



Weak response of saltmarsh infauna to ecosystem-wide nutrient enrichment and fish predator reduction: A four-year study

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ABSTRACT

We examined the effect of whole-ecosystem manipulations of predator removal and nutrient enrichment on saltmarsh macroinfauna in the Plum Island Estuary, Massachusetts. Nitrate and phosphate loading rates were increased 10× above background levels in experimental creeks, and we significantly reduced (by 60%) the abundance of the killifish, *Fundulus heteroclitus*, a key predator in this system. Two creek pairs were manipulated; Creek Pair 1 for three growing seasons and Creek Pair 2 for one. Infaunal responses were examined in four habitats along the inundation gradient: mudflat, creek wall, *Spartina alterniflora*, and *S. patens* habitats. Although benthic microalgae increased synergistically in our treatments, we detected no long-term, population-level numerical response by any taxon. Similarly, no long-term species diversity or community responses were observed. However, nutrient enrichment increased the population biomass of the polychaete *Manayunkia aestuarina* in the creek wall habitat and the oligochaete *Cernosvitoviella immota* in the *S. alterniflora* habitat. No numerical or biomass responses of infauna were detected in predator removal treatments although indirect effects associated with killifish reduction may have contributed to an ephemeral interaction between nutrient addition and predator reduction in *S. patens* habitat. Our data suggest that population and community responses between benthic microalgae and macroinfauna are not tightly coupled even though some species benefit from increased benthic algae biomass by achieving larger body size.

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1. Introduction

As prey for higher trophic 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; Sardá 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), changes in benthic community structure associated with invasive macrophytes (Fell et al., 1998), the effects of contaminated sediment (Carman et al., 1997), long-term nutrient enrichment (Sardá et al., 1995), predator additions (Kneib and Stiven, 1982), and the effect of simultaneous short-term predator removal and nutrient enrichment (Posey et al., 1999, 2002). However, little work has examined the long-term effect of simultaneous alterations of trophic structure and nutrient levels on saltmarsh invertebrates.

Experimental manipulations of nutrients and predators have been used to test the relative importance of top-down (consumer) and bottom-up (resource) controls on benthic food webs (Posey et al.,

1999; Deegan et al., 2007). Although short-term and small spatial-scale (e.g., 1 m²) studies have demonstrated the potential importance of top-down and bottom-up factors on saltmarsh infauna (e.g., Posey et al., 2002), long-term, ecosystem-level experiments may be useful in observing complex food web dynamics such as behavior and feedbacks. For instance, in a four-year, kilometer-scale fertilization of a tundra river, Peterson et al. (1993) found that nutrients increased algal biomass, insect abundances, and fish biomass. In later years, however, insects began exerting strong top-down grazing pressure on epilithic algae (Peterson et al., 1993). Small-scale caging experiments suggest top-down and bottom-up effects operate independently for saltmarsh infauna (i.e., no interaction; 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).

Increased resources (light, nutrients) stimulate benthic microalgae (BMA) biomass and saltmarsh infauna may respond with increased or decreased numbers and/or biomass (Sardá et al., 1996; Posey et al., 2002; Deegan et al., 2007) or remain unchanged (Wiltse et al., 1984). Excessive organic matter in estuaries leads to high decomposition rates, hypoxia and reduced invertebrate densities (Diaz and Rosenberg, 1995; Kemp et al., 2005). Removal of predators typically results in increased infauna numbers (Wiltse et al., 1984; Foreman et al.,

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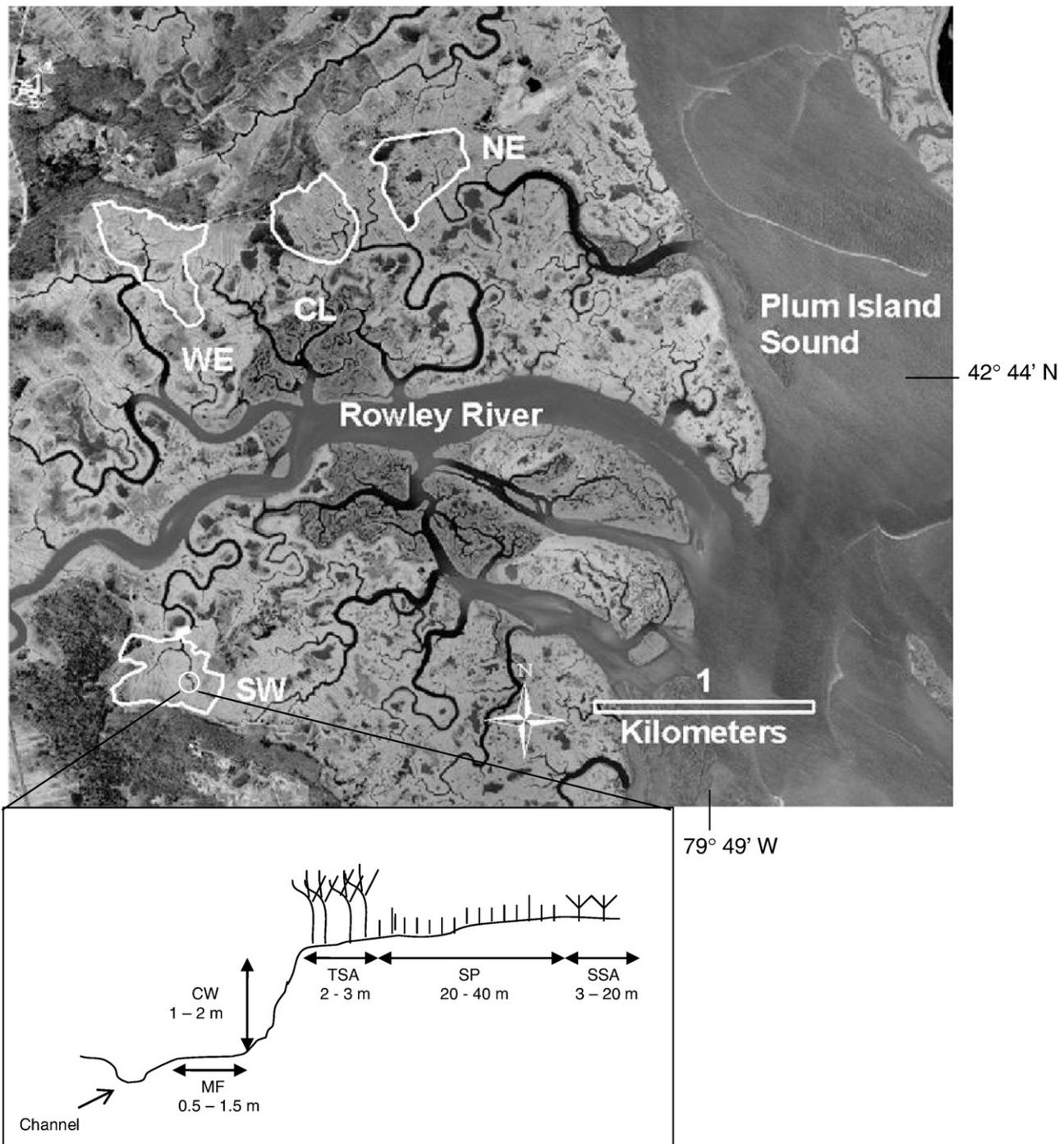


Fig. 1. Upper figure is a satellite photograph (MassGIS Orthophoto 2002) of the Rowley River region salt marshes of the Plum Island Estuary, MA. SW = Sweeney Creek, WE = West Creek, CL = Clubhead Creek, NE = Nelson Creek. Lower figure is a profile of sampled salt marsh habitats (not drawn to scale) with size ranges of each habitat. MF = mudflat; CW = creek wall; TSA = Tall-form *Spartina alterniflora*; SP = *Spartina patens*; SSA = Short-form *S. alterniflora*.

1995; Posey et al., 1999; Posey et al., 2002). Infaunal responses are often taxon- and habitat-specific (e.g., Posey et al., 2006; Fleeger et al., 2008) and the magnitude of consumer and nutrient effects vary along environmental gradients like salinity (Deegan et al., 2007; Fleeger et al., 2008).

The purpose of this paper was to examine top-down and bottom-up effects on saltmarsh infauna at relatively large spatial and long-term 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, we examined the response of the macrofaunal community during three years of manipulation. We examined population-level (density and

biomass of selected taxa) and community-level (similarity and diversity indices) responses of macrofauna. Initial responses of macrofauna to one summer season of treatments are described in Fleeger et al. (2008). Here, we examine the effect of two additional field seasons of manipulation on macrofauna and add a second creek pair to examine replication of short-term responses. We 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).

2. Methods

2.1. Study site

Our 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 saltmarsh creeks—Sweeney, West, Clubhead, and Nelson—in the Plum Island Estuary (PIE), Massachusetts, USA (Fig. 1). These creeks are relatively pristine (background nutrients: $<5 \mu\text{M NO}_3^-$; $\sim 1 \mu\text{M PO}_4^{3-}$). 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). We examined macroinfauna in four habitats along an inundation gradient: two creek habitats and two marsh platform habitats (Fig. 1). The mudflat habitat is a creek habitat of poorly consolidated sediments without macrophytes but with abundant migrating diatoms, chlorophytes and cyanobacteria (Galván, 2008). The creek-wall habitat is a steep, almost vertical wall about 1.5 m in height, with cohesive sediments and an approximately 30-cm wide band of macroalgae and filamentous algae. The creek-bank habitat is dominated by a zone of tall-form *Spartina alterniflora* (>130 cm height in late summer). The marsh platform habitat consists of an expansive area dominated by a dense canopy of *S. patens*. PIE has 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* habitat is infrequently flooded, inundated only during spring tides.

2.2. 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 $70 \mu\text{M NO}_3^-$ and $4 \mu\text{M PO}_4^{3-}$ ($15\times$ 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 $30 \text{ g N m}^{-2} \text{ y}^{-1}$ in 2004 ($\sim 10\times$ 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 (West and Nelson).

A branch of each creek was selected for large-scale removal of the 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 is expected to produce 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 total length (TL)] was greater than small killifish (<40 mm TL); although a 40% reduction of small killifish was observed. 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.

2.3. 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.

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 corer. Cores were placed on ice in the field, and then transported to the laboratory where they were fixed in a 10% formalin and Rose Bengal solution. 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 focus of this study, although the tanaid crustacean *Leptocheilia 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 (\log_e), and Pielou's evenness—of annelids was calculated for each sample using PRIMER (v. 6.1.6).

2.4. Population analysis

We 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). 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). We used a BACI-type ANOVA to analyze changes in annelid densities and diversities for each creek pair separately. 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;

$n/\text{branch} = 3$ in 2003 and $n/\text{branch} = 6$ in 2004–2006.

To detect interactions between fertilization and predator removal, we performed analyses directly on abundance values instead of the differences between the control and treatment values. Data were analyzed as generalized linear mixed models (GLMMs) using Proc GLIMMIX (SAS v. 9.1.3). All data were \log_e -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.

2.5. Biomass analysis

Two levels of infauna biomass were analyzed 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

Table 1
Mean density (individuals m^{-2}) and relative abundance (% in parentheses) of dominant macroinfaunal annelids from sediments in the Plum Island Estuary, MA, USA.

Species	Mudflat	Creek wall	Tall <i>Spartina alterniflora</i>	<i>S. patens</i>
<i>Fabricia sabella</i> (P)	122 (0.81)	7321 (17.19)	602 (1.87)	217 (3.45)
<i>Manayunkia aestuarina</i> (P)	1286 (8.55)	13,755 (32.30)	10,003 (31.01)	1517 (24.19)
<i>Streblospio benedicti</i> (P)	7545 (50.51)	533 (1.25)	537 (1.66)	11 (0.18)
<i>Pygospio elegans</i> (P)	115 (0.77)	285 (0.67)	42 (0.13)	1 (0.02)
<i>Polydora cornuta</i> (P)	297 (1.97)	745 (1.75)	26 (0.08)	0 (0)
<i>Marenzelleria viridis</i> (P)	7 (0.04)	0 (0)	0 (0)	0 (0)
<i>Neries diversicolor</i> (P)	705 (4.69)	95 (0.22)	63 (0.20)	11 (0.18)
<i>Eteone heteropoda</i> (P)	130 (0.86)	7 (0.02)	46 (0.14)	0 (0)
<i>Hobsonia florida</i> (P)	46 (0.31)	6 (0.01)	11 (0.04)	0 (0)
<i>Capitella</i> sp. (P)	34 (0.23)	113 (0.26)	249 (0.77)	11 (0.18)
<i>Paranais littoralis</i> (O)	1028 (6.83)	5934 (13.93)	2781 (8.62)	402 (6.40)
<i>Psammoryctides</i> sp. (O)	13 (0.09)	3 (0.01)	3 (0.01)	1 (0.02)
<i>Tubificoides benedeni</i> (O)	44 (0.30)	8 (0.02)	5 (0.01)	0 (0)
<i>Tubificoides brownie</i> (O)	909 (6.04)	19 (0.04)	11 (0.04)	8 (0.12)
<i>Tubificoides wasselli</i> (O)	1081 (7.18)	80 (0.19)	89 (0.28)	88 (1.40)
<i>Monopylephorus</i> sp. (O)	803 (5.34)	27 (0.06)	21 (0.06)	36 (0.57)
<i>Cernosvitoviella immota</i> (O)	667 (4.43)	12,099 (28.41)	16,829 (52.17)	3287 (52.40)
Other enchytraeids (O)	29 (0.19)	217 (0.51)	423 (1.31)	370 (5.89)
<i>Hargeria rapax</i> (Cr)	25 (0.16)	459 (1.08)	3 (0.01)	0 (0.00)
<i>Tabanus</i> larvae sp. (In)	160 (1.06)	877 (2.06)	511 (1.59)	314 (5.00)

Cr = crustacean, In = insect, O = oligochaete, P = polychaete.
Density values are means from a composite of samples from all sampling sites and sampling dates.

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 represented by only one individual and were not weighed. Specimens of the spionid polychaetes *Streblospio 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 Imaging Software v. 4.5) under 50 \times magnification with a Zeiss StereoLumar stereomicroscope. Regressions for size to mass

Table 2
Population biomass (mg dry weight m^{-2}) and per-capita biomass (μg dry weight $ind.^{-1}$) of dominant macroinfaunal annelids from sediments in the Plum Island Estuary, MA, USA.

Species	Mudflat		Creek wall		Tall-form <i>Spartina alterniflora</i>		<i>S. patens</i>	
	Population biomass	Per capita biomass	Population biomass	Per capita biomass	Population biomass	Per capita biomass	Population biomass	Per capita biomass
	($mg\ m^{-2}$)	($\mu g\ ind^{-1}$)	($mg\ m^{-2}$)	($\mu g\ ind^{-1}$)	($mg\ m^{-2}$)	($\mu g\ ind^{-1}$)	($mg\ m^{-2}$)	($\mu g\ ind^{-1}$)
<i>Fabricia sabella</i> (P)	2.36(0.15)	8.00	77.87(1.65)	53.20	39.82(3.48)	117.56	13.99(4.74)	22.61
<i>Manayunkia aestuarina</i> (P)	22.91(1.47)	49.89	239.48(5.06)	44.69	139.58(12.20)	41.09	63.47(21.53)	33.98
<i>Streblospio benedicti</i> (P)	228.47(14.67)	62.72	343.89(7.27)	199.64	66.53(5.82)	166.07	–	–
<i>Pygospio elegans</i> (P)	–	–	381.73(8.06)	108.65	24.34(2.13)	27.50	–	–
<i>Polydora cornuta</i> (P)	230.08(1.48)	78.00	531.58(11.23)	139.90	46.21(4.04)	156.67	–	–
<i>Marenzelleria viridis</i> (P)	–	–	–	–	–	–	–	–
<i>Neries diversicolor</i> (P)	262.41(16.85)	631.76	2374.60(50.17)	7043.84	202.21(17.67)	685.50	–	–
<i>Eteone heteropoda</i> (P)	62.75(4.03)	111.13	4.72(0.10)	16.00	–	–	–	–
<i>Hobsonia florida</i> (P)	97.58(6.26)	330.79	42.48(0.90)	144.00	30.94(2.70)	104.88	–	–
<i>Capitella</i> sp. (P)	270.90(17.29)	918.35	85.66(1.81)	280.39	130.04(11.37)	348.20	16.78(5.69)	56.88
<i>Paranais littoralis</i> (O)	9.50(0.61)	6.70	47.29(1.00)	115.32	4.73(0.41)	10.56	44.87(15.22)	145.49
<i>Psammoryctides</i> sp. (O)	–	–	–	–	–	–	–	–
<i>Tubificoides benedeni</i> (O)	29.35(1.88)	54.75	3.24(0.07)	10.98	–	–	–	–
<i>Tubificoides brownie</i> (O)	35.56(2.28)	76.69	33.00(0.70)	82.74	4.55(0.40)	13.78	–	–
<i>Tubificoides wasselli</i> (O)	61.57(3.95)	174.13	15.44(0.33)	39.00	78.67(6.88)	266.69	–	–
<i>Monopylephorus</i> sp. (O)	123.83(7.95)	241.24	305.60(6.46)	518.00	89.23(7.80)	232.50	–	–
<i>Cernosvitoviella immota</i> (O)	44.84(2.88)	152.00	68.87(1.45)	38.57	158.54(13.86)	46.42	63.15(21.42)	26.44
Other enchytraeids (O)	–	–	9.69(0.20)	20.84	9.89(0.86)	20.33	10.76(3.65)	21.71
<i>Leptochelia savignyi</i> (Cr)	199.40(12.80)	676.00	94.77(2.00)	130.57	50.22(4.39)	96.63	–	–
<i>Tabanus</i> larvae sp. (In)	83.25(5.34)	237.71	73.45(1.55)	96.82	68.58(5.99)	129.59	81.84(27.76)	197.44

Species' proportion of overall biomass for a particular habitat in parentheses expressed as a percentage (%). Biomass values are means of a composite of samples from all sampling sites from September 2005.

Cr = crustacean, In = insect, O = oligochaete, P = polychaete.

conversions for these species are found in Sardá 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 \log_e 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.

2.6. 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 (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).

3. Results

3.1. 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 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 wall and lowest densities on the marsh platform (i.e., *Spartina patens*) (Table 1). In September 2005, average per-capita biomass ranged from 7–7000 μg DW individual $^{-1}$ across all species and habitats (Table 2). Population biomass ranged from 3×10^{-4} –2.7 g DW m^{-2} across all species and habitats (Table 2). Community biomass ranged from 6.8×10^{-2} –77.7 g DW m^{-2} and was highest in the creek wall and lowest in the *S. patens* sediments.

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).

3.2. 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 3 for statistical summary for abundant taxa). Significant short-term (first year of manipulation) interactive effects (period*nutrient*fish, $p \leq 0.046$) were detected under *S. patens* canopy for *Manayunkia aestuarina*, *Cernosvitoviella immota*, and total annelids in Creek Pair 2. Similar results were seen for *C. immota* and total annelids in Creek Pair 1 (Fleeger et al., 2008). *M. aestuarina* and *C. immota* numerically constitute 80% of the annelids in *S. patens*. Annelid densities in both creek pairs were very low before treatments were initiated (2003) but increased sharply after treatments began. In Creek Pair 2, nutrient enrichment appeared to contribute to numerical increase. However in both creek pairs, increases in annelid abundance were much less when fish abundance was reduced in nutrient enrichment creeks. This similarity in response in two creek pairs suggests the interactive effect was repeatable even though it was not sustained in subsequent years of manipulation. Additionally, after one season of treatment application in Creek Pair 2, nutrient additions significantly increased *S. 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). However, *S. benedicti* abundance did not vary similarly in the short-term in Creek Pair 1 (Fleeger et al., 2008).

3.3. Biomass responses

Treatment effects on population biomass were examined only on the last collection date in 2005. Effects were detected in three habitats and on three species. Nutrients significantly lowered *N. diversicolor* population biomass (a 99% reduction, $p = 0.008$, Table 4A, Fig. 4) in mudflat sediments. However, coring does not adequately sample larger *N. diversicolor*, and estimates may therefore have been unreliable for the population as a whole (e.g., higher growth may have occurred in nutrient additions and more individuals grew out of the size range we could sample). In the creek wall, *M. aestuarina* population biomass increased by 22-fold when nutrients were added without fish removal (nutrient*fish $p < 0.001$, Table 4A, Fig. 4). *M. aestuarina* individual biomass in this habitat also increased with nutrients independent of fish level (data not shown). *C. immota* population biomass was significantly higher in nutrient treatments in *S. alterniflora* sediments ($p = 0.002$, Table 4A, 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 4B).

3.4. 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) were not tightly grouped (Fig. 5). However, in all

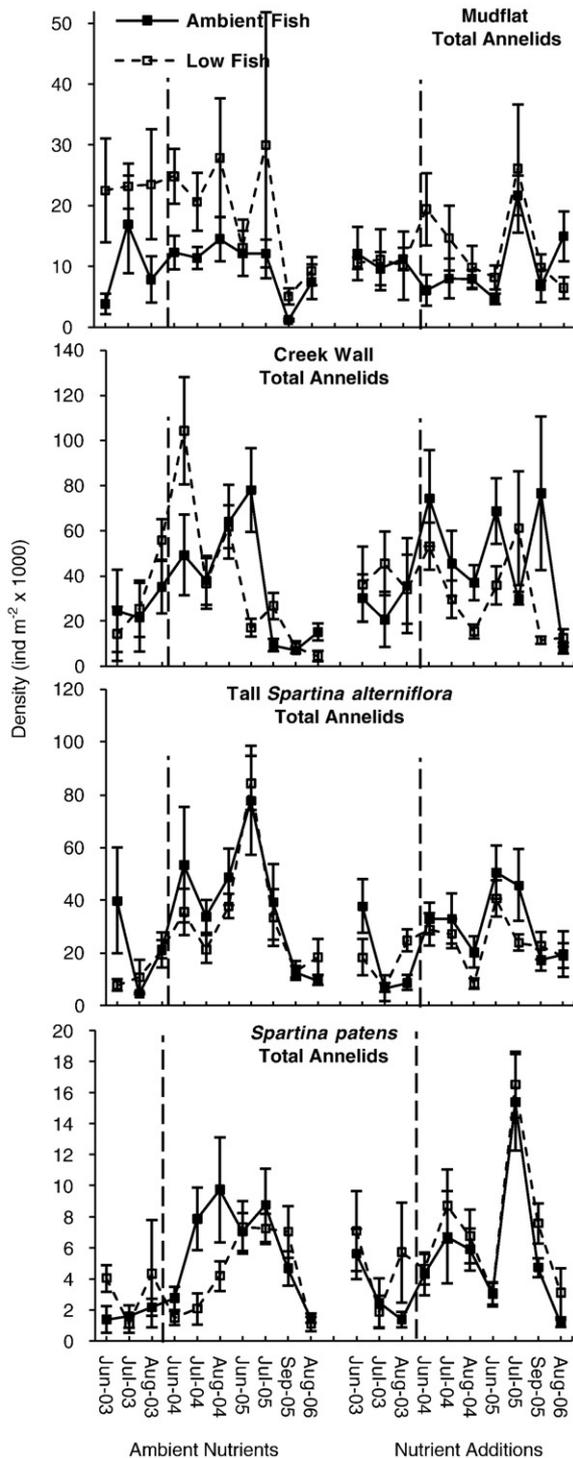


Fig. 2. Mean (S.E.) density of total annelids in experimental Creek Pair 1 (Sweeney and West Creeks) for all four habitats. No significant treatment effects were detected for any taxa in any habitat in Creek Pair 1. The dashed line represents start of treatments.

Table 3
Summary table of *p*-values for abundant macroinfauna species from generalized linear mixed models.

Habitat	Taxon	Creek Pair 1			Creek Pair 2		
		Nutrient	Fish	Nutrient * Fish	Nutrient	Fish	Nutrient * Fish
MF	<i>Streblospio benedicti</i>	0.095	0.162	0.607	0.013	0.690	0.469
	<i>Paranais litoralis</i>	0.884	0.598	0.373	0.982	0.989	0.986
	Total tubificids	0.736	0.666	0.292	0.731	0.055	0.261
	Total annelids	0.691	0.938	0.421	0.105	0.089	0.325
CW	<i>Manayunkia aestuarina</i>	0.926	0.626	0.770	0.051	0.161	0.460
	<i>Fabricia sabella</i>	0.795	0.110	0.361	0.074	0.694	0.665
	<i>Paranais litoralis</i>	0.666	0.660	0.475	0.166	0.304	0.664
	<i>C. immota</i>	0.651	0.769	0.075	0.581	0.586	0.401
	Total annelids	0.843	0.256	0.474	0.030	0.136	0.761
TSA	<i>Manayunkia aestuarina</i>	0.565	0.557	0.408	0.701	0.292	0.986
	<i>Paranais litoralis</i>	0.522	0.842	0.841	0.268	0.944	0.964
	<i>Cernosvitoviella immota</i>	0.277	0.444	0.251	0.640	0.791	0.285
	Total annelids	0.441	0.804	0.458	0.718	0.467	0.650
SP	<i>Manayunkia aestuarina</i>	0.421	0.340	0.952	0.650	0.409	0.046
	<i>Paranais litoralis</i>	0.936	0.070	0.215	0.312	0.545	0.644
	<i>Cernosvitoviella immota</i>	0.998	0.590	0.407	0.895	0.682	0.011
	Other enchytraeids	0.306	0.604	0.470	0.999	0.983	0.999
	Total annelids	0.999	0.638	0.484	0.181	0.333	0.028

In this BACI-type design, only Period * Treatment interactions are of interest and are listed below (e.g., 'Nutrient' = 'Period * Nutrient'). Creek pair 1 represents Sweeney and West Creeks for 2003–2006. Creek pair 2 represents Clubhead and Neslon Creeks for 2003 and 2005. MF = mudflat, CW = creek wall, TSA = tall-form *Spartina alterniflora*, SP = *S. patens*. Significant ($p \leq 0.05$) and marginally significant values ($p = 0.051$) are in bold.

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 (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).

3.5. Response of other trophic levels

Here we summarize the responses of other trophic levels from the TIDE project that may be relevant to understanding the lack of effects on infauna. 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 (Johnson, 2008), possibly due to increased carnivory by shrimp (Galván, 2008). Prior to treatments, within-habitat benthic microalgae (BMA) biomass was similar among creeks, although inter-habitat differences occurred. Significant effects of treatments on BMA biomass were detected only in the second year of treatments in Sweeney and West Creeks in which biomass increased synergistically (Deegan et al., 2007; Fig. 7). In isotopic studies, Galván (2008) found that after one year of fertilization, the annelids *M. aestuarina* and *C. immota* shifted their diet to consume more BMA. Treatments did not alter the abundance or distribution of *S. alterniflora* or *S. patens* over the course of manipulations (Miller, 2006).

4. Discussion

Nutrient additions and fish reductions had few detectable effects on macroinfaunal abundance, species diversity or community structure after three consecutive growing seasons of manipulation. Significant effects of nutrients on population biomass occurred in three species for the single time-point examined, and these effects

were modified by fish reduction for one species. No effects on community biomass were observed. Short-term effects interactive effects were found in replicate creek pairs in *S. patens* habitat that suggest fish reduction initiated an indirect effect mediated by changes in shrimp behavior, which in turn influenced annelid abundance. Below we discuss possible explanations for these relatively mild and limited responses.

4.1. Nutrient (bottom-up) effects

No effects of nutrient enrichment on infauna density, diversity and community structure were observed after three years of summer nutrient enrichment in Creek Pair 1, and no repeatable effects were observed over the short-term in our replicate creek pairs. However, nutrients stimulated increases in benthic algal biomass (up to 4-fold; L.A. Deegan unpublished data) over the same time period. Given that infauna may selectively consume a variety of food sources such as phytoplankton, BMA, and detritus (Galván et al., 2008), our data suggest that annelid abundance was not tightly linked to BMA biomass.

On the one collection examined (September 2005), fertilization significantly increased the population biomass of the small polychaete (~5 mm), *M. aestuarina* (a surface feeder with a diet composed mostly of phytoplankton, Galván et al., 2008), in the creek wall. Increased population biomass for this polychaete may be a function of per-capita biomass, which also increased with nutrient additions. The population biomass of the enchytraeid oligochaete *C. immota* also increased with nutrient additions in the *S. alterniflora* habitat. This typically subsurface-feeding oligochaete consumes mostly *Spartina* detritus in sediments under the vegetation canopy in PIE (Galván, 2008). Galván (2008) found that after one year of fertilization *M. aestuarina* and *C. immota* both altered their diet to consume more benthic microalgae. Thus, both annelids may be responding to increased food supply in these habitats (BMA increased with fertilization) with increased growth and adult body size but without a numerical response (Posey 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. For instance, nutrients had no appreciable effects

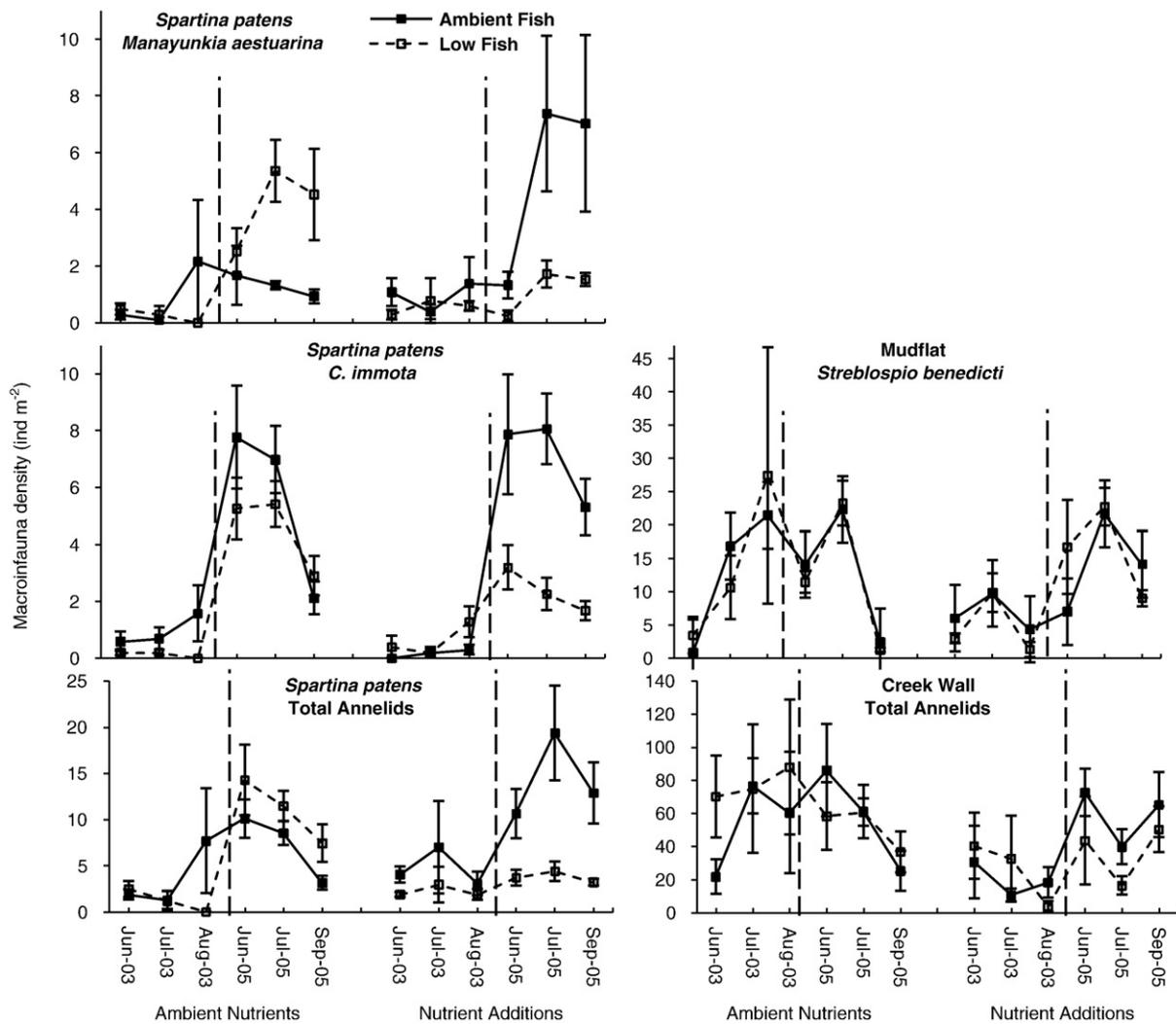


Fig. 3. Mean (S.E.) density of taxa in which treatment effects were detected in experimental Creek Pair 2 (Clubhead and Nelson Creeks). Note the different habitats for each taxa. The dashed line represents start of treatments.

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 nutrient enrichment. Macroinfauna density and population biomass did increase after 15 years of high nutrient loading in a southern New England salt marsh (Sardá et al., 1996). Additionally, the community shifted from a *S. benedicti* (a surface-feeding polychaete) dominated community to one dominated by subsurface-deposit-feeding oligochaetes (e.g., *P. littoralis*, Sardá et al., 1996). Thus, longer time frames (>5 years) and/or higher nutrient loadings [(TIDE nutrient-loading rates were $\sim 10\times$ less than previous dry-fertilizer plot-level experiments (e.g., Sardá et al., 1996)] may be needed to elicit impacts of nutrients on saltmarsh macroinfauna.

It is uncertain why macroinfauna are slow to respond to increases in BMA with numerical responses. Nutrients can stimulate benthic microalgal food resources, particularly Chlorophyta and certain benthic diatoms (Posey et al., 2002), and benthic infauna (e.g., *S. benedicti*) may respond with increased reproductive output (Levin, 1986) and/or increased growth (Posey et al., 2006). Both *M. aestuarina*, an intratubular brooder, and the oligochaete *C. immota*, an asexually reproducing species, have low reproductive output compared to species that broadcast larvae. It is unclear why these species respond with growth (growth should increase fecundity and therefore increase population size) but not abundance. Perhaps these species do not have the same capacity to increase output as broadcast spawners or survivorship of juveniles is reduced. Alternatively,

although studies in this system (e.g., Galván et al., 2008) highlight the importance of algal food resources to infauna, detritus may still be important and likely not affected by our nutrient additions and in turn not impacting infauna.

4.2. 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), we 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 a numerical response. Alternatively, this disparity may be due to the presence of other epibenthic predators that were not excluded by the fish reduction treatment such as grass shrimp (*Palaeomonetes pugio*) or small killifish. In the Great Sippewissett Marsh (Massachusetts, USA), Sardá et al. (1998) observed decreased numbers 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. Beseres and Feller (2007) found that white shrimp predation contributed to seasonal declines in macroinfaunal abundance. Densities of surface-feeding annelids such as *M. aestuarina* and *S. benedicti* significantly increased when all predators were removed in caging experiment conducted by Johnson (2008),

Table 4

Mean (S.E.) (A) population biomass (mg dry weight m⁻²) and (B) mean (S.E.) community biomass (mg dry weight m⁻²) of selected abundant taxa.

Taxa	Ambient nutrients/ambient fish	Ambient nutrients/low fish	Nutrient additions/ambient fish	Nutrient additions/low fish	Nutrient main effect	Fish main effect	Nut*Fish effect
Mudflat							
<i>Streblospio benedicti</i>	0.246(0.096)	0.106(0.047)	0.333(0.109)	0.193(0.029)	0.093	0.284	0.318
<i>Manayunkia aestuarina</i>	0.057(0.054)	0.030(0.016)	0.015 (0.006)	0.014(0.006)	0.579	0.471	0.936
<i>Nereis diversicolor</i>	0.169(0.031)	0.529(0.281)	0.016(0.004)	0.015(0.006)	0.008	0.939	0.767
Creek wall							
<i>Cernosvitoviella immota</i>	0.043(0.015)	0.079(0.016)	0.080(0.025)	0.069(0.019)	0.112	0.182	0.083
<i>Fabricia sabella</i>	0.055(0.023)	0.077(0.025)	0.080(0.024)	0.106(0.039)	0.248	0.943	0.493
<i>Manayunkia aestuarina</i>	0.029(0.014)	0.141(0.048)	0.712(0.398)	0.067(0.019)	0.003	0.779	<0.001
<i>Tabanus sp. Larvae</i>	0.133(0.092)	0.064(0.040)	0.037(0.017)	0.053(0.018)	0.862	0.876	0.454
<i>Spartina alterniflora</i> habitat							
<i>Cernosvitoviella immota</i>	0.140(0.036)	0.091(0.032)	0.201(0.023)	0.200 (0.046)	0.002	0.254	0.603
<i>Manayunkia aestuarina</i>	0.125(0.051)	0.068(0.027)	0.322(0.240)	0.041(0.009)	0.498	0.327	0.273
<i>Capitella sp.</i>	0.084(0.060)	0.203(0.122)	0.064(0.021)	0.166(0.071)	0.235	0.198	0.718
<i>Tabanus sp. Larvae</i>	0.077(0.022)	0.105(0.030)	0.078(0.043)	0.031(0.019)	0.360	0.620	0.053
<i>S. patens</i> habitat							
<i>Cernosvitoviella immota</i>	0.055(0.015)	0.064(0.019)	0.089(0.037)	0.044(0.017)	0.676	0.383	0.503
<i>Manayunkia aestuarina</i>	0.037(0.017)	0.092(0.034)	0.054(0.018)	0.069(0.020)	0.765	0.173	0.337
B)							
Mudflat	134.57(28.45)	133.00(42.31)	105.33(30.91)	75.66(18.09)	0.772	0.981	0.709
Creek Wall	146.72(70.91)	132.21(26.62)	365.03(131.59)	223.91(79.61)	0.147	0.457	0.886
<i>Spartina alterniflora</i>	97.00(19.39)	97.74(22.19)	160.20(65.12)	160.20(65.12)	0.949	0.251	0.680
<i>S. patens</i>	54.26(13.96)	64.14(15.78)	64.82(17.04)	49.18(11.35)	0.734	0.631	0.453

P-values 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.

suggesting large killifish removal alone may not be enough to elicit a numerical response from annelids. 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 caging experiments in PIE suggest that with the removal of large killifish, grass shrimp, but not small killifish, consume more infauna thereby compensating for reductions in consumption by killifish (Galván, 2008; Johnson,

2008). Thus, indirect effects may be important in structuring these infauna communities (Kneib, 1988).

4.3. Interactive effects

Short-term interactive effects were noted only in marsh platform habitats. Top-down and bottom-up treatments interacted on *M. aestuarina*, *C. immota*, and total annelid densities in the *S. patens*

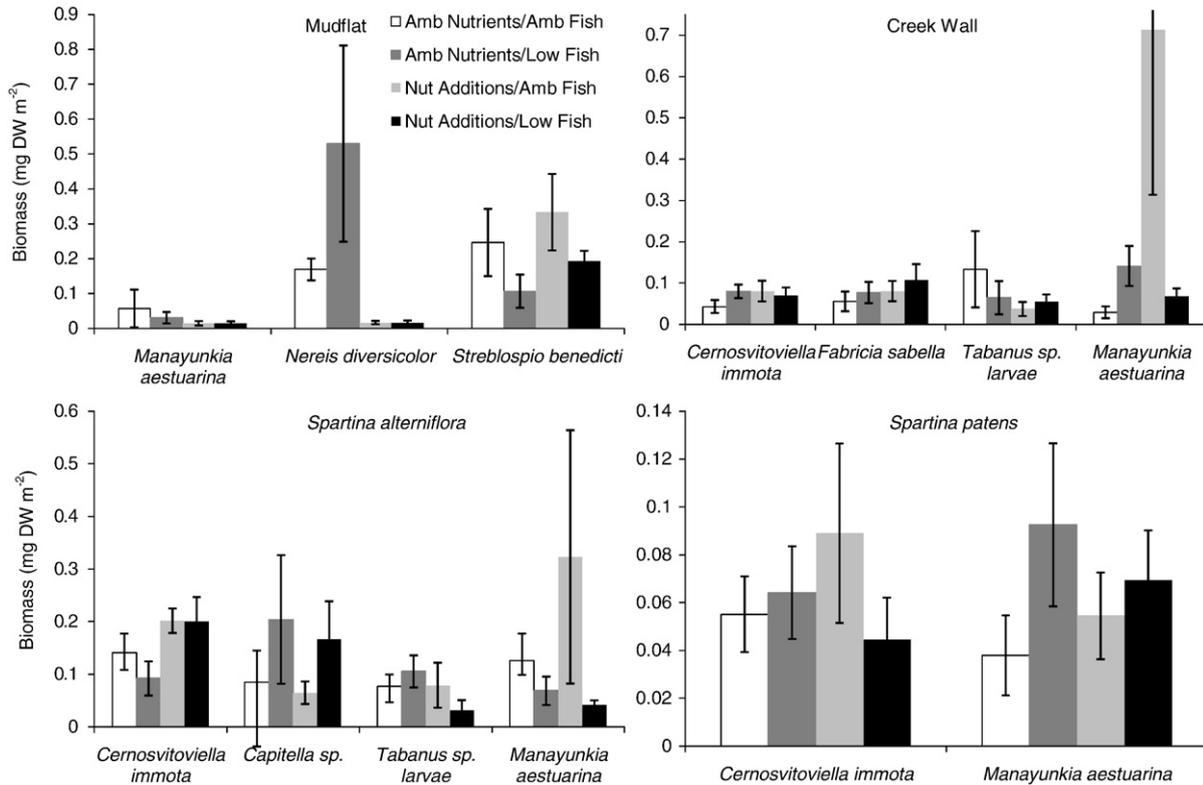


Fig. 4. Mean (S.E.) population biomass (mg DW m⁻²) of selected macroinfauna for September 2005 in the Plum Island Estuary, Massachusetts.

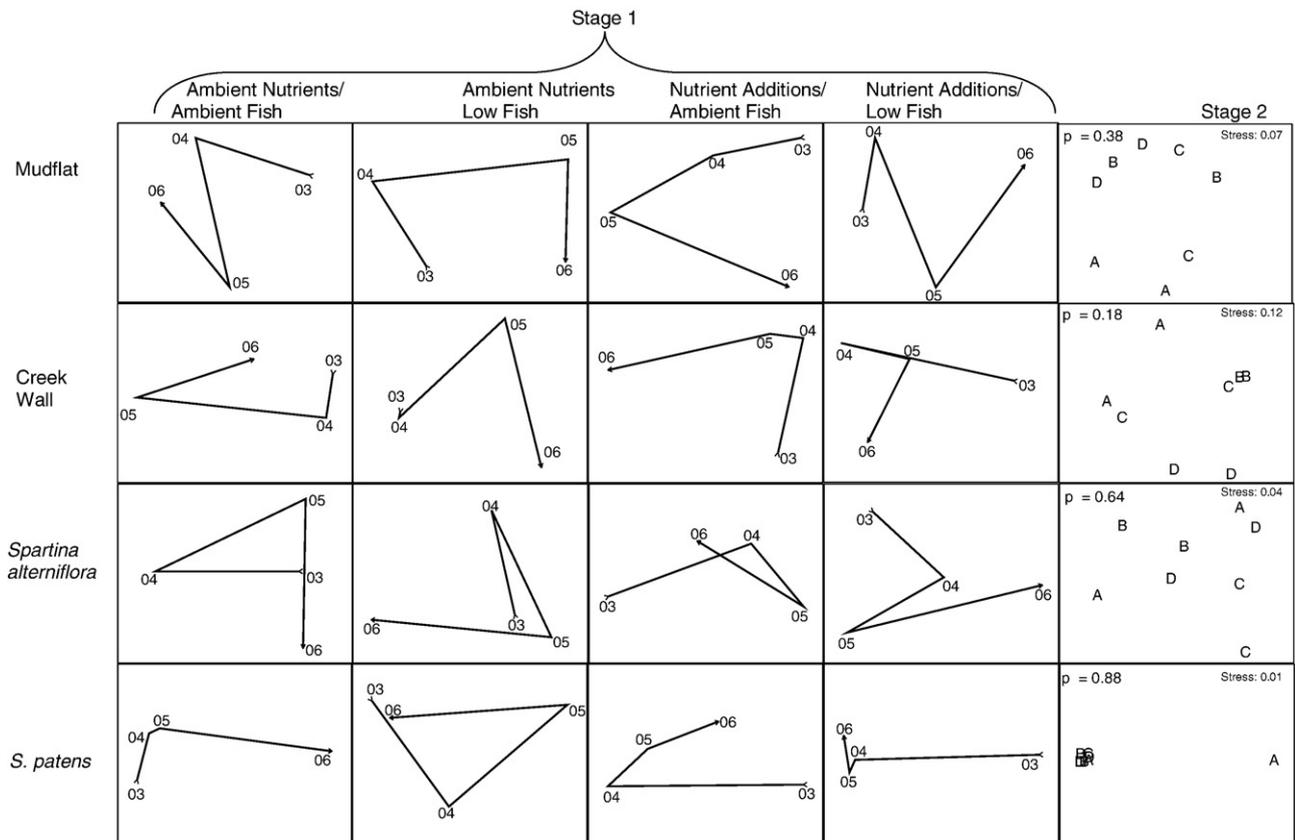


Fig. 5. Second-stage community analysis of Plum Island Estuary infauna by habitat type. Stage 1: First-stage similarities among years (2003–2006) for end of the season samples. Each first-stage MDS are based on Bray–Curtis similarities calculated from $\log(x+1)$ transformed abundances and are averages 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. ANOSIM p -values are listed in the top left corner. A = ambient fish/ambient nutrients, B = fish removal/ambient nutrients, C = ambient fish/nutrient additions, D = fish removal/nutrient additions.

habitat in Creek Pair 2 (this study) and for *C. immota* densities in Creek Pair 1 in the first year of treatments (Fleeger et al., 2008). 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. aestivalis* population biomass in the ambient fish levels but not in the fish reduction areas in the *S. alterniflora* habitats. This observation may result from grass shrimp altering their foraging behavior with the reduction of large killifish (Galván, 2008; Johnson, 2008).

5. Conclusions

The results of our large-scale study confirms the historical findings of small-scale studies that infauna respond weakly, if at all, to top-down and bottom-up field manipulations and responses are limited to a few taxa (Kneib and Stiven, 1982; Wiltse et al., 1984; Posey et al., 2002). Our results also corroborate the notion that predators have little impact on subsurface-dwelling infauna such as oligochaetes (Posey et al., 2002). We 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 three species in two different habitats responded. Increased growth occurred in two species—*M. aestivalis* 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 numerical increases. Indirect effects detected after one season in *S. patens* did not persist perhaps because nutrient-induced alterations of basal

resources (e.g., *Spartina* spp. or benthic algae), which exhibit a time lag after nutrient additions begin. Predation effects are probably not cumulative and interactions with nutrient effects may vary over time.

It is difficult to quantify the relative strength of top-down and bottom-up controls because faunal responses to both treatments were few and mild. Some infauna responded to nutrients but in ways that are difficult to interpret (i.e., increased body size but no density or community change). Top-down control may be obscured due to compensatory predation by other epibenthic predators. 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., 1999, 2002); however, interactions could vary temporally as stronger nutrient effects manifest over time (Sardá et al., 1996). Work continues 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.

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