Response of the benthic food web to short- and long-term nutrient enrichment in saltmarsh mudflats

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ABSTRACT: We examined the responses of biota at or near the base of the benthic food web to nutrient enrichment in salt marsh mudflats in Plum Island estuary (Massachusetts, USA). To simulate eutrophication, nitrate and phosphate loading rates were increased 10- to 15-fold in creeks fertilized for 2 mo (i.e. short-term enrichment) or 6 yr (chronic enrichment). We found that benthic invertebrate community structure was not altered by nutrient enrichment, although the abundance of epifaunal, but not infaunal, grazers increased. Short-term enrichment had no effect on the food web, but significant changes were detected with chronic enrichment. Grazing experiments with ^{15}N-enriched bacteria and ^{13}C-enriched benthic algae revealed higher per capita ingestion rates of benthic microalgae by nematodes, copepods and hydrobiid snails in the creek with chronic nutrient enrichment where isotope composition also indicated that algae increased in dietary importance. The fraction of bacterial biomass grazed was not affected by nutrient enrichment; however, the fraction of benthic algal biomass grazed increased by 235% with chronic enrichment. This higher grazing pressure was partly the result of dietary changes (increases in per capita feeding rate or a change in selection) but was mostly due to an increased abundance of the grazing consumer with the highest biomass, the snail *Nassarius obsoletus*. This increased top-down control partially masked the bottom-up effects of nutrient enrichment on algal biomass and helps explain the slow and inconsistent response of microalgal biomass to chronic nutrient enrichment previously observed in this estuary. Our research shows that eutrophication may subtly affect benthic food webs before large, sustained increases in algal biomass are observed.

INTRODUCTION

Anthropogenic activities have contributed to a doubling of the rate of nitrogen entering the global biogeochemical cycle (Galloway et al. 2008, Gruber & Galloway 2008, Canfield et al. 2010). Through river, groundwater and atmospheric transport, a large fraction of this excess nutrient load passes through coastal ecosystems (Howarth et al. 1996, Nixon et al. 1996) causing harmful algal blooms, hypoxia and fisheries losses (Conley et al. 2009). Nutrient loading and subsequent eutrophication have been documented worldwide in coastal environments (Diaz & Rosenberg 2008), becoming major issues for both scientists and managers. Among coastal environments, salt marshes appear particularly sensitive, experiencing accelerated degradation and habitat loss (Silliman et al. 2010, Deegan et al. 2012).

The effects of eutrophication on salt marsh function are difficult to interpret and predict. One reason is that benthic food webs are complex, and, in general, the nature and strength of interactions are poorly understood. Chronic nutrient enrichment is expected to increase the biomass and/or productivity of the 2 dominant primary producers in salt marshes: cordgrass *Spartina* spp., primarily as aboveground production, and benthic algae (McComb 1995, Bertness et al. 2002, Deegan et al. 2007, Ferreira et al. 2011). This bottom-up stimulation extends to benthic bacteria because bacteria utilize labile organic matter released by benthic microalgae, which increases with nutrient enrichment (Bowen et al. 2009a). Bottom-up stimulation may also extend to infaunal and epifaunal invertebrates that exploit benthic bacteria and algae as food resources (Posey et al. 1999, Johnson & Fleeger 2009, Wimp et al. 2010). An increased per capita consumption by benthic grazers associated with an increased food supply may, over time, stimulate an increase in invertebrate biomass (Bertness et al. 2008, Johnson 2011). Increases in per capita grazing rates and/or grazer biomass both have the effect of increasing grazing pressure on bacteria and algae, possibly enhancing top-down control which, in turn, may limit the increase in biomass of benthic algae caused by eutrophication (Sardá et al. 1996, Posey et al. 2002). Because nutrient enrichment tends to increase primary producer biomass and grazing tends to decrease it, primary producer responses to nutrient enrichment depend on the relative strength of bottom-up and top-down forces (Hillebrand et al. 2000, 2002, Haglund & Hillebrand 2005). Studies comparing the relative importance of these forces as they affect organismal abundance are becoming common (e.g. Posey et al. 2002, Hillebrand et al. 2007, Johnson et al. 2009). Our study however differs from previous work because we also examined the rates of consumption of benthic algae by both infaunal and epifaunal consumers to better elucidate the mechanisms by which benthic food webs change with nutrient enrichment.

We took advantage of a whole-ecosystem manipulation (The USA 'TIDE' project; Deegan et al. 2007, 2012) designed to investigate the effects of nutrient enrichment on salt marshes in the Plum Island estuary, Massachusetts, USA. 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 whole growing seasons since 2004. Excess fertilizer and phytoplankton production are removed on the falling tide due to the local 3 m tidal range and nutrients diluted in the surrounding larger waterways (Drake et al. 2009). The large spatial (60000 m^2) and temporal (>6 yr of seasonal nutrient enrichment) scales over which TIDE is being conducted make this experimental perturbation more similar to human-induced eutrophication than the typical application of dry fertilizer to small plots. These features should contribute to a better understanding of the rate and mechanisms of change associated with chronic nutrient enrichment.

We examined variation in the natural abundance of isotope concentrations and conducted grazing experiments, with simultaneous dual labeling of bacteria and benthic algae (Pascal et al. 2008a), to clarify our understanding of the effects of nutrient enrichment history on mudflat food webs. The results allowed us to estimate per capita and, based on density estimates, population-level grazing rates across the diversity of benthic grazers that occur with a high biomass or a high abundance. Our labeling and isotope studies allowed an evaluation of the effects of nutrient enrichment on the ability of each grazer to select food, quantifying changes in diet. These methods also allowed an appraisal of the influence of nutrient enrichment on top-down control by summing the quantity of bacteria and benthic algae consumed by the total community of benthic grazers. The goal of the present study was therefore to evaluate food web changes induced by nutrient enrichment in the low intertidal marsh environment, the mudflat, in TIDE creeks.

MATERIALS AND METHODS

TIDE project

TIDE stands for 'Trophic cascades and Interacting control processes in a Detritus-based aquatic Ecosystem' and is an ecosystem-level nutrient-enrichment experiment in the 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 *S. patens* are the dominant macrophytes on the creek edge and marsh platform in the seawater-dominated intertidal zone where the salinity ranges from 8 to 28‰. Plant zonation in PIE is considered typical of New England estuaries (Niering & Warren 1980), and PIE has historically been unaffected by anthropogenic nutrient loading (background nutrients: <5 µM NO_3^-; 1 µM PO_4^{3-}). Located in the lower intertidal area, mudflats consist of gently sloping creek habitat of poorly consolidated sediments without macrophytes (Fig. 1).

Nutrient-enrichment experiments were conducted in 3 creeks (West, Clubhead, Sweeney) having similar water chemistry, plant and infaunal communities (details in Deegan et al. 2007, Johnson et al. 2007). Physical dimensions were also similar, with the watershed area (including the marsh platform) ranging between 8.4 and 12.4 ha and the mean tidal exchange volume ranging from 10560 to 21170 m^3. Furthermore, all creeks drain completely at low tide before being replenished with unenriched water from surrounding waterways on the rising tide (Drake et al. 2009). Sweeney (hereafter referred to as the chronically fertilized creek or CFC) was enriched for 6 field seasons (2004 to 2009), Clubhead (hereafter referred to as the short-term fertilized creek or SFC) was enriched for about 2 mo (2009) and West (hereafter referred to as the reference creek or RC) was not enriched. In order to maintain a relatively constant enrichment through each tidal cycle, fertilizer was pumped into the creek at flow rates based on a hydrologic model that estimates the volume of creek flooding water throughout each tidal cycle. In CFC for approximately 150 d during the growing season, nitrate and phosphate levels were increased on each rising tide to approximately 70 µmol l^{-1} NO_3^- and 4 µmol l^{-1} PO_4^{3-}, resulting in a loading rate of ~40 g N m^{-2} yr^{-1}. For SFC, nutrient enrichment was identical to CFC, but began about 2 mo before our experiments were conducted. Nutrient concentrations were chosen because they designate an estuary to be 'moderately to severely eutrophied' (US EPA 2002). Northeastern US estuaries have experienced a large range in anthropogenically derived nutrient enrichment, mostly due to variation in sewage input and differences in the regional frequency and types of agriculture practiced. However, the total inorganic nitrogen concentrations achieved by fertilization in CFC and SFC were similar to levels found in eutrophic Jamaica Bay, New York, USA, although TIDE nitrogen loading rates were about an order of magnitude lower than most plot-level nutrient enrichment studies that add dry fertilizer to salt marsh sediments (Deegan et al. 2012). Detailed background information on experimental design and biogeochemistry of the tidal creeks can be found in Deegan et al. (2007, 2012) and Drake et al. (2009).

Natural abundance isotope analysis

All biological samples (i.e. for isotope analysis, density estimates and grazing experiments) were collected in the mudflats of each creek, at similar tidal elevation in areas with notable physical similarities. Sample sites in nutrient-enriched creeks were located ~100 m upstream from the point at which fertilizer was added. An analogous location was selected for collections in RC. All samples were collected during the second week of July, 2009.

For stable isotope analysis of the natural abundance of meiofauna, the upper 0.5 cm layer of sediment was collected from 3 replicate plots of 900 cm^2 in each creek and frozen. In the laboratory, samples were thawed, centrifuged with Ludox (de Jonge & Bouwman 1977), and ≥700 nematode, ≥300 copepod and ≥10 ostracod specimens were haphazardly removed and retained from each replicate. After centrifugation, foraminifers in the pellet fraction were stained with Rose Bengal, and 300 individuals were picked from each replicate. Each specimen was cleaned of debris and rinsed. Taxa containing high concentrations of calcium carbonate (i.e. foraminifera and ostracods) were decalcified with 2.5% HCl. All specimens from each taxon were pooled from a sample and then dried for isotope analysis ($n = 3$).

Snails were collected by hand near each of the plots used to sample meiofauna. Fifteen specimens of hydrobiid snails were removed from the sediment from each sample, and snail tissue was subsequently separated from their shells using forceps and pooled (mean longer-shell height ± SD = 1.8 ± 0.4 mm). Three groups of 15 small (mean longer-shell height ± SD = 6.7 ± 1.3 mm) and 10 large (mean longer-shell height ± SD = 16.3 ± 1.5 mm) specimens of *Nassarius obsoletus* were also located and pooled by size. Snail tissues were homogenized using a tissue grinder and freeze-dried before isotope analyses ($n = 3$).

Samples were analyzed at the Isotope Facility at the University of California, Davis, using an elemental-analyzer isotope ratio mass spectrometer. Samples were reported relative to the standards atmospheric N_2 and Vienna PeeDee Belemnite carbon. Stable isotope values are reported in δ notation:

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

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

Grazing experiments

^{15}N-enrichment of bacteria was carried out using the methods of Pascal et al. (2008b). Briefly, surficial sediment was sampled to a depth of 0.5 cm during the ebbing tide in RC. Bacteria were cultured in a liquid bacterial medium containing 1 g l^{-1} of ^{15}NH$_4$Cl (>99% ^{15}N-enriched). Cultured bacteria were collected from their medium by centrifugation, frozen in liquid nitrogen and stored in a freezer (–20°C) until used in grazing experiments. Enriched bacteria abundance was 6.4×10^9 bacteria ml^{-1}, and bacterium-produced nitrogen content was 12.28% ^{15}N.

In each creek mudflat, 4 randomly placed plots of 900 cm^2 were staked. NaH^{13}CO$_3$ (>99% ^{13}C-enriched) was dissolved in seawater and applied daily over 4 d with a garden sprayer directly to the sediment surface of each plot in each creek at a concentration of 1 g m^{-2} (Middelburg et al. 2000). After spraying was completed, surficial sediment (0.5 cm) of each plot was collected, pooled from each creek and sieved, and the sediment fraction retained on a 63 µm mesh containing meiofauna was removed. Three subsamples of sediment from each creek aggregate were freeze-dried, and phospholipid-derived fatty acids (PLFA) were extracted and their isotopic composition was determined (n = 3) using a gas-chromatograph combustion-interface isotope-ratio mass spectrometer (GC-c-IRMS) as in Boschker et al. (1999). Concentrations and δ^{13}C PLFA specific to diatoms (C20:4ω6, C20:5ω3, C22:5ω3 and C22:6ω3), green algae and cyanobacteria (C18:2ω6c, C18:3ω3 and C18:4ω3) and bacteria (i14:0, i15:0, ai15:0, i16:0 and cy19:0) were used to estimate the relative contribution of these groups to the total PLFA pool and their weighted-average δ^{13}C composition (Table 1).

^{13}C-enriched sediment from each creek was mixed with ^{15}N-enriched bacteria, and 157 ml of this slurry was amended to each of 3 experimental and 3 control incubation microcosms

(314 cm^2) established for each creek. Bacterial abundance was estimated (see below), and the ratios of total bacteria to ^{15}N-enriched bacteria in slurry sediments from RC, SFC and CFC were, respectively, 1.86, 1.80 and 1.81. Superficial sediment (upper 0.5 cm) was collected in each creek, and 157 ml of this sediment was sieved on a 63 µm mesh. This fraction of sediment containing meiofauna was added to the experimental microcosms from each creek along with 10 specimens of hydrobiid snails, 10 large and 15 small specimens (size previously described) of *Nassarius obsoletus* collected from the same creek. Three controls were also performed from each creek in which fauna were added to microcosms in the quantities described above but after being frozen (–20°C) for 24 h to induce mortality. All incubations were for 5 h and were conducted at the same time, in similar conditions of light and temperature, close to natural ones. To ensure a near-instantaneous cessation of feeding, incubations were ended by sieving sediment on 63 µm mesh and freezing the retained sediment (–20°C). Samples were later thawed, and all isotope samples were prepared and analyzed as previously described.

Isotopic analysis, including the measurement of carbon and nitrogen isotope ratios and carbon and nitrogen content, was conducted on pooled specimens of each microcosm (n = 6). Incorporation of ^{15}N from bacteria into grazers is defined as excess above background ^{15}N (control experiment) and is expressed in terms of specific uptake (I). I was calculated as the product of excess ^{15}N (E) and biomass of N per taxon. I was converted to bacterial carbon grazed using the C/N ratio of bacteria. E is the difference between the background ($F_{\text{background}}$) and the sample (F_{sample}) ^{15}N fraction: $E = F_{\text{sample}} - F_{\text{background}}$, with $F = {}^{15}N/({}^{15}N + {}^{14}N) = R/(R + 2)$ where R is the nitrogen isotope ratio. For $F_{\text{background}}$, we used values measured with the control (frozen) taxa. R was derived from the measured $\delta^{15}N$ values: $R = [(\delta^{15}N / 1000) + 1] \times R_{\text{airN2}}$. The uptake of bacteria was calculated as $[I \times (\%C_{\text{enriched bacteria}} / \%N_{\text{enriched bacteria}}) / (F_{\text{enriched bacteria}} \times \text{incubation time})]$. This uptake was multiplied by the ratio between the abundance of total and enriched bacteria. Incorporation of ^{13}C, and thus benthic microalgae (BMA), into grazers was calculated analogously, with $F = {}^{13}\mathrm{C} / ({}^{13}\mathrm{C} + {}^{12}\mathrm{C}) = R / (R + 1)$, R_{airN2} is replaced by R_{VPDB} and uptake = $I / (F_{\mathrm{enriched\ BMA}} \times \mathrm{incubation\ time})$. $F_{\mathrm{enriched\ BMA}}$ was determined using δ^{13}C of PLFA specific to diatoms.

Taxon-specific, individual weights were derived from stable isotope samples and were used in grazing rate calculations. Weights averaged ± SD were 344.1 ± 122.2 ng nematode^{-1} (a total of **189000** specimens were examined isotopically from which weight was derived), 757.4 ± 276.8 ng copepod^{-1} (**81000** specimens), 17.1 ± 3.4 µg foraminifera^{-1} (**81000** specimens, after acid treatment), 64.5 ± 13.1 µg ostracod^{-1} (270 specimens, after acid treatment), 92.7 ± 32.4 µg hydrobiid^{-1} (405 specimens, after shell removal), 4.2 ± 2.6 mg small *Nassarius obsoletus*$^{-1}$ (405 specimens, after shell removal) and 41.9 ± 10.4 mg large *N. obsoletus*$^{-1}$ (270 specimens, after shell removal).

Population and community grazing rates

In order to estimate the biomass of bacteria and benthic algae on a surface area basis at the time of grazing experiments, the upper 0.5 cm layer of sediment was collected from 3 plots of 900 cm^2 in each creek and homogenized. Three subsamples of this sediment (each 20 ml) were drawn from each homogenate for bacterial and algal analysis. Bacteria were extracted from sediment particles (Carman 1993), stained using DAPI (2500 µg l^{-1}), filtered onto 0.2 µm Nucleopore black filters, and then counted under an epifluorescence microscope to estimate abundance (n = 3 from each creek). Bacterial carbon content was estimated from bacterial abundances using conversion factors (Epstein 1997). Benthic microalgal biomass was assessed using chlorophyll *a* (chl *a*) concentration as a proxy (n = 3 from each creek) and measured after extraction with acetone using a Turner fluorometer (APHA 1992). Values were compared to the studies of Bowen et al. (2009a) and Mitwally & Fleeger (H. M. Mitwally & J. W. Fleeger unpubl. data) who examined the effects of nutrient enrichment on benthic algae in CFC and RC. Algal biomass in units of carbon was estimated using a carbon:chl *a* ratio of 40 (de Jonge 1980). Microalgal production was estimated using empirically developed relationships between benthic chl *a* biomass and microalgal production for estuarine mudflats (Pinckney 1994): production (mg C m^{-1} yr^{-1}) = (3.2087 × biomass) – 0.0903, where biomass is in milligrams of chl *a* per square meter.

For meiofaunal abundance, 2 sediment cores (2.2 cm inner diameter) were sampled at each site, fixed with 10% formalin and stained with Rose Bengal. Nematodes, copepods and ostracods were extracted using Ludox centrifugation and enumerated (de Jonge & Bouwman 1977). Stained foraminifera from the pellet fraction were counted. Mature copepods were identified to species. At least 80 nematode specimens per sample were categorized by trophic group (Jensen 1987). In addition, our abundance estimates were compared to those generated by a long-term study of nutrient-enrichment effects on meiofauna (H. M. Mitwally & J. W. Fleeger unpubl. data) in which meiofaunal samples were collected in late summer in each year of fertilization in CFC and RC from 2003 to 2009.

Hydrobiid snail abundances were estimated by enumerating specimens in the previously described meiofaunal cores. Abundances of the 2 size-class groups of *Nassarius obsoletus* (<14 and >14 mm) in each creek (n = 10 quadrats $creek^{-1}$) were derived from extensive observations in a study of the effects of nutrient enrichment on snail density in 2009 in CFC and RC (Johnson & Short 2012).

Mean meiofaunal (n = 2 in each creek) and epifaunal (n = 10 in each creek) densities were multiplied by mean grazing rates (n = 3 per taxon in each creek) to evaluate the total amount of bacteria and algae grazed by each taxon. Grazing was also summed across all taxa to estimate a community grazing rate. This total amount was compared to bacterial and algal biomasses (n = 3 in each creek) to evaluate the amount of prey standing stock grazed per unit of time.

Data analyses

To examine variation in nematode trophic guild composition and copepod community structure among creeks, analysis of similarity (ANOSIM) was conducted with PRIMER 5.2.9 software (Clarke & Warwick 2001). One-way analysis of variance (ANOVA) was used to test for differences in abundances, individual grazing rates and total amount of prey grazed in each creek.

RESULTS

Population responses and community structure

Bacterial biomass at the time of the study was equivalent in all creeks (Table 2). Microalgal biomass ranged from 15 to 45 mg chl *a* m^{-2} and was less than similar estimates derived from previous studies conducted in the same creeks, i.e. 73 to 88 mg chl *a* m^{-2} (Bowen et al. 2009a) and 51 to 101 mg chl *a* m^{-2} (H. M. Mitwally & J. W. Fleeger unpubl. data). Benthic algal biomass significantly differed among creeks at the time of the experiment, with the highest concentration of chl *a* in CFC and the lowest in SFC.

Mean total abundances of infauna at the time of the grazing experiments were very similar to the long-term averages found by Mitwally & Fleeger (H. M.Mitwally & J. W. Fleeger unpubl. data), and ranges expressed as 10^3 ind. m^{-2} almost completely overlapped for nematodes 287 to 1167 versus 172 to 1783 and ostracods 33 to 98 versus 2 to 112. However, for copepods, means were higher at the time of the grazing experiments (267 to 562 versus 20 to 130). The density of *Nassarius obsoletus* was significantly (4-fold) higher in CFC than RC (Johnson & Short 2012).

We used the composition of trophic groups as a surrogate for the functional diversity of nematodes. Trophic groups did not differ among creeks based on ANOSIM (not significant). Because this analysis did not reveal a creek effect, data from all creeks were pooled, and the overall nematode community was composed of 0.5% deposit feeders, 1.1% scavengers, 41.1% epistrate feeders and 57.3% predators. Similarly, species composition of adult benthic copepods did not differ among creeks (ANOSIM, not significant), and data from each creek were consequently pooled. The relative abundances of the 5 most abundant copepod species in this community were *Stenhelia* sp. at 20%, *Pseudobradya* sp. at 17%, *Enhydrosoma* sp. at 17%, *Scottolana canadensis* at 15% and *Microarthridon littorale* at 8%.

Natural abundance isotopic composition

The natural isotope abundance of large *Nassarius obsoletus* contained relatively more ^{13}C than did other grazers in RC (**Fig. 2**). Small *N. obsoletus*, hydrobiid snails, foraminiferans and ostracods in RC had a similar natural abundance of carbon isotope but copepods were more depleted in ^{13}C compared to the other taxa. Ostracods were depleted in ^{15}N compared to other taxa (Fig. 2).

Confidence intervals for the natural abundance of isotope composition among creeks generally overlapped (**Fig. 3**). However, 2 trends were observed in all creeks: all grazers from RC were more enriched in ^{15}N and, except for small *N. obsoletus*, all taxa from CFC were more depleted in ^{13}C. The most common method of fertilizer production is industrial fixation of atmospheric nitrogen, resulting in fertilizer products with δ^{15}N values close to zero.

Nutrient enrichment in CFC likely led to decreased algal $\delta^{15}N$ ratios, and grazers consuming those algae should be similarly depleted.

Individual grazing rates

Mean per capita grazing rates on bacteria measured in each creek ranged between 12 and 20 pg C ind.$^{-1}$ h^{-1} for nematodes, 78 and 118 pg C ind.$^{-1}$ h^{-1} for copepods, 15 and 26 pg C ind.$^{-1}$ h^{-1} for foraminiferans, 336 and 397 pg C ind.$^{-1}$ h^{-1} for ostracods, 31 and 50 ng C ind.$^{-1}$ h^{-1} for hydrobiid snails, 1.1 and 1.4 µg C ind.$^{-1}$ h^{-1} for small *Nassarius obsoletus* and 3.3 and 3.4 µg C ind.$^{-1}$ h^{-1} for large *N. obsoletus* (**Fig. 4**). Nematodes from CFC grazed bacteria at a significantly lower per capita rate than nematodes from the other creeks (ANOVA, $p < 0.01$). All other taxa ingested bacteria at a similar rate, regardless of nutrient enrichment history (ANOVA, not significant).

Mean per capita grazing rates of benthic algae measured in each creek ranged between 41 and 395 pg C ind.$^{-1}$ h^{-1} for nematodes, 1012 and 2369 pg C ind.$^{-1}$ h^{-1} for copepods, 31 and 77 pg C ind.$^{-1}$ h^{-1} for foraminifera, 344 and 6046 ng C ind.$^{-1}$ h^{-1} for ostracods, 47 and 167 ng C ind.$^{-1}$ h^{-1} for hydrobiid snails, 0.5 and 1.0 µg C ind.$^{-1}$ h^{-1} for small *Nassarius obsoletus* and 1.1 and 2.3 µg C ind.$^{-1}$ h^{-1} for large *N. obsoletus* (**Fig. 5**). Ingestion of algae was significantly higher in CFC than in the other 2 creeks for copepods (ANOVA, $p < 0.05$) and hydrobiid snails (ANOVA, $p < 0.01$). Only nematodes and ostracods differed, with lower per capita ingestion at SFC (ANOVA, $p < 0.05$ and $p < 0.01$, respectively). Foraminiferans and *N. obsoletus* (small and large) ingested benthic microalgae at similar rates in all creeks (ANOVA, not significant).

Specific fatty acids of diatoms and bacteria allow evaluation of their respective biomasses. The ratio of diatoms:bacteria in RC sediment used for grazing experiments was

1.9 ± 1.2 (Table 1). Ingestion rates of bacteria and BMA were measured simultaneously during the grazing experiment. When compared to the ratio of food sources available, the ratio of BMA:bacteria grazed indicates food selection (Pascal et al. 2008a). In RC, this ratio was 10.5 ± 1.9 for nematodes, 17.1 ± 3.1 for copepods, 6.4 ± 4.5 for foraminifera, 16.9 ± 2.4 for ostracods, 2.4 ± 0.6 for hydrobiids, 0.7 ± 0.1 for small *Nassarius obsoletus* and 0.6 ± 0.2 for large *N. obsoletus*. In RC, this ratio was not significantly different between small and large *N. obsoletus* (ANOVA, not significant). This ratio was significantly higher for nematodes and hydrobiids from CFC than from the other 2 creeks (ANOVA, $p < 0.01$).

Population and community grazing rates

Population-level grazing rates (m^{-2}) on bacteria were calculated as the product of the per capita grazing rate and density for each taxon (Table 3). Nematodes and large *Nassarius obsoletus* from CFC and copepods from SFC ingested significantly more bacteria than in the other creeks (ANOVA, $p < 0.05$ and $p < 0.01$, respectively). Hydrobiid snails and small *N. obsoletus* from RC ingested significantly fewer bacteria than in the other creeks (ANOVA, $p < 0.01$ and $p < 0.05$, respectively). The amount of bacteria grazed by total meiofauna, total epifauna and total fauna did not differ significantly in the 3 treatment creeks (ANOVA, not significant). The entire community of benthic invertebrates ingested, respectively, 4.2, 9.3 and 14.5% of bacterial biomass daily in RC, SFC and CFC.

Population-level grazing rates (m^{-2}) on benthic algae were calculated as the product of the per capita grazing rate and density for each taxon (Table 4). Nematodes, hydrobiid snails and large *Nassarius obsoletus* ingested significantly more algae in CFC than in the other creeks (ANOVA, $p < 0.001$, $p < 0.01$ and $p < 0.01$, respectively). Population-level grazing was summed across all taxa, and was significantly higher in CFC than in other creeks

(ANOVA, $p < 0.01$). Together, the community of benthic invertebrates ingested daily 235% more benthic algae in CFC than in RC. Compared to the algal biomass of each creek, the community of grazers ingested daily 1.9, 5.1 and 4.7% of algal biomass, respectively, in RF, SFC and CFC. The grazing community ingested 9.9, 26.3 and 23.9% of benthic algal production, respectively, in RF, SFC and CFC. Overall, hydrobiid snails consumed more benthic algae than any other taxon. However, the fraction ingested by *N. obsoletus* showed the largest increase in CFC, and *N. obsoletus* accounted for about 40% of all benthic microalgae grazed under the conditions of chronic nutrient enrichment.

DISCUSSION

We studied the effects of nutrient enrichment on benthic food webs in mudflats, with emphasis on the potential for top-down control over benthic microalgae and bacteria. Nutrient enrichment may alter the food web in 3 ways that could act singly or in combination: (1) benthic grazers may change their dietary preference by becoming more selective in their diet, (2) per capita grazing rates of individual taxa on bacteria or algae may vary and (3) changes in the population density of grazing species may affect the total consumption of algae or bacteria. We found that the food web was altered in the creek with a long history (6 yr) of nutrient enrichment through all 3 mechanisms. Some grazers became more selective in their diet, increasing their preference for algae over bacteria, and most grazers increased per capita consumption rates of algae with chronic nutrient enrichment. In addition, the epifaunal snail *Nassarius obsoletus* responded to nutrient enrichment with increased abundance (Johnson & Short 2012), and contributed most to the increased grazing pressure in nutrient-enriched creeks. As a result, total community grazing on benthic algae increased with chronic nutrient enrichment.

Diet composition

The diet of most benthic grazers differed in the chronically nutrient-enriched creek. Isotope composition integrates diet over long-time intervals, and benthic invertebrates in PIE have relatively depleted natural abundance δ^{13}C values compared to salt marsh macrophyte detritus (Galván et al. 2008, 2011, present study), indicating an importance of benthic algae to their diet. However, 5 of the 6 taxa we examined had lower δ^{13}C values in CFC, consistent with increased long-term consumption of benthic algae (Fig. 3). We also calculated the ratio of ingested algae and bacteria from our simultaneous, dual-label experiment. This ratio is a function of the ability of the grazing species to select diatoms from the sediment–alga–bacterium aggregate. In the reference creek, ratios observed for nematodes (10.5), copepods (17.1) and ostracods (16.9) indicate that benthic diatoms were the primary carbon source, while ratios observed for hydrobiid snails (2.4) and for *Nassarius obsoletus* (0.6) were smaller, indicating a limited capacity to select algae. Previous research has also found that many meiofauna selectively ingest food (Buffan-Dubau et al. 1996, Moens et al. 1999, Buffan-Dubau & Carman 2000, Wyckmans et al. 2007), often preferring diatoms, while estuarine snails have limited selectivity (Brown 1969, Lopez & Kofoed 1980, Levinton 1987, Haubois et al. 2005). However, nematodes and hydrobiid snails became significantly more selective in their diet in the chronically nutrient-enriched creek, increasing their preference for benthic microalgae over bacteria. Finally, short-term grazing rate experiments revealed that nematodes, copepods and hydrobiid snails ingested more microalgae on a per capita basis under conditions of chronic eutrophication. The per capita grazing rate on bacteria was altered in only 1 taxon (such that nematode grazing rates were significantly lower in CFC).

Previous research has shown that opportunistic species may benefit from nutrient enrichment, leading to communities dominated by generalist feeders (Heip 1995, Keats et al. 2004). However, we found no evidence for a change in benthic community structure in our study creeks, as the macroinfaunal community (Johnson & Fleeger 2009), the relative abundance of nematode trophic groups and the species composition of meiobenthic copepods were all unaffected by nutrient enrichment. Moreover *Nassarius obsoletus* was the most abundant species of epifauna regardless of nutrient enrichment history (Johnson & Short 2012). The dietary changes we observed at the community scale were therefore due to changes in feeding behavior or population density at the species level rather than a change in community composition. Previous studies in estuaries also indicate considerable diet plasticity in benthic grazers. Diet become more varied (Armitage & Fourqurean 2009) and omnivores switched to herbivory (Keats et al. 2004, Fox et al. 2009) when the abundance of the basal resource was altered by nutrient enrichment. In a previous study of nutrient enrichment in PIE, 2 surface-feeding polychaete species switched to a microalgal resource after 10 wk of enrichment (Galván 2008). Estuarine grazers appear to readily adapt to changing food resource availability in their environment under conditions of nutrient enrichment.

Total consumption of bacteria and algae

We calculated population-level grazing rates by multiplying per capita grazing rates with population density. We then employed an estimate of grazing by the benthic community as a whole by summing grazing across the populations of benthic grazers. At the population level, the non-selective epifaunal snails, hydrobiids and *Nassarius obsoletus* were responsible for the greatest grazing pressure on bacteria and microalgae (averaging from 64 to 98% of all grazing) (Figs. 4, 5 & 6). The potential to exert top-down control over benthic algae and bacteria is therefore much greater for epifauna than for meiofauna, as has been found in other mudflats (van Oevelen et al. 2006, Pascal et al. 2009).

Previous research at our study site has shown that nutrient enrichment indirectly benefits the microbial community because of increased algal exudation, although bacterial productivity and community structure are unaffected by nutrient enrichment (Bowen et al. 2009a,b, 2011). A similar indirect nutrient effect was observed on bacteria associated with periphyton (Hepinstall & Fuller 1994, Hillebrand et al. 2002). Our research shows that consumption of bacteria by the grazing community was not affected by nutrient enrichment, even though the most important grazer, *Nassarius obsoletus*, increased in density and population-level consumption of bacteria in CFC. We estimate that the total community of grazers ingested between 3 and 14% of the bacterial standing stock each day. These observations suggest grazing by benthic invertebrates did not mask a bottom-up stimulation of bacterial production and that increased grazing had little effect on the biomass of bacteria. Mudflat bacterial communities appear to be strongly resistant to change from exogenous perturbation to the food web (Bowen et al. 2009b, 2011), including from grazing invertebrates.

Algal biomass in mudflats was subtly (if at all), rather than dramatically, altered in nutrient-enriched creeks after 6 yr of fertilization in PIE (H. M. Mitwally & J. W. Fleeger unpubl. data). This absence of a persistent and easily perceivable change in algal biomass in the chronically nutrient-enriched creek mudflat is of interest because algal biomass is often used as an indicator of eutrophication (Juanes et al. 2008, Giordani et al. 2009, Ferreira et al. 2011). Our research allowed us to determine what role, if any, benthic grazing had in maintaining the levels of algal biomass in mudflats experiencing nutrient enrichment.

Daily grazing by the community of meiofauna and small macroinfauna has been shown to exceed the benthic algal standing stock (Montagna et al. 1995, Carman et al. 1997). Daily consumption by epifauna has also been shown to exceed benthic algal biomass (Connor et al. 1982). Although grazing pressure by benthic invertebrates at our study site did not reach the levels observed in the studies cited above, it was substantial. Meiofauna and epifauna together ingested 2 to 5% of algal stock daily, and the proportion of the daily algal primary production consumed increased from 10% in the reference creek to 24% in the creek with chronic nutrient enrichment. Total grazing pressure on benthic algae in the creek with chronic nutrient enrichment was increased by 235% compared to the reference creek. The difference in algal production grazed between RC and CFC (about 20% more of primary production was grazed per day at CFC) suggests that algal biomass at CFC would increase in the absence of benthic grazers and that algae would reach a higher maximum biomass (Blanchard et al. 2001). This difference is relatively small in the absolute amount of benthic algal biomass, but the increased grazing pressure on benthic algae lessened potential increases in algal biomass associated with chronic eutrophication. Similar results were observed in a sandy environment (Hillebrand et al. 2000). Our results indicate that at least part of the slow and inconsistent response of benthic algae to nutrient enrichment in PIE mudflats was due to top-down control because increased grazing restrained changes in algal biomass.

Our results also show that nutrient-enrichment effects on food webs may take longer than a single growing season to occur. Findings in the creek with short-term nutrient enrichment and the reference creek were very similar and contrasted sharply with those from the chronically fertilized creek. For example, natural abundance isotopes and per capita grazing rates in SFC were not increased compared to RC for any taxon. Similarly, benthic algal biomass did not increase during the first year (2004) of nutrient enrichment in CFC (data in Deegan et al. 2007), but increased by 60% in the second year of enrichment in treatments in which a nektonic grazer was reduced in abundance. Previous research has identified effects of nutrient enrichment on abundance or on consumer diet that occurred within weeks to months after nutrient enrichment was begun (Posey et al. 2006, McFarlin et al. 2008), while other studies have found that food web changes or abundance changes take longer to develop. For example, in a tundra river, fertilization experiment effects were also first noted in the second year of nutrient enrichment (Peterson et al. 1993), and Sardá et al. (1996) found that an infauna community was affected by nutrient enrichment, but over decadal time scales. Although some infaunal species at our study site responded quickly to nutrient enrichment by changes in dietary selectivity (Galván et al. 2008), large non-selective epifauna were the most important grazers at our mudflat site. These large species are slow growing, and changes in abundance occur over long time scales, perhaps explaining the lack of a rapid change in grazing pressure on benthic algae in the nutrient-enriched creeks at our study site.

Other considerations

Salt marsh nekton can consume benthic bacteria and algae but were not considered here. Mummichog *Fundulus heteroclitus* is a key omnivorous fish able to ingest algae, bulk detritus and the microbial community, as well as animal prey (D'avanzo & Valiela 1990). However, adults likely graze filamentous algae not found on mudflats (Lockfield 2011), and juveniles can graze on mudflat microalgae only for a short time as the tide rises over the mudflat before epipelic diatoms vertically migrate. Grass shrimp *Palaemonetes pugio* are not effective grazers on the epipelic microalgal community (Fleeger et al. 1999). Macroinfauna also consume benthic microalgae (Galván et al. 2008), although the biomass of epifaunal snails was >2 orders of magnitude higher than the combined biomasses of all macroinfauna (Johnson & Fleeger 2009, Johnson & Short 2012). Our estimation of total community grazing, therefore, focuses on the most abundant and highest biomass members of the infaunal (the meiofauna) and epifaunal (snail) communities.

Estimates of biomass are needed to evaluate the trophic fate of benthic microalgae and bacteria. Our algal and bacterial biomass estimates were based on 3 large (each 900 cm^2) sediment samples from each creek that were subsequently homogenized and subsampled. This procedure should reduce small-scale variation and increase confidence in the means we generated (Moreno & Niell 2004). To allow comparisons with algal grazing rates, algal abundance must be converted from units of chl *a* to carbon using a conversion factor known to vary (de Jonge 1980, Frost et al. 2005). Bacterial biomass measurements are also potentially biased because a conversion factor from volume to carbon content (Bratbak 1985) is required, and a physical separation of attached bacteria from sediment particles is necessary for accurate counts (Carman 1993). Like all methods previously developed and applied to measure benthic microalgal and bacterial biomasses, the methods used in the present study present potential methodological shortcomings that must be kept in mind when interpreting results.

Our calculation of algal ingestion is based on the assumption that enrichment of grazer tissue is due only to the ingestion of ^{13}C-enriched diatoms. However, other potential food resources may also have become enriched in ^{13}C. Fatty acid isotopic analysis revealed that green algae and cyanobacteria were, on average, 20% more enriched than diatoms. However, their contribution to grazer enrichment was limited because their biomasses were, on average, 1 order of magnitude lower than those of diatoms. Dominance by diatoms is common (Vander Grinten et al. 2004), particularly in muddy sediments (Watermann et al. 1999). PLFA-specific markers for bacteria were also enriched in ^{13}C, but bacterial dietary contributions may be limited because they were, on average, 5-fold less enriched than diatoms. Those potential biases could lead to a small overestimation of diatom importance in the diet of grazers.

However, grazing rates measured using the ^{15}N pre-labeled bacteria method were comparable with those reported for a French intertidal mudflat (Pascal et al. 2008b, 2009).

Another uncertainty in our calculations of the impact of the grazing community on microalgae and bacteria is associated with faunal density estimates. *Nassarius obsoletus* was the most important contributor to changes in the food web in CFC, and its density was surveyed during the time that the present study was conducted (Johnson & Short 2012). However, meiofauna were sampled with minimal replication at the time of our study because density was estimated simultaneously in the same creeks as part of a long-term assessment of nutrient-enrichment effects (H. M. Mitwally & J. W. Fleeger unpubl. data). This long-term assessment could not detect consistent effects on meiofauna associated with nutrient enrichment in the mudflat habitat, and mean values were very similar to those used in our calculations. To determine whether the conclusions of our estimates of grazing would be altered by using the long-term density estimates of meiofauna, we recalculated grazing rates using the meiofauna densities of Mitwally & Fleeger (H. M. Mitwally & J. W. Fleeger unpubl. data). The amount of algae grazed hourly by meiofauna increased by 11 and 10%, whereas the amount of bacteria grazed increased by 24 and 4%, respectively, in RC and CFC. These reduced fluctuations suggest that bias due to low replication of density measurements is limited.

CONCLUSIONS

Increased algal biomass is often used as an indicator of eutrophication (Juanes et al. 2008, Giordani et al. 2009, Ferreira et al. 2011). Similar to the conclusion of Schramm (1999), our research shows that top-down control by an extant benthic invertebrate community may mask the effects of eutrophication when measured by algal biomass.

Although changes in diet preference and increases in species-specific per capita grazing rates both contributed, grazing pressure on benthic algae increased largely because the abundance of the non-selective feeding snail *Nassarius obsoletus* increased with chronic nutrient enrichment. This increased abundance accounted for about 40% of the increase in community grazing rates. Our results also indicate that nutrient-induced changes in the benthic food web were observed sometime after 1 field season and before 6 yr of fertilization, and that large changes in benthic algal biomass were not observed during this time. Thus, we detected changes in food web structure that occurred prior to any large, sustained increases in algal biomass, suggesting that effects of nutrient enrichment on food webs may take place before eutrophication is apparent.

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Table 1. Contribution (%) of fatty acids (FA) specific to diatoms, green algae/cyanobacteria and bacteria to total fatty acid methyl esters (FAME) and weighted $\delta^{13}C$ of each compound in the 3 creeks studied (means ± SD; n = 3). RC: reference creek; SFC: short-term fertilized creek; CFC: chronically fertilized creek

	FAME (%)			Weighted $\delta^{13}C$ (%)		
	RC	SFC	CFC	RC	SFC	CFC
Diatom FA	15.9 ± 1.6	11.3 ± 0.7	17.0 ± 0.9	2973 ± 123	2697 ± 73	2180 ± 38
Green algal/cyanobacterial FA	1.8 ± 0.3	1.8 ± 0.1	1.6 ± 0.0	3164 ± 105	2633 ± 194	3654 ± 217
Bacterial FA	8.3 ± 0.1	10.8 ± 0.2	6.6 ± 0.1	472 ± 7	323 ± 6	808 ± 8

Table 2. Biomasses of bacteria and benthic microalgae (BMA) (in mg C m^{-2}, n = 3) of taxa in the 3 creeks studied. An asterisk indicates significant ($p < 0.01$) differences with the 2 other creeks (ANOVA; Tukey test) for meiofauna (nematodes, copepods, foraminiferans and ostracods, in 10^3 ind. m^{-2} and mg C m^{-2}, n = 2) and epifauna (hydrobiid snails and *Nassarius obsoletus*, in ind. m^{-2} and g C m^{-2}, n = 10). RC: reference creek; SFC: short-term fertilized creek; CFC: chronically fertilized creek

	RC	SFC	CFC
Bacteria (mg C m^{-2})	432 ± 26	408 ± 23	410 ± 53
BMA (mg C m^{-2})	1625 ± 58*	799 ± 72*	2256 ± 52*
Nematodes (10^3 ind. m^{-2})	317 ± 67	287 ± 9	1167 ± 8
Copepods (10^3 ind. m^{-2})	213 ± 74	367 ± 104	199 ± 41
Foraminiferans (10^3 ind. m^{-2})	157 ± 2	213 ± 41	40 ± 33
Ostracods (10^3 ind. m^{-2})	33 ± 22	98 ± 8	58 ± 28
Total meiofauna (mg C m^{-2})	272 ± 81	546 ± 50	392 ± 105
Hydrobiids (10^3 ind. m^{-2})	9 ± 7	18 ± 5	6 ± 8
N. obsoletus small (ind. m^{-2})	22 ± 13	146 ± 87	237 ± 107
N. obsoletus large (ind. m^{-2})	120 ± 74	158 ± 95	515 ± 232
Total epifauna (g C m^{-2})	74 ± 48	135 ± 85	404 ± 164

Table 3. Amount of bacterial carbon grazed daily (µg C m^{-2} d^{-1}) by meiofauna (nematodes, copepods, foraminiferans and ostracods), epifauna (hydrobiid snails, small and large *Nassarius obsoletus*) and total fauna in surficial sediments (0.5 cm) from the 3 creeks studied (means ± SD; n = 3). Percentage values correspond to the fractions of total bacteria ingested by each grazer. An asterisk indicates significant ($p < 0.05$) differences with the 2 other creeks (ANOVA; Tukey test). RC: reference creek; SFC: short-term fertilized creek; CFC: chronically fertilized creek

	RC	SFC	CFC
Nematodes	6.4 ± 1.0	5.8 ± 0.3	15.2 ± 1.3*
Copepods	16.7 ± 3.1	55.4 ± 16.7*	23.5 ± 6.4
Foraminiferans	2.4 ± 1.2	5.5 ± 1.7	0.6 ± 0.5
Ostracods	12.1 ± 5.7	33.0 ± 21.0	22.2 ± 3.5
Total meiofauna	37.7 ± 6.5	99.6 ± 3.7	61.5 ± 9.2
Percent total meiofauna	5	6	2
Hydrobiid snails	285.3 ± 23.7*	838.4 ± 125.7	587.1 ± 137.0
Percent hydrobiid snails	38	53	23
N. obsoletus (small)	28.7 ± 10.9*	193.9 ± 16.7	263.0 ± 77.4
N. obsoletus (large)	399.4 ± 183.6	544.2 ± 106.3	1692.6 ± 616.2*
Percent *N. obsoletus*	57	44	75
Total epifauna	713.4 ± 214.9	1576.5 ± 241.0	2542.7 ± 771.3
Total grazers	751.0 ± 214.3	1676.1 ± 223.6	2604.3 ± 762.1

Table 4. Amount of algal carbon grazed daily (µg C m^{-2} d^{-1}) and fraction of algal standing stock grazed daily (%) by meiofauna (nematodes, copepods, foraminiferans, and ostracods) and epifauna (hydrobiid snails, small and large *Nassarius obsoletus*) in surficial sediment (0.5 cm) of the 3 creeks studied (means ± SD; n = 3). Percentage values correspond to the fractions of total algae ingested by each grazer. An asterisk indicates significant differences ($p < 0.05$) with the 2 other creeks (ANOVA; Tukey test). RC: reference creek; SFC: short-term fertilized creek; CFC: chronically fertilized creek

	RC	SFC	CFC
Nematodes	66.8 ± 17.2	11.8 ± 2.6	493.6 ± 128.3*
Copepods	267.5 ± 63.5	562.2 ± 287.6	470.8 ± 89.2
Foraminiferans	12.1 ± 1.0	6.6 ± 5.1	2.9 ± 1.4
Ostracods	4.0 ± 2.0	33.7 ± 28.1	105.9 ± 15.1*
Total meiofauna	349.3 ± 46.0	614.3 ± 287.4	1073.2 ± 136.9
Percent total meiofauna	27	36	24
Hydrobiid snails	674.5 ± 210.7	842.2 ± 84.9	1977.8 ± 559.0*
Percent hydrobiid snails	51	49	45
N. obsoletus (small)	21.0 ± 12.4	78.2 ± 2.9	222.1 ± 106.8
N. obsoletus (large)	267.3 ± 218.3	170.7 ± 67.5	1112.3 ± 459.1*
Percent *N. obsoletus*	22	15	30
Total epifauna	962.7 ± 434.2	1091.2 ± 153.9	3312.2 ± 1061.7*
Total grazers	1312.0 ± 388.2	1705.5 ± 150.1	4385.4 ± 954.5*

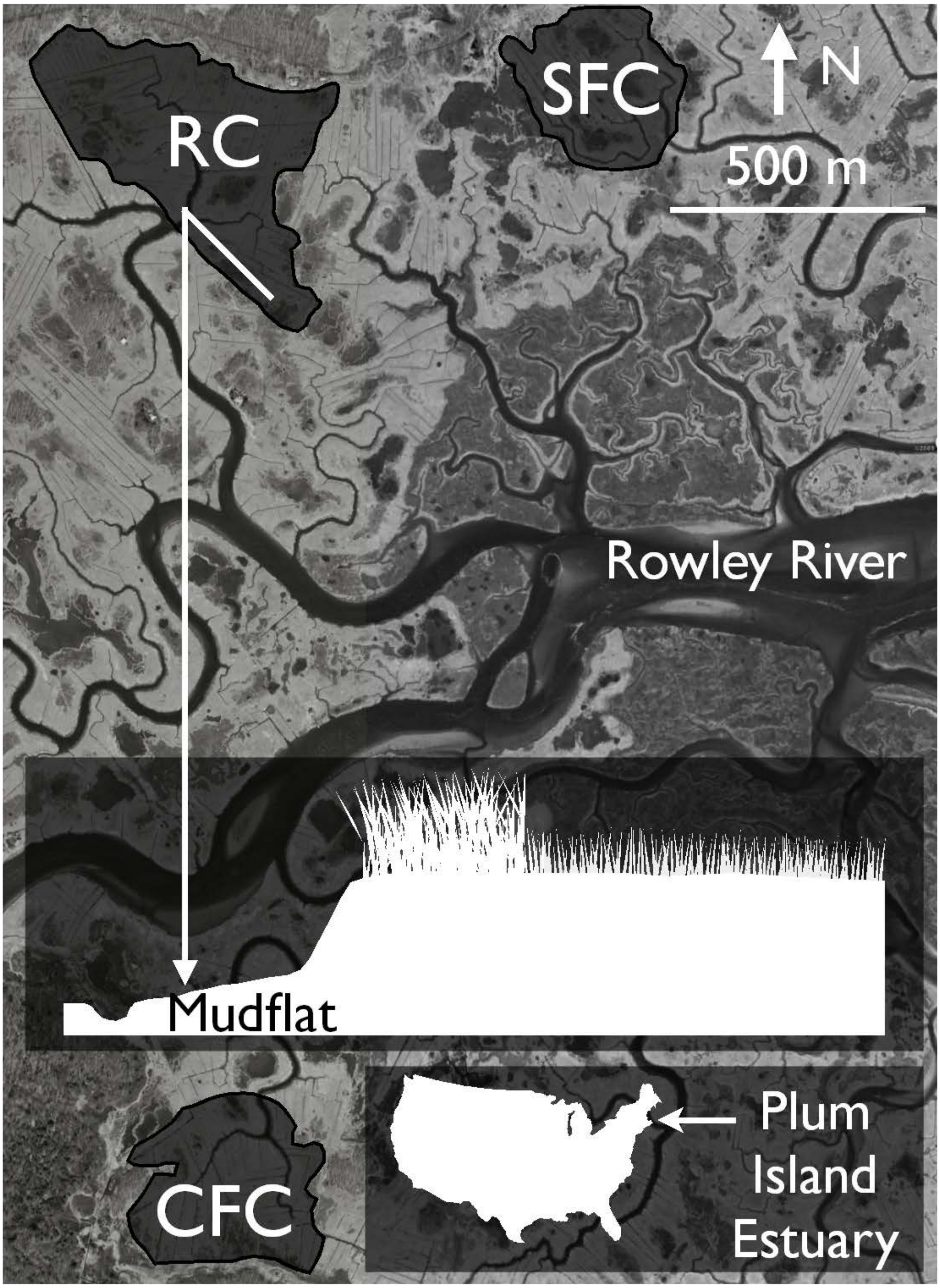

Fig. 1. Rowley River region salt marshes of the Plum Island estuary, Massachusetts, USA, and a profile of salt marsh habitat with the locations of mudflats studied. RC: reference creek; SFC: short-term fertilized creek; CFC: chronically fertilized creek (not drawn to scale)

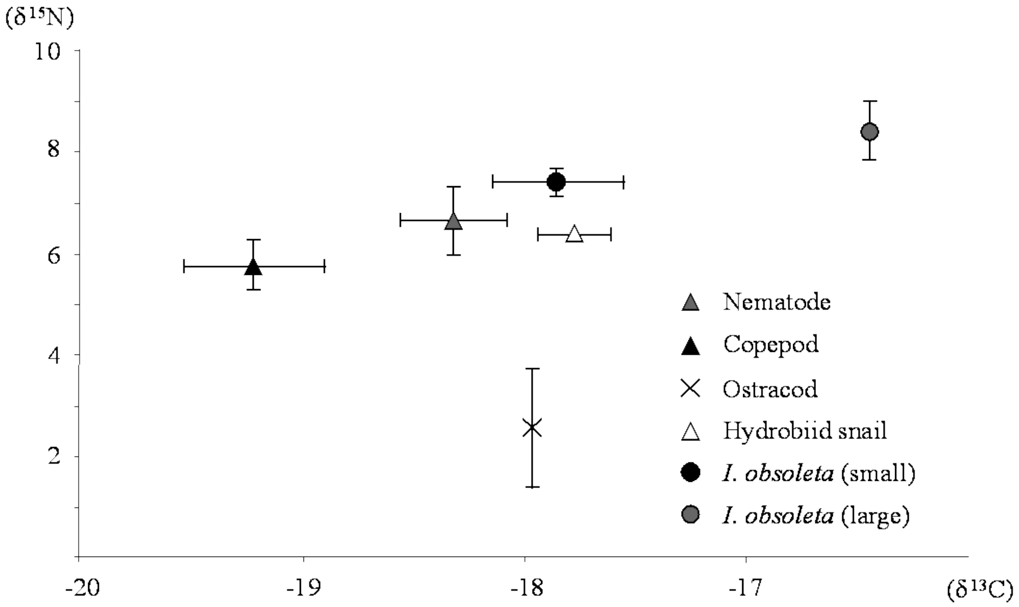

Fig. 2. Natural isotopic composition ($\delta^{15}N$ and $\delta^{13}C$) of meiofauna (nematodes, copepods and ostracods) and epifauna (hydrobiid snails and small and large *Nassarius obsoletus*) in the reference creek (means ± SD; n = 3)

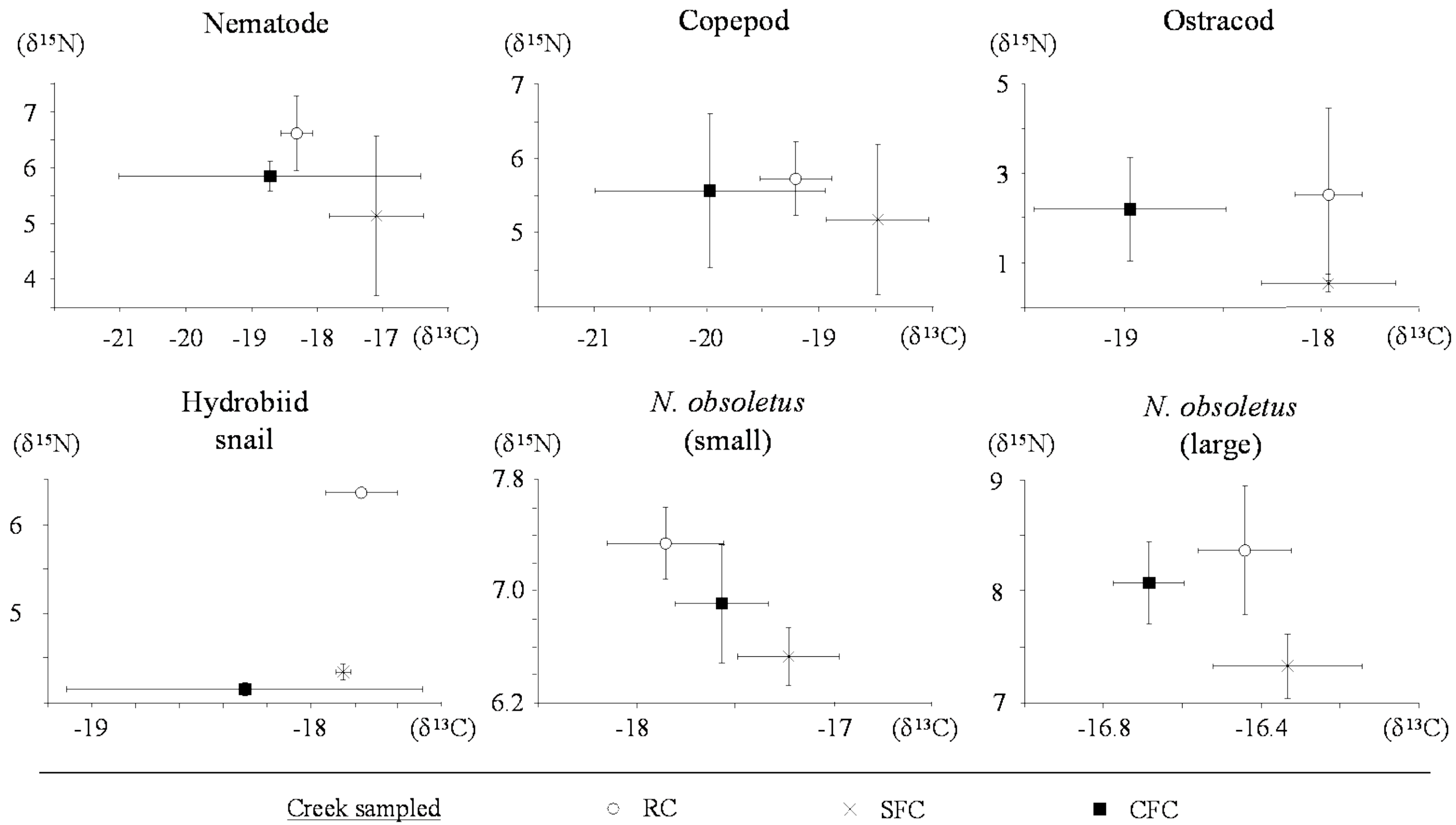

Fig. 3. Natural isotopic composition ($\delta^{15}N$ and $\delta^{13}C$) of meiofauna (nematodes, copepods and ostracods) and epifauna (hydrobiid snails and small and large *Nassarius obsoletus*) in the 3 creeks studied (means ± SD; n = 3). RC: reference creek; SFC: short-term fertilized creek; CFC: chronically fertilized creek

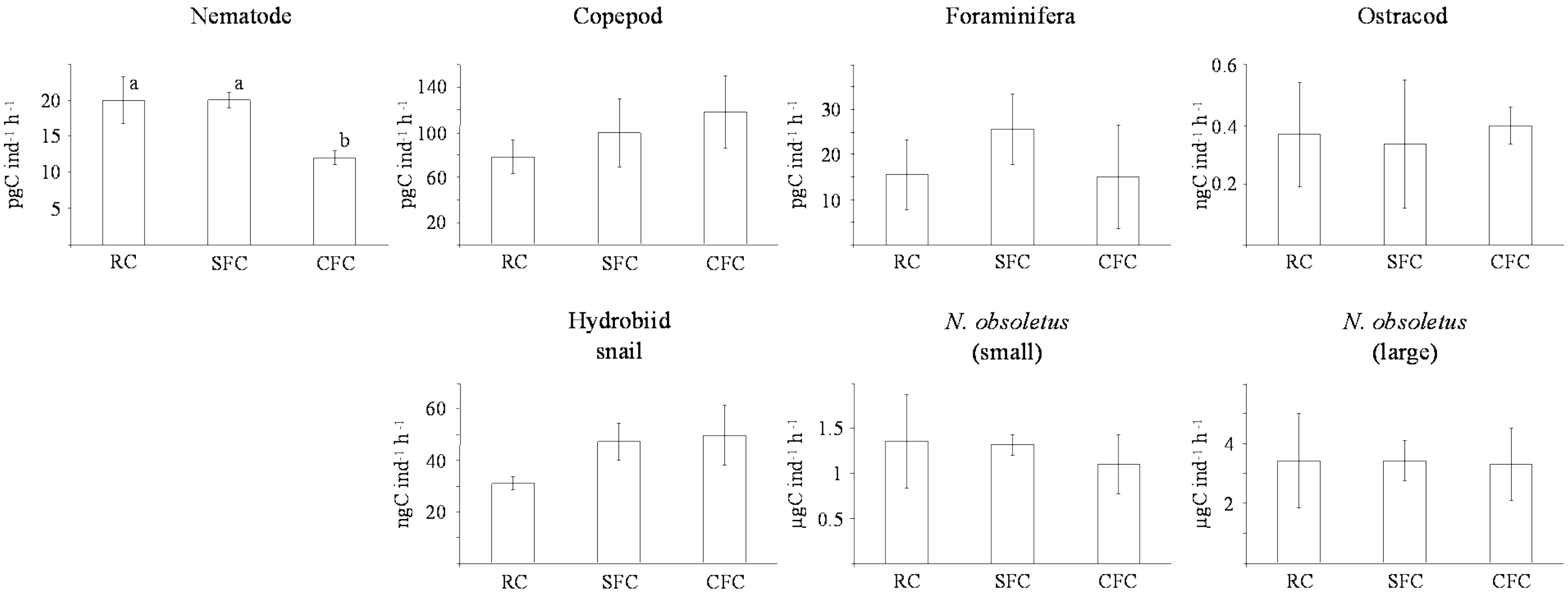

Fig. 4. Grazing rates of bacterial carbon, per individual grazer per hour, of meiofauna (nematodes, copepods, foraminiferans and ostracods) and epifauna (hydrobiid snails and small and large *Nassarius obsoletus*) in the 3 creeks studied (means ± SD; n = 3). Different letters above bars indicate significant differences ($p < 0.05$) between grazing rates (ANOVA; Tukey test). RC: reference creek; SFC: short-term fertilized creek; CFC: chronically fertilized creek

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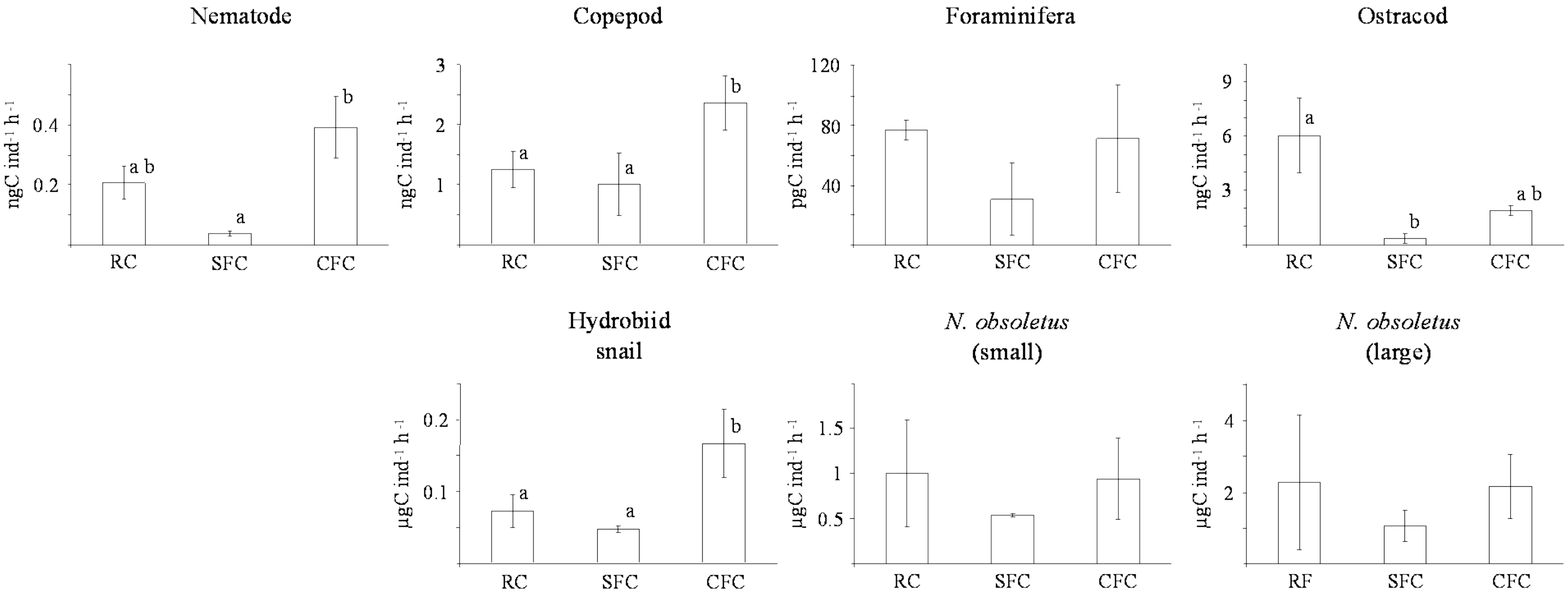

Fig. 5. Grazing rates of algal carbon, per individual grazer per hour, of meiofauna (nematodes, copepods, foraminiferans and ostracods) and epifauna (hydrobiid snails and small and large *Nassarius obsoletus*) in the 3 creeks studied (means ± SD; n = 3). Different letters above bars indicate significant differences ($p < 0.05$) between grazing rates (ANOVA; Tukey test). RC: reference creek; SFC: short-term fertilized creek; CFC: chronically fertilized creek

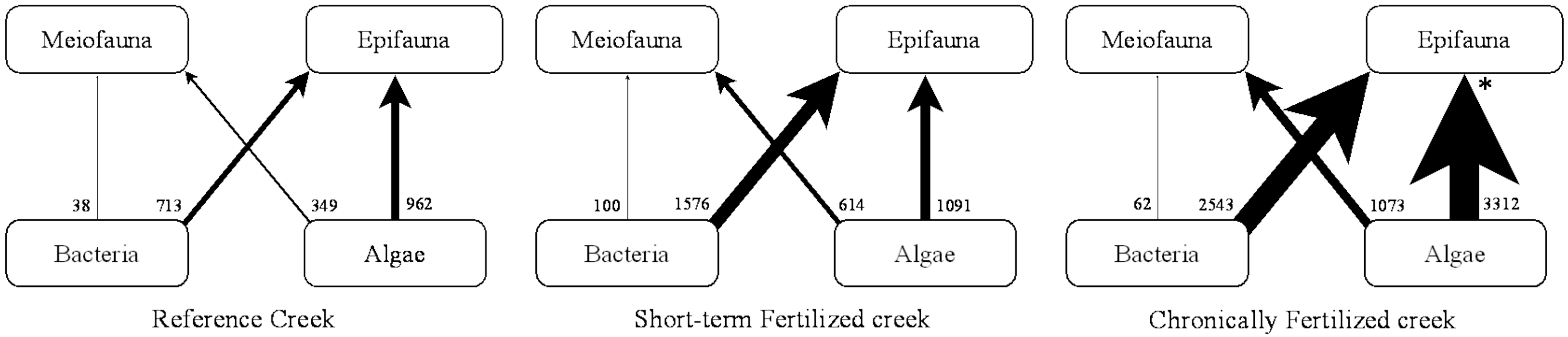

Fig. 6. Fluxes between benthic bacteria and algae and their meiofaunal and epifaunal grazers (g C m^{-2} d^{-1}) in the 3 creeks studied. An asterisk indicates significant ($p < 0.01$) differences in flux (ANOVA; Tukey test)