 5460	1209 ± 341	2063 ± 485
1-99	WW	S+A	-1564 ± 721	22 ± 39	0 ± 0
1-99	Creek	S+A	-469 ± 151	-1326 ± 116	43 ± 336
1-99	Shoal1	S+A	-1284 ± 299	921 ± 1467	-3872 ± 2805
1-99	Hog	S+A	-2428 ± 2368	-2188 ± 2544	-437 ± 432
3-99	WW	S	-737 ± 2818	-2962 ± 850	1278 ± 1266
3-99	Creek	S	2419 ± 2757	-1324 ± 465	-44 ± 22
3-99	Shoal1	S	-2379 ± 658	-1438 ± 999	589 ± 1564
3-99	Hog	S	-3603 ± 127	-2409 ± 1185	602 ± 1188
3-99	WW	S+A	2142 ± 1280	-702 ± 2626	861 ± 503
3-99	Creek	S+A	928 ± 109	-2563 ± 182	1105 ± 1715
3-99	Shoal1	S+A	-1842 ± 388	-1868 ± 711	59 ± 175
3-99	Hog	S+A	-2071 ± 5185	-3000 ± 3763	-97 ± 192
5-99	WW	S	5160 ± 638	-2390 ± 2604	-2259 ± 236
5-99	Creek	S	-4010 ± 1254	-1327 ± 2344	-133 ± 30
5-99	Shoal1	S	-6221 ± 189	-4780 ± 1488	-291 ± 290
5-99	Hog	S	-1282 ± 1205	-3623 ± 3204	357 ± 402
5-99	WW	S+A	-4961 ± 1713	-7726 ± 4534	-2357 ± 364
5-99	Creek	S+A	-2710 ± 2711	-2354 ± 1921	560 ± 1203
5-99	Shoal1	S+A	-8453 ± 524	-4284 ± 3125	1045 ± 1705
5-99	Hog	S+A	-5156 ± 1217	-4367 ± 802	-186 ± 1519
8-99	WW	S	-4257 ± 8016	-3629 ± 938	-1254 ± 1279
8-99	Creek	S	1598 ± 6910	-1490 ± 380	-1292 ± 800
8-99	Shoal1	S	-12385 ± 273	-361 ± 32	-1493 ± 1218
8-99	Hog	S	-3094 ± 1571	-3167 ± 1023	364 ± 581
8-99	WW	S+A	3033 ± 9915	-3316 ± 1302	1205 ± 2346
8-99	Creek	S+A	-4281 ± 3279	-498 ± 180	-2003 ± 336
8-99	Shoal1	S+A	-3318 ± 5495	-166 ± 395	-1014 ± 1287
8-99	Hog	S+A	-3361 ± 1631	-2233 ± 972	-499 ± 298

Mean Daily Flux Rates - DFAA - 2

Date	Site	Core	SER	GLN	HIS
10-98	WW	S	-14487 ± 3561	2621 ± 2653	-84 ± 2855
10-98	Creek	S	-12870 ± 2078	-337 ± 1181	-156 ± 1251
10-98	Shoal1	S	10398 ± 17490	1011 ± 1353	-50 ± 188
10-98	Hog	S	-8113 ± 1986	-1693 ± 485	-76 ± 218
10-98	WW	S+A	-13473 ± 9027	-31 ± 36	-3498 ± 2749
10-98	Creek	S+A	-15731 ± nd	-130 ± nd	444 ± nd
10-98	Shoal1	S+A	-6639 ± 1019	219 ± 1999	-402 ± 1611
10-98	Hog	S+A	-4940 ± 6153	-682 ± 1170	9241 ± 12910
1-99	WW	S	-1668 ± 7369	3681 ± 698	-317 ± 3338
1-99	Creek	S	-6189 ± 2689	-597 ± 1383	-6566 ± 2104
1-99	Shoal1	S	20999 ± 28773	1140 ± 1842	3446 ± 5242
1-99	Hog	S	25551 ± 8924	83 ± 564	3839 ± 1901
1-99	WW	S+A	3554 ± 516	2424 ± 1405	3437 ± 96
1-99	Creek	S+A	-6420 ± 4045	220 ± 1823	-4321 ± 1700
1-99	Shoal1	S+A	-5181 ± 9101	-1157 ± 472	1090 ± 1765
1-99	Hog	S+A	-7641 ± 2599	-2546 ± 790	855 ± 1552
3-99	WW	S	-7330 ± 13859	352 ± 642	-367 ± 1075
3-99	Creek	S	16103 ± 6272	-3139 ± 4214	1016 ± 4601
3-99	Shoal1	S	-14516 ± 3900	-3139 ± 1407	2431 ± 1653
3-99	Hog	S	-306 ± 589	-4919 ± 1091	-7056 ± 5099
3-99	WW	S+A	-15850 ± 9653	-1714 ± 2260	-885 ± 3181
3-99	Creek	S+A	-5094 ± 1902	-4079 ± 2148	884 ± 1182
3-99	Shoal1	S+A	-17708 ± 157	-5123 ± 9	-1114 ± 1534
3-99	Hog	S+A	2085 ± 14373	-5506 ± 85	-2371 ± 6795
5-99	WW	S	-15867 ± 2469	-1230 ± 70	-12411 ± 7228
5-99	Creek	S	-6072 ± 2379	-1593 ± 2405	-2953 ± 47
5-99	Shoal1	S	6201 ± 8029	150 ± 1665	-9804 ± 5647
5-99	Hog	S	1601 ± 976	-955 ± 1140	-1055 ± 3752
5-99	WW	S+A	-23908 ± 14478	-880 ± 475	-13857 ± 12212
5-99	Creek	S+A	-4996 ± 5217	-1561 ± 1416	3417 ± 4032
5-99	Shoal1	S+A	-257 ± 2303	-245 ± 383	-14758 ± 3977
5-99	Hog	S+A	-231 ± 1981	-509 ± 1274	-4257 ± 4985
8-99	WW	S	-7609 ± 2646	2360 ± 2329	-10848 ± 8266
8-99	Creek	S	5472 ± 10870	998 ± 1306	443 ± 16547
8-99	Shoal1	S	-14142 ± 2453	-1409 ± 586	-9173 ± 3449
8-99	Hog	S	-11688 ± 4564	1584 ± 1084	-2216 ± 1604
8-99	WW	S+A	6509 ± 52	4864 ± 2135	-2010 ± 308
8-99	Creek	S+A	4356 ± 5039	-154 ± 322	-6544 ± 632
8-99	Shoal1	S+A	-11234 ± 11209	-1355 ± 419	-4339 ± 12678
8-99	Hog	S+A	-13975 ± 7026	180 ± 269	-769 ± 3665

Mean Daily Flux Rates - DFAA - 3

Date	Site	Core	GLY	THR	ARG
10-98	WW	S	15244 ± 39542	-2209 ± 889	-487 ± 1036
10-98	Creek	S	-3114 ± 6584	-1497 ± 596	-363 ± 453
10-98	Shoal1	S	3097 ± 13369	855 ± 4439	-961 ± 3027
10-98	Hog	S	-20388 ± 3701	-6685 ± 1117	1614 ± 883
10-98	WW	S+A	-6817 ± 4346	-1694 ± 2464	409 ± 2217
10-98	Creek	S+A	-7617 ± nd	-1898 ± nd	437 ± nd
10-98	Shoal1	S+A	-6400 ± 5222	-2181 ± 2643	-4219 ± 630
10-98	Hog	S+A	-14868 ± 6211	-3261 ± 1328	1581 ± 933
1-99	WW	S	-14483 ± 2319	667 ± 1045	2363 ± 3002
1-99	Creek	S	-6253 ± 2708	-1185 ± 719	262 ± 118
1-99	Shoal1	S	10296 ± 11367	4392 ± 4332	-1879 ± 337
1-99	Hog	S	22572 ± 7317	9056 ± 1368	-101 ± 1055
1-99	WW	S+A	-16104 ± 2289	1126 ± 445	216 ± 232
1-99	Creek	S+A	-8897 ± 1831	-1368 ± 384	-86 ± 272
1-99	Shoal1	S+A	-8800 ± 4704	-3165 ± 4315	-2004 ± 536
1-99	Hog	S+A	-10497 ± 3678	-209 ± 1077	902 ± 1127
3-99	WW	S	-5169 ± 16840	-3872 ± 961	326 ± 558
3-99	Creek	S	-8581 ± 1229	447 ± 377	-3253 ± 881
3-99	Shoal1	S	-18537 ± 1335	-6865 ± 1928	1300 ± 2339
3-99	Hog	S	-12531 ± 4159	-1286 ± 1088	-1916 ± 357
3-99	WW	S+A	-18313 ± 9958	-4402 ± 1484	-2180 ± 1031
3-99	Creek	S+A	-23192 ± 4666	-5752 ± 59	-3397 ± 127
3-99	Shoal1	S+A	-19668 ± 7600	-8162 ± 880	124 ± 1417
3-99	Hog	S+A	-10645 ± 5547	-803 ± 1499	-2964 ± 1285
5-99	WW	S	-37923 ± 9505	-3534 ± 1556	5047 ± 11205
5-99	Creek	S	6641 ± 2666	-86 ± 122	625 ± 28
5-99	Shoal1	S	-12163 ± 9137	231 ± 602	5 ± 187
5-99	Hog	S	-6537 ± 18222	0 ± 0	160 ± 1009
5-99	WW	S+A	-31456 ± 19578	-7165 ± 6714	-5380 ± 661
5-99	Creek	S+A	-11306 ± 2379	-1695 ± 2129	3150 ± 2631
5-99	Shoal1	S+A	-21597 ± 4346	-908 ± 1573	494 ± 364
5-99	Hog	S+A	-23698 ± 3420	-138 ± 239	4722 ± 3777
8-99	WW	S	2831 ± 5576	-667 ± 31	-3465 ± 418
8-99	Creek	S	-6289 ± 5435	-198 ± 6058	6142 ± 7660
8-99	Shoal1	S	-7529 ± 3057	-995 ± 1981	-1312 ± 649
8-99	Hog	S	-21198 ± 2776	-240 ± 2575	-570 ± 227
8-99	WW	S+A	11671 ± 445	1392 ± 2953	-3674 ± 9
8-99	Creek	S+A	-5648 ± 7440	1768 ± 7132	1087 ± 12
8-99	Shoal1	S+A	-6862 ± 4749	-1143 ± 1939	3448 ± 4427
8-99	Hog	S+A	-18578 ± 7361	-662 ± 3663	-983 ± 57

Mean Daily Flux Rates - DFAA - 4

Date	Site	Core	ALA	TYR	G-ABA
10-98	WW	S	-5747 ± 3314	299 ± 863	3560 ± 1342
10-98	Creek	S	-2761 ± 1371	376 ± 1088	96 ± 0
10-98	Shoal1	S	-5792 ± 741	-468 ± 251	0 ± 0
10-98	Hog	S	-5059 ± 1416	-819 ± 684	2087 ± 3091
10-98	WW	S+A	-9335 ± 2132	1277 ± 1428	477 ± 80
10-98	Creek	S+A	-2555 ± nd	546 ± nd	96 ± nd
10-98	Shoal1	S+A	-5672 ± 988	-179 ± 348	959 ± 830
10-98	Hog	S+A	-4971 ± 3550	-535 ± 993	308 ± 296
1-99	WW	S	-3117 ± 837	1936 ± 326	-116 ± 1602
1-99	Creek	S	-1988 ± 4358	-863 ± 578	-656 ± 35
1-99	Shoal1	S	2285 ± 10450	3964 ± 5334	4305 ± 2662
1-99	Hog	S	9375 ± 4167	1844 ± 1190	-691 ± 1665
1-99	WW	S+A	-1895 ± 3443	-1110 ± 638	-1422 ± 298
1-99	Creek	S+A	-4118 ± 2449	-186 ± 951	-254 ± 819
1-99	Shoal1	S+A	-3837 ± 3776	9848 ± 6491	3624 ± 1217
1-99	Hog	S+A	-8195 ± 3279	557 ± 776	-984 ± 655
3-99	WW	S	-8684 ± 3472	293 ± 629	130 ± 1113
3-99	Creek	S	2607 ± 860	-477 ± 951	894 ± 2398
3-99	Shoal1	S	-5343 ± 766	-628 ± 1238	-1708 ± 32
3-99	Hog	S	528 ± 2145	996 ± 1047	-1579 ± 501
3-99	WW	S+A	-13000 ± 2871	-274 ± 1115	-2149 ± 367
3-99	Creek	S+A	-6536 ± 1924	-621 ± 1065	-1018 ± 315
3-99	Shoal1	S+A	-7388 ± 1421	-1756 ± 577	-1467 ± 314
3-99	Hog	S+A	-1442 ± 5359	321 ± 1132	-73 ± 1701
5-99	WW	S	-16513 ± 11307	-924 ± 1492	-698 ± 994
5-99	Creek	S	-8844 ± 65	302 ± 1517	1578 ± 2420
5-99	Shoal1	S	-1135 ± 291	-1733 ± 1002	-3132 ± 2496
5-99	Hog	S	1014 ± 987	-2528 ± 166	6226 ± 9031
5-99	WW	S+A	-21099 ± 2948	-2134 ± 1130	500 ± 702
5-99	Creek	S+A	439 ± 2226	-37 ± 1827	-2141 ± 711
5-99	Shoal1	S+A	-1426 ± 721	-2556 ± 155	-3713 ± 1334
5-99	Hog	S+A	-569 ± 1378	-2703 ± 477	-376 ± 375
8-99	WW	S	-8631 ± 1858	-1211 ± 267	161 ± 338
8-99	Creek	S	-4381 ± 3062	1298 ± 3337	84 ± 1827
8-99	Shoal1	S	-6606 ± 195	-475 ± 601	-2676 ± 155
8-99	Hog	S	-11395 ± 1635	844 ± 1178	769 ± 1090
8-99	WW	S+A	-10006 ± 721	-1420 ± 48	486 ± 801
8-99	Creek	S+A	-4403 ± 2312	-958 ± 174	1007 ± 2674
8-99	Shoal1	S+A	-4974 ± 2332	882 ± 1967	-1895 ± 1246
8-99	Hog	S+A	-11045 ± 5442	1569 ± 2766	211 ± 0

Mean Daily Flux Rates - DFAA - 5

Date	Site	Core	MET/TRP	PHE	ILE
10-98	WW	S	301 ± 439	4224 ± 1065	-599 ± 672
10-98	Creek	S	113 ± 0	171 ± 0	-388 ± 0
10-98	Shoal1	S	57 ± 40	0 ± 0	261 ± 453
10-98	Hog	S	-935 ± 451	987 ± 159	-2078 ± 874
10-98	WW	S+A	705 ± 64	3741 ± 1445	-860 ± 1853
10-98	Creek	S+A	113 ± nd	171 ± nd	-388 ± nd
10-98	Shoal1	S+A	36 ± 71	0 ± 0	-112 ± 194
10-98	Hog	S+A	-1803 ± 324	44 ± 1246	2338 ± 5972
1-99	WW	S	-3072 ± 66	375 ± 530	349 ± 265
1-99	Creek	S	-4492 ± 2338	-184 ± 58	-1076 ± 102
1-99	Shoal1	S	889 ± 688	665 ± 1457	1627 ± 1826
1-99	Hog	S	1358 ± 348	1930 ± 226	1931 ± 1010
1-99	WW	S+A	-3155 ± 149	107 ± 186	280 ± 360
1-99	Creek	S+A	-1909 ± 1109	-72 ± 38	-950 ± 87
1-99	Shoal1	S+A	-592 ± 99	-460 ± 566	-97 ± 955
1-99	Hog	S+A	158 ± 707	-275 ± 313	-897 ± 880
3-99	WW	S	-121 ± 0	620 ± 821	-1038 ± 355
3-99	Creek	S	565 ± 799	-2667 ± 5	1381 ± 1828
3-99	Shoal1	S	2166 ± 1782	-1975 ± 787	759 ± 771
3-99	Hog	S	107 ± 0	-1106 ± 696	-369 ± 336
3-99	WW	S+A	-121 ± 0	969 ± 885	-1116 ± 280
3-99	Creek	S+A	-532 ± 752	-1395 ± 370	356 ± 1234
3-99	Shoal1	S+A	551 ± 640	-628 ± 286	-924 ± 202
3-99	Hog	S+A	-2338 ± 642	-124 ± 27	-104 ± 2267
5-99	WW	S	-876 ± 484	-4279 ± 63	-3071 ± 395
5-99	Creek	S	1214 ± 2473	0 ± 0	-284 ± 549
5-99	Shoal1	S	-4344 ± 1448	-2477 ± 40	-5237 ± 444
5-99	Hog	S	1077 ± 179	1863 ± 1064	0 ± 0
5-99	WW	S+A	-250 ± 944	-3272 ± 1417	-2910 ± 356
5-99	Creek	S+A	1842 ± 3354	-1122 ± 1173	-619 ± 708
5-99	Shoal1	S+A	-300 ± 1348	-1982 ± 1484	-5437 ± 199
5-99	Hog	S+A	373 ± 2262	-3097 ± 365	27 ± 799
8-99	WW	S	-233 ± 1070	389 ± 666	-940 ± 151
8-99	Creek	S	-124 ± 214	0 ± 0	336 ± 0
8-99	Shoal1	S	-1120 ± 0	-1913 ± 0	26 ± 718
8-99	Hog	S	916 ± 2599	470 ± 1491	-1474 ± 846
8-99	WW	S+A	-120 ± 859	103 ± 631	-498 ± 737
8-99	Creek	S+A	0 ± 0	1417 ± 2004	336 ± 0
8-99	Shoal1	S+A	-1120 ± 0	-2069 ± 271	550 ± 2020
8-99	Hog	S+A	-880 ± 226	852 ± 2494	-370 ± 1128

Mean Daily Flux Rates - DFAA - 6

Date	Site	Core	LEU
10-98	WW	S	-991 ± 1318
10-98	Creek	S	152 ± 0
10-98	Shoal1	S	-66 ± 188
10-98	Hog	S	-2038 ± 368
10-98	WW	S+A	-1301 ± 937
10-98	Creek	S+A	152 ± nd
10-98	Shoal1	S+A	43 ± 0
10-98	Hog	S+A	-736 ± 910
1-99	WW	S	626 ± 457
1-99	Creek	S	-584 ± 69
1-99	Shoal1	S	959 ± 2399
1-99	Hog	S	2612 ± 1499
1-99	WW	S+A	1204 ± 1337
1-99	Creek	S+A	-223 ± 542
1-99	Shoal1	S+A	417 ± 2119
1-99	Hog	S+A	-1164 ± 335
3-99	WW	S	-1069 ± 1207
3-99	Creek	S	2017 ± 2099
3-99	Shoal1	S	-2556 ± 1081
3-99	Hog	S	-731 ± 2112
3-99	WW	S+A	-1416 ± 196
3-99	Creek	S+A	-539 ± 882
3-99	Shoal1	S+A	-2653 ± 2712
3-99	Hog	S+A	-109 ± 1921
5-99	WW	S	-4218 ± 627
5-99	Creek	S	0 ± 0
5-99	Shoal1	S	-313 ± 0
5-99	Hog	S	-132 ± 18
5-99	WW	S+A	-5792 ± 1428
5-99	Creek	S+A	-54 ± 1391
5-99	Shoal1	S+A	-689 ± 652
5-99	Hog	S+A	125 ± 807
8-99	WW	S	-911 ± 299
8-99	Creek	S	-429 ± 324
8-99	Shoal1	S	731 ± 1227
8-99	Hog	S	1095 ± 636
8-99	WW	S+A	180 ± 287
8-99	Creek	S+A	-123 ± 20
8-99	Shoal1	S+A	456 ± 753
8-99	Hog	S+A	2571 ± 1366

Mean Daily Flux Rates - DCAA - 1

Date	Site	Core	ASP	GLU	SER
10-98	WW	S	11543 ± 5640	12559 ± 8754	19633 ± 7408
10-98	Creek	S	1457 ± 20836	-4235 ± 20553	11849 ± 19335
10-98	Shoal1	S	12098 ± 3051	25379 ± 26795	7597 ± 1925
10-98	Hog	S	8606 ± 2269	5950 ± 1351	7095 ± 3407
10-98	WW	S+A	28896 ± 17433	56804 ± 63173	40854 ± 25010
10-98	Creek	S+A	23306 ± nd	7046 ± nd	28940 ± nd
10-98	Shoal1	S+A	20767 ± 9646	27696 ± 15320	17070 ± 7436
10-98	Hog	S+A	11416 ± 4930	26592 ± 17839	7094 ± 5189
1-99	WW	S	8871 ± 4743	18867 ± 62	10967 ± 1510
1-99	Creek	S	5084 ± 4998	1660 ± 8418	6306 ± 7287
1-99	Shoal1	S	2481 ± 6366	19109 ± 13021	6767 ± 1519
1-99	Hog	S	-1142 ± 2555	1805 ± 5975	-4573 ± 1687
1-99	WW	S+A	18307 ± 5077	52358 ± 5691	25091 ± 9
1-99	Creek	S+A	4083 ± 1276	-853 ± 12203	-12128 ± 3293
1-99	Shoal1	S+A	4514 ± 5007	6203 ± 3479	1403 ± 6968
1-99	Hog	S+A	11568 ± 9553	23129 ± 18084	4679 ± 9350
3-99	WW	S	-26716 ± 12816	-30719 ± 18480	-34790 ± 17453
3-99	Creek	S	-31760 ± 10165	-83451 ± 39596	-22991 ± 17408
3-99	Shoal1	S	134 ± 7773	-365 ± 3417	-12849 ± 7162
3-99	Hog	S	32573 ± 11208	44263 ± 21919	40467 ± 17544
3-99	WW	S+A	14332 ± 12196	14306 ± 14651	-11462 ± 14475
3-99	Creek	S+A	103364 ± 77448	107561 ± 123540	62821 ± 72071
3-99	Shoal1	S+A	37090 ± 40297	52642 ± 49499	13316 ± 25711
3-99	Hog	S+A	61665 ± 29743	80789 ± 59937	61426 ± 35356
5-99	WW	S	16262 ± 7019	16629 ± 5392	20478 ± 6780
5-99	Creek	S	-4619 ± 12155	6304 ± 11192	17597 ± 14651
5-99	Shoal1	S	1816 ± nd	-11202 ± nd	-20593 ± nd
5-99	Hog	S	2290 ± 4722	6115 ± 4945	2178 ± 9990
5-99	WW	S+A	26680 ± 7789	35464 ± 9366	20916 ± 10594
5-99	Creek	S+A	9747 ± 9802	23873 ± 16580	11970 ± 2757
5-99	Shoal1	S+A	7639 ± 1341	3294 ± 5079	-12209 ± 8756
5-99	Hog	S+A	-3497 ± 26611	-2392 ± 29218	-14677 ± 28608
8-99	WW	S	4256 ± 22646	-5036 ± 3327	-18502 ± 29703
8-99	Creek	S	4483 ± 5079	12905 ± 9869	8948 ± 24139
8-99	Shoal1	S	13877 ± 1424	-7683 ± 5777	9842 ± 5484
8-99	Hog	S	3150 ± 17089	-1423 ± 11039	9823 ± 11952
8-99	WW	S+A	14350 ± 11669	-721 ± 4595	-9593 ± 11664
8-99	Creek	S+A	6102 ± 18145	3843 ± 17283	4846 ± 15487
8-99	Shoal1	S+A	8876 ± 11367	3294 ± 18555	13688 ± 547
8-99	Hog	S+A	11139 ± 10547	7353 ± 8441	1554 ± 14952

Mean Daily Flux Rates - DCAA - 2

Date	Site	Core	HIS	GLY	THR
10-98	WW	S	4854 ± 1562	10840 ± 11325	6628 ± 4302
10-98	Creek	S	-1698 ± 9418	-11406 ± 38541	3995 ± 15000
10-98	Shoal1	S	2727 ± 6857	20963 ± 6354	4936 ± 5895
10-98	Hog	S	-2373 ± 5863	17542 ± 8431	4484 ± 2737
10-98	WW	S+A	-1717 ± 2211	37208 ± 30373	14586 ± 7099
10-98	Creek	S+A	2174 ± nd	27456 ± nd	15693 ± nd
10-98	Shoal1	S+A	595 ± 7541	27484 ± 16542	11953 ± 6315
10-98	Hog	S+A	-6750 ± 15432	29138 ± 12294	4628 ± 3540
1-99	WW	S	-8657 ± 11935	8318 ± 21885	3671 ± 5629
1-99	Creek	S	5495 ± 6802	14292 ± 3971	7379 ± 4814
1-99	Shoal1	S	1436 ± 9249	16476 ± 173	753 ± 2615
1-99	Hog	S	302 ± 1120	2817 ± 10397	4967 ± 159
1-99	WW	S+A	1850 ± 7305	43637 ± 14286	26522 ± 3177
1-99	Creek	S+A	11226 ± 2308	13586 ± 4609	5723 ± 6279
1-99	Shoal1	S+A	5205 ± 3361	13498 ± 4295	3077 ± 7681
1-99	Hog	S+A	381 ± 1715	14745 ± 8370	6379 ± 4017
3-99	WW	S	5992 ± 1184	-73324 ± 18847	5135 ± 9782
3-99	Creek	S	-5374 ± 3398	-4849 ± 11284	-19003 ± 6730
3-99	Shoal1	S	-2641 ± 2193	-49065 ± 29126	-20082 ± 7719
3-99	Hog	S	4173 ± 4531	86172 ± 24305	22191 ± 5233
3-99	WW	S+A	14701 ± 16739	31137 ± 5849	23361 ± 8349
3-99	Creek	S+A	24505 ± 34782	79291 ± 76457	73823 ± 57084
3-99	Shoal1	S+A	11069 ± 14638	16659 ± 48609	12496 ± 18581
3-99	Hog	S+A	5033 ± 2641	117297 ± 36368	40872 ± 10163
5-99	WW	S	11283 ± 4721	-11520 ± 97553	40849 ± 30785
5-99	Creek	S	1170 ± 7023	773 ± 9624	1274 ± 5043
5-99	Shoal1	S	-1143 ± nd	-23964 ± nd	-6912 ± nd
5-99	Hog	S	-314 ± 5819	-6692 ± 15934	-3388 ± 5929
5-99	WW	S+A	7337 ± 4015	75785 ± 35553	39269 ± 4861
5-99	Creek	S+A	-1580 ± 6218	27735 ± 16295	5871 ± 6131
5-99	Shoal1	S+A	8139 ± 7682	12634 ± 2022	3092 ± 5241
5-99	Hog	S+A	-37 ± 13192	-4119 ± 53368	-8516 ± 29130
8-99	WW	S	-5021 ± 32294	-63262 ± 42703	-4173 ± 27614
8-99	Creek	S	6700 ± 16087	152594 ± 68516	-990 ± 6455
8-99	Shoal1	S	1199 ± 6744	-8654 ± 10834	3806 ± 3285
8-99	Hog	S	6889 ± 23375	13969 ± 26145	7787 ± 14595
8-99	WW	S+A	20581 ± 1207	2393 ± 10916	14270 ± 4031
8-99	Creek	S+A	17597 ± 3269	103245 ± 11183	4955 ± 5665
8-99	Shoal1	S+A	6986 ± 6957	17400 ± 8410	12700 ± 15573
8-99	Hog	S+A	19073 ± 23376	34216 ± 32226	16404 ± 10386

Mean Daily Flux Rates - DCAA - 3

Date	Site	Core	ARG	ALA	TYR
10-98	WW	S	-510 ± 6064	-1745 ± 5160	-2479 ± 3106
10-98	Creek	S	-6656 ± 4754	-7365 ± 24040	375 ± 5293
10-98	Shoal1	S	7345 ± 5250	14717 ± 3291	559 ± 1258
10-98	Hog	S	1268 ± 7575	-697 ± 5506	628 ± 2041
10-98	WW	S+A	4695 ± 352	25765 ± 5712	1396 ± 2717
10-98	Creek	S+A	-4380 ± nd	19710 ± nd	4495 ± nd
10-98	Shoal1	S+A	8781 ± 2137	16086 ± 12820	1821 ± 1811
10-98	Hog	S+A	6510 ± 5767	2193 ± 9616	3647 ± 1988
1-99	WW	S	-11506 ± 2998	4146 ± 14537	-4535 ± 1167
1-99	Creek	S	-6631 ± 6795	8083 ± 7913	1317 ± 705
1-99	Shoal1	S	8397 ± 2960	646 ± 1842	-760 ± 915
1-99	Hog	S	2176 ± 1113	-11913 ± 11355	1187 ± 1354
1-99	WW	S+A	-3321 ± 2949	5412 ± 4942	1915 ± 478
1-99	Creek	S+A	-794 ± 3618	12398 ± 9645	-1298 ± 3025
1-99	Shoal1	S+A	-6918 ± 19	-3953 ± 2201	-1591 ± 291
1-99	Hog	S+A	4983 ± 4121	5763 ± 25422	-565 ± 293
3-99	WW	S	-7852 ± 9369	-38635 ± 21365	350 ± 2086
3-99	Creek	S	15557 ± 47145	-53920 ± 45472	-3190 ± 2815
3-99	Shoal1	S	2055 ± 3271	-16613 ± 13879	-6845 ± 2391
3-99	Hog	S	-504 ± 3255	43293 ± 14701	1649 ± 2229
3-99	WW	S+A	6903 ± 63	20297 ± 3937	-780 ± 46
3-99	Creek	S+A	22677 ± 24984	92896 ± 68459	3208 ± 5147
3-99	Shoal1	S+A	2290 ± 2711	16768 ± 62453	-3113 ± 2788
3-99	Hog	S+A	11745 ± 7458	81193 ± 37452	2607 ± 2936
5-99	WW	S	5128 ± 7493	31163 ± 6682	764 ± 1564
5-99	Creek	S	-1185 ± 7023	14046 ± 2852	-1256 ± 2074
5-99	Shoal1	S	-14792 ± nd	-18988 ± nd	3353 ± nd
5-99	Hog	S	158 ± 4218	-3341 ± 5835	368 ± 861
5-99	WW	S+A	9578 ± 5807	37455 ± 12310	730 ± 1660
5-99	Creek	S+A	8389 ± 3623	32176 ± 3350	-1078 ± 2169
5-99	Shoal1	S+A	2297 ± 7660	322 ± 7556	855 ± 572
5-99	Hog	S+A	-5361 ± 755	-20073 ± 41072	-401 ± 3163
8-99	WW	S	177 ± 3258	-30863 ± 29496	-1326 ± 1263
8-99	Creek	S	-158 ± 4554	88125 ± 58698	20934 ± 7893
8-99	Shoal1	S	0 ± 0	3629 ± 7280	1213 ± 1859
8-99	Hog	S	2419 ± 4272	-179 ± 20044	-504 ± 1266
8-99	WW	S+A	467 ± 2409	6108 ± 19185	4354 ± 599
8-99	Creek	S+A	701 ± 4007	54160 ± 4197	8874 ± 5888
8-99	Shoal1	S+A	1462 ± 2067	26557 ± 10863	1175 ± 3156
8-99	Hog	S+A	-57 ± 16	10455 ± 25905	-750 ± 1845

Mean Daily Flux Rates - DCAA - 4

Date	Site	Core	G-ABA	MET/TRP	PHE
10-98	WW	S	-7613 ± 325	628 ± 1380	1627 ± 2772
10-98	Creek	S	-2120 ± 1829	905 ± 891	-1061 ± 4041
10-98	Shoal1	S	782 ± 851	2478 ± 1475	1099 ± 791
10-98	Hog	S	-243 ± 868	2131 ± 2459	-1063 ± 1574
10-98	WW	S+A	-2869 ± 2068	624 ± 2310	4767 ± 2782
10-98	Creek	S+A	2953 ± nd	0 ± nd	4437 ± nd
10-98	Shoal1	S+A	2715 ± 1515	2759 ± 1376	3199 ± 618
10-98	Hog	S+A	4008 ± 8099	2178 ± 1174	6152 ± 617
1-99	WW	S	-4117 ± 2036	115 ± 45	1446 ± 1651
1-99	Creek	S	2366 ± 3382	0 ± 0	-660 ± 2690
1-99	Shoal1	S	-6183 ± 3843	0 ± 0	-1472 ± 602
1-99	Hog	S	13224 ± 2136	383 ± 2004	-2184 ± 1052
1-99	WW	S+A	1175 ± 1709	145 ± 28	2607 ± 1587
1-99	Creek	S+A	2458 ± 5014	0 ± 0	921 ± 2622
1-99	Shoal1	S+A	-2943 ± 3325	0 ± 0	-913 ± 68
1-99	Hog	S+A	2098 ± 6021	162 ± 1787	373 ± 1795
3-99	WW	S	2479 ± 7893	508 ± 2979	921 ± 3304
3-99	Creek	S	-1150 ± 7018	-561 ± 5060	-3068 ± 1819
3-99	Shoal1	S	-9377 ± 10472	-687 ± 2315	20 ± 3489
3-99	Hog	S	1404 ± 3733	1460 ± 2313	3914 ± 2190
3-99	WW	S+A	-1961 ± 10951	4200 ± 762	6577 ± 3737
3-99	Creek	S+A	-2680 ± 21406	-618 ± 3371	19602 ± 13594
3-99	Shoal1	S+A	-12517 ± 6518	1189 ± 2266	7985 ± 9420
3-99	Hog	S+A	2490 ± 2089	4247 ± 1748	8439 ± 4594
5-99	WW	S	-6972 ± 804	4102 ± 2670	3494 ± 5786
5-99	Creek	S	5580 ± 9880	-1217 ± 2873	-3034 ± 3296
5-99	Shoal1	S	1384 ± nd	4180 ± nd	881 ± nd
5-99	Hog	S	-2162 ± 3813	-491 ± 339	-4313 ± 1173
5-99	WW	S+A	-7578 ± 2030	271 ± 4123	6195 ± 4106
5-99	Creek	S+A	9198 ± 2875	-1188 ± 2432	1350 ± 1120
5-99	Shoal1	S+A	2744 ± 4178	797 ± 882	912 ± 2734
5-99	Hog	S+A	-2095 ± 5015	277 ± 2032	-1835 ± 4780
8-99	WW	S	-4253 ± 2767	244 ± 1056	-2915 ± 2384
8-99	Creek	S	-2665 ± 737	-9076 ± 3355	6582 ± 720
8-99	Shoal1	S	4296 ± 792	0 ± 0	31 ± 3391
8-99	Hog	S	-5238 ± 760	-796 ± 187	134 ± 1997
8-99	WW	S+A	-2801 ± 1255	6092 ± 532	1940 ± 4401
8-99	Creek	S+A	-960 ± 2500	-11013 ± 0	4446 ± 3717
8-99	Shoal1	S+A	1445 ± 803	0 ± 0	5814 ± 1278
8-99	Hog	S+A	2191 ± 9444	-996 ± 177	-610 ± 3191

Mean Daily Flux Rates - DCAA - 5

Date	Site	Core	ILE	LEU
10-98	WW	S	-792 ± 2004	3920 ± 4565
10-98	Creek	S	-1236 ± 6158	6219 ± 3532
10-98	Shoal1	S	-408 ± 490	-2654 ± 6235
10-98	Hog	S	3098 ± 3311	1868 ± 412
10-98	WW	S+A	4442 ± 473	8975 ± 5837
10-98	Creek	S+A	2378 ± nd	12845 ± nd
10-98	Shoal1	S+A	2092 ± 672	3162 ± 4891
10-98	Hog	S+A	-3973 ± 4133	4323 ± 4444
1-99	WW	S	-2994 ± 6297	5055 ± 6071
1-99	Creek	S	-729 ± 3838	-1175 ± 512
1-99	Shoal1	S	-6127 ± 2325	-2605 ± 4905
1-99	Hog	S	330 ± 2786	-810 ± 943
1-99	WW	S+A	2385 ± 4085	8542 ± 232
1-99	Creek	S+A	1842 ± 354	-976 ± 2515
1-99	Shoal1	S+A	-6004 ± 465	-392 ± 1949
1-99	Hog	S+A	39 ± 2027	3640 ± 2792
3-99	WW	S	-4165 ± 3877	2560 ± 7075
3-99	Creek	S	-13059 ± 882	-21696 ± 2873
3-99	Shoal1	S	-7658 ± 1378	-6051 ± 3054
3-99	Hog	S	3871 ± 4377	1845 ± 4998
3-99	WW	S+A	824 ± 936	6919 ± 1074
3-99	Creek	S+A	11204 ± 12010	11879 ± 8840
3-99	Shoal1	S+A	-2187 ± 7044	1743 ± 9344
3-99	Hog	S+A	13391 ± 4658	6715 ± 5567
5-99	WW	S	2067 ± 1770	7298 ± 3782
5-99	Creek	S	70 ± 2707	-2634 ± 1656
5-99	Shoal1	S	2989 ± nd	-4496 ± nd
5-99	Hog	S	505 ± 2982	897 ± 651
5-99	WW	S+A	4853 ± 801	7048 ± 4990
5-99	Creek	S+A	3441 ± 2532	-1304 ± 3145
5-99	Shoal1	S+A	2501 ± 408	-2069 ± 1263
5-99	Hog	S+A	-1677 ± 3014	-1272 ± 2764
8-99	WW	S	-8320 ± 3804	-10519 ± 7564
8-99	Creek	S	16733 ± 1108	17263 ± 11056
8-99	Shoal1	S	-184 ± 1230	1478 ± 4568
8-99	Hog	S	904 ± 437	2419 ± 1064
8-99	WW	S+A	-1913 ± 4019	-1436 ± 816
8-99	Creek	S+A	13107 ± 7159	13096 ± 2373
8-99	Shoal1	S+A	3133 ± 21	-870 ± 4399
8-99	Hog	S+A	-2089 ± 3607	393 ± 1098

Appendix F: Daily Macroalgal Uptake of Amino Acids

The following tables contain the daily macroalgal release and uptake of DFAA and DCAA measured during each flux experiment. Positive values indicate a release from *U. lactuca*; negative values indicate uptake by *U. lactuca*. Units are nmol AA g dw^{-1} d^{-1} and errors are the standard deviation for n = 3.

Mean Daily Macroalgal Uptake Rates - DFAA - 1

Date	Site	ASP	GLU	ASN	SER
10-98	WW	-45 ± 42	-7 ± 14	-2 ± 7	15 ± 129
10-98	Creek	23 ± nd	9 ± nd	-2 ± nd	-104 ± nd
10-98	Shoal1	-88 ± 53	-9 ± 25	-29 ± 8	-225 ± 46
10-98	Hog	13 ± 43	8 ± 7	-13 ± 3	35 ± 70
1-99	WW	-45 ± 36	13 ± 5	1 ± 1	140 ± 42
1-99	Creek	-6 ± 2	-9 ± 2	-24 ± 5	-33 ± 54
1-99	Shoal1	-55 ± 17	-28 ± 18	-47 ± 37	-370 ± 75
1-99	Hog	-121 ± 40	-56 ± 38	-34 ± 8	-454 ± 50
3-99	WW	61 ± 30	39 ± 36	-11 ± 12	-205 ± 256
3-99	Creek	-25 ± 0	-21 ± 5	21 ± 31	-363 ± 59
3-99	Shoal1	10 ± 7	-8 ± 13	-10 ± 3	-60 ± 6
3-99	Hog	17 ± 72	-13 ± 56	-10 ± 1	19 ± 205
5-99	WW	-194 ± 6	-109 ± 102	-1 ± 7	-175 ± 302
5-99	Creek	6 ± 37	-15 ± 24	15 ± 18	-43 ± 73
5-99	Shoal1	-26 ± 1	4 ± 40	13 ± 15	-75 ± 9
5-99	Hog	-48 ± 3	-14 ± 8	-4 ± 18	19 ± 21
8-99	WW	137 ± 187	5 ± 23	43 ± 40	255 ± 14
8-99	Creek	-128 ± 60	22 ± 2	-15 ± 6	-19 ± 110
8-99	Shoal1	117 ± 79	2 ± 5	7 ± 18	44 ± 154
8-99	Hog	-5 ± 29	16 ± 16	-15 ± 6	-42 ± 125

Date	Site	GLN	HIS	GLY	THR
10-98	WW	-38 ± 0	-49 ± 39	-315 ± 60	7 ± 35
10-98	Creek	7 ± nd	22 ± nd	-163 ± nd	-15 ± nd
10-98	Shoal1	-13 ± 28	-5 ± 19	-133 ± 93	-43 ± 40
10-98	Hog	11 ± 13	105 ± 145	63 ± 69	40 ± 13
1-99	WW	-60 ± 78	183 ± 78	-37 ± 52	17 ± 24
1-99	Creek	9 ± 25	29 ± 23	-44 ± 27	-9 ± 5
1-99	Shoal1	-36 ± 16	-31 ± 19	-273 ± 32	-102 ± 29
1-99	Hog	-34 ± 11	-45 ± 24	-472 ± 50	-127 ± 21
3-99	WW	-39 ± 44	-16 ± 79	-310 ± 278	-14 ± 37
3-99	Creek	-15 ± 35	-2 ± 20	-246 ± 61	-106 ± 7
3-99	Shoal1	-37 ± 2	-68 ± 32	-25 ± 145	-25 ± 18
3-99	Hog	-8 ± 0	61 ± 89	21 ± 77	5 ± 21
5-99	WW	6 ± 8	-45 ± 240	99 ± 362	-79 ± 140
5-99	Creek	16 ± 21	4 ± 52	-248 ± 2	-30 ± 27
5-99	Shoal1	-6 ± 6	-64 ± 62	-125 ± 90	-17 ± 26
5-99	Hog	23 ± 13	-42 ± 43	-223 ± 91	15 ± 6
8-99	WW	46 ± 41	159 ± 4	160 ± 17	39 ± 55
8-99	Creek	-26 ± 10	-155 ± 2	23 ± 167	52 ± 164
8-99	Shoal1	1 ± 5	64 ± 168	7 ± 57	-3 ± 26
8-99	Hog	-25 ± 6	24 ± 63	44 ± 128	-9 ± 65

Mean Daily Macroalgal Uptake Rates - DFAA - 2

Date	Site	ARG	ALA	TYR	G-ABA
10-98	WW	13 ± 32	-51 ± 30	14 ± 21	-44 ± 1
10-98	Creek	29 ± nd	7 ± nd	6 ± nd	0 ± nd
10-98	Shoal1	-44 ± 19	1 ± 12	3 ± 4	11 ± 10
10-98	Hog	0 ± 11	0 ± 42	4 ± 12	-21 ± 2
1-99	WW	-64 ± 35	58 ± 97	-122 ± 36	-47 ± 31
1-99	Creek	-8 ± 4	-102 ± 31	6 ± 13	-3 ± 11
1-99	Shoal1	-3 ± 7	-86 ± 50	88 ± 115	-7 ± 13
1-99	Hog	18 ± 17	-236 ± 36	-16 ± 12	-8 ± 10
3-99	WW	-57 ± 33	-84 ± 28	-17 ± 24	-50 ± 16
3-99	Creek	-3 ± 2	-157 ± 44	-2 ± 18	-33 ± 8
3-99	Shoal1	-23 ± 28	-39 ± 29	-22 ± 12	5 ± 6
3-99	Hog	-17 ± 21	-35 ± 83	-11 ± 18	24 ± 28
5-99	WW	-201 ± 15	-93 ± 70	-25 ± 25	24 ± 17
5-99	Creek	31 ± 32	118 ± 16	-10 ± 23	-47 ± 5
5-99	Shoal1	6 ± 5	-5 ± 11	-10 ± 1	-8 ± 18
5-99	Hog	38 ± 50	-19 ± 21	-2 ± 5	-52 ± 11
8-99	WW	-4 ± 0	-25 ± 14	-4 ± 1	6 ± 15
8-99	Creek	-113 ± 11	-3 ± 52	-51 ± 9	24 ± 62
8-99	Shoal1	56 ± 51	23 ± 33	18 ± 28	10 ± 16
8-99	Hog	-7 ± 1	4 ± 95	12 ± 48	-10 ± 0

Date	Site	MET/TRP	PHE	ILE	LEU
10-98	WW	6 ± 1	-7 ± 21	-4 ± 26	-4 ± 13
10-98	Creek	0 ± nd	0 ± nd	0 ± nd	0 ± nd
10-98	Shoal1	0 ± 1	0 ± 0	-5 ± 4	1 ± 0
10-98	Hog	-10 ± 5	-12 ± 15	55 ± 74	16 ± 12
1-99	WW	-9 ± 7	-10 ± 9	-2 ± 13	30 ± 57
1-99	Creek	37 ± 16	5 ± 1	-1 ± 1	7 ± 7
1-99	Shoal1	-22 ± 5	-17 ± 12	-28 ± 21	-3 ± 30
1-99	Hog	-23 ± 11	-29 ± 5	-39 ± 10	-50 ± 7
3-99	WW	0 ± 0	5 ± 17	-3 ± 6	-7 ± 4
3-99	Creek	-19 ± 14	21 ± 5	-18 ± 22	-44 ± 18
3-99	Shoal1	-30 ± 11	26 ± 7	-32 ± 2	-3 ± 51
3-99	Hog	-36 ± 15	14 ± 2	1 ± 33	7 ± 27
5-99	WW	13 ± 20	18 ± 25	4 ± 7	-32 ± 32
5-99	Creek	12 ± 46	-14 ± 15	-6 ± 9	-2 ± 17
5-99	Shoal1	48 ± 11	3 ± 16	-2 ± 2	-6 ± 10
5-99	Hog	-5 ± 24	-47 ± 12	-14 ± 6	2 ± 10
8-99	WW	2 ± 15	-5 ± 11	8 ± 14	20 ± 6
8-99	Creek	3 ± 0	34 ± 48	0 ± 0	7 ± 0
8-99	Shoal1	0 ± 0	-2 ± 3	8 ± 28	-3 ± 10
8-99	Hog	-32 ± 5	6 ± 44	19 ± 19	26 ± 23

Mean Daily Macroalgal Uptake Rates - DCAA - 1

Date	Site	ASP	GLU	SER	HIS
10-98	WW	198 ± 197	502 ± 717	241 ± 283	-75 ± 24
10-98	Creek	248 ± nd	128 ± nd	194 ± nd	44 ± nd
10-98	Shoal1	99 ± 113	2 ± 218	119 ± 80	-37 ± 101
10-98	Hog	41 ± 65	303 ± 304	4 ± 63	-76 ± 208
1-99	WW	230 ± 75	889 ± 351	368 ± 85	251 ± 127
1-99	Creek	-13 ± 17	-31 ± 166	-252 ± 35	79 ± 35
1-99	Shoal1	16 ± 58	-180 ± 103	-87 ± 116	58 ± 61
1-99	Hog	187 ± 144	304 ± 253	136 ± 141	2 ± 25
3-99	WW	790 ± 51	861 ± 30	421 ± 124	116 ± 277
3-99	Creek	2169 ± 1030	3053 ± 1689	1356 ± 1034	463 ± 521
3-99	Shoal1	677 ± 739	987 ± 926	481 ± 468	260 ± 274
3-99	Hog	393 ± 371	468 ± 796	267 ± 471	15 ± 40
5-99	WW	212 ± 179	377 ± 232	23 ± 206	-71 ± 67
5-99	Creek	175 ± 102	212 ± 207	-75 ± 44	-39 ± 77
5-99	Shoal1	82 ± 35	208 ± 111	104 ± 97	139 ± 132
5-99	Hog	-20 ± 291	-47 ± 312	-146 ± 275	27 ± 155
8-99	WW	176 ± 200	80 ± 88	155 ± 201	462 ± 5
8-99	Creek	16 ± 401	-222 ± 406	-109 ± 355	247 ± 97
8-99	Shoal1	-73 ± 158	157 ± 260	51 ± 4	74 ± 88
8-99	Hog	140 ± 190	153 ± 144	-150 ± 274	210 ± 419

Date	Site	GLY	THR	ARG	ALA
10-98	WW	300 ± 344	91 ± 80	60 ± 3	315 ± 61
10-98	Creek	441 ± nd	133 ± nd	26 ± nd	307 ± nd
10-98	Shoal1	65 ± 204	84 ± 70	18 ± 24	2 ± 165
10-98	Hog	178 ± 214	8 ± 48	79 ± 96	18 ± 135
1-99	WW	963 ± 586	604 ± 221	204 ± 27	18 ± 121
1-99	Creek	-8 ± 63	-21 ± 85	81 ± 53	62 ± 135
1-99	Shoal1	-30 ± 44	14 ± 93	-205 ± 67	-57 ± 9
1-99	Hog	174 ± 125	21 ± 60	41 ± 62	260 ± 381
3-99	WW	2101 ± 642	340 ± 37	300 ± 109	1212 ± 509
3-99	Creek	1324 ± 1110	1486 ± 772	95 ± 401	2372 ± 859
3-99	Shoal1	1216 ± 868	605 ± 328	4 ± 50	604 ± 1140
3-99	Hog	415 ± 463	262 ± 107	171 ± 82	513 ± 464
5-99	WW	1739 ± 925	-24 ± 90	94 ± 125	139 ± 255
5-99	Creek	332 ± 189	53 ± 78	121 ± 42	235 ± 58
5-99	Shoal1	509 ± 131	146 ± 101	225 ± 58	256 ± 50
5-99	Hog	125 ± 634	-8 ± 323	-66 ± 29	-123 ± 421
8-99	WW	1179 ± 128	331 ± 53	4 ± 43	657 ± 307
8-99	Creek	-1089 ± 139	126 ± 113	15 ± 88	-753 ± 17
8-99	Shoal1	354 ± 139	127 ± 218	21 ± 29	313 ± 169
8-99	Hog	351 ± 576	150 ± 184	-44 ± 1	182 ± 468

Mean Daily Macroalgal Uptake Rates - DCAA - 2

Date	Site	TYR	G-ABA	MET/TRP	PHE
10-98	WW	45 ± 32	55 ± 25	0 ± 26	36 ± 31
10-98	Creek	47 ± nd	58 ± nd	-10 ± nd	62 ± nd
10-98	Shoal1	16 ± 20	27 ± 20	7 ± 21	29 ± 13
10-98	Hog	44 ± 40	74 ± 136	-2 ± 18	97 ± 29
1-99	WW	169 ± 52	133 ± 12	1 ± 1	35 ± 48
1-99	Creek	-37 ± 43	3 ± 69	0 ± 0	21 ± 35
1-99	Shoal1	-10 ± 0	36 ± 30	0 ± 0	7 ± 2
1-99	Hog	-25 ± 4	-162 ± 88	-3 ± 27	37 ± 26
3-99	WW	-23 ± 9	-50 ± 190	72 ± 11	101 ± 35
3-99	Creek	101 ± 73	-46 ± 358	2 ± 56	363 ± 183
3-99	Shoal1	69 ± 51	-62 ± 127	35 ± 41	146 ± 172
3-99	Hog	11 ± 41	18 ± 33	39 ± 19	61 ± 57
5-99	WW	2 ± 32	-9 ± 38	-69 ± 69	58 ± 87
5-99	Creek	0 ± 27	50 ± 45	-2 ± 30	57 ± 17
5-99	Shoal1	-35 ± 15	25 ± 62	-46 ± 3	4 ± 38
5-99	Hog	-3 ± 34	10 ± 59	13 ± 27	38 ± 65
8-99	WW	102 ± 5	26 ± 21	106 ± 16	85 ± 74
8-99	Creek	-276 ± 158	35 ± 52	-43 ± 4	-52 ± 88
8-99	Shoal1	-2 ± 42	-38 ± 8	0 ± 0	78 ± 23
8-99	Hog	-4 ± 34	129 ± 160	-4 ± 3	-14 ± 59

Date	Site	ILE	LEU
10-98	WW	60 ± 5	57 ± 66
10-98	Creek	41 ± nd	75 ± nd
10-98	Shoal1	34 ± 17	88 ± 92
10-98	Hog	-97 ± 62	26 ± 53
1-99	WW	128 ± 74	90 ± 15
1-99	Creek	35 ± 3	3 ± 35
1-99	Shoal1	1 ± 6	25 ± 16
1-99	Hog	-5 ± 31	64 ± 41
3-99	WW	98 ± 17	85 ± 10
3-99	Creek	391 ± 153	549 ± 83
3-99	Shoal1	100 ± 128	143 ± 169
3-99	Hog	134 ± 47	65 ± 71
5-99	WW	55 ± 23	2 ± 96
5-99	Creek	41 ± 26	18 ± 42
5-99	Shoal1	-6 ± 4	35 ± 24
5-99	Hog	-20 ± 28	-20 ± 25
8-99	WW	114 ± 66	164 ± 5
8-99	Creek	-73 ± 152	-96 ± 62
8-99	Shoal1	44 ± 3	-29 ± 57
8-99	Hog	-53 ± 65	-36 ± 18

Appendix G: Calculations for N Assimilation and Leakage

The following is an explanation of the calculations used in estimating the assimilation and leakage of N by *G. tikvahiae* as presented in Chapter 4. Following the equations is an example calculation based on the assimilation and release measured in the laboratory experiment conducted in February 2000.

Constants

$\%N_c$ = Critical N content (%N), set at 2.3% (Smit *et al*., 1997)

$\%N_{struct}$ = %N in the structural compartment, set at 0.8% (Hanisak, 1983)

$\%N_{act}$ = %N in the active compartment, set at 1.5% (= $N_c - N_{str}$)

$\%N_{stor}$ = %N in the storage compartment (= actual %N – N_c for all %N > N_c)

$\%^{15}N_{field}$ = atom % ^{15}N measured in field algae, usually ~0.37%

Measured parameters

μ_M = daily growth rate (d^{-1})

μ_N = daily rate of change in %N (d^{-1})

μ_{15} = daily rate of change in atom % ^{15}N (d^{-1})

Using the above parameters and the initial values, the following values were calculated on a daily time-step using an exponential equation:

M = thallus mass (mg)

%N = %N of thallus

$\%^{15}N_{meas}$ = atom % ^{15}N of thallus

Calculations

Total thallus N (N_{total} in mg) was calculated for each day as:

$$N_{total} = M \times \frac{\%N}{100} \quad (1)$$

and from this the amount of ^{15}N and ^{14}N in each thallus ($^{15}N_{total\text{-}meas}$ and $^{14}N_{total\text{-}meas}$ in mg) was calculated for each day based on:

$$^{15}N_{total-meas} = N_{total} \times \frac{\%^{15}N_{meas}}{100} \quad (2)$$

$$^{14}N_{total-meas} = N_{total} \times \frac{100 - \%^{15}N_{meas}}{100} \quad (3)$$

The change in $^{15}N\ d^{-1}$ ($\Delta^{15}N$) and $^{14}N\ d^{-1}$ ($\Delta^{14}N$) was then calculated by difference between time steps:

$$\Delta^{15}N = \left(^{15}N_{total-meas}\right)_{t_{n+1}} - \left(^{15}N_{total-meas}\right)_{t_n} \quad (4)$$

$$\Delta^{14}N = \left(^{14}N_{total-meas}\right)_{t_{n+1}} - \left(^{14}N_{total-meas}\right)_{t_n} \quad (5)$$

Newly assimilated N (N_{new} in mg) was calculated based on the difference between N_{tot} at time t_n and time t_{n+1} using equation 6:

$$N_{new} = \left(N_{total}\right)_{t_{n+1}} - \left(N_{total}\right)_{t_n} \quad (6)$$

Assuming that the newly assimilated N has an atom % ^{15}N equal to $\%^{15}N_{field}$, the predicted amount of ^{15}N added by assimilation ($^{15}N_{new\text{-}pred}$ in mg) should equal:

$$^{15}N_{new-pred} = N_{new} \times \frac{\%^{15}N_{field}}{100} \quad (7)$$

Assuming that all ^{15}N (and all ^{14}N) is retained, the predicted total ^{15}N in the thallus ($^{15}N_{total\text{-}pred}$ in mg) would be equal to:

$$^{15}N_{total-pred} = {}^{15}N_{new-pred} + \left({}^{15}N_{total-meas}\right)_{t_0} \quad (8)$$

$^{15}N_{total\text{-}pred}$ was calculated sequentially at each time step based on Σ $^{15}N_{new\text{-}pred}$ for all previous time steps.

The amount of "missing" ^{15}N ($^{15}N_{leak}$ in mg), assumed to be leaked to the water column, was calculated at each time step from the difference between the predicted and the measured ^{15}N using equation 9:

$$^{15}N_{leak} = {}^{15}N_{total-pred} - {}^{15}N_{total-meas} \quad (9)$$

In order to calculate the total N leaked, it was assumed that all leakage came from the active compartment and that leaked N had a ^{15}N:^{14}N equivalent to that of the total active compartment. The total amount of N in the active compartment (N_{act} in mg) was calculated based on equation 10:

$$N_{act} = M \times \frac{\%N_{act}}{100} \quad (10)$$

The amount of N in the storage compartment (N_{stor} in mg) was calculated in a similar way:

$$N_{stor} = M \times \frac{\%N_{stor}}{100} \tag{11}$$

N_{stor} was only calculated if the total thallus %N > $\%N_c$. The change in N_{stor} d^{-1} (ΔN_{stor} in mg) was calculated based on the difference in stored N between time steps according to:

$$\Delta N_{stor} = \left(N_{stor}\right)_{t_{n+1}} - \left(N_{stor}\right)_{t_n} \tag{12}$$

At t_0 the atom % ^{15}N and atom % ^{14}N in the active and storage pools was assumed to be equal to the atom % $^{15 \text{ or } 14}N$ in the total thallus at t_0. Based on this assumption, the initial ^{15}N or ^{14}N in the active ($(^{15 \text{ or } 14}N_{act})_{t0}$) and storage ($(^{15 \text{ or } 14}N_{stor})_{t0}$) pools was calculated using equations 13 - 16:

$$\left(^{15}N_{act}\right)_{t_0} = N_{act} \times \frac{\left(\%^{15}N_{meas}\right)_{t_0}}{100} \tag{13}$$

$$\left(^{14}N_{act}\right)_{t_0} = N_{act} \times \frac{100 - \left(\%^{15}N_{meas}\right)_{t_0}}{100} \tag{14}$$

$$\left(^{15}N_{stor}\right)_{t_0} = N_{stor} \times \frac{\left(\%^{15}N_{meas}\right)_{t_0}}{100} \tag{15}$$

$$\left(^{14}N_{stor}\right)_{t_0} = N_{stor} \times \frac{100 - \left(\%^{15}N_{meas}\right)_{t_0}}{100} \tag{16}$$

For each subsequent time step, the total ^{15}N ($(^{15}N_{act})_{tn+1}$ in mg) and total ^{14}N ($(^{14}N_{act})_{tn+1}$ in mg) in the active pool was calculated based on $\Delta^{15}N$ and $\Delta^{14}N$ for that time step and, if the overall %N decreased (as was most often the case), any N addition from the storage pool (where $\Delta N_{stor} < 0$) according to:

$$\left(^{15}N_{act}\right)_{t_{n+1}} = \left(^{15}N_{act}\right)_{t_n} + \Delta^{15}N + \left(\Delta N_{stor} \times \frac{\left(\%^{15}N_{meas}\right)_{t_0}}{100}\right) \quad (17)$$

$$\left(^{14}N_{act}\right)_{t_{n+1}} = \left(^{14}N_{act}\right)_{t_n} + \Delta^{14}N + \left(\Delta N_{stor} \times \frac{100 - \left(\%^{15}N_{meas}\right)_{t_0}}{100}\right) \quad (18)$$

The atom % ^{15}N in the active pool ($\%^{15}N_{act}$) was calculated using $(^{15}N_{act})_{tn+1}$ and $(^{14}N_{act})_{tn+1}$ using equation 19:

$$\left(\%^{15}N_{act}\right)_{t_{n+1}} = 100 \times \left(\frac{\left(^{15}N_{act}\right)_{t_{n+1}}}{\left(^{15}N_{act}\right)_{t_{n+1}} + \left(^{14}N_{act}\right)_{t_{n+1}}}\right) \quad (19)$$

The total N leaked (N_{leak} in mg) was then calculated using equation 20:

$$N_{leak} = {}^{15}N_{leak} \times \frac{100}{\%^{15}N_{act}} \quad (20)$$

Sample calculation

Based on data obtained during the February 2000 laboratory incubation experiment, the following set of calculations give the leakage and assimilation of N during the first time step (1 day).

Constants

$\%N_c = 2.3\%$

$\%N_{struct} = 0.8\%$

$\%N_{act} = 1.5\%$

$\%N_{stor}$ = actual %N – 2.3%

$\%^{15}N_{field} = 0.37\%$

Measured parameters

$\mu_M = 0.0472\ d^{-1}$

$\mu_N = -0.0219\ d^{-1}$

$\mu_{15} = -0.0080\ d^{-1}$

Initial values (measured)

$M_{t0} = 169.9$ mg

$\%N_{t0} = 3.556\%$

$\%^{15}N_{meas\ -\ t0} = 0.582\%$

Using the above parameters and the initial values, the following values were calculated on a daily time-step using an exponential equation:

$M_{t1} = 178.1$ mg

$\%N_{t1} = 3.479\%$

$\%^{15}N_{meas\ –\ t1} = 0.557\%$

Calculations

Total thallus N (N_{total} in mg) was calculated for each day as:

$$N_{total-t_0} = 6.042$$
$$N_{total-t_1} = 6.197 \quad (1)$$

and from this the amount of ^{15}N and ^{14}N in each thallus ($^{15}N_{total\text{-}meas}$ and $^{14}N_{total\text{-}meas}$ in mg) was calculated for each day based on:

$$^{15}N_{total-meas-t_0} = 0.0351$$
$$^{15}N_{total-meas-t_1} = 0.0358 \quad (2)$$

$$^{14}N_{total-meas-t_0} = 6.0071$$
$$^{14}N_{total-meas-t_1} = 6.1614 \quad (3)$$

The change in $^{15}N\ d^{-1}$ ($\Delta^{15}N$) and $^{14}N\ d^{-1}$ ($\Delta^{14}N$) was then calculated by difference between time steps:

$$\Delta^{15}N = 0.0006 \quad (4)$$

$$\Delta^{14}N = 0.1543 \quad (5)$$

Newly assimilated N (N_{new} in mg) was calculated based on the difference between N_{tot} at time t_n and time t_{n+1} using equation 6:

$$N_{new} = 0.1549 \quad (6)$$

Assuming that the newly assimilated N has an atom % ^{15}N equal to $\%^{15}N_{field}$, the predicted amount of ^{15}N added by assimilation ($^{15}N_{new\text{-}pred}$ in mg) should equal:

$$^{15}N_{new-pred} = 0.0008 \quad (7)$$

Assuming that all ^{15}N (and all ^{14}N) is retained, the predicted total ^{15}N in the thallus ($^{15}N_{total\text{-}pred}$ in mg) would be equal to:

$$^{15}N_{total-pred} = 0.03596 \quad (8)$$

$^{15}N_{total\text{-}pred}$ was calculated sequentially at each time step based on Σ $^{15}N_{new\text{-}pred}$ for all previous time steps.

The amount of "missing" ^{15}N ($^{15}N_{leak}$ in mg), assumed to be leaked to the water column, was calculated at each time step from the difference between the predicted and the measured ^{15}N using equation 9:

$$^{15}N_{leak} = 0.0002 \quad (9)$$

In order to calculate the total N leaked, it was assumed that all leakage came from the active compartment and that leaked N had a ^{15}N:^{14}N equivalent to that of the total active compartment. The total amount of N in the active compartment (N_{act} in mg) was calculated based on equation 10:

$$N_{act-t_0} = 2.5487$$

$$N_{act-t_1} = 2.6719 \quad (10)$$

The amount of N in the storage compartment (N_{stor} in mg) was calculated in a similar way:

$$N_{stor-t_0} = 2.1342 \quad (11)$$

$$N_{stor-t_1} = 2.1003$$

N_{stor} was only calculated if the total thallus %N > $\%N_c$. The change in N_{stor} d^{-1} (ΔN_{stor} in mg) was calculated based on the difference in stored N between time steps according to:

$$\Delta N_{stor} = -0.0340 \quad (12)$$

At t_0 the atom % ^{15}N and atom % ^{14}N in the active and storage pools was assumed to be equal to the atom % $^{15 \text{ or } 14}N$ in the total thallus at t_0. Based on this assumption, the initial ^{15}N or ^{14}N in the active ($(^{15 \text{ or } 14}N_{act})_{t0}$) and storage ($(^{15 \text{ or } 14}N_{stor})_{t0}$) pools was calculated using equations 13 - 16:

$$\left(^{15}N_{act}\right)_{t_0} = 0.0148 \quad (13)$$

$$\left(^{14}N_{act}\right)_{t_0} = 2.5339 \quad (14)$$

$$\left(^{15}N_{stor}\right)_{t_0} = 0.0124 \quad (15)$$

$$\left(^{14}N_{stor}\right)_{t_0} = 2.1218 \quad (16)$$

For each subsequent time step, the total ^{15}N ($(^{15}N_{act})_{tn+1}$ in mg) and total ^{14}N ($(^{14}N_{act})_{tn+1}$ in mg) in the active pool was calculated based on $\Delta^{15}N$ and $\Delta^{14}N$ for that time step and, if the overall %N decreased (as was most often the case), any N addition from the storage pool (where $\Delta N_{stor} < 0$) according to:

$$\left({}^{15}N_{act}\right)_{t_1} = 0.0153 \tag{17}$$

$$\left({}^{14}N_{act}\right)_{t_1} = 2.6566 \tag{18}$$

The atom % ^{15}N in the active pool (%$^{15}N_{act}$) was calculated using ($^{15}N_{act}$)$_{tn+1}$ and ($^{14}N_{act}$)$_{tn+1}$ using equation 19:

$$\%^{15}N_{act} = 0.5711 \tag{19}$$

The total N leaked (N_{leak} in mg) was then calculated using equation 20:

$$N_{leak} = 0.0363 \tag{20}$$

this value was then divided by the average thallus mass during the time period and converted to μmol N g dw^{-1} d^{-1}, giving a final leakage value of 14.9 μmol N g dw^{-1} d^{-1}. Likewise, daily N assimilation was calculated from the result of equation 6 in the same manner, yielding an assimilation of 63.6 μmol N g dw^{-1} d^{-1}. These numbers represent the values at day 1 in Figure 4-6. Averages over the duration of each experiment are reported as the final results.