

The Effect of Nitrogen Loading on a Brackish Estuarine Faunal Community: A Stable Isotope Approach

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ABSTRACT: Coastal ecosystems worldwide face increased nutrient enrichment from shoreline and watershed development and atmospheric pollution. We investigated the response of the faunal community of a small microtidal estuary dominated by *Ruppia maritima* (widgeon grass) in Maine, United States, to increased nitrogen loading using an in situ mesocosm enrichment experiment. Community response was characterized by assessing quantitative shifts in macroinvertebrate community composition and identifying changes in food web structure using stable carbon and nitrogen isotope ratios of producers and consumers. The community was dominated by brackish water invertebrates including midge larvae, oligochaetes, damselfly larvae, amphipods, and ostracods. Experimental nutrient additions resulted in significantly lower densities of herbivorous chironomids and predatory damselflies and greater densities of deposit feeding oligochaetes. Grazing midge larvae (Chironomidae: *Dicrotendipes*, *Cricotopus*) consumed epiphytic algae under both natural and enriched conditions. Deposit feeding *Chironomus* was dependent on allochthonous sources of detritus under natural conditions and exhibited a shift to autochthonous sources of detritus under enriched conditions. Predatory *Enallagma* primarily consumed grazing chironomids under all but the highest loading conditions. Experimental nutrient loading resulted in an increase in generalist deposit feeders dependent on autochthonous sources of detritus.

Introduction

Nutrient loading and subsequent eutrophication have been documented in estuaries and coastal waters worldwide (Group of Experts on the Scientific Aspects of Marine Pollution 1990; Nixon 1995). Anthropogenic sources of nutrient loading include wastewater, industrial processes, fertilizers, and atmospheric deposition (Valiela et al. 1992; Paerl and Fogel 1994; Arhonditsis et al. 2000). Excessive nutrient loading to estuaries frequently causes a shift in primary producers from submerged vascular plants to algal-dominated communities (Sand-Jensen and Borum 1991; Duarte 1995; Harlin 1995). Fast-growing macroalgae, epiphytes, and phytoplankton will outcompete macrophytes in nutrient enriched estuaries by exploiting the nutrients, proliferating, and reducing light penetration for submerged vegetation (Sand-Jensen and Borum 1991; Short et al. 1995; Taylor et al. 1995; Wear et al. 1999).

Secondary effects of nutrient loading include higher quality organic inputs to benthic detritus, shifts from aerobic to anaerobic conditions in the

sediments, reductions in habitat heterogeneity, decreases in ecosystem stability, and shifts in the faunal community (Heip 1995; Borum 1996; Valiela et al. 1997). The bottom-up effects of nutrients in the system may be complicated by top-down controls. Grazers may help to reduce the effects of nutrients by consuming fast growing algae (Neckles et al. 1993; Williams and Ruckelshaus 1993; Hauxwell et al. 1998), while small predators may amplify nutrient effects by consuming grazers (Gacia et al. 1999; Heck et al. 2000). The response of primary producers to nutrient loading and eutrophication has been well documented, but the influence of this response on food web structure and function is much less well understood. An understanding of the entire ecosystem is necessary to predict the effects of eutrophication (Heck et al. 2000).

Stable isotope analysis is useful for assessing ecological trophic relationships (Fry and Sherr 1984; Peterson and Fry 1987; Lajtha and Michener 1994). In general, carbon (C) isotopes are used to identify the most likely food sources of individual organisms (Fogel and Cifuentes 1993) and nitrogen (N) isotopes are used to determine the trophic level of these organisms (DeNiro and Epstein 1981; Minagawa and Wada 1984; VanderZanden and Rasmussen 2001). Mathematical mixing mod-

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els using the C and N stable isotopes of a consumer and its food sources enable assessment of the relative elemental contribution of these sources to the diet of the consumer (Ben-David et al. 1997; Phillips 2001; Phillips and Koch 2002).

Over the past two decades, stable isotope techniques have been used frequently to assess the food webs of coastal and estuarine systems. Many studies have investigated the relative importance of algae and macrophytes to estuarine consumers (Kitting et al. 1984; Deegan and Garritt 1997; Loneragan et al. 1997). Isotopes have also been used to determine the significance of terrestrially-derived nutrients and organic matter in coastal waters (Simenstad and Wissmar 1985; Cifuentes 1991; Day et al. 1994) and to trace wastewater inputs of N through coastal and estuarine food webs (Hansson et al. 1997; McClelland and Valiela 1998b).

McClelland and Valiela (1998a) used stable isotopes to examine food web changes resulting from increased N loading in three estuaries of Waquoit Bay, Massachusetts. Macroalgae and phytoplankton were major food sources to consumers in all estuaries, and eelgrass (*Zostera marina*) was also an important component of the ecosystem in which N loading was lowest. Their study suggests that losses of eelgrass with increased N loading may eliminate an important pathway through which land-derived N enters the food web. When eelgrass is replaced by algae, the rate at which this N is cycled within the estuarine ecosystem may potentially increase.

Estuarine resources in coastal Maine are threatened by nutrient enrichment associated with atmospheric deposition (Miller 1999) and wastewater from increased residential development in contributing watersheds. Increases in nutrient loading to Maine's estuaries may impact the structure and function of marine ecosystems in the Gulf of Maine and subsequently threaten the resource-based coastal economy (Maine Environmental Priorities Project 1995). Though much work has been done on the impacts of nutrients on estuarine systems, the majority of these studies have focused on large estuaries and coastal embayments. Little baseline research has been done in smaller, microtidal estuaries with euryhaline faunal communities. The Northeast Creek (NEC) estuary of Acadia National Park is a small, microtidal Maine estuary that is threatened by atmospheric pollution and increased residential development. Although NEC estuary is currently a pristine system, lands in the surrounding watershed contain some of the fastest growing areas of single-family homes in the region, and nearby estuarine systems are already moving towards eutrophic conditions (Doering and Roman 1994; Doering et al. 1995; Kinney and Roman 1998). Scant information exists for determining

nutrient loading thresholds or best management practices for these systems. NEC estuary is a site for integrated research by the U.S. Geological Survey (USGS) in collaboration with others. The overall goal of this research is to assess the effects of nutrient loading in order to develop predictive tools for watershed-based planning and monitoring (Nielsen 2002; Neckles et al. 2003). Our specific project characterized the response of the faunal community of NEC estuary to increased N loading by assessing shifts in macroinvertebrate community composition and identifying changes in food web structure using stable isotope techniques.

Materials and Methods

STUDY LOCATION

Northeast Creek (NEC) estuary is located in Acadia National Park, Mount Desert Island, Maine (44°25'N, 68°19'W). This small estuary occupies a drowned river valley fed by a number of freshwater streams. It is approximately 4 km long with a 2,400 ha watershed (Nielsen 2002). The estuarine system includes approximately 286 ha of emergent estuarine wetlands, emergent riverine tidal fresh wetlands, freshwater shrub-scrub wetlands, and subtidal vegetated habitat. NEC estuary averages about 1 m in depth with a narrow tidal range (< 0.5 m). An old rock dam near the mouth of the estuary impedes tidal exchange so that the estuary is generally poorly flushed. In 2001, top and bottom salinities in NEC estuary increased from 0‰ in May and June to around 30‰ in October, indicating that the system was dominated by freshwater inputs in the spring and became increasingly more marine throughout the summer (Culbertson personal communication). Although 2001 was a year of lower than average precipitation, this salinity pattern is typical for the estuary (Culbertson personal communication). The estuarine system is densely vegetated with *Ruppia maritima* along half of its length. In this section, *R. maritima* occurs in dense stands on each side of the creek bordering a narrow channel. The experiments in this study were performed in a particularly dense bed of *R. maritima* approximately halfway up NEC estuary, just downstream of the mouth of Aunt Betsy's Creek. The substrate of the estuary in this location is a silt loam soil containing organic matter of detrital origin.

EXPERIMENTAL DESIGN

In situ N enrichment experiments in field mesocosms were used to assess ecosystem responses to a range of nutrient inputs during the summer growing season of 2001. The mesocosm experiment was part of an ongoing USGS project (Neckles et al. 2003). Experimental units consisted of

1.2 × 1.2 m enclosures with wooden frames and flexible clear vinyl walls to enhance the transfer of physical energy from outside to inside the chamber (Sanford 1997). The mesocosms were designed to mimic natural estuarine conditions as much as possible. They were open to the substrate on the base and to the air on top. Two 1-cm slits were placed on each wall near the base to allow for tidal fluctuation and controlled rates of water exchange. Average residence time of water in the mesocosms was 8.9 d and average water depth was 26 cm (± 4 SD; Kopp personal communication).

Five N treatments were used in a completely random design with four replicates per treatment. The nutrient treatments consisted of unenclosed controls and enclosed ambient, low (8.4 mmol dissolved inorganic nitrogen [DIN] $\text{m}^{-2} \text{d}^{-1}$), moderate (16.8 mmol DIN $\text{m}^{-2} \text{d}^{-1}$), and high (33.8 mmol DIN $\text{m}^{-2} \text{d}^{-1}$) N loading conditions (Neckles et al. 2003). These levels were selected to encompass the existing range of N loading observed in shallow northeastern estuaries (Nixon et al. 2001). N was applied as coated, slow-release fertilizer (Osmocote) contained in diffuser bags made from polyethylene mesh with a 1 mm standard mesh size (Worm et al. 2000). Actual loading rates were determined by measuring weight loss from fertilizer additions. Enrichment commenced on July 3 and continued to August 28, 2001. Mesocosm walls were cleaned of periphyton weekly during this period.

SAMPLE COLLECTION

Macroinvertebrate Community

At the end of the enrichment experiment, we collected one quantitative macroinvertebrate sample down to 10 cm depth in the sediments from each experimental unit using a 10 cm diameter core sampler. Only one sample was taken per unit because units were homogeneous in terms of vegetation and substrate. Sampling locations were chosen randomly from those locations in the units that had not been sampled previously by USGS. Garden shears were used to separate macrophytes associated with the sample from those outside the sampler. The sampling technique was designed to sample the water column fauna, the fauna associated with *R. maritima*, and the infauna. Samples were rinsed through a 500 μm sieve and preserved in 70% ethanol with Rose Bengal dye. All invertebrates were removed and identified to genus when possible using keys by Wiederholm (1983), Peckarsky et al. (1990), Merritt and Cummins (1996), and Epler (2001). Mean densities of the invertebrates were calculated based on the surface area of the core sampler (78.5 cm^2).

Food Web Structure Assessment

Primary producers, terrestrial detritus, herbivores, detritivores, filterers, and predators were also collected from each experimental unit 1 wk before and at the end of the enrichment experiment. *R. maritima* and associated epiphytic material were gently collected using resealable plastic bags and shears. The epiphytic material was separated from *R. maritima* by gently scraping the macrophyte blades with glass slides. This material was then removed to porcelain dishes and dried at 60°C. Suspended particulate organic matter (POM) was filtered from 1 L water samples using 0.7 μm glass fiber filters. Invertebrates were collected using dip nets and benthic grabs and sorted live in the laboratory. Leaves from terrestrial plants were removed from the detritus in the invertebrate grabs. Fish were collected using minnow traps. All items were rinsed in deionized water and frozen. Samples were then dried at 60°C and ground into a homogeneous powder. Osmocote slow-release fertilizer from the same batch used in the mesocosm experiment was also ground into a homogeneous powder for isotopic analysis. Samples were weighed into silver cups and cleaned using vapor phase acidification before stable isotope analysis.

MASS SPECTROMETRY

Stable C and N isotopic composition of animals and primary producers were determined using a Europa Hydra 20/20 continuous flow isotope ratio mass spectrometer system at the University of California Davis Stable Isotope Facility. Isotopic compositions are reported as ppm (‰) deviation from air (N) and Pee Dee Belemnite (PDB) (C). The precision (\pm SD) of replicate analyses of a standard known material was ± 0.05 ‰ for C and ± 0.15 ‰ for N.

DATA ANALYSIS AND INTERPRETATION

To assess nutrient treatment effects on the faunal community and isotopic compositions, analysis of variance was used. An α of 0.05 corrected using the Bonferroni correction factor (final $\alpha = 0.011$) was used to determine significance. All variables were tested for normality and residuals were tested for equality of variance and normality. Invertebrate abundances were $\log_{10}(x+1)$ transformed to satisfy normality assumptions when necessary. Fisher's least significant difference (LSD) multiple comparisons were used to detect significant enclosure effects (control versus ambient) and thresholds for nutrient effects (ambient versus low, moderate, and high loadings). For tests of isotopic compositions, samples from both sampling dates were included.

To determine the relative importance of food

sources to consumers in each nutrient treatment we used visual graphical assessments and model techniques based on C and N stable isotope ratios. Two types of three source mixing models were used: an Euclidean distance index and a linear mass balance mixing model. The Euclidean distance model determines the proportions of three or more food sources in the diet of a consumer based on ratios of the inverse of the Euclidean distance between each food source and the consumer; food sources closer to the consumer are estimated to contribute larger proportions to the diet (Ben-David et al. 1997; Ben-David and Schell 2001). The linear mass balance mixing model quantifies the fractional contribution of three food sources to a consumer's diet based on mass balance (Phillips 2001; Phillips and Koch 2002). The linear mixing model is mathematically unbiased and more accurate than the Euclidean model, but requires organisms to be within the mixing triangle formed by the three food sources. The Euclidean distance model can also be used as an index for ranking the importance of food sources when consumers fall outside this mixing triangle.

Linear mass balance mixing models were used to quantitatively estimate the contributions of each food source to consumers. To correct for trophic fractionation in primary consumers, fractionation values of $-0.41 \pm 1.14\text{‰}$ for $\Delta\delta^{13}\text{C}$ and $2.5 \pm 2.5\text{‰}$ for $\Delta\delta^{15}\text{N}$ were used (VanderZanden and Rasmussen 2001). For secondary consumers, we used a $\Delta\delta^{13}\text{C}$ of $0.47 \pm 1.23\text{‰}$ and a $\Delta\delta^{15}\text{N}$ of $2.92 \pm 1.78\text{‰}$ (VanderZanden and Rasmussen 2001). If the average fractionation correction did not allow for model fit (which we defined as a model prediction of less than -10% for one or more food source), we made slight adjustments of this correction factor within one standard deviation of the average as reported by VanderZanden and Rasmussen (2001). Uncertainty in the linear mixing model was calculated according to the method described by Phillips and Gregg (2001).

The linear mixing model assumes that the proportional contribution of a food source to the consumer is the same for both C and N, that correct food sources have been identified, and that fractionation resulting from metabolic processes has been corrected for accuracy (Schoeller 1999; Phillips 2001; Phillips and Koch 2002). Primary and secondary consumers in NEC estuary satisfied the first model assumption because they rely on food sources that do not vary greatly in C and N concentrations. With the exception of oligochaetes, one potential food source for secondary consumers, we satisfied the second assumption by obtaining isotopic ratios for the most likely food sources in all treatments. To satisfy the third assumption,

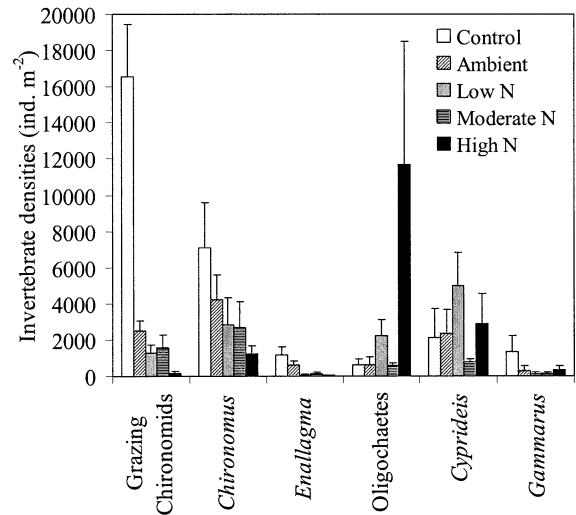


Fig. 1. Densities of the most common invertebrate taxa in the N enrichment experiment. Mean densities (m^{-2}) with standard error bars are shown for all N loading treatments.

we used estimates of trophic fractionation for the consumers from the literature. These literature values are variable (VanderZanden and Rasmussen 2001; Hart and Lovvorn 2002; Post 2002) and may not accurately characterize fractionation in NEC. Use of incorrect fractionation values could result in slightly different model predictions. To account for any errors resulting from incorrect fractionation assumptions in the linear mixing model, we also used visual comparisons of consumers to food sources and the Euclidean distance index. We ran the Euclidean distance model assuming zero fractionation by consumers and used this model to rank the importance of food sources based on the proximity of their isotope values to the values of consumers in each experimental treatment. The use of all three techniques (visual assessment, linear mixing models, and Euclidean distance models) allowed us to accurately assess the relative importance of food sources to consumers.

Results

FAUNAL COMMUNITY CHANGES

Invertebrates found under all N loading treatments include nonbiting midge larvae (Chironomidae: *Dicrotendipes* sp., *Cricotopus* sp., and *Chironomus* sp.), damselflies (Coenagrionidae: *Enallagma* sp.), oligochaetes, ostracods (Cytheridae: *Cyprideis* sp.), and scuds (Gammaridae: *Gammarus* sp.; Fig. 1). Water boatmen (Corixidae: *Trichocorixa* sp.) were found at highest densities in the control mesocosms ($1,400 \text{ m}^{-2}$) and at lower densities ($\sim 200 \text{ m}^{-2}$) in the ambient, moderate, and high N loading mesocosms. Mites (Acari) were found at a very low density (30 m^{-2}) in the moderate loading

TABLE 1. Treatment effects on the most common invertebrate taxa in the N enrichment experiment. Test statistics and probabilities from analysis of variance tests of the effects of treatment on invertebrate abundances (* indicates abundances were $\log_{10}(x+1)$ transformed to satisfy the test assumptions). Mean differences (MD) between ambient (A) and control (C), low (L), moderate (M), and high (H) loading treatments are listed with p values from Fisher's LSD multiple comparisons tests.

Taxa	Treatment Effects		Mesocosm Effects				Threshold of Response to N			
			C-A		L-A		M-A		H-A	
	F	p > F	MD	p	MD	p	MD	p	MD	p
Grazing Chironomidae*	17.03	0.000	0.81	0.002	-0.32	0.162	-0.34	0.143	-0.94	0.001
<i>Chironomus</i> sp.	1.981	0.149	22.50	0.218	-11.00	0.539	-12.25	0.494	-23.50	0.199
<i>Enallagma</i> sp.*	7.51	0.002	0.20	0.302	-0.54	0.011	-0.46	0.028	-0.62	0.005
Oligochaeta*	5.67	0.006	0.10	0.751	0.62	0.056	0.16	0.610	1.21	0.001
<i>Cyprideis</i> sp.	1.12	0.384	-1.75	0.915	20.75	0.216	-12.25	0.458	4.50	0.783
<i>Gammarus</i> sp.*	0.94	0.467	0.43	0.190	-0.09	0.781	-0.09	0.781	0.044	0.890

mesocosms. Snails (Hydrobiidae: *Hydrobia* sp.) were found in the controls (2,000 m^{-2}) and the moderate loading mesocosms (30 m^{-2}).

The presence of enclosures did not have a significant effect on the abundance of oligochaetes, *Enallagma*, *Chironomus*, *Cyprideis*, and *Gammarus* (Table 1). The densities of grazing chironomids significantly declined in the ambient mesocosms (2,900 m^{-2}) relative to the controls (16,600 m^{-2}).

N loading did significantly affect abundances of grazing chironomids, *Enallagma*, and oligochaetes. The abundance of grazing chironomids (*Cricotopus* and *Dicrotendipes*) declined significantly between ambient and high N loading conditions but not between ambient and low and moderate loading conditions (Table 1). The threshold of response to

N by grazing chironomids was between 16.8 and 33.8 $mmol\ DIN\ m^{-2}\ d^{-1}$. *Enallagma* showed a lower threshold of response, significantly declining in all the loading treatments when compared to the ambient mesocosms. Oligochaete abundance increased significantly with high N loading relative to ambient conditions. The abundance of *Chironomus*, *Cyprideis*, and *Gammarus* did not change significantly with N loading. Other taxa were not abundant enough to test for significant treatment differences.

STABLE ISOTOPIC COMPOSITIONS

Average $\delta^{13}C$ values for *R. maritima* decreased from $-16.6 \pm 0.3\text{‰}$ in the control units to $-22.5 \pm 0.4\text{‰}$ in the highest loading treatments (Fig. 2). *R. maritima* $\delta^{15}N$ increased from $-3.4 \pm 0.9\text{‰}$ in the controls to $3.3 \pm 0.7\text{‰}$ in the highest loading mesocosms. Treatment effects were significant on both the C and N isotope ratios (C: $F = 18.18$, $p = 0.000$; N: $F = 14.80$, $p = 0.000$). Enclosures resulted in a significant decrease in $\delta^{13}C$ ($p = 0.000$), but no significant change in the stable N isotope ratios. $\delta^{13}C$ decreased with increased N loading, with the threshold for significant response to loading at the highest level ($p = 0.000$). *R. maritima* $\delta^{15}N$ increased with N loading, with a threshold of response to loading at the moderate loading level ($p = 0.000$).

Epiphytic material associated with *R. maritima* had $\delta^{13}C$ values from $-22.5 \pm 0.5\text{‰}$ under control conditions to $-20.1 \pm 0.3\text{‰}$ under low N loading (Fig. 2). $\delta^{15}N$ increased from $-0.2 \pm 0.3\text{‰}$ in the control units to $2.8 \pm 0.7\text{‰}$ in the highest loading treatments. Increased N loading resulted in significant increases in both $\delta^{13}C$ and $\delta^{15}N$ of epiphytes (C: $F = 4.65$, $p = 0.004$; N: $F = 5.37$, $p = 0.002$). Significant increases in $\delta^{13}C$ were similar at all levels of N loading ($p = 0.011$ – 0.017). Increases in $\delta^{15}N$ were significant at the highest levels of loading ($p = 0.012$). Enclosures did not effect the stable C or N isotope ratios of epiphytes.

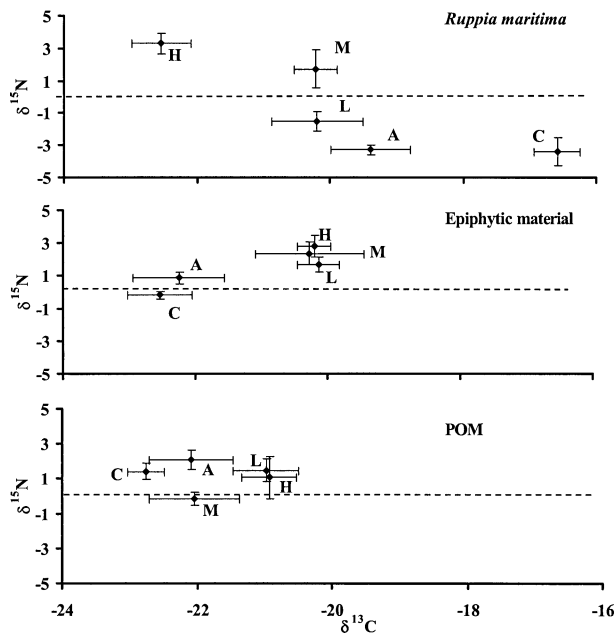


Fig. 2. Average C and N stable isotope values (‰) of primary producers in the N enrichment experiment. C = control, A = ambient, L = low N loading, M = moderate N loading, and H = high N loading. Bars represent 1 standard error.

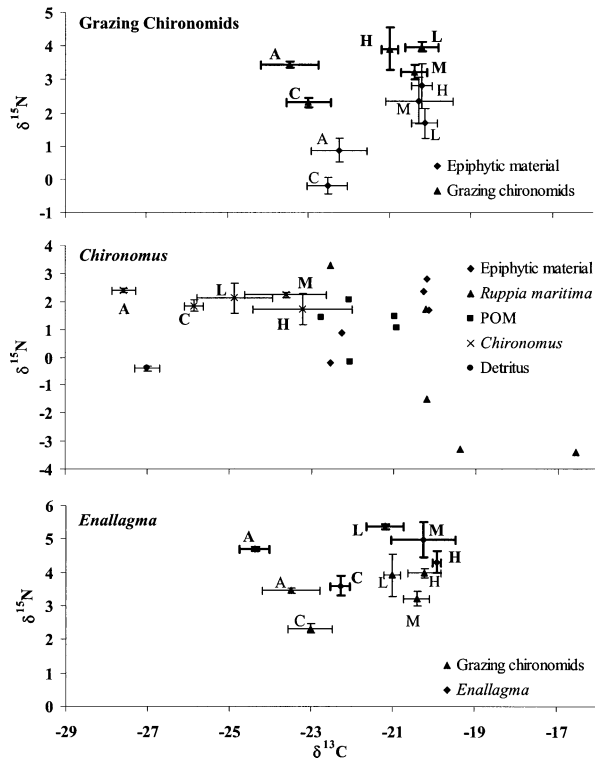


Fig. 3. Average C and N stable isotope values (‰) of consumers and food sources. C = control, A = ambient, L = low N loading, M = moderate N loading, and H = high N loading. Standard error bars are shown for all items except autochthonous food sources in B.

Isotope ratios of suspended POM ranged from $-22.8 \pm 0.3\text{‰}$ (control) to $-21.0 \pm 0.5\text{‰}$ (low loading) and $-20.2 \pm 0.4\text{‰}$ (moderate loading) to $-19.2 \pm 0.6\text{‰}$ (ambient) for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively (Fig. 2). There were no significant changes

in the $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ of POM as a result of N treatments.

The average stable isotopic signatures of terrestrial detritus ($\delta^{13}\text{C} = -27 \pm 0.3\text{‰}$; $\delta^{15}\text{N} = -0.4 \pm 0.1\text{‰}$) were determined by pooling material from all treatments ($n = 20$). The material was pooled because variability seen between treatments resulted from the detrital origin of the samples, not treatment differences. We determined that a pooled value was most representative of available detritus in the system. Average $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of Osmocote slow-release fertilizer were $-30.20 \pm 0.05\text{‰}$ and $1.66 \pm 0.13\text{‰}$, respectively.

Grazing chironomidae (*Cricotopus* and *Dicrotendipes* sp.) $\delta^{13}\text{C}$ values ranged from $-23.5 \pm 0.3\text{‰}$ (ambient) to $-20.2 \pm 0.4\text{‰}$ (low N) and $\delta^{15}\text{N}$ values ranged from $2.3 \pm 0.1\text{‰}$ (controls) to $4.0 \pm 0.1\text{‰}$ (low N; Fig. 3). N loading resulted in a significant increase in $\delta^{13}\text{C}$ even at the lowest level of loading ($p = 0.000$). There were no significant changes in $\delta^{15}\text{N}$ with loading. Graphical comparisons of the C and N isotope ratios of these chironomids and epiphytic material, a potential food source, illustrate relatively similar changes with each treatment. No similar pattern was observed for *R. maritima*, POM or terrestrial detritus. Epiphytic material, *R. maritima*, and terrestrial detritus were considered to be the three most important components of the diets of these chironomids for both the Euclidean distance index and the linear mixing model. The Euclidean distance model ranked epiphytic material as the most important food source in all treatments (Table 2). Terrestrial detritus was of secondary importance under control and ambient conditions and *R. maritima* was of secondary importance in the loading treatments. According to linear mixing models, diets of graz-

TABLE 2. Euclidean distance index values and ranks of important food sources in the diets of consumers in the N enrichment experiment. Index values and ranks are provided for all nutrient treatments for grazing chironomids, *Chironomus*, and *Enallagma*. Higher index values and lower ranks indicate greater importance of the food source to the consumer based on the greater proximity of its C and N isotope values to those of the consumer.

	Control		Ambient		Low N		Moderate N		High N	
	Index	Rank	Index	Rank	Index	Rank	Index	Rank	Index	Rank
Grazing Chironomidae										
Epiphytic material	0.56	1	0.53	1	0.59	1	0.60	1	0.50	1
<i>Ruppia maritima</i>	0.17	3	0.19	3	0.25	2	0.34	2	0.41	2
Terrestrial detritus	0.28	2	0.28	2	0.16	3	0.07	3	0.09	3
<i>Chironomus</i>										
Epiphytic material	0.36	2	0.29	2	0.32	2	0.37	1	0.28	2
<i>Ruppia maritima</i>	0.13	3	0.16	3	0.26	3	0.36	2	0.53	1
Terrestrial detritus	0.51	1	0.54	1	0.43	1	0.27	3	0.20	3
<i>Enallagma</i>										
Grazing Chironomidae	0.50	1	0.60	1	0.54	1	0.48	1	0.59	1
<i>Chironomus</i>	0.18	3	0.23	2	0.18	3	0.19	3	0.16	3
Other	0.31	2	0.17	3	0.28	2	0.33	2	0.25	2

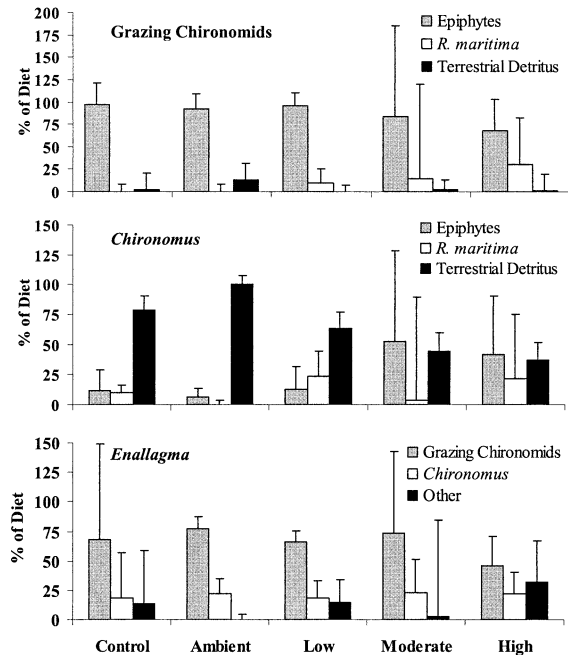


Fig. 4. Linear mass balance mixing model predictions of proportions of food sources in the diets of consumers for all treatments in the N enrichment experiment. Relative importance is indicated by percentage estimates. Bars represent 1 standard error.

ing chironomids ranged from 68.2% (highest loading) to 97.4% (control) epiphytic material (Fig. 4). In the highest loading treatments *R. maritima* was estimated to contribute to 30.6% of the diets of these chironomids. Greater uncertainty surrounds the predictions under moderate and high N loading conditions.

Chironomus $\delta^{13}\text{C}$ values varied from $-27.6 \pm 0.3\text{‰}$ (ambient) to $-23.2 \pm 1.2\text{‰}$ (high N) and $\delta^{15}\text{N}$ values varied from $1.7 \pm 0.5\text{‰}$ (high N) to $2.4 \pm 0.1\text{‰}$ (ambient; Fig. 3). N loading resulted in a significant increase in $\delta^{13}\text{C}$ values even at the lowest levels ($p = 0.003$) although it had no effect on $\delta^{15}\text{N}$ signatures. Mesocosms did not significantly affect either C or N isotope ratios. Comparisons of *Chironomus* C and N stable isotopic values to those of potential food sources illustrate a shift from a dependence on terrestrial detritus to a dependence on more autochthonous sources of C (*R. maritima*, POM, and epiphytic material). The Euclidean distance index ranked terrestrial detritus as the most important food source under control, ambient, and low loading conditions (Table 2). Epiphytes and *R. maritima* ranked as the most important food sources under moderate and high loading conditions. Linear mass balance mixing models also predict that these chironomids are dependent on multiple sources of detritus, including

allochthonous terrestrial detritus and autochthonous detritus derived from *R. maritima* and epiphytic material (Fig. 4). Under control and ambient conditions, the diets of *Chironomus* were composed of 78.9% and 100% terrestrial detritus, respectively. The importance of terrestrial detritus decreased with N loading to 63.2% (low), 44.1% (moderate), and 37.2% (high). Greatest uncertainty in the model was found for *Chironomus* under the moderate and high loading conditions.

Gammarus stable isotope ratios remained relatively constant in all treatments. Values were not obtained for the ambient mesocosms because insufficient numbers of amphipods were collected from these units. $\delta^{13}\text{C}$ ranged from $-20.5 \pm 0.2\text{‰}$ (control) to $-21.4 \pm 0.2\text{‰}$ (high N) and $\delta^{15}\text{N}$ ranged from $2.0 \pm 0.3\text{‰}$ (control) to $2.4 \pm 0.4\text{‰}$ (moderate N).

The $\delta^{13}\text{C}$ values of the predatory damselfly *Enallagma* varied from $-24.4 \pm 0.4\text{‰}$ (ambient) to $-19.9 \pm 0.1\text{‰}$ (high N). $\delta^{15}\text{N}$ varied from $3.6 \pm 0.3\text{‰}$ (control) to $5.4 \pm 0.1\text{‰}$ (low N; Fig. 3). $\delta^{13}\text{C}$ increased significantly at all levels of N, indicating a low threshold of response to loading ($p = 0.000$). N loading had no effect on $\delta^{15}\text{N}$. Mesocosms resulted in significantly lower $\delta^{13}\text{C}$ values ($p = 0.000$) and higher $\delta^{15}\text{N}$ values ($p = 0.002$). Potential prey of *Enallagma* found in NEC estuary included grazing chironomids, *Chironomus*, *Gammarus*, and *Cyprideis*. The C and N isotopic ratios of *Enallagma* shifted with loading in a similar manner to grazing chironomids. No similar shift was seen with any other prey items. The Euclidean distance index consistently ranked grazing chironomids as the primary food source for damselflies under all loading conditions (Table 2). Linear mixing models also identified grazing chironomids as a principal food source for damselflies under all loading treatments (46–77.3%; Fig. 4). Under control, moderate, and high loading conditions there was significant variability in the model predictions, indicating that other prey may also be important.

Discussion

FAUNAL COMMUNITY SHIFT

The macroinvertebrate community of NEC estuary differs from that of other estuaries investigated for community changes with increased nutrient loading. Most studies of faunal responses to nutrient enrichment have focused on bays and higher salinity reaches of estuaries that are dominated by commercial bivalve species, crustaceans, and polychaetes (Valiela et al. 1992; Heip 1995; Raffaelli 1999). The hydrologic and salinity regime of NEC estuary produces an oligohaline-mesohaline system during the spring and summer months

and as a result euryhaline freshwater insects dominate the community of this estuary. Although similar communities have been documented in other tidal freshwater, oligohaline, and mesohaline reaches of estuaries and in *Ruppia* beds (Remane and Schlieper 1971; Verhoeven 1980; Williams and Williams 1998; Williams and Hamm 2002), studies of the effects of nutrient loading on these communities are lacking.

The community shifts seen in NEC estuary follow the model proposed by Heip (1995) regarding eutrophication and community dynamics. As N loading increased, there was a significant increase in numbers of oligochaetes (which are r-selected, small, opportunistic species), and significant decreases in grazer specialists and their predators. With increased eutrophication, the community became dominated by generalist species feeding on a wide range of deposit material. This response was most evident at the highest level of N loading used (33.8 mmol DIN m⁻² d⁻¹). The short duration of the mesocosm experiment may have resulted in an underestimation of the effects that lower N loading would have on the macroinvertebrate community of NEC estuary.

Although for the most part there were no significant differences in invertebrate densities between ambient mesocosms and open estuary controls, we did find lower densities of grazing chironomids in the ambient mesocosms than in the estuary. The mesocosms were designed to mimic physical conditions in the estuary as much as possible. Dye studies of mixing times showed that turbulent energy transfer within the mesocosms was similar to that in the estuary (Kopp personal communication). The average residence time of water in the mesocosms was also similar to that in the estuary. It is likely that mesocosm walls restricted flow during high spring tides and may have caused inconsistent replenishment of emergent insect populations throughout the 2-mo experiment. Grazing chironomids are emergent insects with a short life cycle. They may have emerged from the estuary as adults during the experiment, so populations within the mesocosms may not have been replenished at the same rate as in the open estuary.

HERBIVORES

Epiphytic material was the primary food source for the herbivorous chironomids of NEC estuary under natural and enriched conditions. Others have documented the disproportionate importance of epiphytic material compared to macrophytes in many undisturbed estuarine communities (Kitting et al. 1984; Stephenson et al. 1986; Loneragan et al. 1997). It is assumed that epi-

phytes are preferred by grazers because they have lower C:N ratios and are easier to consume.

Reductions in the quantity and quality of the epiphytes available to grazing chironomids under increased N loading may have been partially responsible for the decline we observed in the densities of these herbivores. Although there was a significant increase in the amount of epiphytic material per unit *R. maritima* biomass with loading, there was an overall decrease in epiphytic biomass due to significant losses of *R. maritima* (Neckles unpublished data). The quality of the epiphytes available to these chironomids did not increase with loading. Easy-to-consume diatoms were found for all treatments, but less desirable filamentous algae became more common under increased N conditions (Keats personal observation).

Changes in physical and chemical habitat structure resulting from increased nutrient loading may also have reduced the survival of herbivorous chironomids under these conditions. Deegan et al. (2002) found that habitat structure changes (losses of macrophyte beds) with increased nutrient loading were the most likely cause of reductions in fish and herbivore abundance. Although *R. maritima* may not be an important food source to herbivores in NEC estuary, it may provide habitat structure and protection from predation. Decreases in dissolved oxygen that often accompany increased N loading may also have degraded living conditions for these herbivores (Heip 1995).

DETRITIVORES

While herbivores did not show a shift in food sources, deposit-feeding *Chironomus* shifted from a dependence on terrestrial sources of detritus under natural conditions to autochthonous sources of detritus such as *R. maritima* and epiphytic material under higher loading conditions. Although caution must be used when assuming that decomposing *R. maritima* and epiphyte biomass have the same isotope ratios as living biomass (Caraco et al. 1998; Cloern et al. 2002), the results of the mixing models make intuitive sense for the mesocosm experiment in NEC estuary. With increased loading, autochthonous sources of detritus increased as algal production increased and *R. maritima* began to die off (Neckles unpublished data).

Similar shifts in detritus have been documented in estuaries exposed to different nutrient regimes (Heip 1995; McClelland and Valiela 1998a). Because consumers can more readily access algal sources of detritus, an increase in cycling usually occurs with this detrital shift. This shift has been associated with losses of stability, as the ecosystem becomes driven by boom and bust cycles of shorter-lived food resources. Although the mesocosm

experiment had a duration of only 2 mo, this shift in detrital sources had already become evident in detritivores in the system. If increased nutrient loading were to occur in NEC estuary, much larger changes would most likely take place.

PREDATORS

Predatory *Enallagma* were largely dependent on grazing chironomids under all conditions and declined in density alongside this primary food source. Herbivorous chironomids were a significant portion of the epifaunal community in all treatments except the highest loading and the importance of chironomids in the diets of damselflies has been found in other studies (Menzie 1981; Hart and Lovvorn 2002). Damselfly densities declined with the reductions in grazing chironomid densities under increased loading conditions. This indicates that loss of prey may have been at least partially responsible for the declines seen in the damselfly population.

Fit of the linear mixing models for *Enallagma* in NEC estuary required adjustments to the average literature fractionation values in all treatments. We used lower fractionation estimates for $\Delta\delta^{15}\text{N}$ in all treatments. It seems reasonable that fractionation by these predators is lower than the literature average. Hart and Lovvorn (2002) found that average N fractionation by *Enallagma* ranged from 1–3.4‰ between saline wetlands. Gut contents of food sources and omnivory could also lower the trophic level enrichment in N (Marguiller et al. 1997; Hart and Lovvorn 2002).

PRIMARY PRODUCERS AND CARBON LIMITATION

The $\delta^{13}\text{C}$ signatures of *R. maritima* and epiphytic material changed in opposite ways with increased N loading. Epiphytes were less depleted in ^{13}C with N loading while *R. maritima* was more depleted in ^{13}C with loading. Greater metabolic activity by producers has been found to result in decreased discrimination against ^{13}C (Fogel et al. 1992; Grice et al. 1996; MacLeod and Barton 1998). This shift is thought to occur because there is an increased uptake of all C under these conditions and increased metabolism may shift the pathway of C fixation and increase internal recycling processes. Increased N loading in the mesocosm experiment may have shifted the metabolic activity of both *R. maritima* and its epiphytic material. *R. maritima* biomass was significantly lower in the increased N treatments, while the ratio of epiphytic material per unit *R. maritima* biomass and phytoplankton chlorophyll *a* concentrations significantly increased with loading (Neckles unpublished data). Under increased N conditions, this greater epiphytic and planktonic algal biomass may have inhibited productivity, and

consequent C demand, of *R. maritima*. This macrophyte discriminated more against the heavier C isotope under these enriched conditions. Epiphytic material, on the other hand, exhibited faster growth and less discrimination against ^{13}C under these increased N conditions. Other possible explanations for the differences seen in $\delta^{13}\text{C}$ are simply high variability in signatures within a single species and signature variability between algal species.

In addition to the changes that occurred with N loading, we observed a significant decrease in the $\delta^{13}\text{C}$ of *R. maritima* with enclosure. Although the average residence time of water in the estuary was approximated by the mesocosms, the design did not incorporate the natural periods of extremely high flushing during spring high tides. Restricted flow into the mesocosms during these events may have limited the movement of external C into the mesocosms. This would have forced the macrophytes to have a greater dependence on lighter C derived from decomposition and recycling (Smith et al. 1976), resulting in lower values of *R. maritima* $\delta^{13}\text{C}$ in the mesocosms than in the open estuary.

PRIMARY PRODUCERS AND NITROGEN

N loading resulted in increased $\delta^{15}\text{N}$ in both *R. maritima* and its associated epiphytic material. The average $\delta^{15}\text{N}$ of Osmocote slow-release fertilizer was 1.66‰, a value that is not high enough to account for the increases in $\delta^{15}\text{N}$ observed for the epiphytes and macrophytes. The addition of fertilizer N in the estuary may have shifted the cycling of N through the system. N additions may have resulted in low dissolved oxygen concentrations due to the stimulation of algal growth in the system and increased organic inputs to the benthos (Heip 1995). Hypoxic conditions would increase denitrification, a process that results in large increases in the $\delta^{15}\text{N}$ of inorganic N left in the system (Lajtha and Michener 1994). As little as 20% total nitrate removal by denitrification will result in an 8‰ increase in $\delta^{15}\text{N}$ (Heaton 1984). Secondary effects of the fertilizer resulting in increased denitrification would result in the trend of increasing $\delta^{15}\text{N}$ observed for both epiphytes and macrophytes in NEC estuary.

IMPLICATIONS

Two months of experimental N loading during the summer growing season resulted in changes in the composition and structure of the faunal community of NEC estuary. The largest shifts in macroinvertebrate densities occurred between the moderate and high levels of loading (16.8–33.8 mmol DIN $\text{m}^{-2} \text{d}^{-1}$), although shifts were observed at low loading levels as well. An increase in the importance of autochthonous detritus was evident

even at low levels of loading. The results of this enrichment experiment indicate that small increases in loading could result in shifts in food web structure. Currently N loading in NEC estuary is slightly higher than $1.3 \text{ mmol DIN m}^{-2} \text{ d}^{-1}$ (Nielsen 2002). If loading in NEC increases to levels observed in other estuaries in the northeast U.S., we would expect that there would be a shift from a community dependent on live epiphytic material to a community dependent on autochthonous detrital material and that, ultimately, benthic deposit feeders would dominate this community. The same predictions can be made for other small, shallow, mesohaline estuaries.

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