

Running head: Saltmarsh response to multiple stressors

SUSCEPTIBILITY OF SALT MARSHES TO NUTRIENT ENRICHMENT AND
PREDATOR REMOVAL

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INTRODUCTION

The sustainability of coastal ecosystems in the face of widespread environmental change is an issue of pressing concern throughout the world (Emeis et al. 2001). Coastal ecosystems form a dynamic interface between terrestrial and oceanic systems and are one of the most productive ecosystems in the world. Coastal systems probably serve more human uses than any other ecosystem and they have always been valued for their rich bounty of fish and shellfish. Coastal areas are also the sites of the nation's and the world's most intense commercial activity and population growth; worldwide, approximately 75% of the human population now lives in coastal regions (Emeis et al. 2001).

Over the past three decades nutrient enrichment of coastal and estuarine waters has become the premier issue for both scientists and managers (National Research Council 2000). Our understanding of coastal eutrophication has been developed principally through monitoring of estuaries, with a focus on pelagic or subtidal habitats (National Research Council 2000, Cloern 2001). Because estuarine systems are usually nitrogen limited, NO_3^- is the most common nutrient responsible for cultural nutrient enrichment (Cloern 2001). Increased nitrogen delivery to pelagic habitats of estuaries produces the classic response of ecosystems to stress (altered primary producers and nutrient cycles and loss of secondary producer species and production; Nixon 1995, Rapport and Whitford 1999, Deegan et al. 2002).

Salt marsh ecosystems have been thought of as not susceptible to nitrogen overloading because early studies found added nitrogen increased marsh grass production (primarily *Spartina* spp., cordgrass) and concluded that salt marshes can adsorb excess nutrients in plants and salt marsh plant-derived organic matter as peat (Verhoeven et al. 2006). Detritus from *Spartina* is important in food webs (Deegan et al. 2000) and in

creating peat that forms the physical structure of the marsh platform (Freidrichs and Perry 2001). However, the accumulation of peat and inputs of sediments and loss of peat through decomposition and sediment through erosion may be altered under high nutrient regimes and threaten the long-term stability of marsh systems.

Nitrogen addition may lead to either net gain or loss of the marsh depending on the balance between increased marsh plant production and increased decomposition. Absolute change in marsh surface elevation is determined by marsh plant species composition, production and allocation to above- and belowground biomass, microbial decomposition, sedimentation, erosion and compaction (Friedrichs and Perry 2001). Levine et al. (1998) suggested that competitive dynamics among plants might be affected by nutrient enrichment, potentially altering relative abundance patterns favoring species with less belowground storage and thus lowering rates of peat formation. When combined with the observation that nutrient additions may also stimulate microbial respiration and decomposition (Morris and Bradley 1999), the net effect on the salt marsh under conditions of chronic nitrogen loading is a critical unknown.

Although most research treats nutrient enrichment as a stand-alone stress, it never occurs in isolation from other perturbations. The effect of nutrient loading on species composition (both plants and animals) and the resultant structure and function of wetlands has been largely ignored when considering their ability to adsorb nutrients (Verhoeven et al. 2006). Recent studies suggest the response of estuaries to stress may depend on animal species composition (Silliman et al. 2005). Animal species composition may alter the balance between marsh gain and loss as animals may increase or decrease primary production, decomposition or N recycling (Pennings and Bertness 2001).

Failure to understand interactions between nutrient loading and change in species composition may lead to underestimating the impacts of these stresses. The 'bottom up or top down' theory originated from the observation that nutrient availability (bottom up)

sets the quantity of primary productivity, while other studies have shown that species composition (top down), particularly of top consumers, has a marked and cascading effect on ecosystems, including controlling species composition and nutrient cycling (Matson and Price 1992, Pace et al. 1999). Most examples of trophic cascades are in aquatic ecosystems with fairly simple, algal grazing pelagic food webs (Strong 1992). The rarity of trophic cascades in terrestrial systems has been attributed to the importance of detrital food webs (Polis 1999). Detritus-based aquatic ecosystems, such as salt marshes, bogs, and swamps, have classically been considered bottom-up or physically controlled ecosystems.

Recent experiments, however, suggest that salt marshes may exhibit top-down control at several trophic levels (Silliman and Zeiman. 2001, Silliman and Bertness 2002, Quiñones-Rivera and Fleeger 2005). One abundant, ubiquitous predator, a small (<10 cm total length) killifish (*Fundulus heteroclitus*, mummichog) has been suggested to control benthic algal through a trophic cascade because they prey on the invertebrates that graze on the benthic algae (Kneib 1997, Sarda et al. 1998). In late summer, killifish are capable of consuming 3-10 times the creek meiofauna production and meiofauna in the absence of predators appear capable of grazing over 60% of the microalgal community per day (Carman et al. 1997). Strong top-down control by grazers is considered a moderating influence on the negative effects of elevated nutrients on algae (Worm et al. 2000). Small-scale nutrient additions and predator community exclusion experiments have demonstrated bottom-up and top-down control of macroinfauna in mudflats associated with salt marsh creeks (Posey et al. 1999, Posey et al. 2002). Together, these observations suggest mummichogs are at the top of a trophic cascade that controls benthic algae (Sarda et al. 1998).

Mummichogs are also omnivorous and ingest algae, bulk detritus and the attached microbial community (D'Avanzo and Valiela 1990). As a result, marsh decomposition

rates may be limited by top-down controls through trophic pathways or by release from competition with algae for nutrients.

Whole-ecosystem experiments have shown that responses to stress are often not predictable from studies of the individual components (Schindler 1998). Developing the information needed to predict the interacting impacts of nutrient loading and species composition change requires experiments with realistic alterations carried out at scales of space and time that include the complexities of real ecosystems. Whole ecosystem manipulation experiments have been used effectively in other ecosystems (Bormann and Likens 1979, Carpenter et al. 1995), but they are rare in coastal research. Experiments in salt marshes have traditionally been less than a few m^2 . Our understanding of the response of salt marsh plants to nutrient enrichment is from small ($<10 \text{ m}^2$), plot-level additions where uniform levels of dry inorganic fertilizer ($20 \text{ to } > 1000 \text{ g N m}^{-2} \text{ y}^{-1}$) are sprinkled on the marsh surface at low tide. Dry fertilizer additions were usually made every two weeks or monthly and the duration of elevated nutrient levels after these additions was usually not determined. Tidal water is the primary vector for N delivery to coastal marshes, suggesting that dry fertilizer addition to the marsh surface may not be the best basis for determining if *Spartina* production responds to nutrient enrichment of tidal waters. Similarly, our understanding of top-down controls in salt marshes also relies on small ($1 - 4 \text{ m}^2$) exclusion experiments that use cages to isolate communities from top consumers. While the design of these cage experiments has improved, there are some remaining drawbacks. For example, it is impossible to selectively exclude single species using cages, and recruitment or size-selective movement into or out of the cages may obscure interpretations. In addition, while these small-scale experiments provide insight into controls on isolated ecosystem processes, they do not allow for interaction among different parts of the ecosystem which may buffer or alter the impacts and are not appropriate for determining the effects of populations of larger more motile animals on whole-ecosystems or the effects of ecosystem changes on populations. For example,

interactions may be caused when a motile species alters its distribution among the habitats available to it because of an experimental treatment. Small-scale experiments generally do not allow such events to happen. Complex feedbacks among physical and biological processes can alter accumulation rates and affect marsh elevation relative to sea level rise making extrapolation of small plot level experiments to whole marsh ecosystems problematic.

We are conducting an ecosystem-scale, multi-year field experiment including both nutrient and biotic manipulations to coastal salt marsh ecosystems. We are testing, for the first time at the ecosystem level, the hypothesis that nutrient enrichment and species composition change have interactive effects across multiple levels of biological organization and a range of biogeochemical processes. We altered whole salt marsh creek watersheds (~60,000 m² of saltmarsh) by addition of nutrients (15x ambient) in flooding waters and by a 60% reduction of a key fish species, the mummichog. Small marsh creek watersheds provide an ideal experimental setting because they have the spatial complexity, species composition and processes characteristic of the larger salt marsh ecosystem, which are often hundreds of thousands of m². Manipulating entire salt marsh creeksheds allowed us to examine effects on large motile animals and the interactive effects of motile species changes on ecosystem processes without cage artifacts. Because our manipulations were done on whole-marsh ecosystems, we are able to evaluate the integrated and interactive effects on all habitats (e.g., water column, tidal creeks and marsh) and on populations. These experiments are similar in many respects to the small watershed experiments carried out in forested catchments.

Our nutrient enrichment is novel compared to past studies in two important ways. We added nutrients (N and P) directly to the flooding tidal creek waters to mimic the way in which anthropogenic nutrients reach marsh ecosystems. All previous experimental salt marsh nutrient enrichment studies used a dose-response design with spatially uniform dry fertilizer loading on small plots (<10 m²). Nutrients carried in water will interact and

reach parts of the ecosystem differently than dry fertilizer. Our enrichment method also creates a spatial gradient of nutrient loading across the landscape that is proportional to the frequency and depth of inundation in the marsh. Spatial gradients in loading within an ecosystem are typical in real world situations in many terrestrial and aquatic ecosystems. Because of our enrichment method, at any location in the ecosystem, nutrient load will be a function of the nutrient concentration in the water, the frequency and depth of tidal flooding and the reduction of nutrients from the flooding waters by other parts of the ecosystem. Uniform loading misses important aspects of the spatial complexity of ecosystem exposure and response.

This work is organized around two questions that are central to understanding the long-term fate of coastal marshes:

1. Does chronic nutrient enrichment via flooding water increase primary production more than it stimulates microbial decomposition?
2. Do top-down controls change the response of the salt marsh ecosystem to nutrient enrichment?

Here we present findings on the first 2 years of these experiments including 1) water chemistry, 2) standing stocks and species composition of benthic microalgae, 3) microbial production, 4) species composition and ecophysiology of macrophytes, 5) invertebrates, and 6) nekton. Because even highly eutrophic waters result in nutrient loading that is an order of magnitude less than most plot level experiments, we expected little stimulation of salt marsh vascular plant growth. However, moderate levels of nutrient enrichment in the water column were expected to increase benthic algal biomass and to stimulate bacterial activity and detrital decomposition throughout the ecosystem because of direct uptake of nitrogen from the water column and availability of more high quality organic matter from increased algal production. We predicted nutrient enrichment would increase invertebrate production because of an increase of high quality microalgal and microbial production at the base of the food web. Finally, we predicted that fish

reduction would reduce predation on benthic invertebrates resulting in increased abundance of benthic invertebrates that would graze down the benthic algae.

SITE DESCRIPTION

The Plum Island Sound estuary in New England where we conducted our experiments is a classic salt marsh estuary that is currently unaffected by nutrient loading (Fig. 1). Tall *Spartina alterniflora* (~200 cm in height; smooth cordgrass) is found in pure stands in low marsh and along creek banks that receive daily tidal inundation, while *Spartina patens* (20 – 60 cm in height; saltmeadow cordgrass) is most abundant in higher elevation areas that are well-drained and flood less frequently. *S. alterniflora* also occurs in a short form (20 – 60 cm in height) in the high marsh, often in pure stands in areas that are poorly drained. About 80% of the total marsh area is high marsh that floods on spring tides. Mean tide is 2.6 m; mean spring tides are 3 m. During a typical growing season, *S. alterniflora* in Plum Island is under water about 35% of the time, while *S. patens* and short form *S. alterniflora* are inundated about 12% of the time. As is typical of most New England marshes, historically the marsh platform was ditched for salt marsh haying and mosquito control. Ditch edges show the same vegetation zonation as creek banks. Currently, the all marshes are periodically sprayed with BTI for mosquito control.

Because characteristics of tidal marsh systems vary along a fresh to salt water gradient, we anticipated that the experimental areas might have to be paired to account for differences associated with position in the estuary. After initial exploratory work, we chose 4 marsh creek systems (Sweeney, West, Clubhead and Nelson) with similar geomorphology along the Rowley River in the central portion of Plum Island Sound. Our experimental areas were at the landward end of the creeks where each creek has 2 roughly equal-sized branches (700 linear m of branch creek channel and 60,000 m² of marsh per treatment). Prior to experimental manipulation (1998 – 2002) we collected baseline temperature, salinity, nutrient and benthic microalgae characteristics on each creek system (4 creeks, 4 years, approximately 8 times per year). Additional baseline data

on total suspended sediments, marsh plant species composition and distribution was collected in 2003.

The four marsh creek watersheds were very similar, but differences in salinity and vascular plant community composition supported pairing the two sets of experimental and reference creeks. Water column temperature, nutrients (NO_3^- , NH_4^+ , PO_4^{3-}), total suspended sediments and phytoplankton did not differ among the four creeks, or in branches within creeks ($p > 0.05$ for all constituents except ammonium; Appendix A), however water column parameters for all creeks were different from Plum Island Sound water (Table 1). Creeks had warmer temperatures, lower salinities, higher nutrients, and higher phytoplankton biomass than the Sound. Sweeney and West are located further upstream along the tidal Rowley River, closer to sources of freshwater, and had slightly lower salinities than Clubhead and Nelson.

Marsh vegetation followed the classic distribution of salt marsh plant communities: tall *Spartina alterniflora* was found along the creek banks in a band that varied in width from 1 – 3 m; short *S. alterniflora*, *S. patens* and *Distichlis spicata* (saltgrass) were found on the high marsh platform. Similarity analysis of the plant community (ANOSIM, Primer-E Ltd., Plymouth, UK) found no difference between Sweeney and West ($P = 0.195$) and between Clubhead and Nelson ($P = 0.152$). Analysis of all other creek pairings found that the plant communities were significantly different (all other $P < 0.004$). Total cover of tall *S. alterniflora* and of high marsh *S. patens* did not differ among creeks. Sweeney and West had less short *S. alterniflora* than did Clubhead and Nelson, and much higher cover of *D. spicata*. *Atriplex patula* (spear saltbush) contributed little to total cover and had higher cover in Sweeney and West compared to Clubhead and Nelson (data not shown). Sweeney and West also had consistently less plant litter and standing dead than did Clubhead and Nelson (data not shown). We also mapped the boundaries of individual plant communities using high precision GPS (± 2 cm horizontal; maps not shown) to provide a baseline from which to

determine long-term shifts in plant community boundaries.

Baseline benthic microalgal biomass differed among habitats (Table 1), and was highest in the intertidal *S. alterniflora* habitat (220 mg chl *a*/m²), intermediate in intertidal mudflat habitat (100 mg chl *a*/m²) and lowest in high marsh *S. patens* (60 μg chl *a*/m²). West had higher mudflat and *S. alterniflora* habitat microalgae biomass compared to Sweeney, while Clubhead and Nelson were very similar to each other. Algal biomass in *S. patens* was highest in Nelson and lowest in Sweeney.

Creek Pairings were made on the basis of plant community structure and position in the estuary. Creek Pair 1 was Sweeney and West. This pair was closest to the freshwater end of the estuary, had slightly lower salinity and very similar marsh plant communities. Creek Pair 2 was Clubhead and Nelson. This pair was more seaward, with higher salinities and marsh plant communities similar to each other and different than Creek Pair 1.

EXPERIMENTAL DESIGN

We manipulated the marsh ecosystem in two ways: 1) Nutrients were added to one creek of each pair (to ~15X reference ambient concentrations); and 2) Fish reduced in one branch of each creek (~60% decline in mummichog) (Fig. 2). This design resulted in 4, ~60,000 m² marsh treatments within each pair: ambient nutrients/ambient mummichog, ambient nutrients/low mummichog, high nutrients/ambient mummichog, and high nutrients/low mummichog. We will refer to the ambient nutrient creeks as 'Nutrient Reference' and the nutrient enriched creeks as 'Nutrient Enrichment'. The fish treatments will be called ambient 'Fish' or reduction 'Low Fish'; these are located in either a 'Reference' or 'Nutrient Enrichment' creek.

We used a BACI-type experimental design in which pairing of experimental units accounts for variability that would contribute to error in a completely randomized design (Underwood 1994). Replication of ecosystem-scale experiments is difficult because it is often hard to find similar ecosystems (Carpenter et al. 1995, Schindler 1998); the

matched-pair approach helps ameliorate this difficulty (Stewart-Oaten and Bence 2001). The use of large experimental units (in our case $\sim 60,000 \text{ m}^2$ per treatment) is both labor-intensive and expensive ($\sim \$20,000$ per year per creek for fertilizer alone). These types of constraints generally necessitate low replication of individual treatments in ecosystem-scale experiments. While subject to the limitations of pseudoreplication (Hurlbert 1984) and small sample size, large-scale manipulative studies provide a realistic environment for examining effects and processes that occur at an ecosystem-scale. Our results include the effects of spatial variation and complexity, interactions between all of the species in the system, gradients across large areas, and large habitat patches.

The experiment was implemented in phases. In 2003 (Year 0), standardized procedures and methods were developed and extensive baseline measurements were made in all creeks and branches. In 2004, we began nutrient and fish reduction manipulations in Creek Pair 1 (Year 1). In 2005, manipulations continued in Creek Pair 1 (Year 2), and began in Creek Pair 2 (Year 1). Nutrients were added to Sweeney Creek (Pair 1) and Clubhead Creek (Pair 2).

Nutrient addition

We implemented an enrichment of $70 \mu\text{M NO}_3^-$ and $4 \mu\text{M PO}_4^{3-}$ (15x over a background of $< 5 \mu\text{M NO}_3^-$; $\sim 1 \mu\text{M PO}_4^{3-}$; Table 1). These concentrations are typical in estuarine systems designated as moderate to highly eutrophic based on the response of the pelagic and subtidal system (NOAA 1999, EPA 2002). We added the NO_3^- and PO_4^{3-} in $\sim 15:1$ molar ratio as anthropogenic increases in NO_3^- in coastal waters is usually accompanied by increased PO_4^{3-} (EPA 2002). We added nutrients by pumping a concentrated solution of NO_3^- and PO_4^{3-} to the flooding water of every tide during the growing season (mid-May – Oct.; ~ 150 d). The pump rate was adjusted every 10 min throughout each incoming tide (based on a hydrologic model) to maintain constant N and P concentrations in incoming waters until a water depth of 3.2 m in the channel (equivalent to ~ 15 cm of water on the high marsh platform) was reached. The cessation

of nutrient addition at water levels >3.2 m affected approximately the last half hour of the incoming tide on less than 20% of the tides between May and October. The solution was distributed through the water column by vertical and horizontal spreader bars and traveled approximately 100 m allowing in-channel mixing to occur before reaching the experimental area boundary.

Fish reduction

Mummichogs in this estuary have been observed to winter in deep water and move up into marsh systems to spawn in May and June, although some may winter in pools on the marsh platform (Raposa 2003). We excluded adult mummichogs from entering 1 branch of each creek by using block nets (installed at the branch mouth and small channel boundaries prior to the spring migration), and by constantly deployed minnow traps that fished throughout the season (selective for mummichogs; Layman and Smith 2001). Previous tagging work in these marshes found mummichogs had a home range of about 300 linear feet of creek and little movement between creek branches (Sweeney et al. 1998). Minnow-trapped mummichogs were released at least 1 km from the treatment area; all other species were returned to the creek. The combination of block netting and minnow trapping reduced mummichog abundance by ~60% (analyses presented below).

Sampling locations

Three benchmark sample areas, or “strata” were established on each branch of each creek in 2003. Each stratum began in the creek channel, was 10 m wide, and extended onto the marsh platform for 50 m. Strata were designed to represent the whole treatment area, and to include potential effects of distance from the nutrient addition point. The strata were spaced along each branch, ~100 m apart, and each included all major habitat types: intertidal mudflat (MF) in the creek, filamentous microalgae (FA) at the top of the creek bank, tall form *S. alterniflora* (TSA) at the top the creek bank, *S. patens* (SP) on the marsh platform, and short form *S. alterniflora* (SSA) on the platform.

From 2003-2005, much of the routine/repeated sample collection, such as benthic chlorophyll and invertebrates, sediments, and plant production, was conducted within the strata; replicates from each habitat type was collected from within each stratum. Specific collection points were haphazardly located within habitats. Additional samples of many types were frequently collected outside of the established strata.

METHODS

Nutrients and suspended sediments

We measured baseline water quality characteristics (NO_3^- , NH_4^+ , PO_4^{3-} , TSS, salinity, etc.) for 4 growing seasons prior to manipulations (May – Sept, 1998 – 2002). We collected water samples during mid-ebb once or twice per month from each creek branch and from ~25m seaward of the confluence of each pair of creek branches. Water samples were also collected 2-4 times each year from 3 locations in the Plum Island Sound to characterize the water that floods the creeks.

In 2003 (pre-manipulation), 2004 and 2005, we conducted intensive, bi-monthly, tidal cycle sampling (hourly sampling over a full, 12-hour tidal cycle). Each month (May – Sept), one spring tide cycle and one neap tide cycle were sampled at each creek branch and confluence location. Tidal cycle sampling allows calculation of nutrient delivery, retention and export (budgets) for whole tidal cycles.

One-liter water samples were collected in acid washed, Nalgene bottles using an autosampler (SIGMA, Loveland, Colorado). Samples were refrigerated and filtered within 24 hours of collection (precombusted 47 mm GFF filter) and frozen until analysis. Chlorophyll *a* (chl *a*) was determined on the filter (foil wrapped; frozen; 90% acetone extraction and fluorometer analysis, Holm-Hansen et al. 1965). NO_3^- was determined colorimetrically on a flow injection analyzer (Lachat Quik Chem 8000, Lachat Zellweger Instruments, Milwaukee, Wisconsin) using the cadmium-copper reduction method (Wood et al. 1967). PO_4^{3-} concentrations were determined colorimetrically using the ascorbic acid method (Parsons et al. 1984). NH_4^+ was determined colorimetrically using the

phenolphthorite method modified for small sample size (Solorzano 1969). Both PO_4^{3-} and NH_4^+ samples were run on a Cary Spectrophotometer (Varian Inc., Palo Alto, California).

Water on the marsh platform at high tide was sampled during 2005. Acid washed, narrow mouth bottles were attached to stakes and placed throughout the marsh at low tide. The flooding tide filled the bottles, which were retrieved after the tide subsided. These samples were treated as above and analyzed for NO_3^- .

In 2003, total suspended solids samples were collected weekly at slack high tide within each branch of the four creeks. One-liter samples were filtered through pre-weighed, ashed filters (47 mm, 0.7 μm GFF). Filters were then ashed and weighed to determine the amount of inorganic material in suspension.

Vascular marsh plants

To characterize the marsh community prior to manipulations (July 2003) plant species occurrence and visual estimates of percent cover by species were recorded in contiguous 1 m² plots (N = 50) along the length of each strata for each creek (N = 3 strata per treatment branch; N = 6 per creek). Plant frequency (percent of plots in which a species occurred) and mean percent cover for each species were determined for each strata (N = 6 for each species per creek).

Shoot growth of tall *S. alterniflora*, short *S. alterniflora*, and *S. patens* was determined in Creek Pair 1 (Year 1 and Year 2). Every two weeks (mid-May to August) haphazardly selected shoots (N = 25) of each type in each stratum were cut at the peat surface and refrigerated within two hours. Each shoot was individually washed to remove sediment, measured for length, and dried (80°C for at least 24 h). Mean shoot length (cm), weight (g) and length specific leaf weight (weight of an individual shoot divided by the total length, g/cm) were determined for each shoot. Values were normally distributed for all three parameters. Nitrogen and carbon content of tall *S. alterniflora*, short *S. alterniflora*, and *S. patens* were determined (June, July and August, 2004; N = 6 per plant

type per creek; PerkinElmer 2400 Series II CHNS/O analyzer, PerkinElmer Life and Analytical Sciences Inc., Wellesley, Massachusetts). Stem density (shoots per unit area) was counted in four plots haphazardly located within 5 m of the centerline of the sample strata in August and means calculated for each plant type for each stratum (N = 6 per plant type per creek). Because of known differences in stem density between species and growth forms, different size quadrats were used for each species: tall *S. alterniflora* square 0.25 m²; short *S. alterniflora* square 0.0625 m²; *S. patens* circle 0.008m².

Microbial production

Surface sediment samples for bacterial production were taken using a core (N = 10; 1.5 cm diameter, < 10 mm depth) monthly during the growing season in tall *S. alterniflora* and *S. patens*. Replicates were homogenized and then subsampled (N = 5) for measurement of bacterial production by uptake of tritiated leucine in sediment slurries (Buesing and Gessner 2003).

Benthic microalgae

Benthic microalgal biomass samples were collected monthly (June-September; 2003, 2004, and 2005; Creek Pair 2 was not sampled in 2004) in mudflat, tall *S. alterniflora*, and *S. patens* habitats. Macroalgae was rare in these systems. Filamentous (and associated epiphytic) algae occur in specific habitats (i.e., the creek wall near the top and associated with the stems of short form *S. alterniflora*) and their responses will be discussed in future reports. In Sept 2005, supplemental samples spaced along the entire creek branch length were taken in each habitat. Cores were taken in each habitat in each sampling area (two per habitat per area; 2.7 cm diameter, 2 cm depth), frozen immediately and total chlorophyll *a* (mg chl *a*/cm²) determined (Lorenzen 1967; acetone extraction and spectrophotometric analysis). Data were log transformed.

Benthic microalgal species composition and numerical response were measured monthly on glass slides (five slides per collection; one collection per stratum per month) located parallel to stream flow in the middle of the creek channel and submerged for 2-3

days. Slides were preserved in 70% ethanol and the species composition and density determined by microscopic examination.

Benthic Invertebrates

Macroinfauna and meiofauna collections were taken coincident with monthly benthic microalgal samples. Macroinfauna samples taken ten-weeks after nutrient addition and fish reduction treatments began (August 2-3, 2004) in tall *S. alterniflora* in Creek Pair 1 were analyzed. Duplicate cores (6.6 cm diameter push core; to 5 cm depth) were collected at low tide and fixed with 10% formalin and Rose Bengal. After a minimum of two days, samples were sieved (500- μ m sieve) and macroinfauna were enumerated and identified to the lowest possible taxon. Permanent meiofauna (e.g., nematodes) were not enumerated. Because annelids constituted 98% of all macroinfauna and not all species have been identified, we report only total annelid abundance. Data met the criteria for normal distribution.

To examine the effects of fish reduction on motile saltmarsh epifauna, passive collectors (litterbags) were placed in the tall *S. alterniflora* habitat of reference creeks in July 2005. The bags (13.5 x 29 cm, with 5-mm delta weave mesh) were filled with ~20 grams of dried, standing dead *S. alterniflora* leaves (Fell et al. 1998). After two weeks litterbags were collected and contents preserved in 50% ethanol and Rose Bengal solution. After at least two days, litter was rinsed over a 0.5 mm sieve and all animals collected using forceps and a hand lens. Animals were identified to lowest possible taxon and enumerated. Here we report on the most abundant species collected, the amphipod *Uholchestia spartinophila*, which comprised about 75% of the animals collected.

Nekton abundance, growth and diet

Nekton were collected on the marsh surface each month (Sept. - Oct. 2003; June-Sept. 2004, 2005) during nighttime spring tides in each stratum (N = 3 per treatment branch). Flume nets (dyed green 1/8" delta mesh) were 3 m wide at the marsh-creek interface and extended perpendicularly 10 m onto the marsh surface (30 m² of marsh

surface). Nets were flush with the marsh surface as the tide rose; at slack high tide, the sides were lifted, and the front and back nets attached to enclose the area. The front net collected fish as they moved off of the marsh surface with the falling tide. All nekton collected were identified to species, and up to 300 individuals of each species measured (total length (TL) \pm 1 mm) and weighed (g \pm 0.01) individually; if we collected more than 300 individuals per species, remaining individuals were weighed as an aggregate by species. Abundance and biomass were square root (n+1) transformed. Mean size (TL \pm 1 mm) of young-of-the-year *Palaemonetes pugio* (daggerblade grass shrimp) was estimated in August and September using length frequency (NORMSEP procedure; Food and Agriculture Organization of the United Nations, 2000. FISAT II. Rome, Italy). Growth was estimated as the change in mean size of the smallest size group. Length frequency was square root (n+1) transformed before ANOVA analysis. Mummichog diet was analyzed on fish (n=20 per treatment/period) captured in flume nets or minnow traps in summer of 2004 and 2006. Gut fullness was estimated and items identified to lowest practical taxa under a dissecting microscope (Hyslop 1980).

Data Analysis

A paired BACI-type ANOVA (Nutrient, Fish Reduction, Before/After) analysis with Type III sums of squares was used to analyze the effectiveness of the fish reduction treatment, and the response of benthic microalgal biomass and grass shrimp abundance to nutrient addition and fish reduction treatments. We present computed *P* values, but infer significance when $P < 0.1$ because in a complex ecosystem experiment background variability is generally high and replication low, and we wanted to identify hypotheses with support in the data (Oksanen 2001, Hobbs and Hilborn 2006). Evidence from several lines of inquiry were used to further examine support for the responses. Because of known differences between creek pairs and because the pairs were manipulated for different lengths of time, creek pairs were analyzed separately. Replicates of response variables were averaged by sample strata within a treatment (N = 3 per treatment). In

exploratory analysis, we found no spatial pattern associated with strata for any variable, therefore they were considered independent estimates of the treatment response. Monthly data were nested within Treatment Year (0, 1, 2) and Before (0)/After (1, 2). Analysis was performed on the mean response variables (appropriately transformed to meet the assumption of normality), not differences, so that we could examine the interactive effects of nutrient addition and fish reduction. A significant interaction term (Nutrient*Fish*Month(Year, Before/After) indicated a difference among treatments over time. If a significant interaction occurred, data were examined visually and Tukey or t-tests used to determine differences (Stewart-Oaten and Bence 2001, Parker and Wiens 2005). A two-way ANOVA (Nutrient, Fish Reduction) was used to analyze benthic microalgal count and macroinfaunal abundance. An ANOVA (Nutrient, Fish Reduction, Month) was used to analyze shrimp body size data. All ANOVA's were calculated using SuperANOVA (1996, Abacus Concepts, Inc., Berkeley, CA). We expected that fish reduction would have no effect on vascular plants, as the overall nutrient regime (enriched or reference) would not be altered by fish abundance. This was confirmed by initial analyses and therefore vegetation data were pooled between fish treatments within a creek (nutrient reference or enrichment) and analyzed using the individual measurements. Because the plant response metrics were calculated from the same samples (height and length specific leaf weight, n=125 for each species in each creek), we used a Bonferroni adjustment of significance from 0.1 to 0.05. Tukey's or t-tests were used to examine differences among treatments. Plant community data was analyzed using the ANOSIM procedure in Primer (Primer 5 for Windows Ver. 5.2.9 © 2002 Primer-E Ltd. Plymouth, England). Microbial production was analyzed by plotting nutrient enriched against reference values and examining the distribution above and below the one-to-one line with Wilcoxon Signed Rank Test.

RESULTS

Experimental manipulations

Nutrient addition

In both creek pairs, we achieved our target NO_3^- enrichment of approximately 15x over reference conditions. Nutrient addition increased the annual average concentration of NO_3^- in the water flooding in Creek Pair 1 by a factor of ~14X (Year 1) to ~19X (Year 2) and ~23X in Creek Pair 2 (Table 2). The nutrient addition increased PO_4^{3-} concentrations in Creek Pair 1 by a factor of ~5X. We added ~3,500 kg of N (as NaNO_3) and 250 kg of P (as NaH_2PO_4) each year to each of the nutrient enriched creeks (Pair 1: N – Yr 1 3251 kg, Yr 2 3546 kg; P - Yr 1 231 kg, Yr 2 252 kg; Pair 2: N – Yr 1 4592 kg, P – Yr 1 327 kg). Average flooding water nutrient concentrations in Year 0 (prior to nutrient addition) in the nutrient enrichment and reference creeks of both pairs (NO_3^- : 2.6 μM ; NH_4^+ 9.1 μM ; PO_4^{3-} 0.9 μM) were similar to the 1998-2002 concentrations (Table 1). In Year 0, as is typical of salt marshes, ebbing waters generally had lower NO_3^- concentrations (1.3 μM), while NH_4^+ was highly variable through the tidal cycle (<1 to 24 μM), with high concentrations generally occurring at low tide. PO_4^{3-} remained at ~1 μM through the tidal cycle.

In the first year of nutrient addition to Creek Pair 1, our addition resulted in an annual average of 70 μM NO_3^- and 5.1 μM PO_4^{3-} for the experimental period. However, examination of specific tidal cycles early in the growing season demonstrated lower than target concentrations during spring tides and higher than target concentrations during neap tides. Consequently, we adjusted the nutrient addition rate in the following year to achieve more uniform nutrient concentrations over a variety of tidal regimes.

The consistent decrease in NO_3^- concentration on ebbing tides in both nutrient enriched creeks suggests uptake of the added NO_3^- by the marsh ecosystem. Significantly lower concentrations of NO_3^- in ebbing water compared to flooding water in Creek Pair 1 (Year 1: 58 vs. 70 μM ; Year 2: 60 vs. 95 μM) and Creek Pair 2 (98 vs. 116 μM) suggest uptake by the marsh. NH_4^+ did not differ substantially between flooding and ebbing tides.

Despite our initial expectation that the marsh far from the addition point might receive water with lower nutrient concentration due to uptake as the water moved across the marsh, nutrient concentrations at high tide were high across the marsh platform (Fig. 3). The mean NO_3^- concentration on the nutrient enriched marsh was $\sim 80 \mu\text{M}$ (range 49 to $105 \mu\text{M}$) for Creek Pair 1. The NO_3^- concentration was $\sim 50 \mu\text{M}$ (range of 2 to $78 \mu\text{M}$) on the nutrient enriched marsh of Creek Pair 2. On highest tides, some dilution of nutrient concentration by water from other creeks outside the study areas was evident on the southern border of Creek Pair 2. These edges were several hundred meters away from the designated sampling areas and were not sampled for any response variables.

Fish reduction

Reduction of mummichog (*Fundulus heteroclitus*) was effective with a decrease in abundance of $\sim 60\%$ between the ambient Fish and Low Fish treatments (Fig. 4; Appendix B). The overall mean was 65 mummichogs/ 30 m^2 in ambient Fish treatments compared to 25 mummichogs/ 30 m^2 in Low Fish treatments. Mummichog was the most abundant fish species captured (19% of total nekton abundance and 92% of fish abundance; 41% of total nekton biomass and 89% of fish biomass over the 3 years). Mummichog abundance and biomass was near zero in June and increased rapidly until peak abundance in September and October. Mummichogs arrive in June to spawn and leave the marsh shortly after October to winter over in deeper water. Mummichog ranged in size from 15 to 110 mm and exhibited size classes associated with spawning and recruitment events (data not shown). Our sampling methodology was most effective on larger mummichogs, suggesting that young-of-the-year, and thus total, mummichog abundances in all treatments may be underestimated. Fish abundance differed between fish treatments (Before/After*Fish Treatment Pair 1: $F = 18.3$, $P = 0.0001$; Pair 2: $F = 2.7$, $P = 0.10$), but there was no additional effect of nutrient addition on mummichog abundance for either Creek Pair (Nutrient addition Pair 1: $F = 1.3$, $P = 0.25$; Pair 2 $F = 0.06$, $P = 0.8$). In Pair 1 prior to manipulations (Year 0), fish abundance in the reference

creek branch randomly chosen for fish reduction was significantly higher (seasonal mean 133 individuals/30 m²) than the paired ambient fish density branch (43 individuals/30 m², t-test, p < 0.001); our removal reduced abundance to below the paired ambient density (Low Fish 17 vs. Fish 46 individuals/30 m²; t-test, P < 0.05). In the nutrient enriched creek of Pair 2, initial fish abundance was slightly, but not significantly, lower in the branch designated for fish reduction (46 individuals/30 m²) compared to the ambient density branch (90 individuals/30 m² with high variation); removal lowered abundance in the reduction branch significantly below the previous density in that branch (22 vs. 46 individuals/30 m²) and well below ambient density in the experimental year (22 vs. 86 individuals/30 m²; t-test, p < 0.05). Reduction was most effective for the larger size individuals (> 40 mm TL) as smaller fish were not retained by the minnow traps or excluded by the block net. Thus, the effect of removing fish made a larger difference in reducing the total biomass compared to the number of individuals of mummichog. The overall seasonal average was ~30 g/30 m² in Low Fish versus ~100g/30 m² in Ambient Fish treatment (Fig. 4).

Ecosystem Response

Vascular marsh plants

Both *S. alterniflora* and *S. patens* growth increased with the nutrient addition, but stem density did not change (Fig. 5, Table 3). Nutrient enrichment increased tall *S. alterniflora* stem length by 20% in Year 1 and increased length specific leaf weight (g/cm of stem) by 25% in both years compared to the nutrient reference creek in Pair 1 (Fig. 5a, b, c, d). Mean short form *S. alterniflora* length specific leaf weight was greater in both years, but the difference was significant only in the first year of nutrient enrichment. *S. patens* length specific leaf weight was indistinguishable between nutrient enriched and reference creek in either year of nutrient enrichment (Fig. 5 c, d). In the first year of nutrient enrichment, mean height of *S. alterniflora* (tall and short) and *S. patens* began to diverge between the enriched and reference creeks in July and by mid-August height

differences were significant. In the second year of nutrient enrichment, by mid-August *S. patens* was again significantly taller in the nutrient enriched creek, however tall *S. alterniflora* height did not differ between creeks and short *S. alterniflora* was taller in the reference.

For both tall and short *S. alterniflora*, nitrogen content in aboveground shoots was higher in plants from the nutrient enrichment creek compared to the reference creek, while nitrogen content of *S. patens* was identical (Fig. 5e.). For belowground biomass, tall *S. alterniflora* in the nutrient enrichment creek had a higher % N than the reference but the short *S. alterniflora* did not differ in its % N. Over the growing season nitrogen content of shoot tissue declined by approximately 35% in tall *S. alterniflora*, 42% in short *S. alterniflora* and 55% in *S. patens*. Nitrogen content in belowground roots and rhizomes was higher in the nutrient enrichment creek for tall *S. alterniflora*, but short *S. alterniflora* had higher N content in the reference creek.

After two years of nutrient enrichment, we found no differences in stem density associated with nutrient enrichment for all plant species (Table 3). West had higher tall *S. alterniflora* stem density compared to all other three creeks, while short *S. alterniflora* stem density did not differ among creeks. Sweeney and West had higher *S. patens* stem densities than Clubhead and Nelson, but only West was significantly different.

Microbial production

The bacterial production assay suggests that the productivity of microbes was enhanced by nutrient enrichment in some marsh habitats, but was unaffected in others. In the tall *S. alterniflora* zone rates of production were significantly higher in nutrient enriched areas compared paired reference areas, but bacterial production in the *S. patens* zone was unaffected by nutrient enrichment (Fig. 6). These results suggest that the microbial response to nutrient enrichment is via some indirect mechanism that has differing effects in each marsh habitat.

Benthic microalgae

Several lines of evidence demonstrate that benthic algae had a cumulative response to the nutrient addition and that the response to nutrient enrichment was greatest in the fish reduction treatment (Fig. 7, 8). By the end of the second year of nutrient enrichment, under ambient Fish, benthic microalgal biomass was 60% higher than initial baseline and the reference creek for both tall *S. alterniflora* and mudflat habitats (Interaction term: mudflat $F = 1.5$, $P < 0.09$; TSA $F = 2.3$, $P < 0.004$; Appendix B). Nutrient enrichment had a strong positive effect on benthic microalgal biomass in the fish reduction treatment in the second year (Fig. 7; t-test, $P < 0.05$). Nutrient enrichment also altered the seasonal pattern and supported a high benthic microalgal biomass in mudflat and tall *S. alterniflora* habitats throughout the summer while in reference creeks and pre-treatment years microalgal biomass was lowest in mid-summer. The pattern (1998-2002) of higher benthic microalgal biomass in tall *S. alterniflora* and mudflat habitats in the reference creek of Creek Pair 1 was also observed in the year immediately prior to manipulations (Year 0). In the first year of nutrient enrichment of Pair 1, microalgal biomass in the tall *S. alterniflora* habitat increased over baseline in the nutrient enriched creek and was about equal to the mean for the reference creek (1998-2003).

The benthic microalgal response was spatially variable. Benthic microalgal biomass in *S. patens* habitat did not differ among any treatment or years ($F = 1.2$, $P = 0.25$). Creek Pair 2 had no detectable change in benthic microalgal biomass associated with any treatment in any habitat (MF, $F = 0.5$, $P = 0.9$; TSA, $F = 1.18$, $P = 0.3$; SP, $F = 0.6$, $P = 0.8$; Appendix B). Fish reduction had no discernable effect on microalgal biomass in the reference creeks (Fig. 7).

Microalgal numerical abundance (Creek Pair 1) followed the same pattern as mudflat microalgal biomass, with a significant increase in the nutrient enriched creek by the end of year 2, although no differences were apparent among treatments in the first year. Again, the greatest response to nutrient enrichment was observed in the Low Fish treatment (Fig. 8a).

Algal species richness increased in response to both the nutrient enrichment and the fish reduction (Fig. 8b). Similar to other measures of microalgal response, species richness increased more in response to the nutrient enrichment in the fish reduction treatment. Species composition differed with nutrient addition, with more diatom species and no cyanobacteria species found in the nutrient enriched creek.

Benthic Invertebrates

Infaunal annelid abundance declined with both nutrient enrichment and fish reduction. Total abundance ranged between 10,000-40,000 individuals/m² (Fig. 9a), with a 50% reduction in abundance in the nutrient enriched creek ($F = 19.13$, $P < 0.001$), and within a nutrient treatment slightly lower abundances in the Low fish treatment ($F = 3.02$, $P = 0.098$); effects were independent ($F = 0.00$, $P = 0.95$). Abundance of the amphipod (*U. spartinophila*) increased about 3-fold (Fig. 9b) with reduction of mummichog (t -value = 4.70, $P < 0.0001$) in reference creeks.

Nekton Community

Daggerblade grass shrimp (*Palaemonetes pugio*; 79% of abundance, 42 % of biomass) and mummichog were the dominant nekton species (combined 98% of total abundance) out of 11 species. The other species were typical of salt marshes: *Crangon septemspinosa* (sand shrimp), *Carcinus maenas* (green crab), Xanthidae sp. (crabs), *Anguilla rostrata* (American eel), Clupeidae sp. (herring), *Menidia menidia* (Atlantic silversides), *Apeltes quadracus* (fourspine stickleback), *Pungitius pungitius* (ninespine stickleback), and *Syngathus fuscus* (northern pipefish). These species were not abundant or captured frequently enough to examine the effects of the experimental treatments.

Grass shrimp responded to the nutrient addition with increased growth, but abundance did not differ among treatments (Fig. 10). Grass shrimp abundance increased over the season, with peak abundance in the fall (data not shown). The mean abundance of *P. pugio* varied between 50 and 1000 individuals per 30 m² (sample range 0 – 1319 individuals per 30 m²). Grass shrimp abundance was variable among years and treatments

and did not appear to be affected by any treatment in either Creek Pair (Appendix B). Although we found a significant interaction term (Pair 1: $F = 2.3$, $P = 0.003$) in Pair 1, this did not appear to be attributable to any treatment effect (Fig. 10). Creek Pair 2 did not have a significant interaction ($F = 0.89$, $P = 0.6$).

Growth of grass shrimp in nutrient addition creeks was 50% higher (~6 mm/month) than growth achieved in the reference creeks (~4 mm/month; Table 4). Shrimp grew between August (overall mean = 21 mm TL) and September (overall mean = 27 mm TL), with an overall effect of larger size in the nutrient enriched marshes (Nutrient: Pair 1 $F = 75.25$, $P < 0.001$; Pair 2 $F = 22.01$, $P < 0.0001$; Appendix C); fish reduction had a small negative effect (Pair 1: $F = 43.89$, $P < 0.0001$, Pair 2: $F = 5.1$, $P = 0.023$) in both reference and nutrient addition creeks.

Mummichog diet was a mixture of detritus, algae, aquatic invertebrates (amphipods, polychaete worms, shrimp, isopods, copepods, nematodes, snails) and terrestrial insects (spiders, flies, grasshoppers) as has been found in other saltmarshes, with an ontogenetic shift from largely carnivorous at small sizes to a more omnivorous, plant based, diet at larger sizes (Smith et al. 2000, Wainright et al. 2000, Fell et al. 2003, Deegan et al. unpublished data). All fish examined had food in their guts; Gut fullness was usually >60%. In 2006, 20% of fish greater than 40 mm TL in the nutrient enriched, fish reduction treatment had algae as the most abundant food item in their stomach. Detritus was ranked the most abundant item in all other fish (data not shown).

DISCUSSION

Our results demonstrate ecological effects of both nutrient addition and predator reduction at the whole-system level, including evidence for synergistic interactions. Our nutrient loading rates were about 10x less than previous dry fertilizer plot-level experiments, yet we detected responses at several trophic levels within 1-2 y, suggesting that application of nutrients via daily water flooding is critical to understanding the impacts of coastal N enrichment. The results also suggest that eutrophication may have

cumulative effects that are not apparent in a single year of nutrient enrichment and interactive effects with species composition. Observed changes in primary producers and decomposers suggest a long-term change in N-processing capacity in response to chronic N-enrichment.

Nutrient loading

Our annual N loadings were low compared to most plot level experiments, despite the high concentrations we achieved in the water column. The simplest approach to estimating the loading rate in our experiment is to divide the total kg of N added by the area of the marsh resulting in a watershed average loading of $30 \text{ g N m}^{-2} \text{ y}^{-1}$. This approach does not account for the spatial gradient of nutrient delivery set up by the differential flooding by the tide and marsh elevation. We can estimate habitat specific loading rates by using a marsh-flooding model (Harris, Vallino and Friedrichs, unpublished data) that estimates the duration and depth of flooding over the experimental addition period of 150 days and measured nutrient concentrations. The intertidal tall *S. alterniflora* habitat experiences a tidally averaged 20 cm of water twice daily, resulting in an annual loading of $\sim 60 \text{ g N m}^{-2} \text{ y}^{-1}$. In the less frequently flooded *S. patens* marsh the annual loading was around $15 \text{ g N m}^{-2} \text{ y}^{-1}$. Nitrogen loading in plot level experiments was generally $100 - 500 \text{ g N m}^{-2} \text{ y}^{-1}$ (Mendelssohn 1979, Valiela 1983, Dai and Wiegert 1997, Boyer and Zedler 1998, Tyler et al. 2003).

We increased the average loading rate about 10x background for this estuary; ambient watershed loading to the whole Plum Island Sound estuary is $\sim 3 \text{ g N m}^{-2} \text{ y}^{-1}$ (Williams et al. 2004). Another perspective on our loading rate is that the amount of N that we added was roughly equivalent to the nutrient loading from the addition of 1000 houses in the upland watershed of each of the nutrient enriched marsh creeks (Cape Cod Commission 1992).

The consistent decrease in NO_3^- concentration in ebbing water in both creek pairs suggests retention of some of the added NO_3^- by the marsh ecosystem. Of course the

entire nutrient content of the water is not removed because at high tides water depths are too deep and residence time too short to allow for complete assimilation by marsh processes. A rough calculation, based on the incoming and outgoing nutrient concentrations and a net water balance, suggests that 30-40% of the added nitrogen did not leave the marsh in the ebbing water. Hydrodynamic modeling and $\delta^{15}\text{N}$ tracer addition experiments are currently being used to obtain a more precise estimate of the retention and fate of the added nutrients.

Question 1 – Response of vascular plants and microbial decomposers

The dominant primary producers, *Spartina* spp. and benthic microalgae generally respond positively to N fertilization (Sullivan and Currin 2000, Mendelsohn and Morris 2000). The combined results of previous studies suggests the dose-response of *Spartina* production was nonlinear with little increase with low N additions (20 g N/m²/y; Valiela 1983), steep increases at medium amounts (100-500 g N/m²/y) and then no further increases at higher N loading rates (Mendelsohn 1979).

Contrary to our expectation that because of the relatively low loading the plant response would not be detectable, *S. alterniflora*, and perhaps *S. patens* production has increased. The first two years of nutrient enrichment in Creek Pair 1 increased the length specific biomass in tall, creek bank *S. alterniflora* resulting in increased aboveground plant production. As in most plot level nutrient loading experiments (e.g., Valiela 1983), we found no difference in stem density for any plant species or growth form. Increased production resulted from greater biomass per unit length of shoot or longer shoots, or in some cases both. We observed a response in year 1 of Creek Pair 1, but not in year 1 of Creek Pair 2. In the first year of some studies researchers found little response (Valiela 1983) while others found differences with nutrient enrichment (Tyler et al. 2003).

The higher aboveground plant nitrogen content in nutrient enriched plants supports the suggestion that both growth forms of *S. alterniflora* responded to our low nutrient loading. Nitrogen content was consistently greater in both tall and short forms of *S.*

alterniflora in the nutrient enriched compared to the reference creek as has been found in many other nutrient enrichment studies (Tyler et al. 2003). Interestingly, despite taller plants in both years, nitrogen content of *S. patens* was identical in the two creeks. Nitrogen content in roots and rhizomes was less clear-cut with a tendency toward greater nitrogen in tall *S. alterniflora* in the nutrient enriched creek compared to the reference, and no difference in short *S. alterniflora* from the high marsh. This result is similar to Boyer et al. (2000) who found that less than 2% of N added was incorporated into root tissue and that there was only a slight trend towards increased belowground biomass with N nutrient enrichment in a created *Spartina foliosa* marsh.

Spartina spp. exhibits low uptake rates of NO_3^- from overlying water (Wright et al. 1996) consistent with *Spartina* obtaining its nutrients from belowground. The probable mechanism for increased growth of *S. alterniflora* near creek edges is tidal pumping (Mendelssohn and Morris 2000), which flushed nutrient rich water through the root zone at the creek edge. The ditching of the marsh increased water inundation and nutrient transport onto the high marsh, potentially increasing the *S. alterniflora* edge effect. The response of *S. patens* was probably limited by the infrequent flooding of the high marsh with the enriched water and because the nutrients do not penetrate deeply into the already water saturated soil of the high marsh during the short period of time (3 - 4 hours) that the marsh is flooded each tide.

Microbial production

Nutrient enrichment was expected to stimulate bacterial activity and decomposition throughout the ecosystem because of direct uptake of N from the water column and availability of more high quality organic matter from increased algal production (Howarth and Hobbie 1982). We expected that in sparsely vegetated salt marsh habitats such as creek bank *S. alterniflora*, where there is sufficient light penetration for benthic microalgae to proliferate (Pinckney and Zingmark 1993), rates of bacterial production would increase as an indirect result of the increase in highly labile

carbon from benthic microalgae. Conversely in more densely vegetated habitats such as *S. patens* high marsh we anticipated that, because of the large store of carbon in marsh peat, bacteria would be nitrogen limited, and bacterial production would be directly enhanced by new nitrogen inputs.

We found a microbial production increase only in the tall *S. alterniflora* habitat, not in the *S. patens* high marsh habitat. If bacteria in these salt marsh sediments were responding directly to the increased nitrogen input we expected to see stimulation of bacterial production in both creek bank *S. alterniflora* and *S. patens* habitats. The lack of a response to the nutrient addition in the *S. patens* high marsh habitat was surprising given the large store of carbon and the importance of microbial decomposition in salt marshes (Howes et al. 1984, Buchan et al. 2003). Nitrate additions in laboratory experiments have stimulated aerobic salt marsh detritus decomposition (Howarth and Hobbie 1982, Morris and Bradley 1999) and anoxic decomposition via NO_3^- reduction (Zumft 1991).

Because we see microbial production stimulation only in the nutrient enriched creek bank *S. alterniflora* habitat where benthic microalgae also increased, this suggests that bacteria are responding primarily to increases in high quality organic matter from benthic microalgae, not to increases in dissolved nitrogen. Although salt marsh macrophytes dominate the total primary productivity in marsh ecosystems, stable isotopic evidence indicates that it may not be a preferred carbon source for marsh sediment bacteria even in the presence of elevated inorganic nitrogen. Stable carbon isotope ratios of bacterial specific polar lipid-derived fatty acids from the Waarde Marsh (The Netherlands), indicated that the dominant carbon source for marsh sediment bacteria was algal (Boschker et al. 1999). Similarly, in a $\delta^{15}\text{N}$ tracer addition experiment in the Rowley River, Massachusetts, the $\delta^{15}\text{N}$ value of the bacterial specific biomarker diaminopimelic acid indicated that the bacteria were more closely linked to benthic microalgae than bulk sediment detritus (Tobias et al. 2003). These findings support the

hypothesis that bacterial production is indirectly stimulated through increased production of benthic microalgae. We are examining this hypothesis through a $\delta^{15}\text{N}$ tracer addition experiment in the nutrient enriched and reference marshes and with litterbag experiments.

Question 2 – Top Down Controls

We found evidence for top-down control on benthic microalgae and on marsh epifauna, but not on benthic infauna. Top-down effects on benthic microalgae were seen only under nutrient enrichment suggesting that killifish influence microalgae via direct effects (e.g., herbivory). The response of the benthic microalgae is suggestive of a synergistic effect of nutrients and top down control and perhaps a self-shading limitation (Hillebrand 2005). Benthic algal biomass and productivity typically increased in response to N additions but these increases were often limited by marsh grass shading and grazing (Pinckney and Zingmark 1993, Sullivan and Currin 2000). The lack of response in microalgal biomass in nutrient enriched *S. patens* was consistent with previous studies that found light limited benthic algal production under the dense *S. patens* canopy; we found average light under the *S. patens* canopy was <3% of ambient (Sheldon, unpublished data).

In the *S. alterniflora* habitat, where light is often not limiting, the simplest microalgal response model assumes a linear increase in biomass with nutrient addition and no consumer control. This model predicts that benthic microalgae would increase from 150 (background) to 2000 mg chl *a*/m² with our 15x increase in nutrient enrichment, if there were no other limiting factors. While we saw an almost doubling of microalgal biomass in Year 2 with nutrient enrichment and fish reduction (to 250 mg chl *a*/m²), the increase was clearly not directly proportional to added nutrients. Species composition also changed, with more diatom species and few cyanobacteria species found in the nutrient enriched creek, suggesting potential increases in the palatability of algae and loss of N fixation. Typical values for benthic microalgal biomass in the *S. alterniflora* habitat are ~100 mg chl *a*/m² (Sullivan and Currin 2000) suggesting that the initial high microalgal biomass might have limited the scope for response before self-shading became limiting

(Hillebrand 2005). The highest microalgal biomass measured in any salt marsh habitat was 800 mg chl *a*/m² in a cyanobacteria mat in a high marsh salt panne (Sullivan and Currin 2000). Our nutrient addition decreased the abundance of cyanobacteria, thus development of a dense cyanobacteria mat is not likely to occur. It is doubtful that we will ever measure 2000 mg chl *a*/ m² unless the algal community changes from domination by diatoms to filamentous algae or macroalgae.

Grazers also clearly play a role in the response of benthic microalgae to nutrient additions (Sullivan and Currin 2000). We found a top-down control on benthic microalgae but only under conditions of nutrient addition. This is consistent with other studies that found the largest increases in benthic microalgae in response to increased nitrogen occurred when grazers were removed (Sullivan and Currin 2000). Killifish is considered a secondary consumer, with a diet of small invertebrates, benthic algae and detritus (Deegan et al. 2000, Currin et al. 2003). Contrary to our expectations, benthic microalgae increased with mummichog reduction suggesting that either mummichog exert top down control by direct grazing or that the trophic cascade operated in ways we do not understand. Based on previous work, we expected that mummichog removal would result in a trophic cascade in which infauna increase and benthic microalgal biomass would decrease in response to increased infaunal grazing. Instead, our results suggest that under conditions of high algal productivity killifish may drop in trophic level from carnivore to herbivorous grazer and become important grazers in a nutrient enriched environment.

Counter-intuitively, our results show that small infauna abundance decreased slightly with mummichog reduction. We did not find the predicted increase in small infauna expected if release from predation by mummichog controlled their abundance. Our methods of lower mummichog abundance were most effective on fish > 40 mm, and larger mummichogs probably prey more heavily on larger epifauna, (e.g., amphipods and grass shrimp) and algae than small infauna (Currin et al. 2003, Fell et al. 2003), perhaps

explaining the absence of direct top-down control on infauna. An increase in the importance of algae in the diet of large mummichogs remaining in the fish reduction treatment supports the potential importance of direct grazing; this idea is also being examined by stable isotope analysis. Additionally, *Spartina* stems may inhibit foraging by *F. heteroclitus* (Walters et al. 1996, Carson and Merchant 2005), thereby precluding strong direct effects in grass habitats.

Our experimental reduction of mummichogs may have affected an intermediate predator of infauna, the grass shrimp, and perhaps initiated an indirect interaction. *P. pugio* graze epiphytic algae and forage for macroinfauna (Fleeger et al. 1999). Smaller mummichogs compete with grass shrimp for infaunal food and larger mummichog prey on grass shrimp (Kneib and Stiven 1982, Cross and Stiven 1999, Weis et al. 2000). Mummichog removal was thus predicted to increase grass shrimp growth and abundance and thereby increase grazing pressure on microalgae and infauna. Although we found no effect on abundance of grass shrimp with mummichog reduction, fish reduction may have allowed grass shrimp to modify their behavior. Carson and Merchant (2005) found that grass shrimp reduce swimming behavior in the absence of large mummichogs, resulting in increased benthic contact time that may facilitate grass shrimp feeding activities. Thus, observed declines in infauna in areas where mummichog abundance was experimentally reduced could be mediated by increased grass shrimp predation. We are currently examining infaunal responses in other marsh habitats and quantifying species composition to determine if responses to mummichog reduction were habitat or species specific (Posey et al., 1999, found species-specific responses).

An important prey species for large mummichog is the intertidal amphipod (*Uholorchestia spartinophila*), a large epifaunal amphipod (Deegan, unpublished, Covi and Kneib 1995). Amphipod abundance in *S. alterniflora* habitats more than doubled with mummichog reduction (Fig. 9), suggesting release from predation. *U. spartinophila*

is widely distributed through the vegetated intertidal marsh, unlike other talitrid amphipods that are restricted to supralittoral zones (Covi and Kneib 1995), and our results demonstrate that their abundances may be regulated by mummichog predation. This may have consequences for long-term gains or losses in marsh elevation as these amphipods are detritivores that can accelerate the decomposition of *S. alterniflora* litter by stimulating microbial activity (Lopez et al. 1977, Bousfield and Heard 1986). These amphipods also feed on benthic microalgae (Galván, unpublished data) and it is possible that they are important grazers in this system. Additional studies are planned to examine treatment effects on amphipod activity patterns.

Unexpectedly, infauna responded to nutrient addition with reduced abundance in the first year of nutrient addition. We are currently examining annelid biomass to determine if there was a per capita increase in biomass with nutrient addition as found by Posey et al. (2006). This first year of nutrient addition did not bring about large increases in benthic microalgae perhaps explaining why infauna did not respond in a bottom-up cascade. Furthermore, the increased body size of grass shrimp in nutrient enriched creeks suggests they exerted increased predation pressure on infauna. Collections from the second year of nutrient addition are currently being processed.

Overall, we found that top-down effects may modify the responses of benthic microalgae to nutrient enrichment, i.e., a synergistic effect on benthic microalgae biomass occurred when nutrient were added and when mummichog was reduced in saltmarsh creeks, but that light limitation prevented similar responses in the high marsh. Although nutrient addition and predator reduction effects noted to date are best expressed as independent on benthic infauna, additional collections across the marsh landscape are

being processed. Most tests for interactions among environmental factors are based on small-scale exclusion (Posey et al., 1999) or mesocosm studies (Breitburg et al. 1999); we anticipate that our whole-ecosystem study will provide a more realistic picture of how nutrient addition and predator reduction interact throughout a salt marsh.

CONCLUSIONS

Human activity is changing local and global environments at unprecedented rates. Some of the most significant changes involve global warming, sea level rise, widespread nutrient enrichment, and species changes. All of these affect salt marsh ecosystems. Understanding the responses of salt marsh ecosystems to stressors, as well as the mechanisms and processes that underlie these responses, is fundamental to the pressing regulatory and cultural decisions that many communities now face. Our project fills a unique role in provision of an ecosystem-scale, multi-year perspective for management decisions, such as installation of sewage treatment plants or regulating housing density that will affect coastal communities and ecosystems for generations.

The initial results and analyses presented here demonstrate ecological effects of both nutrient addition and fish reduction at the whole-system level. Evidence of synergistic interactions between these stressors is also emerging, especially at higher trophic levels. Given the cumulative effects we have observed, it is far too early to make long-term predictions about the relative stimulation of plant production versus microbial decomposition. Although we have identified some early effects of these stressors, we know that salt marsh ecosystems will respond to stress over time periods much longer than two years; some effects may be cumulative, and some, such as plant community change, may take many years to develop. This highlights the importance and potential benefit of continuing the manipulations for several additional years. Related, ongoing studies within the project include sediment dynamics, hydrodynamics, benthic microalgal physiological responses, phytoplankton community and nutrient cycling, and isotopic

tracer studies examining food webs and biogeochemistry; these will add substantially to our understanding of the effects of multiple anthropogenic stressors on salt marsh ecosystems. Our continuing work examines response thresholds and time-lags, the mechanisms regulating salt marsh responses, cumulative responses over longer periods of disturbance, and, we hope eventually, recovery.

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TABLE 1. Salt marsh characteristics (mean \pm 1 SE) prior to experimental manipulations (1998-2003).

	P-value	Creek Pair 1		Creek Pair 2		Plum
		Sweeney	West	Clubhead	Nelson	Island Sound
Marsh creek area (m ² X10 ⁴)		12.5	14.8	11.4	12.1	
Water column						
Salinity (‰)	0.001	20.8 \pm 0.8 ^a	24.0 \pm 0.8 ^{ab}	27.0 \pm 0.8 ^b	25.2 \pm 0.9 ^b	29.1 \pm 0.9 ^b
Temperature (°C)	0.009	18.7 \pm 0.8 ^a	19.4 \pm 0.6 ^a	20.0 \pm 0.7 ^a	19.0 \pm 0.9 ^a	14.1 \pm 1.1 ^b
Nitrate (μ mol/L)	0.023	4.3 \pm 0.3 ^a	3.5 \pm 0.4 ^a	3.2 \pm 0.3 ^a	2.7 \pm 0.3 ^a	0.7 \pm 0.2 ^b
Ammonium (μ mol/L)	0.001	11.7 \pm 1.3 ^a	10.0 \pm 1.6 ^a	13.8 \pm 1.8 ^a	14.9 \pm 2.1 ^a	0.8 \pm 0.2 ^b
Phosphate (μ mol/L)	0.001	1.2 \pm 0.1 ^a	0.9 \pm 0.1 ^a	1.1 \pm 0.1 ^a	1.3 \pm 0.1 ^a	0.4 \pm 0.03 ^b
Phytoplankton (mg chl <i>a</i> /L)	0.001	8.4 \pm 0.9 ^a	8.8 \pm 1.6 ^a	7.4 \pm 0.8 ^a	9.0 \pm 1.1 ^a	2.5 \pm 0.4 ^b
TSS (mg/L)		15 \pm 0.8	18 \pm 1.0	19 \pm 1.3	14 \pm 1.6	
Benthic algae (mg chl <i>a</i> /m ²)						
Mudflat	0.001	62.7 \pm 3.7 ^b	93.6 \pm 7.7 ^a	134.7 \pm 6.7 ^c	110 \pm 11.1 ^{a,c}	
<i>S. alterniflora</i>	0.250	197.5 \pm 33.9 ^a	250.8 \pm 23.9 ^a	215.2 \pm 6.8 ^a	249.0 \pm 27.4 ^a	
<i>S. patens</i>	0.001	94.6 \pm 19.4 ^a	48.6 \pm 6.5 ^a	55.1 \pm 4.3 ^a	135.9 \pm 27.6 ^b	
Vascular Plant Community (% Cover)						

Tall <i>S. alterniflora</i>	0.738	5.5 ± 1.1	6.2 ± 1.1	7.1 ± 1.2	7.1 ± 1.1
Short <i>S. alterniflora</i>	0.001	16.8 ± 1.5 ^a	18.5 ± 1.6 ^a	24.9 ± 1.9 ^b	30.6 ± 1.8 ^b
<i>S. patens</i>	0.078	41.0 ± 1.7	42.3 ± 1.9	43.6 ± 2.0	37.1 ± 1.7
<i>D. spicata</i>	0.001	25.9 ± 1.5 ^a	18.0 ± 1.4 ^b	7.8 ± 1.1 ^c	8.0 ± 1.1 ^c
Number of plant species m ⁻²	0.001	2.7 ± 0.1 ^a	2.5 ± 0.1 ^a	2.1 ± 0.0 ^b	2.1 ± 0.1 ^b

Notes: Water column and benthic algae data are from 1998 – 2002. Total suspended solids and vascular plant community (% cover) is from 2003, prior to experimental treatments. The p-value is for ANOVA main effect for Creek on water column and benthic algae. Differences between creek branches were always $p > 0.05$. Different letters indicate differences among creeks within a row (Tukey, $p < 0.05$). Error degrees of freedom N (Appendix A): salinity = 240; temperature = 159; nitrate = 230; ammonium = 161; phosphate = 250; phytoplankton = 247; mudflat habitat benthic algae = 247; *Spartina alterniflora* habitat benthic algae = 99; *Spartina patens* habitat benthic algae = 102; TSS (Total inorganic suspended sediments) = 109; Vascular plants = 23 for each plant species and number of species.

TABLE 2. Incoming and outgoing nutrient concentrations (mean \pm SE) during the major tidal water flux periods for all creeks in the pre-manipulation year (Year 0) and nutrient addition creeks in treatment years (Year 1, 2). - represents samples that have not yet been analyzed.

	Baseline		Nutrient Addition					
	All Creeks		Creek Pair 1		Creek Pair 1		Creek Pair 2	
	Year 0		Year 1		Year 2		Year 1	
	In	Out	In	Out	In	Out	In	Out
NO ₃ ⁻	2.6 \pm	1.3 \pm	70.7 \pm	58.4 \pm	94.9 \pm	59.2 \pm	116.3	98.2 \pm
(μ mol/L)	0.3	0.3	38.3	38.9	18.4	17.1	\pm 20.8	22.4
NH ₄ ⁺	9.1 \pm	4.7 \pm	9.4 \pm	4.4 \pm	3.7 \pm	6.5 \pm	-	-
(μ mol/L)	3.1	3.4	1.3	0.6	1.0	1.9	-	-
PO ₄ ³⁻	0.9 \pm	1.0 \pm	5.1 \pm	4.2	-	-	-	-
(μ mol/L)	0.05	0.08	2.6	\pm 2.5	-	-	-	-

TABLE 3. Stem density (number of stems/m²; mean \pm SE) in 2005 for tall *Spartina alterniflora*, short *Spartina alterniflora* and marsh platform *Spartina patens*.

	Creek Pair # 1		Creek Pair # 2	
	Year 2 Nutrient addition		Year 1 Nutrient Addition	
	Reference	Nutrient Addition	Reference	Nutrient Addition
Tall <i>Spartina alterniflora</i>	263 \pm 15.9 ^b	206 \pm 12.4 ^a	197 \pm 8.9 ^a	173 \pm 8.7 ^a
Short <i>Spartina alterniflora</i>	1336 \pm 60 ^a	1336 \pm 118 ^a	1358 \pm 71 ^a	1532 \pm 87 ^a
<i>Spartina patens</i>	6938 \pm 2356 ^b	6263 \pm 1246 ^{a,b}	5025 \pm 1314 ^a	5575 \pm 1767 ^a

Notes: Different letters indicate differences among creeks within a row (Tukey, $p < 0.05$).

N = 24 for each species for each creek.

TABLE 4. Young-of-year grass shrimp (*Palaemonetes pugio*) size (mean TL (mm) \pm SE) and growth rate (TL (mm)) between August and September 2005.

Site and month or growth	Reference		Nutrient addition	
	Fish	Low Fish	Fish	Low Fish
Creek Pair 1				
August	21.69 \pm 0.03	21.55 \pm 0.02	23.02 \pm 0.03	21.02 \pm 0.01
September	26.97 \pm 0.01	24.52 \pm 0.01	27.93 \pm 0.01	24.71 \pm 0.01
Growth	5.28	2.97	4.91	6.39
Creek Pair 2				
August	21.85 \pm 0.02	21.74 \pm 0.03	21.53 \pm 0.01	21.25 \pm 0.01
September	26.16 \pm 0.01	26.07 \pm 0.01	28.53 \pm 0.01	27.76 \pm 0.01
Growth	4.31	4.33	7.00	6.01

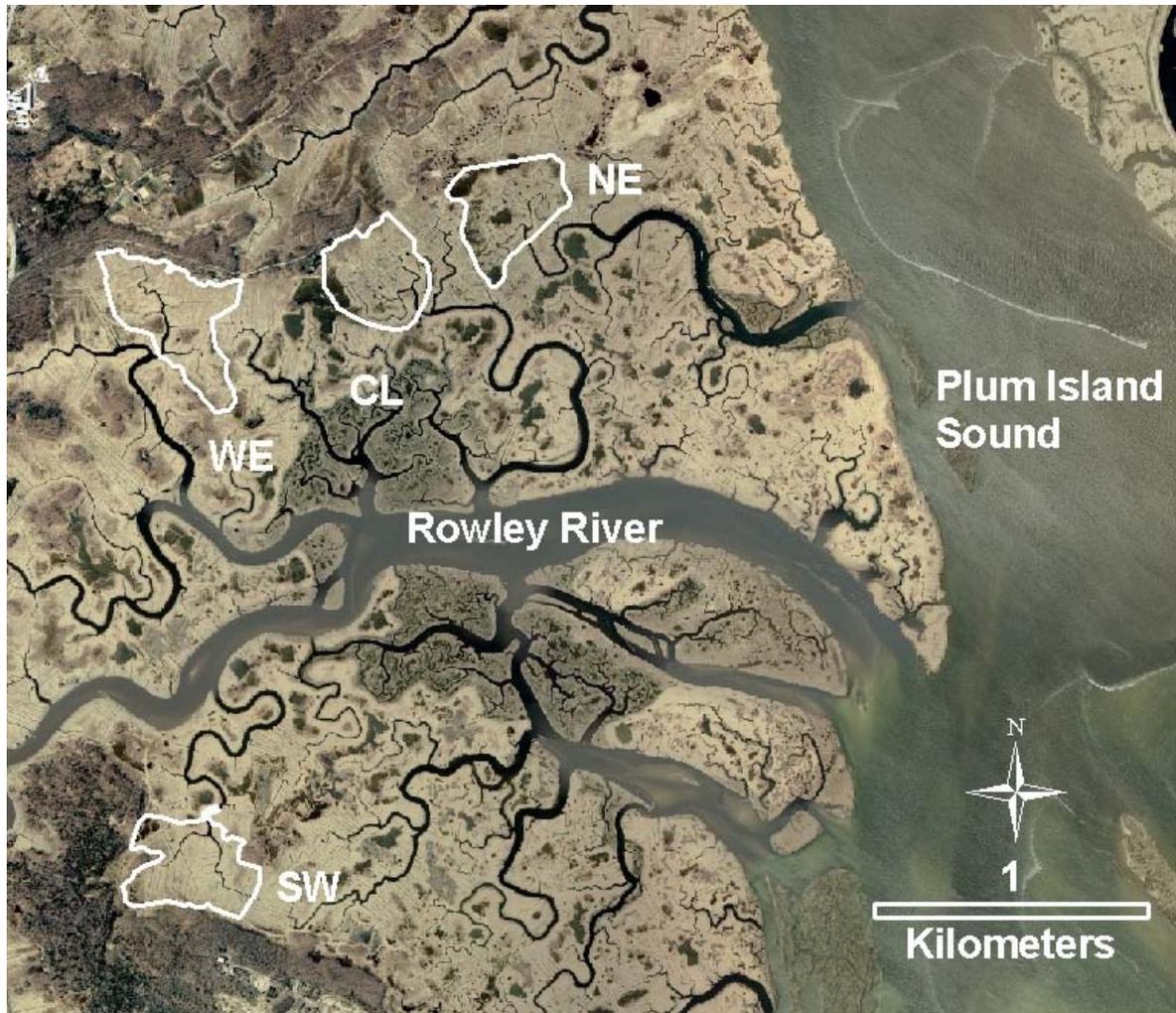


FIG. 1: Aerial photograph

(<http://maps.massgis.state.ma.us/MassGISColorOrthos/viewer.htm>; April 2001) of the Rowley River region salt marshes. Light areas are *Spartina patens* marsh and the darker areas are *Spartina alterniflora* marsh. Mottled dark areas seen in the northwest and southwest corners of the map are upland forest. The areas outlined in white are the experimental marshes (SW=Sweeney Creek, WE=West Creek, CL=Clubhead Creek, NE=Nelson Creek).

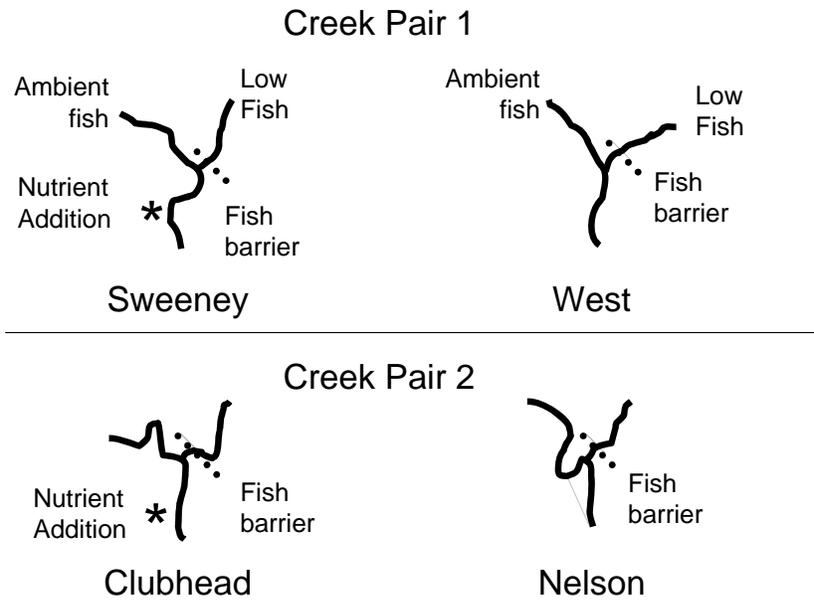


FIG. 2. Diagram of design of creek pairs and experimental treatments. * indicates location of nutrient addition to flooding water; dotted line indicates location of the fish barrier. Minnow traps were placed behind this barrier about every 25 m.

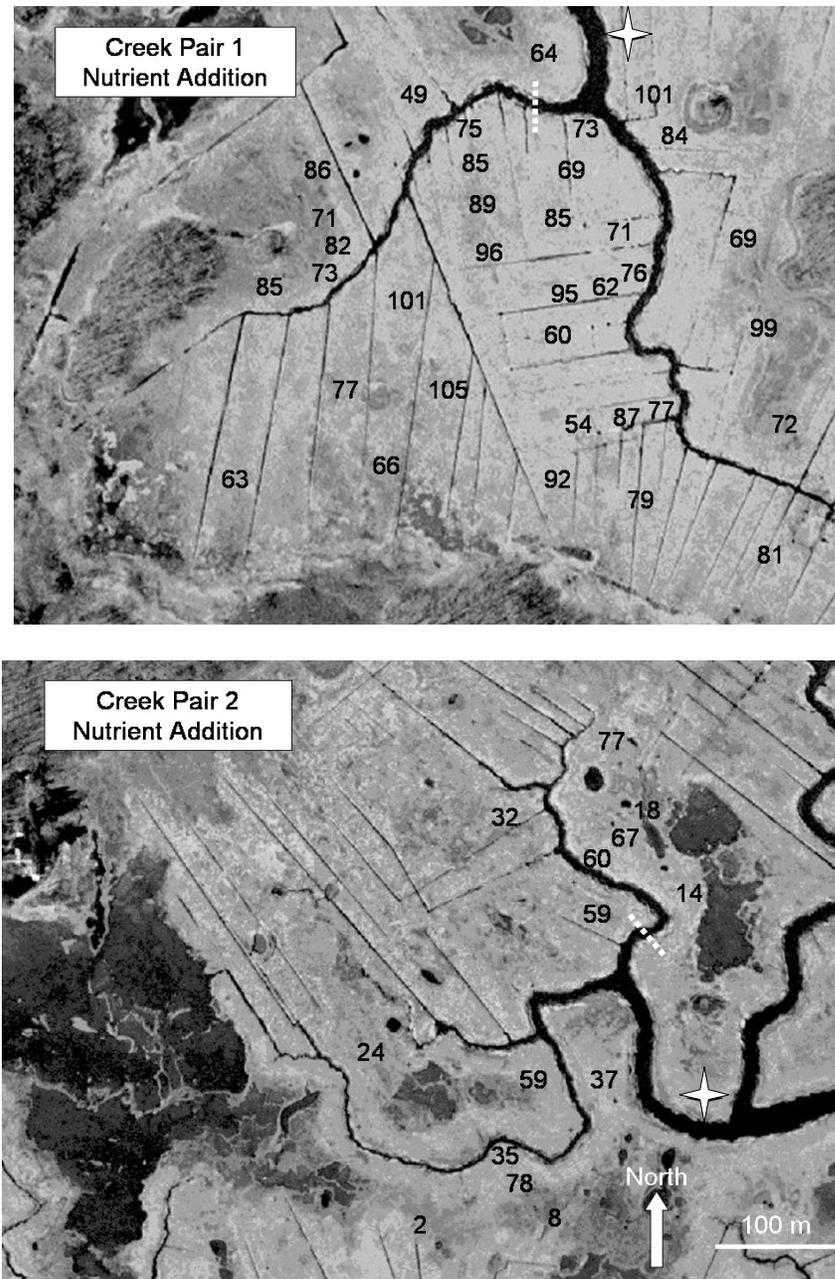


FIG. 3. NO_3^- concentration (μM) on the marsh platform (aerial photograph from <http://maps.massgis.state.ma.us/MassGISColorOrthos/viewer.htm>; April 2001) of the nutrient addition creeks of each creek pair at high tide, July 2005. Creek Pair 1 is Sweeney, Creek Pair 2 is Clubhead. The white star indicates the point of nutrient addition at each study site and the dotted line indicates the location of the fish barrier.

I

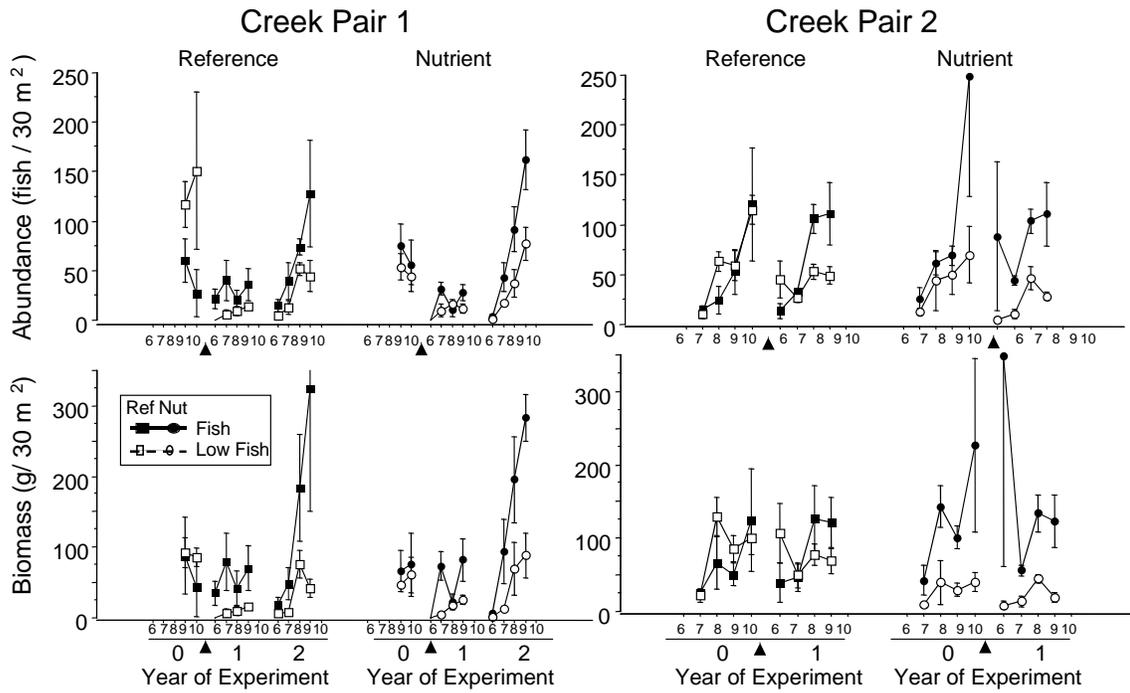


FIG. 4. Abundance (number fish/ 30 m²) and biomass (g/m²) of mummichog (*Fundulus heteroclitus*) in experimental creeks. Nutrient reference creeks are squares; nutrient enrichment creeks are circles. Open symbols are reduction in mummichog density treatments (Low Fish); closed symbols are ambient mummichog density treatments. Along the x-axis, Year 0 corresponds to pre-manipulation year, Year 1 and 2 correspond to first and second years of manipulation, numbers above year are months (6 = June etc.), small triangles indicate when manipulations began.

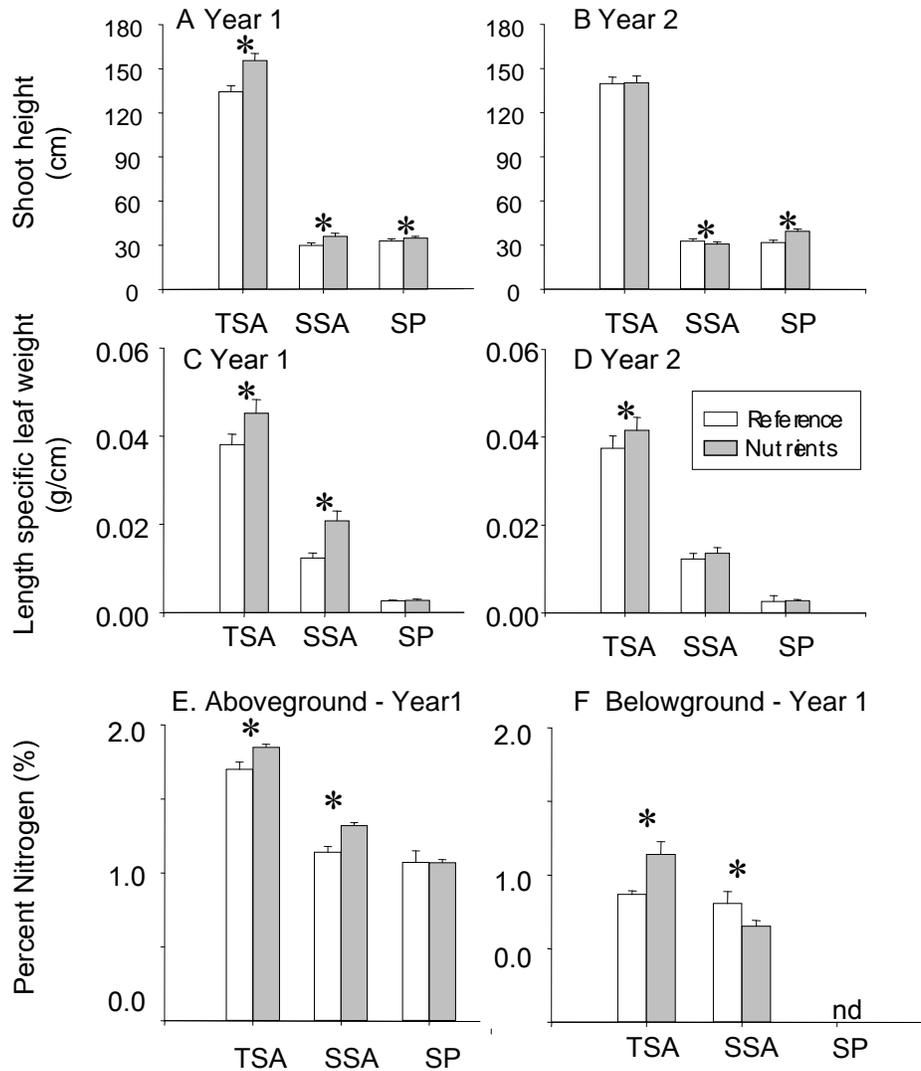


FIG. 5. Growth and % nitrogen content (mean \pm SE). of tall *S. alterniflora* (TSA), short form *S. alterniflora* (SSA), and *S. patens* (SP) of Creek Pair 1 after one and two years of nutrient addition. A, B: mid-August Shoot height (cm); C, D: mid-August length specific leaf weight (dry weight per cm of shoot length); E, F: Nitrogen content (% of dry weight) of above- and belowground biomass in year 1 (SP below ground not measured).

*indicates significantly different pairs (t-test, $P < 0.05$, $n = 125$ for each creek for plant growth and $n = 6$ for each creek for %N).

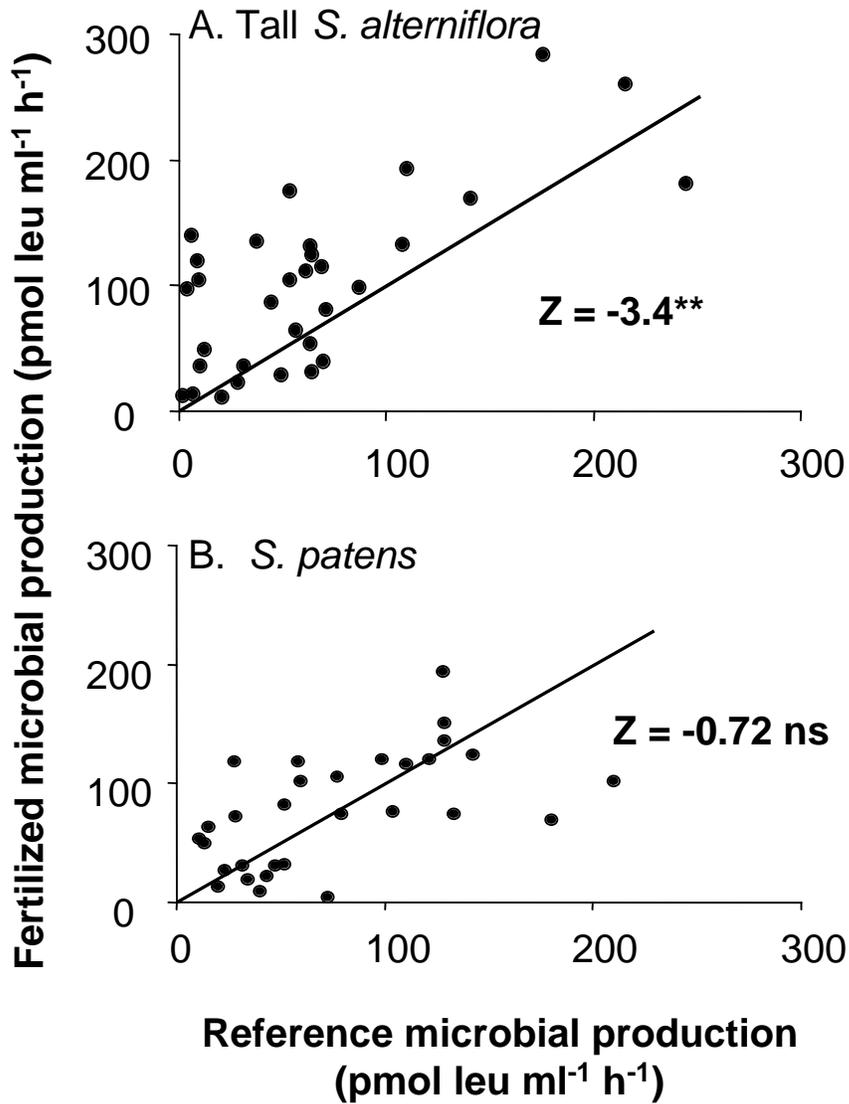


FIG. 6. Benthic microbial production in tall *S. alterniflora* and *S. patens* habitats in Creek Pair 1 ambient fish treatment after 2 years of nutrient enrichment. A significant difference from the one-to-one line was found for *S. alterniflora* (Wilcoxon Signed Rank test $z=3.5$, $P < 0.01$) but not for *S. patens* (Wilcoxon Signed Rank test $z=0.72$, $P > 0.10$).

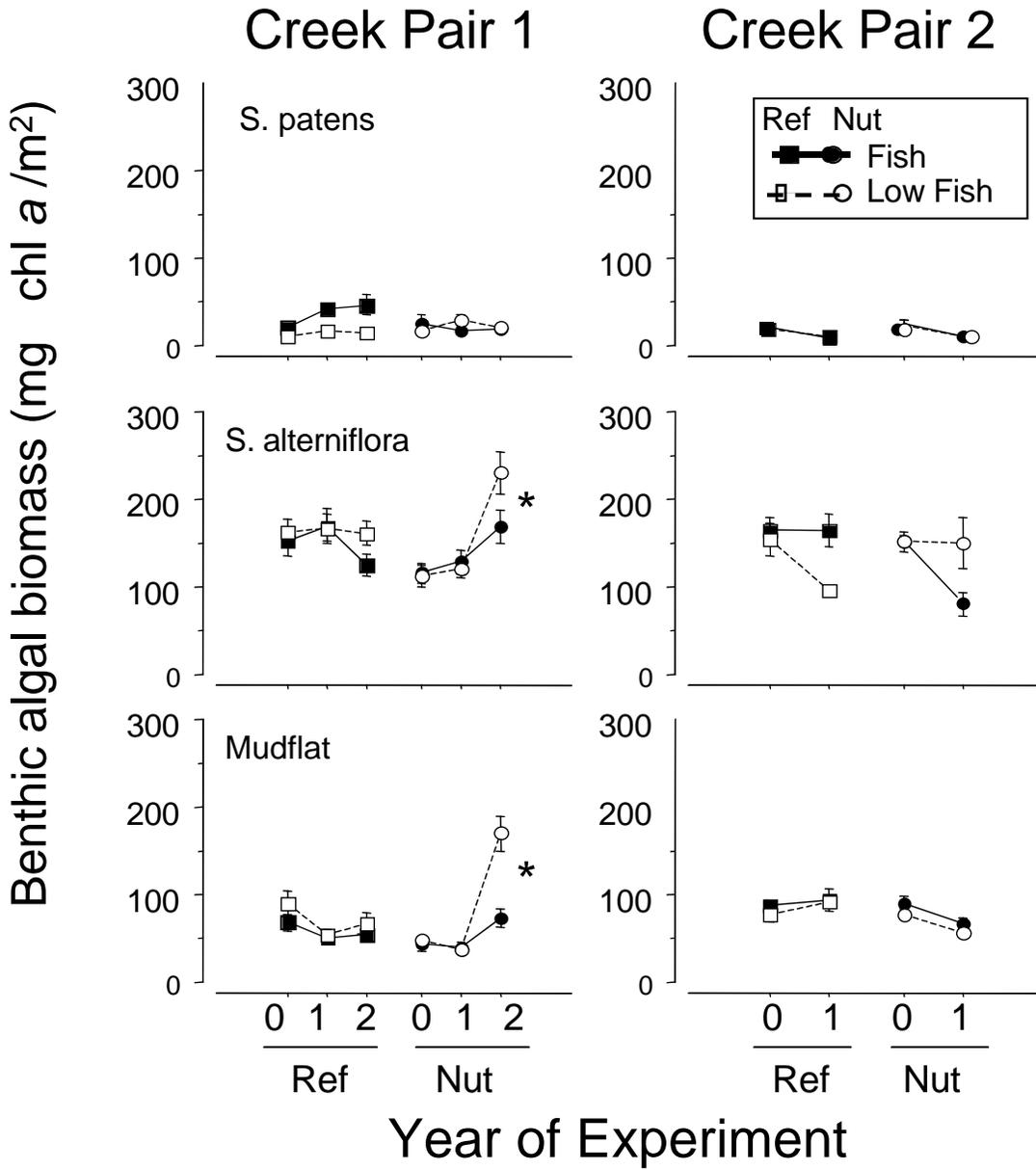


FIG. 7. Biomass of benthic microalgae (mg chl *a* m²) in mudflat, *S. alterniflora* and *S. patens* habitats. Nutrient reference creeks are squares; nutrient enrichment creeks are circles. Open symbols are reduction in mummichog density treatments (Low Fish); closed symbols are ambient mummichog density treatments.

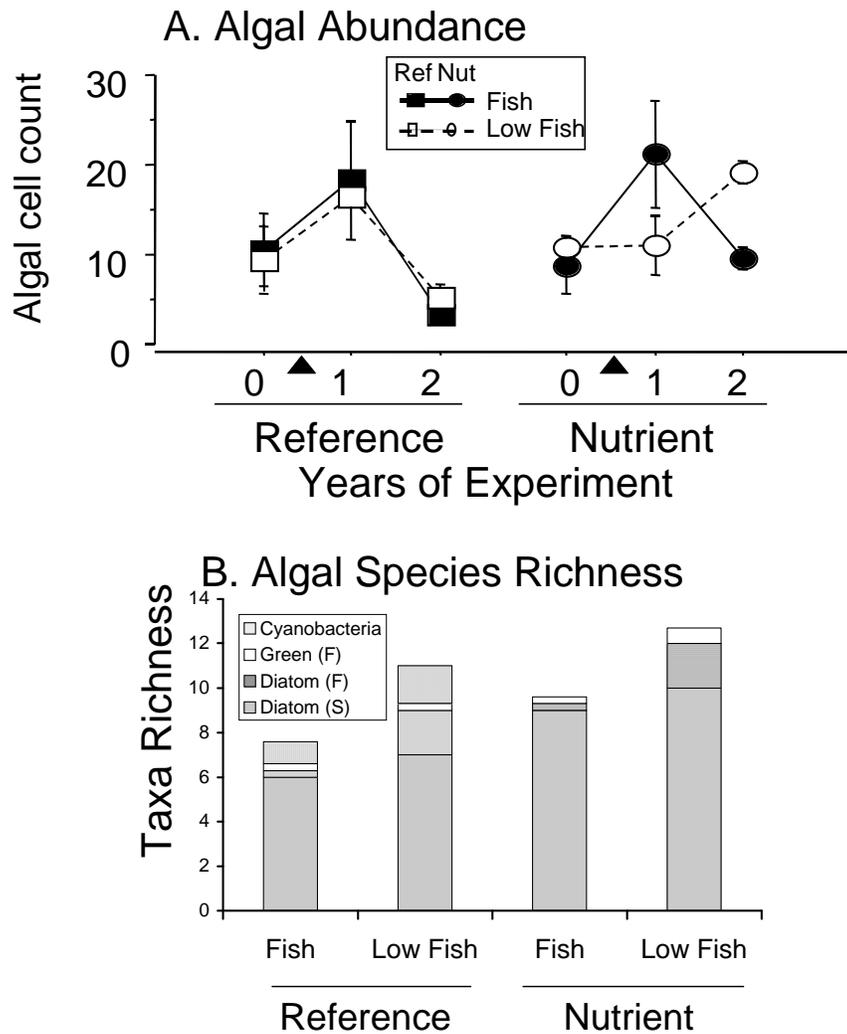


FIG. 8. Cell counts (A, mean \pm SE) and species richness (B, Number of species) of benthic algae in Creek Pair 1. A) Nutrient reference creeks are squares; nutrient enrichment creeks are circles. Open symbols are reduction in mummichog density treatments (Low Fish); closed symbols are ambient mummichog density treatments. Data are from Year 2. Triangle indicates start of manipulations. B) F in parentheses indicates filamentous species, S indicates single cell species. Data are from Year 2.

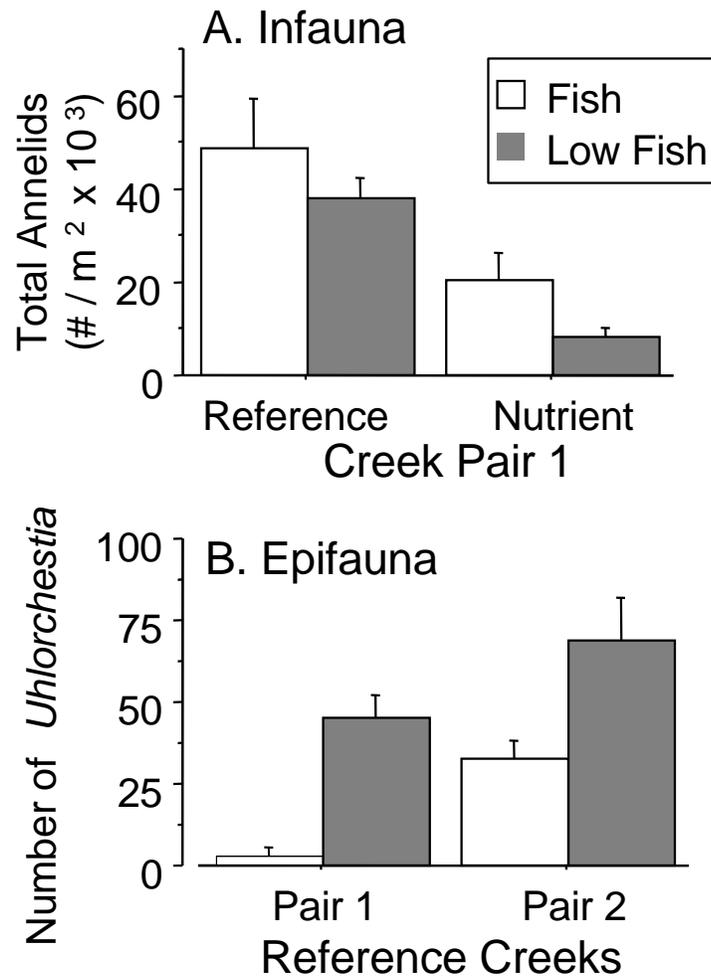


FIG. 9. Abundance of A) infauna (total annelids, number/m²) in Creek Pair 1 after one year of treatments (August 2004) and B) epifaunal (number of *Uhlorchestia*) in ambient Fish and Low Fish treatments in reference creeks (2005).

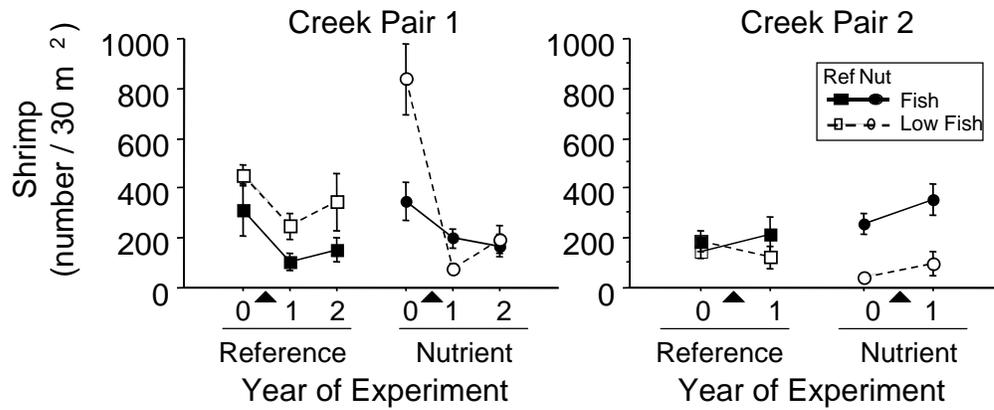


FIG. 10. Abundance of grass shrimp (*Palaemonetes pugio*; individuals/30 m²) in experimental creeks. Nutrient reference creeks are squares; nutrient enrichment creeks are circles. Open symbols are reduction in mummichog density treatments (Low Fish); closed symbols are ambient mummichog density treatments. Triangle on x-axis indicates start of manipulations.