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**TRACING NITROGEN TRANSFORMATIONS THROUGH THE FOOD CHAIN
IN AN URBANIZED TIDAL CREEK**

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ABSTRACT

In order to investigate the fate of nitrogen inputs to tidal creek ecosystems we used an in situ stable isotopic ^{15}N tracer to label the dissolved inorganic nitrogen pool. With this approach we were able to identify dominant N pathways, quantify N transfer rates, and determine overall ecosystem N throughput in a southeastern urbanized tidal creek. Consequently, we were also able to identify the relative importance of attenuators of watershed N at an ecosystem scale as well as quantify the ecosystem dependence on watershed-derived nitrate under base flow conditions.

The study site consisted of a 2 km reach of tidally dominated marsh with relatively high inputs of nitrate (yet uniformly low reach-concentrations of DIN). The tide-mediated addition of $^{15}\text{N-NO}_3$ significantly labeled the DIN pool and it was subsequently taken up, recycled, and stored/exported by the ecosystem. Microautotrophs (phytoplankton and benthic microalgae) were instrumental in the rapid utilization and recycling of DIN, exhibiting a turnover rate of 2 - 3.5 days. Trophic transfer in the phytoplankton-zooplankton pathway was rapid and these two compartments were tightly coupled. Of the higher trophic levels, oysters showed the most significant enrichment and appeared to be a keystone component of the tidal creek N processing at the ecosystem scale.

Bulk sediments were the primary fate of N (61 %) however oysters and marsh macrophytes were also retainers of N (11 % and 5 %, respectively) due to their longer turnover times. Using gas N_2 enrichments we were able to estimate 16 % lost by denitrification and 33 % by tidal export. Based on a creek ^{15}N mass balance, the creek is equal parts transformer of dissolved and particulate N forms and an attenuator of N through denitrification.

(Nitrogen, ^{15}N tracer, denitrification, tidal creek, N transfer)

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SUMMARY AND CONCLUSIONS

The problem of nitrogen (N) over saturation is prevalent in many areas of the United States, and is particularly important in urbanized coastal areas. Tidal creeks function as interceptors for watershed derived N, transforming and recycling it before its eventual fate either into the sediments or exported to the coastal ocean. A 23-day ^{15}N isotopic tracer enrichment experiment was conducted in order to trace the movement of watershed N within an urbanized tidal creek ecosystem. It also provided a method by which N processing rates could be calculated. Finally, we were able to construct a mass balance (with respect to N) of the entire reach, and determine ecosystem N throughput and its dependence on watershed derived N.

Dye studies were executed prior to the enrichment study in order to determine the hydrodynamics of the creek. This creek remained stratified upstream due to freshwater inputs, and during the 23-day experiment the creek exchanged about 85% of its total volume per day (85% of high tide water volume exchanged over two tidal cycles). N content and background isotopic signatures were measured for major N pools in order to give baseline enrichment data. In the 2 km experimental reach, the majority of biotic N was located in marsh macrophytes, second only to storage in the sediments. The addition of labeled NO_3^- enriched the DIN pool between 500 - 12000 ‰. This ensured that both tidal- (per site location in reach over a tidal cycle) and reach- (all site locations per tide) averaged $\delta^{15}\text{NO}_3^-$ enrichment variations were no more than a factor of two for the duration of the study. The ^{15}N that was not lost via tidal export was rapidly taken up by the microautotrophs and subsequently recycled or transformed to higher trophic levels. Phytoplankton showed rapid N uptake demonstrating that the formation of algal blooms is a likely response to an influx of N to the system.

The transfer of ^{15}N in the water column had the greatest impact on the downstream oyster beds. These beds, which constituted roughly 50 % of the total biotic N downstream, retained ~11 % of the total g ^{15}N (second only to sediments: 61 % of total ^{15}N recovered) on the final day of the addition period. Tracer was incorporated into nekton based on feeding strategies, with water column feeders taking up the tracer first. These results, along with spatial distribution patterns, suggest either a movement of some species within the creek, and/or a progressive movement of ^{15}N tracer downstream as it cascades through the ecosystem biota.

Both mud crabs (*Uca pugnax*) and marsh periwinkles (*Littoraria irrorata*) exhibited a lag time in their enrichment trajectories indicating their reliance on recycled N. By the final day of the addition period, ~2% of the tracer could be found in these two pools. Benthic infauna showed similar enrichment, however this N pool was too small to significantly contribute to ecosystem processing of N. Marsh macrophytes retained roughly 5% of the recovered ^{15}N on the final day of the addition experiment. This pool, along with the sediment and oyster pool, exhibited slow turnover times and therefore significantly retained the ^{15}N after the addition experiment.

With this tracer experiment, we were able to examine some fundamental aspects of tidal creek N dynamics not readily attainable without the in situ use of ^{15}N . Uptake of labeled N was rapid in the phytoplankton component, demonstrating that the rapid algal bloom formation that occurs in Hewlett's Creek is related to anthropogenic N inputs. Immediate uptake by phytoplankton was directly followed by spatial redistribution and recycling by benthic microalgae (BMA). This

redistribution of labeled N throughout the entire creek reach fueled secondary and tertiary production, suggested by a tight coupling of N uptake between phytoplankton and zooplankton. Based on the enrichments of finfish and shellfish, < 2 % of their N requirements can be traced back to watershed N. While this watershed contribution (of N) to specific pools was high, the ecosystem as a whole seemed to be maintained predominantly on detrital or microbially-recycled N. By constructing a mass balance of the ecosystem (with respect to N), we estimated that the creek is equal parts transformer of watershed N (via tidal export) and attenuator of watershed N through denitrification.

RECOMMENDATIONS

Research Recommendations: Based on the utility of ^{15}N tracer technology we suggest that a similar experiment be performed during winter. In winter, phytoplankton abundance in tidal creeks is low, marsh emergent plant growth is minimal, and it is likely that the role of the benthic microalgal community and benthic consumers (especially oysters) will be enhanced.

Another useful experiment would be to perform an acute, large-scale loading ^{15}N into the creek sufficient to mimic a rain-event generated influx of DIN that would stimulate algal blooms. As our results indicated rapid uptake of labeled tracer, the fate of an acute N load would be of great management interest in terms of bloom stimulation, food chain impacts and transfer, contributions to water column biochemical oxygen demand (BOD) and sediment oxygen demand (which are hypoxia drivers), marsh uptake, and denitrification.

Management Recommendations: Clearly, the key role of the oyster as an accumulator and transformer of N in the tidal creek ecosystem has been demonstrated. Oysters are not simply sinks of anthropogenic N; they excrete DIN as ammonium that recycles back into the ecosystem. In unimpacted tidal creeks such as in North Inlet, South Carolina, this is important for ecosystem function (Lewitus et al. 1998). In anthropogenically impacted creeks the oyster represents an important product that, when removed by harvest, also removes excess N from the system. This loss can only occur when shellfish beds are open to harvest. When they are polluted by fecal bacteria (as are most of the tidal creeks in this area of the coast), they cannot be harvested and DIN is thus recycled in the creek ecosystem, adding to the anthropogenic N loads from upland runoff, and providing continuing fuel for algal blooms. Thus, strong management efforts to reduce fecal bacteria loads to such creeks will not only allow for harvest of a valuable seafood resource, but will additionally provide a means of removal of excess N from the system.

INTRODUCTION

Salt marsh – dominated tidal creeks are common features of temperate passive continental margins. As conduits for diffuse imports of watershed derived nutrients to the coastal ocean, they also function as highly reactive ecosystems that efficiently convert those nutrients into primary and secondary production. High levels of biological productivity, diversity, and unique geomorphology help define these systems as primary nursery areas for finfish and shellfish species and as feeding grounds for birds and larger fish (Sanger et al, 2004; Mallin & Lewitus 2004).

High rates of ecosystem production are maintained by inputs of dissolved inorganic nitrogen (DIN) from adjacent watersheds and through internal N- cycling (Bronk et al. 1994, Capone et al. 2008). Although high productivity is driven by N availability, excessive N loading can alter ecosystem structure, patterns of trophic transfer, and subsequent function in the landscape (Nixon & Buckley 2002). Human development along these tidal creeks can lead to a variety of pollution inputs, including the nutrients N and phosphorus, which can stimulate algal blooms and cause hypoxia (Rabalais 2002, Mallin et al. 2004; 2006). Quantifying the resilience of tidal creeks to excess N loading is important for understanding system responses to future loadings.

Tidal creeks can be viewed simultaneously as interceptors of watershed N, transformers of N into biomass, and exporters of modified N to the coastal ocean. As N transits tidal creeks (or estuaries), it can be cycled and recycled biologically before it is deposited in the sediment or exported (Boyer et al. 1994). Understanding the efficiency with which tidal creeks perform these functions requires assessment of how they process new N (pathways) and at what rate they recycle N internally among specific trophic levels and as a whole ecosystem. From an experimental perspective, these creeks show many estuarine characteristics (e.g. salinity gradient, species gradient, stratification/destratification patterns) on a compressed spatial scale. Therefore they are analogues for estuarine processes but occur on a scale that can be experimentally manipulated.

Whole-ecosystem isotopic tracer ^{15}N experiments have proven useful in a variety of settings to investigate N processing. In general, the technique involves in situ dosing of an ecosystem with ^{15}N -DIN tracer. The ^{15}N is followed into multiple ecosystem compartments and used to calculate the rate of N movement between pools under natural conditions. This approach eliminates many of the ambiguities associated with natural abundance isotope studies that result from overlapping source endmembers and complex patterns of isotopic fractionation. Although the isotope tracer approach remains somewhat limited in the spatial scale and duration of its application, it has proven well suited for experiments in streams, groundwater, marshes, and small estuaries. In these applications the method has provided simultaneous estimates of DIN cycling, removal via denitrification, and trophic transfer under in situ conditions (Holmes et al. 2000, Hughes et al. 2000, Tobias et al. 2001, Bohlke et al. 2004, Gribsholt et al. 2007,

Mullholland et al. 2000). Of these experiments, the closest analogue to marsh-dominated creeks of the southeastern United States are those conducted in small New England estuaries (Holmes et al. 2000, Hughes et al. 2000, Tobias et al. 2003).

Most recently, ecosystem-scale enriched ^{15}N fertilization of marsh creeks in New England has helped to quantify nitrogen uptake in *Spartina* spp. (Drake et al. 2008). Here we present an application of the ^{15}N in situ tracer approach conducted in a marsh-dominated tidal creek in southeastern North Carolina. These tidal creeks differ substantially from other tidal systems subject to this approach in tidal energy, stratification/destratification, and biologic composition. Application of this common tracer approach to tidal creeks in the Southeastern US will permit comparison with other systems and specifically address the role of some other ecosystem processes (e.g. the role of filter feeders, in situ denitrification) not captured in previous tracer studies.

Hewlett's Creek is one of a set of continental-draining tidal creeks located in New Hanover County, an area that has experienced rapid growth over the past three decades. These creeks receive significant anthropogenic influence and periodically experience phytoplankton blooms and hypoxia in the upper and middle creek sections (Mallin et al. 2004; Johnson 2005). Some of these creeks have also been impaired by sewage spills with consequent nutrient loading and algal blooms (Mallin et al. 2007). Water column biochemical oxygen demand has been strongly correlated with algal blooms (as chlorophyll *a*) in a variety of regional coastal water bodies including this set of tidal creeks (Mallin et al. 2006) and chlorophyll *a* has likewise been positively correlated with sediment oxygen demand in tidal creeks (MacPherson et al. 2007). Based on nutrient addition bioassays phytoplankton production in these tidal creeks is primarily N-limited, but on occasion in upper creek areas phosphorus has been the limiting nutrient; light limitation by algal self shading has also occurred (Mallin et al. 2004).

OBJECTIVES

We assumed that the tidal creek was instrumental in intercepting nitrate from the watershed and using it to fuel production within the creek ecosystem before being exported to the coastal ocean. In order to understand how susceptible these tidal creeks are to nutrient loading, we identified our primary goal as well as several secondary objectives:

1. What is the fate of external nitrogen (N) in a tidal creek ecosystem?
 - What are the dominant pathways of N processing? What are the important ecosystem components?
 - How fast is nitrogen moving through each compartment? What is the overall ecosystem residence time of N?
 - What is the ecosystem dependence on N loading?

The use of an in situ isotopic tracer allowed us the ability to sufficiently label the DIN pool and track this source nitrate through the ecosystem. Due to the dynamic nature of the tidal creek ecosystem there were several variables which were beyond our control. These variables added to the complexity of the experiment and data analysis; however this is not uncommon in full-scale ecosystem experiments and gave us the ability to examine N cycling through a variety of biochemical and geochemical transformations. The experiment began on July 17, 2007 at the peak of a spring tide; thus during these high tides the marsh experiences minor periods of extensive flooding (to a depth of 10 centimeters). The addition experiment continued for 23 days through one and half spring-neap tidal cycles. Also, a major rainfall event on July 28, 2007 (day 12) and July 30 (day 14) led to an influx of watershed-derived unlabeled nitrate and subsequently diluted the isotopic enrichment of the pool.

MATERIALS AND METHODS

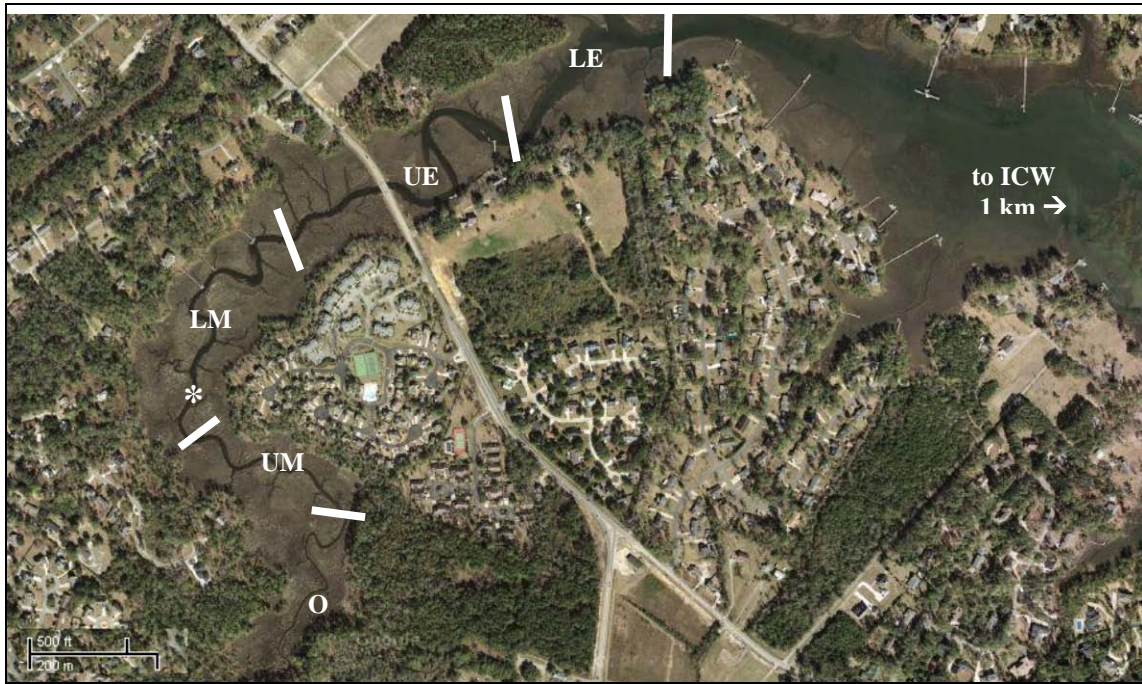
SITE CHARACTERISTICS

Hewlett's Creek is a 3rd order tidal brackish tributary of the Atlantic Intracoastal Waterway in Southeastern North Carolina. Located at 34 deg 11' N latitude, 77 deg 50' W longitude, this creek drains a watershed of 2393 ha with a population of approximately 16,000 (Figure 1; Mallin et al. 2007). Land cover is approximately 22% impervious surface coverage, mostly due to commercial and residential properties along its watershed. It also drains two golf courses (Johnson 2005). The experiment focused on a 2 km reach of the south branch and was divided into subreaches based on salinity for the purpose of this experiment (Figure 2; Table 1). High tide volume of water (tidal prism) in the experimental area is ~ 22,000 m³ with a tidal excursion of ~1.5 km. The creek receives fresh water from the watershed at an average rate of 0.8 m³ sec⁻¹. At low tide, the experimental reach is characterized by an average low tide volume of ~ 6,000 m³ with a creek bed area of 32,500 m².

Figure 1: Regional map of coastal North Carolina highlighting Hewlett's Creek.



Figure 2: South Branch of Hewlett's Creek, New Hanover County, NC. Distance from south branch mouth to the Intracoastal Waterway (ICW) is about 1 km.



White bars divide O, UM, LM, UE, and LE subreaches; the star indicates the ^{15}N addition site.

Table 1: Subreach characteristics of Hewlett's Creek

Sub reach ID	Station numbers	Salinity (ppt)	Length (m)	Average width (m)	Area (m ²)	High Tide Volume (m ³)
oligohaline (O)	1-2	0-5.5	345	7-10.5	3046	1518
upper mesohaline (UM)	2-3	5-18	300	7.5-12	2850	2831
lower mesohaline (LM)	3-4	5-18	535	11-15	6891	5906
upper euryhaline (UE)	4-5	18-35	505	15-32	9913	4598
lower euryhaline (LE)	5-7	18-35	315	18-44.5	9844	7298
total			2000		32544	22151

ISOTOPE ADDITION

The isotope enrichment solution consisted of 0.2M, 20 atom % enriched $K^{15}N-NO_3^-$ (20% of $N-NO_3^-$ was ^{15}N , 80% $N-NO_3^-$ was ^{14}N) that was dripped into the reach at a rate of 16 -18 L d^{-1} at the midpoint of the creek using a series of metering pumps (23 d; 7.4 g $^{15}N-NO_3^- d^{-1}$; 169 g ^{15}N total). Due to the volume change of the tidal prism during a tidal cycle (22,000 m^3 and 6,000 m^3), three metering pumps with varying pump rates (2, 6.5 and 10 $ml\ min^{-1}$) were used. Each was activated by a float switch mounted at different tidal heights located within the channel that turned on or off with the rise and fall of the tide. The overall addition rate therefore increased during the flood tide in order to maintain a more constant concentration of label throughout the tidal prism. This setup provided a much more even distribution of tracer to the creek compared to the single-pump, single rate dripping system used in previous studies. Examination of each ecosystem compartment consisted of field sampling followed by laboratory analysis which yielded the total N content of each pool and its isotopic enrichment ($\delta^{15}N$). These two measures were subsequently combined to yield estimates of ^{15}N mass movement through each pool.

FIELD SAMPLING

During the enrichment phase of the study, the frequency of sampling corresponded to the specific turnover time per pool and ranged from 12 h to 10 day (d) intervals (Table 2). For example, short-turnover pools like dissolved inorganic nitrogen (DIN) and microautotrophs (phytoplankton and benthic microalgae) were sampled daily from the start of the experiment and less frequently as the experiment progressed. Primary consumers (zooplankton, nekton, and benthic invertebrates) were sampled less frequently. Water column N pools quantified during this study included dissolved inorganic nitrogen (NO_3^- and NH_4^+), suspended particulate organic nitrogen (PON) that included phytoplankton and detrital nitrogen, zooplankton (ZOOP) and various fish species. Benthic N pools included bulk sediment organic nitrogen (SED), benthic microalgae (BMA), benthic infauna and epifauna and the fringing marsh grasses *Spartina alterniflora* (SPAR) and *Juncus roemerianus* (JUNC). Estimates of N content and ^{15}N enrichment were calculated for each compartment.

Water Column Sampling. The water column was sampled for DIN, particulate organic matter (POM), phytoplankton, zooplankton, and nekton. DIN and POM samples were collected in 2 L HDPE containers at each station and stored on ice during transport before filtration in the lab (47mm ashed Whatman glass fiber filters (GFF and GFD)). Filters were saved for POM % N and $\delta^{15}N$ analysis. Filtrate was frozen for subsequent NH_4^+ and NO_3^- concentration and isotopic analysis for $\delta^{15}N-NH_4^+$ and $\delta^{15}N-NO_3^-$.

Zooplankton sampling was conducted during timed tows using a nylon tow net (diameter = 23 cm; mesh size = 156 μm ; 30 L) at four locations in the creek (O, UM, UE, LE). Samples were fixed and stained with a mixture of Rose Bengal and Formalin then stored for identification and

quantification. In the laboratory zooplankton were subsampled and counted using a circular counting chamber under a dissecting microscope, with major taxa identified to species when possible.

Nekton were captured using block nets (width = 7 m; mesh opening = ¼ inch) and seines (10 m; mesh opening = ¼ inch). Block nets were deployed in three randomly selected side tributaries to the main channel in the upstream reaches of the creek during high ebb tide. Catch was retrieved at low tide once these tributaries had drained, and identification and quantification was logged in the field. Seining up and downstream collected the fish species that dominated the channel. A sub sample of total catch was collected for IRMS analysis. Fish were euthanized using CO₂ and stored in ethanol until preparation for isotopic analysis.

Benthic Sampling. The benthos was sampled for bulk sediment organic nitrogen, benthic microalgae (BMA), benthic infauna, bivalves, epifauna (*Littoraria* and *Uca*) and marsh macrophytes. Bulk sediment was collected using a series of 2 cm diameter cores (depth = 3 cm) in mud flats during low tide. Cores were sliced at 0-3 and 3-10 mm slabs. Samples were dried, homogenized, and N content and isotopic enrichment were determined during IRMS analysis. Benthic microalgae was collected from a series of sediment cores (diameter = 1.3 cm; depth = 10 cm) in the mudflat, channel, and marsh surface (data not reported). BMA was separated from subtidal sediment based on a density gradient using Ludox BMA isolation (Varela 1986) and collected on an ashed GFA filter for isotopic analysis. Nitex screens (150 cm²; mesh size = 300 microns) were also placed on the mudflats in an attempt to collect surface BMA (Tobias et al. 2003). Screens were washed and filtered onto GFA filters and analyzed as a secondary estimate of BMA-N content and enrichment during IRMS analysis.

Benthic infauna were harvested from sediment core duplicates (diameter = 10 cm; depth = 15 cm) along the creek bed at low tide. Once collected, cores were rinsed through a coarse sieve, then a fine sieve (500 microns) to remove plant matter and sediment particles.

Epifauna were collected at each station by randomly selecting between 2 and 5 individuals and freezing them until isotopic analysis. Quantification of *Littoraria* biomass was determined by a series of individual counts per square meter quadrat of the marsh edge. Similarly, *Uca* population was determined by burrow counts per meter quadrat where mud crab densities are 74% of burrow densities (Mouton and Felder 1996, Genoni 1991). Subsets of grass shrimp caught in seine nets were collected and stored in ethanol until analysis for N content and enrichment. Between 2 and 4 oysters were harvested from each of the four oyster reefs in the creek. Density, area, and oyster count was measured with a series of square meter quadrats.

Marsh macrophytes (*Spartina* and *Juncus*) were collected by carefully removing both roots and shoots of 2-3 individual plants at each station within the edge of a square meter quadrat. At

typical high tides, water flooded the marsh to approximately one lateral meter of marsh fringe so that all fringing marsh sampling occurred within this area. Density of plants was determined by counting individual plants within a series of square meter quadrats.

LABORATORY ANALYSIS FOR N CONTENT AND ^{15}N ENRICHMENT

Depending on the pool, N content was measured with chemical techniques (DIN, BMA, and phytoplankton) or during the isotopic analysis (bulk sediment, zooplankton, fauna). All isotopic analyses were performed using a Thermo Delta-V Plus isotope ratio mass spectrometer (IRMS) located at the Center for Marine Science, University of North Carolina Wilmington. Enrichment was reported as $\Delta\delta^{15}\text{N}$ values in ‰ where:

$$\delta^{15}\text{N} = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000$$

and

$$\Delta\delta^{15}\text{N} = \delta^{15}\text{N}_t - \delta^{15}\text{N}_{\text{background}}$$

where R_{sample} and R_{standard} were the $^{15}\text{N}/^{14}\text{N}$ ratios of the sample and standard air respectively, normalized to known glutamic acid reference materials. Samples of all ecosystem compartments were collected prior to the isotope addition and served to establish baseline (natural abundance) $\delta^{15}\text{N}$. Enrichment was reported as change in $\delta^{15}\text{N}$ values ($\Delta\delta^{15}\text{N}$) above background where N_t is the enrichment at a time interval and $N_{\text{background}}$ is the enrichment prior to the start of the addition (natural abundance). All samples were introduced to the IRMS through a Costech 4050 Elemental Analyzer (EA) interface. In addition to providing isotopic enrichment this also provided N content of each sample.

Water column filtrate was analyzed for DIN concentration and $\delta^{15}\text{N}$. NH_4^+ and NO_3^- concentrations were determined by a colorimetric reaction using a Bran Luebbe segmented flow nutrient auto analyzer (EPA Report 600/R-97/072). Isotopic determination of $\delta^{15}\text{NO}_3^-$ and $\delta^{15}\text{NH}_4^+$ were conducted using diffusion techniques; NH_4^+ was isolated by diffusion onto an acidified filter disk sandwiched between two Teflon membranes after the addition of MgO to raise the sample pH above 10 (Holmes et al. 1998). Determination of $\delta^{15}\text{N}-\text{NO}_3^-$ involved the same treatment of MgO to elevate the pH, after which the sample was boiled to drive off NH_3 . The addition of Devarda's alloy (0.3 g) reduced the dissolved nitrate to ammonia which was collected on the acidified filter and processed like the ammonia diffusions (Sigman et al. 1997). Filter discs were dried in a desiccator with concentrated H_2SO_4 and $\delta^{15}\text{N}$ determined via Elemental Analyzer – Isotope Ratio Mass Spectrometer (EA-IRMS).

Glass fiber filter samples were split and analyzed for chlorophyll *a* and ^{15}N enrichment of PON. A fluorometric analysis of chlorophyll *a* (USEPA standards, Turner 10-AU fluorometer, Welschmeyer 1994) determined concentration of chlorophyll *a* which was then extrapolated to phytoplankton-derived PON. Chlorophyll was converted to phytoplankton-N equivalents by assuming a chlorophyll:carbon ratio of 1:40 and a C:N ratio of 1:7 (Redfield 1958). Isotopic enrichment of PON was performed by analyzing a portion of the filter using the EA-IRMS after drying it at 50°C. N content was determined from calibration against references and the known filtered volume. The EA-IRMS calculated the $\delta^{15}\text{N}$ of bulk POM. Since the filters contained a combination of live phytoplankton plus detrital N, using the concentration of chlorophyll *a* allowed us to calculate the ^{15}N of the phytoplankton fraction according to the following mixing equation:

$$\delta^{15}\text{N}_{\text{phyto}} = \frac{\delta^{15}\text{N}_{\text{POM}} [N_{\text{POM}}] - \delta^{15}\text{N}_{\text{DN}} [N_{\text{DN}}]}{[N_{\text{phyto}}]}$$

where $\delta^{15}\text{N}_{\text{POM}}$ and $\delta^{15}\text{N}_{\text{DN}}$ were isotopic enrichments of respective pools (POM and detrital nitrogen: $\delta^{15}\text{N} = 3 \text{ ‰}$) and $[N_x]$ was the measured N mass of those pools. Similarly, BMA was quantified by analysis of chlorophyll *a* using double extraction and spectrophotometry, where polar differences between two phases of solvent separate degraded pigments from intact chlorophyll *a* (Whitney and Darley 1979). To separate BMA from sediment detrital N, a chlorophyll:C ratio of 1:35 and a C:N ratio of 1:7 were used in order to convert BMA chlorophyll *a* into N mass (N_{BMA}). For isotope analysis BMA was separated from their substrate based on density and isolated in a Ludox silica mixture and trapped on an ashed-GFA filter (Varela 1986, Blanchard et al. 1990). This method was inadequate in that it did not concentrate BMA cells from background detritus as intended. Nitex screens also failed to isolate a clean BMA sample. The BMA enrichment was determined similar to that of $\delta^{15}\text{N}$ phytoplankton using the following mixing equation:

$$\delta^{15}\text{N}_{\text{BMA}} = \frac{\delta^{15}\text{N}_{\text{SON}} [N_{\text{SON}}] - \delta^{15}\text{N}_{\text{SDN}} [N_{\text{SDN}}]}{[N_{\text{BMA}}]}$$

where $\delta^{15}\text{N}_{\text{BMA}}$ was the isotopic enrichment of BMA, $\delta^{15}\text{N}_{\text{SON}}$ was the enrichment of BMA plus sediment detrital N, $\delta^{15}\text{N}_{\text{SDN}}$ was the enrichment of sediment detrital N (background and presumed unlabeled, $\delta^{15}\text{N} - 3.5 \text{ ‰}$), and $[N]$ was the respective N mass of those pools as measured during IRMS analysis. The derivation of $\delta^{15}\text{N}$ phytoplankton and $\delta^{15}\text{N}_{\text{BMA}}$ assumes that the autotrophs held all the enrichment against an unlabeled background of detrital N.

Subsets of stained zooplankton were sorted from detritus under a microscope at 40x and 100x in order to achieve a pure sample of zooplankton then rinsed onto an ashed 25mm GFF Whatman filter and dried for IRMS analysis. Zooplankton counts and species identification were also performed on subsets of each individual sample to determine biomass and normalized to tow volume. N content was calculated as 10 % of total dry body mass where 1 individual = 20 μg (Holmes et al. 2000, Jorgensen 1979).

Samples of nekton and bivalves were dried at 50° F for 4 days. Stomachs were removed before individuals were ground and homogenized, and stomach and body were processed independently for isotopic enrichment. Benthic epifauna were similarly dried and homogenized for analysis (*Littoraria* was removed from its shell). Subsets of infauna were sorted under a dissecting microscope (40x and 100x) and prepared the same way. EA-IRMS provided $\delta^{15}\text{N}$ and % N of all nekton, bivalves, infauna, and epifauna.

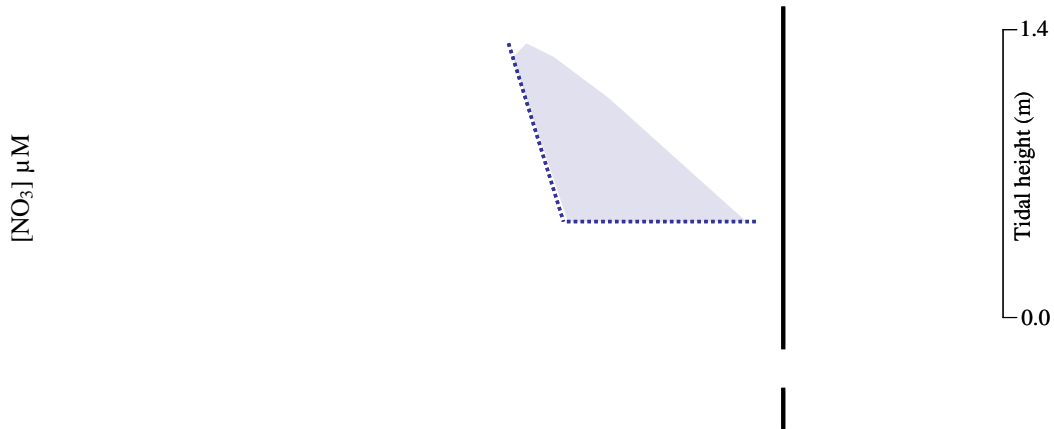
Table 2: Sampling regime of organism pools. “d” refers to day of experiment.

N pool	Week 1	Duration of Addition	Post-tracer sampling	Total
DIN (NO₃⁻, NH₄⁺)	d0.5, d1, d3, d6	d11, d14, d22	d24, d25, d29	10 each
Phytoplankton	d0.5, d1, d3, d6	d11, d14, d22	d24, d25, d29	10
BMA	d3, d7	d14, d22	d24, d31	6
Zooplankton	d4	d8, d16	d25, d35	5
Sediments	d3, d7	d14, d22	d24, d31, d38	7
Macrophytes	d7	d14, d22	d31, d38, d47	6
Infauna/ Epifauna	d3, d7	d13, d22	d24, d31, d38	7
Bivalves	d7	d16, d22	d31, d38, d51	6
Fish	d9	d14, d21	d30, d44	5

RESULTS

High tide water column profiles of the experimental reach indicated vertical stratification upstream where tracer was predominately found. Previous studies indicate that the surrounding watershed is the primary source of new nitrate into the system (Mallin et al. 2004) however in non-rain conditions there were generally low concentrations of NO_3^- throughout the experimental reach during our study. Nitrate concentrations ranged from 0.2 – 9.41 μM , and averaged 1.39 μM , when low tide measurements directly following a day 12 rain event (3.17 – 19.36 μM) were excluded (Figure 3; Table 3). Ammonium concentrations ranged from 0.2 – 31.51 μM and averaged 4.43 μM (Table 3). Headwater NO_3^- concentration was $\sim 4 \mu\text{M}$.

Figure 3: Ambient high tide nitrate concentration in the oligohaline subreach coupled with daily rainfall and tidal data (National Weather Service). The cyclical blue line denotes water level and indicates the creek tidal stage. Black diamonds with the connecting line show measured nitrate values and their fluctuation with response to rainfall. The dotted lines and shaded area indicate uncertainty in the trajectory since no sampling events occurred during this time. Bars indicate rainfall. The vertical black line at day 23 marks the end of the isotope addition period.



Major rainfall events on d12 and d14 caused an increase in ambient NO_3^- into the system. This influx of nitrate stimulated algal growth (reflected by higher chlorophyll *a* concentrations) on day 22. This increase in chlorophyll *a* was presumably within 2-3 days of the rain event, however there were no samples collected during that time period.

Table 3 : Nitrate and ammonium surface concentrations per subreach for all sampling periods throughout the isotope addition experiment. All concentrations are expressed in micromolar (μM). Lines within cells denote concentrations for high, ebb, and low tide when available. As with all subsequent data, d1-d23 is the tracer addition period and the shaded areas indicate pre- and post- addition sampling events. A large rain event on d12 was noteworthy in that it pulsed in a new load of NO_3^- (and NH_4^+ , perhaps from an increase in marsh drainage) during the tracer addition which subsequently affected the source $\delta^{15}\text{NO}_3^-$ enrichment.

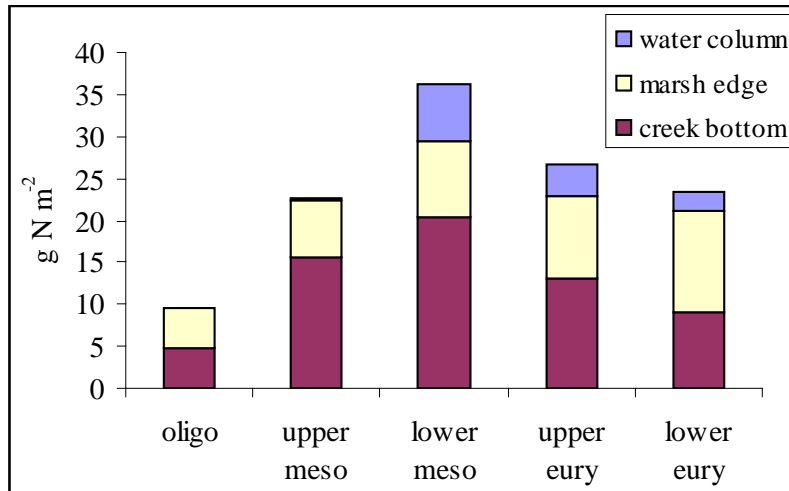
NO_3^-	d0	d1	d3	d6	d11	d14	d22	d24	d25
O	1.8 -----	0.9 -----	0.6 -----	0.8 0.8 2.41	0.7 -----	6.0 2.8 3.9	1.9 -----	2.0 -----	2.3 -----
UM	1.1 -----	0.9 -----	0.7 -----	0.8 0.9 <0.25	0.7 -----	4.9 3.6 12.0	0.8 -----	2.0 -----	1.1 -----
LM	0.9 -----	0.4 -----	0.6 -----	0.8 0.8 <0.25	0.5 -----	4.0 6.9 13.1	0.5 -----	2.0 -----	0.8 -----
UE	0.6 -----	0.3 -----	0.5 -----	0.9 1.0 1.4	0.4 -----	2.3 9.2 8.5	0.3 -----	3.5 -----	0.5 -----
LE	0.8 -----	0.3 -----	0.6 -----	1.0 1.0 2.2	0.4 -----	2.1 7.0 9.8	0.2 -----	1.1 -----	0.4 -----

NH_4^+	d0	d1	d3	d6	d11	d14	d22	d24	d25
O	13.6 -----	5.8 -----	1.0 -----	1.3 1.6 8.8	3.3 -----	10.8 7.1 8.8	3.0 -----	9.6 -----	7.2 -----
UM	7.7 -----	5.6 -----	1.3 -----	1.5 0.6 -----	3.8 -----	10.1 7.7 13.0	2.6 -----	16.0 -----	3.4 -----
LM	9.5 -----	4.0 -----	0.4 -----	1.0 0.6 3.2	4.2 -----	9.9 11.7 12.3	2.0 -----	13.0 -----	2.4 -----
UE	8.6 -----	3.0 -----	0.8 -----	0.6 1.3 2.6	5.5 -----	8.8 14.2 8.6	2.0 -----	13.0 -----	1.6 -----
LE	4.4 -----	1.9 -----	1.2 -----	0.5 1.0 2.4	5.9 -----	7.9 10.8 7.5	1.8 -----	11.0 -----	1.6 -----

GROSS DISTRIBUTION OF N POOLS

The majority of N within the creek was located in the creek benthic environment, including living and dead organic matter and sediments (Figure 4). Bulk sediments contained the most N and comprised between 4.7 g N m^{-2} – 20.2 g N m^{-2} per reach. Marsh plants (1 m fringe) accounted for 1.5 g N m^{-2} – 3.9 g N m^{-2} in the whole creek and made up between 30-40 % of the total marsh edge N inventory (supplemented by *Littoraria* and *Uca*). Creek water column inventory ranged from 0.2 – 6.8 g N m^{-2} .

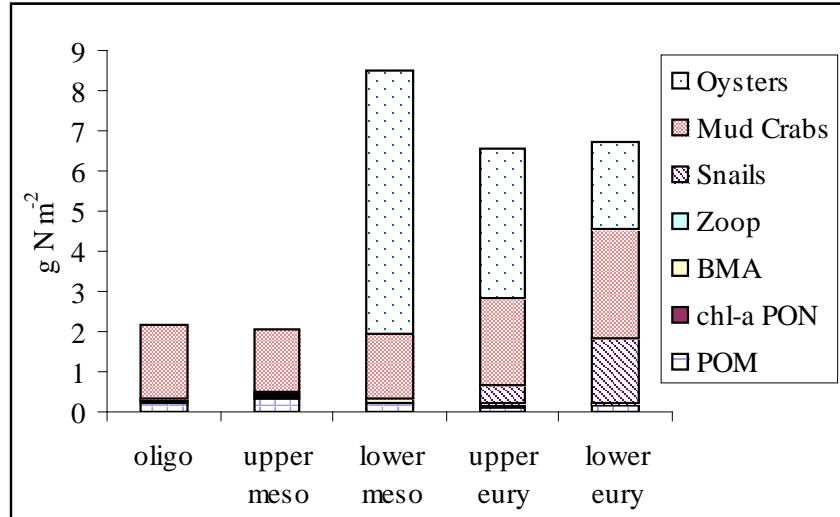
Figure 4: Distribution of N biomass (g N m^{-2}) standardized for area averaged over the whole experiment. Marsh edge includes *Spartina* and *Juncus* spp (1 m of regularly flooded fringe), *Littoraria* and *Uca* (mud crabs). Creek bottom includes benthic microalgae and bulk sediments (0-1 cm). Water column includes PON, zooplankton, fish species and bivalves.



N located in the upstream reaches was evenly split between marsh edge and creek bed compartments with negligible amounts of N in the water column. Water column N contributed more to the total N in the downstream reaches (15-20%). The water-column N contribution peaked mid reach (LM) but then decreased downstream where the marsh edge vegetation (*Spartina alterniflora*) became more important. This increase in water-column N is related to the contribution of oyster biomass found only downstream.

In order to compare “active” N pools (ie. biotic pools) we excluded the massive sediment N pool. We were then able to determine that highly active ecosystem N pools (turnover <1 month, e.g. microautotrophs, zooplankton) comprised 10-30% of this N inventory (Figure 5) and a greater fraction of total N further downstream. Oyster beds in the 3 subreaches downstream (LM, UE, LE) accounted for the majority of biotic N inventory. Since total N inventory peaks mid-estuary and biotic N increases with distance downstream, the faster cycling biotic pools become a proportionally more important part of N inventory downstream relative to the longer turnover plant and sediment detrital pools found throughout.

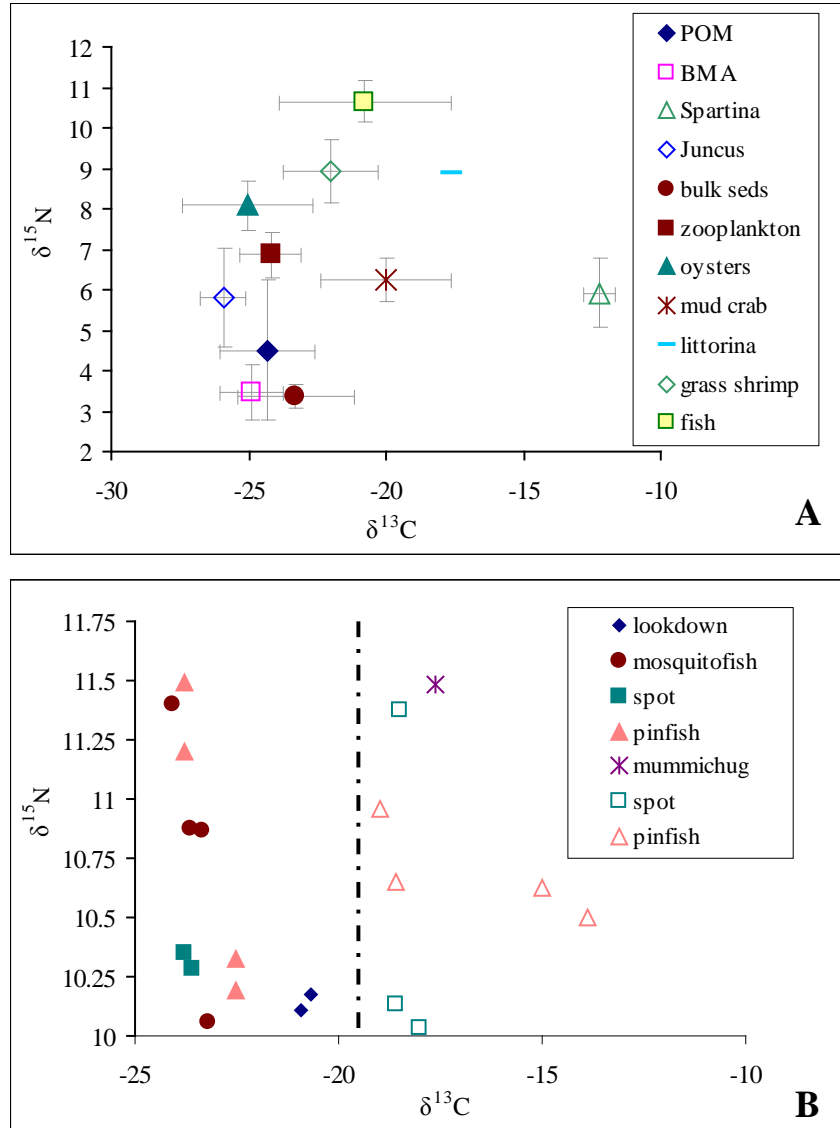
Figure 5: By excluding the contribution of sediment and macrofaunal N pools, we were able to compare the distribution of N (g N m^{-2}) contained in rapidly responding N pools during the first week of the addition. Fish and infauna each comprise less than 0.05 g N m^{-2} . Chl-a PON is the active subset of the POM pool (living phytoplankton). “Zoop” refers to N found in the zooplankton biomass.



NATURAL ABUNDANCE ISOTOPIC SIGNATURES OF BIOTA

Ecosystem pools were analyzed for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ prior to the isotope addition in order to establish accurate isotopic baseline data for each subreach (Figure 6A). Natural abundance isotope values for ^{15}N were uniform throughout the subreaches for all N pools except for grass shrimp, which were slightly heavier upstream by $\sim 2 - 3 \text{ ‰}$. PON $\delta^{15}\text{N}$ ranged from $2 - 7 \text{ ‰}$. The commonly accepted $\sim 3 \text{ ‰}$ $\delta^{15}\text{N}$ increase between trophic level was observed between food sources (POM, BMA) and consumers. A clear separation in $\delta^{13}\text{C}$ between C3 (*Juncus*) and C4 (*Spartina*) plants was also apparent. Higher $\delta^{13}\text{C}$ values in fishes were seen downstream (LE) although nekton $\delta^{15}\text{N}$ changed little through the subreaches (Figure 6B).

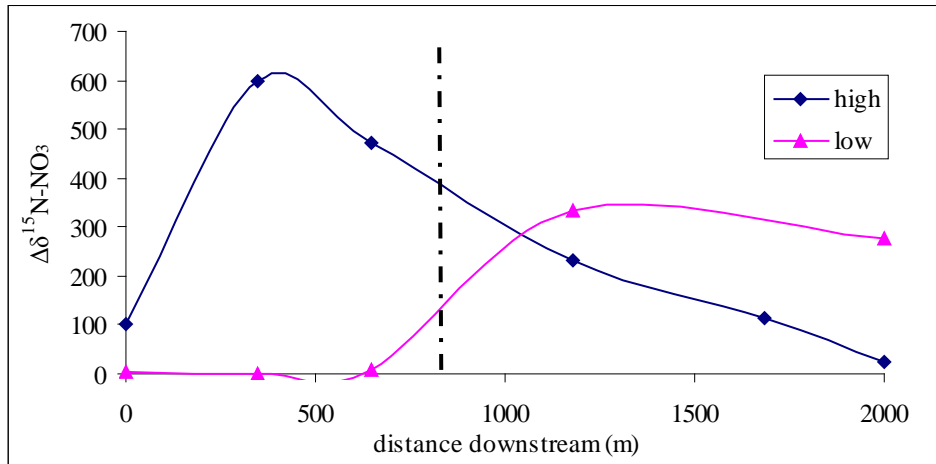
Figure 6: Natural abundance isotopic signatures for sampled pools in Hewlett's Creek (A). Error bars denote one standard deviation from the mean. Isotope values varied within fish populations and indicated a heavier $\delta^{13}\text{C}$ value for individuals captured downstream (B). The dotted line indicates the separation between isotope differences of upstream (UM, to the left of the line) versus downstream (LE, to the right of the line) catches.



ISOTOPIC TRACER ENRICHMENT

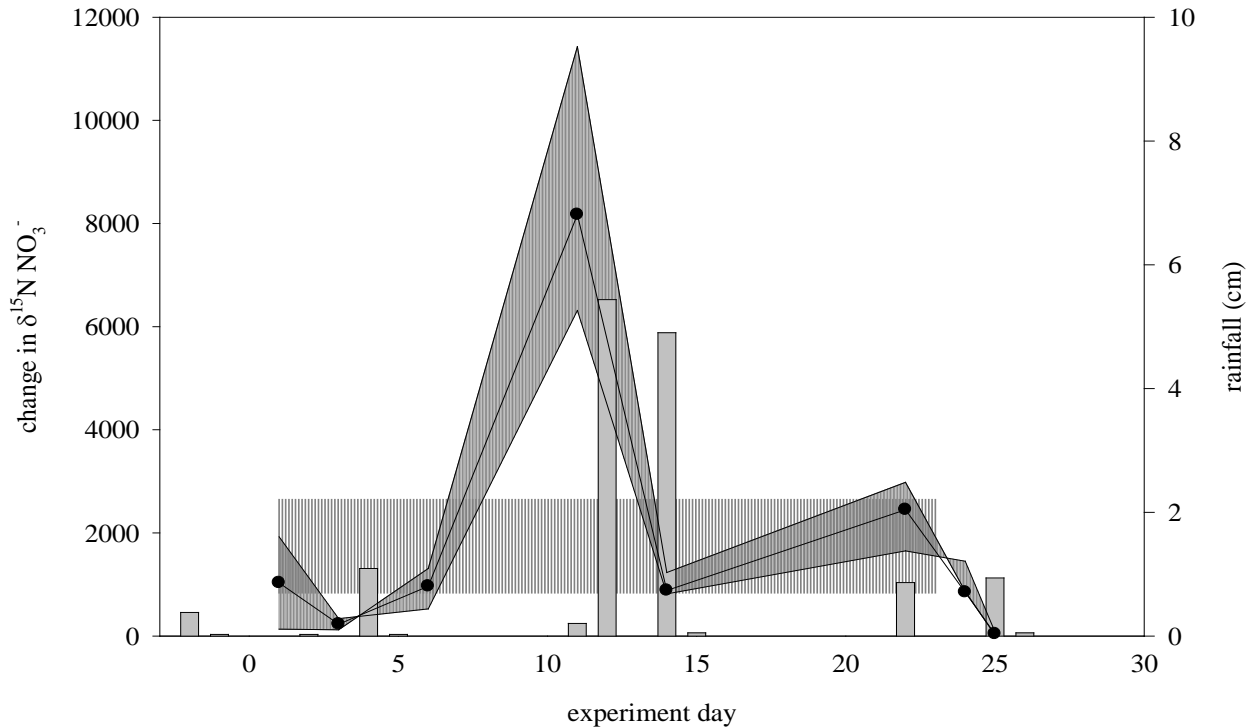
The $\Delta\delta^{15}\text{NO}_3^-$ of the creek ranged between 500 - 2000 ‰ with a time-weighted mean $\Delta\delta^{15}\text{NO}_3^-$ enrichment of ~ 900 ‰. This $\delta^{15}\text{N}$ enriched source of NO_3^- moved back and forth through the reach with the tide. Ebb and flood tide enrichments were in the same range differing only in the location of the peak $\delta^{15}\text{NO}_3^-$ (Figure 7). Due to stratification in the upstream reaches, bottom water enrichment was less than the surface, averaging ~ 200 ‰ (measured on d1, d3, d11, and d22). The isotope addition technique ensured that when the $\delta^{15}\text{NO}_3^-$ enrichment was averaged over a tidal cycle per site (tidal-averaged) and when the $\delta^{15}\text{NO}_3^-$ enrichment of all sites was averaged collectively per tide (reach-averaged), $\delta^{15}\text{NO}_3^-$ enrichment variations were no greater than a factor of two different for the duration of the study.

Figure 7: Spatial distribution of $\delta^{15}\text{N}$ - NO_3^- above background levels for sequential high and low tide creek transects. Data measured on day 14. The dotted line is the location of the isotope addition site (drifter).



The $\delta^{15}\text{NO}_3^-$ enrichment depended on several factors in addition to pumping rate including ambient $[\text{NO}_3^-]$, watershed inputs of NO_3^- , and tidal supply of NO_3^- (concentration and water volume). A major rainfall event on d12 caused an influx of watershed-derived unlabeled nitrate (Figure 1).

Figure 8: Change in $\delta^{15}\text{N-NO}_3^-$ enrichment for the upstream subreaches of the creek at high tide. The black line represents the average of enrichments for O, UM, and LM data. The dark grey shading around this line represents the area of uncertainty (high and low values) of these reaches. The light gray histogram represents rainfall during the experiment, in centimeters. Finally, the horizontal shaded bar represents the most likely value of nitrate pool enrichment, based on isotope addition uptake by primary producers. This bar was calculated as 1 standard error from the mean enrichment (2182 ‰) of all data points from the upstream stations (O, UM, and LM) during the addition. Addition period was day 0 through day 23.

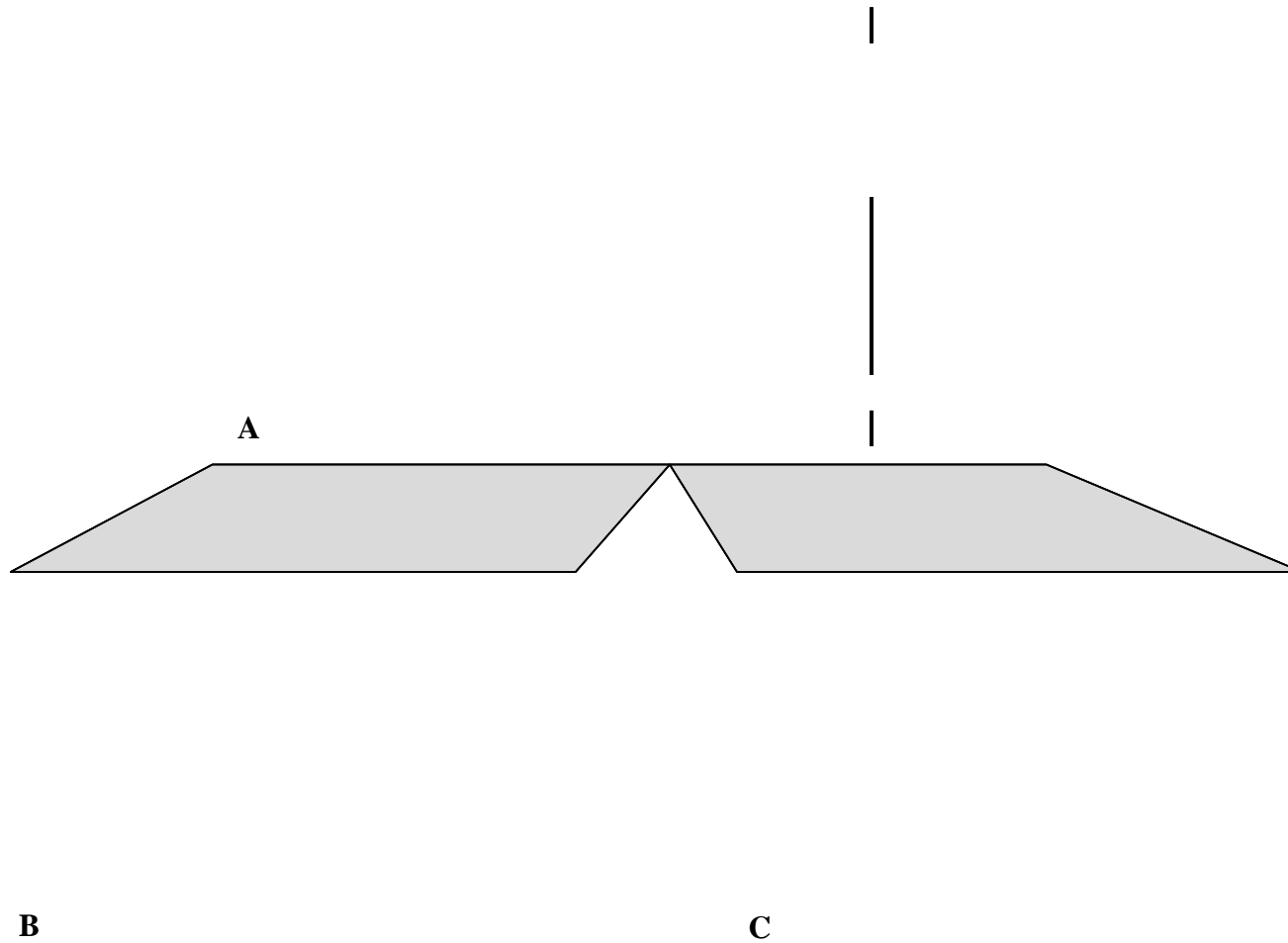


Greater volumes of sample water (than collected) were needed to recover the requisite amount of N to analyze on the IRMS during the beginning of the experiment due to low concentration of nitrate upstream (Figure 3, Table 3). This led to uncertainty in the enrichment trajectory during this time. There was an unusually large spike in enrichment on day 11, however in the subreaches below the addition site, the enrichment was well within the average prior to this spike (1850 – UE, 739 – LE). Given the enrichment of the pool downstream and the enrichment of primary producers, d11 sampling likely sampled the addition pool directly (ie. not proper mixing). Because of sensitivity of sampling technique, sampling the unmixed $\delta^{15}\text{N-NO}_3^-$ pool, and frequency of sampling events surrounding the major rainfall, it is important to note the estimated enrichment of the NO_3^- pool. This estimation was based on average enrichment of all upstream sites during the addition period, +/- 1 standard error. This changing baseline enrichment of $\delta^{15}\text{N}$ was then propagated through faster turnover time organisms like PON and BMA (Figure 9), but the temporal variability in enrichment was smoothed in pools further up the food chain.

¹⁵N INCORPORATION BY AUTOTROPHS

¹⁵N labeled NO₃⁻ was incorporated within the PON pool within 1 day of addition, reflecting the rapid turnover time of the phytoplankton pool. Maximum enrichment for phytoplankton-PON was found consistently in the oligohaline reaches of the creek, and peaked at d11 (1344 ‰ - O, 310 ‰ - LE) for all creek areas (Figure 9B) before sharply declining on d14 in response to the rainfall dilution of δ¹⁵ NO₃⁻. BMA also incorporated tracer following a slight lag time (~3 d), however it reached a lower maximum enrichment relative to phytoplankton – PON of 949 ‰ (Figure 9C). Higher enrichment further upstream was due to closer proximity to tracer.

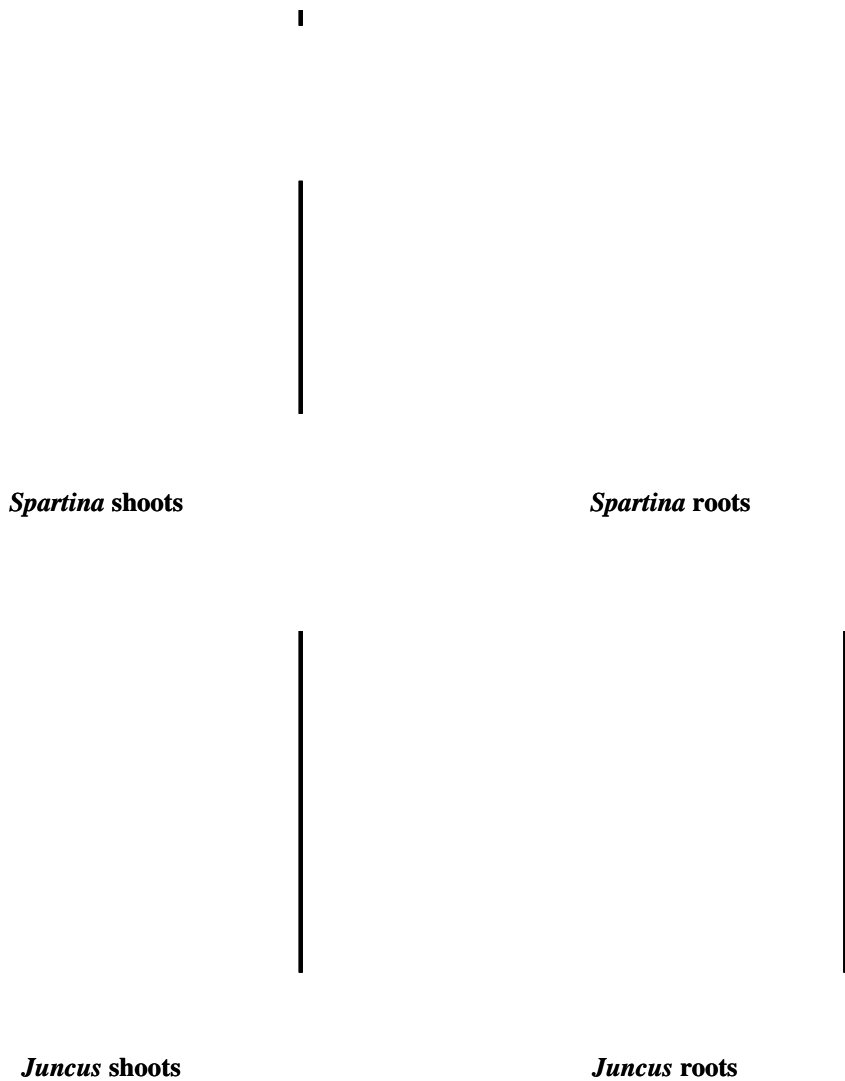
Figure 9: (A) Change in enrichment of BMA and phytoplankton-PON in the oligohaline and upper mesohaline portions of the creek in response to rainfall; the vertical black bar indicates the end of the isotope addition period (d23). (B) Rising enrichment trajectories of phytoplankton-PON in all subreaches prior to rain-induced dilution of tracer; (C) Rising enrichment trajectories of BMA in all subreaches prior to rain-induced dilution of tracer.



Downstream productivity was less affected by the influx of nitrate from the watershed. A lack of sampling for BMA between d7 and d13 caused a skew in the enrichment trajectory; the decline in enrichment most likely does not occur until the first rain event on d12.

Enrichment for creek-edge marsh macrophytes was measured in both shoots and roots and showed a difference in enrichment pattern based on tissue type (Figure 10). Both species of emergent macrophytes incorporated the tracer to the greatest extent directly upstream and downstream of the dripper (UM and LM). For *Spartina*, both locations upstream from the addition site showed immediate incorporation of tracer. Enrichment was less downstream with a maximum $\Delta\delta^{15}\text{N}$ of 5 - 9 ‰ for either marsh plant. *Spartina* roots showed higher peak enrichment at every station (max roots – 41 ‰; shoots – 25 ‰) compared to the shoots. *Juncus* showed comparable peak enrichment in shoots and roots (26 ‰ vs. 18 ‰), but because of a short lag time in enrichment trajectory of shoot samples, ^{15}N was clearly incorporated into *Juncus* roots prior to transfer to shoot tissue.

Figure 10: Enrichment trajectories for *Spartina* and *Juncus* shoots and roots, rinsed to remove mud and epiphytes. The black line indicates the end of the tracer addition period (d23). Data not shown for samples collected at the lowest two subreaches (UE, LE) due to a negligible change in enrichment.



TRANSFER OF ¹⁵N THROUGH THE FOOD WEB

Water Column. Tracer moved from autotrophs into higher trophic levels in both the water column and benthos. Because the time scale of the experiment was shorter than the time required for the seasonal conversion of macrophytic leaf litter to useable detritus, enrichment in higher trophic levels was assumed to originate from the microautotrophs (phytoplankton and BMA) and not from marsh plants.

Enrichment trajectories of the zooplankton pool followed the enrichment trajectories of PON during the first week of addition however they sharply declined on day 8. This decline is

coincident with an increase in zooplankton biomass, potentially diluting the enrichment signal (Figure 11). The increase in zooplankton population on day 8 followed the first rain event (prior to the start of the addition; d-2) by 12 days and the algal response (to this rainfall event) by ten days, a period sufficient to allow the principal zooplankter (*Acartia tonsa*) to pass through its naupliar and copepodite stages to reproductive adulthood. Similarly, the same thing occurred following the large rain event on days 12 and 14 (Figure 1) with the highest biomass of zooplankton measured on day 25. These rain events caused large quantities of unlabeled N to enter the system (Figure 3). It temporarily lowered $\delta^{15}\text{N}$ enrichment, but fueled phytoplankton production which provided a rich food source to spur the zooplankton growth. The increased use of detrital (unlabeled) N to support the larger zooplankton biomass may be another potential cause for the decline in zooplankton enrichment.

For fishes there was a separation in enrichment levels between fish with similar feeding strategies (Figure 12). By d9, all fish species incorporated tracer from 5 to 20 ‰ with water column feeders (menhaden) incorporating the tracer more rapidly. Menhaden and mojarra showed the highest uptake of tracer (d21) with an enrichment of 48 ‰ and 45 ‰, respectively. Highest values for pinfish were 30 ‰ whereas mosquitofish and spot reached a maximum enrichment of only 12 ‰ (d 44) and 18 ‰ (d 21), respectively.

Fish species caught upstream and downstream also showed spatial distribution patterns in enrichment. Mojarra reached maximum enrichment downstream (37 ‰) however pinfish incorporated more of the tracer upstream (24 ‰). The result suggests either movement of some species within the creek with utilization of upstream resources and/or a progressive movement of ^{15}N tracer from upstream to downstream.

Figure 11: Zooplankton biomass and change in enrichment over time (days). Euryhaline subreaches did not show significant enrichment. Lines represent enrichment ($\Delta\delta^{15}\text{N}$) and bars represent biomass (g N).

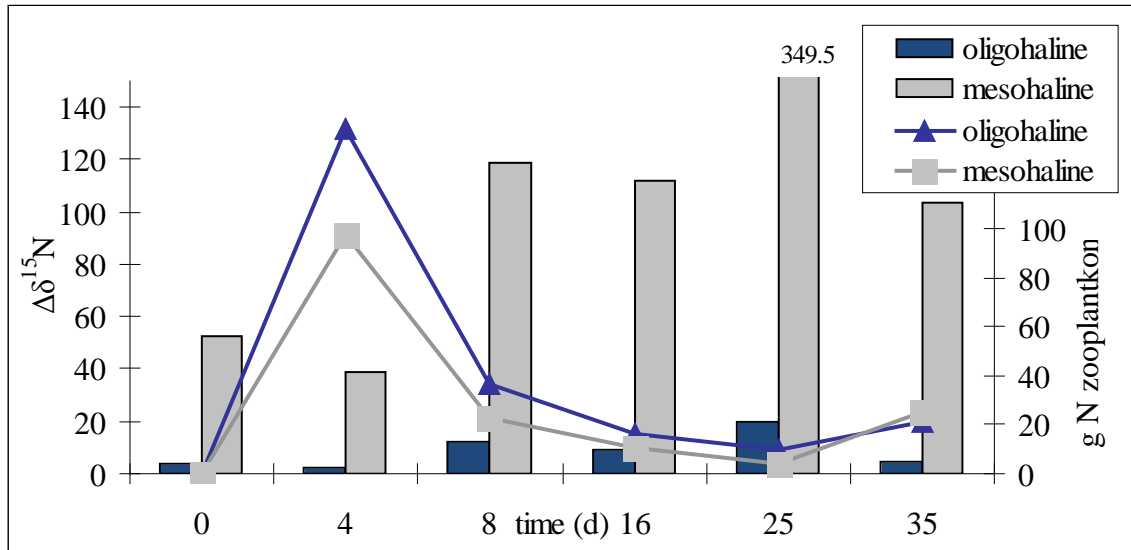
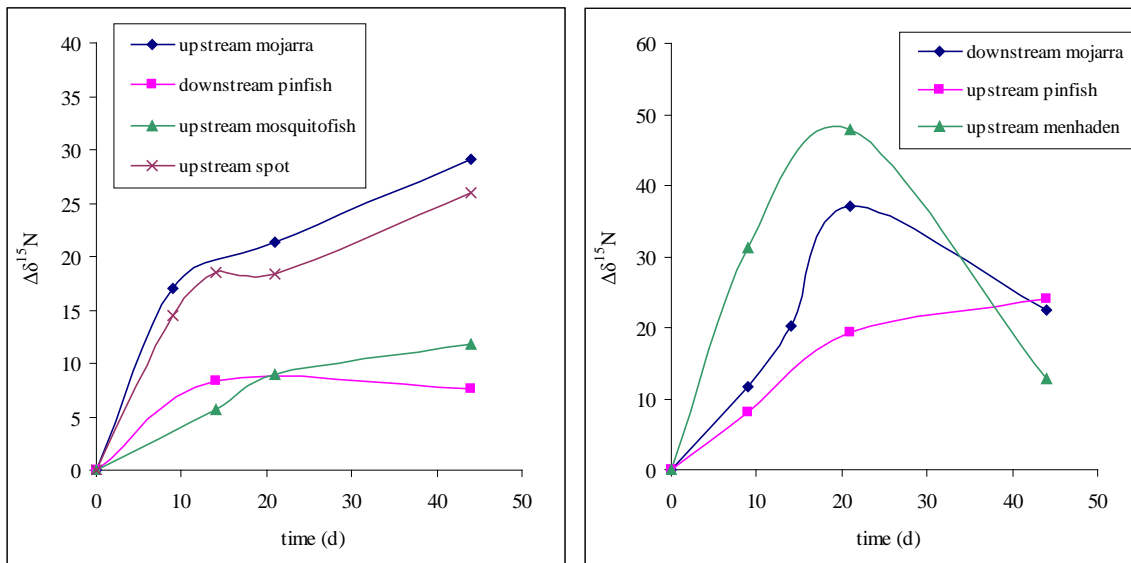
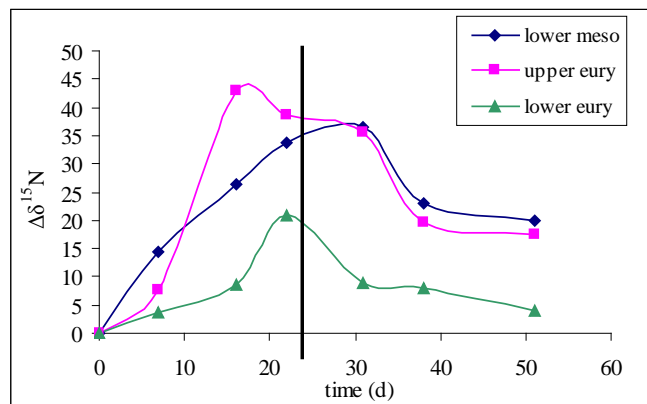


Figure 12: Enrichment trajectories for major fish species.



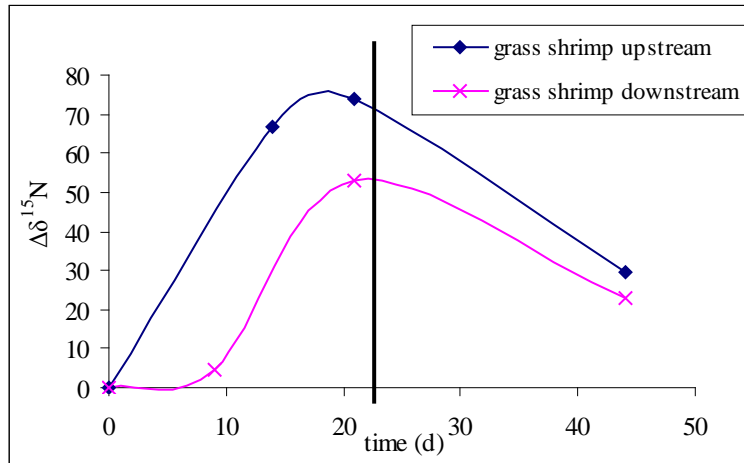
Benthos. Epifauna (oysters, mud crabs, grass shrimp) dominated ^{15}N uptake in the benthic food web. During the first week of the experiment, $\delta^{15}\text{N}$ values increased in oysters as they fed on water column PON (including phytoplankton) and resuspended BMA (Figure 13). Oysters reached a maximum $\Delta\delta^{15}\text{N}$ value of 43.1 ‰ on d16 (UE). There was similar enrichment for LM, and UE, and expectantly lower for LE since these oysters were further from the tracer source. These oyster reefs saw the lowest source of enrichment based on their location both within the water column as well as within the experimental reach. At high tide, the reefs were completely submerged yet saw minimal tracer since the enriched nitrate/PON pool was predominately upstream. As this labeled food source moved downstream with the receding tide, the oyster reefs only encountered some of the label before they became exposed as the water level dropped below the elevation of the live oysters. A moderate enrichment in this pool can also be explained by the fact that the oysters feed on a mix of labeled and unlabeled suspended organic matter (phytoplankton and unlabeled detritus), as well as the extremely large size of the internal N pool.

Figure 13: Enrichment trajectories for oysters composing the three major reefs. The black bar shows the end of the tracer addition period (d23).



Grass shrimp enrichment trajectories were unique between upstream and downstream sites. Maximum enrichment was found upstream (73 ‰ - d21; Figure 14). A lag time in tracer uptake was observed in the downstream samples, most likely indicating tracer relocation.

Figure 14: Enrichment trajectories grass shrimp captured upstream versus downstream. The bar indicates the end of the isotope addition period.



Other epifauna incorporated lower levels of tracer relative to oysters and shrimp; however, these organisms did show enrichment patterns with lag times. *Uca* and *Littoraria* followed very similar enrichment trajectories to each other, exhibiting a delayed enrichment of up to 7 d (Figures 15 and 16). These trajectories differ from the microautotrophs and zooplankton in the duration of uptake lag time indicating a potential reliance on recycled N. Enrichments of *Uca* and *Littoraria* climbed during tracer addition and continued to rise after the tracer was shut off, giving further evidence of their dependence on recycled N (maximum values: 42 ‰, UM, mud crabs; 116 ‰, O, snails).

Benthic infauna showed a maximum enrichment of 28 ‰ after the tracer was shut off (d38, O; data not shown). N biomass was variable between samples, from 0 - 0.5 g N m⁻². Due to this lack of biomass, we were unable to determine turnover time from the samples in this experiment. Even with a rapid turnover time, the N pool was too small to be a dominant pool for processing N for higher trophic levels at the time of the study. The minimal role of infaunal N transfer at an ecosystem scale is in close agreement with results from other N and C isotope tracer experiments (Middleburg et al. 2000).

Figure 15: Enrichment trajectories for *Uca* at each subreach of the creek.

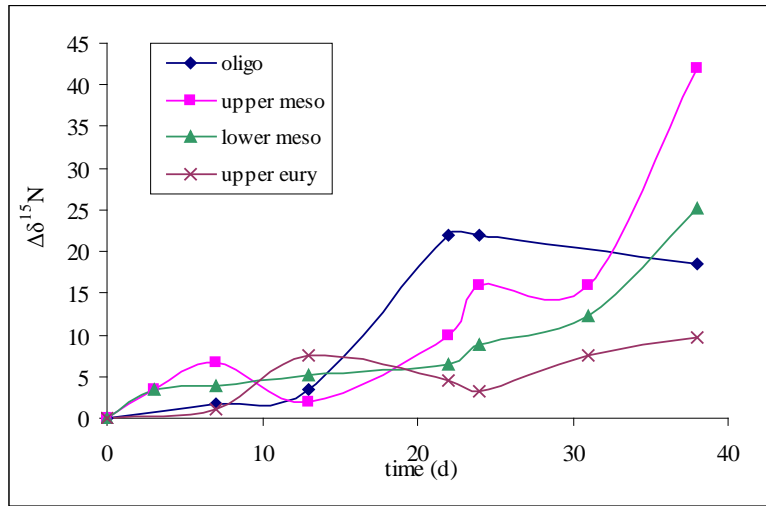
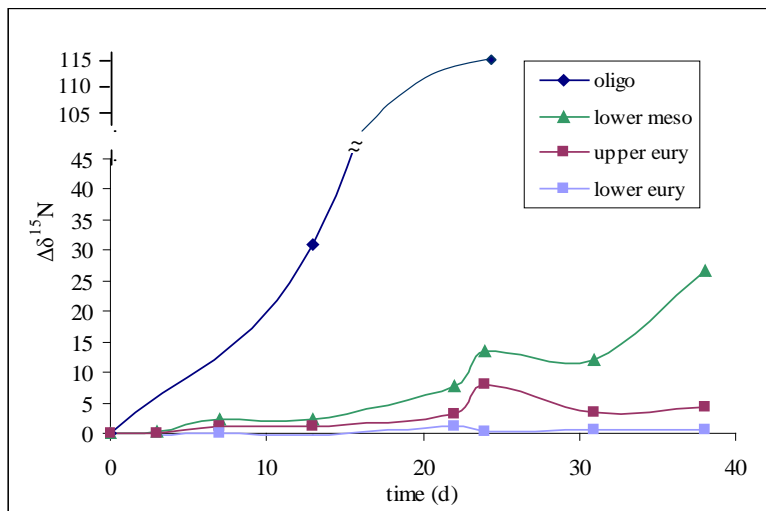
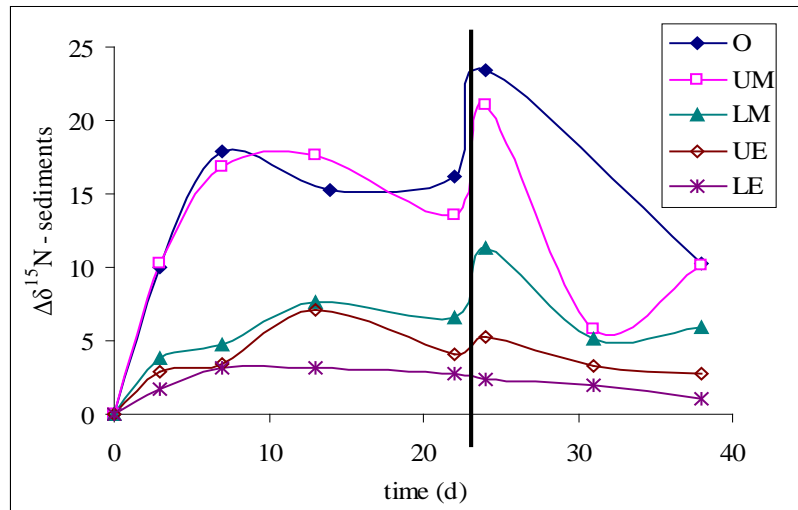


Figure 16: Enrichment trajectories for *Littoraria* per subreach. The trajectory for the oligohaline subreach continues to a maximum value of 116 ‰ on d24.



Bulk Sediments. Subtidal creek bulk sediments contained the majority of N biomass and consisted of a mixture of detritus, BMA, and settled phytoplankton. Bulk sediment exhibited a maximum change in enrichment of 23 ‰ in both subreaches upstream from the addition site (O, UE; Figure 17). Below the isotope dripper, enrichment averaged 3.8 ‰ with a maximum value of 11 ‰ (LM, d24). Peak enrichment was achieved after the dripper stopped indicating slow turnover and contribution from recycled N. On the final day of sediment sampling (d38), creek sediments upstream (O, UM, LM) were still enriched between 6 and 10 ‰.

Figure 17: Enrichment trajectories for bulk sediments. Retention of ^{15}N extending beyond the addition period indicates storage of N within the benthic N pools. The black bar indicates the end of the tracer addition (d23).



TURNOVER TIMES FOR N POOLS

The rising phase of enrichment trajectories was used to calculate turnover time for N pools (Table 4). Rates were calculated by plotting natural log transformed $\delta^{15}\text{N}$ (following normalization to plateau enrichment) against time (days). Turnover time was equal to the inverse slope of the plot (Tobias et al. 2003). Two examples for each trophic level are shown in Figure 18.

Microautotrophs turned over fastest. Phytoplankton and BMA pools turned over N within 2-3 days, and showed decreasing turnover times downstream. The faster rates upstream may be related to the availability of watershed-derived nutrients in the oligohaline subreach of the creek. The calculated rate of turnover for the BMA pool is consistent with previous studies (~3.0 days; Tobias et al. 2003).

The variability in the enrichment trajectories of the zooplankton made calculation of turnover times difficult. The times reported are rough estimates and likely to be high (specifically, LE). Previous studies have reported zooplankton population turnover times of 5 days (Holmes et al. 2000). *Acartia tonsa* and other copepods must pass through several naupliar and copepodite stages to reach reproductive adulthood, which may also impact turnover time calculation.

Uca and *Littoraria* showed similar turnover times averaging ~ 30 days. Similar to BMA and phytoplankton, shorter turnover times were calculated at the upstream reaches. Shrimp turned over more rapidly, ~ 9 days, with minimal difference between upstream and downstream populations (Table 3).

Oyster nitrogen turn-over was approximately 17 days and also showed minimal differences in turnover time and biomass between reefs (Table 4). Fish turnover times were highly variable and ranged from 4 d to 118 d, depending on species and location. High variance may reflect some immigration and emigration effects between the creek and the ICW.

Although previous studies assumed bulk sediments to be a long-term storage pool for N, the observed turnover time in Hewlett's creek was relatively fast (specifically upstream; 4 – 21 days). In order for this sediment N pool to exhibit a fast turnover, fresh microautotroph detritus must comprise a high proportion of the total sediment N (versus old detritus, slow turnover). A sediment pool consisting of a higher proportion of unlabeled 'old' detritus would exhibit less ^{15}N enrichment and subsequently a slower turnover rate. Another possibility for high turnover would be a high bacterial uptake and remineralization of N. These possibilities will be examined further in the Discussion.

Figure 18: Representative linear regression plots used to calculate turnover time of specific N pools. $\delta^{15}\text{N}$ values were log transformed and plotted against time in order to determine the slope of the regression line. PON and BMA were divided into upstream and downstream based on similar turnover times for specific subreaches. Other organisms shown include mud crabs (LM), *Littoraria* (LM), oysters (LE), and upstream pinfish. A complete list of turnover times is found in Table 4.

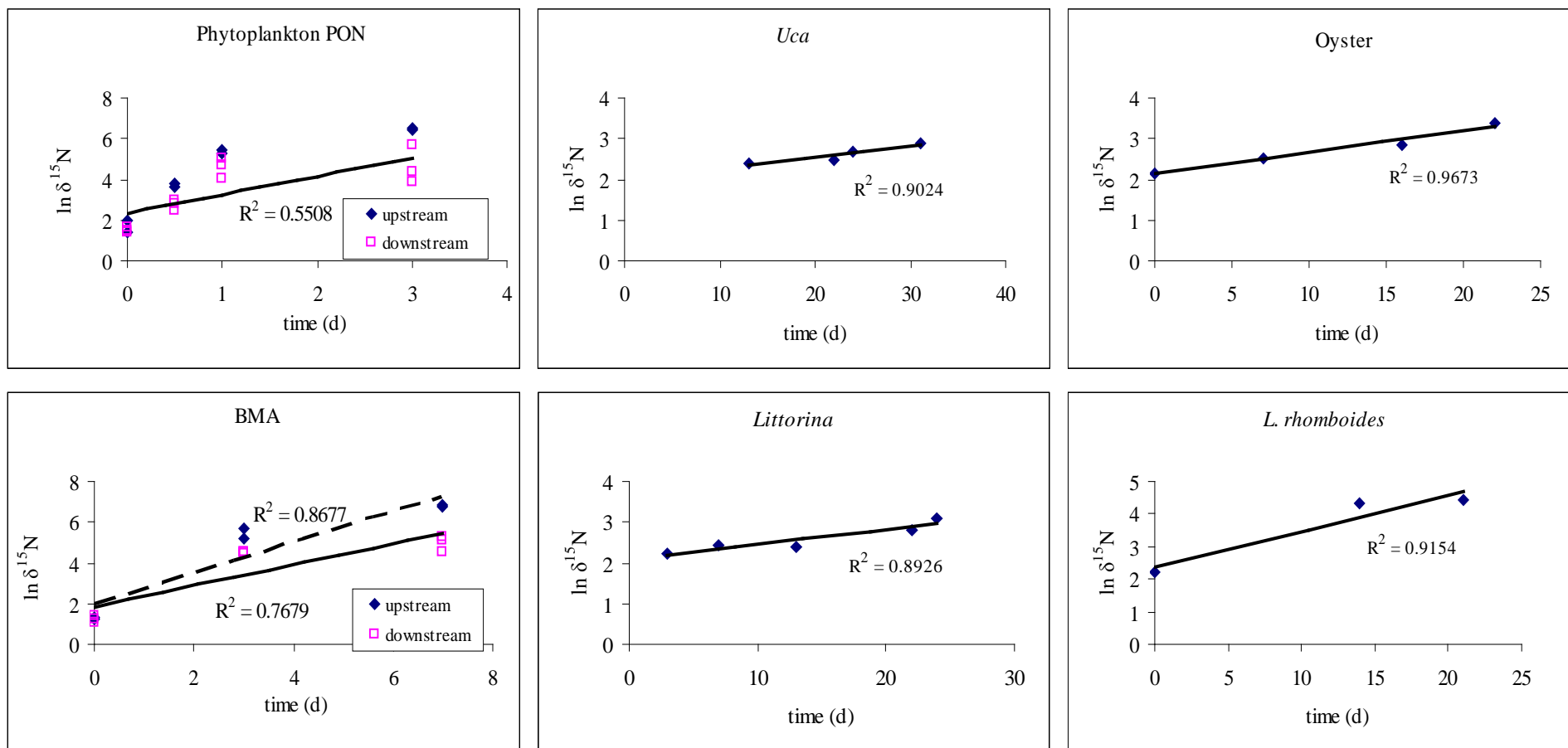


Table 4: Turnover times for the various N pools within the creek. Zooplankton tows were grouped in upstream, downstream, and lower estuary sections due their collected method (two minute tows against the current that extended through subreaches), except for where the width of the creek allowed for transect tows (le).

N pool	Location	Turnover Time	r ²	
phytoplankton	o	2.72 d	0.592	n=6
	um	2.77 d	0.5225	n=6
	lm	2.85 d	0.5766	n=6
	ue	3.41 d	0.607	n=6
	le	3.61 d	0.7352	n=6
BMA	o	1.30 d	0.9112	n=3
	um	1.33 d	0.8274	n=3
	lm	1.70 d	0.8244	n=3
	ue	1.87 d	0.8016	n=3
	le	2.36 d	0.6894	n=3
ZOOB	upstream	4.76 d	0.3219	n=6
	downstream	6.93 d	0.2217	n=6
	le	17.27 d	0.184	n=3
<i>C. virginica</i>	lm	19.21 d	0.8119	n=5
	ue	16.72 d	0.75	n=5
	le	18.94 d	0.9673	n=4
<i>Uca</i>	o	12.33 d	0.9489	n=4
	um	26.67 d	0.4708	n=4
	lm	34.97 d	0.9024	n=4
	ue	32.26 d	0.8505	n=4
	le	28.82 d	0.6872	n=4
<i>Littoraria</i>	o	9.08 d	0.9991	n=3
	lm	27.17 d	0.8926	n=5
	ue	25.97 d	0.7498	n=3
<i>Palaemonetes</i>	um	9.03 d	0.9154	n=3
<i>Palaemonetes</i>	ue	9.90 d	0.9505	n=3
Bulk Seds	o	4.27 d	0.88	n=3
	um	4.22 d	0.86	n=3
	lm	12.79 d	0.8038	n=3
	ue	13.05 d	0.89	n=3
	le	21.51 d	0.7193	n=3
<i>E. argenteus</i> (mojarra)	um	90.1 d	0.9795	n=3
<i>E. argenteus</i> (mojarra)	ue	16.21 d	0.9996	n=3
<i>L. rhomboides</i> (pinfish)	um	44.05 d	0.814	n=4
<i>L. rhomboides</i> (pinfish)	ue	39.37 d	0.7866	n=3
<i>L. xanthurus</i> (spot)	um	117.65 d	0.9725	n=4

¹⁵N STORAGE

Table 5 gives ¹⁵N inventories (in g¹⁵N) on the final day of the addition period (d22) after 3 weeks of isotope addition. These numbers were calculated by converting the measured δ¹⁵N values (on this day) to mole fraction and multiplying by the total N (g) of each subreach pool. Background g¹⁵N (amount naturally occurring within the system) was subtracted to give g¹⁵N excess (ie. g¹⁵N from added tracer). Percent ¹⁵N from within each organism pool per subreach was calculated and denoted in the table beside the mass.

Sediments were the fate of much of the excess ¹⁵N (73 – 90 % in any given subreach, or 61 % of total ¹⁵N found in the creek on day 22), similar to previous in situ tracer studies (Tobias et al. 2003, Holmes et al. 2000), and second only to tidal export of ¹⁵N as DIN, PON, and zooplankton (~2.05 g ¹⁵N d⁻¹, or about 26 % per day). It is important to note that even after the tracer addition period ended, the sediment pool continued to accumulate ¹⁵N. Of the biota measured, the majority of excess ¹⁵N resided in the oyster beds downstream (13-18% per reach, 11% of total ¹⁵N recovered on this day), followed by marsh macrophytes (0.9-6.5%, 5% of total recovered).

Faster turnover time pools such as phytoplankton and BMA retained similar amounts of ¹⁵N, each between 1 - 5 % of the total in any given subreach (~3.7 % of creek total recovered on d22). Phytoplankton ¹⁵N retention decreased downstream related to their decrease in total biomass and coinciding with a longer turnover time. BMA however, retained similar amounts throughout the experimental reach, peaking upstream (O) and downstream (LE). The distribution of BMA populations during sampling was highly variable on the mud flats yet uniform across creek. Zooplankton retained very small amounts of ¹⁵N even though their biomass was greatest during the last week of the experiment.

The dominant epifaunal pool were the oysters downstream, which also comprised a major portion of total storage for the ecosystem, retaining 10.7 % of total ¹⁵N recovered on the final day of the addition period, collectively. The large amount of ¹⁵N found in this pool is indication of N redistribution from upstream throughout the creek. Similarly, other slow-turnover pools such as marsh macrophytes (~5 %) and sediments show ¹⁵N redistribution evenly over the subreaches by the end of the experiment independent of their location in relation to the addition site. This is potential evidence of recycling in other pools prior to incorporation into sediments or plants.

Table 5: ^{15}N inventories for all sampled pools on the final day of the experiment (d22). POM was not included in the calculation; it was represented by ^{15}N of chl-*a* PON. Percent ^{15}N was calculated for each organism pool relative to each subreach. Percentages for the lower euryhaline may be skewed due to the lack of data for the *Spartina* contribution on this day.

	O	UM	LM	UE	LE
POM	0.07	0.08	0.07	0.01	0.02
chl-<i>a</i> PON	0.06 (4.4%)	0.06 (2.3%)	0.06 (1.3%)	0.01 (0.3%)	0.02 (1.1%)
BMA	0.07 (4.9%)	0.01 (0.4%)	0.04 (1.0%)	0.08 (2.8%)	0.07 (4.8%)
<i>Spartina</i>	0.07 (4.9%)	0.12 (4.6%)	0.29 (6.5%)	0.07 (2.4%)	n/a
<i>Juncus</i>	0.02 (1.5%)	0.03 (1.1%)	0.04 (0.9%)	n/a	n/a
Zoop	0.0007 (0.05%)	0.0047 (0.2%)	0.0024 (0.05%)	0.0031 (0.1%)	0.0026 (0.2%)
Snails	n/a	n/a	0.00 (0.01%)	0.01 (0.2%)	0.00 (0.3%)
Mud Crabs	0.10 (7.5%)	0.03 (1.3%)	0.04 (0.9%)	0.04 (1.2%)	0.02 (1.0%)
Oysters	n/a	n/a	0.7315 (17%)	0.3749 (13%)	0.2608 (18%)
Bulk Seds	1.03 (77%)	2.44 (90%)	3.17 (73%)	2.30 (80%)	1.08 (74%)
Total (g)	1.35 (100%)	2.71 (100%)	4.37 (100%)	2.88 (100%)	1.45 (100%)

These slow-turnover pools also retained the tracer well beyond the duration of the experiment. On the final day of sampling (15 days after the end of the addition period), sediments in the lower mesohaline subreach still retained $\sim 3.87 \text{ g } ^{15}\text{N}$ (d38; $8.96 \text{ g } ^{15}\text{N}$ excess in the entire reach). On this same day, oysters contained up to $0.50 \text{ g } ^{15}\text{N}$ (LM; or $0.79 \text{ g } ^{15}\text{N}$ collectively) and still $\sim 0.40 \text{ g } ^{15}\text{N}$ (LM; $0.65 \text{ g } ^{15}\text{N}$ collectively) 4 weeks after the end of the experiment (d51). These longer term repositories and/or exports are potentially important for extensive recycling and eventual burial of N within this ecosystem.

^{15}N REDISTRIBUTION

The incorporation of the tracer was initially located upstream due to uptake by phytoplankton and BMA. By the end of the first week, 66% of the tracer (including storage in sediments) was located above the tracer addition site (O, UM) compared to 11% below (UE, LE). By the third week, however, ^{15}N was dispersed more uniformly, with 41% found upstream and 25% downstream.

The ^{15}N content of the primary producer pool (phytoplankton, BMA, *Spartina*, and *Juncus*), standardized to area (m^2) was predominantly found in phytoplankton and BMA directly surrounding the addition site. As the tracer was incorporated into higher trophic levels, ^{15}N was cycled from rapid-turnover pools to longer-term storage. By the third week of the experiment, 38 % of the added tracer had been incorporated into consumers (Figure 19). Similarly, the ^{15}N was more uniformly distributed between subreaches by the final week of the experiment compared to the initial incorporation in the upstream subreaches (Figure 19). Eventual redistribution of ^{15}N downstream through recycling and trophic transfer caused a more uniform

spatial distribution throughout the creek in these consumers by the end of the study.

¹⁵N RETENTION

While the phytoplankton pool was active in the initial uptake of ¹⁵N, other pools were more instrumental in ¹⁵N retention. Phytoplankton and zooplankton, with their relatively short turnover time, acted as transient processors of N, while pools with longer turnover times acted as N retainers. As observed in previous studies, bulk sediments were the fate of the majority of ¹⁵N and they retained it well beyond the duration of the experiment (Tobias et al. 2003). As the phytoplankton and zooplankton pools flushed ¹⁵N after the addition experiment was terminated, the sediment pool remained fairly steady and at some subreaches even increased in the ¹⁵N content.

Similarly, the BMA pool still retained 84% of its ¹⁵N inventory one week after the tracer was shut off (relative to what was recovered at the end of the addition period, d22) (Figure 20). The ability of this pool to retain ¹⁵N longer than the phytoplankton pool can be related to the deposition of phytodetritus and tight recycling of ¹⁵N between BMA uptake and mineralization back into the ecosystem, similar to previous studies (Tobias et al. 2003). Oysters and marsh plants, because of their long turnover time, were also retainers of N. Marsh macrofauna retained up to 81 % of their assimilated ¹⁵N on day 47 (relative to d22), while the oysters were the predominant epifaunal retainer, containing 48 % of their ¹⁵N inventory on day 51.

Figure 19: Proportional distribution of excess ^{15}N ($\mu\text{g } ^{15}\text{N m}^{-2}$) measured at d7, d14, and d22. Primary producers (microautotrophs and marsh macrophytes) and consumers are further divided by location within the creek.

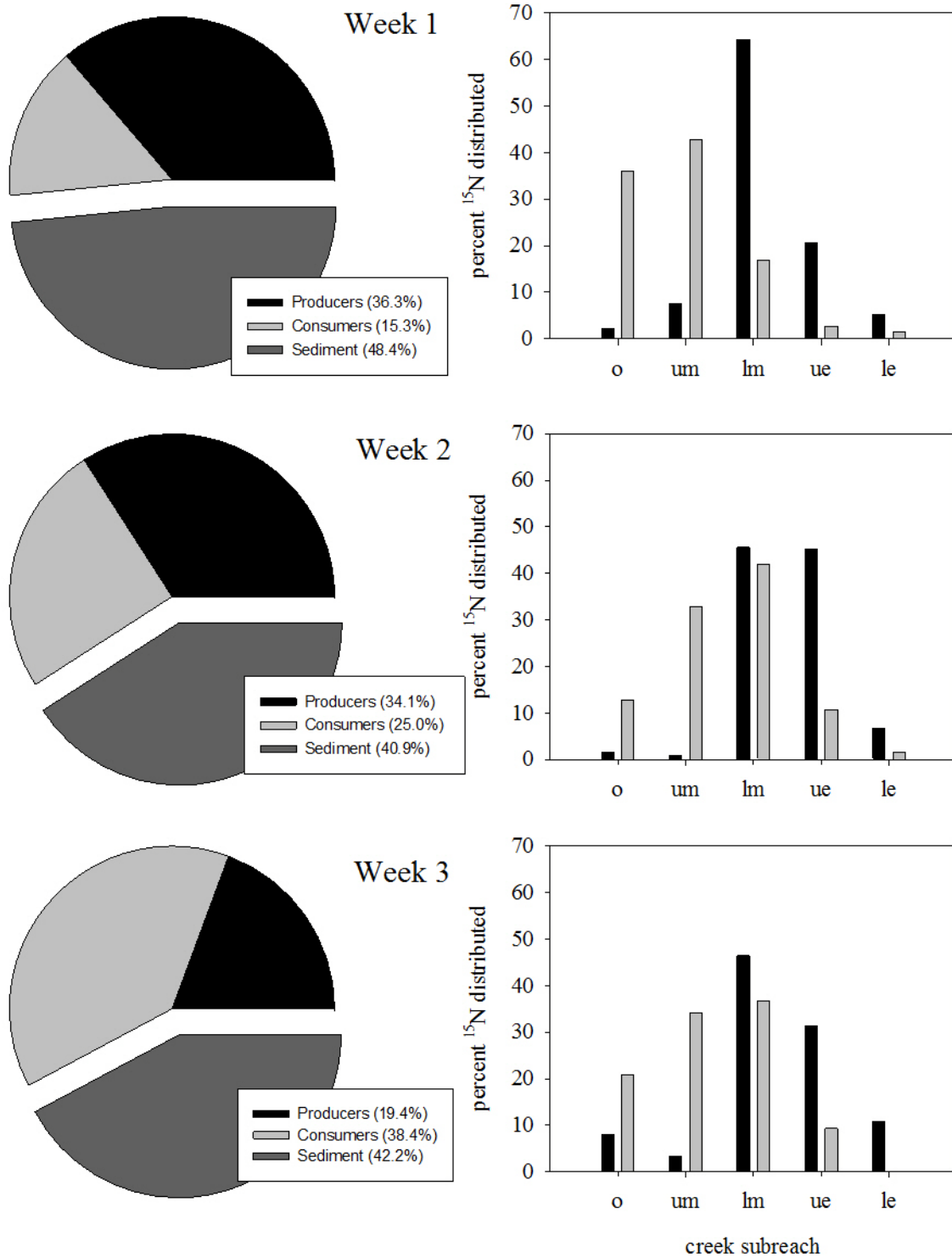
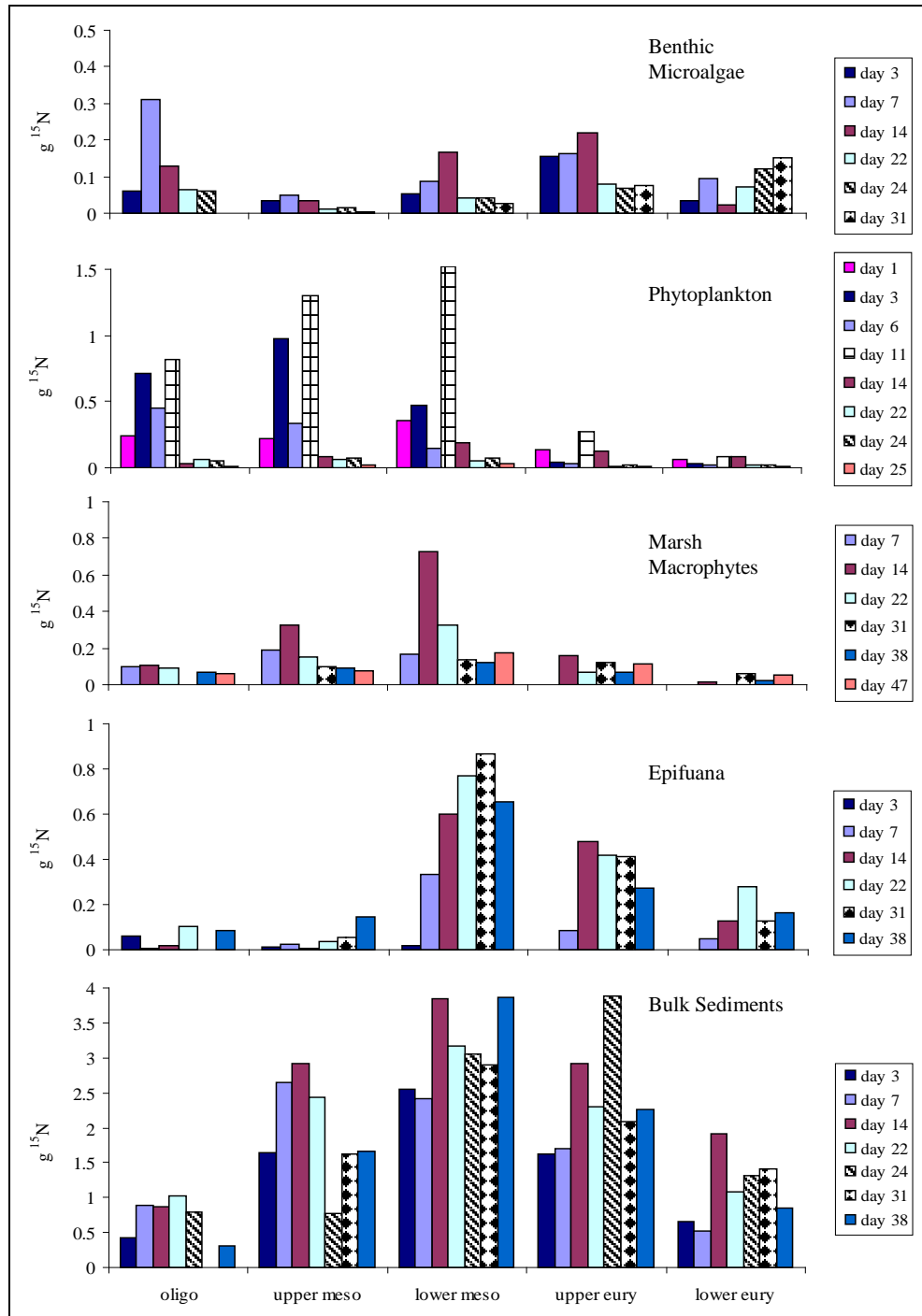


Figure 20: Distribution and collection of ^{15}N within the principle uptake pools (BMA and phytoplankton) and long term retention pools through the course of the isotope addition and post sampling events. Marsh macrophytes include *Spartina* and *Juncus* and epifauna includes *Uca*, *Littoraria*, and oysters. Note the different y axis scales between bar graphs.

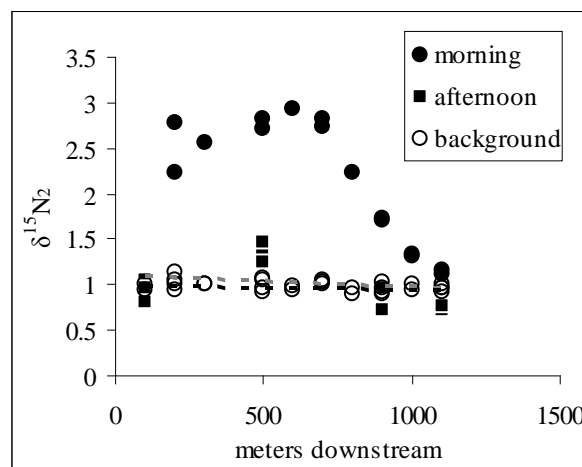


LOSS OF ^{15}N

^{15}N loss was also examined in order to provide a better estimate of ^{15}N cycling. Loss by export to the coastal ocean was calculated based on tidal exchange. The export flux per day is .85, and indicates that 85% of daily tidal volume is exchanged per day. With a tidal export (the sum of all non-motile water column compartments; DIN, PON, zooplankton) of $2.05 \text{ g } ^{15}\text{N day}^{-1}$, 47.12 g of added ^{15}N was lost during the experiment (28 % of total ^{15}N added during the addition experiment). Although dissolved organic nitrogen (DON) was not measured in this study, using a typical creek DON concentration of $20 \mu\text{M N}$ (Dafner et al. 2006) and applying the average enrichment value for the NH_4^+ pool (19 ‰ at high tide), $\sim 8.40 \text{ g } ^{15}\text{N}$ may have been exported tidally as enriched DON.

Another process of N removal is denitrification. ^{15}N denitrified into dissolved $^{15}\text{N}_2$ gas would also be exported on each tide. Isotopic measurements of N_2 gas were taken at low tide in the upstream reaches of the creek on d14 and d21 of the experiment. $\Delta\delta^{15}\text{N}$ values ranged from 0.7 – 1.9 ‰ and 0.3 – 1.5 ‰ for morning and evening low tides, respectively (Figure 21). The N_2 concentration was assumed to be at air saturated equilibrium, or $\sim 1\text{mM N}$ as N_2 . We can use these enrichment values, tidal volume, and tidal exchange to calculate a conservative estimate of ^{15}N lost by denitrification. Converting the $\Delta\delta^{15}\text{N}_2$ value to $\text{g } ^{15}\text{N}$ excess and multiplying it by the tidal volume and molar concentration of N_2 gives an estimate of excess $\text{g } ^{15}\text{N}$ as dissolved N_2 gas exported with the tides. With an export flux of 0.85 per day and an addition period of 23 days, denitrification could account for a loss of $\sim 26.55 \text{ g } ^{15}\text{N}$.

Figure 21: Enrichment of N_2 gas measured from the surface waters of Hewlett's Creek during the final week of the addition experiment. The dotted lines are linear regression lines for the background enrichment values.



DISCUSSION

The addition of isotopically labeled nitrate allowed us to quantify pathways and trophic interactions important in the processing of watershed nutrient inputs. The tracer experiment provided a ~ 1 month analysis during the *high production period* (summer). Lower temperatures, lower primary production, and different species composition (of the ecosystem) observed in the winter may result in a shift in N transformation pathways and/or rates from what are reported here (Mallin et al. 2004, Johnson 2005). Nevertheless, this experiment allowed us to examine some fundamental aspects of tidal creek N dynamics not readily attainable without the in situ use of ^{15}N , including: 1) temporal and spatial patterns of watershed N processing and contributions of watershed NO_3^- to biota; 2) the importance of detrital N and N recycling in fueling creek biota; and 3) the overall ecosystem processing (g N d^{-1}) of watershed-derived N, as well as the mechanisms of overall export.

TEMPORAL AND SPATIAL MOVEMENT OF N AND CONTRIBUTIONS OF WATERSHED N TO BIOTA

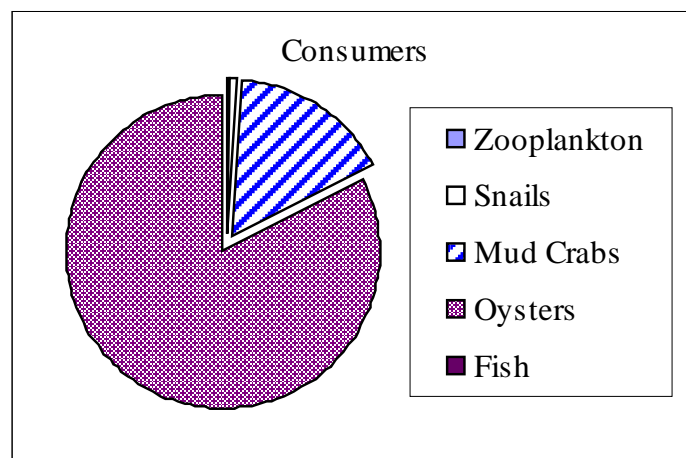
The isotopic enrichment of the phytoplankton within the first day of the experiment (Figure 9B) indicated that this pool quickly incorporated the nitrate tracer. This immediate uptake by phytoplankton is consistent with past estuarine ^{15}N studies with high productivity (Holmes et al. 2000) where pelagic diatoms assimilated > 75% of an ^{15}N - NO_3^- tracer. In Hewlett's Creek, the majority of this rapidly-assimilated N (94 %) was initially retained (d7) locally at or above the addition site (O, UM, LM) where water column productivity was generally highest. The high productivity and chlorophyll *a* biomass in the creek has been noted previously to occur in this region (Mallin et al. 2004; Johnson 2005). The rapid uptake of a pulse of nitrogen and conversion into a phytoplankton bloom was demonstrated in this creek in July 2005 following a large sewer line spill (Mallin et al. 2007).

Enrichment in BMA was on the same scale as phytoplankton at all locations within 7 days of the isotope addition (Figure 9C). Because of their location at the sediment surface it has been accepted that BMA is fueled by porewater DIN (Rizzo and Christian 1990). The magnitude of enrichment in BMA in Hewlett's Creek (200 – 950 ‰ upstream) suggested a highly enriched source of N, possibly NO_3^- from the water column (500 – 2000 ‰). However, the lag time in the initial uptake of tracer (and consequently extended enrichment of the BMA post-addition) suggested that BMA is utilizing recycled N (typically NH_4^+). To satisfy this high *and* prolonged enrichment pattern required both a high enriched source as well as ^{15}N recycling. These criteria are satisfied by BMA preferentially recycling N from freshly deposited highly-labeled phytodetritus. With respect to N uptake by primary producers, Hewlett's Creek behaves like a hybrid of past studies in that both water column and benthic microautotrophs are a sink for water column NO_3^- .

Overall, the $\Delta\delta^{15}\text{N}$ of zooplankton decreased downstream, with a maximum value of 140 ‰ (O, d4; maximum value LE - 21 ‰). The simultaneous decrease in zooplankton $\delta^{15}\text{N}$ with an increase in zooplankton numbers could be attributed to 1) a mixing of creek zooplankton with zooplankton from the ICW (unlabeled), 2) feeding on a less enriched phytoplankton pool downstream, or 3) a shift away from highly-labeled phytoplankton to a more detrital (unlabeled) food source as zooplankton production increases.

Due to tidal flux, the ^{15}N -DIN and ^{15}N assimilated into the phytoplankton and zooplankton pools moved back and forth through the tidal prism. Therefore, the ^{15}N that did not get exported to the coastal ocean was utilized in all subreaches. This redistribution of N fueled secondary and tertiary production in all subreaches of the creek regardless of the point of initial assimilation. The tracer (DIN) was taken up by local water column organisms and subsequently transferred to organisms through the creek indicating a spatial redistribution (net downstream transport) of labeled nitrate by cascading through biota (Figure 19). By the final week of the addition experiment, the ^{15}N shifted from autotrophs to consumers and held a relatively uniform distribution (Figure 19). Of these consumers, mud crabs and oysters made up the majority of N biomass (Figure 22) and retained the most ^{15}N . While the majority of labeled DIN was concentrated upstream, on the final day of the addition 82 % of the total ^{15}N in the consumer pool was incorporated downstream into oyster biomass, (~17 % in mud crabs).

Figure 22: Distribution of ^{15}N ($\mu\text{g m}^{-2}$) within the consumer pool at the end of the addition period (d22). The total ^{15}N inventory is a function of both the enrichment and N biomass.

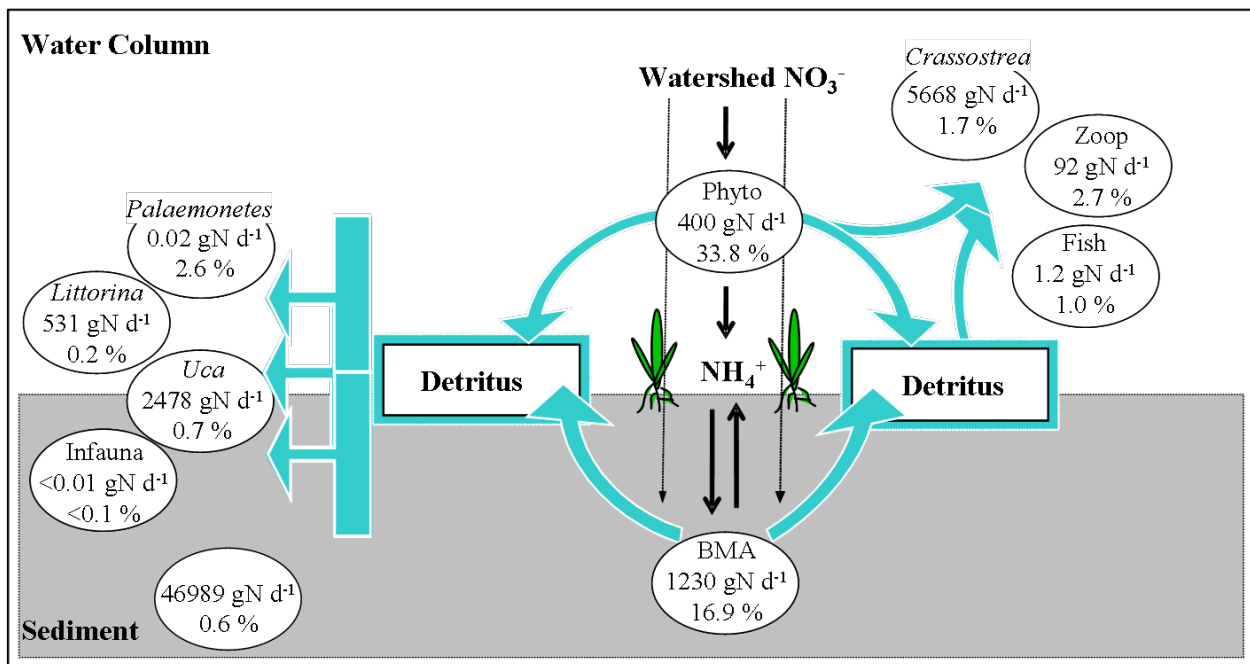


Similar evidence of spatial redistribution was demonstrated by the enrichment trajectories of fish. Tracer incorporation within both upstream and downstream fish is consistent with known species foraging behavior (Levin et al. 1997, Luczkovich 1988, Kerschner et al. 1985). All species studied have a varied diet of detritus, phytoplankton, zooplankton, and benthic infauna. The enrichment trajectories of all fish except mosquitofish show that these fish are not spatially constrained to one subreach (Figure 12), and were using the entire creek to forage.

ECOSYSTEM DEPENDENCE ON N LOADING

Does watershed N contribute to fish and shellfish? Isotope studies have been able to identify proportional contributions of isotopically labeled source material to higher organisms. Cole et al. (2002) for example, was able to identify the contributions of allochthonous carbon to food webs in lakes. For Hewlett's Creek, the ratio of each pool's enrichment relative to the mean source NO_3^- enrichment (2182 ‰) provides an estimate of the fraction of N processed by each pool that was watershed derived (Figure 23). $\Delta\delta^{15}\text{N}$ of shellfish reached a maximum of roughly 50 ‰. This enrichment is about one fortieth of the mean NO_3^- enrichment (2182 ‰), indicating that < 2 % of the fishes and shellfish N requirement can be traced back to the tracer, and thereof watershed-derived NO_3^- (assuming the watershed is the sole source of NO_3^-).

Figure 23: Food web diagram of N cycling within this tidal creek ecosystem. Numbers in the ellipses beside each compartment are the calculated throughput (g N d^{-1}) of the organism pool and the percent of N from the watershed (assuming that labeled NO_3^- is watershed) based on the ratio of pool maximum enrichment to 2182 ‰. An estimate of N throughput for the marsh macrophyte pool is 105 g d^{-1} with a watershed contribution of 3% based on published values of turnover times (Tobias et al. 2003).



At the ecosystem scale, based on the ^{15}N tracer, only about 1.3 % ($\sim 0.7 \text{ kg d}^{-1}$) of the N being turned over in the system was directly derived from watershed nitrate. This value is consistent with the headwater delivery rate of NO_3^- (3.8 kg N d^{-1}) and small tidal exports ($\sim 85 \text{ d}^{-1}$) of creek NO_3^- (0.3 kg N d^{-1}). While the watershed contribution to some pools was high (phytoplankton, BMA, zooplankton, and grass shrimp), as a whole, the ecosystem seems to be fueled predominantly by recycled N within the ecosystem. Specifically, the ecosystem assimilates and reuses nitrogen (as it enters the system from the headwaters) before it eventually

gets exported downstream or buried within sediments. Nitrogen recycling is further supported by a strong correlation between the enrichment trajectories of NH_4^+ (a product of bacterial mineralization) and those of phytoplankton and BMA (p value = 0.001). Presumably, the marsh was the primary source of *this* N however the tracer only indicates that it is not NO_3^- derived.

The biological residence time of ^{15}N was calculated to be ~12 days by multiplying the turnover times of each compartment by the its contribution of ^{15}N to the ecosystem total on the final day of the experiment. This residence time is short compared to other estuarine studies however the length of storage in bulk sediments and marsh macrophytes is also small in comparison (Tobias et al. 2003).

HEWLETT'S CREEK

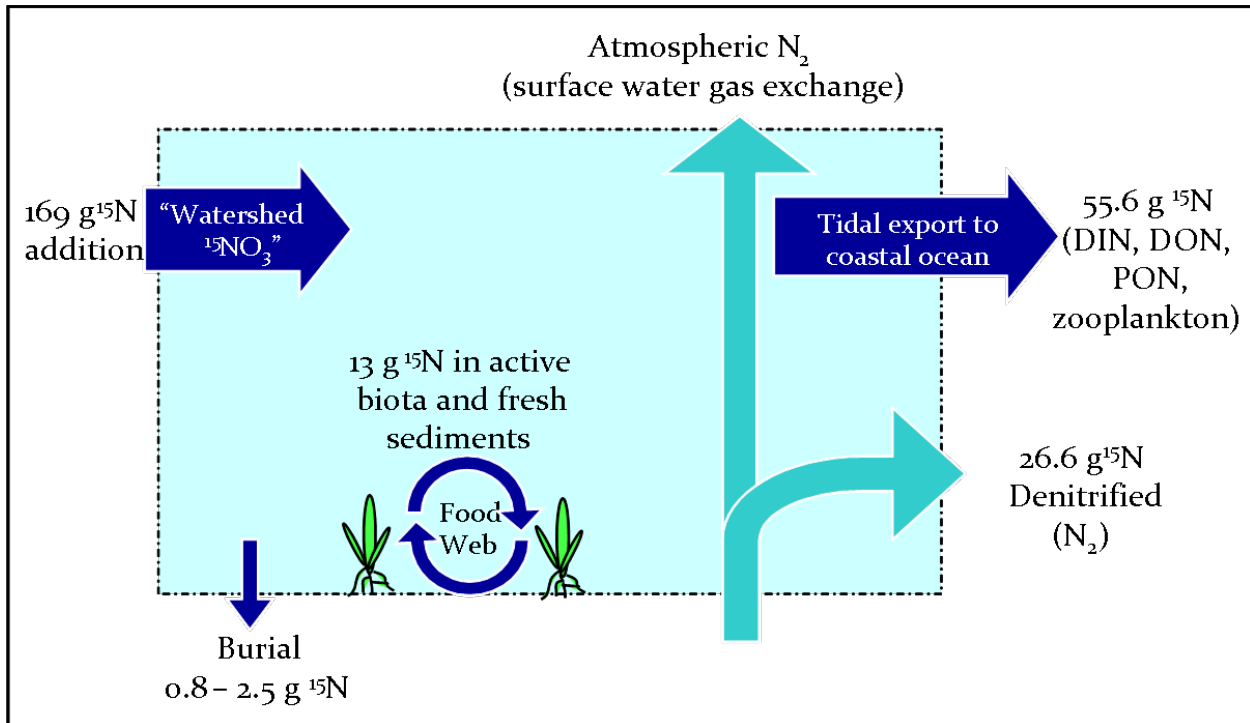
Transformer or Attenuator of watershed N? A total of 169 g of ^{15}N as NO_3^- was added to Hewlett's Creek. On the final day of the addition period 12.76 g (8 % of the total) ^{15}N was measured in the estuary (Table 5). 61 % (7.72 g ^{15}N) of the recovered ^{15}N was found stored in the sediments which acted as both a recycler and as a repository. 9 % (1.11 g ^{15}N) was located in primary producers, and 11 % (1.37 g ^{15}N) in oyster biomass

About ½ of the difference between the ^{15}N released and that recovered on day 22 can be accounted for by a combination of tidal export and denitrification (Figure 24). Tidal export of DIN, PON, DON, phytoplankton, and zooplankton is a pathway by which the creek transforms watershed NO_3^- and exports useable forms of N to the coastal ocean (i.e. little attenuation). ^{15}N loss via denitrification is a mechanism by which tidal creeks attenuate watershed N loads.

Tidal export was measured as ~ 2.42 g ^{15}N lost d^{-1} , or roughly 55.6 g over the experiment (excluding N_2 estimates). If NO_3^- acted conservatively (i.e. not utilized biologically), a tidal exchange of 85 % d^{-1} would cause 85 % of the added tracer to be lost immediately (~144 g total). The measured exchange was far less, indicating that not only was NO_3^- transformed, recycled, etc. as discussed previously, the ^{15}N export was in the form of labeled phytoplankton POM, zooplankton, NH_4^+ , but not NO_3^- . Most of it (the difference between the conservative estimate and measured tidal export) was assimilated by the ecosystem (89 g ^{15}N).

Based on an observed $^{15}\text{N}_2$ enrichment (~ 2 ‰), $[\text{N}_2]$ of ~ 1 μM , a tidal exchange of 85 % d^{-1} and its tidal export, ~ 26.6 g ^{15}N was denitrified and removed. This estimate is likely conservative in that it does not account for gas exchange of $^{15}\text{N}_2$ and is equivalent to a per area denitrification rate of ~ ⅓ of rates typical for these types of systems (Tobias et al. 2003).

Figure 24: Mass balance of ^{15}N in Hewlett's Creek. Amount buried was derived from typical burial rates in these types of systems and an enrichment value of the sediments on 38 days after the experiment (8‰; Tobias et al. 2003, Thomas & Christian 2001).



While $56 \text{ g } ^{15}\text{N}$ gets exported to the coastal ocean in a usable form (tidal export), $27 \text{ g } ^{15}\text{N}$ is attenuated by denitrification (Figure 24), and 13 g remains in the estuary, still not all of the added ^{15}N is accounted for. N burial (considered another route of attenuation) in southeast tidal marsh ecosystems ranges from 9 to $21 \text{ g N m}^{-2} \text{ y}^{-1}$ (Craft et al. 1993, Anderson et al. 1997, Merrill & Cornwell 2000). Using the $\delta^{15}\text{N}$ value of the sediments after 40 days and this yearly estimate, we can calculate a range of roughly $1 - 2.5 \text{ g } ^{15}\text{N}$ buried during the addition experiment. While most addition experiments indicate burial as a large sink for N (Tobias et al. 2003, Thomas & Christian 2001), the highly active ecosystem of this tidal creek and the uncharacteristically rapid turnover time for the sediments could be a reason that this pool may not be a dominant sink for ^{15}N .

CONCLUSIONS

Based on the existing mass balance that accounts for ~ ½ of the added ^{15}N , the creek is equal parts transformer of watershed N via tidal export of useable N, and attenuator of watershed N through denitrification. Because the mass balance is not complete, it is important to identify which N pools may have been underestimated and/or which areas of N assimilation have been missed. Loss of ^{15}N may have occurred through $^{15}\text{N}_2$ gas exchange with the atmosphere, applying typical gas transfer rates could raise the denitrification estimate by a factor of 4. Tracer incorporation during marsh flooding (during spring tides and rain events) was not well characterized. This flooding could have distributed the tracer well beyond the 1 meter marsh fringe sampled in this study and a large area of minimally enriched marsh would account for a large amount of ^{15}N mass. It is an unknown and should be quantified in future ^{15}N studies. Similarly, the major rainfall events during this study and the potential sampling of the unmixed labeled nitrate pool may have over/under estimated the enrichment value of this pool. Better measurement of the labeled nitrate pool would have allowed for a better quantification of its contributions to the ecosystem pools.

For tidal export, uncertainty in DON enrichment might alter the export estimate, but not substantially. Similarly, fish migration (Deegan et al. 2000) from the tidal creek to the coastal ocean could not account for the missing ^{15}N since the fish populations do not retain enough ^{15}N mass within the ecosystem to account for the missing nitrogen. However, oyster ^{15}N mass was significant. These organisms acted as a repository for N that can then be repackaged as feces and pseudofeces that represents a potentially long-term retention pool that was not accounted for in this study (Newell et al. 2002). Future isotope studies should include extensive sampling in the marsh as well as longer addition duration in an attempt to quantify retention in longer storage pools as well as lend the ability to rebound from ecosystem disturbances. We note that oysters also recycle N back into the system as ammonium that is readily available for uptake by phytoplankton and benthic microalgae. We suspect that winter tracer studies would likely further exemplify the importance of oysters and the benthic algal and consumer communities due to lack of significant phytoplankton production in winter in these systems (Johnson 2005).

The role of the oyster as a significant (temporary) N sink in the estuary has water quality implications. In a non-polluted tidal creek in which oysters are harvested by humans, much of this oyster-bound N is transferred to terminal consumer (humans), leaving the estuary entirely. Many of the local tidal creeks (as well as similar urbanizing creeks elsewhere) are closed to shellfishing due to fecal microbial pollution. Thus, pollution of these estuaries will lead to greater N retention and recycling within the system, adding to eutrophication problems.

Despite an open ^{15}N mass balance in this study, Hewlett's Creek appears to attenuate roughly 20 – 40 % of the watershed N it processes. It retains ~ 5 – 10 % on monthly (+) timescales, and exports 20 – 40 % to the coastal ocean as recycled DIN/DON and biological particulates (POM,

phytoplankton, zooplankton, etc.). From an ecosystem perspective, during baseflow events, the creek ecosystem is supported primarily by internally recycled N (about 90%) and relies little on watershed N inputs to support productivity.

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