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Diverse Dietary Responses by Saltmarsh Consumers to Chronic Nutrient Enrichment

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Abstract We examined the effect of whole-ecosystem nutrient enrichment on herbivory in saltmarsh creek-wall habitats in the Plum Island Estuary (Massachusetts, USA). Located between the macrophyte-dominated high marsh and adjoining mudflats, creek walls are steep vertical habitats vegetated with productive filamentous algae and associated epiphytes. Annual nitrate and phosphate loading rates were increased approximately ×10–15 in creeks mimicking short-term (2-month) and chronic (6-year) eutrophication. We assessed the diets of epifaunal invertebrates (three gastropods and one amphipod species) that potentially graze on benthic algae using natural isotope abundance data and per capita grazing rate measurements derived from ^{13}C prelabeled algae. Substantial dietary contributions from benthic algae were observed in all consumers even though previous research has indicated most rely on *Spartina* detritus as the principal food resource. The amphipod *Orchestia grillus* and the snail *Melampus bidentatus* grazed benthic algae in excess of 500 μg algal C g C^{-1} h^{-1}, whereas the snail *Nassarius obsoletus* and hydrobiid snails grazed at lower rates. Few dietary changes were detected with short-term enrichment. Algal grazing rates of *N. obsoletus* and *M. bidentatus* increased with chronic enrichment probably as a functional response to increased algal productivity. *O. grillus* grazed at a high rate and parasitic infection did not affect its consumption of benthic algae. The abundance and frequency of occurrence of *O. grillus* on creek-wall habitats increased with chronic nutrient enrichment suggesting amphipods contribute to top–down control on benthic algae and slow algal growth as nutrient enrichment occurs.

Keywords Nutrient enrichment · Food web · Epiphytes · Filamentous algae · Estuary · Creek-wall habitat

Introduction

Estuaries and associated salt marshes provide important ecological and economic services including those that contribute to nutrient regulation and that support fisheries (Costanza et al. 1997; Valiela and Cole 2002). However, these environments are under increasing anthropogenic pressure, for example, as the amount of nitrogen entering watersheds increases globally (Galloway et al. 2008; Gruber and Galloway 2008; Canfield et al. 2010; Deegan et al. 2012). Nutrient loading and subsequent cultural eutrophication of the coastal environment has been documented worldwide in coastal environments (Diaz and Rosenberg 2008), becoming major issues for both scientists and managers.

Nutrient enrichment can potentially relieve nitrogen or phosphorus limitations affecting biomass and/or production of primary producers in estuaries (Bertness et al. 2002; Deegan et al. 2007; Darby and Turner 2008). Fast-growing macroalgae, epiphytes, and phytoplankton can outcompete macrophytes in nutrient-enriched estuaries (Taylor et al. 1995; Wear et al. 1999). Surface-dwelling invertebrates (epifauna) exerting top–down control on algae (Duffy and Hay 2000) may consequently be affected by resource changes (Rosemond et al. 2001; Johnson 2011). Because services provided by salt marshes depend on these higher trophic levels, it is critical to understand how they will be affected by nutrient addition.

The present study takes advantage of a whole-ecosystem manipulation designed to evaluate the effects of nutrient enrichment on salt marshes in the Plum Island Estuary, Massachusetts, USA (The "TIDE" project; Deegan et al.

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2007). TIDE mimics the nutrient loadings and the mechanism of nutrient delivery typically associated with anthropogenic eutrophication in marsh creeks. Dissolved N and P have been added to creeks with the flooding tide throughout the whole growing seasons since 2004. Large spatial (60,000 m^2) and temporal (6 years of seasonal nutrient enrichment when our study was conducted) scales make this experimental perturbation more similar to human-induced eutrophication than the typical application of dry fertilizer to small plots (Sardá et al. 1996). These features should contribute to a better understanding of the rate and mechanisms of change associated with chronic nutrient enrichment.

TIDE experiments previously revealed that nutrient enrichment modifies grazing pressure on benthic algae by (1) causing dietary shifts in benthic grazers (Deegan et al. 2007; Galván 2008; Johnson et al. 2009; Lockfield 2011), (2) increasing per capita algal consumption (Pascal et al. 2013) and (3) stimulating increases in the density of some grazers (Johnson 2011; Lockfield 2011; Johnson and Short 2013). A detailed understanding of the response of benthic grazers is needed to better understand the process by which nutrient enrichment brings about eutrophication and increased algal biomass in salt marshes.

Creek walls are steep vertical habitats located between the macrophyte-dominated marsh platform and adjoining creek mudflats. Although creek-wall habitats cover a relatively small fraction of the total marsh surface area, this understudied habitat may play a disproportionate role in the structure and function of salt marshes because they harbor productive filamentous algal and associated epiphyte communities and the highest infaunal abundance across the marsh landscape (Johnson et al. 2007; Fleeger et al. 2008). Moreover, the largest response to nutrient addition among benthic infauna was observed in creek-wall habitat (Fleeger et al. 2008).

The aim of the present study was to evaluate changes in herbivory induced by nutrient enrichment in the creek-wall habitat. We focused here on resident and transient epifaunal benthic grazers of this habitat, and we used two approaches: variation in natural abundance of isotopes (δ^{13}C and δ^{15}N) and grazing rate measurements with ^{13}C pre-labeled algae. Chronic and short-term nutrient enrichment were both studied. Our results (1) give a picture of creek-wall habitat herbivory function under natural conditions and (2) facilitate an evaluation of changes induced by nutrient enrichment over time.

Materials and Methods

TIDE Project

TIDE, stands for “Trophic cascade and Interacting control processes in a Detritus based aquatic Ecosystem.” This multi-institutional, multi-disciplinary, multi-year project is a manipulation, at the ecosystem-scale, of the extensive salt marshes of Plum Island Estuary (PIE), Massachusetts (42°44′ N, 70°52′ W), USA (Fig. 1).

PIE is a 25-km long mesotidal estuary with a mean tidal range of 3 m. *Spartina alterniflora* and *Spartina patens* are the dominant macrophytes on the marsh platform in the seawater-dominated intertidal zone where the salinity ranges from 8 to 28‰. Here, we focus on the creek-wall habitat found adjacent to the marsh edge at a lower tidal elevation. It is a steep, almost vertical wall about 1.5 m in height constituted by cohesive sediments (Fig. 1). Creek wall is a distinct habitat with a ~30-cm wide band of dense macroalgae (*Enteromorpha* spp.), filamentous algae (*Rhizoclonium* spp.), and associated epiphytic diatoms that form a distinct and physically complex algal turf extending ~1 cm above the sediment surface. Mudflats of poorly consolidated sediments delimit the lower border of this habitat.

PIE is currently unaffected by eutrophic nutrient loading (creek water background nutrients: <5 μM NO$_3^-$ and 1 μM PO$_4^{3-}$). Along the Rowley River, nutrient enrichment

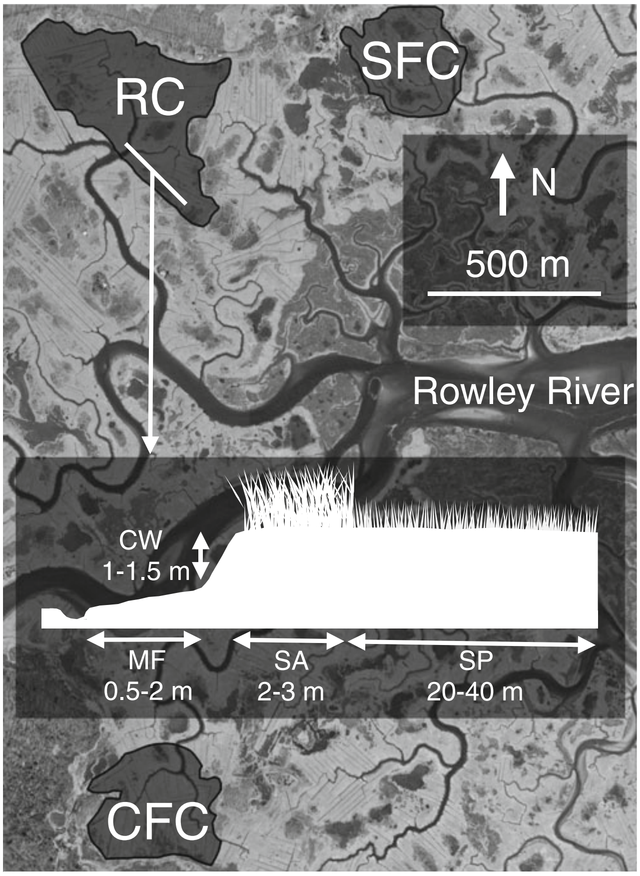

Fig. 1 Map of the Rowley River region salt marshes of the Plum Island Estuary, MA, including figure of a profile of sampled salt marsh habitat (not drawn at scale) with ranges of each habitat. *RC* Reference Creek, *SFC* Short-term fertilized Creek, *CFC* Chronically fertilized Creek, *MF* mudflat, *CW* Creek-wall habitat, *SA Spartina alterniflora*, *SP Spartina patens*

experiments were conducted in three intertidal marsh creeks (West, Clubhead, and Sweeney) having similar physical dimensions, water chemistries, and plant and infaunal communities (details in Deegan et al. 2007; Johnson et al. 2007). Sweeney (hereafter referred to as the chronically fertilized creek or CFC) was fertilized for six field seasons (2004–2009), Clubhead (hereafter referred to as the short-term fertilized creek or SFC) was fertilized for about 2 months (in 2009), and West (hereafter referred to as the reference creek or RC) was unfertilized (Fig. 1). In CFC, for approximately 150 days during the growing season, nitrate and phosphate levels were increased to approximately 70 μmol L^{-1} NO_3^{-} and 4 μmol L^{-1} PO_4^{3-} on each incoming tide. These concentrations were chosen as they designated an estuary to be "moderately to severely eutrophied" (US EPA 2002). In order to maintain a relatively constant enrichment through each tidal cycle, a fertilizer solution was pumped into CFC near its mouth at a flow rate based on a hydrologic model of volume of flooding water. For SFC, fertilization methods were identical to CFC but began about 2 months before our experiments were conducted. Detailed background information of experimental design, biogeochemistry of the tidal creeks, and the effects of fertilization have been previously described (Deegan et al. 2007, 2012; Drake et al. 2009).

Algal Biomass

To assess the effect of nutrient enrichment on surface algae on the creek-wall habitat, ten 2.2-cm inner diameter creek-wall sediment cores from each creek were sampled at the time of grazing experiments and frozen. Benthic algae sampled in each core included filamentous algae and its epiphytes. Algal biomass in the surficial centimeter of sediment of each creek was assessed using chlorophyll *a* as a proxy and measured after extraction with acetone using a Turner fluorometer (APHA 1992). Carbon algal biomass was estimated using a carbon/chlorophyll ratio of 40 (Jonge 1980).

Grazer Collections

Gastropods and amphipods were collected by hand in each of the three creeks in areas with similar tidal elevations ~100 m upstream from the location where fertilizer was added to CFC and SFC and in areas physically similar in RC. Sampling was conducted during the first week of July 2009 for natural isotope analysis and for grazing experiments. Most invertebrates were collected directly on creek walls although some were collected <1 m from creek walls in adjoining habitats.

Stable Isotope Composition

Gastropod taxa selected for natural abundance stable isotope analysis were hydrobiid snails (mean shell height±SD=2.6 mm±0.6), *Melampus bidendatus* (mean shell height±SD=10.9 mm±1.2) and *Nassarius obsoletus* (mean shell height±SD=17.7 mm±2.3). The tissue of five specimens of each was manually removed from their shells and pooled together. In addition, five brown color morphs (mean length ±SD=13.2 mm±3.5) and five orange specimens (mean length±SD=14.0 mm±2.2) of the amphipod *Orchestia grillus* were pooled after being sorted by color. Samples were homogenized using a tissue grinder, freeze-dried, and a fraction of each sample was used for stable isotope analyses. Samples were analyzed at the Marine Biological Laboratory's Stable Isotope Laboratory using a continuous flow isotope ratio mass spectrometer. Samples are reported relative to the standards, atmospheric N_2, and Vienna PeeDee Belemnite carbon. Stable isotope values are reported in δ notation:

$$\delta^{13}C \text{ or } \delta^{15}N = \left[\left(R_{sample}/R_{standard}\right) - 1\right] \times 10^3$$

where R is respectively $^{13}C/^{12}C$ or $^{15}N/^{14}N$.

Grazing Experiments

In each of the three creeks, ten plots of 314 cm^2 were haphazardly located 1 m apart from each other and marked on creek-wall habitat. $NaH^{13}CO_3$ (>99% ^{13}C-enriched) was dissolved in seawater and this solution was applied daily over 6 days during the daytime low tide with a sprayer directly onto the sediment surface. Each plot was sprayed at a concentration of 1 g m^{-2} (Middelburg et al. 2000). After 6 days, sprayed sediment was gently cored (internal diameter=20 cm) and removed from creek-wall habitat at low tide without disturbing its structure. Each cohesive disk of sediment with approximate thickness of 5 cm was placed in an independent mesocosm (inner diameter=20 cm; 314 cm^2). These cores were collected <5 h before the start of grazing experiments.

Five specimens of each consumer of the previously described sizes were placed in each mesocosm. Invertebrates were collected <24 h before grazing experiments and provided with food (sediment and filamentous algae from creek-wall habitat) in order to limit starvation. From each creek, killed controls (n=3) were performed in which all specimens were first frozen (−20°C) for 24 h. All incubations lasted 5 h and were conducted at the same time, in similar conditions of light and temperature close to natural ones. Sediments were not water covered at any time during the experimental period. Incubations were stopped by freezing whole mesocosms (−20°C). Samples were thawed and all isotope samples were prepared and analyzed as previously described.

In each mesocosm, filamentous algae (approximately 1 g wet weight) and their epiphytes were collected at the end of

Table 1 Mean isotopic composition of carbon and nitrogen (‰) of each primary producer previously measured in RC in 2004 and in RC and CFC in 2005 the second year of nutrient enrichment; Galván et al. 2008)

Creek	Year	Epiphytes		Filamentous algae		*S. alterniflora*	
		$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
RC	2004	−20.4	5.8	−19.1	5.5	−13.8	6.7
RC	2005	−18.9	5.8	−19.8	6.1	−13.5	5.9
CFC	2005	−20.3	2.9	−19.8	2.0	−13.7	6.3

In the present study, values from RC in 2004 were used for all SIAR modeling, as interannual variations of sources isotopic compositions were smaller than intergrazers variations in 2013

the experiment, cleaned of foreign debris, pooled, homogenized using a tissue grinder, and freeze-dried. A fraction of each sample was prepared for stable isotope analyses.

Carbon and nitrogen content of each taxon were obtained along with isotopic ratios during isotopic analysis. Incorporation of ^{13}C was defined as excess above background ^{13}C (from control mesocosms) and was expressed in term of specific uptake (I). I was calculated as the product of excess ^{13}C (E) and biomass of C per grazer. E was the difference between the background ($F_{\mathrm{background}}$) and the sample (F_{sample}) ^{13}C fraction: $E=F_{\mathrm{sample}}-F_{\mathrm{background}}$, with $F={}^{13}C/({}^{13}C+{}^{12}C)=R/(R+1)$ and uptake$=I/(F_{\mathrm{enriched\ algae}}\times$ incubation time). $F_{\mathrm{enriched\ algae}}$ was determined using $\delta^{13}C$ of algae from each microcosm.

Data Analyses

One-way analysis of variance (ANOVA) and Tukey a posteriori tests were used to assess differences in individual grazing rates on benthic algae among creeks. Kruskal–Wallis tests were used to compare natural isotopic composition in consumers from different creeks. A Bayesian isotopic mixing model was used to determine possible contribution of primary producers [epiphytes, filamentous algae, *S. alterniflora* (Table 1)] to the diet of epifauna from RC. SIAR (stable isotope analysis in R; Parnell et al. 2010) incorporates the variability of consumers and trophic enrichment factor (TEFs) to produce a mean and a 95 % confidence interval of the percent contribution of each source to a consumer. A TEF of 0.5 % was used for $\delta^{13}C$ (Fry 2006) and 3.4 % for $\delta^{15}N$ for all consumers (Minagawa and Wada 1984).

Results

Algal Biomass and Primary Producer Isotope Composition

Benthic algal biomasses were not significantly different among creeks (ANOVA, $p=0.128$) at the time of the grazing experiment, and means were respectively 6.17±1.05 in RC, 4.47±1.16 in SFC, and 5.96±2.03 g C m^2 in CFC ($n=10$, ±SD). Natural abundance isotope composition of creek-wall habitat primary producers from RC (Table 1) previously sampled at the same time of the year (Galván 2008) was used in dietary comparisons (Fig. 2) and as source values in the SIAR mixing model. Primary producer isotope composition data from Galván (2008) from CFC after 2 years of nutrient enrichment were also used to help interpret consumer diet.

Natural Isotopic Composition

The SIAR model revealed large ranges in the possible dietary contributions of *Spartina* detritus, filamentous algae, and epiphytes in the reference creek (Table 2). The highest basal contribution came from filamentous or epiphytic algae in all consumers. Furthermore, the natural isotope

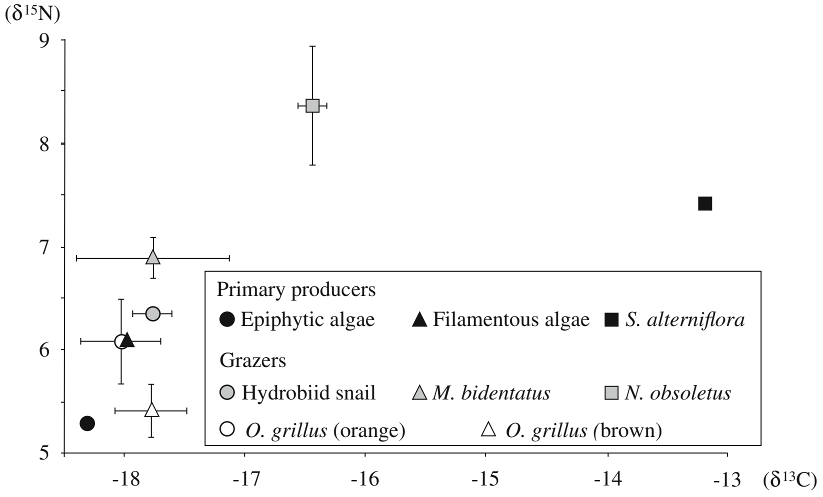

Fig. 2 Natural isotopic composition of nitrogen [$\delta^{15}N$(‰)] plotted against carbon [$\delta^{13}C$(‰)] of primary producers [live epiphytic algae, filamentous algae, and *S. alterniflora* data from Galván et al. (2008)], snails (hydrobiid snail, *M. bidentatus*, and *N. obsoletus*), and amphipods (*O. grillus* orange and brown) in RC (means in ‰±SD; $n=3$)

Table 2 Mean and 95 % confidence intervals of the percent contributions of epiphyte, filamentous algae, and *S. alterniflora* to the diets of various taxa from RC based on a Bayesian mixing model (SIAR) for isotopes of carbon and nitrogen

	Epiphytes	Filamentous algae	*S. alterniflora*
Hydrobiid snail	59 (0–71)	36 (0–71)	5 (0–17)
N. obsoletus	41 (10–73)	34 (1–65)	24 (16–33)
M. bidentatus	50 (11–92)	39 (0–74)	10 (0–33)
O. grillus (Orange)	51 (16–95)	39 (0–74)	10 (0–25)
O. grillus (Brown)	54 (16–96)	39 (0–74)	7 (0–25)

composition of carbon (Kruskal–Wallis test, $p<0.005$) and nitrogen (Kruskal–Wallis test, $p<0.05$) significantly differed among epifaunal species in the reference creek. *N. obsoletus* was distinct from other epifauna with a higher δ^{15}N of 8.4‰ (Fig. 2), and the SIAR mixing model revealed a higher possible mean contribution of *S. alterniflora* in the diet of *N. obsoletus* than other epifauna (Table 2). All other epifauna presented a similar ^{13}C composition (Kruskal–Wallis test, $p=0.68$), indicative of a diet based principally on epiphytes and filamentous algae.

Some differences in isotopic composition were noted in consumers between the nutrient enriched and reference creeks, suggesting that dietary changes occurred with nutrient enrichment. *O. grillus* (both orange and brown color morphs) presented significantly higher δ^{13}C values in CFC compared to RC (Kruskal–Wallis test, $p<0.05$). *M. bidentatus* presented a significantly higher δ^{13}C in SFC compared to RF or CFC (Kruskal–Wallis test, $p<0.05$; Fig. 3).

All epifauna from the reference creek were less depleted in δ^{15}N than epifauna from creeks that were nutrient enriched either recently (2 months) or chronically (6 years) (Fig. 3), and this difference was significant for hydrobiid snails and *M. bidentatus* (Kruskal–Wallis test, $p<0.05$). This difference is probably not related to change in diet but instead to ^{15}N changes induced by the fertilizer used to enrich creek water. The most common method of fertilizer production is industrial fixation of atmospheric nitrogen, resulting in products with δ^{15}N values close to zero.

Grazing Rates

Benthic algae became substantially enriched in ^{13}C in the sprayed plots. At the time of the grazing experiment, enrichment values of algae were (mean±SD) 2.77±0.44, 2.30 ±0.40, and 1.93±0.43 % ^{13}C, respectively, in RC, SFC, and CFC. Expressed in per mill, the algal δ^{13}C was respectively 1,533, 1,009, and 751.

In RC, per capita algal grazing expressed in micrograms algal C/gram grazer carbon/hour were 36±16 (*N. obsoletus*), 64±54 (hydrobiid snails), 551±299 (brown *O. grillus*), 654 ±452 (orange *O. grillus*), and 1,311±556 (*M. bidentatus*). Although per capita grazing rates were increased with chronic nutrient enrichment in all taxa (Fig. 4), rates were significantly increased only for *N. obsoletus* and *M. bidentatus* (ANOVA, $p<0.05$). No taxon differed between RC and SFC (ANOVA, ns).

Discussion

Our natural isotopic composition studies were conducted concurrently with grazing rate measurements, and both suggest that chronic nutrient enrichment increased the contribution of benthic algae in the diet of some benthic consumers. Below, we discuss methodological considerations, trophic relationships in the reference creek, and the time course of changes induced by nutrient enrichment.

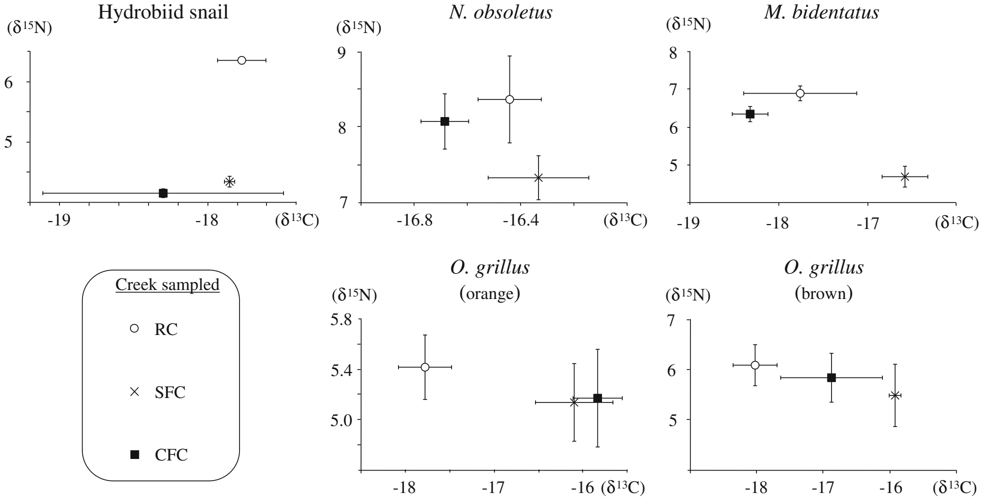

Fig. 3 Natural isotopic composition of nitrogen [δ^{15}N(‰)] plotted against carbon [δ^{13}C(‰)] of snails (*N. obsoletus*, *M. bidentatus*, and hydrobiid snail) and amphipod (*O. grillus* parasitized and non parasitized) in three different creeks (means±SD; $n=7$)

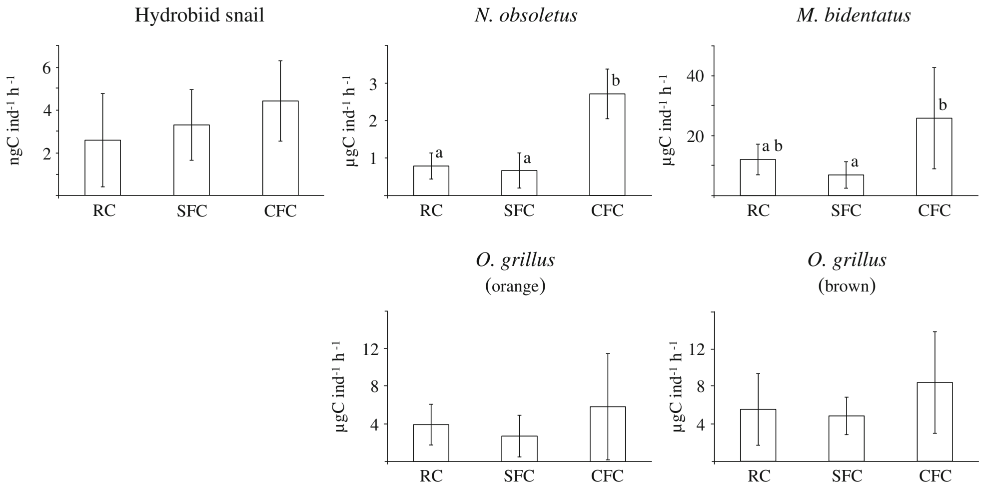

Fig. 4 Individual grazing rates (algal carbon, per individual grazer, and per hour) of snails (*N. obsoletus*, *M. bidentatus*, and hydrobiid snail) and amphipods (*O. grillus* parasitized and nonparasitized) in three different creeks (means ±SD; *n*=7). *Different letters above bars* indicate significant differences between grazing rates (ANOVA; Tukey test)

Methodological Considerations

Ecosystem-scale experiments are useful in that they incorporate a broad range of phenomena (Carpenter 1989). However, replication is challenging because similar ecosystems may be rare (Carpenter et al. 1995), and the maintenance of large-scale treatments is expensive and labor intensive. Our whole-creek nutrient enrichment technique facilitated quantification of trophic dynamics over large spatial (60,000 m^2 creek per treatment) and long temporal (6 years) scales, and studied creeks were similar physically and biologically before nutrient addition began, limiting bias due to intercreek variability (Deegan et al. 2007; Johnson et al. 2007). Treatments (creeks with differing histories of nutrient enrichment) were not replicated in our study and statistical comparisons utilized pseudo-replicates. Previous research in PIE suggests, however, that geochemical, faunal, and floral responses to nutrient enrichment are very similar in replicate creeks (Deegan et al. 2007; Johnson et al. 2007; Drake et al. 2009).

Using similar methods, Galván et al. (2011) found that epiphytes and filamentous algae became equally enriched in ^{13}C in the PIE creek-wall habitat, and we consequently made the assumption that enrichment was equal in our study. Differential enrichment between filamentous algae and their epiphytes could lead to incorrect estimation of grazing rates if epifauna feed selectively.

We used the isotopic composition of primary producers previously sampled in RF and CFC at the same time of the year by Galván (2008, Table 1) to infer the diet of consumers. The level of variation detected by Galván was relatively modest; however, this variation adds additional uncertainty regarding our dietary estimates of consumers. We also found significant variation in the isotope composition of the amphipod *O. grillus* between RC and CFC (amphipods were less depleted in ^{13}C in the creek with nutrient enrichment, and differences between creeks $\delta^{13}C$ were 2.1‰ for brown *O. grillus* and 2.0‰ for orange *O. grillus*). This variation exceeded that found in each potential basal resource (Galván 2008). The maximum $\delta^{13}C$ expressed by epiphytes resulted in a difference of 1.4‰ for $\delta^{13}C$ between creeks and 1.5‰ among years (Table 1), and the variation associated with *Spartina* and filamentous algae was even less. Furthermore, Galván (2008) did not find a significant effect of nutrient enrichment on the $\delta^{13}C$ of primary producers in PIE after 2 years of enrichment (Table 1). Relatively few studies have examined the effect of nutrient enrichment on $\delta^{13}C$ of primary producers although Vermeulen et al. (2011) found that the $\delta^{13}C$ of macroalgae was not affected by nutrient enrichment. We infer from these observations that the changes we detected in the isotope composition of *O. grillus* in CFC were due to changes in diet induced by nutrient enrichment rather than a change in isotope composition of primary producers.

Grazing rates on benthic algae were measured over a 5-h period. Because feeding behavior can potentially vary diurnally and daily, our grazing rates may not be representative of longer time periods. All grazing experiments were performed simultaneously with epifauna collected in the field at the same time in order to limit bias in feeding behavior. Any bias associated with short-term measures should be similar in all creeks, suggesting comparisons among creeks are informative of nutrient-enrichment effects.

Epifaunal Diet in the Reference Creek

SIAR indicated that benthic algae were the principal basal resource for all consumers studied, and the estimated combined mean contributions of filamentous algae and epiphytes ranged from 76 to 95 %. *N. obsoletus* fed at a higher trophic level as evidenced by a higher $\delta^{15}N$ value (DeNiro and Epstein 1981). *N. obsoletus* is an omnivore and scavenger requiring a high protein diet for reproduction (Hurd 1985). The observed higher $\delta^{15}N$ value of this snail is likely due to a mixed diet of animal and plant resources (Curtis and Hurd 1979). Per capita grazing rates in *N. obsoletus* expressed as grams algal C per gram C per hour were more than ten times lower than in *M. bidentatus* and *O. grillus* and 1.8 times lower than hydrobiid snails in the same habitat. Experiments run in the adjacent mudflat environment in PIE lead to a similar range of algal ingestion rates for *N. obsoletus*: 0.7–2.7 μg ind^{-1} h^{-1} in creek-wall habitat versus 1.1–2.3 μg ind^{-1} h^{-1} in mudflat (Pascal et al. 2013). Although the principal habitat of *N. obsoletus* is saltmarsh mudflats, some individuals migrate with the rising tide onto the creek-wall habitat (Johnson, personal communication). However, the low grazing rates, limited foraging time on the creek-wall habitat, and the low density suggest that *N. obsoletus* exerts little grazing pressure on benthic algae on creek walls.

Pascal et al. (2013) found that hydrobiids were responsible for ~50 % of the total grazing pressure exerted by benthic consumers on mudflats adjacent to the creek-wall habitat in PIE. However, per capita grazing rates were much lower (by 28×) on creek walls, presumably due to differences in algal species composition between each habitat. Hydrobiid snails are highly transitory in salt marshes and do not appear to aggregate on creek-wall habitat in reference or nutrient-enriched creeks. The potential for hydrobiid snails to graze significant amounts of benthic algae on creek walls therefore also appears to be low.

M. bidentatus is an herbivore/detritivore that uses a radula to scrape food. Although ingestion of *Spartina* detritus occurs, this species prefers fungi growing on plant material (Lee and Silliman 2006). In addition, benthic algae (i.e., diatoms) occasionally dominate its diet (Thompson 1984). The high per capita algal grazing rates measured here show that *M. bidentatus* can efficiently feed on benthic algae. Natural abundance isotope data on specimens collected from the high marsh platform also indicate that benthic algae provide an important basal resource (Galván 2008). *M. bidentatus* is prone to large fluctuations in density in PIE, and densities were very high in 2009 in all creeks (Fleeger, unpublished data). At least during years of high density, individuals are found on the creek-wall habitat in PIE but in much lower abundance than the high marsh platform.

O. grillus is commonly considered to be a leaf-shredding detritivore (Lopez et al. 1977). In PIE, stable isotope and gut content analyses revealed that the diet of this species was dominated by filamentous algae and epiphytes, at least in our summer studies when benthic algae are most productive in PIE (this study; Galván 2008). The high grazing rates and results of the SIAR mixing model indicate that *O. grillus* consumes mostly filamentous algae and epiphytes, although *Spartina* detritus may contribute up to 25 % of the basal resource (Table 2).

Hydrobiid snails are the initial host of the microphallid trematode *L. byrdii*, and talitrid amphipods are the intermediate host. Infected amphipods develop a bright orange color, lose their strong negative phototaxis (Bousfield and Heard 1986), and migrate from vegetated habitats to exposed creek-walls habitats making them much more frequent and abundant on nutrient-enriched creek walls. These traits also increase amphipod susceptibility to predation by shorebirds, the parasite's definitive host (Johnson et al. 2009). Our experiments showed that infected orange *O. grillus* color morphs fed at rates statistically indistinguishable from brown *O. grillus*. Because it takes up to 30 days for behavioral and morphological changes to manifest after infection (D. S. Johnson, personal communication), some brown amphipods may have also been parasitized without exhibiting symptoms of infection. Feeding rate in parasitized amphipods has been studied in several species and has been shown to be higher (Crompton 1970), unchanged (Fielding et al. 2003), and lower (McCahon et al. 1988; Pascoe et al. 1995) compared to nonparasitized individuals, indicating that the effects of parasites are complex and/or variable among host species.

Effect of Nutrient Enrichment

Previous research in salt marshes (Rosemond et al. 2001; Keats et al. 2004; Fox et al. 2009; Johnson 2011) and seagrass beds (Jaschinski and Sommer 2010) suggests that nutrient enrichment increases dietary contributions from epiphytic or benthic algae to benthic consumers. We examined variation in the diet of consumers based on (1) isotope composition (as a long-term dietary indicator) and (2) grazing rate measurements (as a short-term indicator). The natural isotopic composition of gastropods did not vary significantly with nutrient enrichment. However, significantly higher per capita grazing rates were observed with chronic enrichment in *M. bidentatus* and *N. obsoletus*. Higher algal grazing rates with nutrient enrichment are usually thought to be associated with a functional response to increasing benthic algal production (Jaschinski and Sommer 2010). In addition to higher per capita consumption rates, both species were significantly increased in abundance with chronic enrichment in PIE (Johnson 2011; Johnson and Short 2013), suggesting a greater total consumption of benthic algae with nutrient enrichment by these species.

The diet of the amphipod *O. grillus* was affected by nutrient enrichment in a contrasting fashion. Per capita grazing rates on benthic algae were high (550 μg algal g C^{-1} h^{-1}) and not significantly different between reference and nutrient-enriched creeks (Fig. 4). However, higher δ^{13}C values of *O. grillus* were observed in fertilized creeks, suggesting an increase in the use of *Spartina* detritus as a basal resource. Increased dietary diversity was previously observed in seagrass (Armitage and Fourqurean 2009) and estuarine (Keats et al. 2004) food webs in response to changes in the availability of resources associated with fertilization. In PIE, chronic nutrient addition also enhanced macrophyte root and leaf decomposition rates (Deegan et al. 2012) potentially making *Spartina* detritus and/or associated microbes more attractive or accessible to *O. grillus*. However, the high grazing rates of *O. grillus* on benthic algae and its increased abundance and utilization of the creek-wall habitat with nutrient enrichment (due to increased frequency of parasitic infections) suggest that this species has the potential to impact benthic algal standing crop in areas undergoing chronic enrichment.

Per capita grazing rates of two gastropods were higher in CFC than in SFC, suggesting that dietary changes were slow to develop. A similar trend was observed in local mudflat grazers of PIE (Pascal et al. 2013), suggesting that eutrophication generally may have cumulative effects that are not apparent within a year for algal grazers. Similarly, food web changes in a tundra river fertilization experiment were different between first and following years of fertilization (Peterson et al. 1993). Thus, some effects of nutrient enrichment on food webs may take years to appear.

The intensity and timing of benthic algal responses to long-term nutrient enrichment has been found to differ among subhabitats types within PIE (Mitwally and Fleeger 2013). Benthic algal biomass in tall form *S. alterniflora* (at the marsh-creek edge) increased by the fourth year of fertilization, and responses in mudflat and creek-wall algal communities were smaller, slower, and variable over time (Mitwally and Fleeger 2013). Benthic algal biomass did not differ between the reference and nutrient enriched creeks at the start of our grazing experiments. One explanation for the slow pace of change in benthic algal biomass, even if algal production is increased by nutrient enrichment, is that increased grazing exerts top–down control slowing increases in algal standing crop with nutrient enrichment (Hillebrand et al. 2000, 2002). Increased algal biomass is often used as an indicator of eutrophication (Juanes et al. 2008; Giordani et al. 2009; Ferreira et al. 2011). Top–down control of grazers can potentially mask bottom-up effects of nutrient enrichment in marsh creeks (Posey et al. 1995) and mudflats (Pascal et al. 2013). Grazer control could similarly mask the increase of algal biomass in the creek-wall habitat.

The TIDE project has a unique potential to gauge responses to nutrient enrichment at large spatial scales because nutrient-enriched creek water spreads across the landscape with the rising tide. Benthic grazers may control the rate of change in nutrient-enriched marshes because they can limit or slow the rate of increase in algal biomass. Several taxa (*N. obsoletus*, *O. grillus*, *M. bidentatus*, *Hydrobia* spp., nematodes, ostracods, and mummichogs) have been shown to increase in abundance and/or to increase per capita grazing rates on benthic algae with nutrient enrichment, and TIDE research to date suggests that the species most responsible for increased grazing pressure with nutrient enrichment vary across the landscape. In the creek-wall habitat, *O. grillus* is the largest and most active epifaunal taxon at low tide, and utilization of the creek-wall habitat increases with nutrient enrichment (Johnson and Fleeger 2009). This increased abundance coupled with relatively high per capita algal grazing rates indicate that *O. grillus* contributes most among benthic species to grazing pressure on filamentous and epiphytic algae of nutrient enriched creek walls. In the mudflat habitat, large increases in the population density of *N. obsoletus* and increases in per capita grazing rates by epifaunal and meiofaunal grazers with chronic nutrient enrichment lead to an increase in grazing pressure of 235 % on the epipelic algal community (Pascal et al. 2013). *Fundulus heteroclitus* (the mummichog) also increased in abundance in nutrient enriched creeks, and a surrogate for algal grazing rate (gut content photosynthetic pigment concentration) increased by 115 % with nutrient enrichment (Lockfield 2011). As a result, this abundant nektonic species likely contributed to increased grazing pressure on filamentous algae in creek-wall habitat, marsh edge, and high marsh habitats, and manipulative reductions in abundance led to increases in algal standing crop in PIE (Deegan et al. 2007). On the marsh platform, the abundance of *M. bidentatus* approximately doubled with chronic nutrient enrichment (Johnson 2011). Although usually not considered a grazer of benthic algae, we found high grazing rates and a large increase in per capita grazing rates, suggesting that this species increases grazing pressure on benthic algae in areas experiencing chronic nutrient enrichment. Our research suggests that many species have the potential to contribute to increased grazing pressure with nutrient enrichment in salt marshes.

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