

**Examining the Impact of a Nitrogen Enriched Groundwater Plume Entering West
Falmouth Harbor, Massachusetts by a Comparison of the $\delta^{15}\text{N}$ Signatures of
Macroalgae and Two Nitrogen Sources**

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Abstract

Nutrient loading to coastal ecosystems threatens the biotic stability and function of small harbors and embayments globally. In this study we aimed to address the distribution of nitrogen loading to West Falmouth Harbor, MA from an enriched groundwater plume. We did this by examining the $\delta^{15}\text{N}$ signatures of benthic regenerated nitrogen in three distinct sub-basins and groundwater nitrogen from 9 shoreline wells. Additionally, two species of macroalgae, *Gracilaria tikvahiae* (Link) and *Ulva lactuca* (McLachlan) were tethered to bio-integrate water column nitrogen in 6 sub-basins for 10-12 days. The $\delta^{15}\text{N}$ signal of post-tethered macroalgae was analyzed and compared to the signals from the potential N sources. Nitrogen uptake for both macroalgae species was recorded. Higher $\delta^{15}\text{N}$ values were associated with groundwater taken from wells with high N concentrations. Similar $\delta^{15}\text{N}$ values of regenerated nitrogen were found for the inner most basins; however, regenerated nitrogen for outer basins was heavier. There was no compelling trend found in the $\delta^{15}\text{N}$ of tethered macroalgae, but data from summer 2006 suggested that higher $\delta^{15}\text{N}$ signals are found in inner basins than outer basins. The results of this study suggest that macroalgae may be used as a bio-integrator of nitrogen in coastal waters, however, seasonal variation in algal nutrient uptake may prevent successful use during colder seasons in temperate climates.

Key Words: Nitrogen; Macroalgae; ^{15}N ; Coastal Lagoons; West Falmouth Harbor, Massachusetts

Introduction

Globally, nutrient loading to coastal and estuarine waters has been linked to increased abundance of nuisance macroalgae, loss of economically viable fisheries, and many other alterations to the structure of these aquatic ecosystems (Valiela et al. 1992, Sfriso et al. 1992, McComb and Humphries 1992, Valiela et al. 1997). Recently, anthropogenic inputs of nutrients through groundwater and agricultural run-off have been found to be more significant contributors of nitrogen and phosphorus to nitrogen limited estuarine ecosystems than previously known (Lee and Olson 1985, Valiela et al. 1990, Valiela et al. 1992). Sea-grass habitat loss and declines in economically important shellfisheries are linked to suburban development and nitrogen loaded groundwater in multiple Rhode Island and Massachusetts coastal lagoons (Lee and Olson 1985, Valiela et al. 1992, Short and Burdick 1996). A comprehensive study of rates of nitrogen loading from the sub-watersheds of West Falmouth Harbor (WFH), Falmouth, Massachusetts revealed that most of the transported nitrogen and freshwater flow enters the estuary from groundwater seepage (Howes et al. 2005).

We aimed to assess the impact and spatial distribution of N from the wastewater enriched groundwater plume entering WFH by utilizing macroalgal assimilation of nitrogen. We used two species of macroalgae; *Gracilaria tikvahiae* and *Ulva lactuca* for selective incubation (tethering) within 6 interconnected basins of WFH. Macroalgae in coastal ecosystems readily uptake ammonium and nitrate from both the benthic-pelagic interface and the overlying water column (Peckol et al. 1994, Rivers and Peckol 1995, McGlathery et al. 1997, Phillips and Hurd 2004, Tyler and McGlathery 2006). Recently, Cohen and Fong (2005) found that the accumulation of ^{15}N in macroalgal (*Enteromorpha*

intestinalis) tissue is predictable over a range of $\delta^{15}\text{N}$ water signals and water N concentrations. They suggest that macroalgae may be used successfully as a nondiscriminatory bio-integrator of nitrogen in estuarine environments. For WFH, the duration of the tethering was based on environmental conditions in November 2006 and mooring locations were chosen to best represent major parts of the harbor and areas of incoming groundwater (Fig.1).

Additionally, we analyzed regenerated ammonium from benthic core incubations and groundwater extracted nitrate from shoreline wells for the $\delta^{15}\text{N}$ signal of naturally recycled and allochthonous nitrogen. Initial and post-tether $\delta^{15}\text{N}$ signatures of the macroalgae were compared spatially and with the $\delta^{15}\text{N}$ of source nitrogen in WFH. The goal of this study is three-fold; a) to examine the $\delta^{15}\text{N}$ signal of tethered macroalgae and identify differences within basins in WFH. b) To examine the $\delta^{15}\text{N}$ of regenerated benthic nitrogen in three basins and the $\delta^{15}\text{N}$ of groundwater along gradients of N enrichment, and c) to comparatively analyze the ^{15}N signal of tethered macroalgae with environmental sources of nitrogen.

Methods

The Study Site

We examined the nitrogen (N) signatures of two potential N sources and of tethered macroalgae in West Falmouth Harbor (WFH), Falmouth, Massachusetts during the month of November 2006 (Fig. 1). WFH is a 197 acre, tidal, coastal embayment connected to Buzzard's Bay via a small inlet (Howes et al. 2006). For the purposes of this study, the harbor was divided into 6 separate basins; Snug harbor, Snug/Inner Harbor, Inner Harbor, Inner Harbor South, Middle Harbor, and Outer Harbor (Fig. 1). The harbor contains vast macroalgal mats that are invading ecologically and commercially important eelgrass and salt marsh communities (Howes et al. 2006). We assessed the impact of nutrient enriched groundwater versus internally regenerated N using tethered macroalgae as a bio-integrator of nitrogen in the water column at WFH.

Benthic Regenerated Nitrogen and Groundwater Nitrogen

Benthic regenerated nitrogen and allochthonous groundwater nitrogen were considered as two potential sources of nitrogen in WFH; the latter has been thought to cause nutrient enrichment and ecosystem level alteration (Howes et al. 2006). To obtain regenerated ammonium, benthic cores were taken in triplicate at 3 sites in WFH (1-2 m depth; Inner Harbor, Middle Harbor, and Outer Harbor) and core head-space water was replaced with filtered, low-nutrient seawater (Fig. 2). All cores were incubated for 36 hours at 15-16°C. Ammonium (NH_4^+) concentration was colorometrically derived from samples taken at 0, 17.5, and 36 hours for a final concentration of 2-15 $\mu\text{mol N}$ in 2L of head-space per core (Solarzano 1969). Resulting head-space water from each core was removed and frozen for NH_4^+ extraction and $\delta^{15}\text{N}$ isotopic analysis.

We extracted water (1.5L) from each of 8 shoreline wells (sites SC-1 – SC-9) using a hand-pump (Fig. 2). Each well had as many as 4 depths from 0.5-9.0 m; water from each depth was composited in equal volume depending on number of depths present. Nitrate (NO_3^-) and NH_4^+ concentrations of the groundwater were measured with a LACHAT flow injection analyzer and colorometric analysis respectively (Wood et al. 1967, Solarzano 1969). Samples were frozen for NO_3^- extraction and $\delta^{15}\text{N}$ isotopic analysis.

Macroalgae Tethering

We tethered two species of macroalgae, *Gracilaria tikvahiae* (Link) (Rhodophyta) and *Ulva lactuca* (McLachlan) (Chlorophyta), collected in early-November 2006 from Snug/Inner Harbor, WFH, based on the methods by McGlathery (personal communication). *G. tikvahiae* and *U. lactuca* were prepared for tethering by nutrient starvation in artificial seawater in a diurnal, climate-controlled growth chamber (10°C, 400ppm CO₂, and ~300μE m⁻² s⁻¹) for 3 days and 9 days respectively. There was 5 to 10 g wet weight (ww) of algae per tethering cup (perforated) and an algae sub-sample (5g ww) was taken of each species for an initial δ¹⁵N comparison. We deployed *G. tikvahiae* at 4 mooring sites per basin for 10 days (Fig. 1). Comparatively, we deployed *U. lactuca* at only two of the same mooring sites per basin for 12 days. After collection from WFH tethering, algae samples were cleaned of epiphytes, weighed (ww), dried, and ground for δ¹⁵N isotopic analysis.

Isotopic Analysis

Nitrogen (NH₄⁺ and NO₃⁻) in water samples from benthic core incubations and shoreline groundwater well samples were extracted using modified methods of Holmes et al. (1998, NH₄⁺) and Sigman et al. (1997, NO₃⁻). Volumes and replicates for N extractions were based on N concentrations such that the resulting filter-pack would contain 5-15 μmoles N for δ¹⁵N isotopic analysis. Ammonium extractions spent 10 days shaking at 40°C and NO₃⁻ extractions spent 2 days in a 60°C oven and 8 days shaking at ambient temperatures. Nitrate samples unexpectedly stopped shaking for 1-1.5 days, however, were soon after placed shaking at 40°C with NH₄⁺ samples as compensation. Nitrogen extractions and algae samples were read with a PDZ Europa 20-20 continuous-flow isotope mass spectrometer for δ¹⁵N signatures and comparatively analyzed between the sites and source specific signatures.

Nutrient Uptake

We measured the nitrogen uptake of *G. tikvahiae* and *U. lactuca* to estimate N turnover during the field tethering. Both species of algae were collected from Snug/Inner Harbor, WFH and were kept in low-nutrient (5-10 μM N) natural seawater for four weeks. About three grams of algae was added to 700 mL of natural, filtered (Whatman GF/C) seawater. Known amounts of NH₄⁺ and NO₃⁻ (as NH₄NO₃) were added to give treatments concentrations of 25 μM NH₄⁺ and 25 μM NO₃⁻ plus any background N in the filtered seawater. Samples were incubated in a climate-controlled growth chamber (10°C, 400ppm CO₂, and ~300μE m⁻² s⁻¹) on a gently rotating shaker table for 6 hours. For the first 2 hours seawater NH₄⁺ and NO₃⁻ sub-samples were taken every 30 minutes and thereafter were sub-sampled hourly. *U. lactuca* N uptake was estimated by fitting a modified Michalis-Menten equation to measured data as follows:

$$t = \frac{(C_o - C) + K \left(\ln \left(\frac{C_o}{C} \right) \right)}{V_x}$$

where time (t , hrs.) is expressed as a function of the initial concentration (C_o , μM N), the concentration at t (C , μM N), the constant (K , μM N), and the maximum uptake rate (V_x ,

$\mu\text{M N hr}^{-1}$). *G. tikvahiae* N uptake was modeled with a different, modified Michaelis-Menten equation:

$$t = \frac{C}{A - V_x} + \frac{K(A - V_x) - A(K)}{(A - V_x)^2} \ln(A(K) + (A - V_x)C)$$

Where time (t , hrs.) is expressed as a function of the concentration at t (C , $\mu\text{M N}$), the constant (K , $\mu\text{M N}$), an uptake modifier A , and the maximum uptake rate (V_x , $\mu\text{M N hr}^{-1}$).

Results

The $\delta^{15}\text{N}$ of ammonium extracted from benthic re-mineralization core samples revealed that the signal of N increased (or got heavier) from inner harbor to middle harbor to outer harbor (Fig. 3). Groundwater nitrate extracted from shoreline wells showed lower $\delta^{15}\text{N}$ signals further away from the enriched groundwater wells (SC1-SC4); among all wells there was a trend in the $\delta^{15}\text{N}$ values which followed previously known well-point nitrate concentrations (Foreman and McHorney, unpublished) (Fig. 4).

In the duration of their tethering, the macroalgae, *G. tikvahiae* lost mass at about -20% for all basins except for the outer basin, where mass loss was nearly 80% (Fig. 5). *U. lactuca* only lost mass in two basins and there was never more than 10% mass lost. The most growth by *U. lactuca* was in inner, inner south and outer basins. There was a trend in the change of $\delta^{15}\text{N}$ for *U. lactuca* which showed the most change in basins closest to the groundwater sources of enrichment and less change in the middle and outer basins (Fig. 6). The $\delta^{15}\text{N}$ of tethered *G. tikvahiae* changed less in the inner most and outer most basins and was negligibly increased in the inner and inner harbor south basins. Overall, there was a clear distinction between the $\delta^{15}\text{N}$ values of each species used in the tethering experiment (Fig. 7). *U. lactuca* had slightly higher % N and a lighter $\delta^{15}\text{N}$ values than *G. tikvahiae*. In a comparison of tethered *G. tikvahiae* in the summer of 2006 at similar tethering locations, there was a trend which indicated that higher $\delta^{15}\text{N}$ values are associated with the inner most basins and decreased linearly towards outer basins (Fig. 8).

The rate of uptake demonstrated that under similar environmental conditions to those seen in the 6 basins, ammonium was more readily assimilated by both *G. tikvahiae* and *U. lactuca* than nitrate (Fig. 9). Nitrogen uptake by *U. lactuca* required all of the ammonium (40 μM) in the incubation chamber, whereas, *G. tikvahiae* only assimilated ammonium until an asymptote was reached where there was no uptake beyond a water concentration of $\sim 20 \mu\text{M NH}_4^+$. Comparatively, there was no net uptake of nitrate in the incubation chambers for either species. *G. tikvahiae* may have additionally released a small amount of nitrate into the surrounding water.

Discussion

This study provided clear evidence which suggested that two possible sources of nitrogen to marine biota have distinct ^{15}N signals as compared with each other (allochthonous vs. natural regeneration) and along spatial terms in West Falmouth Harbor. While consequences of nutrient loading include increased water column N concentrations, increased growth of macroalgae, and often subsequent loss of eelgrass

habitat, examining the $\delta^{15}\text{N}$ values of different nutrient sources suggested that we may be able to delineate what source is having an effect in specific regions of a coastal lagoon or embayment (Valiela et al. 1990). This approach, however, may not be entirely clear without the entire processes of nutrient recycling and loading being fully understood. For example; the $\delta^{15}\text{N}$ signature of regenerated NH_4^+ in the inner most harbors (Snug, Snug/Inner, Inner) differed from that of the middle and outer harbors. It was logical to see similar $\delta^{15}\text{N}$ values between groundwater N and regenerated N, however, an increased $\delta^{15}\text{N}$ signal in middle and outer basins suggested that there may be a coupled nitrification-denitrification microbial process which is preferentially selecting for lighter N (Foreman, personal communication). Thus, more investigation of the process of nutrient regeneration may be required before we begin to fully assess the $\delta^{15}\text{N}$ signals in West Falmouth Harbor.

Examination of $\delta^{15}\text{N}$ from shoreline wells further supported that the groundwater entering the northern most part of WFH is part of a plume which derived from the spraying of human effluent into multiple, WFH sub-watersheds. We saw higher $\delta^{15}\text{N}$ values associated with points where NO_3^- concentrations can reach 300 μM N; the current pattern may be stronger than what was seen, as the concentration contour diagram and $\delta^{15}\text{N}$ values do not pair chronologically. By targeting the source of nitrogen loading to the watershed using a comparison between the $\delta^{15}\text{N}$ of the sources (wastewater treatment plant, fertilizer, chemicals, etc...) and the plume entering a particular body of water; this method of investigation may be applicable to other impacted watersheds (particularly those with a history of algal or macroalgal blooms).

There was no compelling trend in the tethered macroalgae $\delta^{15}\text{N}$ data to suggest that we saw differences in N sources within the spatially explicit basins in WFH. We expected that because of the tendency for macroalgae to rapidly and readily take up nutrients (amplified by a short starvation period) from the environment, they would be successful bio-integrators of N in WFH (Cohen and Fong 2005). What we noticed, when our fall 2006 data was compared to preliminary summer 2006 data from McGlathery (unpublished) was that there were clear differences in seasonal macroalgal N uptake in temperate waters. The maximum rates of macroalgal N uptake tested at equivalent environmental conditions for fall 2006 showed that although rates of NH_4^+ uptake for *G. tikvahiae* and *U. lactuca* were similar, *G. tikvahiae* reached a concentration below which there was no uptake (20 μM). As 20 μM is high for even heavily impacted coastal lagoons like WFH, it was likely that while tethered, *G. tikvahiae* was not assimilating NH_4^+ (Valiela et al. 1990). *U. lactuca* was likely a more successful bio-integrator of NH_4^+ as uptake was seen at all concentrations (0-40 μM) during the growth chamber incubation. Additionally, the greatest $\delta^{15}\text{N}$ change was seen with *U. lactuca* tethered in the three inner most basins of WFH which suggested that it was responding to the higher N concentrations from the enriched groundwater plume.

For both *G. tikvahiae* and *U. lactuca*, there was no net uptake of NO_3^- in the N uptake experiment. The primary source of nitrogen in the nutrient plume is NO_3^- , thus if the N uptake was a proxy for what we thought was happening in tethered macroalgae, we may not be examining the N from groundwater in all the basins, but rather regenerated N in the form of NH_4^+ (Howes et al. 2005). The preference of NH_4^+ over NO_3^- for these particular species during different times of the year may merit further study. Knowing

key nutrient dynamics and their seasonal variation would strengthen the use of macroalgae as a bio-integrator of N.

This study provides some preliminary experimental evidence to suggest that there are differences in the isotopic composition of regenerated N and N enriched groundwater with trends moving out of WFH towards Buzzards Bay and north along the shoreline seep of groundwater. We also suggest that using macroalgae as a bio-integrator of N in the water column may be a strong tool; however, seasonal variation in uptake kinetics may compromise the success of a similar study in temperate climates. At this point, using macroalgae as a possible bio-integrator of water column nitrogen is in the infant stages of development and technique, it seems essential that tethering be paired with alternative approaches to measuring the distribution of threatening nutrient addition to coastal ecosystems.

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Appendix

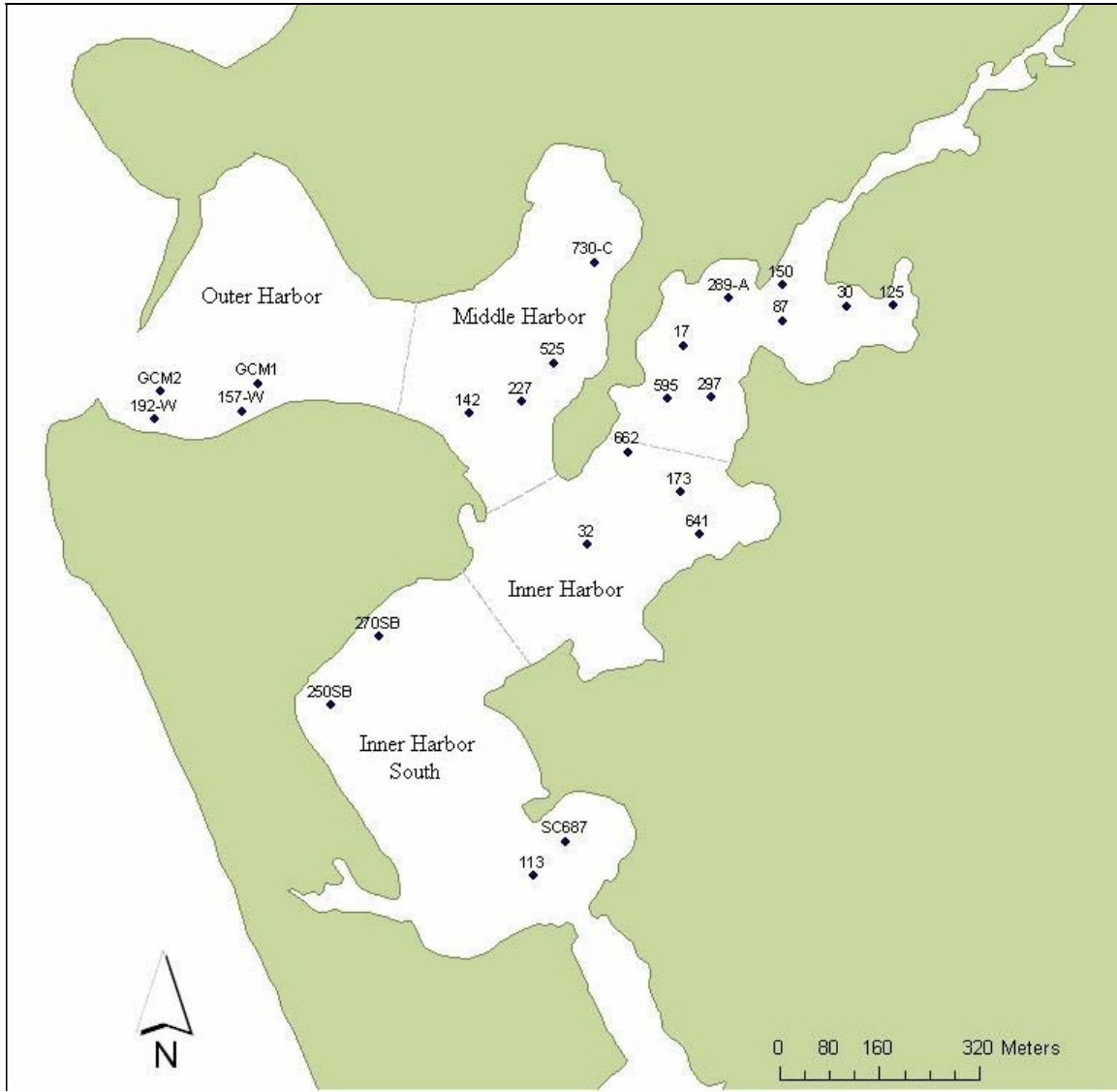


Figure 1. Algal tethering sites for *G. tikvahiae* and *U. lactuca* in West Falmouth Harbor, Flamouth, Massachusetts. Five basins divided by border lines; Snug Harbor (150, 87, 30, 125) and Inner/Snug Harbor (289-A, 17, 595, 297) are considered two separate basins, however are not depicted as such here.

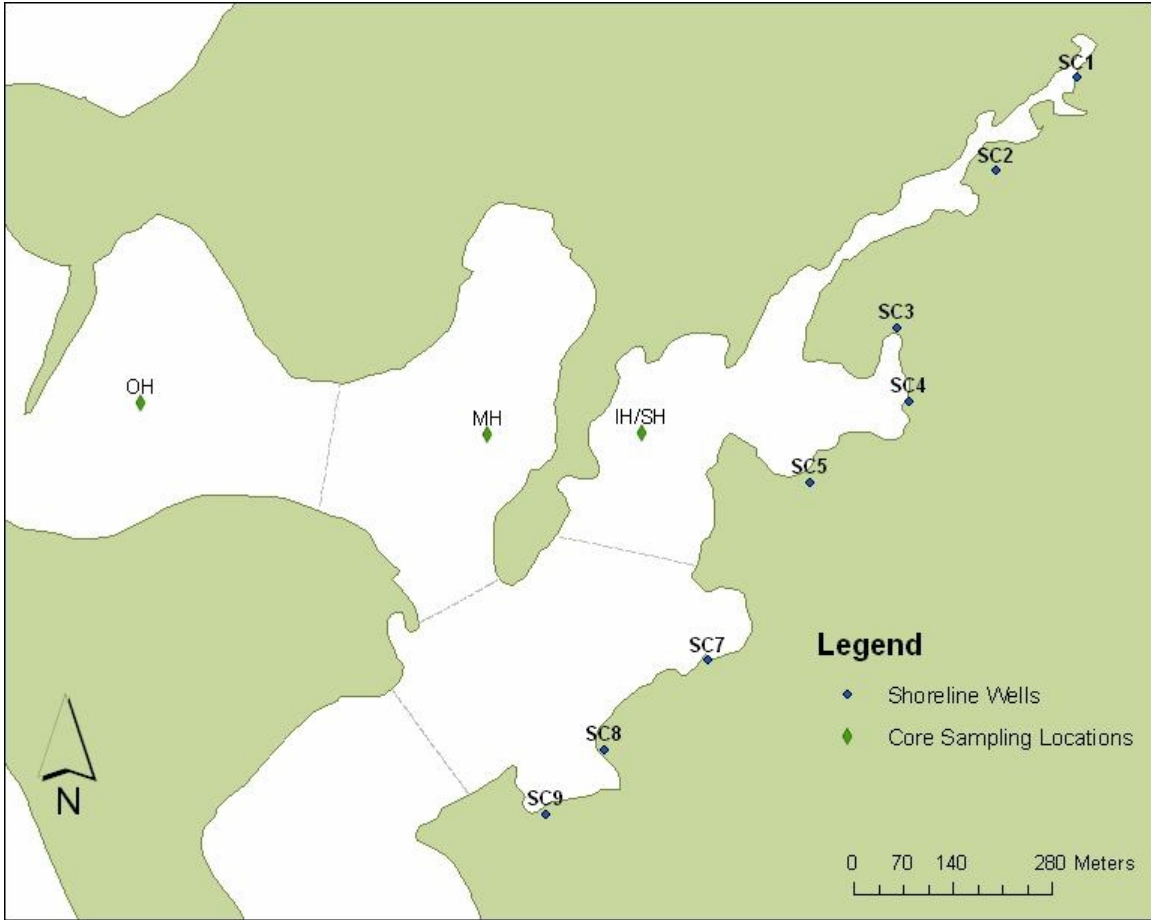


Figure 2. Locations of benthic core samples and shorelines wells in West Falmouth Harbor, Falmouth, Massachusetts. OH means outer harbor, MH means middle harbor, and IH/SH means Inner/Snug Harbor.

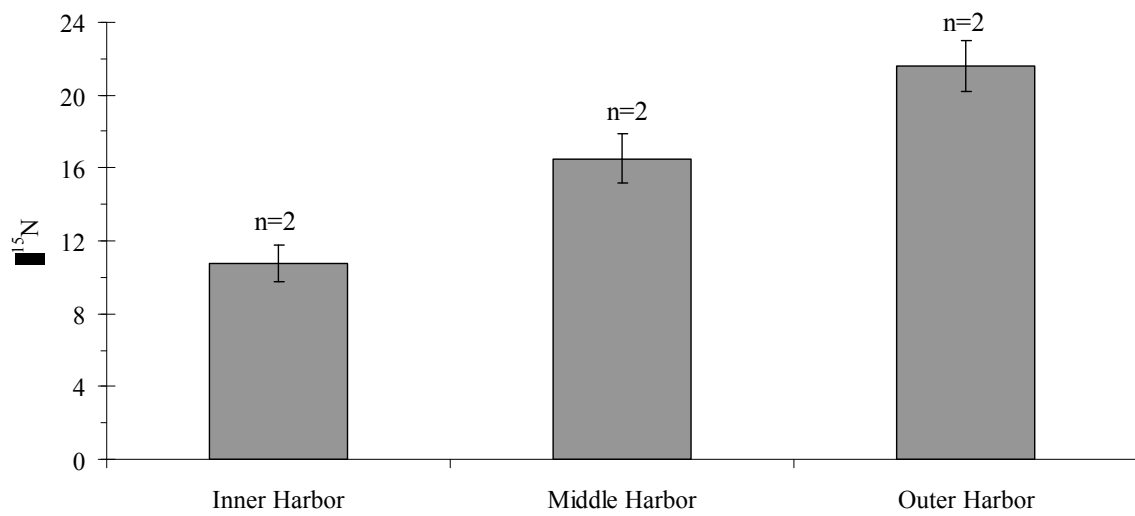


Figure 3. Isotopic signals of regenerated benthic nitrogen in three basins of West Falmouth Harbor, Falmouth, Massachusetts.

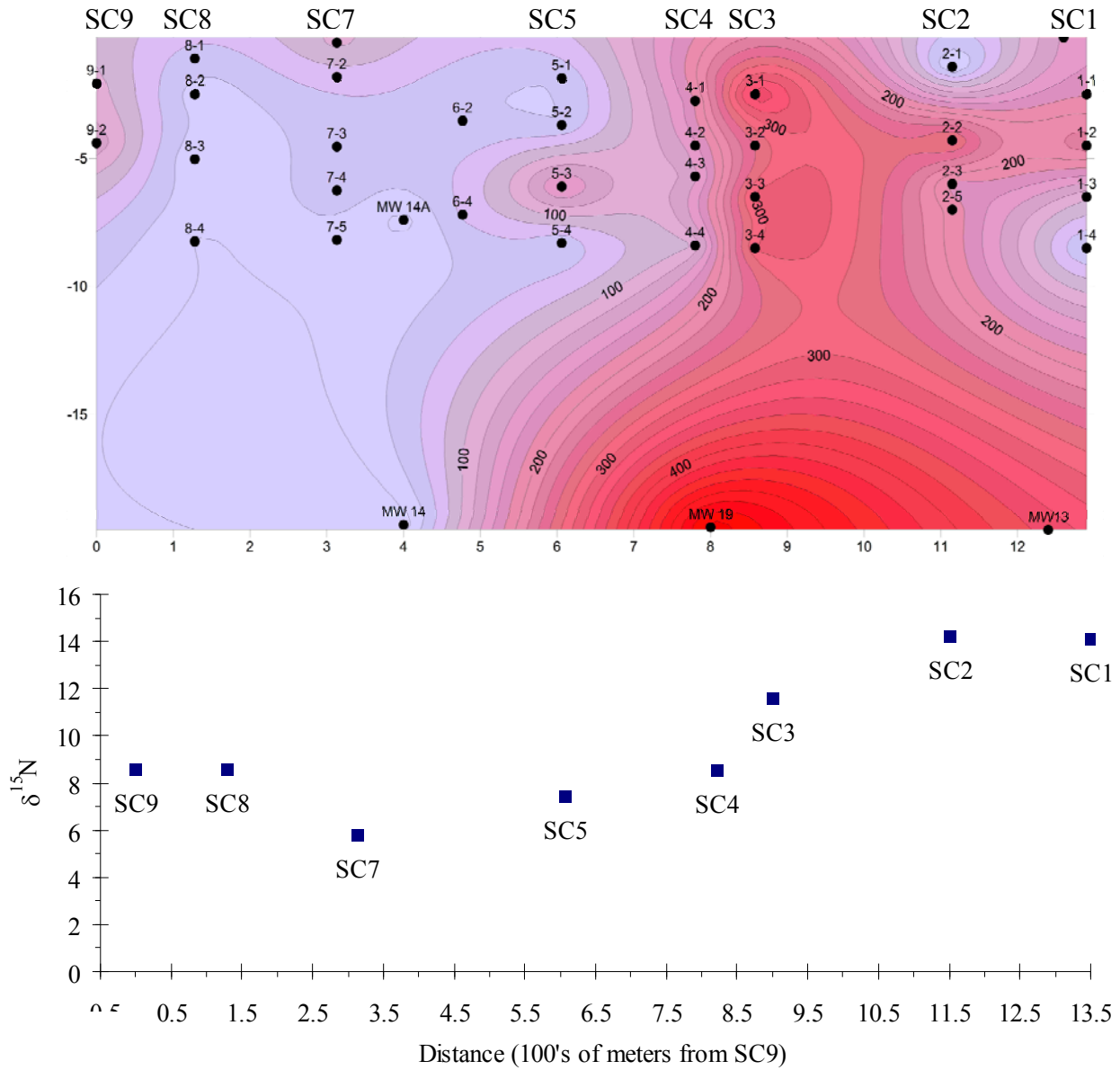


Figure 4. Isotopic signals of groundwater extracted from shoreline wells at West Falmouth Harbor, Falmouth, Massachusetts. Moving north from SC9 to SC1.

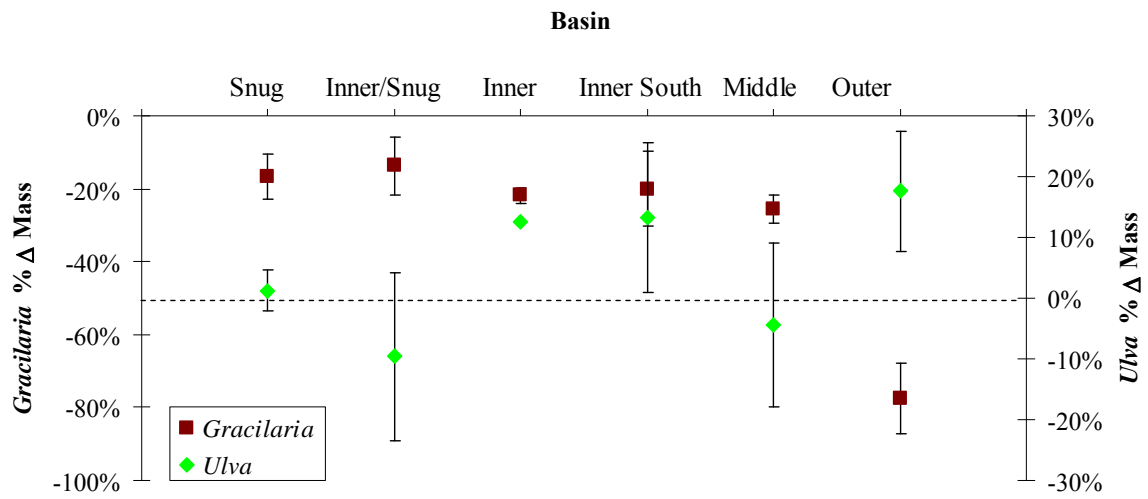


Figure 5. Change in mass (wet weight) of two species of macroalgae, *G. tikvahiae* and *U. lactuca* tethered in West Falmouth Harbor, Falmouth, Massachusetts.

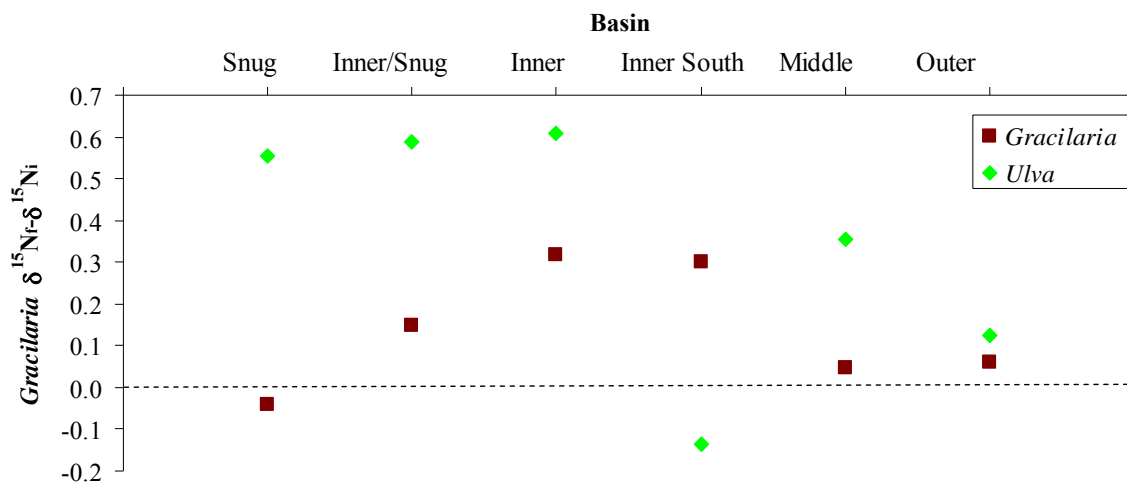


Figure 6. Change in $\delta^{15}\text{N}$ of two species of macroalgae, *G. tikvahiae* and *U. lactuca* tethered in West Falmouth Harbor, Falmouth, Massachusetts.

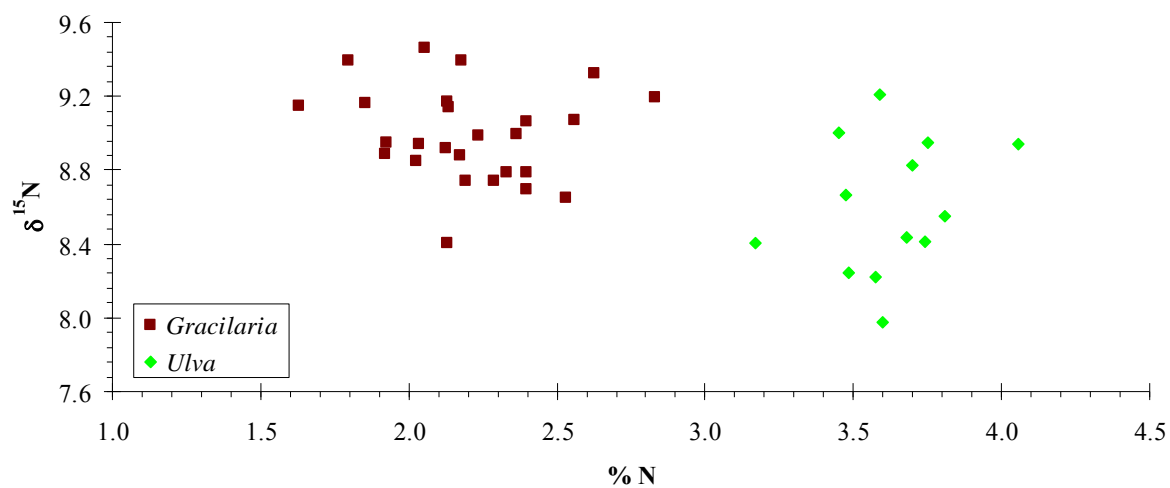


Figure 7. Percent nitrogen versus $\delta^{15}\text{N}$ of two species of macroalgae, *G. tikvahiae* and *U. lactuca* tethered in West Falmouth Harbor, Falmouth, Massachusetts.

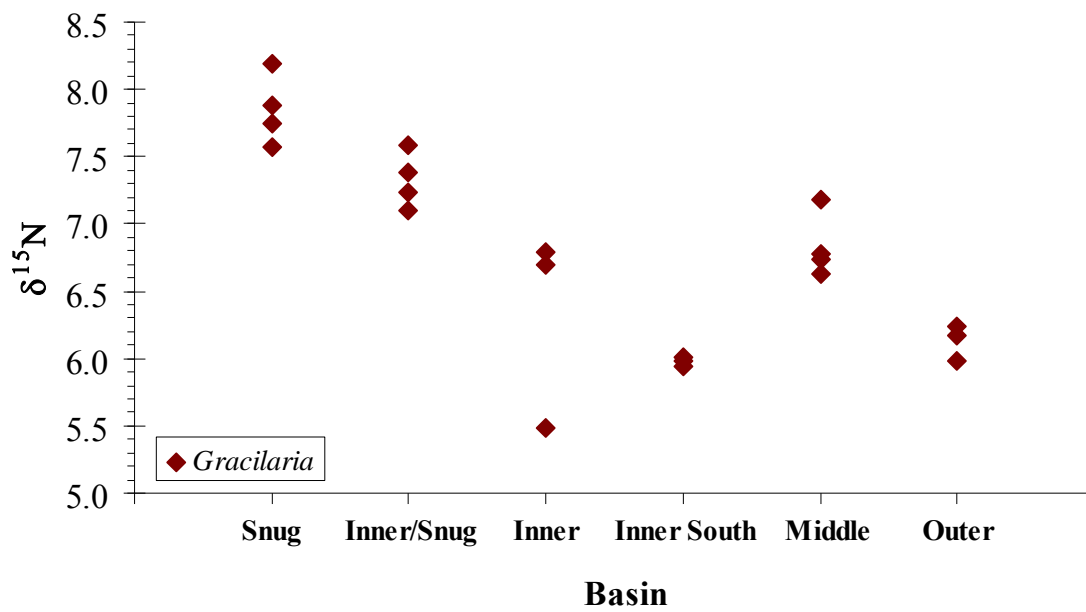


Figure 8. Summer (2006) $\delta^{15}\text{N}$ values of tethered macroalgae *G. tikvahiae* in 6 basins of West Falmouth Harbor, Falmouth, Massachusetts.

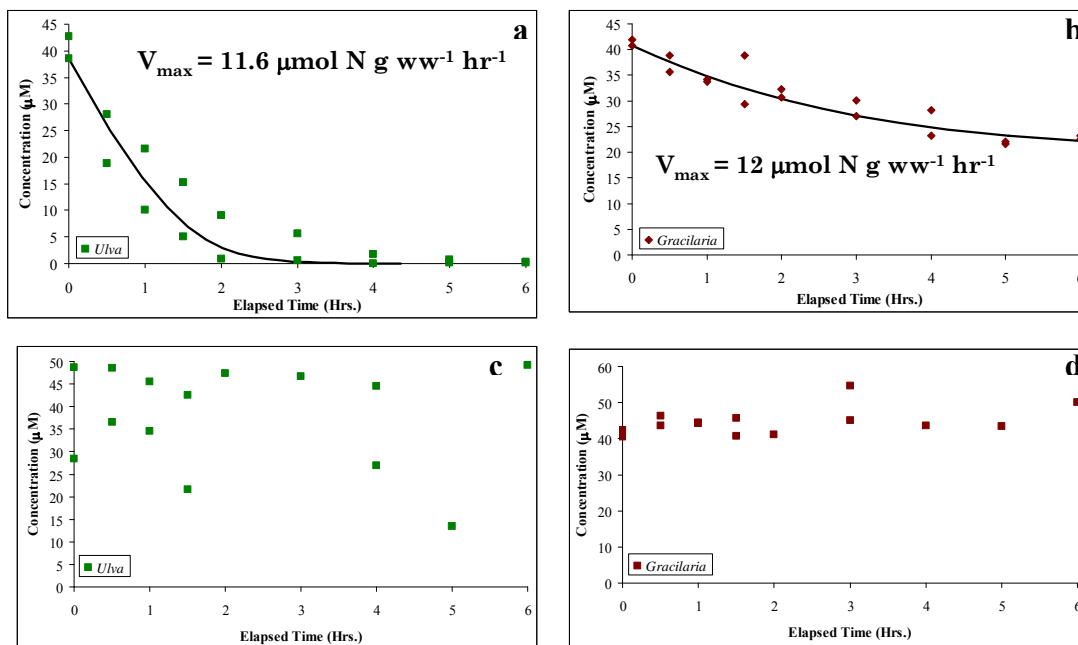


Figure 9. Nitrogen uptake (NH_4^+ and NO_3^-) by two species of macroalgae, *U. lactuca* (a.,c.) and *G. tikvahiae* (b.,d.) at 10 °C and $\sim 300 \mu\text{E m}^{-2} \text{s}^{-1}$ in an environmentally controlled growth chamber. n=2.