High-marsh invertebrates are susceptible to eutrophication

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ABSTRACT: In salt marshes, high-marsh habitats are infrequently flooded (typically only during spring tides). Organisms in these habitats, however, may still be susceptible to the effects of increased nutrients delivered by tidal water (i.e. eutrophication). In a Massachusetts salt marsh, I examined the responses of the epibenthic invertebrates in the *Spartina patens*-dominated high marsh to long-term (7 yr) and landscape-level (4–5 ha) nutrient enrichment. In this ecosystem-level experiment, nutrients (N and P; ~15× reference conditions) were added to the flooding waters of tidal creeks—which flooded the high marsh during spring tides—to mimic cultural eutrophication. Three detritivores: *Melampus bidentatus* (gastropod), *Philoscia vittata* (isopod), and *Orchestia grillus* (amphipod) numerically dominated the benthic invertebrate community (97 % by abundance). These species had higher densities (47 to 199 % increase) in enriched versus reference creeks. *Melampus* size structure shifted to larger individuals with enrichment. End-of-season aboveground biomass and detritus stocks of *S. patens* did not differ between treatments; thus, increased litter quality and/or alternative food-source increases (e.g. microbes) led to increased detritivore density/biomass. Predator densities (spiders and *Tabanus* larvae) increased 125 to 160 % with enrichment, likely due to increased prey densities (including *Orchestia* and *Philoscia*). Analysis of similarities (ANOSIM) revealed that communities were dissimilar between treatments; differences were driven primarily by changes in detritivore abundance. These results suggest that despite being infrequently flooded and thus infrequently exposed to elevated nutrients, high-marsh invertebrates are susceptible to eutrophication. Hence, the high marsh should be integrated into our understanding of how eutrophication impacts saltmarsh functioning.

KEY WORDS: Epifauna · Salt marsh · *Spartina alterniflora* · Plum Island Estuary

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INTRODUCTION

Eutrophication (defined here as increased inorganic nutrient inputs) is a pervasive problem in coastal ecosystems worldwide. Because salt marshes are downstream of often large watersheds that funnel human-derived nutrients from terrestrial inputs, the function and structure of marshes may be particularly susceptible to the effects of nutrient enrichment (Deegan et al. 2007). Plants are dominant organisms in salt marshes and nutrients, par-

ticularly nitrogen, generally stimulate plant production (e.g. Valiela et al. 1975, Mendelssohn 1979, Deegan et al. 2007, Darby & Turner 2008, Wimp et al. 2010). The high levels of primary production in salt marshes fuel food webs; thus, nutrient effects at the primary producer level may propagate throughout the food web (Wimp et al. 2010). In fact, consumers often mirror plant responses to nutrient enrichment with increased densities or biomass (Deegan et al. 2007, Johnson & Fleeger 2009, Wimp et al. 2010).

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A typical New England salt marsh is bounded by an intertidal swath of streamside, tall-form *Spartina alterniflora* (saltmarsh cordgrass), with the high marsh often being dominated by *Spartina patens* (saltmeadow hay) (Miller & Egler 1950, Niering & Warren 1980). There is relatively little work focused on *S. patens* habitats. Recent work has focused on anthropogenic impacts on these habitats including invasive species introductions (Warren et al. 2001), haying (Buchsbaum et al. 2009), and climate change (Charles & Dukes 2009, Gedan & Bertness 2010). The effect of nutrient enrichment on the high marsh remains relatively unknown (although see Fleeger et al. 2008; Johnson & Fleeger 2009).

While low-marsh habitats are inundated during daily (or twice-daily) high tides, high-marsh habitats are often only inundated during spring tides or storm surges. These infrequently flooded habitats may thus not appear to be strongly influenced by nutrient enrichment delivered via tidal water relative to the daily-flooded low-marsh habitats. The few studies that have examined the high marsh for nutrient effects on animal communities have focused on sediment-dwelling fauna (Fleeger et al. 2008, Johnson & Fleeger 2009) or insect communities, which typically occupy the plant canopy (Stiling et al. 1991, Bertness et al. 2008). Thus, no work has examined enrichment effects on high-marsh epibenthic invertebrates (sometimes referred to as 'epifauna').

In New England high-marsh habitats, the epibenthic invertebrate community is dominated by detritivores (e.g. snails, amphipods) (Fell et al. 1982). Predators, such as spiders, are also present within this community. Given that nutrient enrichment can stimulate densities across multiple trophic levels and shift invertebrate community structure in *Spartina alterniflora*-dominated habitats (e.g. Wimp et al. 2010), similar results can be expected in *Spartina patens*-dominated habitats.

For this study, I took advantage of a novel nutrient-enrichment experiment in a northeast Massachusetts (USA) salt marsh to examine the response of epibenthic invertebrates in the *Spartina patens*-dominated high marsh. Nutrients were applied to the flooding waters of tidal creeks that also flood the high marsh providing a large sampling area (4–5 ha), and enrichment had been ongoing for 7 yr at the end of this study. Thus, I was able to examine ecosystem-level responses of invertebrates to long-term enrichment. I hypothesized that nutrient enrichment would stimulate invertebrate densities and alter community structure.

MATERIALS AND METHODS

Study site

This study was conducted in 5 tidal creeks in northeast Massachusetts, USA. These creeks are all located within the broader salt marsh in the Plum Island Estuary (42° 44' N, 70° 52' W; Fig. 1). Four creeks drain into the Rowley River and the remaining creek empties directly into the sound (Fig. 1). All creeks have similar faunal, plant, and physical characteristics (Deegan et al. 2007, Johnson et al. 2007, D. S. Johnson et al. unpubl. data). Vegetation patterns are typical of New England marshes (Miller & Egler 1950, Niering & Warren 1980). Creek-side *Spartina alterniflora* (smooth cordgrass, 160 cm shoot height) dominates the lowest elevation (seaward edge) of the vegetated marsh platform and is replaced by *S. patens* (mixed with *Distichlis spicata*) near mean high water. Patches of stunted *S. alterniflora* (40 cm shoot height) monocultures occupy poorly drained sites of the high marsh. In this system, *S. patens* occupies 60 to 70% of the aerial extent of the vegetated marsh platform. Based on percentage of time inundated, creek-side *S. alterniflora* habitats are inundated ~10% of the time, whereas *S. patens* habitats are inundated only during the highest spring tides or during storm surges, which results in this habitat being inundated ~4% of the time during the growing season (May to September).

Nutrient enrichment

Two creeks were selected to receive nutrient enrichment and 3 creeks remained as references. To mimic anthropogenic nutrient enrichment, nutrients were added in solution to the water column of each creek with a targeted concentration of 50 to 70 μM NaNO_3^- and 6 μM PO_4^{3-} with each flooding tide. This was achieved by pumping a concentrated nutrient solution into the twice-daily flooding waters, with the pump rate being adjusted every 10 min throughout the flooding tide to maintain targeted concentrations. Nutrients were added at least 120 d during the plant growing season (from mid-May to mid-September) of each year from 2004 to 2010. This level of nutrient enrichment was ~15 $\times$ higher than concentrations found in reference creeks. Nutrient enrichment concentrations are typical of those found in estuarine systems designated as moderately-to-severely eutrophic by the Environmental Protection Agency (2002). The current method of enrichment differed from other marsh nutrient enrichment studies using hand-broad-

Fig. 1. Top: aerial photograph of Rowley River marshes (base map from MassGIS 2009); bottom: outline of the state of Massachusetts (USA) with arrow indicating the location of Plum Island Estuary. In the photograph: light colored areas are extensive *Spartina patens* high-marsh habitats; dark areas within these habitats are ponded areas; dark areas near the Rowley River are lower-elevation and extensive *Spartina alterniflora* habitats; white areas are experimental tidal creeks used in this study; E1 and E2 are nutrient enriched creeks; R1, R2 and R3 are reference creeks; and white arrow: point of nutrient addition. Middle: cross-section of typical tidal creek (not to scale). Although *Spartina patens* often extends beyond 100 m from the tidal creek, sampling was conducted within 75 m of the *S. alterniflora*/*S. patens* border

cast dry fertilizer on small plots (m^2) in at least 2 ways: (1) it mimics how anthropogenic nutrients are delivered to the marsh (i.e. via the flooding waters), and (2) the spatial scale of the application is large (4–5 ha). The high-marsh habitats had an average loading rate of $15 \text{ g N m}^{-2} \text{ yr}^{-1}$ and similar concentrations were recorded throughout the high marsh (Deegan et al.

2007). The 2 enriched creeks form a confluence from which nutrients were applied (Fig. 1); however, the distance between sampling areas was at least 200 m. Reference creeks were at least 1 aerial km from the point of nutrient addition (Fig. 1).

Sampling

Late-summer (August–September) invertebrate surveys were completed in Year 6 (2009) and Year 7 (2010) of enrichment. This timing allowed us to collect end-of-season live biomass of *Spartina patens* as an estimate of above-ground production and detrital standing stocks in Year 7. For each survey, 10 quadrats (0.0625 m^2) were haphazardly tossed in the *Spartina patens* habitat of each creek for a total of 50 quadrats. Samples were taken at least 5 m from the creek-side *S. alterniflora*/*S. patens* border, the stunted *S. alterniflora*/*S. patens* border, and the upland terrestrial border. Although the extent of *S. patens* can be >100 m from the *S. alterniflora*/*S. patens* border, samples were taken within 75 m of the *S. alterniflora*/*S. patens* border. Samples were taken on either side of the tidal creek along a 250–300 m length of creek for a sampling area of 4–5 ha creek⁻¹. All grass (live and standing dead shoots) within the quadrat was clipped flush to the sediment surface. Collectors worked quickly from the corners inward to catch fast-moving invertebrates. Some individuals (primarily fast-moving amphipods and spiders) escaped collection but were recorded. Common and readily identifiable species were identified to species level. Spiders, of which there are at least 4 morphospecies (pers. obs.), were lumped into one category because

they often escaped, thus making reliable identification difficult. The 2 different color morphs of *Orchestia grillus* (orange and brown) were also recorded. *Orchestia* is nominally brown in body color, but it becomes orange and alters its behavior when parasitized by a trematode, making it more susceptible to predation by the final host of the trematode—the

shorebirds (Bousfield & Heard 1986). Johnson et al. (2009) showed that enrichment led to increased abundances of parasitized *Ulhorchestia spartino-phila*, which is an amphipod species in low-marsh *Spartina alterniflora* habitats in these marshes.

To determine the potential influence of changes in live *Spartina patens* biomass and detrital stocks on invertebrates, grass (live and standing dead shoots) was collected from each quadrat in 2010. Material was sorted by species and by living vs. dead litter, dried to a constant weight (typically 2 d) at 70°C, and weighed. The 3 numerically dominant invertebrates: the gastropod *Melampus bidentatus*, the amphipod *Orchestia grillus*, and the isopod *Philoscia vittata* (hereafter referred to generically) are considered detritivores (Rietsma et al. 1982, Thompson 1984, Rietsma et al. 1988) and may thus be influenced by changes in *S. patens* biomass. *Orchestia* and *Philoscia* use their mandibles to shred vegetation and *Melampus* uses a radula to scrape the surface of sediments and leaves. *Melampus* and *Orchestia* are known to graze on microbial decomposers (e.g. fungi; Lopez et al. 1977, Graça et al. 2000).

The biomass (g m⁻²) of *Melampus*, *Orchestia*, and *Philoscia* was determined. For *Orchestia* and *Philoscia*, 30 ind. from reference sites and 30 from nutrient enriched sites were randomly selected and dried to a constant mass at 70°C. No difference in average individual weights was detected between treatments (2-sample *t*-test, *p* > 0.05); thus, all 60 ind. species⁻¹ were pooled to generate an average per-capita weight. Biomass (g m⁻²) was determined as the average per-capita dry weight multiplied by the animal density per quadrat for *Philoscia* and *Orchestia*.

For *Melampus*, 50 different sized snails (range: 5.1 to 13.5 mm shell height) were collected haphazardly from reference creeks and used to determine the following statistical relationship between shell height and shell-free dry weight:

$$\text{Shell-free dry weight} = 0.00006(\text{shell height})^{2.65}, \\ R^2 = 0.89$$

This information was used to determine the dry weight of up to 10 *Melampus* individuals that were randomly selected from each quadrat and measured for shell height. Biomass was then determined as the average dry weight of those 10 individuals multiplied by the snail density per quadrat. *Melampus* demographics were also determined based on the shell height of the 10 randomly selected individuals. *Melampus* reproduces at shell heights as small as ~5 mm (Apley 1970). Here, I define sexually reproductive adults as ≥5.5 mm and juveniles as <5.5 mm shell height.

For 2010 surveys, 3 diversity measures were estimated for each quadrat in PRIMER (Clarke & Warwick 2001): richness (*S*), evenness (Pielou's index *J'*), and Shannon's diversity index (*H'*). Diversity indices may be skewed by lumping spiders into one broad category and results should be interpreted with caution.

Statistical analysis

The effects of nutrient enrichment on invertebrate diversity (*S*, *J'*, *H'*), density, biomass, and the percentage of juvenile *Melampus* were analyzed using *t*-tests. Only the densities of the numerically dominant invertebrates (*Melampus*, *Orchestia*, *Philoscia*) and predators (spiders and *Tabanus*) were analyzed. *t*-tests were also employed to examine the effect of nutrient enrichment on live aboveground *Spartina patens* shoots and detrital stocks. Prior to analysis, the average data of the 10 quadrats creek⁻¹ was determined; thus for each parameter, *n* = 2 for nutrient enrichment, and *n* = 3 for the reference creeks. *Melampus* shell heights were binned in 0.5 mm intervals treatment⁻¹ and the effect of enrichment on the size distributions was analyzed using a 2-sample Kolmogorov-Smirnov test for distributions. Each year of treatment was analyzed separately. All univariate analyses were conducted in R (R Development Core Team 2009).

To examine the effect of enrichment at the community level, non-metric multidimensional scaling (nMDS) and analysis of similarities (ANOSIM) were conducted on 2010 survey data based on Bray-Curtis similarity matrices. To determine the percent contribution of species driving any differences between communities, similarity percentages (SIMPER) were calculated. Community analyses were conducted in PRIMER (Clarke & Warwick 2001).

RESULTS

Twelve categories of macroinvertebrates were observed from the surveys conducted in the *Spartina patens* marsh (Table 1). Although insects had the highest categorical richness (*n* = 7), the community was numerically dominated by 3 non-insect taxa: *Melampus*, *Philoscia*, and *Orchestia* (combined relative abundance: 97% of the community; Table 1). These were also the most frequently occurring invertebrates (≥96% of the quadrats; Table 1).

The densities of *Melampus*, *Orchestia*, *Philoscia*, and total invertebrates were significantly higher in

Formula corrected after publication (0.0006 changed to 0.00006)

enriched than in reference creeks in Year 6 (96 to 199 % increase, t -test, $p \leq 0.037$; Fig. 2, Table 2). The same trends were observed in Year 7 except that the effect on *Orchestia* densities was not significant (Fig. 2, Table 2). The density of parasitized *Orchestia* was higher in enriched than in reference creeks in both years, with Year 6 density being significantly higher (t -test, $p = 0.018$; Fig. 2, Table 2). *Tabanus* larvae (adults are commonly known as 'greenhead

flies') densities did not differ between treatments in Year 6 but were significantly higher (238 %) in enriched than in reference creeks in Year 7 (t -test, $p =$

Table 1. Relative abundance and frequency of occurrence of macroinvertebrates collected from *Spartina patens* high marsh in the Plum Island Estuary, MA, USA. Values calculated from quadrats pooled across all creeks ($n = 50$) from Year 7 survey. G: gastropod, I: isopod, A: amphipod, Ar: arachnid, In: insect, B: bivalve

Species	Relative abundance (%)	Frequency of occurrence (%)
<i>Melampus bidentatus</i> ^G	68.96	100
<i>Philoscia vittata</i> ^I	19.72	98
<i>Orchestia grillus</i> ^A	7.39	96
Spiders ^{Ar}	2.04	62
Insect nymphs ^{In}	1.14	26
<i>Tabanus</i> sp. ^{In}	0.27	20
Ladybug ^{In}	0.11	8
Non- <i>Tabanus</i> dipteran larvae ^{In}	0.11	8
Ants ^{In}	0.11	6
<i>Geukensia demissa</i> ^B	0.08	6
Beetle larvae ^{In}	0.05	2
Weevils ^{In}	0.03	2

Fig. 2. Mean densities ($\pm$ SE) of invertebrates collected in late summer (August–September) in the *Spartina patens*-dominated high marsh in Plum Island Estuary, MA. t -test results: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$

Table 2. Mean densities ($\pm$ SE; ind. m⁻²) of macroinvertebrates collected in late summer (August–September) in the *Spartina patens* high marsh in the Plum Island Estuary, MA, USA. Significant values are in **bold**. Means and SEs were calculated from replicates pooled by treatment. t -tests were conducted using values averaged by creek within treatments; thus, $n = 2$ for nutrient enriched creeks and $n = 3$ for reference creeks. Response to enrichment: % change enriched vs. reference creeks

Species	Reference	Enriched	Response to enrichment (%)	t	p
Year 6					
<i>Melampus bidentatus</i>	532 $\pm$ 40	1099 $\pm$ 87	107	3.58	0.037
Parasitized <i>Orchestia grillus</i>	1 $\pm$ 0.7	8 $\pm$ 3.5	700	4.70	0.018
Total <i>Orchestia grillus</i>	18.7 $\pm$ 2.7	36.5 $\pm$ 7.2	96	3.67	0.035
<i>Philoscia vittata</i>	60.0 $\pm$ 9.9	179.5 $\pm$ 35.8	199	15.78	<0.001
Spiders	2.6 $\pm$ 1.1	5 $\pm$ 1.5	92	0.97	0.402
<i>Tabanus</i> sp.	3.3 $\pm$ 1.3	2.5 $\pm$ 1.6	-25	-0.44	0.692
Total inverts	623 $\pm$ 44	1329 $\pm$ 96	113	4.27	0.024
Year 7					
<i>Melampus bidentatus</i>	649 $\pm$ 52	1108 $\pm$ 66	71	4.29	0.023
Parasitized <i>Orchestia grillus</i>	0 $\pm$ 0	4 $\pm$ 3.3	–	2.24	0.111
Total <i>Orchestia grillus</i>	75.2 $\pm$ 10.7	110.4 $\pm$ 12.3	47	1.66	0.195
<i>Philoscia vittata</i>	197.3 $\pm$ 38.6	299.2 $\pm$ 53.0	52	3.79	0.032
Spiders	14.9 $\pm$ 4.1	39.2 $\pm$ 7.2	163	3.95	0.029
<i>Tabanus</i> sp.	2.1 $\pm$ 1.0	7.2 $\pm$ 1.8	238	-3.8	0.032
Total inverts	959 $\pm$ 73	1579 $\pm$ 86	65	4.51	0.020

0.032; Fig. 2, Table 2). Arachnid densities in enriched creeks were at least twice those in reference creeks in both years but were significantly higher only in Year 7 (t -test, $p = 0.029$; Fig. 2, Table 2).

The biomass (g m^{-2}) of *Melampus*, *Orchestia*, and *Philoscia* was significantly higher in enriched than in reference creeks in Year 6 (96 to 200%, t -test, $p \leq 0.047$; Fig. 3, Table 3). In Year 7, the same significant trend in biomass was observed for *Melampus* and *Philoscia* but not for *Orchestia* (Fig. 3, Table 3).

Melampus shell heights ranged from 4 to 11 mm and were not different between treatments in either year (t -tests, $p > 0.213$). The overall proportion of juveniles (<5.5 mm shell height) in the *Melampus* population was low (1.5 to 8.5%) with no differences detected between treatments in either year (t -tests, $p > 0.560$). Although the modal shell height for each treatment was similar each year (6 to 7 mm), there was a significant difference in the size frequency distribution between treatments for each year, with a slight shift to larger snails in enriched creeks (Kolmogorov-Smirnov test, $p < 0.001$; Fig. 4).

No difference in categorical richness, evenness or Shannon's diversity index was detected between treatments (t -test, $p > 0.178$). Community composition significantly differed between treatments (ANOSIM, Global $R = 0.161$, $p = 0.004$; Fig. 5). SIMPER analysis revealed that the 3 numerically dominant species were responsible for 92% of these differences (*Melampus*, 58%; *Philoscia*, 26%; and *Orchestia*, 8%).

In terms of live aboveground biomass and detritus, *Spartina patens* constituted at least 95% of the dry weight in quadrats in Year 7. No differences in live-shoot biomass or detrital stocks were detected between treatments (t -test, $p > 0.469$; Fig. 6).

DISCUSSION

After 7 yr of nutrient enrichment, the density and biomass of the major constituents of the macroinvertebrate community in the *Spartina patens* habitat were significantly higher in the enriched than in ref-

Fig. 3. Mean biomass ($\pm$ SE) of dominant invertebrates collected in late summer (August–September) in the *Spartina patens*-dominated high marsh in Plum Island Estuary, MA. t -test results: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$

Table 3. Mean biomass ($\pm$ SE; g m^{-2}) of detritivores collected in late summer (August–September) in the *Spartina patens* high marsh in the Plum Island Estuary, MA, USA. Significant values are in **bold**. Means and SEs were calculated from replicates pooled by treatment (enriched $n = 20$, reference $n = 30$). t -tests, however, were conducted using values averaged by creek within treatments; thus, $n = 2$ for nutrient enriched creeks and $n = 3$ for reference creeks. Response to enrichment: % change enriched vs. reference creeks

Species	Reference	Enriched	Response to enrichment (%)	t	p
Year 6					
<i>Melampus bidentatus</i>	4.58 $\pm$ 0.35	12.03 $\pm$ 1.38	163	3.25	0.047
<i>Orchestia grillus</i>	0.54 $\pm$ 0.08	1.06 $\pm$ 0.21	96	3.67	0.035
<i>Philoscia vittata</i>	0.30 $\pm$ 0.05	0.90 $\pm$ 0.18	200	15.78	<0.001
Year 7					
<i>Melampus bidentatus</i>	7.36 $\pm$ 0.59	14.75 $\pm$ 1.30	100	10.17	0.002
<i>Orchestia grillus</i>	2.18 $\pm$ 0.31	3.20 $\pm$ 0.36	47	0.454	0.681
<i>Philoscia vittata</i>	0.99 $\pm$ 0.19	1.50 $\pm$ 0.27	52	3.79	0.032

Fig. 4. *Melampus bidentatus*. Size frequency distributions of shell heights for Years 6 and 7 of nutrient enrichment. The effect of nutrient enrichment on distributions was tested using a Kolmogorov-Smirnov test

Fig. 5. Non-metric multidimensional scaling display of high-marsh invertebrate community similarities in enriched and reference creeks based on Bray-Curtis similarity matrices of 2010 surveys

reference creeks. These results agree with previous studies that found increased invertebrate densities and/or biomass in response to nutrient enrichment in marshes (Bertness et al. 2008, Johnson & Fleeger 2009, Wimp et al. 2010). The densities of the 3 dominant taxa (*Melampus*, *Orchestia*, and *Philoscia*) were significantly higher (47 to 199%) in enriched than in

Fig. 6. *Spartina patens*. Mean dry weight ($\pm$ SE) of above-ground detritus and live shoots collected from clip plots in August 2010. ns: not significant (based on *t*-tests)

reference creeks, which drove the significantly higher trend of total invertebrate densities in enriched creeks. After 7 yr of nutrient enrichment, *Tabanus* larvae and spider densities in enriched creeks were at least double those in reference creeks. Both *Tabanus* larvae and spiders are predators of small, high-marsh arthropods (including *Philoscia* and *Orchestia*) and the large increase of potential prey in enriched creeks likely contributed to the density increase in these predators.

No abundance data existed for high-marsh invertebrates prior to nutrient enrichment. Abundances were estimated in Years 2 and 3 of nutrient enrichment using passive collectors (litterbags). These were small mesh bags filled with *Spartina alterniflora* litter placed on the high-marsh surface for 2 wk (see Johnson et al. 2009 for detailed methods). Using this method, I found no differences in abundances of major taxa (amphipods, isopods, and spiders) or community structure (unpubl. data) between treatments. This method, however, could not be used to estimate *Melampus* abundances because snails do not colonize bags. Although the litterbag method used in Years 2 and 3 of enrichment is not directly comparable to the clip-plot methods used in Years 6 and 7, the 2 sets of data both suggest a cumulative effect of enrichment on high-marsh invertebrate abundances that may take several years to detect.

The biomass (g m^{-2}) of *Melampus*, *Philoscia*, and *Orchestia* was significantly higher in enriched than in reference creeks. For *Melampus*, this was a function of increased (by 5 to 8%) individual shell height and a large density increase (by 70 to 110%) in enriched creeks relative to reference creeks. Although the mean shell height for *Melampus* was not significantly different between treatments, the size frequency distributions were significantly different, with a slight shift to larger snails in enriched creeks. Because I did not determine dry weights of indi-

vidual *Orchestia* and *Philoscia* specimens from the quadrats, I can only suggest that the higher dry weights in enriched creeks versus reference creeks for these species are due to higher densities.

There are several mechanisms by which nutrient enrichment may lead to increased invertebrate densities including enhanced food supply and/or food quality. *Melampus*, *Philoscia* and *Orchestia* are considered detritivores that facultatively consume *Spartina* grasses (Lopez et al. 1977, Rietsma et al. 1982, Thompson 1984, Rietsma et al. 1988). Enrichment neither stimulated the end-of-season biomass of live *Spartina patens*, nor did it alter the detrital stocks. Detrital stocks in reference creeks (350 g dry wt m⁻²) was high relative to detritivore biomass (~10 g dry wt m⁻² for *Melampus*, *Orchestia* and *Philoscia* combined). Herjanto & Thomas (1995) determined that *Melampus bidentatus*, which is the most abundant invertebrate found in this study, has a production:biomass ratio of 3:1 in Bermudan mangroves. Using this P:B ratio and the highest biomass (14.75 g m⁻²) from the current study for *Melampus*, production can be estimated at 44.25 g m⁻² yr⁻¹ in these marshes. Thus, it is unlikely that detritivore production is limited by the quantity of *S. patens* detritus. Improved litter quality (increased N, lower lignin content) due to enrichment may have led to increased detritivore density and biomass (Valiela et al. 1984, Rietsma et al. 1988). In 2009, live shoots of *S. patens* had higher %N and lower C:N in enriched vs. reference creeks (D. S. Johnson et al. unpubl. data). Moreover, *Melampus*, *Philoscia*, and *Orchestia* are opportunistic feeders and are thus not strict detritivores (Thompson 1984, Galván 2008). Stimulations of other carbon sources (e.g. algae, bacteria, fungi) by nutrient enrichment may have also led to increased invertebrate densities. Benthic microalgal biomass is relatively low in the high marsh and enrichment has not resulted in increased biomass of this food source (Deegan et al. 2007). Decomposer biomass found on *S. patens* litter, which can be an important carbon source for detritivores such as *Orchestia* (Lopez et al. 1977), was stimulated by enrichment (D. S. Johnson et al. unpubl. data).

My results contrast with those of McFarlin et al. (2008) who found no fertilization effects on *Melampus* densities in a Georgia (USA) salt marsh. The authors noted that their plot size (1 m²) may have been insufficient to elicit a strong aggregative response (McFarlin et al. 2008). The scale of enrichment in the current study encompassed entire tidal creeksheds and I was able to assess ecosystem-wide responses of *Melampus*. Thus, the observed numeri-

cal responses of *Melampus* likely derive from increased production of individuals rather than a net migration of adult snails into the experimental plots. The duration of this experiment may have also been important. In New England, *Melampus* lay eggs on *Spartina alterniflora* and *S. patens* stems in early summer (Spelke et al. 1995, author's pers. obs.). Planktonic veligers emerge 10 to 30 d later, depending on the next tidal submergence (Apley 1970, Russell-Hunter et al. 1972), and remain in the water for 6 wk (Apley 1970). Spats settle at a shell height of 680 µm and grow to only 1 to 2 mm in the first year (Apley 1970). Thus, the small size of first-year cohorts may make detection difficult and these tiny individuals might not have been included in many density estimates. Therefore, it may require >1 yr to detect changes in *Melampus* densities due to enrichment, to allow the first-year cohorts of snails to grow to detectable size classes.

Increased invertebrate densities elicited by nutrient enrichment have potential consequences for vertebrate predators such as fish and birds. High-marsh invertebrates can be important prey for nekton, such as the killifish *Fundulus heteroclitus* (Allen et al. 1994, Deegan et al. 2007) and migratory shorebirds (Buchsbaum et al. 2009, Johnson et al. 2009). An intriguing consequence of nutrient enrichment on trophic interactions involves the parasitism of amphipods. *Uhlorchestia spartinophila* lives in the intertidal low marsh, is normally brown and forages in vegetated, protected habitats (i.e. among *Spartina alterniflora* stems). When parasitized by the trematode *Levinseniella byrdi* (Bousfield & Heard 1986), the amphipod's body color changes from brown to orange and the orange individuals forage in exposed habitats (e.g. unvegetated creek walls; Fig. 1) at low tide (Johnson et al. 2009). This results in increased vulnerability to predation by shorebirds (Johnson et al. 2009), which are the final hosts of the trematode (Bousfield & Heard 1986). *Orchestia*, like *U. spartinophila*, can be parasitized by *L. byrdi* (Bousfield & Heard 1986) and responds similarly. In the current study, I found more parasitized *Orchestia* in the high marsh in enriched than in reference creeks (700% more in Year 6). It is likely that the enrichment-associated increase in the density of parasitized amphipods is partly due to increased densities of the total population; however, sampling only the high marsh underestimates the true densities of parasitized amphipods. Parasitized individuals of both *Orchestia* and *U. spartinophila* migrate to exposed creek-wall habitats (a 1–3 m vertical surface exposed at low tide; Fig. 1) (Johnson et al. 2009, author's pers. obs.). Thus, nutrient enrichment has

consequences for habitat usage of these invertebrates and can alter fundamental trophic interactions (Johnson et al. 2009).

My results suggest that nutrient enrichment has potential consequences for litter decomposition, which is an important ecosystem function in detritus-dominated ecosystems such as salt marshes. *Melampus*, *Orchestia*, and *Philoscia* are the typical dominant invertebrates of the *Spartina patens*-dominated high marsh in New England marshes (Fell et al. 1982, Fell & Williams 1985, present study). Thus, higher detritivore densities may lead to increased litter decomposition. In New England marshes, *S. patens* senesces in the early fall and 'lays down' to form a dense canopy or mat of standing dead shoots near the sediment surface. This standing dead canopy provides both a shaded habitat and a food source for these detritivores (Thompson 1984, Galván 2008). Both *Orchestia* and *Philoscia* are shredders, while *Melampus* scrapes *Spartina* tissue using a radula (Thompson 1984). In nutrient enriched creeks, the turnover of *Spartina* litter may thus be accelerated (Valiela et al. 1984) due to increased detritivores and direct stimulation of decomposers.

Based on ANOSIM results, the community structure of invertebrates differed between treatments. In 4 m² experimental plots, Wimp et al. (2010) observed that fertilizations altered the structure of arthropod communities, with the change being attributed to increased *Spartina alterniflora* production that led to increased species richness. In the current long-term enrichment experiment, changes in end-of-season live biomass of *S. patens* in the high marsh or in invertebrate species richness were not observed. The community differences detected here appear to be driven primarily by abundance increases in the detritivore community (responsible for 92% of the community dissimilarity).

My results highlight that even infrequently flooded high-marsh habitats are susceptible to long-term eutrophication effects through changes in invertebrate community structure and trophic interactions and increasing abundances across multiple trophic levels (detritivores and predators). These results generally add to the evidence that variation in nitrogen inputs can affect community structure and abundance of saltmarsh consumers (Deegan et al. 2007, Bertness et al. 2008, Fleeger et al. 2008, Johnson & Fleeger 2009, Johnson et al. 2009, Wimp et al. 2010). As eutrophication continues to afflict coastal ecosystems, it is important to include high-marsh habitats in our understanding of how eutrophication affects the structure and function of salt marshes.

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