Macroalgae, often the dominant primary producers in shallow estuaries, can be important regulators of nitrogen (N) cycling. Like phytoplankton, actively growing macroalgae release N to the water column; yet little is known about the quantity or nature of this release. Using 15N labeling in laboratory and field experiments, we estimated the quantity of N released relative to assimilation and gross uptake by Gracilaria vermiculophylla (Ohmi) Papenfuss (Rhodophyta, Gracilariales), a non-native macroalga. Field experiments were carried out in Hog Island Bay, a shallow back-barrier lagoon on the Virginia coast where G. vermiculophylla makes up 85%–90% of the biomass. There was good agreement between laboratory and field measurements of N uptake and release. Daily N assimilation in field experiments (32.3 ± 7.2 μmol N g dw⁻¹ d⁻¹) was correlated with seasonal and local N availability. The average rate of N release across all sites and dates (65.8 ± 11.6 μmol N g dw⁻¹ d⁻¹) was 67% of gross daily uptake, and also varied among sites and seasons (range: 33%–99%). Release was highest when growth rates and nutrient availability were low, possibly due to senescence during these periods. During summer biomass peaks, estimated N release from macroalgal mats was as high as 17 mmol N m⁻² d⁻¹. Our results suggest that most estimates of macroalgal N uptake severely underestimate gross N uptake and that N is taken up, transformed, and released to the water column on short time scales (minutes–hours).

Key index words: dissolved organic compounds; invasive species; lagoons; macroalgae; nitrogen; organic matter; Virginia Coast

Abbreviations: BOD, biological oxygen demand; C, carbon; DIN, dissolved inorganic nitrogen; DO, dissolved oxygen; DOC, dissolved organic carbon; DOM, dissolved organic matter; DON, dissolved organic nitrogen; LTER, Long Term Ecological Research; N, nitrogen

In shallow estuaries, benthic macroalgae are important primary producers, particularly in estuaries impacted by nutrient enrichment. As non-native invaders, macroalgae can also have a strong influence on ecosystem function and detrimentally impact local biodiversity (Ruiz et al. 1997, 1999). The uptake and storage of nutrients (and carbon) by macroalgae represents a significant temporary sink where biomass is high (Smith 1981, Viali et al. 1997, McGlathery et al. 2001). Following senescence, decomposition is rapid because most bloom-forming algae are highly labile (Buchschbaum et al. 1991, Enríquez et al. 1993). This rapid release of organic matter and nutrients following the collapse of a macroalgal bloom can dramatically impact ecosystem processes by increasing heterotrophic oxygen consumption in the water column, which may in turn lead to anoxia, dystrophy, and overall ecosystem instability (Sfriso et al. 1992, Viaroli et al. 1995, Rysgaard et al 1996).

Less well understood is the quantity of dissolved inorganic nitrogen (DIN) and organic matter (DOM) that living macroalgae, like phytoplankton, release to the water column. The release of DOM by living macroalgae has been acknowledged, with estimates of dissolved organic carbon (DOC) release ranging from 0.5% up to 40% of total C fixed by photosynthesis (Khailov and Burlakova 1969, Harlin and Craigie 1975, Brylinsky 1977, Penhale and Capone 1981, Carlson and Carlson 1984). However, until recently, the concomitant release of dissolved organic nitrogen (DON) has largely been ignored. We have shown previously in microcosm incubations that by releasing DON during active growth, the macroalga Ulva lactuca may substantially increase the total flux of DON from the benthos to the water column (Tyler et al. 2001). Much of the DON released by U. lactuca may be comprised of combined amino acids (Tyler et al. 2003), as has been shown for phytoplankton (Flynn and Berry 1999). Macroalgae may also release some nitrogen as DIN and free amino acids (Naldi and Wheeler 2002).

The release of N by growing macroalgae demonstrates that estimates of macroalgal N demand based solely on biomass and tissue N content significantly underestimate the actual quantity of N passing through the macroalgal pool. Because 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 senescing macroalgae is important in determining the overall impact of mac-
roalgal on ecosystem production and metabolism. In the work presented here, we used 15N in laboratory and field experiments to estimate the total N uptake, as the sum of assimilated and "leaked" N, by the macroalga *Gracilaria vermiculophylla* (Ohmi) Papenfuss (Rhodophyta, Gracilariaceae) along a nutrient gradient in a shallow lagoon on the Virginia coast. The identification of *G. vermiculophylla*, a non-native from the Eastern Pacific, is based on recent molecular and morphological studies (Gurgel and Frederico 2004, Rueness 2005, Thomsen et al. 2006a). Our results indicate that accounting for N release will provide improved estimates of the overall impact of macroalgae on N retention in shallow, macroalgal-dominated estuaries.

**Materials and Methods**

*Site description.* Fieldwork was conducted in Hog Island Bay, a shallow, back-barrier lagoon located within the Virginia Coast Reserve Long-term Ecological Research (LTER) site. The 100 km² lagoon extends eastward from the southern portion of the Delmarva Peninsula, VA, and meets the Atlantic Ocean at the southern end of Hog Island. The shallow areas of the lagoon (<2 m deep at mean low water) make up more than 80% of the total area (Oertel 2001). The water residence time is spatially variable, and varies from days at the inlet to weeks near the mainland (Fugate et al. in press).

There is a gradient of water column N across the lagoon, with the highest concentrations closest to the mainland (McGlathery et al. 2001, Tyler et al. 2001, 2003). Much of the external input of N is via enriched groundwater from the small, agricultural watershed (Lee and Olsen 1985, Reay et al. 1992, Neikirk 1996); however, atmospheric deposition (Paerl et al. 1990, Paerl and Fogel 1994) and runoff after storm events are also important.

Macroalgae, benthic microalgae, and phytoplankton are the dominant primary producers in Hog Island Bay as sea grasses have been absent from the lagoon since the wastewater disease of the 1950s. *G. vermiculophylla* is the dominant macroalga (78% of total biomass), but *Ulva lactuca* L. (Chlorophyta) and *Bryopsis plumosa* (Hudson) C. Agardh (Chlorophyta) are occasionally important (Thomsen et al. 2006b). Biomass varies greatly across the lagoon and generally peaks in the mid-summer. We chose three sites that represent the range of environmental conditions across the lagoon: "Creek," a muddy tidal creek close to the mainland, "Shoal," a sandy site adjacent to a relict oyster reef in the mid-lagoon, and "Hog," a sandy site at the southern tip of Hog Island. Water column dissolved N was highest at Creek (DO, 1–7 μM; DON, 10–17 μM, maxima in late summer/early fall) than Shoal or Hog (DON <2 μM, maxima in October; DON, 7–13 μM, late summer maxima; K. J. McGlathery et al. unpublished data). Macroalgal biomass at Creek (monthly mean = 1–30 g dw m⁻², maxima in fall) and Hog (monthly mean = 2–300 g dw m⁻², maximum in mid-summer) is generally an order of magnitude lower than at Shoal (monthly mean = 2–300 g dw m⁻²; Thomsen et al. 2006b). The reeds at Shoal act as a barrier, trapping floating macroalgae and also providing attachment points. During the mid-summer macroalgal bloom at Shoal, biomass reached >650 g dw m⁻² in patchily distributed mixed-species mats (McGlathery et al. 2001). In 1999 and 2000, biomass at this site declined gradually as temperatures became warmer; in other years, isolated "crashes" of the mats led to the release of large quantities of dissolved N (Tyler et al. 2001).

*Macroalgal collection and pre-treatment.* *G. vermiculophylla* was collected from the Shoal site 10 days before the initiation of field experiments in October 1999 and February, April, and July 2000. The algae were returned to the laboratory in Charlottesville, VA, and grown in a Conviron® environmental growth chamber (Conviron Environmental Inc., Pembina, ND, USA) at ambient field temperatures and a light-dark cycle appropriate for each season. "Day-time" light intensity was approximately 550 μmol photons 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 NH₄Cl:KH₂PO₄ to ensure sufficient N to meet the growth demand (Atkinson and Smith 1983). The rate of N addition was estimated to maintain tissue N at 3% of dry weight at a growth rate of 10% d⁻¹ in October, April, and July; in February, we assumed 2.5% N and 3% 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 dissolved oxygen (DO) production measurements were fertilized with 14NH₄Cl; tubs with algae intended for the field assimilation and release experiments were fertilized with a solution of 15NH₄Cl and 15NH₄Cl (98 atom % 15N, Sigma Chemical Co., St Louis, MO, USA) at ~2 atom % 15N in October, February, and April. To increase the resolution, the solution was increased to ~50 atom % 15N in July. Macroalgae were grown for 10 days and then transferred to the LTER laboratory on the Eastern Shore of Virginia where they were prepared for field incubation. The night before the initiation of the field experiments, individual thalli were cut by breaking off the apical branches and kept in seawater overnight to minimize the potential impact of a wound response on DO production and release measurements.

*DO production.* Water collected from each site was filtered through 0.2 μm Nuclepore filters to remove bacteria, phytoplankton, and other microorganisms. Glass biological oxygen demand (BOD) bottles (300 mL) were filled with filtered water and the initial DO concentration and temperature were measured. Three light and three dark bottles were placed at random in a rack, which was secured on top of a cinder block to prevent the bottles from sinking into the sediment. Individual thalli of *G. vermiculophylla* (approximately 0.3 g wet weight [g ww]) were placed into the bottles immediately before placing the rack in the water at each site. The racks were retrieved 2–4 h later, and the macroalgae were immediately retrieved from the bottles and placed in individual bags. The bottles were kept in a cooler in the dark, until final oxygen concentrations and temperatures were recorded. Measurements were generally made within 2 h of collection, and it is unlikely that changes associated with residual microbial activity altered the DO concentrations substantially relative to macroalgal production or consumption. Macroalgae were patted dry and the wet weight was recorded. Thalli were later freeze-dried and the dry weight was measured. Net daily O₂ production was calculated based on the numbers of light and dark hours for each date. Because all measurements were made at or near low tide on clear, sunny days, the production estimates we report probably represent maximum rates. However, even on cloudy days, the light intensity at the bottom (usually >100 μmol photons m⁻² s⁻¹) was likely sufficient for maximum photosynthesis (Lobban and Harrison 1997). DO production in the light was analyzed using a two-way ANOVA by site and season (SPSS 8.0).

*Light extinction.* Water column light intensity was measured at each site concurrent with the DO production using a Li-Cor model 193SA meter with a 4π spherical quantum sensor (Li-Cor Lincoln, NE, USA). The light extinction coeffi-


cient \( (k) \) was calculated from duplicate profiles, with point measurements at the surface and at depths of 10, 25, 50, 75, 100, 125, and 150 cm. These results were analyzed using a one-way ANOVA by site (SPSS 8.0).

Estimation of \( N \) assimilation and release. Nitrogen assimilation, release, and total uptake by \( G. \) vermiculophylla were measured by labeling macroalgal tissue in the laboratory and measuring growth rates, nitrogen content, and the decrease in total \( ^{15}N \) over time in field and laboratory incubations. Labeled macroalgal thalli (1.00 ± 0.05 g ww) were placed in the field at each of the three sites for 10–16 days. At periodic intervals (approximately 1, 3, 6, 10, and 16 days), three to five thalli were collected from each site, and the change in biomass, total \( N \) content (\%N), and atom \% \( ^{15}N \) was measured. Release and assimilation were then calculated, as described below, from the changes in these values over time.

Field incubations. At the initiation of each experiment, several thalli were retained for initial weight and \( N \) measurements. The remaining thalli were placed in cylindrical cages (30 cm long × 10 cm diameter) with sides constructed of a 0.5 mm clear Nyrex\textsuperscript{TM} mesh and clear plastic ends. The cages were attached to stakes and suspended horizontally approximately 50 cm above the sediment surface. Two cages were used at each site, and collections were made from both cages at each sampling. The mesh decreased light availability minimally (<20\%). Sediment, 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. At the end of the field incubation, thalli were rinsed briefly 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 an electric coffee mill. The University of California at Davis Stable Isotope Facility using a Europa Scientific Integra Isotope Ratio Mass Spectrometer performed all \( ^{15}N \) and \%N analyses. Field \( G. \) vermiculophylla samples \( (n = 3) \) were collected from each site and analyzed as described above to obtain baseline \( ^{15}N \) and tissue \%N content at each sampling time. The \%N of field samples were analyzed using a two-way ANOVA, with site and season as fixed factors.

Laboratory experiment. Coincident with the February field experiment, we conducted an additional experiment under controlled conditions 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 \( ^{14}\text{NH}_4\text{Cl}:\text{KH}_2\text{PO}_4 \) solution at a rate calculated to sustain 5\% growth and a tissue \%N content of 2.5\%, and assuming that all \( N \) taken up is retained in the tissue. The water was changed daily to prevent uptake of released \( N \); 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 days and analyzed as described above.

Calculations. A detailed description of the calculations used to estimate \( N \) uptake, assimilation, and release is provided in Appendix A. Briefly, our model was based on the idea that the total \( N \) content of the thalli at a particular point in time \( (T_{15+14}) \) is the sum of the initial tissue \( N \) \( (I_{15+14}) \) plus new \( N \) \( (U_{15+14}) \) taken up from the environment, less any \( N \) released back to the environment \( (R_{15+14}) \), i.e.:

\[
T_{15+14} = I_{15+14} + U_{15+14} - R_{15+14}
\]

Equation (1) was split into two separate equations, for \( ^{15}N \) and \( ^{14}N \) individually, and solved for the unknowns \( U_{15} \), \( U_{14} \), \( R_{15} \), and \( R_{14} \). The model rests on two primary assumptions: (1) the \( ^{15}N: ^{14}N \) of released \( N \) is equivalent to the \( ^{15}N: ^{14}N \) of the tissue at the previous time step \( (\text{constant } = b) \), and (2) the \( ^{15}N: ^{14}N \) of new \( N \) taken up from the environment is equivalent to the natural abundance \( ^{15}N: ^{14}N \) of algae growing in the field at each site and season \( (\text{constant } = k) \). The potential error associated with these assumptions is discussed below.

Non-linear regression analysis (SPSS 8.0) was used to fit an exponential equation to the measured change in thallus mass, \%N, and atom \% \( ^{15}N \) in order to obtain the model parameters for the relative growth rate \( (\mu_b) \), and change in \%N \( (\mu_s) \) and atom \% \( ^{15}N \) \( (\mu_5) \). The standard errors associated with each of these model parameters were used to estimate propagated error, as described below. When an increase in \%N occurred, the data were fitted to a logistic equation with the measured field \( N \) concentration as the upper bound. 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. Instead, we used the site-specific linear regression for \( D \) production versus thallus growth obtained during other months to estimate the growth rate using the measured \( D \) production for April. The errors associated with this estimate were also incorporated into the bootstrap procedure to estimate the propagated errors. Thallus mass, total thallus \%N content, and total thallus \( ^{15}N \) and \( ^{14}N \) were calculated on a 1-day time step using these model parameters. Newly assimilated \( N \), i.e. new \( N \) that has been incorporated into macroalgal tissue, was estimated from the difference in total thallus \%N between time steps. Daily release, uptake, and assimilation values were calculated for each thallus as a whole, also on daily time steps. These values were then divided by the average thallus mass between time steps, and the resulting values were averaged over the 14 days of simulation to obtain release as \( \mu \text{mol N g dry wt}^{-1} \text{ d}^{-1} \).

Errors for daily release, uptake, and assimilation were calculated using a bootstrap procedure (Efron and Tibshirani 1986). We assumed that the mean and standard deviation for each measured parameter were normally distributed, and the combined results for release 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. We performed a sensitivity analysis on the constant \( k \) to assess the performance of the model relative to the assumption that newly taken up \( N \) has the same \( ^{15}N: ^{14}N \) as macroalgal tissue. Error associated with this assumption could be introduced if there is a significant fractionation associated with \( N \) uptake and incorporation into macroalgal tissue. We therefore adjusted \( k \) to test the sensitivity of the model to this parameter, assuming the minimum (−9.7\%) and maximum (+12.1\%) fractionations observed for marine phytoplankton (Goericke et al. 1994).

A rough estimate of the total areal impact of \( N \) uptake and release by macroalgae in Hog Island Bay was obtained by multiplying the release and assimilation values obtained in this experiment by monthly field biomass at each site (Thomsen et al. 2006b). Because \( G. \) vermiculophylla was consistently 80–95\% of the total macroalgal biomass in the field during this period, using the \( G. \) vermiculophylla values for all algae will give a reasonable estimate of total algal uptake and release.

RESULTS

Light. The mean light extinction coefficient, \( k \) (Fig. 1), decreased along the mainland-island gradient, 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 overall significant difference between sites \( (F = 3.6, P = 0.072, df = 2) \) due to the interseasonal variation. The highest \( k \) at all sites was measured in

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February, with relatively constant rates across the other months.

**DO production.** Daily DO production ranged from 28.2 to 152.5 mg O₂·g⁻¹·d⁻¹, but did not differ significantly between sites \((F = 0.6, P = 0.55, df = 2;\) Fig. 2). There was, however, a significant interaction between site and season \((F = 2.7, P = 0.04, df = 6)\) and significant seasonal effects, with February<April and October<April and July \((F = 36.2, P < 0.001, df = 3)\).

**Field N content.** Macroalgal tissue N reflected the gradient in N availability across the lagoon (Fig. 3), with significantly higher \((F = 124.4, P < 0.001, df = 2)\) percentages closest to the mainland at the Creek (3.7 ± 0.2 SE), intermediate at Shoal (2.6 ± 0.2 SE), and significantly lower at Hog (2.0 ± 0.1 SE). There was also a significant interaction between site and season as well as differences between dates \((F = 1.7, P < 0.001)\). The mean value in October (3.4 ± 0.3 SE) was significantly higher, by at least 0.75%, than all other dates.

**Growth, assimilation, and release.** The results of the laboratory incubation experiment are shown in Fig. 4; a similar pattern was observed for all field incubations (data not shown). Thallus mass increased at approximately 5%·d⁻¹, whereas the %N decreased at 2%·d⁻¹ and atom %¹⁵N decreased at 1%·d⁻¹ (Table 1). Even though the total tissue N continued to increase, the assimilation rate declined nearly 25% over the simulation period; this is likely due to insufficient N supply. 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 release such that the fertilization rate and assimilation were insufficient to keep pace with N uptake and release. The mean assimilation of N was 85.6 ± 12.1 μmol N·g⁻¹·d⁻¹. The average daily release rate in the laboratory experiment was 119.1 ± 45.0 μmol N·g⁻¹·d⁻¹. Release averaged 58% of total uptake (assimilation + release), and increased from 33% to 75% over the course of the experiment as the daily assimilation rate declined.

Growth rates, change in N content, and change in atom %¹⁵N during the field experiments are shown in Table 1. The growth rates, which ranged from 1.4% to 4.4%·d⁻¹, varied between site and date. Growth rates were the lowest in February at all sites; maximum rates at Creek and Shoal were in July, whereas 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 G. vermiculophylla tissue decreased 0.8%–2.9%·d⁻¹ throughout the course of the field incubation 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 at each site within 1 week. The atom %¹⁵N decrease, which ranged from 0.6% to 3.7%·d⁻¹, was the greatest at Hog during all seasons and lowest at Creek, with the exception of July 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 ($r = 0.73$, $F = 10.5$, $P = 0.01$, $n = 12$). There was no significant relationship between the decrease in $^{15}$N and the change in %N.

The gross uptake of N across sites and dates was $98.1 \pm 14.1 \mu$mol N·g dw$^{-1}$·d$^{-1}$ (SE, $n = 12$), with similar values across sites (Fig. 5a). In contrast, N assimilation was generally the highest at Creek and the lowest at Shoal (Fig. 5b), except in February, when Hog was the lowest. The mean daily N assimilation across sites was $32.3 \pm 7.2 \mu$mol N·g dw$^{-1}$·d$^{-1}$ (SE, $n = 12$). Assimilation varied seasonally, with the highest rates measured at all sites in October and the lowest in February. The average rate of N release across all sites and dates was $65.8 \pm 11.6 \mu$mol N·g dw$^{-1}$·d$^{-1}$ (SE, $n = 12$) (Fig. 5c). The Creek always had the lowest release rates ($36 \pm 10 \mu$mol N·g dw$^{-1}$·d$^{-1}$ SE, $n = 4$), Shoal had the highest ($84 \pm 20 \mu$mol N·g dw$^{-1}$·d$^{-1}$ SE, $n = 4$), and Hog had intermediate rates ($78 \pm 23 \mu$mol N·g dw$^{-1}$·d$^{-1}$ SE, $n = 4$), except in October, when Hog was the highest. Overall, release of N was 70% of gross uptake (= release + assimilation), and varied substantially between sites and seasons. Release was 33%–69% of total daily uptake at Creek, 75%–93% at Shoal, and 57%–99% at Hog. The inverse relationship between field N content and release ($R = 0.73$, $F = 11.3$, $P = 0.007$, $n = 12$) indicates...
higher release under low N conditions. Increasing \( k \) to reflect an uptake fractionation of \(-9.7\%\) resulted in a mean release of 67.9 \( \mu \text{mol N \cdot g dw}^{-1} \cdot \text{d}^{-1} \) and a mean uptake of 100.2 \( \mu \text{mol N \cdot g dw}^{-1} \cdot \text{d}^{-1} \), with release equal to 67% of total uptake; decreasing \( k \) to reflect an uptake fractionation of \(+12.1\%\) resulted in a mean release of 59.6 \( \mu \text{mol N \cdot g dw}^{-1} \cdot \text{d}^{-1} \) and a mean uptake of 91.7 \( \mu \text{mol N \cdot g dw}^{-1} \cdot \text{d}^{-1} \), with release equal to 66% of uptake. When the local biomass of macroalgae at each site was taken into consideration, the areal rates ranged from a low of 0.008 and 0.005 \( \text{mmol} \cdot \text{m}^{-2} \cdot \text{d}^{-1} \) for nitrogen release and assimilation at Hog in October, respectively, to a high of 17.0 and 6.56 \( \text{mmol} \cdot \text{m}^{-2} \cdot \text{d}^{-1} \) at Shoal in July (Fig. 6).

**DISCUSSION**

The considerable release of N (65.8 \( \mu \text{mol N \cdot g dw}^{-1} \cdot \text{d}^{-1} \)) relative to assimilation (32.3 \( \mu \text{mol N \cdot g dw}^{-1} \cdot \text{d}^{-1} \)) from actively growing macroalgae indicates that considerably more N 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. This rapid uptake and release of dissolved N by macroalgae clearly has substantial impacts for N dynamics in shallow, macroalgal-dominated estuaries.

The mean percent of total uptake released (all sites) in the field in February (74%) was similar to the release measured in the laboratory at the same time of the year (58%). Even though the water was changed daily to prevent mineralization and re-uptake of \(^{15}\text{N}\) during the laboratory experiment, bacterial processing of exuded \(^{15}\text{N}\) may have been sufficiently rapid that mineralized N was available for re-uptake (Flynn and Berry 1999) and may have caused us to underestimate release. Re-uptake in the field incubations is less likely because of continual refreshment of local water. Our model rests on the assumption that new N taken up from the environment has a \(^{15}\text{N}:^{14}\text{N}\) similar to field algae at that point in time (\( k \)) and that this value represents an integrated estimate of the \(^{15}\text{N}:^{14}\text{N}\) of DIN and DON available for uptake. Changing the value of \( k \) to represent the extremes of available \(^{15}\text{N}:^{14}\text{N}\) has only a small impact on the calculated rates of uptake and release, and therefore does not introduce a great deal of error into our measurements.

### Table 1. Growth rates and changes in %N and atom %\(^{15}\text{N}\) during the first 14 days of each experiment.

<table>
<thead>
<tr>
<th>Site</th>
<th>Change (d(^{-1}))</th>
<th>Thallus weight</th>
<th>%N</th>
<th>Atom %(^{15}\text{N})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lab</td>
<td>5.6 ± 0.5</td>
<td>120</td>
<td>16</td>
<td>0.92</td>
</tr>
<tr>
<td>October 1999</td>
<td>2.4 ± 0.3</td>
<td>56</td>
<td>16</td>
<td>0.97</td>
</tr>
<tr>
<td>Creek</td>
<td>2.3 ± 0.3</td>
<td>47</td>
<td>16</td>
<td>0.94</td>
</tr>
<tr>
<td>Shoal</td>
<td>4.4 ± 0.3</td>
<td>194</td>
<td>10</td>
<td>0.99</td>
</tr>
<tr>
<td>Hog</td>
<td>2.0 ± 0.2</td>
<td>106</td>
<td>14</td>
<td>0.88</td>
</tr>
<tr>
<td>February 2000</td>
<td>2.9 ± 0.3</td>
<td>121</td>
<td>3</td>
<td>0.95</td>
</tr>
<tr>
<td>Creek</td>
<td>2.9 ± 0.3</td>
<td>2112</td>
<td>3</td>
<td>1.00</td>
</tr>
<tr>
<td>Shoal</td>
<td>3.7 ± 0.2</td>
<td>192</td>
<td>20</td>
<td>0.93</td>
</tr>
<tr>
<td>Hog</td>
<td>4.0 ± 0.4</td>
<td>98</td>
<td>19</td>
<td>0.96</td>
</tr>
</tbody>
</table>

All values are expressed as percent change per day ± SE.

*This value was estimated using the logistic equation, rather than the exponential equation because of the 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. The errors reported for these values are for the regression between DO production and growth rate.

DO, dissolved oxygen.
The differences in the relative magnitude of assimilation, growth, and release between sites suggest spatially and temporally variable controls on these three parameters. The gradient in N inputs across the lagoon is reflected in tissue N values from field samples, in high N assimilation rates at Creek, and in the paler color of *Gracilaria vermiculophylla* at Hog relative to Creek (personal observation), which is likely the result of a lack of stored phycocerythrin, a common N storage compound in the rhodophyta (Ryther et al. 1981, Horrocks et al. 1995). The higher assimilation of N at Hog relative to Shoal, in spite of slightly lower standing stock concentrations, may be due in part to a greater overall nutrient flux because of faster current velocities at Hog (Thomsen 2004). In addition, the cages were suspended above the macroalgal mat at Shoal, so that only water column nutrients were available to the caged macroalgae. Rapid recycling at the bottom of macroalgal mats can support new growth, even in the absence of new nutrient input (Lavery and McComb 1991, McGlathery et al. 1997, Trimmer et al. 2000, Astill and Lavery 2001), and this may contribute to the higher tissue N of field algae but the low assimilation rates of caged algae at Shoal. Further, because much of the macroalgal biomass is below the photic zone within the mat, growth rates are low and N accumulation high relative to the caged algae. 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. 2003).

Our values for release rates are similar to those reported for NH$_4^+$ (2.9–28.5 μmol N·g dw$^{-1}·d^{-1}$) and free amino acids (90.1 μmol N·g dw$^{-1}·d^{-1}$; estimated from reported 12% of NH$_4^+$ uptake of 31.30 μmol N·g dw$^{-1}·h^{-1}$ on the first day of exposure to 1 mM NH$_4^+$) by *G. pacifica* (Naldi and Wheeler 2002). In their study, the release was small relative to the very high uptake rates stimulated by the high external N concentration (5.12–31.76 μmol N·g dw$^{-1}·h^{-1}$ (Naldi and Wheeler 2002); 2.2 μmol N·g dw$^{-1}·h^{-1}$, this study). The high release at low N sites in the current study suggests that release may, to some extent, be a function of nutrient availability. In phytoplankton, the magnitude of N release is proportional 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 the highest. *Gracilaria* sp. generally use free amino acids, proteins, and pigments as storage compounds, rather than small compounds such as NO$_3^-$ or NH$_4^+$ (Naldi and Wheeler 1999). Although some free amino acids may pass freely across the gradient at the cell surface, diffusion-driven loss of small ions may not be as important in *Gracilaria* sp. as in species with larger inorganic N storage pools that may lose N across the cell membrane (Chapman and Craigie 1977, McGlathery et al. 1996, Naldi and Wheeler 1999). High release at low nutrient sites may also be explained by the release of extracellular enzymes such as alkaline phosphatase for nutrient procurement (Weich and Graneli 1989). Ambient phosphate concentrations were consistently higher at Creek (~2 μM) than at Shoal or Hog (~1 μM, K. J. McGlathery et al., unpublished data), perhaps contributing to greater release at the lower nutrient sites. This does not, however, explain the high release rates found in the laboratory experiment, where phosphate was added in presumably sufficient amounts. If uptake of exuded $^{15}$N occurred in the field, the probability of real absorption would likely be related to the thickness of the boundary layer surrounding the thallus, which is in turn related to the local flow conditions. The low current speeds at Creek (Thomsen 2004) may aid in explaining the lower release measured there. Finally, our calculation of release assumed that leaked N had a $^{15}$N/$^{14}$N equivalent to the total tissue N pool ("old" N plus "new" N). In reality, the released N represents the integrated $^{15}$N/$^{14}$N value of all released compounds, which can vary substantially, particularly for amino acids (Macko et al. 1987). If the released N was actually derived more from newly absorbed N (low $^{15}$N) or from isotopically lighter compounds, rather than from the total pool (higher $^{15}$N), then in using the higher $^{15}$N/$^{14}$N total pool to calculate the $^{14}$N released, we may have underestimated the $^{14}$N leaked, and thereby underestimated the total N released. If the former were the case, the underestimate would be the greatest where N availability is the highest—at Creek. Conversely, if isotopically heavier compounds were released, either preferentially or at the onset of the experiment, we would have overestimated the total N released.

To some extent, the magnitude of release may be controlled by the differences in macroalgal productivity across the lagoon. Productivity, in turn, is related to a combination of factors, including temperature and light and nutrient availability, that vary seasonally and spatially. At Creek, nutrients, light, and growth rate were high; at Hog, light was high, but nutrients were low and growth rates were still high. At Shoal, intermediate in both nutrients and light, we found the lowest growth rate. There are two theories regarding the loss of DOC from phytoplankton. One states that the loss is passive and unavoidable ( Bjornden 1988); the other describes an “income tax” concept, where loss is tightly coupled to photosynthesis (Zlotnik and Dubinsky 1989). The mean release across sites was the lowest during July, when DO production was the highest and Shoal algae had the lowest growth rates and highest release. Although we observed high release rates where growth was the highest (Hog), our results do not clearly support the idea that the release of N is positively related to the photosynthetic rate and suggest instead that poorly growing or possibly senescent algae may have the highest release rates.
The storage of N for later use may confer a competitive advantage to certain macroalgal species in systems with pulsed or seasonal N delivery (Chapman and Craigie 1977, Rosenberg and Ramus 1982, Asare and Harlin 1983, Lapointe and Duke 1984, Fujita 1985, Trimmer et al. 2000). Generally, in temperate western Atlantic estuaries, storage follows late winter and early spring nutrient pulses (Rosenberg and Ramus 1982, Asare and Harlin 1983, Fujita 1985), and enables high growth in summer, when temperature and light availability are more favorable for growth, but nutrient concentrations are lower. In Hog Island Bay, N availability was generally the highest during late summer to early fall due to mineralization of increased organic matter input (Anderson et al. 2003, Tyler et al. 2001). Macrotidal systems with pulsed or seasonal N delivery (Chapman and Craigie 1977, Lapointe and Duke 1984, Chapman 1981) demonstrated that exuded DOM by Ulva lactuca is capable of releasing 2–5 mmol N m⁻² d⁻¹ as urea-free DON. These measurements, based on the difference in benthic fluxes observed in cores with and without macroalgae, are lower than the values presented here, but rapid uptake by water column heterotrophs may have decreased the observed release and DIN may be a significant portion of the released N.

The release of DOM 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 (Penhale and Smith 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 a large variation in the amount of photosynthate released. Up to 30%–40% release has been reported (Khalilov and Burlakova 1969), but the majority of studies have found less than 5% release (Harlin and Craigie 1975, Brylinsky 1977, Penhale and Capone 1981, Carlson and Carlson 1984). The comitment of release of N has not been as fully investigated. In estuarine phytoplankton, reported DON release rates ranged from 11% to 28% of gross N uptake (see Table 2 in Bronk and Ward 2000). Our values for N release by G. vermiculophylla, 33%–99%, represent a higher proportion and 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, perhaps due to senescence, along with periods of active growth and lower release. Tyler et al. (2001) reported that Ulva lactuca was capable of releasing 3.5 μmol N · g dw⁻¹ d⁻¹ as urea-free DON. These measurements, based on the difference in benthic fluxes observed in cores with and without macroalgae, are lower than the values presented here, but rapid uptake by water column heterotrophs may have decreased the observed release and DIN may be a significant portion of the released N.

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 up to 17 mmol N · m⁻² d⁻¹. This indicates that the non-native G. vermiculophylla may have a significant impact on ecosystem dynamics in Hog Island Bay. As has been shown following the senescence of seagrasses (Kemp et al. 1997), this increased production of available organic matter is likely to fuel heterotrophic metabolism in the water column (Brylinsky 1977, Valiela et al. 1997) and if exported from the estuary may be an important source of organic matter for coastal ocean food webs. 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), attraction of the toxic dinoflagellate Prymnesium parvum to mats of Cladophora sp. (Johnsen and Lein 1989), and stimulation of epiphytization (Santelices and Varela 1993).

This study shows that estimates of new N assimilation based solely on growth rate and N content would, in some cases, underestimate the actual N uptake by nearly 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 uptake and release still occur. Several authors have suggested that the rapid release of DON to solution may cause an underestimate of DON uptake in 15N uptake experiments (Collos 1992, Bronk and Ward 2000). The results of our experiments, while on a longer time scale than most phytoplankton experiments, also indicate that DON release may result in an underestimate of total uptake in 15N uptake experiments (Collos 1992, Bronk et al. 1994). The release of DON to solution may cause an underestimate of total uptake in 15N uptake experiments (Collos 1992, Bronk et al. 1994).

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MEASURED PARAMETERS

- \( \mu_M \) = daily growth rate (day\(^{-1}\))
- \( \mu_N \) = daily rate of change in \( \%N \) (d\(^{-1}\))
- \( \mu_{15} \) = 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
- \( %^{15}N \) = atom \( %^{15}N \) of thallus

CALCULATIONS

The total amount of \( N \) in the thallus at time \( t \) (\( N_t \)) is equal to the initial amount of \( N \) in the thallus (\( N_i \)) plus new \( N \) taken up from the environment (\( N_u \)) minus \( N \) released (\( N_r \)):

\[
N_t = N_i + N_u - N_r \quad (A.1)
\]

Equation (A.1) was split into separate equations for \( ^{15}N \) and \( ^{14}N \):

\[
^{15}N_t = ^{15}N_i + ^{15}N_u - ^{15}N_r \quad (A.2)
\]

\[
^{14}N_t = ^{14}N_i + ^{14}N_u - ^{14}N_r \quad (A.3)
\]

Total thallus \( N \) (\( N_t \) in mg) was calculated for each day as:

\[
N_t = M \times \frac{\%N}{100} \quad (A.4)
\]

From Equation (A.4) the amount of \( ^{15}N \) and \( ^{14}N \) in each thallus (\( ^{15}N_t \) and \( ^{14}N_t \) in mg) was calculated individually using equations (A.5) and (A.6) for each time step:

\[
^{15}N_t = N_t \times \frac{\%^{15}N}{100} \quad (A.5)
\]

\[
^{14}N_t = N_t \times \frac{100 - \%^{15}N}{100} \quad (A.6)
\]

We assumed that the \( N \) released from the thallus has a ratio of \( ^{15}N/^{14}N \) equivalent to the ratio found in the thallus at the initial time step (\( b \)), such that:

\[
\frac{^{15}N_i}{^{14}N_i} = b \quad \text{or} \quad ^{15}N_r = ^{14}N_r b \quad (A.7)
\]

Likewise, we have assumed that newly acquired \( N \) has the same ratio of \( ^{15}N/^{14}N \) as field algae present at the time of the experiment (\( k \)):

\[
\frac{^{15}N_u}{^{14}N_u} = k \quad \text{or} \quad ^{15}N_u = ^{14}N_u k \quad (A.8)
\]

Substituting the ratios given in equations (A.7) and (A.8) into equation (A.2) gives:

\[
\frac{^{15}N_i - ^{15}N_t}{^{14}N_u} = k \quad \text{or} \quad ^{15}N_i - ^{15}N_t = ^{14}N_u k - ^{14}N_r b \quad (A.9)
\]

Equation (A.9) may be rearranged after dividing all terms by \( k \):

\[
\frac{^{15}N_i - ^{15}N_t}{^{14}N_u} = \frac{^{14}N_u - ^{14}N_r b}{k} \quad (A.10)
\]

Subtracting equation (A.10) from equation (A.3) gives:

\[
{^{14}N_t} - {^{14}N_i} = \frac{^{15}N_i - ^{15}N_t}{k} = \left( ^{14}N_u - ^{14}N_r \right) - \left( ^{14}N_u - ^{14}N_r \frac{b}{k} \right) \quad (A.11)
\]

After canceling \( ^{14}N_u \) terms and some rearrangement, Equation (A.11) may be rewritten as:

\[
k \left( ^{14}N_t - ^{14}N_i \right) - ^{15}N_i + ^{15}N_t = ^{14}N_r \quad (A.12)
\]

Substituting \( ^{14}N_t \) derived in equation (A.12) back into equation (A.7) allows us to solve for \( ^{15}N_r \). This can then be inserted into equation (A.2) to solve for \( ^{15}N_u \) and finally into equation (A.8) to solve for \( ^{14}N_u \).