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

Rachel A. Keats

THE EFFECT OF NITROGEN LOADING ON AN ESTUARINE FAUNAL COMMUNITY: A STABLE ISOTOPE APPROACH

By

Rachel A. Keats

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Advisory Committee:

Laurie J. Osher, Associate Professor of Plant, Soil and Environmental Sciences

Hilary A. Neckles, U.S. Geological Survey, Patuxent Wildlife Research Center

Les Watling, Professor of Oceanography

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Thesis Advisor: Dr. Laurie J. Osher

An Abstract of the Thesis Presented
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Coastal ecosystems worldwide face increased nutrient enrichment from shoreline and watershed development and atmospheric pollution. To gain an understanding of the effects of nitrogen loading on the natural faunal community of *Ruppia maritima* beds in Northeast Creek estuary (Acadia National Park, Maine, USA), we (1) assessed the response of the faunal community to increased nitrogen loading using an *in situ* enrichment experiment during the summer growing season of 2001 (Chapter 1), and (2) completed a description of the natural macroinvertebrate community in the estuary in 2001 with qualitative (May-July) and quantitative (August-October) monthly sample collections (Chapter 2). This study formed part of a larger study by the U.S. Geological Survey of the relationship between increased nutrient enrichment and ecosystem integrity in the estuary. Faunal community response to increased nitrogen loading was characterized by (1) assessing quantitative shifts in macroinvertebrate community

composition and (2) identifying changes in food web structure using stable C and N isotope ratios of producers and consumers.

Salinity in the estuary indicated that the system was dominated by freshwater inputs in the spring and became increasingly more marine throughout the summer (reaching 30 ‰ in the fall). Euryhaline freshwater fauna dominated Northeast Creek estuary throughout 2001. The most common invertebrates were non-biting midge larvae (Chironomidae: *Dicrotendipes*, *Cricotopus* and *Chironomus*), damselfly larvae (Coenagrionidae: *Enallagma*), gastropods and ostracods. Less common invertebrates included oligochaetes, water boatmen (Corixidae: *Trichocorixa*), water mites (Acari), and amphipods (Gammaridae: *Gammarus*). Brackish water fish, *Fundulus heteroclitus*, were also common in the estuary. Total macroinvertebrate densities were 31100 m^{-2} in August, 23200 m^{-2} in September and 27700 m^{-2} in October. Freshwater insects composed between 50 and 80% of the macroinvertebrate community in the estuary during this time period. The estuary was characterized by low taxa richness, diversity and evenness.

Experimental nutrient additions resulted in significantly lower densities of herbivorous chironomids and predatory damselflies and higher densities of deposit feeding oligochaetes. Both *R. maritima* and epiphytic algae were more enriched in ^{15}N under higher N conditions possibly due to increased denitrification. *R. maritima* was more depleted in ^{13}C with loading while epiphytic material showed the opposite trend, indicating shifts in metabolic activity with increased loading. Mixing models showed a dependence of grazing chironomids on epiphytic algae under both natural and enriched conditions. *Chironomus* was dependent on allochthonous sources of detritus under natural conditions but under enriched conditions exhibited a shift to autochthonous

sources of detritus. Predatory *Enallagma* was found to be largely dependent on grazing chironomids for prey although under the highest loading conditions this link may be broken.

Experimental nutrient loading altered the composition and structure of the natural community in this estuary. This study provides a baseline on faunal community composition and community response to nitrogen loading in NEC estuary for use in developing predictive tools for watershed-based planning and monitoring.

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Chapter Summary

Coastal ecosystems worldwide face increased nutrient enrichment from shoreline and watershed development and atmospheric pollution. The response of the faunal community to increased nitrogen loading in a small estuary dominated by *Ruppia maritima* (widgeon grass) in Acadia National Park, Maine, was investigated using an *in situ* mesocosm enrichment experiment during the summer growing season of 2001. This study formed part of a larger study by the U.S. Geological Survey of the relationship between increased nutrient enrichment and ecosystem integrity in the estuary. Faunal community response to increased nitrogen loading was characterized by (1) assessing quantitative shifts in macroinvertebrate community composition and (2) identifying changes in food web structure using stable C and N isotope ratios of producers and consumers. The estuarine faunal community is dominated by brackish water invertebrates including midge larvae (Chironomidae: *Dicrotendipes* sp., *Cricotopus* sp., *Chironomus* sp.), oligochaetes, damselfly larvae (Coenagrionidae: *Enallagma* sp.), amphipods (Gammaridae: *Gammarus* sp.), ostracods, and water boatmen (Corixidae: *Trichocorixa* sp.), and fish (Fundulidae: *Fundulus heteroclitus*). Experimental nutrient additions resulted in significantly lower densities of herbivorous chironomids and predatory

damselflies and greater densities of deposit feeding oligochaetes. Both *R. maritima* and epiphytic algae were more enriched in ^{15}N under increased N conditions possibly due to increased denitrification. *R. maritima* was more depleted in ^{13}C with loading while epiphytic material showed the opposite trend, indicating shifts in metabolic activity with increased loading. Mixing models showed a dependence of grazing chironomids on epiphytic algae under both natural and enriched conditions. *Chironomus* was dependent on allochthonous sources of detritus under natural conditions but under enriched conditions exhibited a shift to autochthonous sources of detritus. Predatory *Enallagma* was found to be largely dependent on grazing chironomids for prey although under the highest loading conditions this link may be broken. Experimental nutrient loading altered the composition and structure of the natural community in this estuary.

Introduction

Nutrient loading and subsequent eutrophication have been documented in estuaries and coastal waters worldwide (Orth and Moore 1983, GESAMP 1990, Nixon 1995, Rafaelli 1999, Arhonditis et al. 2000). Anthropogenic sources of nutrient loading include wastewater, industrial processes, fertilizers and atmospheric deposition (Culliton et al. 1989, Valiela et al. 1992, Paerl and Fogel 1994, Arhonditsis et al. 2000). The relative importance of these sources differs depending on watershed land use, hydrology, and geographic location. Today, 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. Because Maine's estuaries merge into the Gulf of Maine, increases in nutrient loading may impact the

structure and function of marine ecosystems in the Gulf and subsequently threaten the resource-based coastal economy (MEPP 1995).

Excessive nutrient loading to estuaries frequently causes a shift in primary producers from submerged vascular plants to algal-dominated communities (Orth and Moore 1983, Day 1989, Valiela at al. 1992, Sand-Jensen and Borum 1991, Duarte 1995, Harlin 1995, Short and Wyllie-Echeverria 1996). Fast-growing macroalgae, epiphytes and phytoplankton will out compete macrophytes in nutrient enriched estuaries by exploiting the nutrients, proliferating and reducing light penetration for submerged vegetation (Harlin and Thorne-Miller 1981, Sand-Jensen and Borum 1991, Short et al. 1995, Taylor et al. 1995, Short and Burdick 1996, 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 (Valiela et al. 1992, Heip 1995, Borum 1996, Valiela et al. 1997, McClelland and Valiela 1998a). The bottom up effects of nutrients in the system may be complicated by the top down effects of grazers and small predators (Bronmark 1985, Hootsmans and Vermaat 1985, Howard and Short 1986, Borum 1987, Williams and Ruckelshaus 1993, Neckles et al. 1993, Hauxwell et al. 1998, Gacia et al. 1999, Heck et al. 2000). In general, while the response of primary producers to nutrient loading and eutrophication has been well documented, the influence of this response on food web structure and function is much less well understood. Because of this, 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 estuarine trophic relationships (see reviews by Fry and Sherr 1984, Peterson and Fry 1987, Lajtha and Michener 1994, Gannes et al. 1997). Carbon isotopes are useful because estuarine primary producers tend to differ in their $^{13}C/^{12}C$ ratios due to different metabolic pathways, making it possible to distinguish the origin of food sources by examining these ratios (Fogel and Cifuentes 1993). Nitrogen is useful in determining the trophic level of each organism evaluated because $^{15}N/^{14}N$ ratios are enriched by approximately 3‰ at each level in the food chain (DeNiro and Epstein 1981, Minagawa and Wada 1984, VanderZanden and Rasmussen 2001). Sulfur isotopes are also useful in estuarine systems because the primary producers derive sulfur from different sources, thus making it possible to distinguish between them (Peterson et al. 1985). By using multiple stable isotopes it is possible to improve the resolution of food web structure over the use of C isotopes alone (Fry 1991). Knowledge of the stable isotope compositions for two elements in a consumer and three food sources allows for use of mixing models to assess the 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 (Gearing et al. 1984, Peterson et al. 1985, Rau et al. 1990, Sholto-Douglas et al. 1991, Hobson and Welch 1992, Wainright et al. 1993, Lajtha and Michener 1994). Several studies have addressed the issue of preference by estuarine consumers for algae over less digestible macrophytes (Kitting et al. 1984, Peterson et al. 1985, Yelenik et al. 1996, Loneragan et al. 1997). Other studies have shown that both macrophytes and algae may be important food sources in estuarine ecosystems, depending on the location, size, feeding mode and trophic position of the

consumers (Peterson and Howarth 1987, Deegan and Garritt 1997, Hughes et al. 2000). Isotopes have also been used to determine the significance of terrestrially derived nutrients and organic matter in coastal waters (Thayer et al. 1983, Peterson and Howarth 1987, Simenstad and Wissmar 1985, Conkright and Sackett 1986, Cifuentes 1991, Day et al. 1994, Riera 1998). In addition, stable isotope analysis has been used to trace wastewater inputs of N through coastal and estuarine food webs (Hansson et al. 1997, McClelland et al. 1997, McClelland and Valiela 1998b).

Recent studies have utilized stable isotope analysis to investigate the effect of carbon and nitrogen limitation on the isotopic signatures of primary consumers. In general, under lower nitrogen conditions there is less fractionation against ^{15}N because in times of nutrient stress animals will use all available nutrients as well as internally recycle nutrients (Peterson et al. 1993, Pennock et al. 1996, Gannes et al. 1998, McClelland and Valiela 1998b, Waser et al. 1999). This observation is true for carbon isotopes as well. When metabolic activity is high and carbon limitation occurs, there is less discrimination against ^{13}C as a result of increased uptake of all carbon under these conditions, shifts in the pathways of C fixation, and increased internal recycling processes (Smith et al. 1976, Osmond et al. 1981, Guy et al. 1989, Fogel et al. 1992, Maberly et al. 1992, Grice et al. 1996, MacLeod and Barton 1998, Finlay et al. 1999, Hemminga and Duarte 2000). Thus, differences in C and N isotopes may indicate nutritional stress as well as trophic position.

McClelland and Valiela (1998a) used stable isotopes to examine food web changes resulting from increased N loading in three estuaries of Waquoit Bay, Massachusetts. Diets of herbivores, suspension feeders and detritivores were found to be

influenced by the dominant forms of production they were exposed to. While macroalgae and phytoplankton were major food sources in all estuaries, eelgrass (*Zostera marina*) was found to be an important component of the ecosystem where it was present (under low loading conditions). 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 this eelgrass is replaced by algae, the rate at which this N is cycled within the estuarine ecosystem may potentially increase.

Though much work has been done on the impacts of nutrients on estuarine systems, little research has been done on the estuarine systems typical of much of Maine's coast (Kahl et al. 1999). Scant information exists for determining nutrient loading thresholds or best management practices for these systems. The Northeast Creek estuary of Acadia National Park is a current site for integrated research by the U.S. Geological Survey in collaboration with others. The overall goal of this research is to assess the effects of nutrient loading in a small Maine estuary in order to develop predictive tools for watershed-based planning and monitoring (Nielsen 2002, Nielsen et al. in progress, Neckles et al. in progress). Northeast Creek estuary is a pristine system threatened by atmospheric pollution and increased residential development. It is located in an area where nearby estuarine systems are already moving towards eutrophic conditions (Doering and Roman 1994, Doering et al. 1995, Kinney and Roman 1998, Farris and Oviatt 1999).

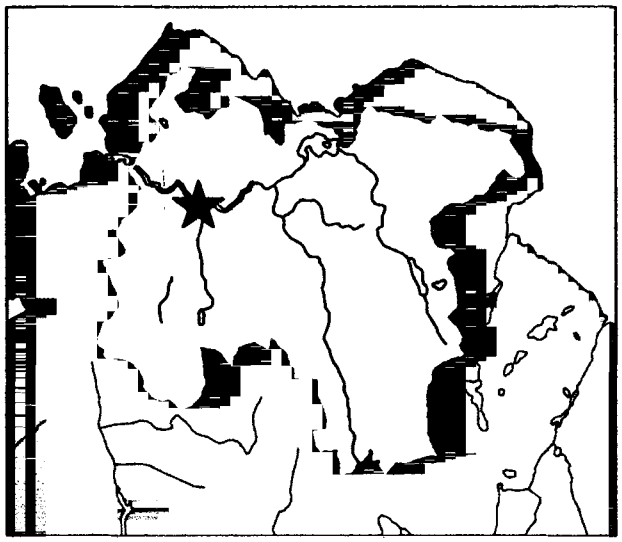
Our research focused on characterizing the response of the faunal community of Northeast Creek estuary to increased nitrogen loading by (1) assessing shifts in macroinvertebrate community composition and (2) identifying changes in food web

structure using C and N stable isotope techniques. *In situ* nitrogen enrichment experiments associated with the USGS integrated research project were used to accomplish both objectives.

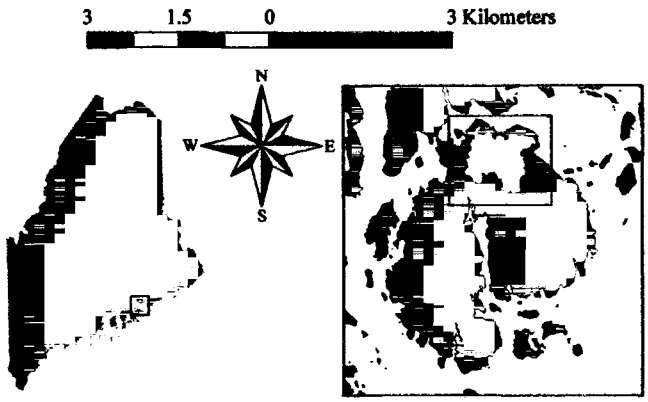
Methods

Study Site

Northeast Creek estuary is located in Acadia National Park, Mount Desert Island, Maine (Figure 1.1). This small estuary occupies a drowned river valley fed by a number of freshwater streams. It is approximately 4 km long with a watershed of 6400 acres. Lands in the surrounding watershed contain some of the fastest growing areas of single-family homes in the region. The estuarine system includes approximately 500 acres of emergent estuarine wetlands, emergent riverine tidal fresh wetlands, palustrine shrub-scrub wetlands and subtidal vegetated habitat. Northeast Creek estuary averages about 1m in depth with a narrow tidal range (<0.5m). 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 (Figure 1.2, USGS unpublished data). The estuarine system is densely vegetated with *Ruppia maritima* along most of its length. The experiments in this study were performed in a particularly dense bed of *R. maritima* approximately halfway up Northeast Creek, just downstream of the mouth of Aunt Betsy's Creek (Figure 1.1). The substrate of the estuary in this location is a silt loam soil containing organic matter of detrital origin.



Northeast Creek Watershed



Maine, USA Mt. Desert Island

Figure 1.1. Map of Northeast Creek watershed and study site. Northeast Creek is located on Mt. Desert Island in Maine, USA. Study site location is indicated by the star.

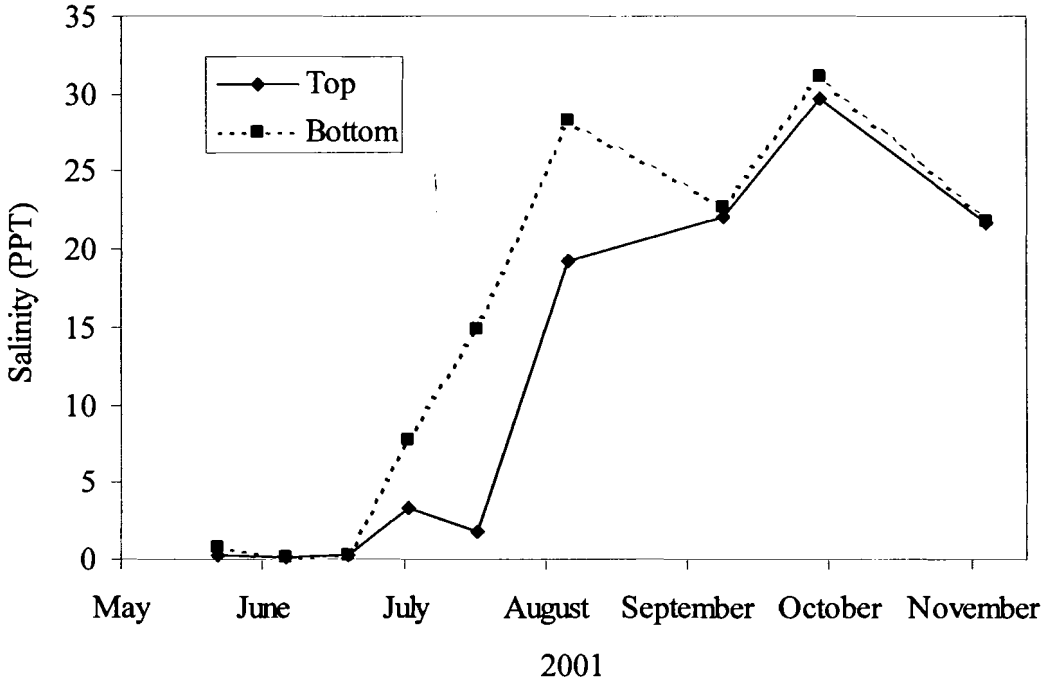


Figure 1.2. Top and bottom water salinities (PPT) in Northeast Creek estuary from May to mid-November 2001. USGS unpublished data.

Experimental Design

In situ nitrogen 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. in progress). Experimental units consisted of 1.2 m by 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). Mesocosms 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 days (B.S. Kopp, USGS, personal communication). Average water depth was 26 cm (± 4 S.D.) (B.S. Kopp, USGS, personal communication). Mesocosm walls were cleaned of phytoplankton on a weekly basis. Five nitrogen treatments were used in a completely random design with four replicates per treatment. The nutrient treatments were: unenclosed controls, and enclosed ambient, low (8.4 mmol DIN $m^{-2}d^{-1}$), moderate (16.8 mmol DIN $m^{-2}d^{-1}$) and high (33.8 mmol DIN $m^{-2}d^{-1}$) nitrogen loading conditions (B.S. Kopp, USGS, personal communication). Nitrogen 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 3rd and continued to August 28th 2001.

Sample Collection

At the end of the enrichment experiment, we collected one quantitative faunal sample down to 10 cm depth from each experimental unit using a 10 cm diameter core sampler. The core sampler was modified from the sampler described by Miller and Bingham (1987) by reducing the handle to 15 cm and using the size dimensions of the sampler described by Davis and Steinman (1998). The sampler 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 from the samples and identified to genus when possible (using keys in Weiderholm 1983, Peckarsky et al. 1990, Merritt and Cummins 1996, Epler 2001). Mean densities of the invertebrates were calculated for the quantitative samples based on the surface area of the core sampler (78.5 cm^2).

Primary producers, terrestrial detritus, herbivores, detritivores, filterers, and predators were also collected from each experimental unit one week before and at the end of the enrichment experiment for use in stable isotope analyses. *R. maritima* and associated epiphytic material were gently collected using ziploc 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 for drying in the oven (where excess water was able to evaporate). Suspended particulate organic matter was filtered from 1L 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.

Freezing has been shown to have no effect on isotope signatures (Kaehler and Pakhomov 2001). 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. Vapor phase acidification was used to clean all samples before analysis.

Mass Spectrometry

Stable C and N isotopic composition of animals and primary producers were determined using the isotope ratio mass spectrometer (IRMS) system interfaced to an elemental analyzer (EA/IRMS) at UC Davis' stable isotope laboratory. Isotopic compositions are reported as parts-per-mil (‰) deviation from air (N) and PDB (C). The precision (standard deviation) of replicate analyses of a standard known material was ± 0.05 ‰ for carbon and ± 0.15 ‰ for nitrogen.

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 LSD multiple comparisons were used to detect significant enclosure effects (control vs. ambient) and thresholds for nutrient effects (ambient vs. low, moderate and high loading). 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: 1) a Euclidean distance index and 2) 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 such that 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). To quantitatively assess food source contributions, both models assume that consumers partition food sources equally, that correct food sources have been identified, and that fractionation has been corrected for accurately (Ben-David and Schell 2001, Phillips 2001, Phillips and Koch 2002). However, in reality animals may assimilate dietary components differently, fractionate the nutrients differently and allocate nutrients in their diet to different body tissues (Steele and Daniel 1978, Macko et al. 1982, Hobson et al. 1993, Peterson et al. 1993, Lajtha and Michener 1994, Hobson et al. 1996, Gannes et al. 1997, Schoeller 1999, Adams and Sterner 2000) and careful consideration must be used in the application of these models. 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.

For the most part, consumers in NEC estuary fit within the assumptions of the three source mixing models. Both the primary and secondary consumers rely on food sources that do not vary greatly in carbon and nitrogen content. We obtained isotopic ratios for the most common food sources, except for oligochaetes in the highest loading treatment. However, we were concerned about the fractionation assumption because we relied on literature estimates of trophic fractionation for the consumers in NEC estuary and because variability in N fractionation has been so well documented in the literature (Macko et al. 1982, Hobson et al. 1993, Lajtha and Michener 1994, Pennock et al. 1996, Gannes et al. 1998, VanderZanden and Rasmussen 2001, Hart and Lovvorn 2002, Post 2002). To address this concern, we ran the Euclidean distance model assuming zero fractionation by consumers in order to rank the importance of food sources based on the proximity of their isotope values to the values of consumers in each experimental treatment. This index was then compared to the quantitative results of the linear mass balance mixing models that relied on literature averages of trophic fractionation.

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 ± 1.14 (SD) ‰ for $\Delta\delta^{13}C$ and 2.5 ± 2.5 (SD) ‰ for $\Delta\delta^{15}N$ were used (VanderZanden and Rasmussen 2001). For secondary consumers, we used a $\Delta\delta^{13}C$ of 0.47 ± 1.23 (SD) ‰ and a $\Delta\delta^{15}N$ of 2.92 ± 1.78 (SD) ‰ (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 the 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). This method calculates standard errors for each proportion estimate based on standard deviations and sample sizes associated with each food source and consumer.

Results

Faunal Community Changes

Brackish water invertebrates and fish dominate the faunal community of Northeast Creek estuary. The most common invertebrates include non-biting midge larvae (Chironomidae: *Dicrotendipes* sp., *Cricotopus* sp., and *Chironomus* sp.), damselflies (Coenagrionidae: *Enallagma* sp.), oligochaetes, and ostracods (Figure 1.3, Table 1.1). Less common organisms include scuds (Gammaridae: *Gammarus* sp.), water boatmen (Corixidae: *Trichocorixa* sp.), mites (Acari), snails (Gastropoda), and fly larvae (Table 1.1). Mummichugs (*Fundulus heteroclitis*) were the most common fish in the estuary.

Mesocosms did not have a significant effect on the abundance of oligochaetes, damselflies, *Chironomus* and ostracods (Table 1.2). However, the densities of grazing chironomids significantly declined from 16584 m^{-2} in the controls to 2483 m^{-2} in the ambient mesocosms. Nitrogen loading did significantly affect abundances of some of the most common invertebrates. There was a significant decline in the abundance of grazing chironomids (*Cricotopus* and *Dicrotendipes*) between ambient and high nitrogen loading conditions but not between ambient and low and moderate loading conditions (Table 1.2), indicating that the threshold of response by grazing chironomids is between 16.8 and 33.8 mmol DIN $m^{-2}d^{-1}$.

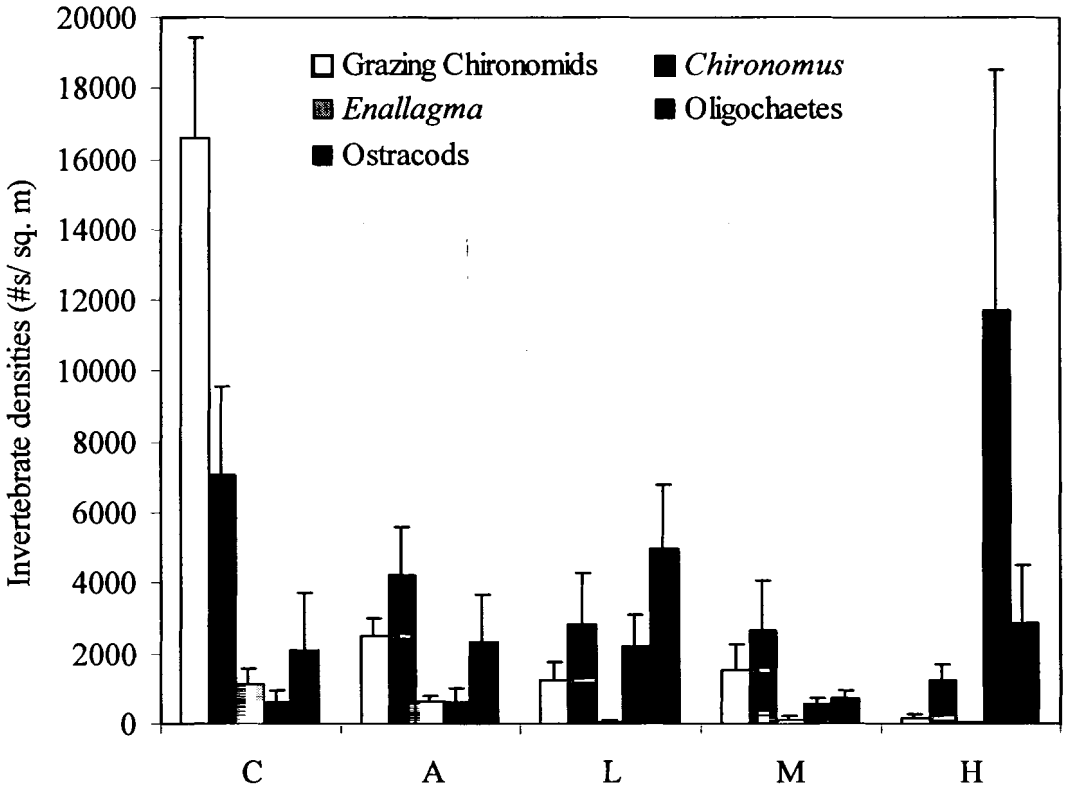


Figure 1.3. Densities of the most common invertebrate taxa in the N enrichment experiment. Mean densities (#s/ square meter) with standard error bars are shown for the different nutrient treatments. C = control, A = ambient, L= low, M = moderate, H = high loading.

Table 1.1. Macroinvertebrate densities in the N enrichment experiment. Mean densities (#s per square meter) of macroinvertebrate taxa for the five loading treatments. Means are of four replicates and standard errors (italicized) are presented.

	Densities (#s/ square meter)									
	Control		Ambient		Low N		Moderate N		High N	
Invertebrate Taxa	mean	*S.E.*	mean	*S.E.*	mean	*S.E.*	mean	*S.E.*	mean	*S.E.*
Insecta										
Chironomidae										
Dicrotendipes	14260.3	*2802.0*	2419.2	*493.1*	1209.6	*410.9*	1496.1	*694.2*	191.0	*82.2*
Cricotopus	2323.7	*393.7*	63.7	*63.7*	63.7	*63.7*	31.8	*31.8*	0.0	*0.0*
Chironomus	7098.3	*2477.8*	4233.5	*1372.6*	2833.0	*1486.1*	2673.8	*1401.5*	1241.4	*448.3*
Zygoptera										
Enallagma	1145.9	*456.1*	604.8	*210.3*	63.7	*36.8*	127.3	*73.5*	31.8	*31.8*
Corixidae										
Trichocorixa	1432.4	*523.3*	191.0	*151.5*	0.0	*0.0*	191.0	*191.0*	286.5	*245.9*
Crustacea										
Malacostraca										
Gammaridae										
Gammarus	1305.1	*936.2*	286.5	*245.9*	127.3	*90.0*	127.3	*90.0*	318.3	*241.0*
Ostracoda	2100.8	*1615.1*	2323.7	*1343.8*	4965.6	*1840.7*	763.9	*201.3*	2896.6	*1624.2*
Hydrachnidia										
Acari	0.0	*0.0*	0.0	*0.0*	0.0	*0.0*	31.8	*0.0*	0.0	*0.0*
Gastropoda	2069.0	*1168.5*	0.0	*0.0*	31.8	*31.8*	0.0	*0.0*	0.0	*0.0*
Oligochaeta	604.8	*330.3*	604.8	*442.2*	2228.2	*889.0*	541.1	*167.4*	11713.8	*6785.8*
Others	191.0	*36.8*	127.3	*73.5*	191.0	*36.8*	159.2	*80.1*	63.7	*36.8*
Total	32531.3	*3835.7*	10854.4	*2229.3*	11713.8	*1909.8*	6143.4	*1854.1*	16743.1	*8296.8*

Table 1.2. 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 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.

	Treatment Effects		Mesocosm Effects		Threshold of Response to N					
			C-A		L-A		M-A		H-A	
Taxa	*F*	*p > F*	*M.D.*	*p*	*M.D.*	*p*	*M.D.*	*p*	*M.D.*	*p*
Grazing Chironomidae*	17.03	0.000	0.81	0.002	-0.32	0.162	-0.34	0.143	-0.94	0.001
Chironomus sp.	1.981	0.149	22.50	0.218	-11.00	0.539	-12.25	0.494	-23.50	0.199
Enallagma 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
Ostracoda	1.12	0.384	-1.75	0.915	20.75	0.216	-12.25	0.458	4.50	0.783

Damselflies (*Enallagma*) showed a lower threshold of response, significantly declining in all the loading treatments when compared to the ambient mesocosms (Table 1.2). The abundance of oligochaetes increased significantly between the ambient and the highest loading treatments, although they did not show a significant response to lower levels of loading. The abundance of the benthic feeding *Chironomus* did not change significantly with N loading although average densities decreased from 4230 m^{-2} under ambient conditions to 1240 m^{-2} under the highest loading conditions (Table 1.2). Ostracod abundance did not change significantly with nitrogen 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 ± 0.3 ‰ in the control units to –22.5 ± 0.4 ‰ in the highest loading treatments (Table 1.3, Figure 1.4A). $\delta^{15}N$ of *R. maritima* increased from –3.4 ± 0.9 ‰ in the controls to 3.3 ± 0.7 ‰ in the highest loading mesocosms (Table 1.3, Figure 1.4A). There were significant treatment effects on both the C and N isotope ratios of this aquatic macrophyte (Table 1.4). Enclosures resulted in a significant decrease in $\delta^{13}C$, although they did not have a significant effect on the stable N isotope ratios. The C isotope ratios decreased with increased N loading (Figure 1.4A), although the threshold for a significant response to loading was the highest loading level (Table 1.4A). N isotope ratios of *R. maritima* increased with increased loading (Figure 1.4A), showing a lower threshold of response to N loading at the moderate loading level (Table 1.4B).

Epiphytic material associated with *R. maritima* had $\delta^{13}C$ values from –22.5 ± 0.5 ‰ under control conditions to –20.1 ± 0.3 ‰ under low loading (Table 1.3, Figure 1.4B). $\delta^{15}N$ increased from –0.2 ± 0.3 ‰ in the control units to 2.8 ± 0.7 ‰ in the highest loading treatments. Increased nitrogen loading was associated with significant increases in both $\delta^{13}C$ and $\delta^{15}N$ of epiphytes (Table 1.4). The C isotope ratios responded significantly and similarly to all levels of N loading (Table 1.4A). N isotope ratios showed a significant response to the highest levels of loading (Table 1.4B). Enclosures did not affect the stable C or N isotope ratios of epiphytes.

Table 1.3. Average primary producer and consumer stable C and N isotope values (‰) in the N enrichment experiment. Averages are provided for each of the five nutrient treatments. Numbers of replicates and standard errors are given.

	Control					Ambient				
		$\delta^{13}C$		$\delta^{15}N$			$\delta^{13}C$		$\delta^{15}N$	
	N	mean	*S.E.*	mean	*S.E.*	N	mean	*S.E.*	mean	*S.E.*
Primary Producers										
Ruppia maritima	8	-16.6	*0.3*	-3.4	*0.9*	8	-19.4	*0.6*	-3.3	*0.3*
Epiphytic material	8	-22.5	*0.5*	-0.2	*0.3*	8	-22.2	*0.7*	0.9	*0.4*
POM	8	-22.8	*0.3*	1.4	*0.5*	8	-22.1	*0.6*	2.1	*0.6*
Invertebrates										
Grazing chironomidae	6	-23.0	*0.5*	2.3	*0.1*	7	-23.5	*0.7*	3.5	*0.1*
Chironomus	6	-25.9	*0.2*	1.9	*0.2*	8	-27.6	*0.3*	2.4	*0.1*
Enallagma sp.	6	-22.3	*0.2*	3.6	*0.3*	6	-24.4	*0.4*	4.7	*0.1*
Amphipods	4	-20.5	*0.2*	2.0	*0.3*	0	NA	*NA*	NA	*NA*
Fish										
Fundulus	8	-22.8	*0.2*	5.7	*0.1*	6	-25.1	*0.7*	5.3	*0.5*

	Low Loading					Moderate Loading					High Loading				
		$\delta^{13}C$		$\delta^{15}N$			$\delta^{13}C$		$\delta^{15}N$			$\delta^{13}C$		$\delta^{15}N$	
	N	mean	*S.E.*	mean	*S.E.*	N	mean	*S.E.*	mean	*S.E.*	N	mean	*S.E.*	mean	*S.E.*
Primary Producers															
Ruppia maritima	8	-20.2	*0.7*	-1.5	*0.6*	8	-20.2	*0.3*	1.7	*1.2*	8	-22.5	*0.4*	3.3	*0.7*
Epiphytic material	8	-20.1	*0.3*	1.7	*0.5*	8	-20.3	*0.8*	2.4	*0.7*	8	-20.2	*0.3*	2.8	*0.7*
POM	7	-21.0	*0.5*	1.5	*0.6*	8	-22.0	*0.7*	-0.2	*0.4*	8	-20.9	*0.4*	1.1	*1.2*
Invertebrates															
Grazing chironomidae	7	-20.2	*0.4*	4.0	*0.1*	10	-20.4	*0.3*	3.2	*0.2*	6	-21.0	*0.2*	3.9	*0.6*
Chironomus	4	-24.9	*0.9*	2.1	*0.6*	3	-23.6	*1.0*	2.2	*0.1*	3	-23.2	*1.2*	1.7	*0.5*
Enallagma sp.	3	-21.2	*0.5*	5.4	*0.1*	2	-20.2	*0.8*	5.0	*0.5*	3	-19.9	*0.1*	4.3	*0.3*
Amphipods	2	-20.8	*1.3*	2.2	*0.3*	2	-20.6	*0.2*	2.4	*0.4*	4	-21.4	*0.2*	2.0	*0.4*
Fish															
Fundulus	6	-20.9	*0.5*	5.7	*0.2*	8	-21.2	*0.8*	4.8	*0.2*	4	-20.5	*0.4*	5.5	*0.2*

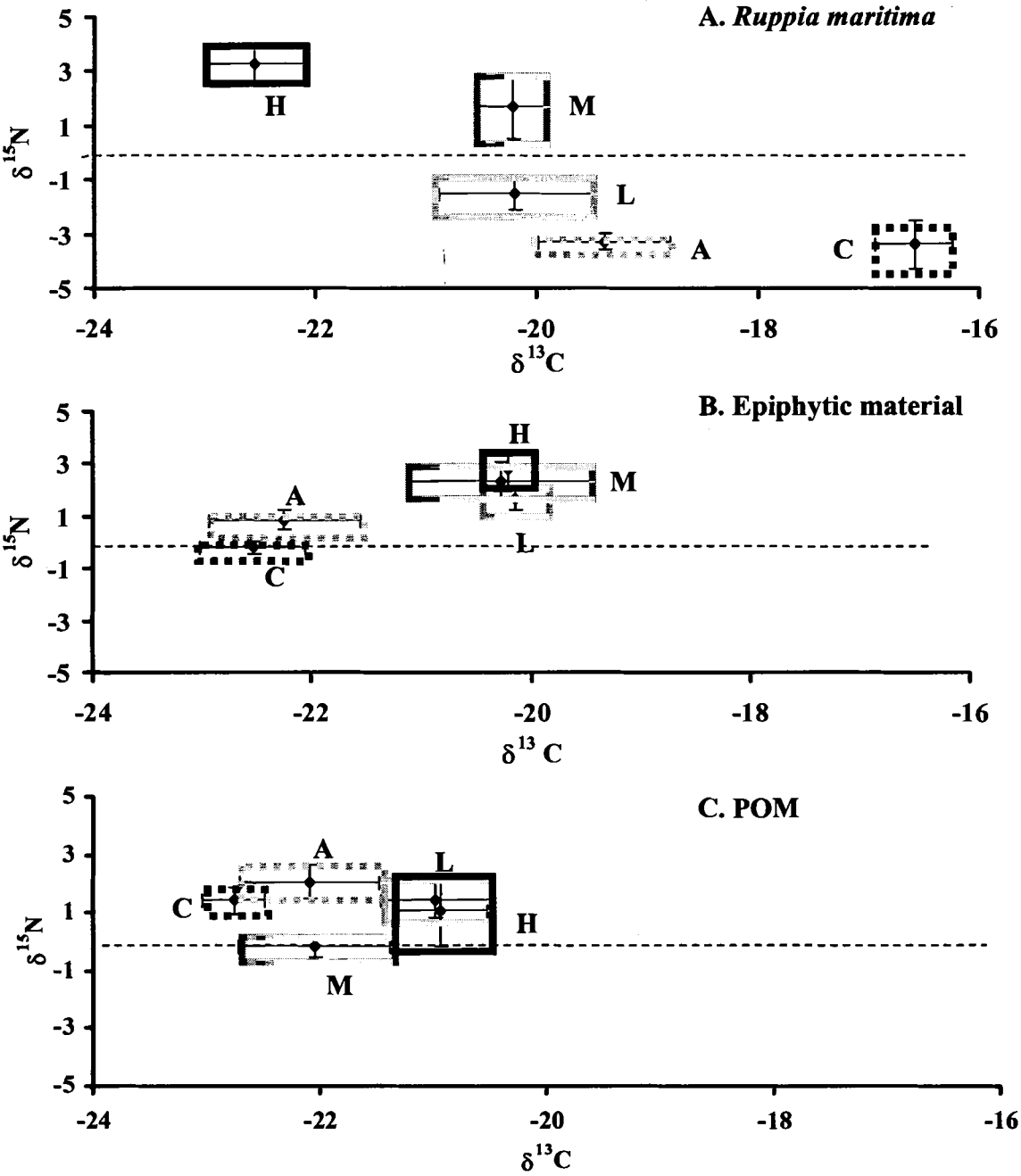


Figure 1.4. Average C and N stable isotope values (‰) of primary producers in the N enrichment experiment. C = control, A = ambient, L = low N loading, M = moderate loading and H = high loading. A. *Ruppia maritima*, B. epiphytic material, and C. Particulate organic matter. Boxes represent 1 standard error.

Table 1.4. Treatment effects on the C and N stable isotope values (‰) of primary producers and consumers in the N enrichment experiment. Test statistics, df and probabilities from analysis of variance tests of the overall effects of treatment are presented. In addition, mean differences 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. A. statistics for $\delta^{13}C$ values and B. statistics for $\delta^{15}N$ values.

A. $\delta^{13}C$

	Treatment Effect			Mesocosm Effect		Threshold of Response to N					
				C-A		L-A		M-A		H-A	
	df	F	p > F	*M.D.*	*p*	*M.D.*	*p*	*M.D.*	*p*	*M.D.*	*p*
Primary Producers											
Ruppia maritima	4	18.18	0.000	2.80	0.000	-0.80	0.267	-0.83	0.252	-3.15	0.000
Epiphytic material	4	4.65	0.004	-0.29	0.719	2.11	0.011	1.98	0.017	2.05	0.014
POM	4	2.36	0.073	-0.67	0.357	1.11	0.143	0.04	0.953	1.16	0.114
Invertebrates											
Grazing chironomidae	4	10.82	0.000	0.47	0.502	3.27	0.000	3.08	0.000	2.48	0.001
Chironomus	4	9.48	0.000	1.71	0.023	2.70	0.003	3.95	0.000	4.36	0.000
Enallagma sp.	4	24.96	0.000	2.11	0.000	3.21	0.000	4.14	0.000	4.46	0.000
Fish											
Fundulus	4	8.63	0.000	2.36	0.009	4.22	0.000	3.91	0.000	4.58	0.000

B. $\delta^{15}N$

	Treatment Effect			Mesocosm Effect		Threshold of Response to N					
				C-A		L-A		M-A		H-A	
	df	F	p > F	*M.D.*	*p*	*M.D.*	*p*	*M.D.*	*p*	*M.D.*	*p*
Primary Producers											
Ruppia maritima	4	14.80	0.000	-0.11	0.925	1.77	0.119	5.00	0.000	6.58	0.000
Epiphytic material	4	5.37	0.002	-1.07	0.154	0.82	0.270	1.49	0.049	1.94	0.012
POM	4	1.37	0.266	-0.62	0.515	-0.61	0.563	-2.25	0.032	-1.021	0.318
Invertebrates											
Grazing chironomidae	4	4.88	0.004	-1.13	0.012	0.53	0.201	-0.24	0.534	0.47	0.277
Chironomus	4	1.06	0.405	-0.54	0.113	-0.28	0.462	-0.16	0.700	-0.67	0.117
Enallagma sp.	4	7.35	0.002	-1.1	0.002	0.67	0.086	0.27	0.527	-0.39	0.306
Fish											
Fundulus	4	2.42	0.073	0.37	0.305	0.33	0.391	-0.55	0.135	0.13	0.770

Suspended particulate organic matter $\delta^{13}C$ ranged from –22.8 ± 0.3 ‰ under control conditions to –21.0 ± 0.5 ‰ under low loading and $\delta^{15}N$ ranged from –0.2 ± 0.4 ‰ under moderate loading to 2.1 ± 0.6 ‰ under ambient conditions (Table 1.3, Figure 1.4C). Nutrient treatments did not have any significant effects on stable C or N isotope ratios (Table 1.4).

The stable isotopic signatures of terrestrial detritus were pooled for all treatments (N = 20) since no significant mesocosm and nutrient treatment effects were found on the $\delta^{13}C$ and $\delta^{15}N$ values of this material. $\delta^{13}C$ was –27 ± 0.3 ‰ and $\delta^{15}N$ was –0.4 ± 0.1 ‰. The average $\delta^{15}N$ of Osmocote™ slow release fertilizer was 1.66 ± 0.13 ‰. Average $\delta^{13}C$ of the fertilizer was –30.20 ± 0.05 ‰.

Grazing chironomidae (*Cricotopus* and *Dicrotendipes* sp.) $\delta^{13}C$ values ranged from –23.5 ± 0.3 ‰ in ambient units to –20.2 ± 0.4 ‰ under low N while $\delta^{15}N$ values ranged from 2.3 ± 0.1 ‰ in controls to 4.0 ± 0.1 ‰ under low N (Table 1.3, Figure 1.5). Significant treatment effects were found for both C and N stable isotope ratios (Table 1.4). Nitrogen loading resulted in a significant increase in $\delta^{13}C$ even at the lowest level of loading (Table 1.4A) but no changes in $\delta^{15}N$ (Table 1.4B). Enclosures had a significant effect on $\delta^{15}N$ but no effect on $\delta^{13}C$. Graphical comparisons of the C and N isotope ratios of these chironomids with those of potential food sources indicated that the ratios shifted consistently with epiphytic material in each loading treatment (Figure 1.5), but not with *R. maritima*, POM or terrestrial detritus. For both the Euclidean distance index and the linear mixing model we assumed that epiphytic material, *R. maritima* and terrestrial detritus were the three most important components of the diets of these chironomids.

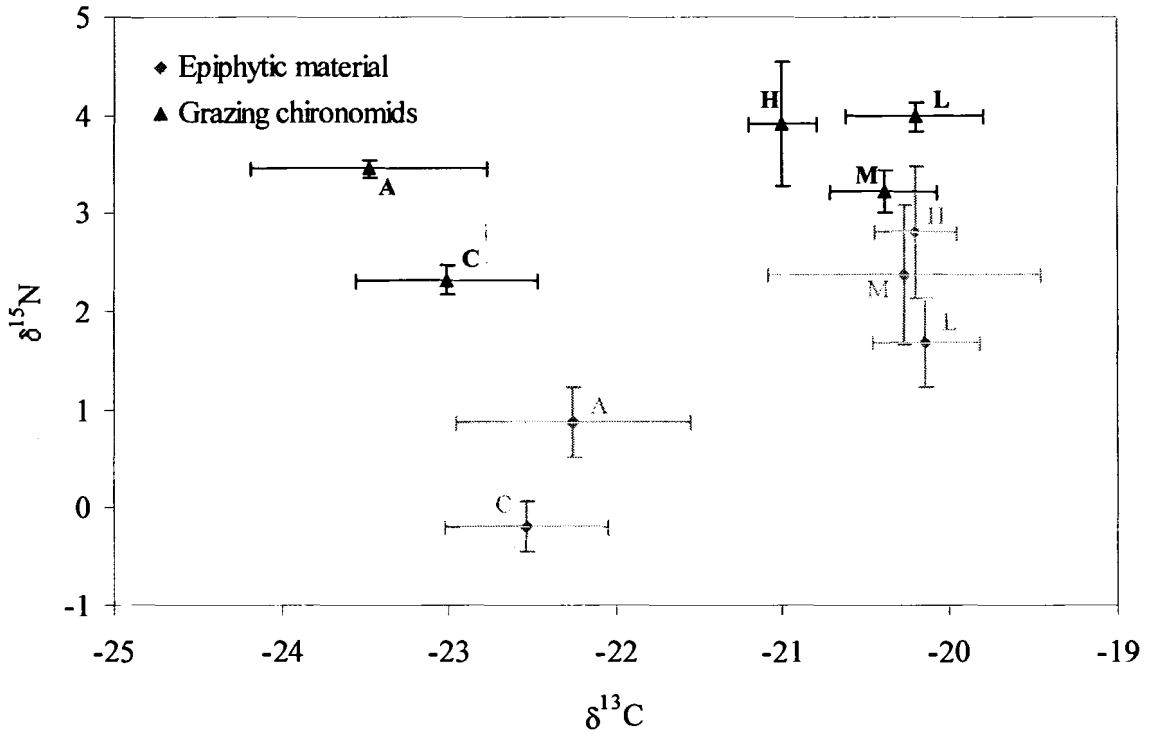


Figure 1.5. Average C and N stable isotope values (‰) of grazing chironomidae and epiphytic material in the N enrichment experiment. C = control, A = ambient, L = low N loading, M = moderate loading and H = high loading. Standard error bars are shown.

The Euclidean distance model ranked epiphytic material as the most important food source for grazing chironomids in all treatments. Terrestrial detritus was of secondary importance under control and ambient conditions and *R. maritima* was of secondary importance in the loading treatments (Table 1.5). According to linear mixing models, diets of grazing chironomids consisted of between 68.2% (highest loading) and 97.4% (control) epiphytic material (Table 1.6, Figure 1.6A). *R. maritima* and detritus were less important although in the highest loading treatments *R. maritima* was estimated to contribute to 30.6% of the diets of these chironomids (Table 1.6). Average literature fractionation values were used in the linear mixing models for control, ambient and low loading conditions. Because of the proximity of the isotope ratios of *R. maritima* and epiphytic material under moderate and high loading conditions, slightly higher $\Delta\delta^{13}C$ values (0‰) and lower $\Delta\delta^{15}N$ values (1‰) were used to provide fit to the linear model. Greater uncertainty surrounds the predictions under these loading conditions (Table 1.6).

Chironomus $\delta^{13}C$ values varied from –27.6 ± 0.3 ‰ in ambient mesocosms to -23.2 ± 1.2 ‰ under highest loading (Table 1.3, Figure 1.7). $\delta^{15}N$ values varied from 1.7 ± 0.5 ‰ in high loading to 2.4 ± 0.1 ‰ under ambient conditions. Nitrogen loading resulted in a significant increase in $\delta^{13}C$ values even at the lowest levels (Table 1.4A) although it had no effect on $\delta^{15}N$ signatures (Table 1.4B). Mesocosms did not significantly affect either C or N isotope ratios (Table 1.4).

Table 1.5. 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*, *Enallagma* and *Fundulus heteroclitus*. 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
Ruppia maritima	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
Chironomus										
Epiphytic material	0.36	2	0.29	2	0.32	2	0.37	1	0.28	2
Ruppia maritima	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
Enallagma										
Grazing Chironomidae	0.50	1	0.60	1	0.54	1	0.48	1	0.59	1
Chironomus	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
Fundulus heteroclitus										
Grazing Chironomidae	0.25	2	0.22	2	0.16	2	0.25	2	0.33	2
Chironomus	0.17	4	0.14	3	0.05	4	0.13	4	0.12	4
Enallagma	0.39	1	0.56	1	0.71	1	0.44	1	0.41	1
Other	0.19	3	0.08	4	0.08	3	0.18	3	0.15	3

Table 1.6. Linear mass balance mixing model predictions of proportions of food sources in the diets of consumers in the N enrichment experiment. Proportions are provided for all nutrient treatments for grazing chironomids, *Chironomus*, *Enallagma* and *Fundulus heteroclitus*. Importance is indicated by percentage estimates. Standard errors (S.E.) of these estimates are also reported.

	Control		Ambient		Low N		Moderate N		High N	
	mean	*S.E.*	mean	*S.E.*	mean	*S.E.*	mean	*S.E.*	mean	*S.E.*
Grazing Chironomidae										
Epiphytic material	97.4	*23.7*	92.1	*16.4*	95.2	*14.7*	83.3	*102.0*	68.2	*34.6*
Ruppia maritima	0.2	*8.2*	-5.8	*8.6*	9.8	*15.4*	14.7	*105.4*	30.6	*51.3*
Terrestrial Detritus	2.5	*18.6*	13.7	*17.6*	-5.1	*7.6*	1.9	*10.9*	1.3	*17.5*
Chironomus										
Epiphytic material	11.5	*17.2*	6.4	*7.3*	12.8	*18.7*	52.6	*76.0*	41.4	*49.1*
Ruppia maritima	9.6	*6.9*	-6.6	*3.9*	23.9	*20.3*	3.3	*86.6*	21.4	*53.5*
Terrestrial Detritus	78.9	*11.3*	100.3	*7.6*	63.2	*14.0*	44.1	*15.6*	37.2	*14.7*
Enallagma										
Grazing chironomidae	67.9	*81.4*	77.3	*10.0*	66.6	*8.9*	73.9	*69.0*	46.0	*25.2*
Chironomus	18.1	*38.5*	22.3	*13.0*	18.3	*14.9*	22.9	*29.0*	22.2	*17.9*
Others	14.0	*44.6*	0.4	*4.2*	15.1	*18.6*	3.1	*81.7*	31.9	*35.7*
Fundulus heteroclitus										
Grazing chironomidae	48.6	*24.9*	17.0	*39.7*	64.3	*26.0*	40.9	*49.4*	76.8	*137.3*
Chironomus	28.9	*14.4*	28.1	*17.7*	9.4	*10.6*	53.7	*30.4*	5.8	*39.7*
Enallagma	22.5	*13.5*	54.8	*36.4*	26.3	*18.4*	5.5	*21.7*	17.4	*99.8*

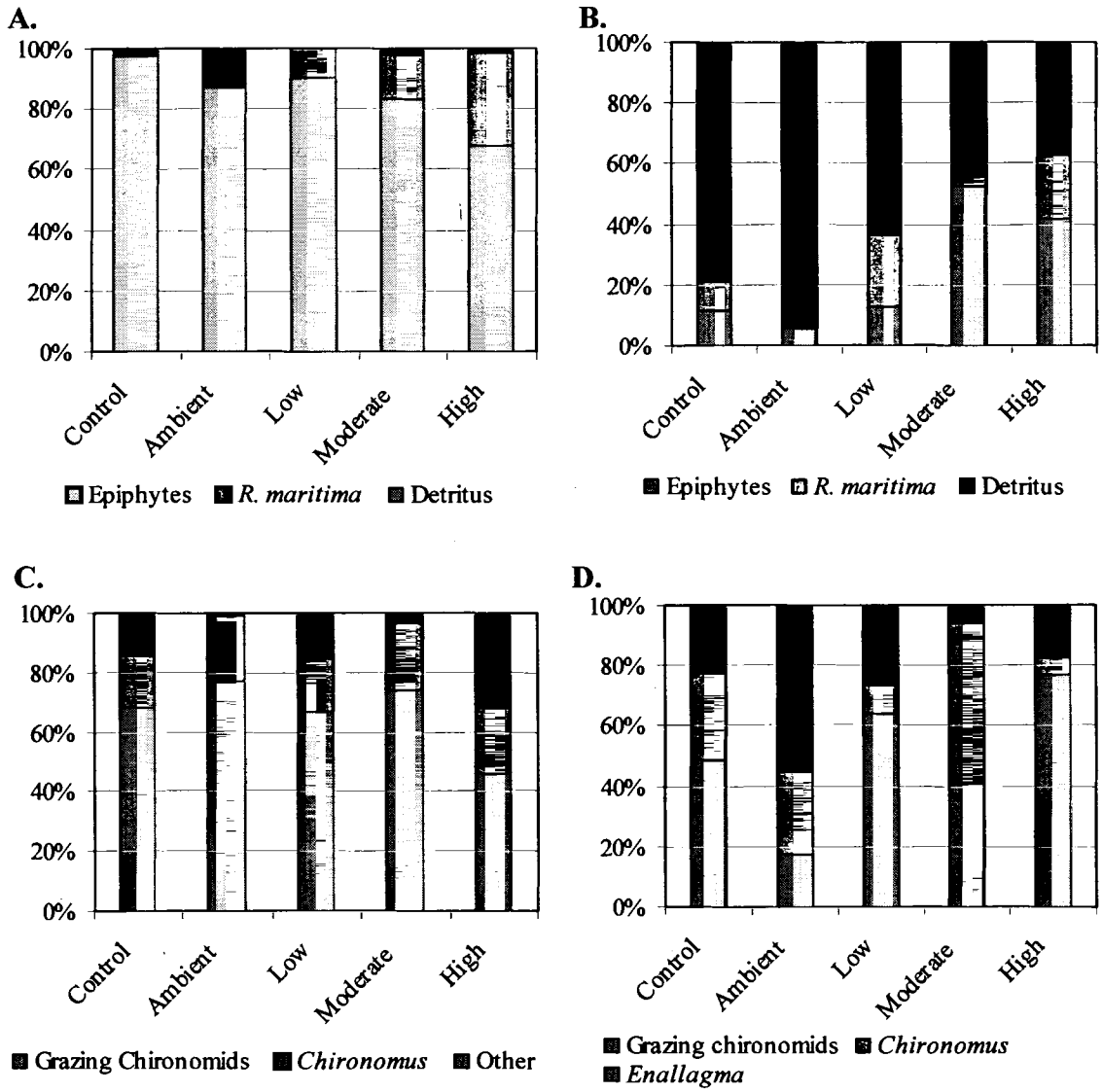


Figure 1.6. Relative importance of food sources to consumers in the N enrichment experiment based on three-source linear mixing models. All treatments are shown. A. Grazing chironomidae. B. *Chironomus* sp. C. *Enallagma* sp. and D. *Fundulus heteroclitus.*

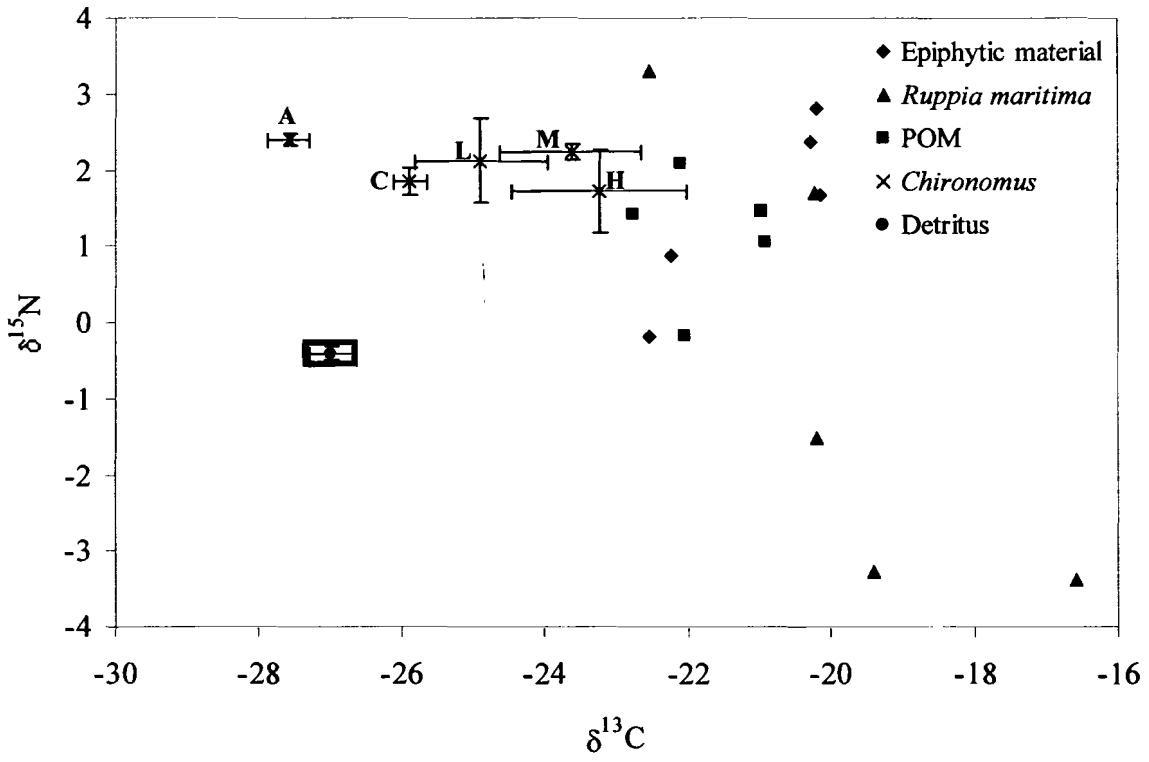


Figure 1.7. Average C and N stable isotopic values (‰) of *Chironomus* and possible food sources in the N enrichment experiment. Standard errors are shown for *Chironomus* and terrestrial detritus (which is boxed). C = control, A = ambient, L = low, M = moderate and H = high loading.

Comparisons of *Chironomus* C and N stable isotopic values to those of potential food sources provides evidence for a shift from a dependence on terrestrial detritus for carbon to a dependence on more autochthonous sources of carbon such as *R. maritima*, POM and epiphytic material (Figure 1.7). Terrestrial detritus was ranked as the most important food source under control, ambient and low loading conditions using the Euclidean distance index (Table 1.5). Epiphytes and *R. maritima* ranked as the most important food sources under moderate and high loading conditions (Table 1.5). 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 (Table 1.6, Figure 1.6B). Under control and ambient conditions, the diets of *Chironomus* were composed of 78.9 and 100% terrestrial detritus respectively. The importance of terrestrial detritus decreases in the loading treatments (63.2%, 44.1% and 37.2% respectively in the low, moderate and high loading treatments). Literature average fractionation values were used for control, ambient and low loading conditions. Because of the overlap in the C and N isotope ratios of *R. maritima* and epiphytic material under moderate and high loading conditions, a $\Delta\delta^{15}N$ of 1‰ was used to create model fit under moderate conditions and a $\Delta\delta^{13}C$ and $\Delta\delta^{15}N$ of 0‰ were used to create fit under high loading conditions. Greatest uncertainty in the model was found for these moderate and high loading conditions (Table 1.6).

Amphipod stable isotope ratios remained relatively constant in all treatments. Values were not obtained for the ambient mesocosms because the numbers of amphipods collected from these units was insufficient for stable isotopic analysis. $\delta^{13}C$ ranged from -20.5 ± 0.2 ‰ in the control mesocosms to -21.4 ± 0.2 ‰ under high N loading while

$\delta^{15}N$ ranged from 2.0 ± 0.3 ‰ in the controls to 2.4 ± 0.4 ‰ under moderate loading. Statistical tests of nutrient treatment effects were not performed for amphipods.

The $\delta^{13}C$ values of the predatory damselfly *Enallagma* varied from –24.4 ± 0.4 ‰ in ambient mesocosms to –19.9 ± 0.1 ‰ under highest loading conditions. $\delta^{15}N$ varied from 3.6 ± 0.3 ‰ under control conditions to 5.4 ± 0.1 ‰ under low loading (Table 1.3, Figure 1.8A). $\delta^{13}C$ increased significantly at all levels of nitrogen loading, indicating a low threshold of response to loading (Table 1.4A). $\delta^{15}N$ was not significantly affected by N loading (Table 1.4B). Mesocosms resulted in significantly decreased $\delta^{13}C$ values and increased $\delta^{15}N$ values (Table 1.4). The C and N isotopic ratios of *Enallagma* shifted with loading in a similar manner to grazing chironomids, although this link was least strong under the highest loading conditions (Figure 1.8A). Grazing chironomids consistently ranked first in importance to damselflies under all loading conditions according to the Euclidean distance index (Table 1.5). Linear mixing models indicated that under all loading treatments grazing chironomids composed the majority of the diets of these damselflies (46-77.3%, Table 1.6, Fig 1.6C). However, other prey may be equally important, especially under control, moderate and high loading conditions, where there is significant variability in the model results (Table 1.6). In addition, because different fractionation values were used to create model fit for each treatment, caution must be used in interpreting the results of these models. Differential fractionation could be part of the reason for the high variability in the model results, although other sources of model failure such as the importance of unmeasured food sources and variability in isotope ratios due to small sample sizes cannot be ruled out.

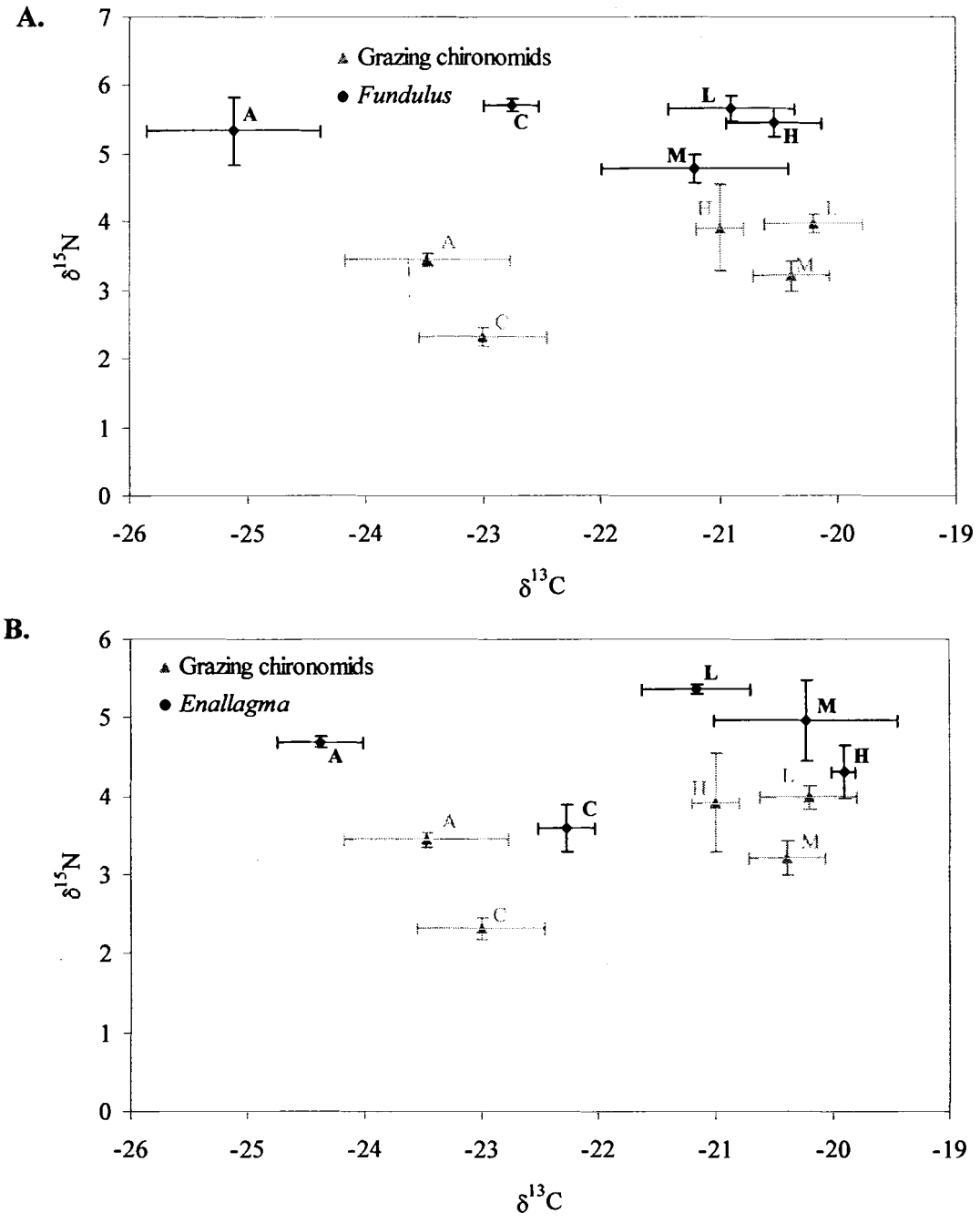


Figure 1.8. Average stable C and N isotope values (‰) of predators and prey in the N enrichment experiment. A. Presents values for *Enallagma* and potential grazing chironomid prey and B. presents values for *Fundulus heteroclitus* and potential grazing chironomid prey for each treatment (Control (C), Ambient (A), Low (L), Moderate (M) and High (H) loading). Error bars represent 1 standard error.

Fundulus heteroclitus $\delta^{13}C$ values ranged from –25.1 ± 0.7 ‰ in ambient enclosures to –20.5 ± 0.4 ‰ under high loading and $\delta^{15}N$ values varied from 4.8 ± 0.2 ‰ under moderate loading to 5.7 ± 0.1 ‰ under control conditions (Table 1.3, Figure 1.8B). Although the N isotope ratios did not significantly vary with treatment (Table 1.4B), the C isotope ratios were significantly and consistently higher under all levels of increased nitrogen loading (Table 1.4A). Mesocosms resulted in a significant decrease in $\delta^{13}C$ (Table 1.4A). Graphical comparisons of the C and N isotope ratios of *F. heteroclitus* and possible prey indicated that shifts in the isotope values of this predator paralleled shifts in grazing chironomids (Figure 1.8B) and *Enallagma* with nutrient loading. The Euclidean distance index consistently ranked *Enallagma* as the most important prey and grazing chironomids as the second most important prey for all treatments (Table 1.5). The quantitative estimates from the linear mixing models were highly variable for *F. heteroclitus*, especially for the highest loading treatments (Table 1.6). These model results must be interpreted with care because one or more of the assumptions may have been violated. Different fractionation values (within one standard deviation of the average literature values) were used to attain model fit for each treatment. In addition, because one of the prey items most likely consumes another (*Enallagma* feed on grazing chironomidae), the model is not able to properly distinguish between these sources. The mixing models predict that both *Enallagma* and grazing chironomids are the most important prey for these predatory fish, although *Chironomus* was estimated to be the largest proportion of the diet (53.7%) under moderate loading conditions (Table 1.6, Figure 1.6D).

Although oligochaetes were common under the highest loading treatments, we did not obtain sufficient biomass for stable isotope analysis of these organisms. Oligochaetes may have been an important source of prey for predators under these nutrient conditions and without knowing their isotope ratios, caution should be used in interpreting the results of mixing models for both *Enallagma* and *F. heteroclitus* in the high loading treatments.

Discussion

Faunal Community Shift

Increased nitrogen loading resulted in significant changes in the densities of the macroinvertebrate community, including reductions in grazing chironomidae and predatory *Enallagma*, and increases in deposit feeding oligochaetes. These responses were most evident at the highest level of loading used in the experiment. The densities of deposit feeding *Chironomus* and ostracods did not change significantly even at the highest loading level. In general, the community shifted from a grazer dominated to a benthic deposit-feeder dominated community.

The macroinvertebrate community of this 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, Rafaelli 1999). However, the hydrologic and salinity regime of Northeast Creek 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. While 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 1998a, Williams and Hamm 2002), studies of the effects of nutrient loading on similar communities are lacking. In general, the community shifts seen in Northeast Creek do follow the model proposed by Heip (1995) regarding eutrophication and community dynamics. As nitrogen 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.

The faunal community shifts observed in this experiment imply that N additions to NEC estuary would result in shifts in the faunal community of the entire estuary. However, use of the mesocosm experiment for predicting the full impacts of large-scale eutrophication on this community is limited by the experimental design. The experiment was designed by USGS investigators primarily to quantitatively assess plant community response to nitrogen and quantitative assessments of the faunal community are limited by the fact that mesocosms were not fully enclosed. The mesocosms had significantly lower densities of grazing chironomids and in general resulted in lower species richness. Insect larvae such as chironomids and damselflies may have emerged from the mesocosms as adults during the experiment and replenishment of these populations may not have been consistent between the open estuary controls and inside the enclosures. Changes to experimental design to assess invertebrate response would require complete closure of

the mesocosms and use of stocked numbers of invertebrates, or the use of emergence traps to correct for losses due to emergence. In addition, the duration of the experiment was only two months. While this time period allowed organisms with shorter generation times (such as oligochaetes) to respond to the increased nutrients, it may have underestimated the response of organisms with longer generation times. Overall, the experiment most likely underestimated the effects that increased eutrophication would have on the macroinvertebrate community of NEC estuary.

Primary Producers and Carbon Limitation

Primary producer carbon stable isotope ratios varied substantially with nutrient treatment. The decrease in *R. maritima* $\delta^{13}C$ associated with the mesocosms is most likely an indication that the enclosures caused a change in C sources available to these macrophytes. The enclosures may have limited the movement of C by reducing flushing rates. While the average flushing rate of the estuary was approximated by the mesocosms, the design did not incorporate the natural periods of extremely high flushing during spring high tides. Reductions in flushing rates could cause these macrophytes to have a greater dependence on C derived from decomposition or recycled C (which is lighter than other C sources). Smith et al. (1976) found that seagrasses grown in a greenhouse were 5-9 ‰ more depleted in ^{13}C compared to seagrasses in the field due to the increased use of recycled C.

The $\delta^{13}C$ signatures of *R. maritima* and epiphytic material changed in opposite ways with increased N loading. Epiphytes were less depleted in ^{13}C with nitrogen loading while *R. maritima* was more depleted in ^{13}C with loading. Under natural conditions, it is

generally thought that aquatic macrophytes are carbon limited while algae are carbon saturated and that increased productivity will increase carbon limitation (Hemminga and Duarte 2000). In general, greater metabolic activity by producers has been found to result in decreased discrimination against ^{13}C (Smith et al. 1976, Osmond et al. 1981, Guy et al. 1989, Fogel et al. 1992, Maberly et al. 1992, Grice et al. 1996, MacLeod and Barton 1998, Finlay et al. 1999, Hemminga and Duarte 2000). This shift is thought to occur because (1) there is an increased uptake of all carbon under these conditions, and (2) 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 nitrogen treatments, while the ratio of epiphytic material per unit *R. maritima* biomass and phytoplankton Chl *a* concentrations significantly increased with loading (Neckles et al. unpublished data). Under increased N conditions, this greater epiphytic and planktonic algal biomass may have inhibited growth of *R. maritima*. As a result, this macrophyte discriminated more against the heavier C isotope under these enriched conditions. In addition, the thicker growth of epiphytes on *R. maritima* under high N conditions may have increased the dependence of this macrophyte on isotopically lighter recycled C. 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 $\delta^{13}C$ are simply high variability in signatures within a single species (as documented by Boyce et al. (2001) in *Ruppia megacarpa*) and signature variability between algal species (algal community composition was not measured but was observed to have a more flocculent community

under ambient conditions and a highly filamentous community with N additions (R. Keats personal observation)).

Primary Producers and Nitrogen

Nitrogen loading resulted in increased $\delta^{15}N$ in both *R. maritima* and its associated epiphytic material. These increases in $\delta^{15}N$ most likely occurred because the addition of fertilizer resulted in significant increases in the N stable isotope ratios of inorganic nitrogen in the system, either directly or indirectly by altering nitrogen cycling within the mesocosms.

The average $\delta^{15}N$ of Osmocote™ slow release fertilizer was 1.66 ‰. Fertilizers tend to have $\delta^{15}N$ similar to atmospheric N (0 ‰) because they are manufactured by conversion of these sources (Freyer and Aly 1974, Gormly and Spalding 1979, Macko and Ostram 1994). While increases in $\delta^{15}N$ with nutrient loading have been documented for primary producers in other studies (Grice et al. 1996, Hansson et al. 1997, McClelland et al. 1997, McClelland and Valiela 1998b), the high $\delta^{15}N$ of sewage discharge and other wastewater sources is thought to have been responsible for these shifts. The N stable isotope signature of the fertilizer is not high enough to account for the increases in $\delta^{15}N$ 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 in organic inputs to the benthos (Heip 1995). Hypoxic conditions would increase denitrification, a process that results in large increases in the $\delta^{15}N$ of inorganic N left in

the system (Macko and Ostram 1994, Michener and Schell 1994). As little as 20% total nitrate removal by denitrification will result in an 8‰ increase in $\delta^{15}N$ (Heaton 1984). Thus, secondary effects of the fertilizer resulting in increased denitrification would result in the trend of increasing $\delta^{15}N$ observed for both epiphytes and macrophytes in NEC estuary.

Food Source Shifts in Consumers

Several shifts in the food sources and abundances of the most common invertebrates and fish were observed with experimental N loading in NEC estuary. Grazing chironomids were dependent on epiphytic material under natural as well as increased nitrogen loading conditions, although they declined in abundance under increased N conditions. Predatory *Enallagma* were largely dependent on grazing chironomids under all conditions and declined in density alongside this primary food source. *Fundulus heteroclitus* were largely dependent on both *Enallagma* and chironomid prey except in the highest loading units. *Chironomus* did not change in abundance with increased loading but shifted from a dependence on allochthonous sources of detritus under natural conditions to autochthonous sources of detritus such as *R. maritima* and epiphytic material under higher loading conditions. Although oligochaetes increased in density with N loading, we were unable to obtain enough material for stable isotope analysis. In general, oligochaetes are deposit feeders (Gosner 1971) and their isotopic compositions probably followed a similar pattern to *Chironomus*. Oligochaetes may have been the important food source for predatory *Enallagma* and *Fundulus heteroclitus* under high loading conditions although they were not included in the mixing model predictions.

In general, experimental N loading led to a shift from a community dependent on live epiphytic material to a community dependent on autochthonous detrital material.

Evidence from C and N stable isotope ratios indicates that live *R. maritima* is of limited importance to the herbivorous chironomids of Northeast Creek estuary under both natural and enriched conditions and that epiphytes are the preferred food source. Investigators have documented the disproportionate importance of epiphytic material compared to macrophytes in many undisturbed estuarine communities (Kitting et al. 1984, Stephenson et al. 1986, Sullivan and Moncreiff 1990, Loneragan et al. 1997). Epiphytes in general are preferred by grazers because they have lower C:N ratios and are easier to consume.

Changes in the food resources available to grazing chironomids under increased N loading may have been partially responsible for the decline in 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 the biomass of this epiphytic material due to significant declines in *R. maritima* biomass (Neckles et al. unpublished data). In addition, inspection of the epiphytic material indicated that the quality of the epiphytes to these chironomids probably did not increase with loading. Easy to consume diatoms were found in all treatments but less desirable filamentous algae appeared to become more common under increased N conditions (R. Keats personal observation). Reductions in the quantity and quality of the epiphytic material may have been responsible for the decline in herbivores. However, losses of *R. maritima* associated with increased loading (Neckles et al. unpublished data) as well as increases in organic matter deposition may also have been important in determining survival. Deegan

et al. (2002) found that changes in the physical and chemical habitat structure of estuaries in Waquoit Bay, Massachusetts resulting from losses of macrophyte beds with increased nutrient loading were the most likely cause of reductions in fish abundance and diversity and herbivore abundance. While *R. maritima* may not be an important food source to herbivores in NEC estuary, it may provide habitat structure and protection from predation by *Enallagma* and *F. heteroclitus*. In addition, decreases in dissolved oxygen that often accompany increases in organic matter deposition with increased loading may also have degraded living conditions for these herbivores (Heip 1995).

While herbivores did not show a shift in food sources, deposit-feeding *Chironomus* appeared to become less dependent on terrestrial detritus under increased N loading conditions, indicating a shift in the sources of detritus. 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 et al. unpublished data). Similar shifts in detritus have been documented in estuaries exposed to different nutrient regimes (Heip 1995, McClelland and Valiela 1998a). In general, because consumers can more readily access algal sources of detritus, an increase in cycling occurs with this detrital shift and there is a loss in stability in the system. Although the mesocosm experiment had a duration of only two months, this shift in detrital sources had already begun to be evident in detritivores in the system. If increased nutrient loading were to occur in Northeast Creek, much larger changes would most likely take place.

Fit of the linear mixing models for both of the important predators in NEC estuary required adjustments to the average literature fractionation values in all treatments. We used lower fractionation estimates for $\Delta\delta^{15}N$ in all treatments for both predators. It seems reasonable that fractionation by these predators is lower than the literature average. Hart and Lovvorn (2002) found that average fractionation by *Enallagma* ranged from 1-3.4 ‰ between saline wetlands. In addition, gut contents of food sources and omnivory could also lower the trophic level enrichment in N (Marguiller et al. 1997, Hart and Lovvorn 2002). However, because we do not know the actual fractionation values for these predators, we interpret the results of these models with the simple comparisons of the C and N isotope ratios of these predators to those of their potential prey using the Euclidean distance index and visual assessments. Together, these methods allowed us to determine the primary importance of grazing chironomid prey to *Enallagma* and the importance of both grazing chironomids and *Enallagma* as prey to *F. heteroclitus*. It is reasonable that chironomid larvae were important food sources to predators in NEC estuary under both natural and low loading conditions. 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). In addition, 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.

Implications

Two months of experimental nitrogen loading resulted in changes in the composition and structure of the faunal community of Northeast Creek estuary. The largest shifts in macroinvertebrate densities occurred between the moderate and high levels of loading (16.8-33.8 mmol DIN $m^{-2}d^{-1}$). 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. We expect that with increased N loading the community would become more dependent on autochthonous sources of detritus, and that, ultimately, benthic deposit feeders would dominate this community. Based on the results of this experiment, we would predict that even greater increases in loading or longer periods of low loading could cause similar or larger changes in faunal community composition and food web structure in Northeast Creek estuary.

Chapter 2

FAUNAL COMMUNITY COMPOSITION IN *RUPPIA MARITIMA* BEDS IN A NORTHEASTERN USA ESTUARY

Chapter Summary

Estuaries are some of the most threatened ecosystems today, both by increased coastal development and human impacts and by the relatively poor knowledge base upon which management decisions are currently being made. Very little information exists on faunal community composition in Maine estuaries. We completed qualitative and quantitative assessments of the macroinvertebrate community of *Ruppia maritima* beds in Northeast Creek estuary (Acadia National Park, Maine) from May to October in 2001. Salinity in the estuary indicated that the system was dominated by freshwater inputs in the spring and became increasingly more saline throughout the summer (reaching 30 ‰ in the fall). Euryhaline freshwater invertebrates dominated Northeast Creek estuary throughout 2001. The most common invertebrates were non-biting midge larvae (Chironomidae: *Dicrotendipes*, *Cricotopus* and *Chironomus*), damselflies (Coenagrionidae: *Enallagma*), gastropods and ostracods. Less common invertebrates included oligochaetes, water boatmen (Corixidae: *Trichocorixa*), water mites (Acari), and amphipods (Gammaridae: *Gammarus*). Total macroinvertebrate densities were 31100 m^{-2} in August, 23200 m^{-2} in September and 27700 m^{-2} in October. Freshwater insects composed between 50 and 80% of the faunal community in NEC estuary between August and October 2001.The estuary was characterized by low species richness, diversity and

evenness, which is typical of brackish water environments. This study provides a baseline on faunal community composition in NEC estuary for use in future monitoring studies.

Introduction

Estuaries and coastal waters worldwide are continually threatened by increased coastal development and its associated human impacts. These waters are some of the most nutrient enriched ecosystems on earth (Nixon 1995). Unfortunately, estuaries are also some of the most variable and least well-studied ecosystems. In 1991, the International Estuarine and Coastal Sciences Association placed spatial and temporal comparisons of estuaries as the highest research priority in order to improve our knowledge of the natural variability of these systems for making educated management decisions (Elliot and Ducrotoy 1991).

The Maine coast has 5,500 miles of coastline and 1,633 square miles of estuaries (MEPP 1995). Close to 5% of Maine's estuaries have been filled and nearly 80% have been altered (MEPP 1995). Today, estuarine resources in coastal Maine are threatened by nutrient enrichment associated with atmospheric deposition (Miller 1999) and increased development in contributing watersheds. Though much work has been done to investigate the effects of anthropogenic impacts on estuarine systems, little research has been done on estuarine systems in Maine (Kahl et al. 1999). Scant information exists on the faunal communities of Maine's estuaries. Without prior knowledge of the natural communities, it will be impossible to detect changes in these communities and to design and direct restoration projects should environmental conditions deteriorate.

Faunal communities of estuaries are highly variable, due to the variable physical conditions of these systems. The distribution of species is driven mainly by salinity and

salinity fluctuations (Sanders et al. 1965, Remane and Schlieper 1971, Ristich et al. 1977, Verhoeven 1980, Lopez 1988, Diaz 1989), although size of the estuary, substrate size and stability, and past and present connections to other water bodies for colonization are also important in determining distributions (Verhoeven 1980, Williams and Hamm 2002). In general, four types of organisms inhabit estuaries: holeuryhaline species (which can tolerate the whole salinity range), euryhaline freshwater species (which originate in freshwater but tolerate some salinity), euryhaline marine species (which originate in the ocean but tolerate lower salinities) and brackish water specialist species (Remane and Schlieper 1971). In general, there are more euryhaline freshwater species than marine species in brackish waters because freshwater organisms are more tolerant of a range of salinities. The salt tolerance of freshwater species is attributed to the greater chemical variability in lake and stream habitats compared with more constant salinity marine habitats (Lopez 1988). However, estuarine systems tend to have fewer species than both marine and freshwater systems (Remane and Schlieper 1971, Verhoeven 1980). Cheng (1976) suggests that estuaries serve as "bridging" habitats for the speciation of freshwater organisms (insects in particular) into marine systems. Significant numbers of euryhaline insects have been found in some estuarine systems (Williams and Williams 1998a, Williams and Hamm 2002) and saltmarsh pools (Sutcliffe 1961).

Submerged vascular plant beds are generally thought to contribute disproportionately to species diversity and productivity in estuarine systems (Orth et al. 1984, Fredette et al. 1990, Heck et al. 1995, Mattila et al. 1999). These aquatic macrophytes provide habitat complexity, abundant food sources, sediment stability and refuge from predation compared to surrounding soft substrates. Three types of organisms

live in macrophyte beds: epifauna, infauna, and mobile species (Orth et al. 1984). In general, seagrass faunal communities tend to be dominated by crustaceans, polychaetes, mollusks, oligochaetes and gastropods (Fredette et al. 1990, Heck et al. 1995, Knowles and Bell 1998, Mattila et al. 1999). *Ruppia maritima* is a submerged vascular plant that is common in estuaries but not restricted to areas of high salinity. In contrast, seagrasses are defined as submerged vascular plants that are restricted to marine waters and high salinity reaches of estuaries. To date, very few studies of the fauna of *Ruppia maritima* beds exist. The studies that have been done have found faunal communities in *Ruppia* beds in high salinity waters to be similar to those in seagrass beds (Verhoeven 1980, Wenner and Beatty 1988, Heck et al. 1995, Knowles and Bell 1998). However, because *R. maritima* beds extend over a wide range of salinities, community species composition is more variable. While seagrass beds are always dominated by marine species, *R. maritima* may support a range of euryhaline marine and freshwater species as well as brackish water specialists (Verhoeven 1980).

Estuaries are widely variable environments, physically, chemically and biologically. To detect variability in faunal communities due to anthropogenic causes, we must first understand the natural variability of these systems (Elliot and Ducrotoy 1991). Inter-estuary variability also makes it difficult to rely on between estuary faunal comparisons to detect significant environmental changes (Schmitt and Osenberg 1996, Rafaelli 1999). Before and after comparisons are a much more substantial monitoring tool. However, long term monitoring provides the best tool for detecting environmental changes early on (Rafaelli 1999).

The Northeast Creek (NEC) estuary of Acadia National Park is a current site for integrated research by USGS in collaboration with scientists from the University of Maine and the National Park Service. One goal of this research is to develop a monitoring program for detecting environmental change (Nielsen et al. in progress, Neckles et al. in progress). NEC estuary is a relatively pristine system threatened by atmospheric pollution and increased residential development. It is located in an area where nearby estuarine systems are already moving towards eutrophic conditions (Doering and Roman 1994, Doering et al. 1995, Kinney and Roman 1998, Farris and Oviatt 1999).

Little baseline research has been done in estuaries similar to Northeast Creek estuary. The combination of the estuary's small size, salinity regime, high latitude and submerged macrophyte community make it a relatively unique system. As a result, scant information exists for monitoring the faunal community. We completed qualitative and quantitative assessments of the faunal community in the *R. maritima* beds of Northeast Creek estuary from May to October of 2001 in order to describe and document the community of this system for future monitoring.

Methods

Study Site

Northeast Creek estuary is located in Acadia National Park, Mount Desert Island, Maine (Figure 1.1). This small estuary occupies a drowned river valley fed by a number of freshwater streams. It is approximately 4 km long with a watershed of 6400 acres. Lands in the surrounding watershed contain some of the fastest growing areas of single-family homes in the region. The estuarine system includes approximately 500 acres of

emergent estuarine wetlands, emergent riverine tidal fresh wetlands, palustrine shrub-scrub wetlands and subtidal vegetated habitat. The estuary averages about 1m in depth with a narrow tidal range (<0.5m). An old rock dam impedes tidal exchange so that the estuary is generally poorly flushed. Mean top and bottom salinities throughout the 2001 period of this study show that salinity levels are highly seasonal and are strongly influenced by stream discharge (Figure 1.2, USGS unpublished data). The estuarine system is densely vegetated with *Ruppia maritima* along most of its length. This study was performed in a particularly dense bed of *R. maritima* just downstream of the mouth of Aunt Betsy's Creek (Figure 1.1). The substrate of the estuary in this location is a silt loam soil containing organic matter of detrital origin.

Sample Collection

Qualitative samples of the fauna associated with *R. maritima* were collected by grabbing samples of the macrophytes using 1 gallon plastic bags on May 25, June 28, July 12 and July 27 in 2001. On the same dates, four quantitative samples of the infauna down to 10 cm depth were taken using a 10 cm diameter core sampler. The core sampler was modified from the sampler described by Miller and Bingham (1987) by reducing the handle to 15 cm and using the size dimensions of the sampler described by Davis and Steinman (1998). This core sampler was used to quantitatively sample the infauna, epifauna and mobile fauna associated with *R. maritima* beds on August 24, September 13 and October 19 in 2001 by modifying the sampling technique. This modified technique involved the following steps: 1) random placement of the core sampler in a dense macrophyte bed, 2) use of hand held garden shears held parallel to the sides of the sampler to gently separate shoots and roots of *R. maritima* not associated with the

sample, 3) insertion of the sampler 10 cm into the estuarine soil, 4) use of a stopper to seal the sampler, and 5) removal of the sample into a large bucket. Without trimming *R. maritima* during the sampling procedure, it was not possible to push the sampler into the soil and maintain the seal needed to successfully pull the sample out. This technique allowed for the sampling of the entire water column of this shallow estuary, including the macrophytes and the benthos. Seven replicate samples were collected per sampling date.

All qualitative and quantitative samples were rinsed through a 500 μm sieve and preserved in 70% ethanol with Rose Bengal dye. Invertebrates were removed from the samples and identified to genus when possible (using keys in Weiderholm 1983, Peckarsky et al. 1990, Merritt and Cummins 1996, Epler 2001). Information on presence and absence of taxa were used to qualitatively compare samples throughout the seasons. Mean densities of the invertebrates were calculated for the quantitative samples based on the surface area of the core sampler (78.5 cm^2). Biodiversity (H') and taxa evenness (J') were calculated using the Shannon-Weiner Biodiversity Index (Pielou 1966) for the quantitative August, September and October samples. Taxa richness (the total number of taxa) was also calculated for these samples.

Results

The faunal community of NEC estuary was dominated by euryhaline freshwater taxa, especially insects, throughout the 2001 sampling period (Table 2.1). During this period, water salinity increased substantially (Figure 1.2). Both top and bottom salinities increased from 0 ‰ in May and June to around 30 ‰ in October.

A total of 14 macroinvertebrate taxa, including nine insect taxa and two crustacean taxa, were found in the *Ruppia maritima* beds of NEC estuary between May

and October 2001 (Table 2.1). Larval *Dicrotendipes*, *Cricotopus* and *Chironomus,* three genera of the Dipteran family Chironomidae (non-biting midges), were observed throughout the collection period (Table 2.1). Dipteran larvae from other families were less common in the estuary, including Ceratopogonidae (found in August and October only) and Tabanidae (found in August only). The damselfly larvae *Enallagma* (Coenagrionidae) was observed during every collection period except late June (Table 2.1). One dragonfly larvae (Odonata: Anisoptera) was found in the August 24th collection. Water boatmen (Corixidae: *Trichocorixa*) were observed in the estuary from the end of June to October. Water mites (Acari) were found in all of the samples collected between May and the end of July in 2001, and again in the September samples. Amphipods (Gammaridae: *Gammarus*), ostracods and oligochaetes were present throughout the sampling period. Gastropods were only found in samples taken after the middle of July.

Average total faunal densities were 31100 m^{-2} in August, 23200 m^{-2} in September and 27700 m^{-2} in October. The densities of individual taxa changed during this three month period in different ways (Figure 2.1). Total numbers of chironomids declined between August and October, with the majority of this decline due to decreased numbers of *Cricotopus* (1600 m^{-2} to 90 m^{-2}) and *Chironomus* (8200 m^{-2} to 1600 m^{-2}) (Figure 2.1). *Dicrotendipes* increased from 14800 m^{-2} in August to 25500 m^{-2} in September with a decrease to 9700 m^{-2} in October (Figure 2.1). *Enallagma* decreased from 1500 m^{-2} in August to 90 m^{-2} in October (Figure 2.1). *Trichocorixa* almost disappeared in October (20 m^{-2}) after they were observed at similar higher levels in August and September (approximately 500 m^{-2}) (Figure 2.1).

Table 2.1. Presence of macroinvertebrate taxa in *Ruppia maritima* beds in Northeast Creek estuary from May to October 2001. Presence of taxa is indicated by an X.

	2001						
Taxa	**May 25**	**June 28**	**July 12**	**July 27**	**Aug. 24**	**Sept. 13**	**Oct. 19**
Insecta							
Chironomidae							
Dicrotendipes	X	X	X	X	X	X	X
Cricotopus	X	X	X	X	X	X	X
Chironomus	X	X	X	X	X	X	X
Ceratopogonidae					X		X
Tabanidae					X		
Other Diptera	X	X		X	X	X	
Coenagrionidae							
Enallagma	X		X	X	X	X	X
Other Odonata					X		
Corixidae							
Trichocorixa		X	X	X	X	X	X
Acari	X	X	X	X		X	
Crustacea							
Malacostraca							
Gammaridae							
Gammarus	X	X	X	X	X	X	X
Ostracoda	X	X	X	X	X	X	X
Gastropoda				X	X	X	X
Oligochaeta	X	X	X	X	X	X	X

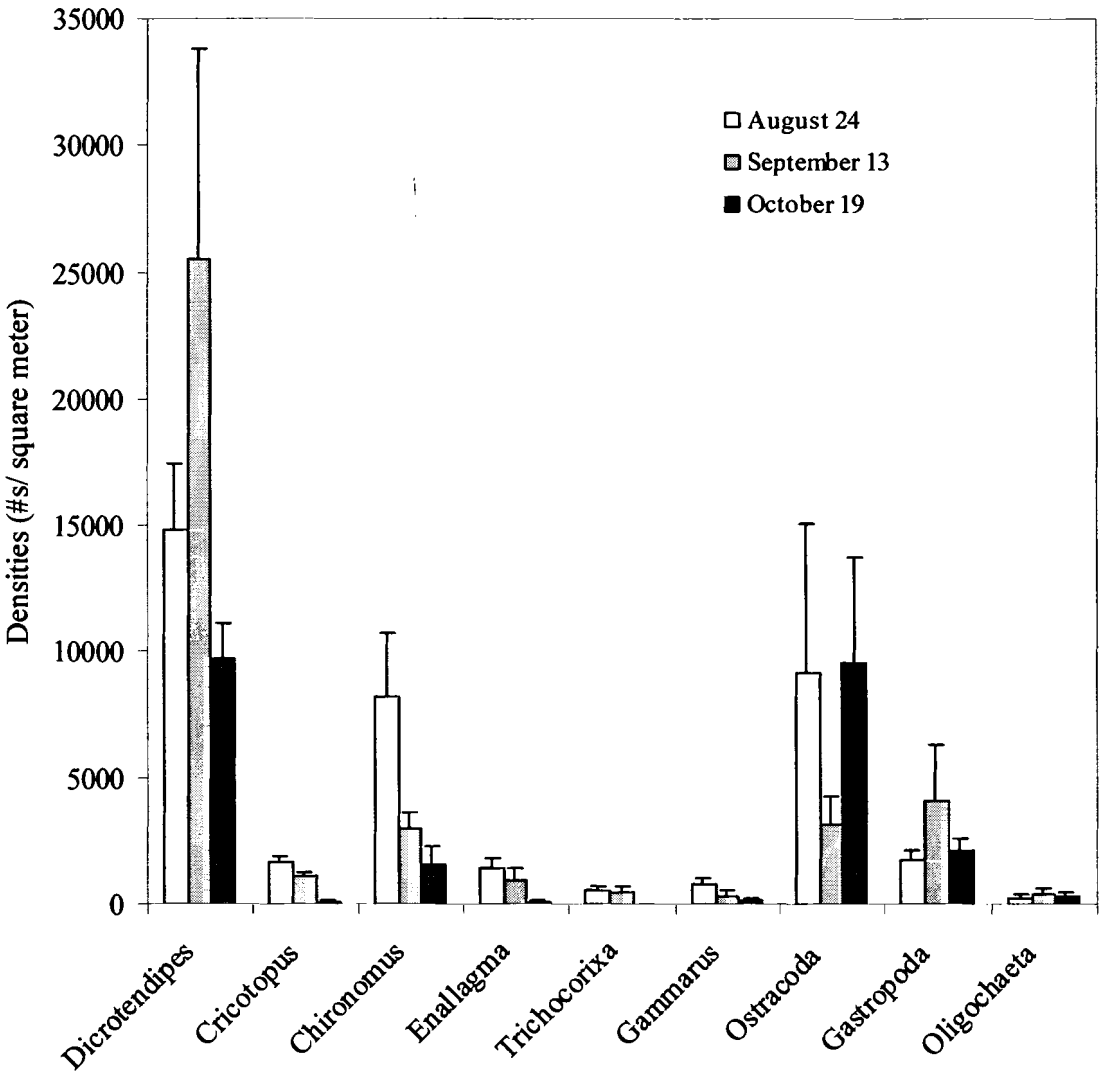


Figure 2.1. Macroinvertebrate densities in *Ruppia maritima* beds in Northeast Creek estuary in August, September and October 2001. Densities are in #s per square meter and error bars represent 1 standard error.

Gammarus decreased from 800 m^{-2} to 100 m^{-2} between August and October (Figure 2.1). Ostracods varied in density from 9200 m^{-2} in August, 3100 m^{-2} in September, and 9500 m^{-2} in October (Figure 2.1). Gastropods and oligochaetes were most common during September with average densities of 4100 m^{-2} and 400 m^{-2} respectively (Figure 2.1).

Taxa richness in NEC estuary was 13 in August, 11 in September and 10 in October 2001 (Table 2.2). Biodiversity (H', Shannon-Weiner Biodiversity Index) was 1.62 in August, 1.25 in September and 1.28 in October (Table 2.2). Taxa evenness (J') ranged from 0.57 in September to 0.67 in August (Table 2.2).

Table 2.2. Taxa richness (S), diversity (H') and evenness (J') in August, September and October 2001 in Northeast Creek estuary.

Month	S	H'	J'
August	13	1.62	0.67
September	11	1.25	0.57
October	10	1.28	0.58

Discussion

Northeast Creek estuary was dominated by euryhaline freshwater fauna from May to November 2001. The most common invertebrates were non-biting midge larvae (Chironomidae: *Dicrotendipes*, *Cricotopus* and *Chironomus*), damselflies (Coenagrionidae: *Enallagma*), gastropods and ostracods. Less common invertebrates were oligochaetes, water boatmen (Corixidae: *Trichocorixa*), water mites (Acari), and amphipods (Gammaridae: *Gammarus*).

Most of the macroinvertebrates found in NEC estuary are able to tolerate 2nd degree euryhalinity and mesohaline conditions (8-15 ‰) (Remane and Schlieper 1971). All have been documented in estuaries and/or *Ruppia* beds by other studies. Non-biting midge larvae (Chironomidae) are important insects in freshwater, brackish water and marine systems. Some Chironomidae are tolerant of very high salinities and new species have evolved in marine systems (Remane and Schlieper 1971, Cheng 1976, Colbo 1996). The three genera we found are typically euryhaline freshwater. *Dicrotendipes* has been found in New Brunswick estuaries (Williams and Hamm 2002). *Cricotopus* and *Chironomus* have been documented for many saline ecosystems (Sutcliffe 1961, Menzie 1981, Frid and James 1989, Colbo 1996, Williams and Williams 1998a, Williams and Hamm 2002). Some species of *Chironomus* are very tolerant to salt water and are actually brackish water specialists (Remane and Schlieper 1971, Verhoeven 1980). *Enallagma* and *Trichocorixa* are also freshwater insect genera known to be tolerant of brackish water (Merritt and Cummins 1996), and closely related species have been recorded in European *Ruppia* beds (Verhoeven 1980) and other saline habitats (Remane and Schlieper 1971, Cheng 1976, Hart and Lovvorn 2002). Acari are known to be euryhaline freshwater, while ostracods, oligochaetes and gastropods may be euryhaline freshwater or euryhaline marine (Remane and Schlieper 1971, Verhoeven 1980). *Gammarus* may be euryhaline marine or brackish water specialists, depending on the species (Remane and Schlieper 1971, Verhoeven 1980).

This macroinvertebrate community is most similar to communities in *Ruppia* beds in other estuaries and to communities in freshwater streams and ponds with similar habitats. In an extensive survey of *Ruppia* beds in European estuaries, Verhoeven (1980)

found a range of taxa, including many chironomidae (*Chironomus*), several Odonata, Oligochaetes, Amphipoda (*Gammarus*), Corixidae, Gastropoda and Acari. NEC estuary is most similar in terms of faunal composition to a site in the Baltic sampled by Verhoeven (1980) with sparse euryhaline freshwater fauna, *R. maritima* and organic mud. Other studies of *Ruppia* beds have found less similar communities (mollusks, polychaetes, crustaceans, some insects and oligochaetes), most likely due to a more constant marine influence at the sites studied (Wenner and Beatty 1988, Heck et al. 1995, Knowles and Bell 1998). The NEC estuary faunal community was quite different from communities of true seagrass beds, which are generally dominated by more marine species such as polychaetes, mollusks, and crustaceans (Fredette et al. 1990, Heck et al. 1995, Mattila et al. 1999). Soft-bottomed rivers and small streams with high organic matter contents have more similar communities to NEC estuary than marine seagrass beds. These communities tend to be dominated by Chironomidae and Oligochaeta (Benke et al. 1984, Rolauffs et al. 2001, Brunke et al. 2002).

Freshwater insects were 70% of the faunal community in NEC estuary in August, 80% in September and 50% in October. Very few estuarine studies have documented such dominance by insect taxa. Insects have been found to constitute 17-54 % of the macroinvertebrate fauna of New Brunswick estuaries (Williams and Hamm 2002) and 32% of this fauna in Aber estuary in North Wales (Williams and Williams 1998a). However, many researchers that have documented the presence of insects in estuaries do not identify them past the taxonomic level of Order (e.g., Sanders et al. 1965, Wenner and Beatty 1988, discussed by Williams and Hamm 2002). Classically, insects have been thought to be restricted to the upstream freshwater sections of estuaries based entirely on

salinity (Sanders et al. 1965, Remane and Schlieper 1971, Ristich et al. 1977). Recent studies have shown this idea to be too simplistic and that some insects are significant throughout estuaries (Williams and Williams 1998a, Williams and Hamm 2002).

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. This shift in salinity was accompanied by changes in the community of organisms. Water mites were scarcer in the later summer and early fall samples. Gastropods increased in importance in the late summer and early fall under more marine conditions. In addition, during the period of high and increasing salinity in the estuary from August to mid-October, insects declined from 70-80% to 50% of the total community. Total insect densities also decreased during this time period.

Possible reasons for the shifts observed include increased salinity over an extended time, increased larval emergence, reductions in egg-laying and other seasonal factors. Laboratory salinity trials by Williams and Williams (1998a) indicated that all insects found in the freshwater sections of Aber estuary in North Wales could tolerate short inundations by salt water but only some could tolerate extended periods of high salinity. In addition, seasonal shifts in the faunal community were found for Aber estuary, with the extension of chironomid larvae downstream during the more freshwater dominated months of April and July (1998) (Williams and Williams 1998b). The distribution of insects in estuaries is most likely based on a combination of factors, including salinity fluctuation, water depth, bottom substrate, estuarine size, and current and historical connections to other water bodies for colonization (Verhoeven 1980,

Williams and Hamm 2002). We could be observing the early stages of insects moving into salt water through bridging habitats (Cheng 1976, Williams and Williams 1998a).

Under conditions of fluctuating salinity, which we observed in NEC estuary, freshwater species would be expected to be more common because most marine species require a narrow range of salinities (due to the constant salinities of ocean water), while freshwater species are more tolerant of a wider range of salinities and anoxia (due to the variable chemical compositions of freshwater lakes and streams) (Lopez 1988). Because NEC estuary is dominated almost entirely by freshwater inputs during the spring, it is unlikely that even euryhaline marine species could tolerate these conditions. However, while water salinities were variable in NEC estuary, sediment salinities may have been more constant. We estimated sediment salinities to be 24.70 ± 1.59 (SE) ‰ in October 2001. In general, the greater stability of sediment salinities is thought to provide more constant conditions for the infauna while more variable water salinities are problematic for the epifauna (Sanders et al. 1965).

The estuary was characterized by low taxa diversity (H` ranged from 1.25 – 1.62), evenness (J' ranged from 0.57 – 0.67) and richness (a total of 14 taxa were found during 2001), an overall poverty of taxa typical of brackish water systems (Remane and Schleiper 1971, Verhoeven 1980). Although we did not identify organisms to species and diversity, evenness and richness may be slightly underestimated, it is unlikely that more specific identifications would yield large changes in these numbers for this species poor system. Verhoeven (1980) found that *Ruppia* beds in the Mediterranean and N.W. Europe also had low species diversity (H` ranged from 0.13 to 2.12), evenness (J' ranged from 0.15 - 0.76) and richness (5 – 36 species, depending on the location). However, Williams

and Hamm (2002) found higher species richness (53 – 78 species) in course-bottomed estuaries in North Wales and New Brunswick. The small size of NEC estuary could be a possible reason for its species impoverishment since species richness has been found to decrease with estuary size (Williams and Hamm 2002). Faunal densities in NEC estuary were 31100 m^{-2} in August, 23200 m^{-2} in September and 27700 m^{-2} in October. These densities are within the range of densities found by Verhoeven (1980) in European *Ruppia* beds (2000 – 44000 m^{-2}).

Conclusions

This study is the first to document the fauna of the *Ruppia maritima* beds of a Maine estuary. The macroinvertebrate community of Notheast Creek estuary was dominated by tolerant euryhaline freshwater invertebrates from May to November 2001. The community was composed largely of insect taxa and was most similar to communities in other *Ruppia* beds and in freshwater soft-bottomed organic streams. Our study provides baseline ecological data on the faunal community of NEC estuary that can be used in future monitoring studies.

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APPENDICES

Appendix A

DATA FROM NITROGEN ENRICHMENT EXPERIMENT

Table A.1. Macroinvertebrate abundances in the N enrichment experiment. Raw abundances from core samples. Sampler area = 78.54 cm^2.

Nutrient Treatment	Experimental Unit	*Dicrotendipes* sp.	*Cricotopus* sp.	*Chironmus* sp.	Other Diptera	*Enallagma* sp.	*Trichocorixa* sp.	Acari	*Gammarus* sp.	Ostracoda	Gastropoda	Oligochaeta	others
Control	2	143	22	0	0	13	10	0	0	0	0	1	1
Control	8	93	25	82	0	17	23	0	32	2	35	12	0
Control	10	153	13	58	0	4	8	0	6	10	29	5	0
Control	19	59	13	83	0	2	4	0	3	54	1	1	0
Ambient	3	16	0	14	1	1	0	0	1	0	0	1	1
Ambient	15	28	2	25	0	4	0	0	0	44	0	3	0
Ambient	17	10	0	64	2	5	5	0	0	2	0	0	0
Ambient	18	22	0	30	0	9	1	0	8	27	0	15	0
Low N	1	16	2	23	1	1	0	0	0	48	1	3	0
Low N	6	14	0	55	1	0	0	0	0	16	0	36	0
Low N	12	3	0	6	0	0	0	0	1	76	0	12	0
Low N	13	5	0	5	2	1	0	0	3	16	0	19	0
Moderate N	5	1	0	3	0	0	0	0	0	9	0	4	0
Moderate N	9	6	0	8	3	0	6	0	3	8	0	8	0
Moderate N	16	14	0	21	0	2	0	0	1	5	0	2	0
Moderate N	20	26	1	52	1	2	0	1	0	2	0	3	0
High N	4	1	0	8	0	0	0	0	0	3	0	17	0
High N	7	2	0	2	1	0	8	0	2	58	0	246	0
High N	11	0	0	10	0	1	1	0	0	25	0	81	0
High N	14	3	0	19	1	0	0	0	8	5	0	24	0

Table A.2. Stable isotope ratios for all material in the N enrichment experiment.

Reported as ratios to PDB for C and to N_2 in air for N.

Treatment	Unit	Date	Material	$\delta^{13}C$	$\delta^{15}N$
C	2	08/17/2001	*Ruppia maritima*	-17.69	-8.09
C	2	08/28/2001	*Ruppia maritima*	-15.74	-5.94
C	8	08/17/2001	*Ruppia maritima*	-17.55	-3.15
C	8	08/28/2001	*Ruppia maritima*	-15.5	-3.74
C	10	08/17/2001	*Ruppia maritima*	-16.84	-2.52
C	10	08/28/2001	*Ruppia maritima*	-15.73	-2.03
C	19	08/17/2001	*Ruppia maritima*	-17.77	-0.96
C	19	08/28/2001	*Ruppia maritima*	-15.85	-0.66
A	3	08/17/2001	*Ruppia maritima*	-17.09	-2.76
A	3	08/28/2001	*Ruppia maritima*	-16.74	-4.14
A	15	08/17/2001	*Ruppia maritima*	-20.12	-2.75
A	15	08/28/2001	*Ruppia maritima*	-20.24	-2.46
A	17	08/17/2001	*Ruppia maritima*	-20.77	-3.12
A	17	08/28/2001	*Ruppia maritima*	-19.38	-2.54
A	18	08/17/2001	*Ruppia maritima*	-21.63	-4.76
A	18	08/28/2001	*Ruppia maritima*	-19.11	-3.72
L	1	08/17/2001	*Ruppia maritima*	-19.39	-1.66
L	1	08/28/2001	*Ruppia maritima*	-18.33	-1.9
L	6	08/17/2001	*Ruppia maritima*	-18.98	-2.19
L	6	08/28/2001	*Ruppia maritima*	-18.57	-3.27
L	12	08/17/2001	*Ruppia maritima*	-20.67	-1.18
L	12	08/28/2001	*Ruppia maritima*	-19.38	-2.83
L	13	08/17/2001	*Ruppia maritima*	-22.79	-1.47
L	13	08/28/2001	*Ruppia maritima*	-23.38	2.41
M	5	08/17/2001	*Ruppia maritima*	-20	-0.82
M	5	08/28/2001	*Ruppia maritima*	-20.01	0.13
M	9	08/17/2001	*Ruppia maritima*	-18.58	-0.98
M	9	08/28/2001	*Ruppia maritima*	-19.54	-2.08
M	16	08/17/2001	*Ruppia maritima*	-20.54	4.19
M	16	08/28/2001	*Ruppia maritima*	-20.41	6.44
M	20	08/17/2001	*Ruppia maritima*	-21.75	0.91
M	16&20	08/28/2001	*Ruppia maritima*	-20.86	5.92
H	4	08/17/2001	*Ruppia maritima*	-23.38	2.91
H	4	08/28/2001	*Ruppia maritima*	-23.06	2.8
H	7	08/17/2001	*Ruppia maritima*	-21.37	4.22
H	7	08/28/2001	*Ruppia maritima*	-21.88	4.88
H	11	08/17/2001	*Ruppia maritima*	-20.2	3.82
H	11	08/28/2001	*Ruppia maritima*	-23.55	-0.77
H	14	08/17/2001	*Ruppia maritima*	-23.74	3.45
H	14	08/28/2001	*Ruppia maritima*	-23.12	5.05

Table A.2 continued.

Treatment	Unit	Date	Material	$\delta^{13}C$	$\delta^{15}N$
C	2	08/17/2001	Epiphytic material	-22.09	-1.05
C	2	08/28/2001	Epiphytic material	-21.25	-0.8
C	8	08/17/2001	Epiphytic material	-24.24	0.5
C	8	08/28/2001	Epiphytic material	-21	-0.08
C	10	08/17/2001	Epiphytic material	-21.63	0.63
C	10	08/28/2001	Epiphytic material	-22.17	-0.19
C	19	08/17/2001	Epiphytic material	-24.77	0.52
C	19	08/28/2001	Epiphytic material	-23.13	-1.05
A	3	08/17/2001	Epiphytic material	-21.14	1.49
A	3	08/28/2001	Epiphytic material	-19.06	0.52
A	15	08/17/2001	Epiphytic material	-22.61	1.56
A	15	08/28/2001	Epiphytic material	-20.42	-0.44
A	17	08/17/2001	Epiphytic material	-24.09	1.77
A	17	08/28/2001	Epiphytic material	-22.51	-0.04
A	18	08/17/2001	Epiphytic material	-25.09	2.24
A	18	08/28/2001	Epiphytic material	-23.07	-0.09
L	1	08/17/2001	Epiphytic material	-21.27	1.72
L	1	08/28/2001	Epiphytic material	-19.8	-1.33
L	6	08/17/2001	Epiphytic material	-19.51	2.57
L	6	08/28/2001	Epiphytic material	-19.39	2.07
L	12	08/17/2001	Epiphytic material	-20.28	1.65
L	12	08/28/2001	Epiphytic material	-18.96	1.7
L	13	08/17/2001	Epiphytic material	-21.55	2.43
L	13	08/28/2001	Epiphytic material	-20.33	2.76
M	5	08/17/2001	Epiphytic material	-19.41	1.86
M	5	08/28/2001	Epiphytic material	-18.4	1.2
M	9	08/17/2001	Epiphytic material	-18.73	1.82
M	9	08/28/2001	Epiphytic material	-17.67	1.6
M	16	08/17/2001	Epiphytic material	-23.66	1.79
M	16	08/28/2001	Epiphytic material	-21.12	6.94
M	20	08/17/2001	Epiphytic material	-23.65	0.52
M	20	08/28/2001	Epiphytic material	-19.52	3.23
H	4	08/17/2001	Epiphytic material	-20.78	0.25
H	4	08/28/2001	Epiphytic material	-20.71	0.92
H	7	08/17/2001	Epiphytic material	-19.42	5.38
H	7	08/28/2001	Epiphytic material	-19.58	5.02
H	11	08/17/2001	Epiphytic material	-20.64	3.4
H	11	08/28/2001	Epiphytic material	-20.18	1.74
H	14	08/17/2001	Epiphytic material	-21.07	1.98
H	14	08/28/2001	Epiphytic material	-19.22	3.81

Table A.2 continued.

Treatment	Unit	Date	Material	$\delta^{13}C$	$\delta^{15}N$
C	2	08/17/2001	POM	-23.84	-0.7
C	2	08/28/2001	POM	-23.25	1.87
C	8	08/17/2001	POM	-21.93	1.16
C	8	08/28/2001	POM	-21.93	1.16
C	10	08/17/2001	POM	-22.63	0.44
C	10	08/28/2001	POM	-21.96	1.93
C	19	08/17/2001	POM	-23.68	1.76
C	19	08/28/2001	POM	-22.83	3.71
A	3	08/17/2001	POM	-21.85	2.97
A	3	08/28/2001	POM	-19.75	2.45
A	15	08/17/2001	POM	-22.15	-0.99
A	15	08/28/2001	POM	-22.45	0.81
A	17	08/17/2001	POM	-19.73	3.26
A	17	08/28/2001	POM	-24.77	1.06
A	18	08/17/2001	POM	-22.23	3.67
A	18	08/28/2001	POM	-23.78	3.4
L	1	08/28/2001	POM	-21.89	-0.77
L	6	08/17/2001	POM	-21.85	-0.31
L	6	08/28/2001	POM	-19.1	1.02
L	12	08/17/2001	POM	-21.22	1.42
L	12	08/28/2001	POM	-19.42	1.77
L	13	08/17/2001	POM	-22.43	3.23
L	13	08/28/2001	POM	-20.94	3.93
M	5	08/17/2001	POM	-20.65	0.25
M	5	08/28/2001	POM	-22.05	0
M	9	08/17/2001	POM	-20.4	-0.51
M	9	08/28/2001	POM	-19.84	0.82
M	16	08/17/2001	POM	-24.83	-1.61
M	16	08/28/2001	POM	-24.83	-1.87
M	20	08/17/2001	POM	-21.88	0.53
M	20	08/28/2001	POM	-21.89	1.04
H	4	08/17/2001	POM	-20.45	-5.04
H	4	08/28/2001	POM	-21.6	-1.31
H	7	08/17/2001	POM	-20.74	3.9
H	7	08/28/2001	POM	-18.57	6.09
H	11	08/17/2001	POM	-22.02	1.23
H	11	08/28/2001	POM	-21.66	-0.52
H	14	08/17/2001	POM	-21.87	1.28
H	14	08/28/2001	POM	-20.52	2.83

Table A.2 continued.

Treatment	Unit	Date	Material	$\delta^{13}C$	$\delta^{15}N$
NA		08/28/2001	Osmocote	-30.10	1.54
NA		08/28/2001	Osmocote	-30.29	1.99
NA		08/28/2001	Osmocote	-30.29	1.41
NA		08/28/2001	Osmocote	-30.11	1.68
ALL		08/28/2001	Terrestrial Detritus	-28.75	-1.36
ALL		08/28/2001	Terrestrial Detritus	-25.59	-0.12
ALL		08/28/2001	Terrestrial Detritus	-26.14	0.37
ALL		08/28/2001	Terrestrial Detritus	-28.71	-0.73
ALL		08/28/2001	Terrestrial Detritus	-28.59	-0.75
ALL		08/28/2001	Terrestrial Detritus	-26.05	0.55
ALL		08/28/2001	Terrestrial Detritus	-25.19	0.24
ALL		08/28/2001	Terrestrial Detritus	-26.13	0.47
ALL		08/28/2001	Terrestrial Detritus	-25.31	-0.78
ALL		08/28/2001	Terrestrial Detritus	-26.28	-0.16
ALL		08/28/2001	Terrestrial Detritus	-26.42	-0.35
ALL		08/28/2001	Terrestrial Detritus	-26.39	0.39
ALL		08/28/2001	Terrestrial Detritus	-28.81	-0.66
ALL		08/28/2001	Terrestrial Detritus	-26.52	-0.15
ALL		08/28/2001	Terrestrial Detritus	-28.06	-1.07
ALL		08/28/2001	Terrestrial Detritus	-28.09	-1.13
ALL		08/28/2001	Terrestrial Detritus	-26.19	0.00
ALL		08/28/2001	Terrestrial Detritus	-28.05	-0.75
ALL		08/28/2001	Terrestrial Detritus	-28.02	-1.14
ALL		08/28/2001	Terrestrial Detritus	-26.10	-0.32
ALL		08/28/2001	Woody Detritus	-26.98	0.90
ALL		08/28/2001	Woody Detritus	-27.11	0.24
ALL		08/28/2001	Woody Detritus	-27.80	-0.90
ALL		08/28/2001	Woody Detritus	-28.02	-0.71
ALL		08/28/2001	Woody Detritus	-27.04	0.69
ALL		08/28/2001	Woody Detritus	-27.90	-2.94
ALL		08/28/2001	Woody Detritus	-27.17	0.42
ALL		08/28/2001	Woody Detritus	-27.98	-2.78

Table A.2 continued.

Treatment	Unit	Date	Material	$\delta^{13}C$	$\delta^{15}N$
C	2	08/28/2001	Grazing Chironomidae	-22.21	2.4
C	8	08/17/2001	Grazing Chironomidae	-25.02	2
C	8	08/28/2001	Grazing Chironomidae	-23.13	2.94
C	10	08/28/2001	Grazing Chironomidae	-23.87	2.31
C	19	08/17/2001	Grazing Chironomidae	-21.19	2.03
C	19	08/28/2001	Grazing Chironomidae	-22.61	2.27
A	3	08/17/2001	Grazing Chironomidae	-22.9	3.59
A	3	08/28/2001	Grazing Chironomidae	-19.67	3.51
A	15	08/28/2001	Grazing Chironomidae	-25.08	3.05
A	17	08/17/2001	Grazing Chironomidae	-25.04	3.28
A	17	08/28/2001	Grazing Chironomidae	-23.41	3.61
A	18	08/17/2001	Grazing Chironomidae	-23.66	3.71
A	18	08/28/2001	Grazing Chironomidae	-24.55	3.42
L	1	08/17/2001	Grazing Chironomidae	-20.95	3.54
L	1	08/28/2001	Grazing Chironomidae	-19.25	3.61
L	6	08/17/2001	Grazing Chironomidae	-20.15	4.43
L	6	08/28/2001	Grazing Chironomidae	-19.07	3.93
L	12	08/28/2001	Grazing Chironomidae	-19.57	3.8
L	13	08/17/2001	Grazing Chironomidae	-22.21	4.05
L	13	08/28/2001	Grazing Chironomidae	-20.24	4.53
M	5	08/28/2001	Grazing Chironomidae	-19.38	2.04
M	9	08/17/2001	Grazing Chironomidae	-19.25	3.88
M	9	08/28/2001	Grazing Chironomidae	-19.09	3.82
M	16	08/17/2001	Grazing Chironomidae	-20.72	3.88
M	16	08/28/2001	Grazing Chironomidae	-21.8	3.7
M	16	08/28/2001	Grazing Chironomidae	-22.08	3.64
M	20	08/17/2001	Grazing Chironomidae	-20.49	2.28
M	20	08/28/2001	Grazing Chironomidae	-20.21	3.19
M	20	08/28/2001	Grazing Chironomidae	-20.68	2.67
M	20	08/28/2001	Grazing Chironomidae	-20.22	3.07
H	7	08/17/2001	Grazing Chironomidae	-20.52	6.1
H	7	08/28/2001	Grazing Chironomidae	-21.3	4.87
H	11	08/17/2001	Grazing Chironomidae	-21.89	2.95
H	11	08/28/2001	Grazing Chironomidae	-20.78	1.7
H	14	08/17/2001	Grazing Chironomidae	-20.74	4.41
H	14	08/28/2001	Grazing Chironomidae	-20.76	3.5

Table A.2 continued.

Treatment	Unit	Date	Material	$\delta^{13}C$	$\delta^{15}N$
C	2	08/28/2001	*Chironomus*	-26.9	1.12
C	8	08/28/2001	*Chironomus*	-25.55	2.48
C	10	08/28/2001	*Chironomus*	-25.27	1.91
C	19	08/28/2001	*Chironomus*	-25.76	1.57
C	19	08/28/2001	*Chironomus*	-25.77	2.08
C	19	08/28/2001	*Chironomus*	-25.99	1.99
A	3	08/28/2001	*Chironomus*	-26.12	1.95
A	15	08/28/2001	*Chironomus*	-27.86	2.65
A	17	08/28/2001	*Chironomus*	-28.27	2.35
A	17	08/28/2001	*Chironomus*	-28.38	2.38
A	17	08/28/2001	*Chironomus*	-28.53	2.21
A	18	08/28/2001	*Chironomus*	-27.16	2.53
A	18	08/28/2001	*Chironomus*	-27.13	2.61
A	18	08/28/2001	*Chironomus*	-27.19	2.52
L	1	08/28/2001	*Chironomus*	-22.42	3.25
L	6	08/28/2001	*Chironomus*	-24.54	2.75
L	12	08/28/2001	*Chironomus*	-26.04	1.71
L	13	08/28/2001	*Chironomus*	-26.52	0.78
M	5	08/28/2001	*Chironomus*	-21.79	2.3
M	16	08/28/2001	*Chironomus*	-23.93	2.04
M	20	08/28/2001	*Chironomus*	-25.18	2.38
H	4	08/28/2001	*Chironomus*	-22.01	2.72
H	11	08/28/2001	*Chironomus*	-22.01	0.86
H	14	08/28/2001	*Chironomus*	-25.65	1.61
C	2	08/17/2001	*Enallagma*	-22.19	2.76
C	2	08/28/2001	*Enallagma*	-22.34	4.47
C	8	08/17/2001	*Enallagma*	-21.86	3.51
C	10	08/17/2001	*Enallagma*	-23.36	2.75
C	10	08/28/2001	*Enallagma*	-21.66	3.93
C	19	08/28/2001	*Enallagma*	-22.19	4.17
A	3	08/28/2001	*Enallagma*	-22.83	4.66
A	15	08/28/2001	*Enallagma*	-24.38	4.55
A	17	08/28/2001	*Enallagma*	-24.62	4.78
A	17	08/28/2001	*Enallagma*	-25.24	4.88
A	18	08/17/2001	*Enallagma*	-25.17	4.47
A	18	08/28/2001	*Enallagma*	-24	4.84
L	6	08/17/2001	*Enallagma*	-21.21	5.24
L	6	08/28/2001	*Enallagma*	-20.34	5.46
L	13	08/28/2001	*Enallagma*	-21.94	5.4
M	9	08/28/2001	*Enallagma*	-19.45	4.45
M	20	08/28/2001	*Enallagma*	-21.01	5.49
H	4	08/28/2001	*Enallagma*	-19.98	4.12
H	7	08/28/2001	*Enallagma*	-20.04	3.85
H	11&14	08/28/2001	*Enallagma*	-19.71	4.96

Table A.2 continued.

Treatment	Unit	Date	Material	$\delta^{13}C$	$\delta^{15}N$
C	2	08/31/2001	*Fundulus heteroclitus*	-22.96	6.09
C	2	08/31/2001	*Fundulus heteroclitus*	-22.95	6.05
C	8	08/31/2001	*Fundulus heteroclitus*	-22.1	5.6
C	8	08/31/2001	*Fundulus heteroclitus*	-21.91	5.63
C	10	08/31/2001	*Fundulus heteroclitus*	-23.42	5.33
C	10	08/31/2001	*Fundulus heteroclitus*	-23.86	5.62
C	19	08/31/2001	*Fundulus heteroclitus*	-22.52	5.82
C	19	08/31/2001	*Fundulus heteroclitus*	-22.37	5.53
A	3	08/31/2001	*Fundulus heteroclitus*	-22.76	3.78
A	3	08/31/2001	*Fundulus heteroclitus*	-23.11	3.85
A	15	08/31/2001	*Fundulus heteroclitus*	-25.45	5.63
A	15	08/31/2001	*Fundulus heteroclitus*	-25.5	5.91
A	17	08/31/2001	*Fundulus heteroclitus*	-26.79	6.49
A	17	08/31/2001	*Fundulus heteroclitus*	-27.09	6.36
L	1	08/31/2001	*Fundulus heteroclitus*	-20.15	5.71
L	1	08/31/2001	*Fundulus heteroclitus*	-20.49	5.59
L	6	08/31/2001	*Fundulus heteroclitus*	-22.46	5.21
L	6	08/31/2001	*Fundulus heteroclitus*	-22.64	5.12
L	12	08/31/2001	*Fundulus heteroclitus*	-19.87	6.18
L	12	08/31/2001	*Fundulus heteroclitus*	-19.79	6.2
M	5	08/31/2001	*Fundulus heteroclitus*	-19.46	4.74
M	5	08/31/2001	*Fundulus heteroclitus*	-19.63	4.66
M	9	08/31/2001	*Fundulus heteroclitus*	-18.91	3.95
M	9	08/31/2001	*Fundulus heteroclitus*	-18.75	3.97
M	16	08/31/2001	*Fundulus heteroclitus*	-22.65	5.27
M	16	08/31/2001	*Fundulus heteroclitus*	-22.67	5.19
M	20	08/31/2001	*Fundulus heteroclitus*	-24.02	5.35
M	20	08/31/2001	*Fundulus heteroclitus*	-23.58	5.18
H	4	08/31/2001	*Fundulus heteroclitus*	-19.79	5.2
H	4	08/31/2001	*Fundulus heteroclitus*	-19.9	5.03
H	7	08/31/2001	*Fundulus heteroclitus*	-21.21	5.86
H	7	08/31/2001	*Fundulus heteroclitus*	-21.25	5.76

Table A.2 continued.

Treatment	Unit	Date	Material	$\delta^{13}C$	$\delta^{15}N$
C	2	08/28/2001	*Gammarus*	-21.04	1.22
C	8	08/28/2001	*Gammarus*	-20	2.29
C	10	08/28/2001	*Gammarus*	-20.4	1.94
C	19	08/28/2001	*Gammarus*	-20.63	2.59
L	12	08/28/2001	*Gammarus*	-19.49	2.45
L	13	08/28/2001	*Gammarus*	-22.17	1.94
M	9	08/28/2001	*Gammarus*	-20.44	2.85
M	5&16&20	08/28/2001	*Gammarus*	-20.8	1.96
H	7	08/28/2001	*Gammarus*	-21.78	2.8
H	7	08/28/2001	*Gammarus*	-21.66	1.99
H	11	08/28/2001	*Gammarus*	-21.01	0.74
H	7&14	08/28/2001	*Gammarus*	-21.04	2.31
L	1	08/28/2001	Ostracoda	-15.8	0.07
L	12	08/28/2001	Ostracoda	-18.7	-1.45
H	11	08/28/2001	Ostracoda	-19.74	-3.47
C	2	08/28/2001	*Trichocorixa*	-18.62	-1.15
C	10	08/28/2001	*Trichocorixa*	-22.7	0.95
C	19	08/28/2001	*Trichocorixa*	-19.25	-0.87
C	19	08/28/2001	*Trichocorixa*	-18.06	-1.33
C	19	08/28/2001	*Trichocorixa*	-20.58	-0.04
A	17	08/28/2001	*Trichocorixa*	-22.11	1.34
A	18	08/28/2001	*Trichocorixa*	-17.5	-1.86
H	7	08/28/2001	*Trichocorixa*	-18.41	3.05
H	11	08/28/2001	*Trichocorixa*	-19.92	-0.49

Appendix B

MACROINVERTEBRATE ABUNDANCE DATA

Table B.1. Macroinvertebrate Abundances: August-October 2001. Raw abundances from core samples. Sampler area = 78.54 cm^2.

Sampling Date	Sample Replicate	*Dicrotendipes* sp.	*Cricotopus* sp.	*Chironmus* sp.	Ceratopogonidae	Other Diptera	*Enallagma* sp.	Other Odonata	*Trichocorixa* sp.	Acari	*Gammarus* sp.	Ostracoda	Gastropoda	Oligochaeta
08/24/2001	1	146	12	69	0	0	9	0	3	0	3	10	8	0
08/24/2001	4	114	7	124	1	1	5	0	5	0	15	335	22	1
08/24/2001	5	80	11	30	0	0	10	0	0	0	8	111	13	1
08/24/2001	6	98	10	46	0	0	12	0	5	0	6	9	5	0
08/24/2001	7	211	23	148	0	1	27	1	10	0	9	24	10	9
08/24/2001	9	39	16	18	0	0	3	0	5	0	2	4	11	0
08/24/2001	10	128	11	18	0	1	14	0	2	0	1	13	28	0
09/13/2001	1	286	12	0	0	0	9	0	1	0	0	3	27	1
09/13/2001	2	553	11	26	0	0	30	0	13	0	13	16	134	14
09/13/2001	4	145	4	29	0	0	6	0	4	0	1	75	6	1
09/13/2001	5	65	2	42	0	1	3	0	1	0	0	21	18	1
09/13/2001	6	90	12	31	0	1	1	0	0	1	5	34	2	0
09/13/2001	7	180	8	20	0	2	1	0	1	0	0	15	1	1
09/13/2001	10	82	11	18	0	0	2	0	5	0	0	8	37	5
10/19/2001	1	68	0	38	1	0	0	0	0	0	0	39	16	4
10/19/2001	2	121	2	12	0	0	0	0	0	0	1	21	18	2
10/19/2001	3	63	0	7	0	0	0	0	0	0	0	13	5	1
10/19/2001	4	85	0	4	0	0	1	0	0	0	1	8	12	1
10/19/2001	5	100	0	1	1	0	1	0	0	0	5	101	30	3
10/19/2001	6	30	1	3	1	0	1	0	1	0	0	87	7	0
10/19/2001	8	67	2	24	0	0	2	0	0	0	0	255	29	7

BIOGRAPHY OF THE AUTHOR

Rachel A. Keats was born in Albany, New York on February 29, 1976. She was raised in Niskayuna, New York, and graduated from Niskayuna High School in 1994. She attended Cornell University's College of Agriculture and Life Sciences and graduated in 1998 with a Bachelor of Science degree in Entomology. After working for a year for Cornell University as a research assistant, she then moved to Maine and entered the Ecology and Environmental Sciences graduate program at the University of Maine in the fall of 1999.

After receiving her degree, Rachel plans to pursue further educational opportunities by beginning a graduate program in Spatial Information Science and Engineering at the University of Maine. In the future, she hopes to use her knowledge of ecology and the environment as well as her enthusiasm for data management and statistics to help solve important environmental problems. Rachel is a candidate for the Master of Science degree in Ecology and Environmental Sciences from The University of Maine in December, 2002.