Trophic control of saltmarsh invertebrates

David Samuel Johnson
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TROPHIC CONTROL OF SALTMARSH INVERTEBRATES

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Biological Sciences

by

David Samuel Johnson
B.S., University of Central Arkansas, 2003
August 2008
ACKNOWLEDGEMENTS

The path to my Ph.D. started in the mountains of Arkansas, where as a kid I was a hillbilly vagabond roaming barefoot over the mountainside filling my plastic bucket with woodland treasures such as wildflowers, doodle bugs, and frog eggs. A watershed moment occurred in my professional development as a scientist when I filled my bucket with a poisonous copperhead snake. Mama knew she couldn’t stop her little amateur naturalist, so she bought me a book on snakes because if I was going to fill my bucket, I needed to know who didn’t belong in there. And so my journey began.

I have to thank Mama for letting me breed spiders in her mayonnaise jars, keep hognose snakes in her flower beds, and house snapping turtles in her ice chests. I thank my sister, Melody, for sharing in my woodland adventures. I thank my little brother Cody, who is twenty years my junior, for recently showing me that it’s okay to chase grasshoppers without thinking of them as data points. I thank the rest of my family including Jamie, Logan, Aunt Nancy, and my mullet-wearing Uncle Bobby for their love, support, and not ever letting me forget where I came from because even if I am the first Ph.D. in the family, I’m not above teasing banter.

I have to acknowledge the Morris family – Kim, Keriann, Kristin, Marty, David, Grandpa and Grandma – who took in an awkward and angry 16-year-old and helped him find his path again. I thank Jon, Janet, and Grandma Crabbe who pushed me when I didn’t want to be pushed.

As with my genetic and extended family, I’ve been blessed with an excellent academic family. I recognize the intellectual spark and sass of Dr. Kristin Keteles at the University of Central Arkansas, who showed me as an undergrad that if you’re interested in science you’ve got to get out of the lab sometimes.
My dissertation, like myself, has been molded intellectually by many hands over the past five years. In 2003, the brave or foolhardy Dr. John Fleeger, with his nodding head and seemingly infinite patience that I tested more than once took in my independent and sometimes irascible spirit and navigated it down a tortuous, yet productive path. I thank him for reading (and re-reading and re-reading) every word I’ve written as a graduate student, for swatting and cursing mosquitoes with me in the marsh, and for always having his door and mind open. I thank Dr. Kyle Harms for his incredible sense of duty and for diligently reading and editing every word I handed him. I thank Drs. Kevin Carman and Ken Brown for stimulating conversations about science and how I might improve as a scientist. I thank Dr. John Day for his review of my dissertation.

I have to acknowledge the crew at the Marshview field station in Massachusetts including Brita, Lynsey, Erin (Amazon), Mike, Christian, Deanne, Kari and numerous others. I thank Dr. Linda Deegan at the Marine Biological Laboratory for her skills at herding cats as the head of the TIDE project and for her determination in the face of great and daunting responsibilities. I give special thanks to Kari Galván for arguing with me while standing crotch-deep in marsh mud, for 1 A.M. stats talks, and for being my academic sister in this journey.

Finally, the love and support I’ve received from friends nationwide (from Arkansas to Massachusetts including Adam, Iova, Brita, Terence, Kevin, Travis, Jenn, Margaret, Duley, and Idiz) and here in Baton Rouge (including the LSB 3rd Floor Mafia: Jeff, Jerry, Barry, Mark, Kari, and Wes) has been incredibly uplifting. I never thought this shoeless hillbilly vagabond would make it this far but now that I’m outfitted excellent academic shoes I’m excited to see where I roam next. And so my journey continues.
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ABSTRACT

Top-down (consumer) versus bottom-up (resource) control of food webs has long interested ecologists. Here, I take advantage of a full-factorial design of ecosystem-wide manipulations of nutrient additions (loading rates 10x above background) and the significant reduction (~60%) of a key predator, the killifish *Fundulus heteroclitus*, in the tidal creeks of the Plum Island Estuary, Massachusetts. Prior to manipulations, annelids numerically constituted 97% of the infaunal community and the largest scale (creeks) accounted for little spatial variability in annelid populations and diversities. Tidal creeks were similar based on diversity indices, abundance, and community patterns, suggesting the tidal creeks are appropriate replicates/experimental units for manipulations. Using data collected before (2003) and after (2004-2006) manipulations began, I observed little evidence of top-down or bottom-up control on infaunal densities, biomass, or community structure in four different habitats along an inundation gradient.

Using exclusion cages to remove all predators (primarily killifish and the grass shrimp *Palaemonetes pugio*) within fish removal treatments (in non-nutrient creeks), I found top-down control of surface feeding polychaetes including *Manayunkia aestuarina* and *Streblospio benedicti*. Shrimp body size increased with killifish reduction but not shrimp density, suggesting that shrimp may alter their behavior and exert stronger top-down control on infauna when killifish are removed. No corresponding decrease in benthic microalgae (BMA) occurred when infauna abundance increased, suggesting a weak infauna-BMA interaction.

For epifauna on the marsh platform, I found that hydrobiid snails increased in the creek bank *Spartina alterniflora* with fish removal and treatments interacted antagonistically on the amphipod, *Uhlorchestia spartinophila*. The interaction likely
resulted from the parasite-induced movement of _U. spartinophila_ to the creek wall habitat. This movement, in turn, made the amphipod more susceptible to predation by the semipalmated sandpiper, _Calidris pusilla_.

Top-down and bottom-up control has been thought to operate independently on saltmarsh invertebrates. I demonstrate that food-web phenomena such as trophic omnivory, behavioral modification and indirect effects increase complexity and preclude simple predictions of trophic control on benthic invertebrates. If these trends are widespread, then long-term, large spatial-scale studies may be required to more completely understand the relative importance of top-down and bottom-up control on benthic invertebrates.
The concept of food webs and the potential effects of species interactions was an early focus in ecology (e.g., Elton 1927), but it was Hairston et al.’s assertion in 1960 that predators prevent runaway consumption of terrestrial primary producers by regulating herbivores which spurred much research and debate about whether top-down or bottom-up forces regulate food webs. That is, do consumers regulate the system from the top of the food web or do resources regulate the system from the bottom? The ‘system’ is often the distribution and control of biomass at various trophic levels. The debate is not about the presence of top-down and bottom-up control in nature, but centers around which one of these controls has primacy (Ecology Special Section1992). Top-down and bottom-up (TD/BU) control has been studied in a variety of ecosystems including those in terrestrial (Schmitz et al. 2000), freshwater (Carpenter et al. 1985, Brett and Goldman 1997), and marine (Micheli 1999) environments.

While there has been much debate over which control has primacy (Carpenter et al. 1985, Hunter and Price 1992, Shurin et al. 2002, Heck and Valentine 2007), a consensus is forming among ecologists that TD/BU forces likely operate simultaneously and may interact in complex ways (Menge 2000, Denno et al. 2003, Deegan et al. 2007). Thus, a TD/BU-experiment must employ simultaneous manipulation of consumers and resources in a fully crossed design to examine the relative effects of each control and any possible interactions. Additionally, the identification of the response variable must be considered. Generally, primary production is the response variable of interest, but any response variable such as density, diversity, and relative abundance of any trophic level (e.g., herbivores) may be of interest. Consideration of different response variables is important because TD/BU controls may act on one and not another. For instance, Posey et al. (2006) found that nutrient additions stimulated
annelid growth/biomass in a North Carolina estuary, but not annelid densities. In addition to differentially affecting a suite of response variables, TD/BU controls may vary along environmental gradients (Fleeger et al. 2008) so that environmental variation (e.g., elevation, salinity, predation) may interact with TD/BU controls to modify their strength. The relative strength of TD/BU controls may also vary with scale (Menge 2000), time (Sarda et al. 1995, Boyer et al. 2003), and food-web complexity (Polis and Strong 1996, Finke and Denno 2004). Thus, the question of TD/BU control may be context dependent and generalities may prove difficult to construct (Hillebrand et al. 2007).

Salt marshes are highly productive, but low-diversity ecosystems that present many advantages in examining TD/BU questions. Historically, salt marshes have been considered bottom-up systems in which primary production is limited by resources (nutrients/light) (Odom and de la Cruz 1967, Mitsch and Gosselink 2001). Recently, this bottom-up paradigm has been challenged as consumers have been demonstrated to have strong effects on primary production (Silliman and Bertness 2002, Silliman et al. 2005, reviewed in Valiela et al. 2004). For example, the marsh periwinkle *Littoraria irrorata* may limit marsh primary productivity and may in turn be limited by predation from blue crabs (*Callinectes sapidus*) (Silliman and Bertness 2002). Similarly, top-down control of marsh cordgrass *Spartina alterniflora* by insects has been demonstrated and the magnitude of control can be modified by the presence of insect predators and/or altered nutrient levels (Denno et al. 2002, 2003). Thus, top-down and bottom-up forces may function in tandem in salt marshes.
Since 2003, I have participated in the TIDE (Trophic Cascades and Interacting Control Processes in a Detritus-based Ecosystem, http://ecosystems.mbl.edu/Tide/) project. TIDE is a multi-institutional, multi-disciplinary project using ecosystem-wide manipulations to examine the effect of nutrient addition and the reduction of a key predator (the killifish Fundulus heteroclitus) on saltmarsh ecosystems in the Plum Island Estuary (PIE), Massachusetts, USA. These marsh systems are excellent for top-down and bottom-up examinations because they are relatively pristine (low background nutrients) and have relatively low consumer diversity (e.g., no Littoraria irrorata, blue crabs or bottom-feeding sciaenid fishes). Furthermore, Johnson and Jessen (2008) suggest that grasshoppers and other insects do not limit macrophytes in this system as demonstrated in southern Atlantic marshes in the US (e.g., Denno et al. 2003), thus emphasize the need to examine trophic controls in the aquatic compartment of marshes in PIE.

Briefly, TIDE achieved significant reduction of killifish (~60%) and increased nutrient loading (~10x background nitrogen) (more detailed methodology and results can be found in Deegan et al. 2007). Because nutrient and predator treatments were crossed for a full factorial design, I was able to contribute to the project by examining TD/BU questions regarding the intermediate consumers in this system, benthic macroinvertebrates. Previous plot-level (e.g., 1 m²) experiments suggest strong top-down control and weak-to-moderate bottom-up control (Wilde et al. 1984, Posey et al. 1999, 2002) and that the controls operate independently (i.e., no interactive effects). The large spatial scale of the TIDE project allowed me to examine how these effects on the macrobenthos might vary along an inundation gradient, and allowed for the more natural movements of animals among habitats, which is restricted by previous plot-level
caging studies. Manipulations began in 2004 and are currently (2008) ongoing; but this
dissertation encompasses the years 2003 – 2006.

In Chapter 2, I characterize the spatial and temporal distributions of the benthic
macroinfauna – a class of invertebrates that live within the sediment and are retained on
a 500-µm sieve – in the four study creeks in 2003 prior to manipulations. This
characterization of the benthos provides a reference as an unperturbed system. I also
examine the level of variability associated with different spatial scales (from core-to-core
to creek scale) for annelid populations and diversities. Chapter 2 is published in
Estuaries and Coasts (hereafter referred to as Johnson et al. 2007).

In Chapter 3, I examine the effect of three consecutive field seasons of nutrient
additions and predator removal on macroinfauna in four habitats from the mudflats to
the marsh platform. Annelids numerically constitute 97% of the infauna community in
PIE and are the focus of this chapter, spanning 2003 – 2006. Fleeger et al. (2008)
reported no effect of treatments on macroinfauna abundance or diversity in the first year
(2004) of treatments. Here, I ask how the effects change with additional years of
treatments.

In Chapter 4, I narrow my focus to predator control of macroinfauna. Using a
two-stage removal of predators, I examine how omnivory influences the cascading
effects of two omnivorous predators. In Stage 1, Fundulus heteroclitus abundances
were significantly reduced as part of the overall TIDE project and in Stage 2 I removed
all predators including the grass shrimp Palaemonetes pugio, a numerically dominant
but intermediate predator that interacts with killifish as a prey and a competitor.
Previous work suggests killifish may impact both shrimp density and behavior (Kneib
and Stiven 1982, Posey and Hines 1991), which may indirectly impact infauna. Here, I examine the responses of lower trophic levels – infauna (primary consumers) and benthic microalgae (primary producers) – to detect potential cascading effects of different levels of predator removal.

In Chapter 5, I examine the effect of nutrient additions and predator removals on another class of benthic macroinvertebrates, epifauna. Epifauna live on the sediment surface and have greater mobility and body mass than infauna. In PIE, the epifauna community is dominated by hydrobiid and pulmonate snails, talitrid amphipods, and isopods. Few previous studies have examined TD/BU effects on saltmarsh epifauna, in comparison to algal-based ecosystems such as seagrasses (Duffy and Hay 2000, Heck et al. 2000, Bruno and O’Connor 2005, Gil et al. 2006). In these ecosystems, nutrients may alter density, biomass, and behavior of epifauna (Kraufvelin et al. 2006, Gil et al. 2006). In salt marshes, epifauna are a major component of killifish diets (Allen et al. 1994); thus killifish may exert top-down effects on epifauna at high tide. Shorebirds are also known to prey on epifauna at low tide (Wilson 1991). In this system, both top-down and bottom-up effects may influence epifauna. I examine epifaunal densities in different habitats along the inundation gradient to detect possible movements of these mobile organisms in response to TD/BU treatments. I also examine the potential effect of treatments on shorebirds, which are epifaunal predators.

Finally, in Chapter 6 I summarize the findings presented in this dissertation and provide general conclusions.

**LITERATURE CITED**


CHAPTER 2

WORM HOLES AND THEIR SPACE-TIME CONTINUUM: SPATIAL AND TEMPORAL VARIABILITY OF MACROINFAUNAL ANNELIDS IN A NORTHERN NEW ENGLAND SALT MARSH*

INTRODUCTION

Salt marshes are highly productive and ecologically important coastal ecosystems that function as nursery grounds for fishes, shrimps, and crabs; nesting areas for birds; and protective buffers from erosion and anthropogenic nutrient loading (Bertness 1999). Sediment-dwelling fauna (mostly infaunal invertebrates) are a diverse and abundant component of salt marshes that serve as nutrient recyclers, consumers of primary productivity and prey for fishes and crustaceans (Levin and Talley 2000).

Approximately 75% of the human population worldwide lives near coastal areas, and as a result, coastal systems serve many human uses and are highly susceptible to nutrient loading and removal of top predators (Emeis et al. 2001, Von Bodungen and Turner 2001). Macroinfauna are often used as key indicators in experiments designed to examine the effects of anthropogenic activities (Posey et al. 2006). To enhance the ability to detect the effects of anthropogenic activities, it is therefore important to understand how infaunal abundance and community patterns vary naturally within ecosystems.

For many benthic invertebrates, variability is scale dependent and the scale may differ among species (Benedetti-Cecchi 2001). For example, barnacles in the Mediterranean Sea are most variable at 10s to 100s of km (Benedetti-Cecchi et al. 2000), whereas, brown mussels in South Africa are most variable at small scales (< 50 cm) (Lawrie and McQuaid 2001). Thus, a hierarchical understanding (at the landscape level) of the scale most responsible for spatial heterogeneity of populations is essential. Once the relationship between heterogeneity and scale is characterized, selection of the most appropriate scale for manipulative or census studies can be made, thus increasing confidence in the interpretation of results (Rafaelli 2006).
Characterization of the saltmarsh macrobenthos over large spatial scales (e.g., km scale) is uncommon and examination of infaunal variation among creek systems with similar salinity regimes within an estuary is rare (West 1985, Posey et al. 2003). Additionally, studies examining the tidal inundation gradient often focus on vegetated habitats (Kneib 1984, Whaley and Minello 2002); however, Coull et al. (1979) examined the zonation of meiofauna across a complete inundation gradient (i.e., from mudflat to vegetated high marsh platform) in South Carolina. Infaunal studies that have focused on unvegetated versus vegetated habitats have examined natural abundance patterns (Netto and Lana 1999), the effects of invasive species (Posey et al. 2003), and succession in created marshes (Levin et al. 1996). These comparisons in salt marshes have revealed variable results with vegetation having positive, negative or neutral impacts on invertebrate densities (Levin and Talley 2000). This result is inconsistent with the established paradigm of seagrass communities in which the presence of vegetation increases invertebrate densities and diversities (Orth 1977, Virnstein et al. 1983, Orth et al. 1984, Orth et al. 1991, Heck et al. 1997). Although unvegetated and vegetated habitats have been compared in salt marshes, we can find no previous comprehensive studies in the primary literature of macroinfauna that encompass an entire tidal inundation gradient along the US Atlantic coast.

Studies of macrobenthic communities, ranging from community descriptions to anthropogenic effects, have been conducted in *Spartina* spp. marshes along most of the US coastline (Kneib 1984, Wardle et al. 2001, Moseman et al. 2004). However, no extensive studies exist for northern New England marshes (i.e., north of Cape Cod, Massachusetts). Salt marshes north of Cape Cod are typically small with a few notable exceptions: Scarboro marshes in Maine, Hampton marshes in New Hampshire, and
Parker River marshes (Plum Island Estuary) in Massachusetts (Teal 1986).

Furthermore, because of zoogeographic barriers, species including *Callinectes sapidus* (greater blue crab), *Littoraria irrorata* (marsh periwinkle), and *Uca* spp. (fiddler crabs) that have recently been assigned important keystone or facilitator functions in marshes south of Cape Cod are absent from these northern systems (Bertness 1985, Silliman and Bertness 2002, Teal 1986, DSJ personal observation).

The purpose of this study was to describe the macroinfaunal community within and among tidal creeks in the Plum Island Estuary (PIE), Massachusetts, USA. This paper focuses on annelids because they numerically comprised 97% of total infauna. Specific null hypotheses of this study were that no differences exist in annelid populations or assemblages in terms of: (1) temporal trends, (2) distribution and abundance patterns along the tidal inundation gradient, or (3) natural variability associated with spatial scale.

**METHODS**

**Study Site**

This study was conducted in PIE from June – October 2003 in four intertidal creek systems: Sweeney, West, Clubhead and Nelson. All creeks except Nelson drain into the Rowley River (42°44’N, 70°52’W), which opens into Plum Island Sound (at about 7-m inland from where Plum Island Sound enters the Atlantic Ocean) (Fig. 1). Nelson Creek drains directly into Plum Island Sound (Fig. 1). Sweeney Creek, the creek farthest inland, opposes West and Clubhead Creeks on the Rowley River (Fig. 1). PIE has a mean tidal amplitude of ~2.6 m during normal tides and ~3 during spring tides.
Figure 2.1. Upper figure is a satellite photograph (MassGIS Orthophoto 2002) of the Rowley River region salt marshes of the Plum Island Estuary, MA. SW=Sweeney Creek, WE=West Creek, CL=Clubhead Creek, NE=Nelson Creek. Lower figure is a profile of sampled salt marsh habitats (not drawn to scale) with size ranges of each habitat. MF = mudflat; CW = creek wall; TSA = Tall-form Spartina alterniflora; SP = Spartina patens; SSA = Short-form S. alterniflora.
The marsh platform edges are dominated by tall-form *Spartina alterniflora* (smooth cordgrass) (>130 cm in August 2003) which receives twice daily tidal inundation. The marsh platform floods to a depth of ~10 cm on spring tides and consists of a zone of *S. patens* (saltmeadow cordgrass), which is mixed with smaller, less demarcated patches of various plants (e.g., *Distichlis spicata*.) Salt pannes mottle the marsh platform landscape and a zone of short (stunted)-form *S. alterniflora* (< 40 cm in August 2003) occurs along the perimeter of these pannes. The terrestrial edge of the marsh is dominated by *Iva frutens* (marsh elder).

**Creek Dimensions and Physical Properties**

Temperature and salinity were measured monthly from April – October 2003 within a meter of the creek bottom in the center of the channel. Salinity was measured in each branch and temperature was recorded at the confluence of the two branches; water samples were taken at mid-ebbing tide (~2.5 hours after peak high tide). Temperature was measured with a thermistor (YSI model 9600, YSI Environmental) and salinity was measured with a handheld refractometer. Sediment cores were taken at each habitat within each creek branch with a 2.2-cm inner diameter plastic corer (3-cm depth) and sediment particle sizes were analyzed using a slightly modified version of a protocol described by Folk (1980). Creek branch lengths were measured in the field and each branch was divided into 50-m segments from the confluence to the terminus. The cross-sectional area and volume were measured for each segment. The cross-sectional area was measured as the width of the channel multiplied by the maximum depth and the volume was calculated by multiplying the cross-sectional area by 50 m (segment length). The volume of all 50-m sections in each creek were summed to calculate the total volume of a creek. Creek distances from the Rowley River and Plum
Island Sound were calculated to the nearest 5 m using aerial USGS maps (www.usgs.gov). Sinuosity was calculated as the ratio of creek branch length to the straight-line distance (to the closest 5 m) of the creek branch from the confluence to its terminus.

**Benthic Sampling**

To determine infaunal variability associated with spatial scale, all four tidal creeks (> 1 km) were sampled in June (17-19), July (9-10), August (4-5), and October (3-4), 2003. In each branch (100s of m) of each creek, three transects were selected at ~50, 100, and 150 m (≥50 m) from the confluence of the two branches. Each transect (50 m in length and 20 m in width) was stratified along an inundation gradient into five habitat zones (from lowest to highest elevation): 1) unvegetated creek mudflat of unconsolidated sediment (MF), 2) creek wall (CW) – a vertical wall with a band of filamentous algae, 3) tall-form *S. alterniflora* (TSA), 4) *S. patens* (SP), and 5) short-form *S. alterniflora* (SSA) (Fig. 1). The tidal regime of PIE inundates the MF, CW, and TSA habitats twice daily and the SP and SSA habitats only during spring tides.

The hierarchical nested design of 4 creeks x 2 branches per creek x 3 transects per branch x 5 habitats per transect yielded 480 sample sites for all four months. At each sample site, a single macroinfauna core (6.6-cm inner diameter push corer) was taken to a depth of 5-cm. This method may inadequately sample larger, more mobile infauna (e.g., *Nereis diversicolor*) and surface-dwelling epifauna (e.g., amphipods). Cores were placed on ice in the field and fixed with 10% formalin and Rose Bengal in the laboratory. After a minimum of two days, cores were sieved through a 1-mm sieve stacked on top of a 500-µm sieve. Large debris and roots retained on the 1-mm sieve were discarded after visual inspection and removal of large invertebrates. Annelids
constituted 97% of macroinfaunal abundances and are the focus of this study. All annelids were sorted and identified to species, although some were assigned a nominal species designation rather than a formal taxonomic status. Shannon-Weiner diversity ($H'$ log base $e$), evenness (Pileou’s diversity $J$), and species richness (species number) were calculated for the annelid community for each sample with PRIMER 5.2.9 software (Clarke and Warwick 2001).

**Statistical Analyses**

To determine the variation of the diversity indices and annelid abundances at different spatial scales, a GLIMMIX macro was used to fit a Generalized Linear Mixed Model (GLMM) in SAS (v. 9.1, Cary, NC, USA). The different spatial scales [Creek (> 1 km), branches within creeks (100s of m), and transects within branches (50 – 200 m)] were assigned as random variables and variance component estimates were calculated using Restricted Maximum Likelihood (REML) estimates with a small sample (i.e., 1 replicate per site) size correction (Kenward-Rogers adjustment) for the error term. The error term or residual is equivalent to the variation among cores (< 50 m). The primary goal of variance component estimation is to estimate the covariation between random factors and the dependent variable (Statsoft, Inc 2006). For instance, an estimate of covariance between the creek factor and a population indicates the amount of variation due to creek for that population. Fixed effects of habitat, month, and their interaction were tested in the GLMM with a Type 3 Test for Fixed Effects. All data were log$_e$-transformed using the Link=log function and the errors were assumed to have a Poisson distribution (Manly 2001).
To detect patterns in annelid communities among creeks for each habitat (spatial trends), among months for each habitat (temporal trends), and among habitats for each month (trends along an inundation gradient), communities were analyzed using analysis of similarities (ANOSIM) in PRIMER 5.2.9 software (Clarke and Warwick 2001). Non-metric multidimensional scaling (nMDS) was used to visualize trends in significant community differences. If any significant differences occurred for global (whole test) sample statistics ($R$) in ANOSIM, pairwise sample statistics ($r$) were tested between factor pairs (i.e., creek, month, or habitat pairs). Significantly dissimilar pairwise combinations were further analyzed with similar percentages (SIMPER) analysis to determine the species contributing most to the dissimilarity. All data were log$_e$ (x+1) transformed and Bray-Curtis similarity was used to generate a distance matrix.

**RESULTS**

**Creek Dimensions and Physical Properties**

From April – October 2003, all four creeks had broadly overlapping salinity concentrations, which ranged from 14.0 – 34.0 ‰, and similar temperature ranges (9.0 – 26.0 ºC; Table 1). However, Sweeney Creek had the lowest average salinity (22.98 ‰) and Clubhead Creek had the highest average salinity (27.76 ‰) (Table 1). Nelson Creek’s right branch was the most sinuous (3.33), due to its oxbow (Table 1; Fig. 1). Both Sweeney Creek’s right and West Creek’s left branches were the straightest (1.1) (Table 1). Excluding Nelson Creek’s right branch (630 m long), all branches were similar in length (230 – 410 m) (Table 1). Despite having the longest creek branch, Nelson Creek had the lowest volume ($4.1 \times 10^6$ L) and Sweeney Creek had the highest
Table 2.1. Physical properties and dimensions of tidal creeks in the Plum Island Estuary, Massachusetts. Salinity and temperature values are ranges from April –October 2003 with the average of all months in parentheses. Silt-clay % is the range across all habitats (e.g., mudflats to high marshes) within each creek. L = Left creek branch and R = Right creek branch.

<table>
<thead>
<tr>
<th>Creek</th>
<th>Salinity Range (ppt)</th>
<th>Temperature Range (°C)</th>
<th>Silt-Clay %</th>
<th>Distance from Plum Island Sound (km)</th>
<th>Distance of Confluence from Rowley River (km)</th>
<th>Creek Volume (L X 10^6)</th>
<th>Branch Length (m)</th>
<th>Cross-sectional area (m²) at 50 m from confluence</th>
<th>Branch Sinuosity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clubhead</td>
<td>21.00 – 34.00 (27.76)</td>
<td>10.00 – 27.00 (18.71)</td>
<td>79.3 – 91.6</td>
<td>3.90</td>
<td>1.90</td>
<td>5.9</td>
<td>360 240</td>
<td>22.12 17.55</td>
<td>1.7 1.5</td>
</tr>
<tr>
<td>Nelson</td>
<td>20.00 – 32.50 (25.93)</td>
<td>9.00 – 26.00 (17.93)</td>
<td>79.0 – 88.8</td>
<td>2.19</td>
<td>-</td>
<td>4.1</td>
<td>270 630</td>
<td>5.27 8.89</td>
<td>1.7 3.3</td>
</tr>
<tr>
<td>Sweeney</td>
<td>14.00 – 30.50 (22.98)</td>
<td>9.50 – 26.00 (18.36)</td>
<td>77.5 – 86</td>
<td>4.43</td>
<td>1.31</td>
<td>7.5</td>
<td>300 335</td>
<td>12.42 10.73</td>
<td>1.1 1.2</td>
</tr>
<tr>
<td>West</td>
<td>19.00 – 32.00 (25.62)</td>
<td>11.50 – 25.00 (18.64)</td>
<td>80.9 – 88.4</td>
<td>3.89</td>
<td>1.43</td>
<td>6.1</td>
<td>410 230</td>
<td>5.27 8.89</td>
<td>1.3 1.1</td>
</tr>
</tbody>
</table>
volume \((7.5 \times 10^6\) L). All creeks except Nelson Creek drain into the Rowley River and are between 1.31 and 1.90 km away (measured from the confluence of creek branches) (Table 1). The creeks had broadly similar silt-clay fractions of sediment, which ranged from 77.5 – 91.6% across all habitats (Table 1).

**Annelid Assemblages**

All four creeks supported similar annelid species assemblages. Small annelids were most common, and 30,168 individuals were collected representing 17 species (Table 2). The five most common species (defined as representing > 5% of the number of total annelids) were *Fabricia sabella* (Ehrenberg, 1837) (8%), *Manayunkia aestuarina* (Bourne, 1883) (39%), *Streblospio benedicti* (Webster, 1879) (7%), *Paranais litoralis* (Müller, 1784) (9%), and *Cernosvitoviella immota* (Knöllner, 1935) (24%), together comprising 87% of the total annelid community. Overall, polychaetes and oligochaetes comprised 56 and 44% of the annelid community, respectively.

**Temporal Trends**

Total annelid density (individuals m\(^{-2}\)) increased from June – October for the CW, TSA, and SP habitats, whereas annelid density decreased for the MF and SSA habitats (Fig. 2). Although *M. aestuarina* populations varied relatively little for the first three months, abundances increased significantly (GLMM; \(p < 0.0001\); Table 3) with 7.5, 2, and 4-fold increases in abundance for October relative to June in the CW, TSA and SP habitats, respectively (Fig. 2). *S. benedicti* and *P. litoralis* both experienced significant population declines (GLMM; \(p < 0.0001\); Table 3) in October and August, respectively (Fig. 2), though this effect was habitat dependent for *P. litoralis* but not *S. benedicti* (Table 3; see *Trends along the inundation gradient* section).
Figure 2.2. Temporal trends of mean ±1 SE (n=24) densities (individuals m$^{-2}$) dominant annelids from June – October 2003 for each habitat. Total macroinfaunal annelid density (individuals m$^{-2}$) of each month-habitat combination is shown above each set of means.
Table 2.2. Species composition and mean ± 1 SE annelid density (indm$^{-2}$) at each habitat type along the inundation gradient in the marshes of Plum Island Estuary, Massachusetts, USA for June – October 2003. Each mean is calculated across the 96 cores collected from all creeks and months for each habitat. MF=mudflat; CW=creek wall; TSA=Tall-form *Spartina alterniflora*; SP=*S. patens*; SSA=Short-form *S. alterniflora*.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>MF</th>
<th>CW</th>
<th>TSA</th>
<th>SP</th>
<th>SSA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Annelids</strong></td>
<td>14,850±</td>
<td>44,661±</td>
<td>21,204±</td>
<td>4,974±</td>
<td>7,005±</td>
</tr>
<tr>
<td></td>
<td>1,349</td>
<td>4,821</td>
<td>2,305</td>
<td>836</td>
<td>1,129</td>
</tr>
<tr>
<td><strong>Polychaeta</strong></td>
<td>9,126±</td>
<td>29,108±</td>
<td>11,701±</td>
<td>2,231±</td>
<td>1,143±</td>
</tr>
<tr>
<td></td>
<td>942</td>
<td>4,280</td>
<td>1,922</td>
<td>415</td>
<td>213</td>
</tr>
<tr>
<td><strong>Capitellidae</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Capitella</em> sp.</td>
<td>40±20</td>
<td>181±35</td>
<td>194±39</td>
<td>22±13</td>
<td>27±25</td>
</tr>
<tr>
<td><strong>Nereididae</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Nereis diversicolor</em></td>
<td>1,192±186</td>
<td>120±36</td>
<td>22±8</td>
<td>37±21</td>
<td>12±6</td>
</tr>
<tr>
<td><strong>Phyllodocidae</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Eteone heteropoda</em></td>
<td>369±53</td>
<td>12±6</td>
<td>147±124</td>
<td>0±0</td>
<td>27±28</td>
</tr>
<tr>
<td><strong>Sabellidae</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Manayunkia aestuarina</em></td>
<td>913±</td>
<td>22,274±</td>
<td>10,684±</td>
<td>1,668±</td>
<td>943±</td>
</tr>
<tr>
<td><em>Fabricia sabella</em></td>
<td>205</td>
<td>4,009</td>
<td>1,871</td>
<td>295</td>
<td>175</td>
</tr>
<tr>
<td></td>
<td>135</td>
<td>5,841±</td>
<td>436±</td>
<td>495±</td>
<td>116±</td>
</tr>
<tr>
<td><strong>Spionidae</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Streblospio benedicti</em></td>
<td>5,660±</td>
<td>243±</td>
<td>154±</td>
<td>6±</td>
<td>12±</td>
</tr>
<tr>
<td><em>Polydora cornuta</em></td>
<td>900</td>
<td>83</td>
<td>61</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td><em>Pygospio elegans</em></td>
<td>584±105</td>
<td>280±88</td>
<td>31±12</td>
<td>0±0</td>
<td>3±3</td>
</tr>
<tr>
<td><strong>Oligochaeta</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Paranais littoralis</em></td>
<td>5,724±</td>
<td>15,554±</td>
<td>9,504±</td>
<td>2,744±</td>
<td>5,863±</td>
</tr>
<tr>
<td></td>
<td>718</td>
<td>2,032</td>
<td>1,219</td>
<td>549</td>
<td>1,071</td>
</tr>
<tr>
<td><strong>Enchytraeidae</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Cernosvitoviella</em></td>
<td>218±</td>
<td>10,238±</td>
<td>8,084±</td>
<td>1,475±</td>
<td>2,541±</td>
</tr>
<tr>
<td><em>immota</em></td>
<td>184</td>
<td>1,579</td>
<td>1,167</td>
<td>456</td>
<td>455</td>
</tr>
<tr>
<td>Enchytraied 3</td>
<td>55±55</td>
<td>166±90</td>
<td>363±179</td>
<td>473±115</td>
<td>608±190</td>
</tr>
<tr>
<td>Enchytraied 2</td>
<td>0±0</td>
<td>206±86</td>
<td>178±154</td>
<td>40±24</td>
<td>80±39</td>
</tr>
<tr>
<td>Enchytraied 4</td>
<td>3±3</td>
<td>37±25</td>
<td>95±43</td>
<td>12±7</td>
<td>200±71</td>
</tr>
<tr>
<td><strong>Naididae</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Paranais</em></td>
<td>974±</td>
<td>4,689±</td>
<td>615±</td>
<td>412±</td>
<td>2,108±</td>
</tr>
<tr>
<td><em>litoralis</em></td>
<td>216</td>
<td>1,326</td>
<td>192</td>
<td>76</td>
<td>923</td>
</tr>
<tr>
<td><strong>Tubificididae</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Tubificoides browniae</em></td>
<td>2,006±360</td>
<td>12±6</td>
<td>12±7</td>
<td>6±4</td>
<td>9±7</td>
</tr>
<tr>
<td><em>Tubificoides</em></td>
<td>1,601±</td>
<td>92±</td>
<td>108±</td>
<td>237±</td>
<td>215±</td>
</tr>
<tr>
<td><em>wasselli</em></td>
<td>482</td>
<td>39</td>
<td>49</td>
<td>58</td>
<td>42</td>
</tr>
<tr>
<td><em>Monoplylephorus</em></td>
<td>833±</td>
<td>101±</td>
<td>37±</td>
<td>89±</td>
<td>101±</td>
</tr>
<tr>
<td>sp.</td>
<td>295</td>
<td>86</td>
<td>22</td>
<td>41</td>
<td>46</td>
</tr>
<tr>
<td><em>Tubificoides benedeni</em></td>
<td>34±13</td>
<td>9±9</td>
<td>3±3</td>
<td>0±0</td>
<td>0±0</td>
</tr>
</tbody>
</table>
All species diversity indices exhibited significant variation among sampling dates (GLMM; $p \leq 0.0354$) and these effects were habitat dependent (GLMM; $p \leq 0.0007$; Table 3). Average species diversity and richness were highest in October mudflats (1.37 and 5.7, respectively), whereas creek walls had the lowest evenness (0.40; Fig. 4). SP and SSA habitats exhibited the lowest average species richness (2.25) for July and August, respectively. Annelid communities were most even (0.85) in SP in August (Fig. 4). In general, June exhibited the highest values for all three diversity indices (Fig. 4).

Annelid communities within a habitat were significantly affected by month (ANOSIM; $p = 0.001$; data not shown). However, communities in SP were not different from June through August and in SSA, communities did not differ from July to August.

**Trends along the Inundation Gradient**

The ratio of polychaetes to oligochaetes varied across the inundation gradient with a general decrease from MF to SSA. Large shifts in annelid species composition occurred across the habitat landscape and there was a significant (GLMM; $p < 0.0001$) habitat effect on abundance for all species tested (Table 3; Fig. 3). Variation among habitats was especially evident for *S. benedicti* and *F. sabella*, which were abundant only in MF and CW habitats, respectively (Figs. 3). Month-by-habitat interactions were not significant for either of these species. Generally, *C. immota* was ubiquitous in space and time, but its abundance fluctuated with habitat and time (Figs. 2 and 3), producing significant habitat-by-month interactions (Table 3). *M. aestuarina* and *P. litoralis*, when abundant, were similarly widely distributed across the marsh landscape and experienced significant (GLMM; $p < 0.0001$) habitat-by-month interactions (Table 3).
Table 2.3. Summary table of p-values for tests of fixed effects on annelid diversity indices and selected annelid abundances in four intertidal creeks in Plum Island Estuary, MA. J’ = Pielou’s evenness index, H’ = Shannon’s diversity index.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Habitat</th>
<th>Month</th>
<th>Habitat x Month</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fabricia sabella</td>
<td>p &lt; 0.0001</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Manayunkia aestuarina</td>
<td>p &lt; 0.0001</td>
<td>p &lt; 0.0001</td>
<td>p=0.0029</td>
</tr>
<tr>
<td>Streblospio benedicti</td>
<td>p &lt; 0.0001</td>
<td>p &lt; 0.0001</td>
<td>NS</td>
</tr>
<tr>
<td>Paranais litoralis</td>
<td>p &lt; 0.0001</td>
<td>p &lt; 0.0001</td>
<td>p=0.0002</td>
</tr>
<tr>
<td>Cernosvitoviella immota</td>
<td>p &lt; 0.0001</td>
<td>NS</td>
<td>p &lt; 0.0001</td>
</tr>
<tr>
<td># spp</td>
<td>p &lt; 0.0001</td>
<td>p &lt; 0.0001</td>
<td>p=0.0007</td>
</tr>
<tr>
<td>J’</td>
<td>p &lt; 0.0001</td>
<td>p=0.0354</td>
<td>p=0.0004</td>
</tr>
<tr>
<td>H’</td>
<td>p &lt; 0.0001</td>
<td>p &lt; 0.0001</td>
<td>p &lt; 0.0001</td>
</tr>
</tbody>
</table>

Figure 2.3. Mean ±1 SE (n=24) densities (individuals m-2) of dominant annelids for habitats along an inundation gradient for each month. MF = mudflat; CW = creek wall; TSA = Tall-form Spartina alterniflora; SP = Spartina patens; SSA = Short-form S. alterniflora.
Figure 2.4. Mean (n=24) evenness ($J'$), species diversity ($H'$) and species richness for benthic annelid communities along an inundation gradient by month for intertidal creeks in the Plum Island Estuary, MA. MF = mudflat; CW = creek wall; TSA = Tall-form *Spartina alterniflora*; SP = *Spartina patens*; SSA = Short-form *S. alterniflora*. 
experienced significant (GLMM; p < 0.0001) habitat-by-month interactions (Table 3). These interactions resulted from a sharp population increase in the CW and TSA habitats in October for *M. aestuarina* and a precipitous population decline for *P. litoralis* in the SSA habitat in July (Fig. 2). Highest total annelid densities (33,418 – 65,535 individuals m$^{-2}$) consistently occurred in the CW habitat (Fig. 2); lowest densities (2,421 – 10,668 individuals m$^{-2}$) were found in the SP habitat.

Habitat significantly affected all species diversity indices (GLMM; p < 0.05; Table 3). Habitat effects were evident for $H'$ and species richness, which decreased with decreasing tidal inundation (Fig. 4). For all months, $H'$ tracked species richness along the inundation gradient. Habitat effects on species evenness were evident as $J'$ increased with decreasing tidal inundation, with communities most even in the SP habitat (Fig. 4). Overall, highest species diversity consistently occurred in the MF habitat and decreased with increasing elevation (Fig. 4).

**Spatial Trends**

For the five most abundant annelid species and species diversity indices analyzed in GLMM, there was very little variation associated with creek, branch, or transect location (Table 4). Greater than 90% of the variability exhibited by annelid populations or diversity indices was attributable to mesoscale (i.e., < 50 m) variability (Table 4).

In contrast, there was a significant effect of creek location (i.e., among creek variation) on annelid communities for some habitats (ANOSIM; p ≤ 0.014; Table 5). Significant differences among the frequently inundated MF, CW, and TSA habitats were observed in all months, except the CW habitat in August and the TSA habitat in October.
Table 2.4. Variance component estimates at different scales from GLMM (see text for details) for selected annelid population abundances and species diversity indices in intertidal creeks in Plum Island Estuary, MA for June – October 2003. Percentage of the total variance component is in parentheses, indicating the amount of variation attributable to that scale. J’ = Pielou’s evenness index, H’ = Shannon’s diversity index.

<table>
<thead>
<tr>
<th>Creek</th>
<th>Branch (100s m)</th>
<th>Transect (50-200 m)</th>
<th>Error (&lt;50m)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fabricia sabella</td>
<td>0.326</td>
<td>0.256</td>
<td>13.888</td>
<td>14.447</td>
</tr>
<tr>
<td></td>
<td>(2.3%)</td>
<td>(0%)</td>
<td>(1.8%)</td>
<td>(95.9%)</td>
</tr>
<tr>
<td>Manayunkia aestuarina</td>
<td>0.205</td>
<td>0</td>
<td>28.707</td>
<td>29.139</td>
</tr>
<tr>
<td></td>
<td>(0.7%)</td>
<td>(0%)</td>
<td>(0.007%)</td>
<td>(98.5%)</td>
</tr>
<tr>
<td>Streblospio benedicti</td>
<td>0.454</td>
<td>0.233</td>
<td>6.672</td>
<td>7.380</td>
</tr>
<tr>
<td></td>
<td>(6.2%)</td>
<td>(0.2%)</td>
<td>(3.5%)</td>
<td>(90.1%)</td>
</tr>
<tr>
<td>Paranais litoralis</td>
<td>0</td>
<td>0.104</td>
<td>15.292</td>
<td>15.797</td>
</tr>
<tr>
<td></td>
<td>(0%)</td>
<td>(0.7%)</td>
<td>(2.5%)</td>
<td>(96.7%)</td>
</tr>
<tr>
<td>Cernosvitoviella immota</td>
<td>0.012</td>
<td>0.170</td>
<td>29.198</td>
<td>29.380</td>
</tr>
<tr>
<td></td>
<td>(0.1%)</td>
<td>(0.6%)</td>
<td>(99.3%)</td>
<td>(100%)</td>
</tr>
<tr>
<td># spp</td>
<td>0.001</td>
<td>0.001</td>
<td>0.7122</td>
<td>0.714</td>
</tr>
<tr>
<td></td>
<td>(0.1%)</td>
<td>(0.1%)</td>
<td>(0%)</td>
<td>(99.8%)</td>
</tr>
<tr>
<td>J’</td>
<td>0.007</td>
<td>0</td>
<td>0.1031</td>
<td>0.110</td>
</tr>
<tr>
<td></td>
<td>(6.4%)</td>
<td>(0%)</td>
<td>(0%)</td>
<td>(93.6%)</td>
</tr>
<tr>
<td>H’</td>
<td>0.005</td>
<td>0</td>
<td>0.1841</td>
<td>0.189</td>
</tr>
<tr>
<td></td>
<td>(2.6%)</td>
<td>(0%)</td>
<td>(0%)</td>
<td>(97.4%)</td>
</tr>
</tbody>
</table>

(Table 5). Pairwise comparisons between creeks revealed that Sweeney and Nelson Creeks were most frequently dissimilar (Table 5). MDS plots showed distinct separation of Sweeney Creek relative to Nelson Creek in specific monthly comparisons (Fig. 5). SIMPER analysis showed the species contributing the most to community dissimilarity for significant pairwise creek comparisons varied with no trend evident (data not shown). Conversely, high marsh habitats (i.e., SP and SSA) communities did not differ among creek systems in any monthly collection based on ANOSIM (Table 4). Overall, annelid communities among the four creek systems studied differed in low-marsh habitats (i.e., MF, CW, and TSA); communities in high marsh habitats (i.e., SP and SSA) did not differ.
DISCUSSION

Our study is the first to examine the distribution of infauna across a complete saltmarsh tidal inundation gradient on the US Atlantic coast and is the first comprehensive study of macroinfaunal annelids in a northern New England salt marsh. Temporal and spatial variation in annelid communities were common and variation was scale and species specific. We found that (1) some annelid populations (i.e., *M. aestuarina* and *P. litoralis*) experienced large population fluctuations associated with time of year from June – October, (2) species composition strongly varied along the inundation gradient, (3) there was little spatial variability in annelid abundances and diversity indices associated with (≥ 50 m) or among creek systems (> 1 km) in the same region of the estuary, and (4) annelid communities were often dissimilar among creeks for those habitats that experience twice-daily inundation [i.e., mudflat (MF), creek wall (CW), and Tall-form *S. alterniflora* (TSA) habitats (TSA)], but not the high marsh habitats that are flooded only during spring tides [i.e., *S. patens* (SP) and short-form *S. alterniflora* (SSA) habitats].

Temporal Trends

Pronounced temporal variation of annelid populations was evident in three of the five annelid species analyzed. *P. litoralis* populations declined regardless of habitat beginning in August. Sharp summer population declines of this species on the Atlantic coast are common (Cheng et al. 1993, Sarda et al. 1996). Though temporal variations in macroinfauna densities (i.e., population crashes) may be attributed to predation
Table 2.5. One-way Analysis of Similarity (ANOSIM) for total annelid communities among creeks by month and habitat; among months by habitat; and among habitats by month. If model was significant, then pairwise comparisons which were significant (p < 0.05) are listed. NS = no significant differences. SW=Sweeney Creek, WE=West Creek, CL=Clubhead Creek, NE=Nelson Creek.

<table>
<thead>
<tr>
<th>Habitat</th>
<th>June</th>
<th>July</th>
<th>August</th>
<th>October</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mudflat</td>
<td>p = 0.002</td>
<td>p = 0.003</td>
<td>p = 0.001</td>
<td>p = 0.014</td>
</tr>
<tr>
<td></td>
<td>SW-NE, SW-CL, SW-WE</td>
<td>SW-NE, SW-CL</td>
<td>SW-NE, CL-NE, WE-NE, SW-WE</td>
<td>SW-NE</td>
</tr>
<tr>
<td>Creek Wall</td>
<td>p = 0.035</td>
<td>p = 0.028</td>
<td>NS</td>
<td>p = 0.004</td>
</tr>
<tr>
<td></td>
<td>SW-NE, SW-WE</td>
<td>SW-NE, WE-NE</td>
<td>NS</td>
<td>CL-NE, CL-WE, NE-WE, SW-WE,</td>
</tr>
<tr>
<td>Tall-form</td>
<td>p = 0.056</td>
<td>p = 0.032</td>
<td>p = 0.01</td>
<td>NS</td>
</tr>
<tr>
<td>Spartina alterniflora</td>
<td>SW-NE, WE-NE</td>
<td>SW-NE, CL-NE, WE-NE</td>
<td>SW-NE, CL-NE, SW-WE</td>
<td></td>
</tr>
<tr>
<td>S. patens</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Short-form</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>S. alterniflora</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>
Figure 2.5. MDS plots of macroinfaunal samples (i.e., creek replicates = cores within creeks) for each month by habitat for collections with a significant global $R$ (ANOSIM, $p < 0.05$). Data were log ($x+1$) transformed prior to analysis. NS = not significant. SW=Sweeney Creek, WE=West Creek, CL=Clubhead Creek, NE=Nelson Creek.
(Kneib 1984, Valiela 1995), Cheng et al. (1993) suggest that the *P. litoralis* decline in Flax Pond (New York, USA) is due to depleted nutritional value of the sediment in late summer.

Although overall *C. immota* densities peaked in August, densities in the *S. patens* (SP) and short-form *S. alterniflora* (SSA) habitats peaked in October with average densities an order of magnitude higher than June densities (Fig. 2). The increase in *C. immota* densities coincided with the population decline of *P. litoralis* (Fig. 2). This “replacement” of *P. litoralis* by *C. immota* suggests a competitive relationship between these two subsurface deposit feeders, in which *P. litoralis* outcompetes *C. immota* during the summer and is replaced by *C. immota* when its population crashes in late summer. Additional study is necessary to determine the mechanism of this relationship.

*M. aestuarina* is a dominant, but small bodied, tube-building polychaete in Atlantic and Gulf of Mexico salt marshes (Bell 1982, Bishop 1984, Stocks and Grassle 2003). After remaining relatively constant during the summer, *M. aestuarina* densities increased dramatically in the fall (October 2003), particularly in the creek wall (CW) and tall-form *S. alterniflora* (TSA) habitats (Fig. 2). *M. aestuarina* young are brooded in tubes for ~8 weeks before they are released into the environment; thus, a large reproductive event in mid to late summer (mid July to early August) may explain the increase in October (Bick 1996). Alternatively, the increase may be a result of decreased temperature stress in the fall or a decrease in size-selective predation by epifaunal predators (Bell 1982). *M. aestuarina* reproduction is discontinuous for South Carolina populations, with densities highest in the fall followed by spring and winter peaks (Bell 1982). We cannot determine if a summer decline occurred because we did
not sample in the winter or spring. We doubt there is peak in winter densities as northern New England marshes are subject to ice sheets that scour the marsh surface (Whitlach 1981, DSJ personal observation).

*S. benedicti* is a dominant polychaete in Atlantic, Gulf of Mexico, and Pacific coastal marshes (Levin 1984). *S. benedicti* was most abundant in PIE mudflat (MF) habitats with highest mean densities occurring in July and August (8,456 and 8,960 individuals m$^{-2}$, respectively) and lowest in October (2,593 individuals m$^{-2}$) (Fig. 2), which is similar to October densities (2,875 individuals m$^{-2}$) found by Levin et al. (1998) in *S. foliosa* marshes in Southern California, USA. Our results suggest a late spring recruitment. Laboratory-reared *S. benedicti* have a life span of 6 – 12 months (Levin 1984). Thus, post-settlement mortality such as predation may be responsible for autumnal declines (Posey and Hines 1991).

**Zonation Patterns**

Zonation patterns of temperate saltmarsh plant communities along inundation/elevation gradients are discrete and the mechanisms of zonation are well studied (Bertness 1999). However, few ecologists have attempted to synthesize patterns of zonation for invertebrates along the same gradient (Kneib 1984, Levin and Talley 2000). The research portrayed here was designed to describe infaunal abundance patterns and did not include experiments to test mechanisms (but see Deegan et al. 2007 for experiments examining nutrient enrichment and predator exclusion). Although the patterns of macroinfauna are not as discrete as trends in saltmarsh plant communities, trends did emerge for macroinfauna in PIE.

First, of the five numerically dominant annelids, *S. benedicti* and *F. sabella* were abundant only in MF and CW habitats, respectively. *M. aestuarina, P. litoralis*, and *C.
immota were widely distributed across the gradient when abundant. Second, there was a striking community difference between the MF and short-form S. alterniflora habitats owing to a few key taxa. That is, S. benedicti dominated the MF and P. litoralis or C. immota dominated the SSA habitat, depending on time of the year.

Intuitively, the presence of vegetation in soft-sediment communities should increase benthic invertebrate densities and diversities by providing sediment stabilization and protection from predators. Although this has been established as a paradigm in seagrass communities (Orth 1977, Orth 1991), studies of the saltmarsh benthos have produced inconsistent results with vegetation having positive, negative or neutral effects on benthic invertebrates (Levin and Talley 2000). In contrast to seagrass communities, the highest diversities in the PIE creeks generally occurred in the unvegetated mudflats (MF). This higher diversity results from higher oligochaete diversity in mudflats, a result that may be more prominent in soft-sediment communities but missed because studies may not identify individual oligochaete species. In contrast to annelid diversities, annelid densities in the unvegetated mudflat (MF) were typically lower than creek bank S. alterniflora (TSA), except in July 2003. Although this result may suggest that vegetation enhances annelid densities in vegetated habitats in PIE creeks, the highest mean annelid densities were found consistently in the creek wall. The extreme vertical wall feature of the CW habitat, found only in northern US marshes with high tidal amplitude, but may be important in PIE. A distinct band of filamentous and macroalgae (e.g., Enteromorpha spp.) is common on CW habitats ~1 m from creek bottom and higher infaunal densities there may result from added protection or increased food availability. In addition, CW sediment is more compacted than TSA sediments and unconsolidated MF sediments, which may inhibit predation from digging.
and sediment-biting predators. The ecological function of this habitat is not well understood and merits further investigation.

The lowest densities occurred in the *S. patens* (SP) habitat. Low annelid densities found in the SP habitat may result from high stem densities, which inhibit light penetration to the sediment surface. Light may therefore limit benthic microalgae (an annelid food source), which may reduce infaunal abundances (Stocks and Grassle 2001). Additionally, the SP habitat floods only during spring tides, and thus sediment desiccation between spring tides may occur, particularly in the summer months. Although the short-form *S. alterniflora* (SSA) habitat is less frequently inundated, it typically retains shallow (1 – 2 cm) standing water, which may facilitate the persistence of generalist surface-deposit feeders (e.g., *M. aestuarina*).

Our findings support the Levin and Talley (2001) generalization that oligochaetes, particularly Enchytraeidae, comprise a greater fraction of the infaunal community in vegetated versus unvegetated sediments. However, this trend may be a function of tidal inundation rather than the presence of vegetation. PIE oligochaetes are best described as subsurface feeders (Cook and Brinkhurst 1973) and, consistent with other studies (e.g., Whaley and Minello 2002), surface-deposit-feeder densities (i.e., polychaetes) declined relative to subsurface deposit feeder densities in habitats farther from the marsh edge (i.e., SP and SSA). Excluding *Capitella* sp, all polychaetes in PIE are classified as facultative surface-deposit feeders (Fauchald and Jumars 1979) and require tidal inundation to replenish food resources. Because reduced flushing may lower densities of surface-deposit-feeders, the decreased tidal inundation of the higher marsh habitats may limit surface deposit feeder (i.e., polychaete) densities (Stocks and Grassle 2003). Also consistent with other findings regarding oligochaetes (Levin et al.
we found that tubificids and naidids dominated MF sediments whereas
enchytraeids dominated higher marsh sediments.

The dominance of *S. benedicti* in the creek MF habitat is consistent with Levin et al. (1998) who judged this polychaete common in mudflats but relatively rare in the adjacent *S. foliosa* marsh. However, our findings contrast with other studies in Atlantic and northern Gulf of Mexico marshes that found *S. benedicti* abundant in vegetated *S. alterniflora* habitats (Kneib 1984, Sacco et al. 1994, Whaley and Minello 2002). This difference may be associated with morphology of PIE creeks, which have a vertical wall creating ~1.5 m vertical distance (Fig. 1) between the mudflats and vegetated zones versus the gentle gradation of mudflat into vegetated zones (i.e., no vertical wall) of most Atlantic marshes.

**A Question of Scale**

Population variation across the environmental landscape has received increased attention in the past 25 years (Levin 1992, Benedetti-Cecchi 2001). In a literature review of studies describing distribution patterns of marine populations and assemblages from both hard substrates and soft sediments, Fraschetti et al. (2005) concluded that abundance patterns were most variable at meso- to small scales (10s of m to 10s of cm). Our results contribute to this general trend of patchiness at smaller scales (< 50 m) for macroinfauna. Food patchiness (Lopez and Levinton 1987, Kelaher and Levinton 2003), local biological interactions (Levin 1981, 1982) and small-scale physical processes (Fleeger et al. 1995, Rossi and Underwood 2002) are pervasive in soft sediments, causing high levels of spatial variation. Conversely, we found little variation among macroinfauna populations at larger scales [within creeks (≥ 50 m) or among creeks (> 1 km) in a similar salinity regime]. The mechanism for this trend may
be related to recruitment or post-recruitment dispersal. Larval (e.g., *Streblospio benedicti*, Levin 1984), juvenile or adult stages (e.g., *P. litoralis*, Nilsson et al. 2000) of many annelids in PIE are known to disperse via the water column, and this dispersal ability may be enhanced by the relatively large tidal flux of PIE. Our findings are congruent with Posey et al. (2003), who found infaunal abundances were more variable associated with local microhabitat (topographical) differences than over large-scale distances (>1 km).

Although it is well known that the marine benthos is patchy at small spatial scales (Sun and Fleeger 1991, Bergstrom et al. 2002), soft-sediment benthic ecologists typically conduct experiments that manipulate the environment on similarly small scales (<10 m) and extrapolate those results to an ecosystem (Posey et al. 1999, Sarda 1995). Our work suggests that experiments conducted at small spatial scales may be misleading in at least two important ways. First, some processes and interactions may require large spatial and temporal scales to be operative. For example, treatment effects on epifauna (e.g., isopods and amphipods) may be impossible to ascertain because exclusion or inclusion cages do not allow for the natural movement of these organisms that are capable of migrating across habitats within the saltmarsh landscape (Deegan et al. 2007). Second, high variability reduces statistical power and the confidence in conclusions (Fraschetti et al. 2005).

Though all PIE creeks exhibited similar macroinfaunal assemblages, community differences existed among creeks but only in twice-daily flooded habitats (i.e., MF, CW and TSA). The relatively large tidal flux of this system may be responsible for these differences by affecting recruitment, predation, competition, or food supply in areas that receive daily flooding. Macroinfaunal communities in Sweeney and Nelson Creeks
were most frequently dissimilar (Table 4). Sweeney Creek has lower salinity than Nelson Creek (Table 1) due to freshwater inputs from the surrounding watershed. Nelson Creek is only 2.19 km from the sound whereas Sweeney Creek is 4.43 km (Fig. 1; Table 1). Differential salinities and proximities to the sound may influence the macroinfaunal community structure of these two creeks. Alternatively, the supply of larvae may be important. Planktotrophic larvae (e.g. *S. benedicti*) may settle at the first available and acceptable location, and thus fewer larvae from PI Sound or Rowley River may travel to more inland locations (Butman 1987). Nevertheless, (Olafsson et al. 1994) suggested that post-settlement processes (e.g., predation and disturbance) are more important in regulating soft sediment communities than larval supply. Because the high marsh habitats (SP and SSA) are flooded infrequently, effects of predation and disturbance are limited, perhaps contributing to observed community stability.

**Implications for Future Work**

In experimental ecology, two contrasting approaches exist: (1) experiments conducted at small, and often viewed as inadequate, spatial and temporal scales to achieve replication, or (2) experiments conducted at ecosystem-wide scales without replication (Oksanen 2001). Ecosystem-wide experiments in aquatic environments began in limnological studies and are useful because they incorporate a broad range of ecosystem phenomena (Carpenter 1989). However, due to logistical and fiscal restraints, they rarely allow for replication, which is essential for the use of inferential statistics (Oksanen 2001). Our results indicate that PIE creeks exhibit minimal variation at the creek scale (i.e., ecosystem-wide, >1 km) with most of the variability found at meso-spatial scales (< 50 m). Therefore, PIE creeks may afford one the opportunity to combine ecosystem-wide experiments with replication. High variability associated with
small scales (i.e., patchiness) may reduce the power of statistical tests (Fraschetti et al. 2005); thus the loss of the number of degrees of freedom associated with ecosystem-wide experiments may be offset by the benefits of examining a scale with low variability. Despite measurable community differences among the creek systems, the four creek systems studied may represent adequate replicates for ecosystem-wide studies. Physically, all creeks studied have similar tidal regimes in addition to having comparable volumes and creek branch lengths (excluding the right branch of Nelson Creek) with corresponding similarities in sediment particle size distributions (Table 1). All creeks exhibited similar annelid species composition, distribution and abundance patterns across the landscape. Therefore, whole-ecosystem manipulations conducted at the creek level in PIE would be appropriate and allow for replication.

**LITERATURE CITED**


CHAPTER 3

RESPONSE OF SALTMARSH INFAUNA TO FERTILIZATION AND PREDATOR REMOVAL: A FOUR-YEAR STUDY
INTRODUCTION

Marine ecosystems serve many human uses, and as a result most of the world’s marine ecosystems are impacted by human activities, with at least 40% of these systems affected by multiple insults (Halpern et al. 2008). Thus, there is a need in marine research to examine multiple impacts simultaneously. Salt marshes are often impacted by multiple stressors (Valiela et al. 2004, Deegan et al. 2007) and are excellent coastal ecosystems for the study of anthropogenic activities due to their importance to humans. For instance, salt marshes buffer adjacent aquatic ecosystems from land derived nutrients and serve as nurseries and a prey-source for important commercial and recreational species (Pennings and Bertness 2001).

As prey for higher tropic levels and consumers of primary producers that respond to nutrient enrichment, saltmarsh benthic invertebrates may be key indicators for examining the effect of anthropogenic activities (Posey et al. 1999, 2002, Sarda et al. 1998, Fleeger et al. 2008). Examinations of saltmarsh invertebrates have focused on spatial and temporal distributions (Kneib 1984, Rader 1984, Johnson et al. 2007), patterns of succession in created marshes (Craft and Sacco 2003, Moseman et al. 2004), changes in benthic community structure associated with invasive macrophytes (Fell et al. 1998, Levin et al. 2006, Neira et al. 2007), the effect of long-term nutrient enrichment (Sarda et al. 1995) and the effect of short-term predator removal and nutrient enrichment (Posey et al. 1999, 2002).

Human-induced nutrient loading and trophic alterations both impact salt marshes, often simultaneously (Deegan et al. 2007). Experimental manipulations of nutrients and predators have been used to gain understanding of the relative importance of top-down (consumer) and bottom-up (resource) controls on benthic food webs (Posey et al. 1995,
Deegan et al. 2007), which has been a major focus of research in aquatic ecosystems (McQueen et al. 1989, Deegan et al. 1997, Menge 2000). Although small temporal and spatial-scale (i.e., plot-level) studies have demonstrated the potential importance of top-down and bottom-up factors on saltmarsh infauna (e.g., Posey et al. 2002), ecosystem-level experiments may be useful in observing complex food web dynamics such as behavior and feedbacks. For instance, in a kilometer-scale fertilization of a tundra river for four years, Peterson et al. (1993) found that nutrients increased algal biomass, insect abundances, and fish biomass. However, in later years, insects began exerting strong top-down grazing pressure on epilithic algae (Peterson et al. 1993). Plot-level (e.g., caging) experiments suggest top-down and bottom-up effects operate independently for saltmarsh infauna (i.e., no interactions; Foreman et al. 1995, Posey et al. 1999, 2002, Fleeger et al. 2008). However, scale may affect trophic interactions (Van de Koppel et al. 2006) and processes may operate at different spatial and temporal scales, making the detection of interactive effects problematic (Posey et al. 1999). Small-scale experiments may limit the natural movements of animals, (Carpenter et al., 1995) and there is a need for coastal ecosystem-level experiments (Heck and Valentine 2007).

Increased resources (light, nutrients) stimulate benthic microalgae biomass and saltmarsh infauna may respond with increased abundances and/or biomass (Sarda et al. 1996, Posey et al. 2002) or remain unchanged (Wiltse et al. 1984). More generally, studies of benthic communities in estuaries and continental shelves suggest that excessive organic matter leads to high decomposition rates that reduce oxygen levels and invertebrate abundances (Diaz and Rosenberg 1995, Kemp et al. 2005). Oxygen stress is typically found in salt marshes where organic matter is added, e.g., sewage
outfalls. Increased nutrient input results in decreased infaunal abundances. Removal of predators typically results in increased infauna abundance (Wiltse et al. 1984, Foreman et al. 1995, Posey et al. 1999, Posey et al. 2002). Predation may strongly affect macroinfauna abundances, whereas nutrient enrichment may have stronger impacts on biomass (Sarda et al. 1996, Posey et al. 2006). Abundance and biomass may therefore need to be examined simultaneously to observe differential effects. Moreover, infaunal responses are often taxon- and habitat-specific (e.g., Posey et al. 2006, Fleeger et al. 2008). The magnitude of consumer and nutrient effects vary along environmental gradients like salinity (Pennings and Bertness 2001, Denno et al. 2005, Deegan et al. 2007, Fleeger et al. 2008).

The purpose of this chapter was to examine top-down and bottom-up effects on saltmarsh infauna at relatively large spatial and temporal scales. As part of a larger, multi-disciplinary study examining the effect of ecosystem-wide manipulations of trophic structure and nutrient availability on a Massachusetts salt marsh (the TIDE study described by Deegan et al. 2007), I examined the response of the macroinfaunal community during three years of manipulation. I examined population-level (density and biomass of selected taxa) and community-level (similarity and diversity indices) responses of macroinfauna. Initial responses of macroinfauna to one summer season of treatments are described in Fleeger et al. (2008). Here, I examine the effect of two additional field seasons of manipulation on macroinfauna.
I predicted (i) nutrient additions would stimulate infauna abundance and biomass as their food source, benthic microalgae, became more abundant or nitrogen-enriched; (ii) predator removal would elicit increased infauna abundances and biomass as predation pressure was reduced on the community; and (iii) the effect of combined treatments would be additive (i.e., no interactions).

METHODS

Study Site

My study is part of the TIDE (Trophic Cascades and Interacting Control Processes in a Detritus-based Ecosystem, http://ecosystems.mbl.edu/Tide/) project. TIDE is a multi-institutional, multi-disciplinary project using ecosystem-wide manipulations to examine the effect of nutrient addition and the reduction of a key predator (the killifish Fundulus heteroclitus) on saltmarsh ecosystems. These manipulations were conducted in four intertidal creeks – Sweeney, West, Clubhead, and Nelson – in the Plum Island Estuary (PIE), Massachusetts, USA (Fig. 1). PIE is a saltmarsh estuary that is relatively unaffected by nutrient loading (background nutrients: < 5 µM NO$_3^-$; ~1 µM PO$_4^{3-}$). The four intertidal creeks exhibit similar physical dimensions, water chemistry, plant and infaunal communities (details in Deegan et al. 2007 and Johnson et al. 2007). I examined macroinfauna in four habitats along an inundation gradient: two creek habitats and two marsh platform habitats (Fig. 1). Mudflats are creek habitats of poorly consolidated sediments without macrophytes but dominated by migrating diatoms, chlorophytes and cyanobacteria in the sediment-dwelling algae (K. A. Galván, unpublished). Creek walls are steep, almost vertical walls about 1.5 m in height, with cohesive sediments and an approximately 30-cm wide band
Figure 3.1. Upper figure is a satellite photograph (MassGIS Orthophoto 2002) of the Rowley River region salt marshes of the Plum Island Estuary, MA. SW=Sweeney Creek, WE=West Creek, CL=Clubhead Creek, NE=Nelson Creek. Lower figure is a profile of sampled salt marsh habitats (not drawn to scale) with size ranges of each habitat. MF = mudflat; CW = creek wall; TSA = Tall-form *Spartina alterniflora*; SP = *Spartina patens*; SSA = Short-form *S. alterniflora.*
of macroalgae and filamentous algae. The creek bank is dominated by a zone of tall-
form *Spartina alterniflora* (>130 cm height in late summer). The marsh platform consists
of an expansive area dominated by a dense canopy of *S. patens*. PIE experiences a
mean tidal amplitude of ~3 m during spring tides, and mudflat, creek wall and creek-
bank *S. alterniflora* habitats are inundated twice daily while the *S. patens* habitats is
inundated to a depth of ~10 cm during spring tides.

**Experimental Design**

A matched-pair design was used to pair Sweeney and West Creeks (Pair 1) and
Clubhead and Nelson Creeks (Pair 2). Pair 1 creeks were manipulated for three years
(2004 – 2006) and Pair 2 creeks were manipulated for 1 year (2005). In Sweeney and
Clubhead Creeks, nutrient enrichment of 70 µM NO$_3^-$ and 4 µM PO$_4^{3-}$ (15x over
background) was achieved by pumping a concentrated solution of nutrients to the water
of every flooding tide during the growing season (mid-May – Oct.; ~150 d). The pump
rate was adjusted, based on a hydrologic model, every 10 min throughout each
incoming tide to maintain constant N and P concentrations in incoming waters (Deegan
et al., 2007). Watershed nutrient loading averaged 30 g N m$^{-2}$ y$^{-1}$ in 2004 (~10x
background loading) but spatial variation across the landscape was significant. Creek-
bank *S. alterniflora* experienced a higher nutrient loading than the less frequently
flooded *S. patens* (Deegan et al. 2007). Fertilizer was not added to reference creeks.

A branch of each creek was selected for large-scale removal of killifish, *F.
heteroclitus*. This was achieved by stretching a Vexar (6.35-mm mesh) block net across
the entrance of the branch from June – September 2004, coupled with continuous fish
trapping and removal. This method of exclusion produces fewer artifacts than
traditional small-scale exclusions (Virnstein 1978). A 60% reduction in killifish density
was achieved (Deegan et al. 2007). Reduction of large killifish (> 40 mm) was greater than small killifish (< 40 mm); although a 40% reduction of small killifish was observed. Thus, a full factorial design was employed with four treatments: (1) ambient nutrients/ambient fish (control) (2) ambient nutrients/low fish, (3) nutrient additions/ambient fish, and (4) nutrient additions/low fish.

**Benthic Sampling**

Macroinfauna were sampled by hand coring at low tide. Pre-treatment collections were taken in June (17-19), July (9-10), and August (4-5) 2003 and post-treatment collections were taken in June (14-15), July (12-13) August (2-3) 2004; June (26-28), July (28-31) and September (12-15) 2005; and August (15-16) 2006. In each creek branch, three transects were selected at ~50, 100, and 150 m from the confluence of the two branches. Each transect (50 m in length and 20 m in width) was stratified along an inundation gradient into the four habitat zones discussed above. Thus, a sample site in the hierarchical design consisted of a habitat nested within a transect nested within a branch nested within a creek.

In 2003 collections, a single macroinfauna sample was taken at each sampling site (habitat within a transect within a branch within a creek), whereas two samples were taken at each site in 2004-2006. Macroinfauna cores (6.6-cm inner diameter) were taken to a depth of 5 cm. This method inadequately samples larger, more mobile infauna. For instance, the relatively large polychaete *Nereis diversicolor* can reach up to 30 cm in body length and large size classes may not be sampled by the core. Cores were placed on ice in the field and fixed with 10% formalin and placed in a Rose Bengal solution in the laboratory. After a minimum of two days, cores were sieved through a 1-mm sieve stacked on top of a 500-µm sieve. Large debris and roots retained on the 1-
mm sieve were discarded after visual inspection and removal of large invertebrates. Animals were sorted to the lowest possible taxon. Annelids constituted 94% of the infauna community and are the predominate focus of this study, although the tanaid crustacean *Leptochelia savignyi* and greenhead fly larvae *Tabanus* sp. – low density, but large prey items for killifish – were also included in biomass analyses. Species diversity – estimated as richness, Shannon’s value (logₑ) and Pielou’s evenness – of annelids was calculated for each sample using PRIMER (v. 6.1.6).

**Population Analysis**

I used a before-after, control-impact (BACI) experimental design which pairs experimental units and accounts for variability that may contribute to error in a completely randomized design (Underwood 1994). Because of natural differences between ecosystems, replication of ecosystem-scale experiments is difficult (Carpenter et al. 1995) but the matched-pair approach helps ameliorate this difficulty (Stewart-Oaten and Bence 2001). Although this design entails pseudoreplication, the BACI design is a powerful method for detecting impacts because it incorporates both temporal and spatial variation by observing reference and impact sites over time (Parker and Wiens 2005). I used a BACI-type ANOVA (based on a level-by-time “parallelism” design) to analyze changes in annelid densities and diversities for each creek pair separately. Level-by-time designs are ineffective if many zeroes are present (Parker and Wiens 2005), and I analyzed taxa only in habitats where they were abundant. Previous analysis (Johnson et al. 2007) suggested that variance associated with transects for macroinfauna populations did not contribute significantly to spatial variation.
in PIE (i.e., no spatial autocorrelation within branches). Transects were therefore considered replicates and pooled; n / branch = 3 in 2003 and n / branch = 6 in 2004 – 2006.

To detect interactions between fertilization and predator removal, I performed analyses directly on abundance values instead of deltas (differences between reference and impact sites) (Stewart-Oaten and Bence 2001). Data were analyzed as generalized linear mixed models (GLMMs) using Proc GLIMMIX (SAS v. 9.1.3). GLMMs are extensions of mixed models and can accommodate non-normal errors (Littell et al. 1996). All data were loge-transformed and errors were assumed to have a Poisson distribution (Littell et al. 1996). Period, nutrient level, fish level and all possible interactions were set as fixed factors, whereas month within period was defined as a random factor. Only significant period*treatment interactions were of interest because they suggest that change over time occurred due to treatment effects. One assumption using this type of analysis is that although response variables at different sites may differ spatially, those differences track each other over time. This assumption, however, may be violated, reducing confidence in results (Wiens et al. 2004). To bolster confidence in results and to identify the direction of changes for significant interactions, I visually inspected graphic representations of data in pre-treatment and treatment periods.

**Biomass Analysis**

I focused on two levels of infauna biomass for treatment effects: population biomass (mg dry weight m⁻² of a selected population) and community biomass (mg dry weight m⁻² of all species combined). Population biomass was determined for most individual species from each sample taken in September 2005 via dry weights of pooled
individuals of each species after drying for two days at 70° C. Occasionally small (< 5 mm) and rare species were only represented by one individual and were not weighed. Specimens of the spionid polychaetes *Streblopio benedicti* and *Polydora cornuta* (=ligni) were often damaged during sieving and biomass was determined via measurement of a morphometric feature (width of widest setiger of *S. benedicti* and the fifth setiger of *P. cornuta*) using digital imaging software (SPOT Imagining Software v. 4.5) under 100x magnification with a Zeiss StereoLumar stereomicroscope. Regressions for size to mass conversions for these species are found in Sarda et al. (1996). Average individual (per capita) biomass for abundant species was also recorded by dividing the total biomass of a sample by the number of individuals weighed. Population and community biomass data were analyzed using two-way ANOVA with nutrient and fish levels as fixed effects. Data were loge transformed to meet assumptions of normality. When treatments had significant effects on population biomass, individual biomass (average biomass of individuals within a sample) was examined to determine if effects were due to changes in per-capita biomass.

**Community Analysis**

Second-stage community analysis was used to determine if changes in annelid communities over time were due to treatment effects. Second-stage community analysis does not test for naturally occurring differences in communities between different areas, but rather tests whether temporal variations in areas show a different temporal pattern (i.e., trajectory) as a result of treatments (a treatment by time interaction; Clarke and Gorley 2006). In second-stage community analysis, similarity matrices of community changes in time for a given area (in this case, treatment branch) are first generated and then are compared to determine similarity in the temporal
trajectories of community assemblages are (an MDS of multiple first-stage MDSs). Clarke and Gorley (2006) and Clarke et al. (2006) provide details and examples for use of this analysis.

Using data from end-of-year sampling (2003-2006 August/September samples) for Sweeney and West Creeks only, four Bray-Curtis similarity (log_{10} x+1 transformation) matrices were generated for species that contributed at least 1% by abundance to the community for each treatment branch. Within each habitat, this generated eight first-stage MDSs with the four sampling dates as factors of interest. A second stage similarity matrix was then generated comparing the time trajectories of community assemblages in the four treatments, which become the factors of interest. An analysis of similarities (ANOSIM) was then performed on the second-stage matrix values to determine if there was a difference in communities over time due to treatments (Clarke and Gorley 2006). This was done separately for each habitat type. Analyses were conducted in PRIMER (v. 6.1.6, Clarke and Warwick 2001).

RESULTS

General Trends for Macroinfauna

A total of 105,958 macroinfauna individuals were collected representing at least 30 species. Annelids numerically constituted 93.8% of the overall invertebrate community with 99,438 individuals representing at least 10 polychaete and 8 oligochaete species. Total annelid density ranged from 300 – 200,000 individuals m^{-2} across the landscape and across the years of study. Highest densities were consistently observed on the creek walls and lowest on the marsh platform (i.e., Spartina patens). In September 2005, average per-capita biomass ranged from 7 – 7000 µg DW individual^{-1} across all species and habitats (Tables 1-4). Population
biomass ranged from $3 \times 10^{-4} – 2.7$ g DW m$^{-2}$ across all species and habitats (Tables 1-4). Community biomass ranged from $6.8 \times 10^{-3} – 77.7$ g DW m$^{-2}$ and was highest in the creek wall and lowest in the *Spartina patens* sediments (Tables 1-4).

Average species richness for annelids ranged from 2.5 (*S. patens*) to 4.8 (mudflat) across time and treatments. Average evenness (Pielou’s $J'$) for annelids ranged from 0.6 (*S. alterniflora*) to 0.8 (*S. patens*). Average species diversity ($H'$) for annelids ranged from 0.7 (*S. patens*) to 1.0 (mudflat). For comparison, annelid diversity (particularly richness) in PIE is similar to (Stocks and Grassle 2003) or lower than (Sarda et al. 1995, Posey et al. 1999) values reported in other studies of the saltmarsh benthos in the western Atlantic. Fleeger et al. (2008) reported that annelid richness is generally lower than meiobenthic copepod diversity in PIE.

As is often observed in soft-sediment communities, numerically dominant species in PIE did not dominate the total biomass of the community (Levin and Talley 2000). For instance, although *Manayunkia aestuarina* numerically dominated the creek wall (average 13,755 ind m$^{-2}$, 32% of the community), it only accounted for 5% of the community biomass. Instead, with only 95 individuals m$^{-2}$ on average (5% of the community), *Nereis diversicolor* dominated the community biomass in the creek wall with 50% (Table 2).

**Density Responses**

After three growing seasons of predator removal and nutrient addition, no annelid taxon (individual species or total annelids) density in any habitat responded to either treatment in Creek Pair 1 (see Fig. 2 for total annelids; Table 5 for statistical summary for abundant taxa). However, short-term interactive effects on the density may have
Table 3.1. Mean density (ind m$^{-2}$), population biomass (mg dry weight m$^{-2}$), and per-capita biomass (µg dry weight ind. $^{-1}$) of dominant macroinfauna from mudflat sediments in the Plum Island Estuary, MA, USA. Density values are means from a composite of samples from all sampling sites and sampling dates. Biomass values are means of a composite of samples from all sampling sites from September 2005. Collected epifauna (e.g., amphipods and spiders) are not included.

<table>
<thead>
<tr>
<th>Mudflat taxa</th>
<th>Density (ind. m$^{-2}$)</th>
<th>% fauna density</th>
<th>Total biomass (mg m$^{-2}$)</th>
<th>% of total biomass</th>
<th>Per capita biomass (µg ind.$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fabricia sabella (P)</td>
<td>122</td>
<td>0.81</td>
<td>2.36</td>
<td>0.15</td>
<td>8.00</td>
</tr>
<tr>
<td>Manayunkia aestuarina (P)</td>
<td>1,286</td>
<td>8.55</td>
<td>22.91</td>
<td>1.47</td>
<td>49.89</td>
</tr>
<tr>
<td>Streblospio benedicti (P)</td>
<td>7,545</td>
<td>50.15</td>
<td>228.47</td>
<td>14.67</td>
<td>62.72</td>
</tr>
<tr>
<td>Pygospio elegans (P)</td>
<td>115</td>
<td>0.77</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Polydora cornuta (P)</td>
<td>297</td>
<td>1.97</td>
<td>230.08</td>
<td>1.48</td>
<td>78.00</td>
</tr>
<tr>
<td>Marenzelleria viridis (P)</td>
<td>7</td>
<td>0.04</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Neres diversicolor (P)</td>
<td>705</td>
<td>4.69</td>
<td>262.41</td>
<td>16.85</td>
<td>631.76</td>
</tr>
<tr>
<td>Eteone heteropoda (P)</td>
<td>130</td>
<td>0.86</td>
<td>62.75</td>
<td>4.03</td>
<td>111.13</td>
</tr>
<tr>
<td>Hobsonia florida (P)</td>
<td>46</td>
<td>0.31</td>
<td>97.58</td>
<td>6.26</td>
<td>330.79</td>
</tr>
<tr>
<td>Capitella sp. (P)</td>
<td>34</td>
<td>0.23</td>
<td>270.90</td>
<td>17.39</td>
<td>918.35</td>
</tr>
<tr>
<td>Paranais litoralis (O)</td>
<td>1,028</td>
<td>6.83</td>
<td>9.50</td>
<td>0.61</td>
<td>6.70</td>
</tr>
<tr>
<td>Psammoryctides sp. (O)</td>
<td>13</td>
<td>0.09</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tubificoides benedeni (O)</td>
<td>44</td>
<td>0.30</td>
<td>29.35</td>
<td>1.88</td>
<td>54.75</td>
</tr>
<tr>
<td>Tubificoides brownae (O)</td>
<td>909</td>
<td>6.04</td>
<td>35.56</td>
<td>2.28</td>
<td>76.69</td>
</tr>
<tr>
<td>Tubificoides wasselli (O)</td>
<td>1,081</td>
<td>7.18</td>
<td>61.57</td>
<td>3.95</td>
<td>174.13</td>
</tr>
<tr>
<td>Monopylephorus sp (O)</td>
<td>803</td>
<td>5.34</td>
<td>123.83</td>
<td>7.95</td>
<td>241.24</td>
</tr>
<tr>
<td>Cernosvitoviella immota (O)</td>
<td>667</td>
<td>4.43</td>
<td>44.84</td>
<td>2.88</td>
<td>152.00</td>
</tr>
<tr>
<td>Other enchytraeids (O)</td>
<td>29</td>
<td>0.19</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Leptochelia savignyi (Cr)</td>
<td>25</td>
<td>0.16</td>
<td>199.40</td>
<td>12.80</td>
<td>676.00</td>
</tr>
<tr>
<td>Tabanus larvae sp. (In)</td>
<td>160</td>
<td>1.06</td>
<td>83.25</td>
<td>5.34</td>
<td>237.71</td>
</tr>
<tr>
<td>Total Community</td>
<td>14,859</td>
<td>100</td>
<td>107.22</td>
<td>100</td>
<td>189.04</td>
</tr>
</tbody>
</table>

Cr = crustacean, In = insect, O = oligochaete, P = polychaete.
Table 3.2. Mean density (ind m⁻²), population biomass (mg dry weight m⁻²), and per-capita biomass (µg dry weight ind⁻¹) of dominant macroinfauna from creek wall sediments in the Plum Island Estuary, MA, USA. Density values are means from a composite of samples from all sampling sites and sampling dates. Biomass values are means of a composite of samples from all sampling sites from September 2005. Collected epifauna (e.g., amphipods and spiders) are not included.

<table>
<thead>
<tr>
<th>Creek wall taxa</th>
<th>Density (ind. m⁻²)</th>
<th>% fauna density</th>
<th>Population biomass (mg m⁻²)</th>
<th>% of total biomass</th>
<th>Per capita biomass (µg ind⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fabricia sabella (P)</td>
<td>7,321</td>
<td>17.19</td>
<td>77.87</td>
<td>1.65</td>
<td>53.20</td>
</tr>
<tr>
<td>Manayunkia aestuarina (P)</td>
<td>13,755</td>
<td>32.30</td>
<td>239.48</td>
<td>5.06</td>
<td>44.69</td>
</tr>
<tr>
<td>Streblospio benedicti (P)</td>
<td>533</td>
<td>1.25</td>
<td>343.89</td>
<td>7.27</td>
<td>199.64</td>
</tr>
<tr>
<td>Polydora cornuta (P)</td>
<td>745</td>
<td>1.75</td>
<td>381.73</td>
<td>8.06</td>
<td>108.65</td>
</tr>
<tr>
<td>Pygospio elegans (P)</td>
<td>285</td>
<td>0.67</td>
<td>531.58</td>
<td>11.23</td>
<td>139.90</td>
</tr>
<tr>
<td>Nereis diversicolor (P)</td>
<td>95</td>
<td>0.22</td>
<td>2,374.60</td>
<td>50.17</td>
<td>7,043.84</td>
</tr>
<tr>
<td>Eteone heteropoda (P)</td>
<td>7</td>
<td>0.02</td>
<td>4.72</td>
<td>0.10</td>
<td>16.00</td>
</tr>
<tr>
<td>Hobsonia florida (P)</td>
<td>6</td>
<td>0.01</td>
<td>42.48</td>
<td>0.90</td>
<td>144.00</td>
</tr>
<tr>
<td>Capitella sp. (P)</td>
<td>113</td>
<td>0.26</td>
<td>85.66</td>
<td>1.81</td>
<td>280.39</td>
</tr>
<tr>
<td>Paranais litoralis (O)</td>
<td>5,934</td>
<td>13.93</td>
<td>47.29</td>
<td>1.00</td>
<td>115.32</td>
</tr>
<tr>
<td>Psammoryctides sp. (O)</td>
<td>3</td>
<td>0.01</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tubificoides benedeni (O)</td>
<td>8</td>
<td>0.02</td>
<td>3.24</td>
<td>0.07</td>
<td>10.98</td>
</tr>
<tr>
<td>Tubificoides brownae (O)</td>
<td>19</td>
<td>0.04</td>
<td>33.00</td>
<td>0.70</td>
<td>82.74</td>
</tr>
<tr>
<td>Tubificoides wasselli (O)</td>
<td>80</td>
<td>0.19</td>
<td>15.44</td>
<td>0.33</td>
<td>39.00</td>
</tr>
<tr>
<td>Monopylephorus sp (O)</td>
<td>27</td>
<td>0.06</td>
<td>305.60</td>
<td>6.46</td>
<td>518.00</td>
</tr>
<tr>
<td>Cernosvitoviella immota (O)</td>
<td>12,099</td>
<td>28.41</td>
<td>68.87</td>
<td>1.45</td>
<td>38.57</td>
</tr>
<tr>
<td>Other enchytraeids (O)</td>
<td>217</td>
<td>0.51</td>
<td>9.69</td>
<td>0.20</td>
<td>20.84</td>
</tr>
<tr>
<td>Leptochelia savignyi (Cr)</td>
<td>459</td>
<td>1.08</td>
<td>94.77</td>
<td>2.00</td>
<td>130.57</td>
</tr>
<tr>
<td>Tabanus sp. Larvae (In)</td>
<td>877</td>
<td>2.06</td>
<td>73.45</td>
<td>1.55</td>
<td>96.82</td>
</tr>
<tr>
<td>Total Community</td>
<td>41,244</td>
<td>100</td>
<td>215.54</td>
<td>100</td>
<td>315.59</td>
</tr>
</tbody>
</table>

Cr = crustacean, In = insect, O = oligochaete, P = polychaete.
Table 3.3. Mean density (ind m^{-2}), population biomass (mg dry weight m^{-2}), and per-capita biomass (µg dry weight ind.^{-1}) of dominant macroinfauna from *Spartina alterniflora* sediments in the Plum Island Estuary, MA, USA. Density values are means from a composite of samples from all sampling sites and sampling dates. Biomass values are means of a composite of samples from all sampling sites from September 2005. Collected epifauna (e.g., amphipods and spiders) are not included.

<table>
<thead>
<tr>
<th>S. alterniflora taxa</th>
<th>Density (ind. m^{-2})</th>
<th>% fauna density</th>
<th>Population biomass (mg m^{-2})</th>
<th>% of total biomass</th>
<th>Per capita biomass (µg ind.^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fabricia sabella (P)</td>
<td>602</td>
<td>1.87</td>
<td>39.82</td>
<td>3.48</td>
<td>117.56</td>
</tr>
<tr>
<td>Manayunkia aestuarina (P)</td>
<td>10,003</td>
<td>31.01</td>
<td>139.58</td>
<td>12.20</td>
<td>41.09</td>
</tr>
<tr>
<td>Streblisio benedicti (P)</td>
<td>537</td>
<td>1.66</td>
<td>66.53</td>
<td>5.82</td>
<td>166.07</td>
</tr>
<tr>
<td>Pygospio elegans (P)</td>
<td>42</td>
<td>0.13</td>
<td>24.34</td>
<td>2.13</td>
<td>27.50</td>
</tr>
<tr>
<td>Polydora cornuta (P)</td>
<td>26</td>
<td>0.08</td>
<td>46.21</td>
<td>4.04</td>
<td>156.67</td>
</tr>
<tr>
<td>Nereis diversicolor (P)</td>
<td>63</td>
<td>0.20</td>
<td>202.21</td>
<td>17.67</td>
<td>685.50</td>
</tr>
<tr>
<td>Eteone heteropoda (P)</td>
<td>46</td>
<td>0.14</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hobsonia florida (P)</td>
<td>11</td>
<td>0.04</td>
<td>30.94</td>
<td>2.70</td>
<td>104.88</td>
</tr>
<tr>
<td>Capitella sp. (P)</td>
<td>249</td>
<td>0.77</td>
<td>130.04</td>
<td>11.37</td>
<td>348.20</td>
</tr>
<tr>
<td>Paranais litoralis (O)</td>
<td>2,781</td>
<td>8.62</td>
<td>4.73</td>
<td>0.41</td>
<td>10.56</td>
</tr>
<tr>
<td>Psammaryctides sp. (O)</td>
<td>3</td>
<td>0.01</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tubificoides benedeni (O)</td>
<td>5</td>
<td>0.01</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tubificoides brownae (O)</td>
<td>11</td>
<td>0.04</td>
<td>4.55</td>
<td>0.40</td>
<td>13.78</td>
</tr>
<tr>
<td>Tubificoides wasselli (O)</td>
<td>89</td>
<td>0.28</td>
<td>78.67</td>
<td>6.88</td>
<td>266.69</td>
</tr>
<tr>
<td>Monopylephorus sp. (O)</td>
<td>21</td>
<td>0.06</td>
<td>89.23</td>
<td>7.80</td>
<td>232.50</td>
</tr>
<tr>
<td>Cernosvitoviella immota (O)</td>
<td>16,829</td>
<td>52.17</td>
<td>158.54</td>
<td>13.86</td>
<td>46.42</td>
</tr>
<tr>
<td>Other enchytraeids (O)</td>
<td>423</td>
<td>1.31</td>
<td>9.89</td>
<td>0.86</td>
<td>20.33</td>
</tr>
<tr>
<td>Leptochelia savignyi (Cr)</td>
<td>3</td>
<td>0.01</td>
<td>50.22</td>
<td>4.39</td>
<td>96.63</td>
</tr>
<tr>
<td>Tabanus sp. larvae (In)</td>
<td>511</td>
<td>1.59</td>
<td>68.58</td>
<td>5.99</td>
<td>129.59</td>
</tr>
<tr>
<td>Total Community</td>
<td>31,740</td>
<td>100</td>
<td>106.19</td>
<td>100</td>
<td>123.04</td>
</tr>
</tbody>
</table>

Cr = crustacean, In = insect, O = oligochaete, P = polychaete.
Table 3.4. Mean density (ind m$^{-2}$), population biomass (mg dry weight m$^{-2}$), and per-capita biomass (µg dry weight ind. $^{-1}$) of dominant macroinfauna from *Spartina patens* sediments in the Plum Island Estuary, MA, USA. Density values are means from a composite of samples from all sampling sites and sampling dates. Biomass values are means of a composite of samples from all sampling sites from September 2005. Collected epifauna (e.g., amphipods and spiders) are not included.

<table>
<thead>
<tr>
<th><em>S. patens</em> taxa</th>
<th>Density (ind. m$^{-2}$)</th>
<th>% fauna density</th>
<th>Population biomass (mg m$^{-2}$)</th>
<th>% of total biomass</th>
<th>Per capita biomass (µg ind $^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fabricia sabella (P)</td>
<td>217</td>
<td>3.45</td>
<td>13.99</td>
<td>4.74</td>
<td>22.61</td>
</tr>
<tr>
<td>Manayunkia aestuarina (P)</td>
<td>1,517</td>
<td>24.19</td>
<td>63.47</td>
<td>21.53</td>
<td>33.98</td>
</tr>
<tr>
<td>Streblus sp. benedicti (P)</td>
<td>11</td>
<td>0.18</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Nereis diversicolor (P)</td>
<td>11</td>
<td>0.18</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pygospio elegans (P)</td>
<td>1</td>
<td>0.02</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Capitella sp. (P)</td>
<td>11</td>
<td>0.18</td>
<td>16.78</td>
<td>5.69</td>
<td>56.88</td>
</tr>
<tr>
<td>Paranais litoralis (O)</td>
<td>402</td>
<td>6.40</td>
<td>44.87</td>
<td>15.22</td>
<td>145.49</td>
</tr>
<tr>
<td>Psammoryctides sp. (O)</td>
<td>1</td>
<td>0.02</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tubificoides brownie (O)</td>
<td>8</td>
<td>0.12</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tubificoides wasseili (O)</td>
<td>88</td>
<td>1.40</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Monopylephorus sp. (O)</td>
<td>36</td>
<td>0.57</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cernosvitoviella immota (O)</td>
<td>3,287</td>
<td>52.40</td>
<td>63.15</td>
<td>21.42</td>
<td>26.44</td>
</tr>
<tr>
<td>Other enchytraeids (O)</td>
<td>370</td>
<td>5.89</td>
<td>10.76</td>
<td>3.65</td>
<td>21.71</td>
</tr>
<tr>
<td>Tabanus sp. larvae (In)</td>
<td>314</td>
<td>5.00</td>
<td>81.84</td>
<td>27.76</td>
<td>197.44</td>
</tr>
<tr>
<td>Total Community</td>
<td>5,960</td>
<td>100</td>
<td>58.04</td>
<td>100</td>
<td>46.55</td>
</tr>
</tbody>
</table>

*In = insect, O = oligochaete, P = polychaete.*
occurred in both creek pairs. Fleeger et al. (2008) found interactive effects of treatments on *Cernovitoviella immota* densities in the *S. patens* habitat after one year of treatment application. Significant interactive effects (period*nutrient*fish, p ≤ 0.046) were detected for *Manayunkia aestuarina*, *Cernosvitoviella immota*, and total annelids in the *Spartina patens* sediments also in Creek Pair 2 (Fig. 3, Table 6). For these three taxa, nutrient additions in ambient fish treatment increased densities (*M. aestuarina* and *C. immota* numerically constitute 80% of total annelids in this habitat), however, this increase was not observed with nutrient addition in the fish-removal treatment. In addition after one season of treatment application in Creek Pair 2, nutrient additions significantly increased *Streblospio benedicti* abundance (period*nutrient, p = 0.0134) in the mudflat and total annelid abundances (period*nutrient, p = 0.0303) in the creek wall (Fig. 3).

**Biomass Responses**

Population biomass was examined only on the last collection date. Effects were detected in three habitats. Nutrients significantly lowered *N. diversicolor* population biomass (a 99% reduction, p = 0.008, Table 7, Fig. 4) in mudflat sediments. The individual biomass of *N. diversicolor* was lower with nutrient enrichment (data not shown), suggesting that changes in population biomass were due to changes in individual body size. In the creek wall, *M. aestuarina* population biomass increased dramatically (by 2300%) when nutrients were added without fish removal (nutrient*fish p < 0.001, Table 7, Fig. 4). *M. aestuarina* individual biomass in this habitat also increased with nutrients independent of fish level (data not shown). Though not significant, a similar trend for *M. aestuarina* population biomass was seen in *S. alterniflora* sediments (Fig. 4). *C. immota* population biomass was significantly higher in nutrient treatments in
Table 3.5. Creek pair 1: Sweeney and West. Dates: 2003 – 2006. Summary table of p-values for abundant macroinfauna species from GLMM. In this BACI-type design, only Period*Treatment interactions are of interest. MF = mudflat, CW = creek wall, TSA = tall-form *Spartina alterniflora*, SP = *S. patens*.

<table>
<thead>
<tr>
<th>Habitat</th>
<th>Taxon</th>
<th>Period(B/A)</th>
<th>Nutrients</th>
<th>Fish</th>
<th>Period*Nutrient</th>
<th>Period*Fish</th>
<th>Nutrient*Fish</th>
<th>Period<em>Nutrient</em>Fish</th>
</tr>
</thead>
<tbody>
<tr>
<td>MF</td>
<td><em>S. benedicti</em></td>
<td>0.069</td>
<td>0.030</td>
<td>0.096</td>
<td>0.095</td>
<td>0.162</td>
<td>0.939</td>
<td>0.607</td>
</tr>
<tr>
<td></td>
<td><em>P. litoralis</em></td>
<td>0.553</td>
<td>0.017</td>
<td>0.262</td>
<td>0.884</td>
<td>0.598</td>
<td>0.620</td>
<td>0.373</td>
</tr>
<tr>
<td></td>
<td>Total Tubificid</td>
<td>0.278</td>
<td>0.000</td>
<td>0.041</td>
<td>0.736</td>
<td>0.666</td>
<td>0.618</td>
<td>0.292</td>
</tr>
<tr>
<td></td>
<td>Total Annelids</td>
<td>0.998</td>
<td>0.198</td>
<td>0.021</td>
<td>0.691</td>
<td>0.938</td>
<td>0.105</td>
<td>0.421</td>
</tr>
<tr>
<td>CW</td>
<td><em>M. aestuarina</em></td>
<td>0.181</td>
<td>0.755</td>
<td>0.649</td>
<td>0.926</td>
<td>0.626</td>
<td>0.272</td>
<td>0.770</td>
</tr>
<tr>
<td></td>
<td><em>F. sabella</em></td>
<td>0.666</td>
<td>0.048</td>
<td>0.611</td>
<td>0.795</td>
<td>0.110</td>
<td>0.709</td>
<td>0.361</td>
</tr>
<tr>
<td></td>
<td><em>P. litoralis</em></td>
<td>0.205</td>
<td>0.013</td>
<td>0.241</td>
<td>0.666</td>
<td>0.660</td>
<td>0.813</td>
<td>0.475</td>
</tr>
<tr>
<td></td>
<td><em>C. immota</em></td>
<td>0.956</td>
<td>0.428</td>
<td>0.571</td>
<td>0.651</td>
<td>0.769</td>
<td>0.321</td>
<td>0.075</td>
</tr>
<tr>
<td></td>
<td>Total Annelids</td>
<td>0.325</td>
<td>0.658</td>
<td>0.998</td>
<td>0.843</td>
<td>0.256</td>
<td>0.701</td>
<td>0.474</td>
</tr>
<tr>
<td>TSA</td>
<td><em>M. aestuarina</em></td>
<td>0.542</td>
<td>0.314</td>
<td>0.593</td>
<td>0.565</td>
<td>0.557</td>
<td>0.288</td>
<td>0.408</td>
</tr>
<tr>
<td></td>
<td><em>P. litoralis</em></td>
<td>0.129</td>
<td>0.185</td>
<td>0.709</td>
<td>0.522</td>
<td>0.842</td>
<td>0.900</td>
<td>0.841</td>
</tr>
<tr>
<td></td>
<td><em>C. immota</em></td>
<td>0.002</td>
<td>0.156</td>
<td>0.248</td>
<td>0.277</td>
<td>0.444</td>
<td>0.677</td>
<td>0.251</td>
</tr>
<tr>
<td></td>
<td>Total Annelids</td>
<td>0.001</td>
<td>0.458</td>
<td>0.225</td>
<td>0.441</td>
<td>0.804</td>
<td>0.686</td>
<td>0.458</td>
</tr>
<tr>
<td>SP</td>
<td><em>M. aestuarina</em></td>
<td>0.735</td>
<td>0.406</td>
<td>0.142</td>
<td>0.421</td>
<td>0.340</td>
<td>0.082</td>
<td>0.952</td>
</tr>
<tr>
<td></td>
<td><em>P. litoralis</em></td>
<td>0.313</td>
<td>0.680</td>
<td>0.563</td>
<td>0.936</td>
<td>0.070</td>
<td>0.204</td>
<td>0.215</td>
</tr>
<tr>
<td></td>
<td><em>C. immota</em></td>
<td>0.000</td>
<td>0.576</td>
<td>0.555</td>
<td>0.998</td>
<td>0.590</td>
<td>0.649</td>
<td>0.407</td>
</tr>
<tr>
<td></td>
<td>Other enchytraieds</td>
<td>0.332</td>
<td>0.091</td>
<td>0.336</td>
<td>0.306</td>
<td>0.604</td>
<td>0.598</td>
<td>0.470</td>
</tr>
<tr>
<td></td>
<td>Total Annelids</td>
<td>0.000</td>
<td>0.549</td>
<td>0.598</td>
<td>0.999</td>
<td>0.638</td>
<td>0.498</td>
<td>0.484</td>
</tr>
</tbody>
</table>
Table 3.6. Creek pair 2: Clubhead and Nelsom Creeks. Dates: 2003, 2005. Summary table of p-values for abundant macroinfauna species from GLMM. In this BACI-type design, only Period*Treatment interactions are of interest. MF = mudflat, CW = creek wall, TSA = tall-form *Spartina alterniflora*, SP = S. patens.

<table>
<thead>
<tr>
<th>Habitat</th>
<th>Taxon</th>
<th>Period(B/A)</th>
<th>Nutrients</th>
<th>Fish</th>
<th>Period*Nutrient</th>
<th>Period*Fish</th>
<th>Nutrient*Fish</th>
<th>Period<em>Nutrient</em>Fish</th>
</tr>
</thead>
<tbody>
<tr>
<td>MF</td>
<td><em>S. benedicti</em></td>
<td>0.033</td>
<td>0.115</td>
<td>0.772</td>
<td>0.013</td>
<td>0.690</td>
<td>0.801</td>
<td>0.469</td>
</tr>
<tr>
<td></td>
<td><em>P. litoralis</em></td>
<td>0.995</td>
<td>0.987</td>
<td>0.986</td>
<td>0.982</td>
<td>0.989</td>
<td>0.987</td>
<td>0.986</td>
</tr>
<tr>
<td></td>
<td>Total Tubificid</td>
<td>0.040</td>
<td>0.005</td>
<td>0.004</td>
<td>0.731</td>
<td>0.055</td>
<td>0.628</td>
<td>0.261</td>
</tr>
<tr>
<td></td>
<td>Total Annelids</td>
<td>0.939</td>
<td>0.142</td>
<td>0.084</td>
<td>0.105</td>
<td>0.089</td>
<td>0.603</td>
<td>0.325</td>
</tr>
<tr>
<td>CW</td>
<td><em>M. aestuarina</em></td>
<td>0.111</td>
<td>0.002</td>
<td>0.506</td>
<td>0.051</td>
<td>0.161</td>
<td>0.633</td>
<td>0.460</td>
</tr>
<tr>
<td></td>
<td><em>F. sabella</em></td>
<td>0.090</td>
<td>0.154</td>
<td>0.635</td>
<td>0.074</td>
<td>0.694</td>
<td>0.869</td>
<td>0.665</td>
</tr>
<tr>
<td></td>
<td><em>P. litoralis</em></td>
<td>0.648</td>
<td>0.265</td>
<td>0.783</td>
<td>0.166</td>
<td>0.304</td>
<td>0.052</td>
<td>0.664</td>
</tr>
<tr>
<td></td>
<td><em>C. immota</em></td>
<td>0.424</td>
<td>0.046</td>
<td>0.635</td>
<td>0.581</td>
<td>0.586</td>
<td>0.981</td>
<td>0.401</td>
</tr>
<tr>
<td></td>
<td>Total Annelids</td>
<td>0.163</td>
<td>0.004</td>
<td>0.942</td>
<td>0.030</td>
<td>0.136</td>
<td>0.536</td>
<td>0.761</td>
</tr>
<tr>
<td>TSA</td>
<td><em>M. aestuarina</em></td>
<td>0.040</td>
<td>0.001</td>
<td>0.763</td>
<td>0.701</td>
<td>0.292</td>
<td>0.179</td>
<td>0.986</td>
</tr>
<tr>
<td></td>
<td><em>P. litoralis</em></td>
<td>0.080</td>
<td>0.325</td>
<td>0.484</td>
<td>0.268</td>
<td>0.944</td>
<td>0.599</td>
<td>0.964</td>
</tr>
<tr>
<td></td>
<td><em>C. immota</em></td>
<td>0.023</td>
<td>0.185</td>
<td>0.623</td>
<td>0.6400</td>
<td>0.791</td>
<td>0.219</td>
<td>0.285</td>
</tr>
<tr>
<td></td>
<td>Total Annelids</td>
<td>0.009</td>
<td>0.008</td>
<td>0.964</td>
<td>0.718</td>
<td>0.467</td>
<td>0.795</td>
<td>0.650</td>
</tr>
<tr>
<td>SP</td>
<td><em>M. aestuarina</em></td>
<td>0.001</td>
<td>0.549</td>
<td>0.208</td>
<td>0.650</td>
<td>0.409</td>
<td>0.222</td>
<td>0.046</td>
</tr>
<tr>
<td></td>
<td><em>P. litoralis</em></td>
<td>0.456</td>
<td>0.469</td>
<td>0.372</td>
<td>0.312</td>
<td>0.545</td>
<td>0.141</td>
<td>0.644</td>
</tr>
<tr>
<td></td>
<td><em>C. immota</em></td>
<td>&lt;0.001</td>
<td>0.705</td>
<td>0.229</td>
<td>0.895</td>
<td>0.682</td>
<td>0.134</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td>Other enchytraed</td>
<td>0.983</td>
<td>0.998</td>
<td>0.985</td>
<td>.999</td>
<td>0.983</td>
<td>0.998</td>
<td>0.999</td>
</tr>
<tr>
<td></td>
<td>Total Annelids</td>
<td>&lt;0.001</td>
<td>0.621</td>
<td>0.005</td>
<td>0.181</td>
<td>0.333</td>
<td>0.139</td>
<td>0.028</td>
</tr>
</tbody>
</table>
Figure 3.2. Mean (S.E.) density of total annelids in experimental Creek Pair 1 (Sweeney and West Creeks) for all four habitats. No significant treatment effects were detected for any taxa in any habitat in Creek Pair 1. The dashed line represents start of treatments.
Figure 3.3. Mean (S.E.) density of taxa in which treatment effects were detected in experimental Creek Pair 2 (Clubhead and Nelson Creeks). Note the different habitats for each taxa. The dashed line represents start of treatments.
S. alterniflora sediments ($p = 0.002$, Table 7, Fig. 4). No effects were detected in the S. patens sediments. No effect of treatments was detected for macroinfauna community biomass for any habitat (Table 8).

**Community and Species Diversity Responses**

First-stage MDS plots indicated that macroinfauna community patterns changed over time (i.e., inter-annual variability) because points (i.e., years) was not tightly grouped (Fig. 5). However, in all habitats along the inundation gradient, second-stage community analysis revealed no changes in community patterns over time as a result of local (i.e., treatment) effects ($\text{ANOSIM } p > 0.05$) because replicate time trajectories for each treatment (represented by A,B,C,D) did not tightly cluster to each other. No treatment effects were detected for any annelid diversity indices (data not shown).

**DISCUSSION**

Contrary to my predictions, neither nutrient addition nor fish reduction had detectible effects on macroinfaunal abundance, species diversity or community structure after three consecutive growing seasons of manipulation. Some ephemeral interactive effects of nutrient addition and fish reductions on density were observed in both creek pairs in *Spartina patens*. Contrary to the prediction that nutrient addition (~10 fold increase in loading) would stimulate benthic microalgae and in turn increase infauna densities and/or biomass, neither variable responded to fertilization. Similarly, my prediction that killifish removal (60% reduction) would lead to increased infauna densities and/or diversity due to reduced predation pressure was not observed. Treatment effects on population biomass in three species were observed for the single timepoint (September 2005) examined in this study. Significant effects of nutrients on population biomass occurred in these instances, but these effects were modified by fish
Table 3.7. Mean (S.E.) population biomass (mg dry weight m\(^{-2}\)) of selected abundant taxa and statistical results based on two-way ANOVAs. Data from specimens collected in Clubhead, Nelson, West, and Sweeney Creeks in the Plum Island Estuary, MA, in September 2005. Bold indicates significant effects. P=polychaetes, O=oligochaete, In=insect.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Ambient Nutrients/</th>
<th>Ambient Nutrients/</th>
<th>Nutrient Additions/</th>
<th>Nutrient Additions/</th>
<th>Nutrient Main Effect</th>
<th>Fish Main Effect</th>
<th>Nut*Fish Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ambient Fish</td>
<td>Low Fish</td>
<td>Ambient Fish</td>
<td>Low Fish</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mudflat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streblospio benedicti (P)</td>
<td>0.246(0.096)</td>
<td>0.106(0.047)</td>
<td>0.333(0.109)</td>
<td>0.193(0.029)</td>
<td>0.093</td>
<td>0.284</td>
<td>0.318</td>
</tr>
<tr>
<td>Manayunkia aestuarina (P)</td>
<td>0.057(0.054)</td>
<td>0.030(0.016)</td>
<td>0.015 (0.006)</td>
<td>0.014(0.006)</td>
<td>0.579</td>
<td>0.471</td>
<td>0.936</td>
</tr>
<tr>
<td>Nereis diversicolor (P)</td>
<td>0.169(0.031)</td>
<td>0.529(0.281)</td>
<td>0.016(0.004)</td>
<td>0.015(0.006)</td>
<td>0.008</td>
<td>0.939</td>
<td>0.767</td>
</tr>
<tr>
<td>Creek Wall</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cernosvitoviella immota (O)</td>
<td>0.043(0.015)</td>
<td>0.079(0.016)</td>
<td>0.080(0.025)</td>
<td>0.069(0.019)</td>
<td>0.112</td>
<td>0.182</td>
<td>0.083</td>
</tr>
<tr>
<td>Fabricia sabella (P)</td>
<td>0.055(0.023)</td>
<td>0.077(0.025)</td>
<td>0.080(0.024)</td>
<td>0.106(0.039)</td>
<td>0.248</td>
<td>0.943</td>
<td>0.493</td>
</tr>
<tr>
<td>Manayunkia aestuarina (P)</td>
<td>0.029(0.014)</td>
<td>0.141(0.048)</td>
<td>0.712(0.398)</td>
<td>0.067(0.019)</td>
<td>0.003</td>
<td>0.779</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tabanus sp. Larvae (In)</td>
<td>0.133(0.092)</td>
<td>0.064(0.040)</td>
<td>0.037(0.017)</td>
<td>0.053(0.018)</td>
<td>0.862</td>
<td>0.876</td>
<td>0.454</td>
</tr>
<tr>
<td>Spartina alterniflora habitat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cernosvitoviella immota (O)</td>
<td>0.140(0.036)</td>
<td>0.091(0.032)</td>
<td>0.201(0.023)</td>
<td>0.200 (0.046)</td>
<td>0.002</td>
<td>0.254</td>
<td>0.603</td>
</tr>
<tr>
<td>Manayunkia aestuarina (P)</td>
<td>0.125(0.051)</td>
<td>0.068(0.027)</td>
<td>0.322(0.240)</td>
<td>0.041(0.009)</td>
<td>0.498</td>
<td>0.327</td>
<td>0.273</td>
</tr>
<tr>
<td>Capitella sp.(P)</td>
<td>0.084(0.060)</td>
<td>0.203(0.122)</td>
<td>0.064(0.021)</td>
<td>0.166(0.071)</td>
<td>0.235</td>
<td>0.198</td>
<td>0.718</td>
</tr>
<tr>
<td>Tabanus sp. Larvae</td>
<td>0.077(0.022)</td>
<td>0.105(0.030)</td>
<td>0.078(0.043)</td>
<td>0.031(0.019)</td>
<td>0.360</td>
<td>0.620</td>
<td>0.053</td>
</tr>
<tr>
<td>S. patens habitat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cernosvitoviella immota (O)</td>
<td>0.055(0.015)</td>
<td>0.064(0.019)</td>
<td>0.089(0.037)</td>
<td>0.044(0.017)</td>
<td>0.676</td>
<td>0.383</td>
<td>0.503</td>
</tr>
<tr>
<td>Manayunkia aestuarina (P)</td>
<td>0.037(0.017)</td>
<td>0.092(0.034)</td>
<td>0.054(0.018)</td>
<td>0.069(0.020)</td>
<td>0.765</td>
<td>0.173</td>
<td>0.337</td>
</tr>
</tbody>
</table>

Table 3.8. Mean (S.E.) community biomass (mg dry weight m\(^{-2}\)) for each habitat and statistical results based on two-way ANOVAs. Data from specimens collected in Clubhead, Nelson, West, and Sweeney Creeks in the Plum Island Estuary, MA, in September 2005.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Ambient Nutrients/</th>
<th>Ambient Nutrients/</th>
<th>Nutrient Additions/</th>
<th>Nutrient Additions/</th>
<th>Nutrient Main Effect</th>
<th>Fish Main Effect</th>
<th>Nut*Fish Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ambient Fish</td>
<td>Low Fish</td>
<td>Ambient Fish</td>
<td>Low Fish</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mudflat</td>
<td>134.57(28.45)</td>
<td>133.00(42.31)</td>
<td>105.33(30.91)</td>
<td>75.66(18.09)</td>
<td>0.772</td>
<td>0.981</td>
<td>0.709</td>
</tr>
<tr>
<td>Creek Wall</td>
<td>146.72(70.91)</td>
<td>132.21(26.62)</td>
<td>365.03(131.59)</td>
<td>223.91(79.61)</td>
<td>0.147</td>
<td>0.457</td>
<td>0.886</td>
</tr>
<tr>
<td>Spartina alterniflora</td>
<td>97.00(19.39)</td>
<td>97.74(22.19)</td>
<td>160.20(65.12)</td>
<td>160.20(65.12)</td>
<td>0.949</td>
<td>0.251</td>
<td>0.680</td>
</tr>
<tr>
<td>S. patens</td>
<td>54.26(13.96)</td>
<td>64.14(15.78)</td>
<td>64.82(17.04)</td>
<td>49.18(11.35)</td>
<td>0.734</td>
<td>0.631</td>
<td>0.453</td>
</tr>
</tbody>
</table>
Figure 3.4. Mean (S.E.) population biomass (mg DW m\textsuperscript{-2}) of selected macroinfauna for September 2005 in the Plum Island Estuary, Massachusetts.
Figure 3.5. Second-stage community analysis of Plum Island Estuary infauna by habitat-type. Stage 1: First-stage similarities among years (2003 – 2006) for end of the season samples. Each first-stage MDS are based on Bray-Curtis similarities calculated from Log (x+1) transformed abundances and are averages of all replicates within each area. Stress values are all 0. Stage 2: Second-stage MDS ordination. Labels represent data from two sites within each treatment and each label represents the pattern of community change at a site through time. ANOSIM p-values are listed in the top left corner. See text for details. A=ambient fish/ambient nutrients, B=fish removal/ambient nutrients, C=ambient fish/nutrient additions, D=fish removal/nutrient additions.
reduction for one species. No effects on community biomass were observed. Below I
discuss possible explanations for these limited responses.

**Nutrient (Bottom-Up) Effects**

I observed no effect of nutrients on infauna density, diversity and community
structure after three years of summer fertilization. Although nutrients stimulated
*Streblospio benedicti* densities in the mudflat and total-annelid densities in the creek
wall in Creek Pair 2 after one season of additions, these results were not consistent in
the replicate creek pair (Fleeger et al. 2008). No effect of nutrients was detected for
Creek Pair 1 after three years of nutrient enrichment, which agrees with other studies
that have found limited-to-no response of infauna density to nutrient additions (Wiltse et
al. 1984, Posey et al. 2002). Nutrients stimulated increases in benthic algal biomass
(up to 4-fold; L.A. Deegan *unpublished data*). The lack of a numerical response in
suggests that macroinfauna were typically not food limited in PIE. Given that infauna
may selectively consume a variety of food sources such as phytoplankton, BMA, and
detritus (Galván et al. 2008), BMA and macroinfauna may not be tightly coupled. In this
system, a potential trophic cascade stops at the macroinfauna-to-BMA level, further
suggesting weak interactions between infauna and BMA (Chapter 4).

However, on the one collection examined (September 2005), fertilization
increased the population biomass of the small polychaete (~5 mm), *Manayunkia
eaestuarina*, significantly in the creek wall and as a non-significant trend in *S. alterniflora*
habitats. Population biomass is influenced by the number of individuals (density) and
individual biomass or their combination. Increased population biomass for this
polychaete may be a function of per-capita biomass, which also increased with nutrient
additions. *M. aestuarina* feeds on a mixture of benthic microalgae and phytoplankton in
these habitats (Galván et al. 2008). The population biomass of the enchytraeid oligochaete *Cernosvitoviella immota* also increased with nutrient additions in the *S. alterniflora* habitat. This typically subsurface-feeding oligochaete consumes mostly *Spartina* detritus in sediments under vegetated canopy in PIE (Galván 2008). Galván (2008) also found that after one year of fertilization *Manayunkia aestuarina* and *Cernosvitoviella immota* both altered their diet to consume more benthic microalgae under conditions of fertilization. Thus, both annelids may be responding to increased food supply in these habitats (BMA increased with fertilization) with increased growth but without increases in abundance. Increased growth may lead to increases in secondary production in annelids (Seitz and Schaffner 1995). Since biomass measurements were a snapshot in time, more time points are required to validate this trend, and formal measurement of secondary production is recommended in future studies. My results are similar to Posey et al. (2006) in which numerically dominant infauna (primarily annelids) found in North Carolina (U.S.) estuaries did not exhibit numerical increases in response to nutrient additions, but did increase in individual size (biomass). Nutrients can stimulate benthic microalgal food resources, particularly Chlorophyta and certain benthic diatoms (Posey et al. 2002) and taxa may differentially respond with increased reproductive output (Levin 1986) and/or with increased growth (Posey et al. 2006). In a mesocosm study, Levin (1986) showed that *S. benedicti*, a broadcast spawner, responded to nutrient enrichment with increased brood size, which has consequences for population size. If higher quality resources were stimulated by nutrients, *M. aestuarina* and *C. immota* may have allocated energy to growth but not reproduction. *M. aestuarina* is an intratubular brooder and the oligochaete *C. immota*
reproduces asexually; thus any increases in reproductive output may take longer to manifest as increased abundances than those species with planktotrophic larvae.

Contrary to expectations, *N. diversicolor* population biomass decreased significantly with nutrient additions in the mudflats. The results suggest that the decrease in population biomass was due to the presence of smaller individuals, although this result may be an artifact of sampling. The 6.6-cm diameter core does not adequately sample larger *N. diversicolor* (which can reach lengths up to at least 30 cm) and thus the biomass and density estimates may have been unreliable for the population as a whole. If nutrients stimulate *N. diversicolor* growth, then a larger portion of individuals may grow to a size class that is inadequately sampled.

Similar in design to this study but contrary in results, Cross et al. (2006) used a paired-catchment design and found increased consumer abundance and secondary production with large-scale nutrient additions in a detritus-based stream. Species studied by Cross et al. (2006) directly consumed detritus and levels of organic matter were significantly enhanced by fertilization. Although salt marshes have been traditionally been considered detritus-based ecosystems (Teal 1962), most of the macroinfauna studied in PIE have a diet principally composed of microalgae (either benthic or pelagic) and significant consumption of macrophyte detritus was limited to *C. immota* (except with nutrient enrichment when consumption of benthic algae became predominant) (Galván 2008). Furthermore, levels of sediment organic matter as a result of decomposition of *Spartina* were probably not impacted by nutrient addition during the 3-year study (Deegan, personal communication). In PIE, only species that consumed benthic microalgae responded to fertilization (by changes in biomass, not abundance). Thus, bottom up control in salt marshes may be more complex or not driven by detritus
levels compared to systems in which detritus fuels consumer growth (e.g., Cross et al. 2006). Regardless of the food-web base (detritus or algae), nutrient additions may enhance secondary production of macroinvertebrates in a variety of ecosystems (lakes: Blanco et al. 2008; streams: Cross et al. 2005, 2006; seagrasses: Gil et al. 2006).

Several studies have demonstrated that despite stimulation of benthic algae, effects on infauna abundance/biomass remain minimal or are slow to develop in western Atlantic salt marshes. For instance, nutrients had no appreciable effects on macroinfauna density after 4-weeks (Posey et al. 2002), two years (Foreman et al. 1995), three years (this study), and five years (Wiltse et al. 1984) of enrichment. Macroinfauna density and population biomass did increase after 15 years of high nutrient loading in a southern New England salt marsh (Sarda et al. 1996). Increases in population biomass were due to increases in abundances, not individual biomass (Sarda et al. 1996), a trend not observed here. Additionally, the community shifted from a *Streblospio benedicti* (a surface-feeding polychaete) dominated community to one dominated by subsurface-deposit-feeding oligochaetes (e.g., *Paranais litoralis*, Sarda et al. 1996). Thus, longer time frames (> 5 years) and/or higher nutrient loadings [(TIDE nutrient-loading rates were ~10x less than previous dry-fertilizer plot-level experiments (e.g., Sarda et al. 1996)] may be needed to elicit impacts of nutrients on saltmarsh macroinfauna. Alternatively, predation by small epibenthic predators such as the grass shrimp *Palaemonetes pugio* that were able to penetrate the mesh of the exclusion blocks may have dampened bottom-up effects (see Top-down effects).

Although certain response variables of macroinfauna appear to respond slowly to nutrient treatments, the meiobenthos (animals ranging in size from 63 µm – 500 µm) may be more sensitive to fertilization effects. In an earlier study in PIE, Fleeger et al.
(2008) found that nutrients stimulated ostracod densities and the frequency of gravid female copepods after only one year of application. Similarly, in a two year study Foreman et al. (1995) found that nutrients stimulated algal growth, which coincided with increased meiobenthic copepod densities, suggesting strong bottom-up control of these meiofauna. Work is currently underway to examine the long-term effects of nutrients on meiofauna in PIE (J.W. Fleeger unpublished data).

Macroinfauna densities and communities vary more among habitats (e.g., creek wall vs. mudflat) in tidal creeks than among creek systems (e.g., West vs. Sweeney) in PIE (Johnson et al. 2007). The changes observed for macroinfauna due to treatments observed in this study were small; much smaller than those differences in macroinfauna across habitats. For example, infauna density and diversity is lowest in the S. patens habitat, but highest in the creek wall (Johnson et al. 2007, Fleeger et al. 2008). Although not studied here, macroinfauna densities were low in the stunted S. alterniflora habitat – a habitat higher in elevation and infrequently inundated (Johnson et al. 2007). Thus, effects on the habitat or vegetation composition may be required before large changes in macroinfauna are observed.

**Predation (Top-Down) Effects**

In contrast to other studies that observed strong predation effects on infauna densities in a relatively short timeframe (e.g., within a growing season; Posey et al. 2002), I observed no effect of killifish removal on the density of infauna after three growing seasons. Killifish were reduced by 60% in a size-specific fashion and although killifish are predators, a full reduction of predators may be needed to elicit an infaunal response. Alternatively, this disparity may be due to the presence of another epibenthic predator, the grass shrimp *Palaeomonetes pugio*, which was not excluded by the fish
reduction treatment. Densities of surface-feeding annelids such as *M. aestuarina* and *S. benedicti* significantly increased when all predators were removed (Chapter 4), thus complex interactions among a suite of predators may operate to exert top-down control on these infauna. In the Great Sippewissett Marsh (Massachusetts, USA), Sarda et al. (1998) observed decreased densities of small infauna in predator exclusion cages, which they attributed to the presence of small epibenthic predators such as *P. pugio*, which were permeable to the large mesh size of their cages. Grass shrimp are prey for large killifish and their impact on infauna may be enhanced by the removal of large killifish (> 40 mm) via increased foraging (Kneib and Stiven 1982, Posey and Hines 1991). Isotope and plot-level caging experiments in PIE suggest that with the removal of large killifish, grass shrimp may feed more actively on infauna thereby preventing infauna densities from increasing (Galván 2008, Chapter 4). Thus, indirect effects may be important in structuring these infauna communities (Kneib 1988).

Fish reduction treatments selectively excluded large (> 40 mm) killifish, which may have a larger impact on larger-bodied benthic invertebrates than small-bodied invertebrates. Sarda et al. (1998) found that increases in population biomass in cages that excluded larger-sized predators was due to increased density of larger-bodied polychaetes (e.g., *Nereis* spp.), indicating that these larger predators tend to consume larger prey. *N. diversicolor* was found in 18% of large killifish guts collected from PIE, suggesting they consume these large polychaetes (Galván 2008). Although not significant, the average individual biomass of *N. diversicolor* increased 3-fold with predator removal in mudflat sediments (data not shown). Furthermore, epifauna such as amphipods and snails significantly increased with killifish removal in this system.
(Chapter 5), suggesting that large killifish are able to impact some large invertebrates in this marsh system.

**Interactive Effects**

Top-down and bottom treatments interacted on *M. aestuarina*, *C. immota*, and total annelid densities in the *S. patens* habitat in Creek Pair 2 and for *C. immota* densities in Creek Pair 1 in the first year of treatments (Fleeger et al. 2008, this study). Because densities of these taxa responded to nutrient additions (as noted above, both species also responded to fertilization with increased biomass and with a diet shift to utilize BMA in parts of the PIE landscape) in ambient fish levels but not when fish were reduced, possible indirect effects initiated by killifish on infauna may explain this trend. Similarly, nutrients increased *M. aestuarina* population biomass in the ambient fish levels but not in the fish reduction areas in the *S. alterniflora* habitats. This observation may also be a result of an indirect effect. Indirect effects are often mediated by an intermediate predator (Kneib 1991) and intermediate predators such as grass shrimp on the marsh platform may have been responsible for the observed interactions. Grass shrimp enter the marsh platform at high tide with killifish and may forage with killifish in all areas including *S. patens* habitats. The foraging behavior of grass shrimp may be altered with the reduction of large killifish. Killifish reduction led to increases in grass shrimp body size (but not density) that could be caused by increased consumption of more or larger infauna by grass shrimp (Chapter 4, Deegan et al. 2007). Moreover, $^{15}$N isotopic analysis of grass shrimp indicates an increased trophic level when killifish are removed suggesting a more carnivorous diet (i.e., more carnivory) (Galván 2008). After one year of treatments in Creek Pair 1, a similar interaction was observed by Fleeger et al. (2008) for *C. immota* densities in *S. patens* habitat where fish reduction lead to
decreased annelid densities. Although there is support for this indirect effect, it is limited to one habitat and it is not sustained beyond one year for densities (i.e., no effects in Creek Pair 1 after three growing seasons of treatments). Benthic algae in PIE did not respond significantly until at least the second year of nutrient additions (Deegan et al. 2007), thus the interactive effects observed on infauna may be modified as bottom-up effects increase. This suggests that longer-term studies are required to understand these effects.

**Spatial and Temporal Effects**

In salt marshes, zonation patterns of plants including biotic and abiotic interactions are well studied (see Bertness and Pennings 2000 for review), but distribution patterns along an inundation gradient are relatively understudied for infauna (but see Coull et al. 1979, Kneib 1984, Johnson et al. 2007). Even less studied are the drivers of these patterns. Typically, studies examining the impact of anthropogenic activities on infauna focus on unvegetated mudflats and rarely focus on effects along a gradient (Posey et al. 1999). Fleeger et al. (2008) reported that fertilization and fish removal effects on meiofauna in the first treatment year of this study occurred primarily in the mid range of the inundation gradient in this system. Differential effects of treatments that were habitat and species specific were observed for infauna across the inundation gradient. Thus, the strength of top-down and bottom-up controls on infauna vary spatially across the landscape. Effects may vary not only spatially, but also temporally. As discussed above, interactive effects of treatments were observed for the dominant species *M. aestuarina* and *C. immota* in *S. patens* after a year of treatments, but not after three seasons of treatments (this study, Fleeger et al. 2008).
Sensitivity of Response Variables

In any experiment, the selection of the appropriate response variable(s) is important. To my knowledge, long-term studies of predator removal on saltmarsh infauna are unavailable in the literature so the long-term effect of predator removal on infauna community remains unknown. However, previous work suggests that predators significantly impact infauna densities in relatively short time frames (e.g., Posey et al. 2002). Because the manipulations done in the TIDE project were unable to remove all predators or selectively remove all killifish, it is difficult from this study to make statements about the sensitivity of the response variables to killifish removal. Grass shrimp are important predators of infauna under certain conditions (Kneib and Stiven 1982, Posey and Hines 1991, Galván 2008) and may be preventing any changes in infauna.

The stochastic nature and high environmental stress of the Plum Island Estuary may influence the recruitment of individuals and in turn the numerical response of infauna to treatments. For instance, treatment-induced increases in the reproductive output of species that produce planktonic larvae may not be observed if the larvae are simply advected away by high tidal flows. Those offspring without a pelagic life stage should not be as affected by tidal flow, and thus may not be as recruitment limited as planktonic larvae. *M. aestuarina* is an intra-tubular brooder that releases juveniles (Bick 1996) whereas *S. benedicti* releases planktonic larvae that settle onto the sediment after a few days of development (Levin 1984). Limited effect of treatments was seen on these two polychaetes with different reproductive strategies suggesting that recruitment was not limited in this system. *M. aestuarina* is abundant in both macroinfauna and meiofauna size classes and Fleeger et al. (2008) found that treatments had no effect on
the abundance of either size class suggesting top-down and bottom-up effects don’t impact recruitment of juveniles into the larger size class. Effects of predator removal and nutrient additions have been observed in as little as three weeks (Posey et al. 1999, 2002). This suggests that saltmarsh infauna are not recruitment limited and post-settlement processes are important in structuring these communities (Olafsson et al. 1994).

A lack of response by the annelid communities in this study is surprising given that after one year of manipulation Fleeger et al. (2008) suggested a potential treatment effects on communities. However, Fleeger et al. (2008) did not use a second-stage community analysis which examines changes in an impacted community over time relative to a control community to account for natural temporal differences in the community, thus their observations may be the result of naturally occurring differences not accounted for in their analyses.

Experiments yielding few to no negative results (i.e., negative results) bring into question the possibility of Type II errors. While other large-scale studies have opted to increase their alpha (up to 0.20) to minimize Type II errors (Steinbeck et al. 2005, Deegan et al. 2007), I selected an alpha = 0.05 to offset the inflation of Type I error rate due to a high number of univariate analyses. Macroinfauna are notoriously patchy, making their variability extremely high and detection of effects with low sampling size difficult (Levin and Talley 2000, Johnson et al. 2007). Studies that report significant changes in infauna densities typically detected changes of at least 100%, and at times no statistical difference at large density changes (say 600%) (Wiltse et al.1984, Sarda et al. 1996). Variations in abundance among treatment creeks were frequently below 100% for the most abundant species in this study.
Response of Other Trophic Levels

The effect of treatments on other taxa (e.g., killifish and benthic microalgae) relevant to explaining potential top-down and bottom-up effects on infauna are detailed for the first two years of experimental treatments in Deegan et al. (2007). Killifish abundance varied temporally and spatially in experimental creeks. Despite the variation, fish removal did lead to significant reductions in killifish abundance (see Deegan et al. 2007, Fig. 4). Killifish reduction did not lead to increased grass shrimp density (another potential predator of infauna) (Deegan et al. 2007), but did lead to enhanced growth of shrimp (Chapter 4). Prior to treatments, within-habitat BMA biomass was similar among creeks, although inter-habitat differences occurred. Significant effects of treatments on BMA biomass was detected only in the second year of treatments in Sweeney and West Creeks in which biomass increased synergistically (Deegan et al. 2007, Fig. 7). Treatment effects on BMA over longer time periods have not yet been analyzed. Treatments did not alter the abundance or distribution of Spartina alterniflora or S. patens over the course of manipulations (Miller 2006).

Spartina alterniflora acts as a foundation species in salt marshes and facilitates the establishment of faunal communities by stabilizing and oxygenating sediments, baffling water flows, and providing shade (Pennings and Bertness 2001, Alitieri et al. 2007) and can influence macroinfauna communities and distributions (Kneib 1984, Rader 1984, Levin and Talley 2000). Changes in macrophyte composition may lead to changes in the macroinfaunal community. For instance, S. alterniflora is an invasive plant in California marshes and significantly alters the macroinfauna community (Neira et al. 2005, Levin et al. 2006, Neira et al. 2006). In seagrass beds, macrophyte loss can lead to reduced secondary production and changes in the infauna community (Dolbeth
et al. 2003). However, even with large changes in macrophyte composition, it may take years for the macroinfauna community to shift. For instance, in restored *Spartina* spp. marshes differences among restored and natural marshes can persist up to 25 years after restoration (Moseman et al. 2004, Craft and Sacco 2003). Nutrient availability can alter the competitive interactions among marsh macrophytes and in turn modify macrophyte distributions across the landscape (Bertness and Pennings 2000). However, altered competitive dynamics among macrophytes may not have been detectable within the short time frame of my studies. The nutrient treatments did not alter the relative abundance or distribution of *S. alterniflora* or *S. patens*, although it increased the nitrogen content of *S. alterniflora* leaves and increased *S. alterniflora* biomass (Deegan et al. 2007, Drake et al. 2008), which may have important consequences for detritivorous infauna such as *C. immota* (Galván 2008). Given that the landscape of macrophytes (*Spartina* spp.) did not change, a lack of response by macroinfauna may be due to the lack of a large change in foundation species.

Conclusions

I observed no effects of nutrient additions and killifish removal on annelid density, diversity, or community structure after three consecutive seasons of whole-ecosystem manipulation. Effects on population biomass were examined as a snapshot in time and only three species in two different habitats responded with mixed results (i.e., nutrients stimulated one species but dampened another). However increased growth occurred in two species – *M. aestuarina* and *C. immota* – that altered their diet to take advantage of rapidly responding benthic algae (Galván 2008) and these species may have had increased rates of secondary production that were not manifested by increases in abundance. Indirect effects detected after one season in *S. patens* did not persist
perhaps because nutrient-induced alterations of basal resources (e.g., *Spartina* sp. or benthic algae), which exhibit a time lag after nutrient additions begin. Predation effects are probably not cumulative, but interactions with nutrient effects may vary over time.

It is difficult to compare the relative importance of top-down vs. bottom-up effects from this study because all epibenthic predators were not excluded. Few interactions occurred in this study, a result that corresponds to other studies that suggest no interactions between trophic manipulations and nutrient additions on infauna (Foreman et al. 1995, Posey et al. 1995, 1999, 2002); however interactions could vary temporally as stronger nutrient effects manifest over time. Although limited responses of treatments were seen on macroinfauna, strong top-down and bottom-up effects have been observed on other invertebrates and trophic levels in this system, sometimes resulting in complicated interactions (Deegan et al. 2007, Fleeger et al. 2008, Chapter 5). Cumulative effects of nutrients were seen on benthic microalgae, but the effect was modified by consumer control (Deegan et al. 2007). Thus, effects of treatments, particularly nutrient effects, on infauna may be cumulative and consumer controls may interact strongly over longer time periods and this possibility underscores the importance of continuing manipulations for several additional years. The TIDE project will continue to examine potential cumulative responses, feedbacks and interactions over longer time periods. As estuarine systems continue to be chronically impacted by multiple human activities, long-term multistressor studies are important in understanding the effects of these activities.

**LITERATURE CITED**


Minello, T.J. and J.W. Webb. Use of natural and created Spartina alterniflora salt marshes by fishery species and other aquatic fauna in Galveston Bay, Texas, USA. 151:165-179.


CHAPTER 4

OMNIVORY COUNTERACTS A TROPHIC CASCADE IN A MARINE FOOD WEB
INTRODUCTION

Increased worldwide exploitation of top predators (e.g., overfishing) and increased anthropogenic nutrient loading make understanding the relative contributions of top-down and bottom-up control of food webs an imperative in ecology (Vitousek et al. 1997, Jackson et al. 2001, Deegan et al. 2007). Although top-down and bottom-up control of ecosystems has been long debated (Hairston et al. 1960, Murdoch 1966, Carpenter et al. 1985, Strong 1992), trophic cascades result from top-down control, in which predators regulate prey populations; disturbance at higher trophic levels propagates to lower levels with alternating positive and negative effects (Carpenter et al. 1985, Pace et al. 1999, Fleeger et al. 2003). Trophic cascades occur in terrestrial (Schmitz et al. 2000), freshwater (Carpenter et al. 1985), marine (Halpern et al. 2006), and intertidal systems (Silliman and Bertness 2002).

The strength of trophic cascades across ecosystems has recently been examined and debated (Strong 1992, Shurin et al. 2002, Borer et al. 2005). Trophic cascades are typically found in food webs that exhibit some combination of the following characteristics: structural simplicity, small spatial scale, low species diversity, distinct separation in body size among biota in different trophic levels, or algal-based food webs. Therefore, the strongest cascades are thought to occur in aquatic ecosystems because they exhibit many of these qualities (Strong 1992). In support of this notion, a recent meta-analysis of six different ecosystems determined that the strongest trophic cascades occur in benthic marine food webs (Shurin et al. 2002).

Trophic-cascade theory is built on linear food chains (Fig. 1a); however, trophic omnivory (i.e., predation on > 1 trophic level) creates a reticulated (non-linear) food web that has the potential to dampen or disrupt trophic cascades by diffusing predation
effects over several trophic levels (Fig. 1b; Polis and Strong 1996). Thus, the potential exists for omnivory to prevent trophic cascades even in ecosystems where the strongest cascades exist (i.e., the marine benthos; Shurin et al. 2002). In algal-based food webs, the effects of omnivorous top predators may cascade if strong interactions exist (e.g., Lodge et al. 1994 – freshwater benthos) or omnivorous predators may decouple a potential trophic cascade by simultaneously influencing multiple trophic levels (e.g., Bruno and O’Connor 2005 – marine benthos).

In the salt marshes of the Plum Island Estuary, Massachusetts, the killifish *Fundulus heteroclitus* (L.), is considered a top predator and thus may exert top-down control that may cascade to the benthic algal community (Deegan et al. 2007). In Chapter 2, I found no evidence of top-down control of killifish on infauna and suggested that the presence of another abundant predator the grass shrimp *Palaemonetes pugio* (Holthuis), may have become the primary predator of infauna when killifish were removed. Although cascading effects of predator removal have been demonstrated on the saltmarsh benthos (e.g., Posey et al. 2002), these studies have utilized total predator removal so the contributions of killifish and other predators to cascading effects is unknown. Additionally, killifish and other predators such as the grass shrimp are omnivorous (Morgan 1980, Allen et al. 1994, Posey and Hines 1991) and therefore may not exert strong top-down control in saltmarsh food webs. In this study, I deconstruct the food web and examine how omnivory may have influenced the cascading effects of killifish and grass shrimp. To isolate the potential cascading effects of killifish, I used a 2-stage omnivorous predator removal in the field.
In Stage 1, I took advantage of the TIDE manipulations that significantly reduced killifish by 60% at large (ecosystem-wide) scales, which promoted grass shrimp (an intermediate omnivore) to the role of dominant consumer. In Stage 2, I removed all predators/omnivores at small (plot level) scales.

**METHODS**

**Study Site**

My experiment was conducted in two saltmarsh tidal creeks (West and Nelson Creek) that are a part of the Plum Island Estuary (PIE) located in Northeast Massachusetts, USA (42°44’N, 70°52’W; Fig. 2). West Creek drains into the Rowley River, which empties into the Plum Island Sound, whereas Nelson Creek drains directly into the sound. Both creeks are characterized by two branches, 3-m tidal fluxes, and distinct habitat zonation, and may be considered replicate ecosystems (see Fig. 2).
(Johnson et al. 2007). Additionally, the creeks have similar macroinfauna (predominately annelids) species composition, and the density of individual species differ little between creeks (i.e., low spatial variability) (Johnson et al. 2007). *Spartina alterniflora* (hereafter called *Spartina*) habitats in PIE tidal creeks are narrow (2 – 3 m) bands of vegetation along the platform edge. Mudflats are unvegetated areas 0.5 – 2 m wide in the creek channel. Additionally, these two habitats are separated by a 1.5-m vertical creek wall (Fig. 2), and thus time of inundation of *Spartina* habitats is less than that of mudflats.

**Food Web Constituents**

The killifish *Fundulus heteroclitus* (Linnaeus) and the grass shrimp *Palaemonetes pugio* (Holthuis) are abundant nekton in US Atlantic Coast salt marshes (Kneib 1985, Kneib 1986). Both are predators/omnivores that share common resources, including benthic invertebrates (infauna) and benthic algae and both are subject to ontogenetic diet shifts (Morgan 1980, Currin et al. 2003). Medium to large (> 40 mm total length) killifish also consume larger crustaceans such as amphipods and grass shrimp (Kneib and Stiven 1982, personal observation). Additionally, large killifish may influence the foraging activities of grass shrimp (Posey and Hines 1991). Infauna consume and may be limited by algal resources such as benthic microalgae (Stocks and Grassle 2001, Galván et al. 2008). Thus, the food web had four levels: an omnivorous top predator (killifish), an omnivorous intermediate predator (grass shrimp), obligate herbivores (infauna), and autotrophs (benthic microalgae - BMA). Although I recognize there are other algal grazers in the marsh (e.g., amphipods, snails), I chose
infaunal annelids as the primary consumers because they numerically dominate the study habitats (Johnson et al. 2007) and in the absence of predators, infauna may be able to daily graze 60% of the microalgal community (Carman et al. 1997). PIE has a low diversity nektonic community (11 fish and large invertebrate species), which is dominated by grass shrimp and killifish (98% combined abundance of total nekton) (Deegan et al. 2007), and thus I focus on the effects of these two main predators.
**Experimental Design**

My experiment took advantage of a larger, multidisciplinary project examining anthropogenic impacts on coastal marshes (the TIDE project, described in Deegan et al. 2007), including the effects of large spatial-scale reduction of the killifish *F. heterolcitus* on ecosystem functioning. This was achieved by stretching a Vexar (6.35-mm mesh) block net across the entrance of one branch of each creek from June – September 2005 and augmented with fish removal by trapping, and together resulted in a 60% reduction in killifish (Deegan et al. 2007). Thus, each creek had two fish treatments: Ambient Fish and Low Fish. Although the mesh diameter of the block net selectively excluded larger killifish (≥ 40 mm), small killifish (< 40 mm) densities were reduced by ~40%. The block net did not alter shrimp abundances, allowing them to persist in the creeks (Deegan et al. 2007). Grass shrimp constitute 79% of the total nekton abundance in this system (Deegan et al. 2007), and are considered the principal predator of infauna in fish removal sites (Posey and Hines 1991). Because the other species of potential fish and invertebrate predators (e.g., green crabs) were so low in abundance, I considered their predation/disturbance effects on infauna as negligible.

Within each fish level, three cage types – full, partial (cage controls), and open cage plots - were placed in two habitats of the two creeks: creek bank *Spartina* and mudflats. Full cages excluded all remaining predators (e.g., grass shrimp, small killifish) to determine a total predator effect. Partial cages were used to test for possible caging artifacts (e.g., water baffling) (Virnstein 1978). Open cage plots were used as a control in which predators had unrestricted access to infauna. Thus, I had a 2 x 3 factorial experimental design, with two levels of fish (ambient fish and low fish) and three levels of cages (full, partial, and open) within each of two replicate creeks. Each treatment
combination had six replicates (3 replicates/creek) for 36 treatment plots per habitat. Creeks were considered replicates of each other because they exhibited similar infauna assemblages, benthic algal biomass, and physical attributes (Deegan et al. 2007, Johnson et al. 2007) and were pooled for analysis.

Exclusion devices (cages) were constructed from Vexar (3.2-mm mesh) attached with cable ties to corner posts (0.5-inch diameter PVC pipes), but with differences between habitats. All plots in the Spartina habitat were 0.76 m x 0.76 m. For full cages in the Spartina habitat, Vexar was attached to a PVC frame that was 1.5-m tall with a 0.76 m x 0.76 m square top frame. The top frame added stability to the tall structure and because the cage was higher than the highest spring tides, a mesh top was not used. Partial cages had only two sides and open cage plots consisted of only 4 PVC posts. Cage corner posts and attached mesh were buried 20 cm into the sediment. Full cages (50 x 50 x 50 cm) in mudflats consisted of mesh attached to four corner posts, and because these cages would be completely immersed during high tides, mesh tops were added. Partial cages consisted of two sides and a top, and open cage plots were marked with 4 PVC corner poles. An additional 50-cm length of PVC was added to each pole of full and partial cages, which was necessary to secure the cages. Cages were buried 60 cm (50 cm of the pole and 10 cm of the mesh) into the sediment. I duplicated the disturbance associated with full and partial cage deployment (e.g., trampled vegetation) around open cage plots. No predators were observed in any full cage plot and debris did not accumulate on any of the cages; no maintenance was conducted once emplaced.

Spartina predator exclusion cages were deployed July 14-15, and mudflat devices were deployed July 27-28 (6 - 7 weeks after fish removal began). This timing
allowed us to incorporate other indirect effects of killifish on infauna [increased foraging due to increased shrimp density (a density mediated indirect interaction – DMII) or altered habitat use by shrimp (a trait mediated indirect interaction - TMII)] (Werner and Peacor 2003). After three weeks, three macroinfauna cores (6.6-cm inner diameter push corer to a 5-cm depth) were taken within each plot. This sampling method may inadequately sample larger, more mobile infauna (e.g., *Nereis diversicolor*) and epifauna (e.g., amphipods). Cores were placed on ice in the field and fixed with 10% formalin and Rose Bengal in the laboratory. After a minimum of two days, cores were sieved through a 1-mm sieve stacked on top of a 500-µm sieve. Large debris and roots retained on the 1-mm sieve were discarded after visual inspection and removal of large invertebrates. Infaunal annelids constituted 97% of macroinfaunal abundances and are the focus of this study. All annelids were sorted, identified to species and enumerated.

Two cores (2.7-cm inner-diameter plastic corer to a 2-cm depth) were taken for BMA biomass in each plot. Chl a concentration, as a proxy for BMA biomass, was determined in each core (Lorenzen 1967; acetone extraction and spectrophotometric analysis).

Killifish removal did not lead to increased shrimp abundances (Deegan et al. 2007); however, grass shrimp may forage more actively when killifish are reduced due to lower predation risk (a TMII; Posey and Hines 1991). This increased foraging should lead to increased per capita tissue production (i.e., growth). To determine if fish treatment affected grass shrimp growth, shrimp abundance and size class data from Deegan et al. (2007) of monthly (June – August 2005) collections using flume nets that sampled the marsh platform were analyzed. Flume nets did not capture juvenile grass shrimp (< 14-mm total length). Grass shrimp were separated into two size classes:
adult (large, ≥ 30 mm TL) and subadult (small, 14-30 mm TL) following Fleeger et al. (1999) and the relative abundance of adult grass shrimp was analyzed between fish levels using t-tests for each monthly collection with SigmaStat (SSI v 3.1, Richmond, CA). Assumptions of normality and equal variance were met except for June, which was analyzed with a Mann-Whitney rank sum test. Data were plotted and inspected to determine how body size differed. Significantly higher proportions of adult grass shrimp in fish removal sites may indicate increased per capita growth due to increased foraging.

**Statistical Analyses**

Prior to analysis of plots, data from individual cores (species counts of infauna and chl a concentrations) were averaged for each plot so that each plot represented a replicate. The response of chlorophyll a (chl a) concentration and selected infaunal taxa in terms of abundance to fish and exclusion treatments was determined. Data were analyzed using GLIMMIX, which is a SAS macro for fitting non-normal data to generalized linear mixed models (GLMM) (SAS v. 9.1.3, Cary, NC). GLMMs produce Type III F-statistics and P-values, which are based on likelihood estimations rather than sums of squares as in ANOVAs. The GLIMMIX macro allows one to analyze fixed and random effects and set the error distribution of the data. Data were analyzed in a block ANOVA-type design, with cage type and fish level assigned as fixed factors and creek location and the interaction of fish level and creek location assigned as random factors to account for spatial dependence of creek location (i.e., blocking by creek). All data were loge-transformed and errors were assumed to have a Poisson distribution (Littell et al. 1996). To further account for possible spatial dependence of data on creek location, plots of results from each creek were examined visually to ensure that responses were
consistent between creeks. Pairwise comparisons of interest were assessed using least square means (LSM). Mudflat and Spartina habitats were analyzed independently.

RESULTS

Infauna

The 30,174 annelids collected represented 15 species. The annelid assemblages in Spartina and mudflat habitats were similar (11 shared species out of 15), but differed in relative abundance (Table 1). Because grass shrimp may only be able to consume macroinfauna in the top 2 cm of the sediment (Posey and Hines 1991), I separated macroinfauna (i.e., annelids) into two functional groups (subsurface and surface deposit feeders) and selected representative taxa from each habitat for analysis. The spionid polychaete Streblospio benedicti (79% of mudflat community) and sabellid polychaete Manayunkia aestuarina (24% of the Spartina community) were considered surface deposit feeders (Fauchald and Jumars 1979) (Table 1) and were selected for analysis. All oligochaetes sampled were considered subsurface deposit feeders (Cook and Brinkhurst 1973), and I selected total oligochaetes (13% of the mudflat community) and the enchytraeid Cernosvitoviella immota (66% of the Spartina community) for analysis (Table 1).

Mudflat Habitat

In the mudflat habitat, chl a concentration was unaffected by fish removal (GLMM, p = 0.5819), but was affected significantly by cage type (GLMM, p = 0.0148), which depended on fish level (GLMM, Fish x Cage p = 0.0122). In the ambient fish level, full cage treatments had significantly higher chl a concentrations (up to 1.6x) than partial cage and open treatments (LSM, p < 0.05, Fig. 3a); however, chl a
concentrations did not differ among treatments in areas experiencing fish removal. *S. benedicti* densities were unaffected by fish removal (GLMM, p = 0.588), but densities were significantly greater (up to 1.5x) in full cage plots relative to partial and open cage plots (LSM, p < 0.05; Fig. 3b) and no difference between partial and open cage plots was detected (LSM, p = 0.481) (i.e., no caging artifacts). This increase was independent of fish level (GLMM, Fish x Cage p = 0.9052). Pairwise comparison of open cage plots between fish levels revealed no difference in *S. benedicti* densities (LSM, p = 0.6705) suggesting that fish removal had no effect on density. There was no effect of either treatment on total oligochaete density (GLMM, p > 0.05).

**Creek Bank Spartina alterniflora Habitat**

In the creek bank *S. alterniflora* habitat, *C. immota* density and chl a concentration were unaffected by either treatment or their interaction (GLMM, p > 0.05). Fish removal had no effect on *M. aestuarina* densities (GLMM, p = 0.1020), but densities increased significantly (2.5 – 4.6x) in full cage treatments relative to open plots and partial cages (LSM p < 0.05; Fig. 4b) regardless of fish level (GLMM, Fish x Cage, p= 0.6477). Pairwise comparison of open plots between fish levels revealed no difference in *M. aestuarina* densities (LSM, p = 0.1835) further suggesting that fish removal had no effect on density.

**Grass Shrimp**

Because fish removal did not influence grass shrimp densities (Deegan et al. 2007), the analysis here is focused on effects of fish removal on grass shrimp body size. Adult grass shrimp constituted a majority (>97%) of the total grass shrimp abundances for June and July regardless of fish treatment (t-test, Mann-Whitney U, p > 0.05, Fig. 5). In August, adults comprised a minor portion (<34%) of total grass shrimp
Table 4.1. Annelid community composition for (A) mudflat habitat and (B) creek bank *Spartina alterniflora* habitat for the Plum Island Estuary MA. Annelid class and family names are in parentheses. P = polychaete and O = oligochaete.

### A Mudflat habitat

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Relative Abundance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class</td>
<td></td>
</tr>
<tr>
<td>Total Oligochaetes</td>
<td>13.1</td>
</tr>
<tr>
<td>Total Polychaetes</td>
<td>87.1</td>
</tr>
<tr>
<td>Total</td>
<td>100.0</td>
</tr>
</tbody>
</table>

**Species**

- *Streblospio benedicti* (P: Spionidae) 79.4
- *Tubificoides brownae* (O: Tubificidae) 4.7
- *Monopylephorus* sp. (O: Tubificidae) 4.0
- *Tubificoides wasselli* (O: Tubificidae) 3.8
- *Manayunkia aestuarina* (P: Sabellidae) 3.8
- *Eteone heteropoda* (P: Phyllodocidae) 1.5
- *Nereis diversicolor* (P: Nereididae) 1.4
- *Polydora cornuta* (=ligni) (P: Spionidae) 0.7
- *Hobsonia florida* (P: Ampharetidae) 0.4
- *Capitella* sp. (P: Capitellidae) 0.2
- *Enchytraied 2* (O: Enchytraiedae) 0.1
- *Paranais litoralis* (O: Naididae) 0.1
- *Fabricia sabella* (P: Sabellidae) 0.1
- *Cernosvitoviella immota* (O: Enchytraiedae) 0.01
- Total                        100.0

### B Creek Bank *Spartina alterniflora*

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Relative abundance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class</td>
<td></td>
</tr>
<tr>
<td>Total Oligochaetes</td>
<td>69.0</td>
</tr>
<tr>
<td>Total Polychaetes</td>
<td>31.0</td>
</tr>
<tr>
<td>Total</td>
<td>100.0</td>
</tr>
</tbody>
</table>

**Species**

- *Cernosvitoviella immota* (O: Enchytraiedae) 66.3
- *Manayunkia aestuarina* (P: Sabellidae) 24.3
- *Fabricia sabella* (P: Sabellidae) 3.1
- *Paranais litoralis* (O: Naididae) 2.4
- *Pygospio elegans* (P: Spionidae) 1.2
- *Hobsonia florida* (P: Ampharetidae) 1.1
- *Capitella* sp. (P: Capitellidae) 1.1
- *Enchytraied 2* (O: Enchytraiedae) 0.3
- *Streblospio benedicti* (P: Spionidae) 0.2
- *Monopylephorus* sp. (O: Tubificidae) 0.02
- *Tubificoides brownae* (O: Tubificidae) 0.01
- *Tubificoides wasselli* (O: Tubificidae) 0.01
- Total                        100.0
Figure 4.3. Response of (A) benthic microalgae (BMA) (B) the surface deposit feeding polychaete *Streblospio benedicti* and (C) total oligochaetes to fish reduction and exclusion treatments in the mudflat habitat. Vertical bars represent untransformed data (mean ± SE). Open = open cage plots, Full = full cage plots. Bars with the same letter do not differ significantly (LSM methods). Partial cages are omitted because they do not differ from open cages.
abundances, indicating that a major recruitment of juvenile grass shrimp (14 – 30 mm TL) to the subadult size class occurred concurrent with the experiments in late-July and early August. The adult component of grass-shrimp abundances in August was ~1.6x greater in fish removal sites (34%) than in ambient fish sites (22%) (t-test, p=0.036, Fig. 5). Deegan et al. (2007) reported a slight negative effect of fish removal on shrimp growth. The analyses here differ in that I included only data from summer months (when the experiments were conducted), whereas, their analyses included only fall data when shrimp growth rate declined slightly in fish removal sites.

**DISCUSSION**

The purpose of this study was to determine if omnivore removal would initiate a trophic cascade in a low diversity, intertidal ecosystem. No trophic cascade was detected regardless of the level of omnivore removal, although top-down effects were indicated on surface-feeding annelids when all omnivores were removed. Below I decompose the food web to discuss both stages of the 2-stage omnivore removal and propose mechanisms by which omnivory may have modified food webs.

**Stage 1: Removal of an Omnivorous Top Predator**

**Killifish-Grass Shrimp**

Although killifish consumes grass shrimp (Kneib and Stiven 1982), shrimp densities were unaffected by killifish removal (Deegan et al. 2007). Therefore, no density-mediated trophic cascade was initiated because there was no direct top-down control of grass shrimp by killifish. Nevertheless, the relative abundance of adult grass shrimp increased when killifish were removed (Fig. 5). At least two explanations are possible. Because killifish affect the behavior of grass shrimp (Posey and Hines 1991), shrimp may have foraged more actively when fish were removed allowing recently
Figure 4.4. Response of (A) benthic microalgae (BMA) (B) the surface-deposit feeding polychaete *Manayunkia aestuarina* and (C) the subsurface deposit feeding oligochaete *Cernosvitoviella immota* to fish removal and exclusion treatments in the creek bank *Spartina alterniflora* habitat. Vertical bars represent untransformed data (mean ± SE). Open = open cage plots Full = full cage plots. Bars with same letter do not differ significantly (LSM methods). Partial cages are omitted because they did not differ from open cages.
recruited juveniles to reach the adult stage more quickly with killifish removal (Fig. 5).

Furthermore, isotopic evidence suggests that grass shrimp feed at higher trophic levels (i.e., are more carnivorous) when killifish are removed suggesting a shift in foraging behavior to consume more infauna (Galván 2008). Alternatively, juvenile shrimp in the fish removal sites may have been consumed at higher rates by remaining killifish. Sixty percent of the standing stock of primarily large (>40 mm) killifish were removed and it seems unlikely that the remaining killifish increased predation rates enough to create differential mortality among grass shrimp size classes. Thus, increased growth in grass shrimp may have resulted from a change in foraging behavior. This may be even more significant because this experiment was conducted concurrent with a large recruitment event of subadult/juvenile shrimp into adult sizes (Fig. 5).

**Killifish-Infauna**

Although killifish consume a variety of infaunal annelids (Kicklighter et al. 2004) and infaunal annelids may comprise up to 65% of the killifish diet (Sarda et al. 1998),
killifish removal did not affect infaunal densities in either habitat (Figs. 2b,c, 3b,c). This result suggests that killifish do not exert direct top-down control on infauna and thus killifish may not be strong grazers of infauna (Kneib and Stiven 1982).

**Killifish-BMA**

Killifish removal alone did not result in significant changes in BMA biomass by either direct or indirect means (Figs. 2a, 3a). Although killifish graze on micro- and macroalgae (Allen et al. 1994, Deegan et al. 2007), I know of no studies which have examined the direct effect of grazing by killifish on BMA.

**Summary of Stage 1 Effects**

If the food web was linear, one would expect a trophic cascade to be initiated with the reduction of a top predator due to strong interactions among trophic levels (Fig. 1a). Killifish, however, feed at various trophic levels (e.g., Allen et al. 1994) and I suggest that omnivory operated to prevent a trophic cascade by diffusing predation effects of a top predator over several trophic levels thereby weakening its impact on any one level. Thus, I suggest that killifish are weak interactors as a result of their omnivorous diet (Fig. 1b).

Alternatively, one might argue that killifish removal was not sufficient to elicit significant effects on lower trophic levels. Fish reduction was statistically significant (including up to a 40% reduction in small killifish, which probably prey more heavily on benthic invertebrates than do large killifish). Related experiments in PIE show that this level of killifish reduction elicits top-down control of other saltmarsh invertebrates; meiobenthic copepods (another important prey of small killifish, Kneib 1986, Fleeger et
al. 2008) and epifaunal amphipods (prey of larger killifish) (Deegan et al. 2007). Thus, the level of fish reduction achieved has the potential to impact other invertebrates in this system.

**Stage 2: Total Omnivore Removal**

**Killifish/Grass Shrimp-Infauna**

Total exclusion of both killifish and grass shrimp led to increased densities of surface-feeding infauna, regardless of habitat type (Figs. 3b, 4b), suggesting top-down control over this functional group, a result observed in other Atlantic marshes (Posey et al. 1999). Subsurface oligochaetes were not affected; suggesting that living position in the sediment has an important influence on predator efficacy.

At least two explanations may be invoked as to why surface-feeding infauna responded to total omnivore removal only: (1) killifish alone do not significantly affect infaunal densities, but the additive effect of both consumers (fish and shrimp) does or (2) grass shrimp and/or remaining killifish assumed a greater role as predators when killifish were reduced (Posey and Hines 1991; Galván 2008) thereby maintaining infaunal densities. My goal was to determine the existence of a trophic cascade in the food web, but I did not test for specific mechanisms. To test the mechanism of additive predation effects of the two predators (mechanism 1), one could use a 2x2 factorial of high and low fish levels with and without shrimp (i.e., no predators, fish only, shrimp only, both fish and shrimp). Because of the technical difficulty of excluding shrimp while not excluding fish in the field, this test would be most appropriate as an inclusion study.

Mechanism 2 relates to compensatory predation responses (CPRs) of the remaining predators once killifish are reduced, where the reduced predation on infauna by killifish in fish removal sites is offset by an increase in predation by other predators.
For instance, it is possible that the remaining killifish simply exhibited a CPR and consumed more invertebrates due to reduced intraspecific competition. However, no increase in carnivory was detected for small killifish in areas of killifish reduction using $^{15}$N isotope analysis (Galván 2008). As consumers of infauna, grass shrimp may have also exhibited a CPR (Posey and Hines 1991). This CPR can be the result of a change in shrimp density or a behavioral shift. Although killifish consume grass shrimp, shrimp abundance did not increase with killifish removal (Deegan et al. 2007) but per capita growth likely increased (Fig. 5) and grass shrimp exhibited a higher trophic level with fish removal (Galván 2008); therefore, a shrimp CPR may be a function of behavior. Grass shrimp adopt defensive postures (individually or collectively) or seek refuge in the presence of large killifish (Posey and Hines 1991, Carson and Merchant 2005), and this behavioral change can weaken predator-prey (in this case, shrimp-infauna) interaction strength (Schmitz et al. 1997), thereby limiting grass shrimp growth. Large killifish ($>40$ mm) were most effectively reduced (Deegan et al. 2007), and increased grass shrimp growth and trophic level in fish removal sites suggests grass shrimp fed more actively where killifish were reduced (Fig. 5; Galván 2008). Trait (behavior) mediated effects on species interactions are now recognized as important mechanisms in food web dynamics (Werner and Peacor 2003) and thus behavior may play a significant role in this food web.

**Infauna-BMA**

BMA biomass did not exhibit a reciprocal decrease when infauna increased in either habitat. This response may be explained by at least two reasons: (1) weak plant-herbivore interactions and/or (2) the response of unobserved herbivores. In the *Spartina* habitat, no change in BMA biomass was observed for any level of
predator/omnivore removal. Recent stable-isotope analysis in PIE suggests that *M. aestuarina* (one of the most abundant macroinfaunal species) feeds primarily on phytoplankton (Galván et al. 2008); therefore, this herbivore is not likely a strong grazer of BMA. Additionally, the relatively large (~10 mm) herbivorous amphipod *Uhlorchestia spartinophil*a was able to enter cages in the *Spartina* habitat at low tide and may have continued to graze despite the manipulation of fish and grass shrimp abundance (Bousfield and Heard 1986; personal observation). Although Deegan et al. (2007) found significantly higher amphipod abundances with fish removal, a killifish-amphipod-BMA trophic cascade was not indicated.

In mudflats, BMA biomass increased within full cages in both fish levels, but only significantly in ambient fish levels. This increase may be explained by a nontrophic interaction between infauna and BMA in which increases in infauna lead to increased inorganic N and P excretion, which in turn stimulates algal growth (Polis and Strong 1996). Alternatively, isotopic analysis suggests *S. benedicti* feeds on both phytoplankton and BMA (Galván et al. 2008) and therefore this annelid may not consume significant amounts of BMA. Also, removal of omnivorous small grass shrimp and/or small killifish may have contributed to increased BMA biomass in the mudflat habitats. The small omnivores in this study (especially young killifish and grass shrimp) consume microalgae and small herbivores (Galván et al. 2008), while adults feed on macroalgae and much larger herbivores (e.g., macroinfauna) (Kneib 1985, Sarda et al. 1998, Allen et al. 1994). Grass shrimp may graze a significant proportion of microalgal production (Morgan 1980, Fleeger et al. 1999, Quiñones-Rivera and Fleeger 2005).
Because BMA increased when all predators were excluded in the mudflat habitat (Fig. 3a) I suggest that killifish and/or grass shrimp may have short-circuited a potential trophic cascade by grazing directly on BMA.

**Summary of Stage 2 Effects**

Manipulations did not elicit reciprocating cascading effects in alternating trophic levels, which is the classic indication of a trophic cascade (Fig. 1a) (Pace et al. 1999). My results suggest two reasons why a trophic cascade was not observed after all potential predators were removed: weak herbivore-plant interactions (infauna-BMA) and short-circuiting effect of omnivory. Omnivores in mudflats may provide two major effects on the food web; simultaneous top-down regulation of (1) herbivores and (2) primary producers via direct consumption (Fig. 1b); a phenomenon seen in seagrass beds and marine hard bottoms (Heck et al. 2000, Bruno and O’Connor 2005). Thus, food webs with omnivory may have consumers that directly regulate multiple trophic levels and have a short-circuiting effect that prevents trophic cascades.

**Implications for Saltmarsh Ecology**

Predator control of BMA biomass mediated by infauna in a trophic cascade has been suggested in previous studies in other marshes (often in unvegetated habitats); however, these studies used a mesh size up to 4x larger than the mesh size used here (e.g., Posey et al. 1999). Taken together with previous work, the data above suggests deposit feeding infauna (annelids) are not strong grazers of BMA (Galván et al. 2008, this study), and furthermore, because small epibenthic grazers (e.g., small grass shrimp) may have been able to enter cages in previous field studies, top-down control may have been mediated through an unidentified consumer of BMA and not through infauna.
Two lines of evidence support the suggestion that grass shrimp, although an intermediate omnivore, may be more important in saltmarsh functioning than generally thought. First, because infauna increased only with the additional removal of grass shrimp, *P. pugio* may function as a high-level predator and control lower trophic levels. Shrimp are becoming increasingly recognized as important predators of macroinfauna (Kneib and Stiven 1982, Posey and Hines 1991, McTigue and Zimmerman 1998, Beseres and Feller 2007). My findings are consistent with those of Kneib and Stiven (1982), whose inclusion of different sized killifish in cages allowed them to conclude that grass shrimp are important predators of infauna. Second, if small grass shrimp are important grazers of microalgae, then these animals may exert strong top-down control on saltmarsh primary productivity (Fleeger et al. 1999). Killifish are usually considered to be the most important nektonic species in salt marshes because they are typically the dominant vertebrate predator in both abundance and biomass (Wiltse et al. 1984, Deegan et al. 2007). However, previous studies of the salt marsh benthos that have demonstrated top-down control often used small-scale (e.g., 1 m²) exclusion cages that excluded all predators and were unable to isolate the effects of individual predators (Sarda et al. 1998, Posey et al. 1999) so it is difficult to determine the role of killifish predation in these studies.

**Other Ecological Implications**

While omnivory may be responsible for generating disparity in trophic cascade strength across ecosystems (Strong 1992), it may also generate disparity within ecosystems. As the interface between continents and oceans, salt marshes can be partitioned into terrestrial and aquatic compartments. I found no trophic cascade in the aquatic compartment of a salt marsh; however, Silliman and Bertness (2002) found a
strong trophic cascade in the terrestrial compartment. The trophic cascade found by Silliman and Bertness (2002) resulted from a linear food chain with a specialist consumer and no direct consumption of macrophytes by predators (i.e., no omnivory). The nonlinear food web architecture in the aquatic compartment I studied appears to have muted a trophic cascade. My results, in addition to those of Silliman and Bertness (2002), contravene ecological theory that predicts the strongest trophic cascades occur in aquatic, algae-based food webs (Strong 1992). Similarly, Duffy et al. (2001) suggests that seagrass macrophytes are susceptible to cascading consumer effects; however, a simple trophic cascade may not be observed on epiphytic algae on seagrasses when omnivorous pinfish (*Lagodon rhomboides*) are present (Heck et al. 2000). Therefore, different cascading effects may be observed within ecosystems depending on the presence or absence of omnivores.

Exploitation of top consumers (i.e., overfishing) is a pervasive human activity that can lead to ecosystem decline (Jackson et al. 2001), and examinations of actual or simulated overfishing have demonstrated strong trophic cascades in marine communities (Silliman and Bertness 2002, Frank et al. 2005). Strong omnivory, however, may reduce the possibility of trophic cascades after overfishing events (Bascompte et al. 2005). In my experiment, the significant reduction of top predator (killifish) densities represents a major perturbation (i.e., overfishing), particularly for a low diversity system, and my results demonstrate that mechanisms such as short-circuiting omnivory and compensatory responses may protect against ecosystem collapse. If grass shrimp are able to exhibit a CPR, then the ecosystem may have some level of ‘functional redundancy’ in that taxonomically distinct species have similar ecological functions (Walker 1992). In many ecosystems, small changes in species
diversity may result in loss of key ecosystem processes (Micheli and Halpern 2005); however, my results demonstrate that in this low diversity ecosystem, omnivory and/or functional redundancy may be sufficient to mitigate the effects of species loss. I am not suggesting that decreased biodiversity (i.e., killifish eradication) in salt marshes would result in no loss in ecosystem functioning, but instead that in low-diversity and dynamic ecosystems such as salt marshes, mechanisms exist to increase resistance to perturbations. Thus, although increased biodiversity may increase community stability (Tilman et al. 2006), my results suggest that omnivory may buffer against the effects of certain human activities and thus may be an important stabilizing force in low diversity ecosystems (McCann and Hastings 1997, Borrvall et al. 2000). Therefore, an understanding of food web structure (degree of omnivory and functional redundancy) is necessary for management and conservation decisions because food webs with omnivory are not rigid chains susceptible to cascading effects, but pliable, reticulate webs that may be resistant to perturbations.

In conclusion, my experiment in the marine benthos revealed that the significant reduction of an omnivorous top predator did not elicit a trophic cascade. This suggests that killifish are not strong interactors with lower trophic levels as a function of their omnivorous diet. In addition, the intermediate omnivore, grass shrimp, may have counteracted cascading predator effects by feeding at more than one trophic level, thereby preventing the top-down control of invertebrates by killifish and short-circuiting a trophic cascade by direct BMA grazing. Direct limitation of primary producers by omnivores may be a common occurrence in aquatic systems (Dorn and Wojdak 2004, Bruno and O’Connor 2005). Omnivory may interrupt a potential trophic cascade even in a system thought to exhibit the strongest cascades (Shurin et al. 2002) and contribute to
the growing evidence that omnivory can weaken or disrupt trophic cascades in simple, aquatic algae-based food webs (Bruno and O'Connor 2005). The results of this study also emphasize the need for understanding the complex interactions among species in food webs to make predictions about the effects of anthropogenic activities.

**LITERATURE CITED**


CHAPTER 5

TOP-DOWN AND BOTTOM-UP CONTROLS INTERACT TO ALTER HABITAT
UTILIZATION OF SALTMARSH FAUNA
INTRODUCTION

Of great interest to ecologists is predicting how ecosystems respond to anthropogenic-induced perturbations. Two anthropogenic activities that impact many aquatic systems – often concurrently – are nutrient loading (e.g., land-derived nutrients) and food web alterations (e.g., predator removal) (Deegan et al. 2007). These anthropogenic activities simultaneously embody the classic ecological debate over whether the functioning or community structure of ecosystems are regulated by top-down (consumer driven) or bottom-up (resource driven) processes (Hairston et al. 1960, Power 1992). Understanding how these processes operate is critical in predicting how ecosystems respond to human activities.

Top-down and/or bottom-up processes may operate independently (Posey et al. 1999, Posey et al. 2002) or interactively (Russell and Connell 2005). Because predictions about interactive effects cannot be made by examining each process in isolation, it is important to examine both effects simultaneously. Another difficulty in predicting top-down and bottom-up effects is that they may vary across the landscape (Fleeger et al. 2008). Predictions about top-down and bottom-up effects on the entire ecosystem based on plot-level experiments, which are typically conducted in one part of the landscape (e.g., Posey et al. 2002), may therefore only be applicable to that habitat (Fleeger et al. 2008).

Benthic macroinvertebrates, as consumers of primary production and food for higher trophic levels, may be excellent organisms to study anthropogenic effects in many environments, including salt marshes. Although it is known that saltmarsh infauna (sediment-dwelling invertebrates such as annelids) are regulated by both top-down and bottom-up factors (Posey et al. 1999, Posey et al. 2002, Deegan et al. 2007),
the effects of these factors on epifauna (surface-dwelling invertebrates such as amphipods) remains relatively unknown, possibly because of the mobility of epifauna necessitates large-scale manipulations to effectively capture their responses. Top-down control of epifauna such as amphipods may be exerted by the killifish Fundulus heteroclitus L. (Kneib 1982, Allen et al. 1994, Fell et al. 1998), a common predator known to consume epifauna in the tidal marshes of the western Atlantic (Allen et al. 1994). Top-down control may also be exerted on epifauna by aerial predators (i.e., birds). For instance, in the Bay of Fundy birds exert top-down control on the amphipod Corophium volutator during low tide (Wilson 1991). Because birds may have access to exposed epifauna at low tide and fish may have access to epifauna during inundation periods, an indirect interaction between fish and bird predators may exist (Crowder et al. 1997). Benthic microalgae, an epifaunal food source, which responds rapidly to nitrogen input with increased biomass and/or productivity (Sarda et al. 1998) and may exert bottom-up control on epifauna. Epifauna such as amphipods commonly exert top-down control on algae in marine systems (Duffy and Hay 2000), and may be important algal consumers in salt marshes.

I present the results of whole-ecosystem manipulations of key predator (Fundulus heteroclitus) removal (top-down) and nutrient addition (bottom-up) on saltmarsh epifauna. My results represent traditional examination of top-down and bottom-up influences on the abundances of saltmarsh invertebrates (e.g., Posey et al. 1999), where density changes may result from altered survivorship or reproductive success of a species (whether from reduced predation pressure or increased food supply). Here, I focus on individual taxon responses instead of ecosystem or community responses because individual taxa may vary in their response to top-down
and bottom-up factors (Posey et al. 1999, 2002, Fleeger et al. 2008). The scale of the experiments allowed me to examine possible treatment-induced movements of epifauna across the landscape. As a result of treatments, I found that amphipods moved from vegetated habitats to unvegetated habitats, which in turn increased their susceptibility to a rarely encountered predator, *Calidris pusilla*, a migratory shorebird. This was an unexpected consequence observable only in a whole ecosystem study because of the size of area needed to influence bird behavior.

**METHODS**

**Study Site**

This study was conducted in the Plum Island Estuary (PIE), Massachusetts during two growing seasons (May – September), 2005 and 2006, in four bifurcated intertidal creeks: Sweeney, West, Clubhead and Nelson. These creeks drain into the 14.5-km Rowley River estuary (42°44'N, 70°52'W), which opens into Plum Island Sound at about 7-km inland from where Plum Island Sound enters the Atlantic Ocean (Fig 1). All first order creeks are characterized by two similarly sized second order creeks, 3-m tidal fluxes, and distinct habitat and biotic zonation. The creeks have similar macroinfauna (predominately annelids) species composition, and the density of individual species differ little between creeks (i.e., low large-scale spatial variability) (Johnson et al. 2007). The physical attributes of the creeks are similar and there was no difference among creeks in terms of sediment-dwelling algal biomass and infaunal abundances before treatments were applied (Deegan et al. 2007, Johnson et al. 2007). First-order creeks are 300 – 500 m long and sections of the second-order creek channels selected for data collection are 3 – 5 m wide and have a depth of 1.5 – 2.5 m. Like many northwestern Atlantic salt marshes, PIE creeks are replete with mosquito ditches. Ditches are smaller
channels than creek channels and connect perpendicular to the tidal creeks and range from 0.25 – 1.0 m in width and have steep vertical walls typically to a depth of 0.5 – 1.0 m. Marsh ditches are little studied, but have been determined to be important for killifish foraging (Allen et al. 1994). In this study I discuss three habitat types. (1) ‘Creek-bank Spartina alterniflora’ is a 2-3 m swath of S. alterniflora monoculture at the edge of the marsh platform, (2) ‘creek wall’ is a vertical habitat immediately adjacent to the creek-bank S. alterniflora in the main creek channels, and (3) ‘ditch wall’ is a vertical habitat in mosquito ditches.

**Treatments**

To simulate nutrient loading, nutrients were added in solution to the water column with a targeted concentration of $70\,\mu\text{M/L NaNO}_3$ and $6\,\mu\text{M/L PO}_4^{+}$ with each flooding tide at the confluence of Sweeney and Clubhead creeks from May 15 – October 1, 2005. West and Nelson creeks received no nutrient enrichment. In one second-order creek of each first-order creek (ambient nutrients and nutrients additions), *F. heteroclitus* was reduced in abundance by 60% by stretching 7-mm mesh Vexar across the creek channel to exclude fish and using mummichog-selective traps behind the exclusion. A full factorial design of the two treatments with replication (n=2) was therefore implemented in 2005: ambient fish/ambient nutrients (control), ambient fish/nutrient additions, low fish/nutrient additions, and low fish/ambient nutrients. Maintenance of such large scale treatments is expensive and labor intensive, and as a result the TIDE project only manipulated Sweeney and West Creeks in 2006 for the same factorial design, but without first-order, creek-scale replication.
Figure 5.1. Map of Plum Island Estuary showing location of experimental creeks (MassGIS orthophoto 2002). SW=Sweeney Creek, WE=West Creek, CL=Clubhead Creek, NE=Nelson Creek. In 2005, all four creeks were manipulated for true replication, but only SW and WE were manipulated in 2006.

**Epifauna Collection**

To quantify the response of saltmarsh epifauna to fish reduction and nutrient addition, passive collectors (litterbags; after Fell et al. 1998) were deployed in the creek bank *Spartina alterniflora* for two weeks in all treatment creeks in 2005 and 2006. Each litterbag (29 x 13.5 cm, 5-mm Delta weave mesh) was filled with 15 – 20 g of dried, standing dead *S. alterniflora* leaves and stems. These are passive collectors that allow
epifauna to enter and exit through the mesh throughout the time of deployment. I did not attempt to estimate the absolute abundance of epifauna from litterbags, but instead examined the relative abundance patterns of epifauna among treatments. At low tide, 8-10 litterbags were haphazardly placed among the stems of creek-bank S. alterniflora plants within 50 to 100 m from the confluence the secondary creeks. Garden staples held litterbags in place. I recognize some biases of the litterbags as epifaunal collectors; some visually abundant species such as the coffee bean snail, Melampus bidentata (DSJ personal observation) did not enter the bags and the mesh size may have precluded collection of unusually large amphipods. Litterbag collection dates were similar in the two years; July 8 and August 12 in 2005 and July 15 and August 21 in 2006.

At the time of retrieval, each litterbag was placed individually into a 4-L plastic bag to minimize escape of epifauna, and then placed in a cooler for transport. In the laboratory, the contents of each litterbag were emptied into the plastic bag, with care taken not to allow active epifauna to escape. A 50% ethanol and Rose Bengal solution was then added. After at least two days, litter was rinsed over a 0.5 mm sieve and all animals were collected using forceps and a hand lens. Animals were identified to lowest possible taxon, enumerated and preserved in 95% ethanol. Recent studies have demonstrated disproportionate predation on male Corophium volutator, an intertidal amphipod, due to active mate searching (McCurdy 2005). Therefore, Uhlorchestia spartinophila, an intertidal amphipod and major constituent of the salt marsh community, was separated into two categories (males and female/juveniles) to examine differential effects of the treatments on the abundances of the sexes.
Amphipod identification procedures in *S. alterniflora* habitat differed between the two years. In 2005, talitrid amphipods, *U. spartinophila* and *Orchestia grillus*, were identified as a single talitrid amphipod group. 2005 samples were discarded after species identification. In 2006 I distinguished between the two species. I found that *U. spartinophila* comprised 99.99% (1160 *U. spartinophila* and 8 *O. grillus* were collected) of the individuals of two species for July and August 2006 samples combined. This distribution between the two species is consistent with previous work on habitat distributions that found *U. spartinophila* prefers low marsh habitats (e.g., creek bank *S. alterniflora*) and *O. grillus* prefers more terrestrial, higher marsh habitats (e.g., *S. patens*) (Bousfield and Heard 1986, Covi and Kneib 1995). Thus, statements about amphipods in the *S. alterniflora* habitat refer to *U. spartinophila*, though I recognize a few *O. grillus* are probably included in 2005. Another important epifaunal group, hydrobiid snails, was pooled into a single category although at least two species of hydrobiid snails are found in the system (Mandracchia and Ruber 1990).

Although *U. spartinophila* is thought to be primarily a detritivore that inhabits the vegetated marsh (Kneib et al. 1997), I noted unexpectedly high densities of *U. spartinophila* on the steep, almost vertically oriented, algae-covered creek-wall habitat adjoining the *S. alterniflora* habitat, and on the walls of mosquito ditches (hereafter ditch walls) in some creek systems near the end of the field season in 2005 (DSJ personal observation). Intertidal amphipods have been shown to move to algal enriched areas (Kraufvelin et al. 2006) and I considered the possibility that some *U. spartinophila* may have been making foraging forays to creek wall or that some individuals emigrated to this algal-rich habitat. Therefore, I conducted direct counts of amphipods on the creek and ditch walls in 2006 to determine their use of these habitats in experimental creeks.
Thirty quadrats (0.25 m²) were haphazardly placed in each treatment between 50 – 200 m from the confluence of the secondary creeks. For ditch walls, 10 quadrats were placed in three of the ditches found in each treatment for a total of 30 per treatment. Amphipod counts were conducted after at least one day after quadrat placement to minimize disturbance. Two color morphs – orange and brown – were found on the wall habitats and each morph was quantified. In addition to quantifying amphipods, the percent cover of algae was determined visually within each quadrat. All counts and percent cover estimates were conducted by the same investigator (DSJ).

Because movement into unvegetated habitats potentially increases the susceptibility of amphipods to bird predation at low tide, I visually censused birds in the creek and ditch channels. For creek channels, this was accomplished by walking 150m along the platform edge at low tide and each bird seen in the creek channels typically foraging on mudflats was identified and counted. All creek sections had similar widths (3 – 5 m) and foraging areas for birds. When birds were flushed and landed further down the creek channel, care was taken not to count the same bird twice. For bird counts in ditch channels, 50 m of three ditches within a second order creek (i.e., treatment) were walked (total of 150m/secondary creek). Four bird walks were conducted between August 17 – September 17, 2006. Each creek walk and ditch walk (pooled from three ditches) took approximately 15 minutes. To determine if birds consumed amphipods, bird foraging behavior in the creek wall habitats in Sweeney was monitored visually using binoculars during low tide on three separate days (August 30, September 5 and 6, 2006) for a total of six hours.
Statistical Analysis

To examine the effect of fish reductions and nutrient additions on epifauna abundances, selected taxa were examined using Proc GLIMMIX to generate a generalized linear mixed model (GLMM) (SAS v9.1.3 Cary, NC, USA). For each collection timepoint, fish (low and ambient fish) and nutrient (addition and ambient nutrients) levels were analyzed as fixed factors while primary creek nested within nutrient treatment and secondary creek nested within nutrient and fish treatment were set as random factors. When random factors contributed little to the variability, they were dropped from the model. I used a Poisson distribution for the error with the appropriate log transformation. LSMeans were used to examine pairwise comparisons of interest between treatments. For direct counts of amphipods and birds, log-transformation to approximate normality and homogeneity of variances was applied and data were examined with Gaussian distributions in a GLMM as above with only the residual as the random factor. Again, LSMeans were used to examine pairwise comparisons of interest. To determine if the percent algal cover differed between the two nutrient treatments, t-tests were conducted.

RESULTS

Epifauna in Creek-Bank *Spartina alterniflora*

Overall, 5801 individuals were collected, representing at least 14 epifaunal species. For 2005 samples, hydrobiid snails and the amphipod *Uhlorchestia spartinophila* were selected for analysis because they represented major constituents of the epifauna community (15% and 76%, respectively) (Table 1). In 2006, *U. spartinophila* was again examined, however; fewer than 0.35 hydrobiid snails were collected per litterbag (down from 4.6/litterbag in 2005) and were judged to be too low in
Table 5.1. Relative abundance (% of community) of epifauna in the creek-bank *Spartina alterniflora* habitat in the Plum Island Estuary, MA. Percentages are a composite of all samples taken per year.  A = amphipod, I = isopod, S = snail, C = Chelicerate, In = insect.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>2005</th>
<th>2006</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male <em>Uhlorchestia spartinophila</em> (A)</td>
<td>12.5</td>
<td>15.4</td>
</tr>
<tr>
<td>Female/juvenile <em>U. spartinophila</em> (A)</td>
<td>63.0</td>
<td>50.2</td>
</tr>
<tr>
<td>Total <em>U. spartinophila</em> (A)</td>
<td>75.5</td>
<td>65.7</td>
</tr>
<tr>
<td>Male <em>Orchestia grillus</em> (A)</td>
<td>-</td>
<td>0.4</td>
</tr>
<tr>
<td>Female <em>O. grillus</em> (A)</td>
<td>-</td>
<td>0.1</td>
</tr>
<tr>
<td><em>Gammarus</em> sp. (A)</td>
<td>4.2</td>
<td>21.7</td>
</tr>
<tr>
<td><em>Philosica vittata</em> (I)</td>
<td>0.0</td>
<td>0.7</td>
</tr>
<tr>
<td><em>Hydrobia</em> spp. (S)</td>
<td>15.4</td>
<td>1.7</td>
</tr>
<tr>
<td>Mites (C)</td>
<td>1.0</td>
<td>2.5</td>
</tr>
<tr>
<td>Spiders (C)</td>
<td>1.2</td>
<td>2.0</td>
</tr>
<tr>
<td>Psuedoscorpion (C)</td>
<td>0.3</td>
<td>0.4</td>
</tr>
<tr>
<td><em>Tabanus</em> sp. larvae (In)</td>
<td>1.8</td>
<td>1.1</td>
</tr>
<tr>
<td>Other insect larvae (In)</td>
<td>0.1</td>
<td>2.0</td>
</tr>
<tr>
<td>Beetles (In)</td>
<td>0.3</td>
<td>1.4</td>
</tr>
<tr>
<td>Collembola (In)</td>
<td>0.0</td>
<td>0.1</td>
</tr>
</tbody>
</table>

abundance for analysis. Because all categories of amphipod sex and/or maturity exhibited similar trends statistically and graphically, I concluded that male and female amphipods responded similarly to treatments and discussion of amphipods hereafter will refer to the entire population.

In June 2005, relative abundance of hydrobiid snails was significantly higher in fish removal treatments (up to 3.3-fold relative to controls; fish main effect: p=0.0074) (Fig. 2), but there were no treatment effects on *U. spartinophila*. In August 2005, there was a highly significant fish effect on hydrobiid snails, with enhanced relative abundance, and an interactive effect of nutrient additions and fish removals on *U. spartinophila* (fish x nutrient: p < 0.0001). *A posteriori* tests were used to examine main effects on *U. spartinophila* because of the significant interactive effect. Relative to controls, amphipods increased significantly with fish removals alone (ambient fish/ambient nutrient vs. low fish/ambient nutrients LSMeans: p < 0.0001; a 225%
increase). Nutrient additions alone resulted in a non-significant increase in amphipod relative abundance (ambient fish/ambient nutrient vs. ambient fish/nutrient additions LSMeans p > 0.05; a 108% increase). The joint effect of treatments resulted in a slight increase in *U. spartinophila* abundance relative to controls, but the increase was significantly lower than that predicted by an additive response (i.e., an antagonism).

In both July and August 2006, there was a significant interactive effect of nutrient additions and fish removals on *U. spartinophila* relative abundance (fish x nutrient interaction: p ≤ 0.0167, Fig 2). Nutrient addition alone resulted in an average of 15% increase in amphipod abundance compared to controls and fish removal resulted in a 33% increase (Fig. 2). However, these main effects were non-significant in both collections based on *a posteriori* testing (LSMeans p > 0.05). An antagonistic effect was observed in both collections because amphipod relative abundance in creeks with joint treatments was lower than controls and therefore much lower than that predicted by an additive response.

**Creek-Wall and Ditch-Wall Amphipod Density**

In 2006, direct counts of amphipods were conducted to test the hypothesis that amphipod abundances on creek wall and ditch wall habitats were affected by treatments. In the creek walls, nutrient additions significantly (p=0.030) increased amphipod densities regardless of fish level (i.e., no interaction), whereas there was no indication of a fish effect. In the ditch walls, the interaction term was significant for amphipod density (fish x nutrient: p > 0.001). While fish and nutrient treatments alone did not affect amphipod density (LSMeans p > 0.05), the combination of treatments
Figure 5.2. Epifauna collected in the creek bank *Spartina alterniflora* habitat in the tidal creeks of the Plum Island Estuary, MA. Hydrobiid snails collected during (A) June and (B) August 2005. Total (males + females/juveniles) *Uhlorchestia spartinophila* collected during (C) June 2005, (D) August 2005, (E) July 2006 and (F) August 2006. Values are means (+/- 1 SE). For 2005 (n=16), for 2006 (n=10) per treatment.
resulted in a significant increase in amphipod density relative to controls and the effect was strongly synergistic (LSMeans ambient nutrients/ambient fish vs nutrient addition/low fish p = 0.0001; a 2420% increase). Additionally, amphipod densities in joint treatments in the ditch wall were >10-fold higher than densities for either treatment in the creek wall (Fig. 3).

**Color Morphs of Amphipods**

Overall, the orange color morphs constituted 89% of the creek-wall and 98% ditch-wall amphipods noted from direct counts. In the ambient nutrient treatments, 100% of the amphipods were of the brown color morph, regardless of type of wall habitat. In the nutrient treatments, 100% of the ditch-wall amphipods and 86% of creek-wall amphipods were of the orange color morph.

**Bird Sightings**

Thirty eight of the 39 birds counted in the two creeks examined were the semipalmated sandpiper, *Calidris pusilla*, a migratory shorebird; there was one seaside sparrow, *Ammodramus maritimus*. A significant treatment interaction occurred (fish x nutrient: p = 0.015 Fig. 3) and both treatments significantly increased bird sightings in the creek bottoms relative to controls (a 2900% increase in low fish/nutrient additions vs. ambient fish/ambient nutrient; LSMeans p = 0.002). No birds were observed in the ditches of non-nutrient (West) creek and an average of ~2 birds/walk were observed in nutrient (Sweeney) ditches. *C. pusilla* was observed foraging (i.e., probing the sediment with their beaks) when in creeks; ~20% of their time was spent on the creek wall, with the remainder of their time on mudflats. Birds were directly observed feeding upon amphipods on two occasions on the creek wall.
Figure 5.3. (A) *Uhlorchestia spartinophila* (amphipods) collected in litterbags for the *Spartina alterniflora* habitat and (B) amphipod density for the adjoining creek wall habitat. (C) *Calidris pusilla* (birds) counted in the main creek channel at low tide over 150m. (D) Amphipod density for ditch wall habitat and (E) Birds counted in 150m of ditch channels. All collections are from August 2006.
Percent Algal Cover

Nutrient additions significantly increased algal cover by 1.5-fold on creek walls (mean ± 1-SE; ambient nutrients: 39.3±3.7%; nutrient additions: 58.8±3.7%; t-test; p < 0.001). Algal cover was only slightly and non-significantly higher on ditch walls in nutrient creeks (mean ± 1-SE; ambient nutrients: 48.0±3.6%; nutrient additions: 53.8±3.4%; t-test; p > 0.05).

DISCUSSION

Large-scale, multi-stressor studies are rare in coastal ecosystems (Deegan et al. 2007), but are becoming increasingly recognized as important tools to predict the impacts of anthropogenic activities (Heck and Valentine 2007). Due to the landscape approach of the experiment, I was able to quantify changes in habitat utilization of organisms, and my results suggest that at least two saltmarsh fauna, amphipods and sandpipers, alter their foraging behavior as a result of nutrient addition, fish removal or both in combination.

I observed strong predation effects on hydrobiid snails, a common component of killifish gut contents (Allen et al. 1994, Fell et al. 1998), in the vegetated (S. alterniflora) creek-bank habitat. Given the small size and limited ability of hydrobiid snails to escape predation, I consider changes in relative abundance for this taxon were due to predation by effects of killifish suggesting strong top-down regulation of this taxon by killifish.

Although top-down and bottom-up effects were difficult to isolate and compare in terms of intensity, my results suggest very that strong interactions between the two forces did occur in one species of epifauna. Treatments strongly interacted on U. spartinophila in the S. alterniflora habitat such that killifish removal and nutrient additions acted antagonistically (both factors tended to increase abundance alone but
antagonistically lowered abundances when combined). Because increased amphipod densities in the creek-wall and ditch-wall habitats corresponded to decreased amphipod abundances in *S. alterniflora* (Fig. 3a and 4b), the mechanism for the observed antagonism in *S. alterniflora* may have been movement of amphipods between the two habitats. This interaction was unexpected and shows that a commonly held assumption in ecological studies (that multiple factors act independently, Agrawal et al., 2007) was not met in this system for this species. The interaction notwithstanding, I observed weak bottom-up and top-down effects in the *S. alterniflora* habitat for *U. spartinophila*, but I cannot make statements about the entire amphipod population across habitats because the collection techniques are not additive and would need to be pro-rated for the spatial extent of each habitat.

In creek-bank *S. alterniflora*, when fish removals were combined with nutrient additions, fewer amphipods were detected than predicted by additive responses to each treatment (i.e., an antagonism). However, there was not a corresponding synergistic abundance increase in the creek wall as might be expected if amphipods emigrate from the *S. alterniflora* habitat. Predation by the semipalmated sandpier, *Calidris pusilla* may explain this observation. During low tide, *C. pusilla* may replace killifish as the principal predator of amphipods when nutrients are increased and fish removed. Creek walls have small outcroppings which birds may perch on to forage for infauna (e.g., annelid worms) and epifauna (e.g., amphipods). Thus at low tides, sandpipers may be able to exert top-down control on amphipods in creek channels, a notion supported by two lines of evidence: (1) direct observation of amphipod predation by sandpipers and (2) a 10-fold increase in amphipod densities in ditch walls compared to creek walls (Fig. 3). Ditches may naturally exclude bird predators because the steep walls provide no
perches, there is often overhanging vegetation, and the narrow width may prevent quick escape from predators (e.g., hawks). To compare the two types of wall habitats (creek vs. ditch wall), amphipod densities were highest on ditch walls where all predators were excluded (fish: artificially; birds: naturally) and nutrients were added (Fig 3). Therefore, the abundance of amphipods on creek or ditch walls may be a complex function of immigration and removal by predation suggesting that top-down (fish) and bottom-up effects both contribute to the changes in abundance. Although various factors have been implicated for determining the zonal distribution of intertidal, gammaridean amphipods on the Atlantic west coast including predation by killifish (Vince et al. 1976), intraspecific competition (Van Dolah 1978) and physical/habitat conditions (Kneib 1982), my results suggest that inter-habitat movement and predation risk may also be important in determining amphipod distributions. Further, I suggest that an unanticipated form of top-down control on a mobile amphipod species by birds is present under some conditions in this ecosystem.

In combined treatments, amphipods densities on the ditch wall were 10x those on the creek wall in combined treatments and I suggest this difference is due to bird predation. Sandpipers were observed over a 150-m linear distance, and an average of 6 more birds were seen in creeks compared to ditches over the same distance-time period. If amphipods are standardized to the same linear distance (150 x 1 m), then an estimate of 90 amphipods were on the creek wall, and 1020 on the ditch wall. The difference in abundance between the two channel types is then 930 per 150 m for amphipods. Thus, for the large difference between amphipod densities between habitats to occur, 6 birds would have to consume 930 amphipods (or each bird consuming 155 amphipods) if amphipods visit creek and ditch walls equally. Sandpiper
abundances peak between mid July to mid August, a time during which direct counts were conducted. Using a 30 day time period, each bird would then have to consume 5.2 amphipods d\(^{-1}\) and using a conservative foraging period (low tide) per day of 3 hours, each bird would have to consume at least 1.7 amphipods h\(^{-1}\) d\(^{-1}\). Anecdotal observations suggest sandpipers can consume up to 4 amphipods h\(^{-1}\) on the creek wall at low tide (DSJ personal observation). Thus, it plausible that bird predation over the course of the summer season is enough to limit amphipod densities on the creek wall at the end of the season.

There are several possible explanations for movement of amphipods from *S. alterniflora* to unvegetated wall habitats including enriched food supply (Kraufvelin et al. 2006), predator release (Vince et al. 1976) or density-dependent migration (Van Dolah 1978). Preliminary isotopic evidence from PIE (K. A. Galvan unpublished data) suggests that amphipods forage for algae while on creek and ditch walls. Benthic algae was increased synergistically in creeks with nutrient enrichment and fish reduction during the time of the studies (Deegan et al., 2007) suggesting that amphipods may migrate to wall habitats to exploit a food resource. Alternatively, it is possible that the movement of amphipods was parasite induced. For instance, infaunal amphipods, *Corophium volutator*, in the Bay of Fundy that are parasitized by the nematode *Skrjabinoclava morrisoni* crawl more actively on the sediment surface thereby making them more susceptible to predation by *C. pusilla*, the parasite’s final host (McCurdy et al. 1999). In the life cycle of the microphallid trematode *Levinseniella byrdi*, the initial hosts are hydrobiid snails and intermediate hosts are talitrid amphipods (including *U. spartinophila*); shorebirds (including *C. pusilla*) are the final host. Amphipods parasitized by *L. byrdi* develop a bright orange color due to the dissociation of
carotenoids from proteins and do not exhibit strong negative phototaxis as do un-
parasitized individuals (Bousfield and Heard 1986). These trematode-induced trait
modifications (behavior and color) make the amphipod more vulnerable to predation by
shorebirds, the final host of the trematode (Bousfield and Heard 1986). 89 – 98% of
amphipods found on the wall habitats during the experiments were bright orange and
trematode metacercariae (Levinseniella sp.) were found in examined specimens of the
orange morph (R. W. Heard, personal communication) suggesting that U. spartinophila
on walls were heavily parasitized. Parasitized amphipods may move into more exposed
habitats such as the creek walls where they are preyed upon by C. pusilla and other
shore birds (e.g., seaside sparrows). If so, the mechanism explaining the increased
incidence of parasitized amphipods in the combined treatments is unclear but could be
associated with factors that influence survivorship or growth of hyrdrobiid snails (the first
host) or the intermediate stages (cecariae) of the parasite that infect the U.
spartinophila. The abundance of hydrobiid snails was increased by fish removals but
not by nutrient addition, thus increases in snail abundance do not likely explain
increased amphipod infection. Alternatively, infected birds may be attracted to fertilized
creeks for some other reason (e.g., another prey species becomes abundant due to
fertilization) and the increased frequency of use of fertilized creeks by infected birds
may lead to an increase in the infection rate of amphipods. Almost all amphipods found
in the creek/ditch wall habitats of the nutrient creek were orange, whereas all
amphipods found in non-nutrient creek were brown suggesting that nutrient enrichment
influenced the frequency of parasitism. The Plum Island Estuary is an important
stopover for many migratory birds including C. pusilla, and my results suggest human
activities such as nutrient loading and predator removal may result in increased incidence of parasitized birds.

The relative importance of top-down and bottom-up effects has long been of interest in ecology (Hairston et al. 1960, Power 1992), particularly in marine systems (e.g., kelp forests: Halpern et al. 2006, Foster et al. 2006, coral reefs: LaPaointe 1997, Hughes et al. 1999). Recent work suggests that top-down forces may hold primacy over bottom-up forces in marine systems (Silliman and Bertness 2002, Shurin et al. 2002, Heck and Valentine 2007); however, many recognize the connectedness between top-down and bottom-up forces in marine ecosystems (e.g., rocky interidal: Menge et al. 2003, coral reefs: Lapointe 1997, kelp forests: Russell and Connell 2005). My results suggest that the two forces act in concert and that in this system behavior-mediated effects were important. Previous examinations of the saltmarsh benthos which have combined top-down/bottom-up manipulations on infauna (sediment-dwelling fauna that are less motile) have typically not found interactive effects (Posey et al. 1999, Posey et al. 2002). The interactive effects I found for epifauna were a function of changes in behavior and the mobility of organisms because the amphipods in this study appear to have non-random, inter-habitat movements.

My results suggest that single-factor experiments are poor predictors of multifactor effects for mobile species. It is possible that stronger single factor effects occurred but were difficult to detect due to at least two complicating factors in this system: (1) single factor effects are difficult to detect for mobile epifauna because of their movement between habitats, and thus knowledge of true effects requires a landscape, multiple habitat approach, and (2) even with the knowledge of total population effects (i.e., all habitats combined), top-down effects may be difficult to
identify due to the functional redundancy between birds and fish wherein birds exhibit compensatory predation when fish are removed. I was initially limited in my assessment of single factor effects because I was myopic in the sampling effort by sampling only one habitat, and thus the single factor effects appear mild or non-existent. Sampling an additional habitat (wall habitats) enhanced the understanding of treatment effects. Therefore, to increase the predictive power of single factor effects and/or interactive effects, a better understanding of the basic ecology and distribution of saltmarsh fauna, including their parasites, is needed.

My work shows that large-scale experiments can aid in the understanding of anthropogenic effects. Although replication of experimental units was low in this study, I found similar responses by epifauna to treatments in temporally replicated observations within seasons and between field seasons, suggesting that the patterns observed are repeatable and related to the treatments of interest. My approach more appropriately simulates anthropogenic activities than plot-level experiments and allowed me to pragmatically examine the relative importance of top-down and bottom-up factors in a real world context. My results add to the growing body of evidence that the traits of prey, specifically behavior, play an important role in ecological interactions (Werner and Peacor 2003) and that behavior is important in saltmarsh ecology. The behavior (i.e., movement) of amphipods also indicated that top-down and bottom-up forces may act simultaneously in this system and make it more difficult to identify single-factor effects. My results revealed not only an unexpected connectedness between habitats as a function of amphipod movement, but also of a possible unexpected connectedness between trophic levels (e.g., fish-bird interaction mediated by amphipods). Because of the interactions between top-down and bottom-up controls and resulting movement of
fauna (i.e., amphipods and birds), my work underscores the inability to predict effects based on examining each control in isolation.

**LITERATURE CITED**


Table 5.2. Summary of p-values from Generalized Linear Mixed Model for selected taxa in the creek bank *Spartina alterniflora* habitat. In 2006, too few snails were collected and were not analyzed.

<table>
<thead>
<tr>
<th></th>
<th>June</th>
<th>August</th>
<th>July</th>
<th>August</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>F(P-value)</td>
<td>df</td>
<td>F(P-value)</td>
</tr>
<tr>
<td>Total</td>
<td>N</td>
<td>1,2 6.72(0.110)</td>
<td>1,2 0.11(0.776)</td>
<td>1,28 6.49(0.017)</td>
</tr>
<tr>
<td>U. spartinophila</td>
<td>F</td>
<td>1,48 2.71(0.107)</td>
<td>1,58 6.78(0.012)</td>
<td>1,28 2.44(0.130)</td>
</tr>
<tr>
<td></td>
<td>NxF</td>
<td>1,48 3.74(0.059)</td>
<td>1,58 21.01 (0.000)</td>
<td>1,28 8.67(0.006)</td>
</tr>
<tr>
<td>Hydrobiid snails</td>
<td>N</td>
<td>1,2 2.07(0.262)</td>
<td>1,2 0.02(0.898)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>1,48 7.90(0.007)</td>
<td>1,58 7.42(0.009)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>NxF</td>
<td>1,48 2.21(0.145)</td>
<td>1,58 0.05(0.832)</td>
<td>-</td>
</tr>
</tbody>
</table>

N=nutrient treatments, F=fish treatments.
Table 5.3. Summary of p-values from Generalized Linear Mixed Model for direct counts of the amphipod *Uhlorchestia spartinophila* vertical wall habitats (creek and ditch) and the shorebird *Calidris pusilla* in two channel types (creek and ditch).

<table>
<thead>
<tr>
<th></th>
<th>Creeks</th>
<th></th>
<th>Ditches</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>F(P-value)</td>
<td>df</td>
<td>F(P-value)</td>
</tr>
<tr>
<td>Amphipods</td>
<td>N</td>
<td>1,116</td>
<td>1, 87</td>
<td>21.58(&lt;0.001)</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>1,116</td>
<td>1, 87</td>
<td>18.39(&lt;0.001)</td>
</tr>
<tr>
<td></td>
<td>NxF</td>
<td>1, 116</td>
<td>1, 87</td>
<td>19.95(&lt;0.001)</td>
</tr>
<tr>
<td>Birds</td>
<td>N</td>
<td>1,12</td>
<td>1,12</td>
<td>21.77(&lt;0.001)</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>1,12</td>
<td>1,12</td>
<td>0.87(0.3691)</td>
</tr>
<tr>
<td></td>
<td>NxF</td>
<td>1,12</td>
<td>1,12</td>
<td>0.87(0.3691)</td>
</tr>
</tbody>
</table>

N=nutrient treatments, F=fish treatments.
CHAPTER 6

SUMMARY AND CONCLUSIONS
My dissertation focuses on the effects of the nutrient addition and the removal of a key predator (the killifish *Fundulus heteroclitus*) on benthic macroinvertebrates in a Massachusetts salt marsh to understand top-down and bottom-up controls on these fauna. In Chapter 2, I characterize the spatial and temporal distribution of infauna prior to any manipulations as a baseline to compare with treatment effects. Results suggest that variability is highest at the smallest spatial scales examined (< 50 m) and that the four creek systems exhibited similar annelid assemblages. Macroinfauna also varied strongly among habitat types along the inundation gradient, e.g., populations and communities differed between creek and marsh platform habitats. Although informative in its own right (e.g., brooding species such as *Manayunkia aestuarina* and species with larval dispersal such as *Streblospio benedicti* are similarly dispersed among the creeks), this study also justifies the use of these creeks are replicates in whole-ecosystem manipulative experiments. In Chapter 3, I examine the effects of treatments on the infaunal community that was dominated by small bodied, semi-sedentary annelids (mass range: $7.0 \times 10^{-3} – 7.0$ mg). Overall, little evidence for top-down or bottom-up control was observed in this study for annelid density, biomass, diversity or community structure, although three instances of interactive effects occurred for annelid densities, and the population biomass of some species was affected. In Chapter 4, I narrow my focus to help elucidate why removal of *Fundulus heteroclitus* did not elicit top-down control on infauna. Using exclusion cages within fish removal areas, I show that removal of all predators elicited top-down control of infauna and suggest that an intermediate omnivorous consumer, the grass shrimp *Palaemonetes pugio*, may alter its behavior to increase its per-capita consumption of infauna as a response to the reduced predation by killifish.
In Chapter 5, I examine the effect of treatments on epifauna, a mobile class of benthic macroinvertebrates (mass range: 0.1 – 4.0 g). In the creek-bank *Spartina alterniflora* habitat, top-down control was exhibited on hydrobiid snails and the amphipod *Uhlorchestia spartinophila*, but this control was modified by bottom-up effects on amphipods. This interaction of combined treatments in *S. alterniflora* result in lower than expected abundances from an additive response (i.e., an antagonism) and I suggest that this was a function of inter-habitat movements by amphipods and further suggest that this movement was parasite-induced. The incidence of parasitized amphipods was higher in nutrient enriched creeks, but the mechanism by which this occurred is unknown. A consequence of this amphipod movement is that it makes them more susceptible to predation by the semipalmated sandpiper, *Calidris pusilla*, which is the final host of the parasite. Thus, top-down control on amphipods is exerted by birds when nutrients are added but by killifish in conditions without fertilization.

While this dissertation focuses on benthic invertebrates, it may be helpful to discuss the effects on other trophic levels to understand how interactions among these levels may have influenced the infaunal responses observed. Below I synthesize the results from the above studies with pertinent results from the overall TIDE study.

**TOP-DOWN CONTROL**

Top-down control occurred for surface-feeding infauna in the *Spartina alterniflora* creek bank and the mudflat when all predators were removed (Chapter 4) and hydrobiid snails and weakly for the amphipod *U. spartinophila* on the creek bank when killifish were reduced (Chapter 5). Further suggestion of top-down control of invertebrates in PIE is conferred by Fleeger et al. (2008) who observed predator limitation of meiobenthic copepod densities in creek wall with fish removal. Similar to my results,
top-down effects on infauna have been observed in the western Atlantic marshes with predator removal (Wiltse et al. 1984, Posey et al. 1995, 1999). While there has been little work on the top-down bottom-up control of epifauna in salt marshes with which to compare this study, there is evidence of top-down control of epifauna grazers in seagrass and algal-dominated communities (Heck et al. 2000, Williams and Heck 2001, Bruno and O’Connor 2005).

Although direct top-down control was detected on invertebrates in some cases, other trophic levels were not as strongly impacted. For instance, killifish removal did not alter the densities of grass shrimp (Deegan et al. 2007), an important predator of infauna, but shrimp feeding behavior may have been altered, contributing to an increase in carnivory (Chapter 4, Galván 2008). Thus, top-down control of infauna may involve complex interactions among a suite of predators/omnivores. Trait-mediated effects have been found in various systems (terrestrial: Beckerman et al. 1997, rocky shores: Trussell et al. 2003) and TIDE studies in aggregate (Deegan et al. 2007, Fleeger et al. 2008, Galván and Johnson unpublished data, Chapter 4) suggest they may be as important as density-mediated effects (Peacor and Werner 2001).

Recently, evidence for top-down primacy of saltmarsh primary producers has emerged (Silliman and Bertness 2002, Finke and Denno 2004); however, little evidence for top-down control of potentially important food resources for infauna was observed in PIE. Although complete predator removal elicited increases in surface-dwelling infauna, top-down effects did not cascade to benthic microalgae (BMA) (Chapter 4). Taken together with Deegan et al.’s (2007) findings of no change in BMA biomass with fish removal, our results suggest weak direct or indirect control of BMA by killifish. However, interaction strengths between BMA and other grazers such as grass shrimp...
and amphipods have not been studied and the synergism seen for BMA could be influenced by these taxa.

Other consumers implicated as strong grazers of primary productivity (primarily macrophytes) in salt marshes such as littorinid snails (Silliman and Bertness 2002) and delphacid planthoppers (Finke and Denno 2004) are absent from PIE. Johnson and Jessen (2008) found no limitation of creek-bank *Spartina alterniflora* biomass in PIE by large acridid grasshoppers (30 – 60-mm body length) even at densities an order of magnitude higher than ambient (34 vs. 3 ind m$^{-2}$). Thus, there appears to be weak consumer control of primary productivity in PIE by both aquatic and terrestrial herbivores. Future studies in PIE are planned to examine killifish, grass shrimp and amphipod grazing effects on BMA to determine if this trend is true for epifauna and nekton.

**BOTTOM-UP CONTROL**

Weak bottom-up control was seen for infauna and epifauna, a result similar to other studies of saltmarsh invertebrates in the western Atlantic (Wiltse et al. 1984, Posey et al. 1999, 2002). Nutrient additions alone did not stimulate BMA biomass, but BMA was stimulated when nutrients were added and fish were removed after two field seasons (Deegan et al. 2007) (see *Interactive effects*). This suggests a cumulative effect of nutrients on food resources for infauna. In the Great Sippewissett Marsh (Massachusetts, U.S.A.), nutrients did not stimulate infauna biomass, densities, or community structure after five years (Wiltse et al. 1984), but did after fifteen years of nutrient additions (Sarda et al. 1996). Together, bottom-up effects on the majority of infauna may not become apparent for years after nutrient additions begin and longer-
term studies may be required to observe effects. Plans are underway to continue fertilization in PIE and to monitor longer-term effects.

Marsh biota are subjected to high environmental stress such as constant and rapid daily fluctuations in salinity, temperature, and inundation; a condition not conducive to the survival of many organisms. As a result, marshes are relatively low diversity ecosystems and perhaps this favors broad ecological niches to cope with constantly changing environmental conditions, which in turn minimizes the interaction strengths among species. The animals examined here exhibit wide-ranging diets with multiple food resources (e.g., omnivory), which could inhibit changes associated with bottom-up control as individual species do not strongly interact with one particular food source. However, two species responded to nutrient addition by shifting their diet to benthic microalgae (from phytoplankton or detritus) and these species increased their individual biomass under fertilization (Galván 2008). This observation suggests that these species may have increased secondary production without increasing abundance and future studies may wish to examine this possibility. Comparison to other studies in detritus based ecosystems (Cross et al. 2006) suggest that species that consume detritus respond to nutrient-induced increases in organic matter in a detritus-based ecosystem. Perhaps the broad diet of saltmarsh macroinfauna mutes bottom-up responses for all but a very limited number of species.

**INTERACTIVE EFFECTS**

Interactive effects of top-down and bottom-up control were observed on the amphipod, *Ulorchestia spartinophila*. Because of the large spatial scale of the manipulations, I was able to observe the inter-habitat movements of *U. spartinophila* from the creek-bank *S. alterniflora* habitat to the creek wall. I suggest that this movement is
parasite-induced. Interactive effects were observed for infauna in the S. patens habitat in both experimental creek pairs after one season of manipulation (Chapter 3, Fleeger et al. 2008), but these effects disappear after one season. Previous examinations of saltmarsh infauna have typically not found interactive effects (Posey et al. 1999, Posey et al. 2002) or that interactive effects were not found equally across the landscape (Fleeger et al. 2008). Interactive effects found for infauna in the above study were limited and infauna may generally be regulated by top-down and bottom-up controls independently.

Let us consider that strong interactive effects occur for mobile epifauna such as U. spartinophila but not for semi-sedentary infauna such as annelids. It is then interesting to note that the difference in the presence or absence of interactive effects between these two classes of saltmarsh invertebrates may be attributed to the relative mobility of these organisms. The interactive effects on amphipods occurred because of their movement from the creek-bank S. alterniflora to the creek wall when treatments were combined. Annelids do not exhibit the same level of non-random movement.

If we consider that interactive effects occurred for both infauna and epifauna, then all interactive effects occurred as a function of modified behavior and the interaction with another organism. For amphipods, their behavior and morphology was modified by the presence of a parasitic trematode. For infauna, because densities increased with nutrient additions in ambient fish levels but not when fish were reduced in S. patens, possible indirect effects of killifish on infauna mediated by altered grass shrimp behavior may explain this result (a trait-mediated indirect interaction). Behavioral effects are often studied in microcosms that can introduce confounding factors (e.g., restriction of movement), which should be minimized in whole-ecosystem
studies. Because of the large scale of the TIDE project, I was able to detect changes in behavior that would not have been detectable in microcosm studies (e.g., amphipod movement seen in Chapter 5).

Saltmarsh food webs are viewed as relatively simple systems, but food web features such as indirect effects, behavior modifications, and trophic omnivory preclude simple predictions about top-down and bottom-up control. Here, I found top-down control of infauna, which corresponds to other short-term studies of saltmarsh infauna (Wiltse et al. 1984, Foreman et al. 1995, Posey et al. 1999, Posey et al. 2002) but only with the complete removal of all predators. Limited responses of infauna to bottom-up influence was observed, similar to other studies (Wiltse et al. 1984, Foreman et al. 1995, Posey et al. 1999), but the duration of the study may not have been sufficient to detect effects (Sarda et al. 1996). Treatments interacted on amphipods and resulted in movements across the landscape, suggesting that large spatial-scale studies are required to more fully understand effects on fauna.

As mentioned above, the broad feeding habits of marsh fauna may make their responses to bottom-up control slow. Broad feeding habits may also complicate the detection of top-down control. In this system, diets among species overlap resulting in redundant predators. For instance, birds and large killifish are redundant predators of amphipods, and grass shrimp and killifish are redundant predators of infauna. This functional redundancy masks potential top-down effects, thereby making it difficult to understand the consequence of treatments. In this study, the use of both fish removal and nutrient additions allowed for a deeper understanding of single factor effects that would not have been evident had each treatment been observed in isolation from the other.
Limited effects of treatments on fauna, particularly nutrient effects may be related to changes in foundation species (*Spartina alterniflora*). Changes in macrophyte composition may lead to changes in the macroinfaunal community (Levin et al. 2006, Neira et al. 2006, Neira et al. 2007). Nutrient availability can alter the competitive interactions among marsh macrophytes and in turn modify macrophyte distributions across the landscape (Bertness and Pennings 2000). However, altered competitive dynamics among macrophytes may not have been detectable within the short time frame of my studies. Given that the landscape of macrophytes (*Spartina* spp.) did not change, a lack of response by macroinfauna may be due to the lack of a large change in foundation species. Even with large changes in macrophyte composition, it may take years to decades for the macroinfauna community to shift (Moseman et al. 2004, Craft and Sacco 2003).

**MANAGEMENT IMPLICATIONS**

Anthropogenic activities often alter the top and the bottom of marine food webs (e.g., nutrient alterations and overfishing) (Virstein et al. 1997, Jackson et al. 2001), thus understanding how the alterations propagate throughout food webs is important. For instance, consumer pressure (top-down) is exacerbated by increased droughts that may result from anthropogenically induced climate change. For instance, reduced abundances of large predators such as sea otters as a result of anthropogenic activities can lead to significant numbers of sea urchins that in turn graze kelp forests to barren substrate via overgrazing (Estes et al. 1998). An example of an anthropogenically induced bottom-up effect is the ‘Dead Zone’ in the Gulf of Mexico (GOM) where fertilizer runoff from the Mississippi River watershed stimulates algal blooms in the GOM which leads to anoxic conditions and extinctions of invertebrates and fishes (Rabalais and
In salt marshes, the marsh perwinkle *Littoraria irrota* can limit the macrophyte *Spartina alterniflora* and the magnitude of the consumer effect can be exacerbated by human-induced climate change and overfishing (Silliman and Bertness 2002, Silliman et al. 2005). Thus, understanding how anthropogenic activities alter top-down and bottom-up controls in food webs will help ecologists and managers predict how humans are impacting ecosystems.

My work suggests that weak TD/BU control over macroinfauna exists, but stronger top-down control with interactive effects occurs for epifauna. Overall, the invertebrates appear to be resilient to treatments in this system and given this resiliency, salt marshes may continue to operate under certain conditions of anthropogenic stress without major changes in ecosystem function. However, stresses may pass a threshold of resiliency with increasing nutrient loads such as those seen near sewage outfalls. It is therefore important for managers to understand these thresholds and how species alterations and nutrient loadings may interact.

**CONCLUSIONS**

Although top-down and bottom-up control has been previously demonstrated as operating independently on saltmarsh invertebrates, I demonstrate that simple predictions of trophic control on benthic invertebrates are contingent and interactive effects may occur. If these trends are widespread then long-term, large spatial-scale studies may be required to more completely understand the relative importance of top-down and bottom-up control on benthic invertebrates in salt marshes.

**LITERATURE CITED**


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VITA

David Samuel Johnson was born to Ethel Jane Tyrney in September 1976, in Folkston, Georgia. He was reared on a mountain in northcentral Arkansas where his love of the outdoors was born. He graduated from the University of Central Arkansas as an Undergraduate Scholar with a Bachelor’s of Science in environmental science in May 2003. He joined the laboratory of Dr. John Fleeger at Louisiana State University to pursue his doctorate in June 2003. He successfully defended his dissertation on June 23, 2008, and was awarded the Degree of Philosophy in biological sciences in August 2008.