Top-Down and Bottom-Up Control of Infauna Varies Across the Saltmarsh Landscape

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Abstract

Responses of infaunal saltmarsh benthic invertebrates to whole-ecosystem fertilization and predator removal were quantified in Plum Island Estuary, Massachusetts, USA. Throughout a growing season, we enriched an experimental creek on each flooding tide to 70 μM NO_3^- and 4 μM PO_4^{-3} (a 10 x increase in loading above background), and we reduced *Fundulus heteroclitus* density by 60% in a branch of the fertilized and a reference creek. Macroinfauna and meiofauna were sampled in creek (mudflat and creek wall), marsh edge (tall form *Spartina alterniflora*) and marsh platform (*Spartina patens* and stunted *S. alterniflora*) habitats before and after treatments were begun; responses were tested with BACI-design statistics. Treatment effects were most common in the mid-range of the inundation gradient. Most fertilization effects were on creek wall where ostracod abundance increased, indices of copepod reproduction increased and copepod and annelid communities were altered. These taxa may use epiphytes (that respond rapidly to fertilization) of filamentous algae as a food source. Killifish reduction effects on meiobenthic copepod abundance were detected at the marsh edge and suggest predator limitation. Fish reduction effects on annelids did not suggest top-down regulation in any habitat; however, fish reduction may have stimulated an increased predation rate on annelids by grass shrimp. Interactions between fertilization and fish reduction occurred under *S. patens* canopy where indirect predator reduction effects on annelids were indicated. No effects were observed in mudflat or stunted *S. alterniflora* habitats. Although the responses of infauna to fertilization and predator removal were largely independent and of similar mild intensity, our data suggests that the effects of ecological stressors vary across the marsh landscape.

Keywords: saltmarsh gradient; fertilization; predator removal; *Fundulus heteroclitus;* macroinfauna; meiofauna; impact assessment; indirect effects

Introduction

Agrawal et al. (2007) recently pointed out that three assumptions are implicit in most ecological research; (1) the effects of multiple factors are independent (2) traits of interacting species are uniform and unchanging and (3) feedbacks inherent to ecological interactions may be ignored without diminishing the understanding of complex interactions. Few experiments have been conducted to test these assumptions across large spatial scales and in different ecological contexts; however, conditional outcomes of species interactions (Bronstein, 1994), indirect effects (Wootton, 1994) and trait-mediated interactions (Preisser et al., 2005) have been reported. Coastal ecosystems are becoming increasingly threatened as humans exploit resources and alter habitats (Vituosek et al., 1997; Jackson et al., 2001), and understanding multifactor anthropogenic-induced change is a priority (Riedel and Sanders, 2003; Wiegner et al., 2003). It is therefore important for coastal resource managers, conservationists and ecologists alike to know if the assumptions listed above compromise our understanding of anthropogenic effects.

The salt marsh is an appropriate model system to examine how ecological effects vary across physical gradients in coastal systems. Salt marshes exhibit complex habitat structure and biotic zonation. Marsh landscapes include unvegetated mudflats, a creek-marsh ecotone between vegetated and non-vegetated sediments and a densely vegetated high marsh platform. Inundation, aerial exposure, flow, light, and sediment chemistry, along with biotic factors, vary across the elevation/inundation gradient. Because of this variation, traits of interacting species may differ across the gradient and variable responses to environmental challenges are possible. Studies of saltmarsh benthos have examined abiotic (e.g., flow, Fleeger et al., 1984, and nutrients Valiela et al., 2004) and biotic factors (Silliman and Zieman, 2001) or both (Posey et al., 1999; Novak et al., 2001; Posey et al., 2002). Most studies, however, have been conducted in only one habitat type (but see Palmer, 1986 for an exception) and/or in small experimental plots. Small-scale manipulations are susceptible to artifacts that may limit ecosystem phenomena, e.g., natural movements of animals may be restricted or cage artifacts may occur, limiting the generality of findings (Carpenter et al., 1995). Little is known about gradients of predation pressure on saltmarsh animals although Pennings and Bertness (2001) posit that predation pressure is highest at creek-marsh interface. Thus, how the landscape responds as a whole to human impacts may be poorly addressed by most previous studies.

Identifying the most informative bioindicators (Walker, 1992) is also an important consideration in assessment studies. As relatively sedentary consumers of primary production and prey for higher trophic levels, benthic infauna are often used to assess the impact of anthropogenic activities (Warwick et al., 1990; Levin and Talley, 2002). Although there is limited information regarding benthic infaunal communities along the marsh tidal inundation gradient (although see Coull et al., 1979 and Johnson et al. 2007), two size classes, meiofauna (63 µm – 500 µm) and macroinfauna (> 500 µm), are often used to monitor benthic environments. Although meiofauna taxonomy may be daunting to non-specialists, meiofauna may be advantageous for monitoring because of their: (1) relative ease of sample collection and processing (2) short generation times (3) intimate association with sediments throughout life history (without dispersing larvae) that increases the likelihood that changes in abundance are due to effects of the factor of interest and (4) high density and biodiversity that provide exceptional information content regarding community responses. Meiofauna have been implicated as the more sensitive indicator (Boucher, 1980; Coull and Chandler, 1992; Warwick, 1993; Christie and Berge, 1995; Schratzberger et al., 2003) but may not be sensitive to all ecological stressors. For instance, meiofauna disperse quickly via resuspension (Chandler and

Fleeger, 1983; Palmer, 1988) and may not be as sensitive to mechanical disturbance as sedentary, tubiculous macroinfauna (e.g., amphipods, annelids) (Austen et al., 1989). Thus, the responses of these two biotic groups may provide complementary information. Studies rarely examine ecological responses of macroinfauna and meiofauna simultaneously (exceptions include Bell and Woodin, 1984; Netto et al., 1999; Gobin and Warwick, 2006).

As trophic intermediates in food webs, infauna may shed light on the relative importance of top-down and bottom-up control and reveal interactions between these human-induced stressors. Nutrient loading increasingly threatens coastal systems and predator reductions by overfishing are common; as a result, both often occur simultaneously (Heck et al., 2000; Deegan et al., 2007). The purpose of this report is to discuss the effects of whole-ecosystem experimental nutrient addition (bottom-up effect) and predator reduction (top-down effect) on saltmarsh macroinfauna and meiofauna. To this end, we conducted fertilization and predator removal manipulations in tidal creeks of the Plum Island Estuary (PIE), Massachusetts, USA in such a way that treatment effects were exerted across the marsh landscape. Thus, we are able to test assumptions of independence between multiple factors across a landscape and examine the possibility that trait-mediated responses, such as those associated with trophic cascades, vary across locations. Our null hypotheses are: (1) top-down and bottom-up responses (and interactions) by infauna do not differ across the landscape and (2) meiofauna and macroinfauna respond equally to our treatments.

Materials and Methods:

Site Description

Two bifurcated intertidal creek systems, Sweeney and West, were studied; both open into the Rowley River (42°44'N, 70°52'W), which opens into Plum Island Sound at about 7-km inland from where Plum Island Sound enters the Atlantic Ocean (Fig. 1). Sweeney Creek, the creek farthest inland, opposes West Creek across the Rowley River.

Infaunal invertebrates were examined in five habitats that span the inundation gradient: two creek habitats, the creek-marsh interface, and two marsh platform habitats. Mudflats are gently sloping unvegetated creek habitats consisting of poorly consolidated sediments in the creek floor near the creek wall. Migrating diatoms, chlorophytes and cyanobacteria dominate sediment-dwelling algae (hereafter referred to as edaphic algae) in mudflat (Galván, unpublished). Creek walls are steep, almost vertical walls about 1.5 m in height with cohesive sediments and an approximately 30-cm wide band of macroalgae and filamentous algae. Marsh edge is dominated by a zone of tall-form *Spartina alterniflora* (>130 cm in late summer) that baffles water flow and shades sediment. The marsh platform consists of an expansive area dominated by a dense canopy of *S. patens* that greatly reduces light penetration to the sediment and a smaller zone of stunted *S. alterniflora* (< 40 cm in late summer) adjacent to saltmarsh pannes. PIE experiences a mean tidal amplitude of ~3 m during spring tides, and mudflat, creek wall, and tall-form *Spartina alterniflora* habitats are inundated twice daily while *S. patens* and stunted-form *S. alterniflora* habitats are inundated (to a depth of ~10 cm) during spring tides.

A faunal baseline survey was conducted before manipulations were initiated. Four creeks (including West and Sweeney) exhibited similar macroinfauna abundance, species composition and assemblages, although large faunal differences were found among habitats (Johnson et al., 2007). Preliminary analysis suggests similar trends for meiofauna major taxon abundance and composition, and for copepod species and assemblages (Fleeger unpublished).

Experimental Design

Long-term, whole-ecosystem manipulations of fertilization and predator removal were initiated in 2004 (Deegan et al., 2007). Here we report results from the first year of treatment application. In mid-May 2004, an enrichment of 70 μM NO_3^- and 4 μM PO_4^{-3} (15x over background) was implemented in Sweeney Creek downstream of the confluence of the two branches. Background nutrient values prior to fertilization were < 5 μM NO_3^- ; ~1 μM PO_4^{3-} , indicating Plum Island Estuary is a relatively low nutrient system, favoring a response to fertilization (Posey et al., 2006). Nutrients were added by pumping a concentrated solution of NO_3^- and PO_4^{-3} to the water of every flooding tide during the growing season (mid-May – Oct.; ~150 d) using a computer-controlled automated peristaltic pump. The pump rate was adjusted, based on a hydrologic model, every 10 min throughout each incoming tide to maintain constant N and P concentrations in incoming waters (Deegan et al., 2007). Watershed nutrient loading averaged 30 g N m^{-2} y^{-1} in 2004 (~10x background loading) but spatial variation across the landscape was significant. The tall *S. alterniflora* habitat experienced a higher nutrient loading than the less frequently flooded *S. patens* (Deegan et al., 2007). Fertilizer was not added to West Creek which is considered a reference creek.

The killifish, *Fundulus heteroclitus*, is considered a top predator in US salt marshes (Kneib 1986), and was selected to examine top-down effects on infauna. We opted to reduce rather than enhance killifish density because the marsh drains at low tide to only a few cm of standing water in creek channels, and concentrating a larger than normal number of fish into a small volume of water may have unexpected consequences. Thus, we considered density reduction a more tenable option. Although not commercially harvested, killifish reduction allowed us to mimic overfishing effects common in the real world.

A branch of each creek (downstream of the nutrient addition in Sweeney) was selected for large-scale removal of killifish. This was achieved by stretching a Vexar (6.35-mm mesh) block net across the entrance of the branch from June – September 2004, coupled with continuous fish trapping and removal. This method of exclusion should produce fewer artifacts than traditional small-scale exclusions (Virnstein, 1978). A 60% reduction in killifish density was achieved (Deegan et al., 2007). Reduction of large killifish (> 40 mm) was greater than small killifish (< 40 mm); however, a 40% reduction of small killifish was observed. Killifish are omnivorous, consuming a range of food including primary producers, infauna and larger prey (Allen et al., 1994). Killifish diet changes with size (Currin et al., 2003) and diet varies among habitats within salt marshes (James-Pirri et al., 2001). Further, different habitats within salt marshes offer unique trade-offs between predation and growth of killifish (Halpin, 2000), although little is known of the variation in foraging intensity of killifish across the marsh landscape. The species richness of nekton in experimental creeks is low (11 species) and killifish and grass shrimp comprised ~98% of the total abundance (19% and 79%, respectively) (Deegan et al., 2007). The mesh size of the block nets prevented larger killifish from entering, but allowed access by grass shrimp and small killifish. Because any other potential consumers (e.g., green crabs) were found in such low relative abundance, it is unlikely that their exclusion impacted infauna significantly.

The full factorial design of our experiment included two creek systems with four branches. Creek branches with each of the following treatments were examined; (1) nutrient addition (NA) and no fish reduction (FR), (2) no nutrient addition but with fish reduction, (3) NA and FR, and (4) no NA or FR. Because these treatments impacted the entire marsh landscape as a function of tidal flux, we were able to assess their impact across the landscape.

Benthic Sampling and Laboratory Analysis

Macroinfauna and meiofauna were sampled by hand coring at low tide. Pre-treatment collections were taken in June (17-19), July (9-10), and August (4-5) 2003 and post-treatment collections were taken in June (14-15) and August (2-3) 2004. In each creek branch, three transects were selected at ~50, 100, and 150 m from the confluence of the two branches. Each transect (50 m in length and 20 m in width) was stratified along an inundation gradient into the five habitat zones discussed above. Thus, a sample site in our hierarchical design consisted of a habitat nested within a transect nested within a branch nested within a creek. Meiofauna samples from marsh platform habitats and from all locations in August, 2003 were not examined due to resource limitations.

In 2003 collections, a single macroinfauna sample was taken at each sampling site (habitat within a transect within a branch within a creek), whereas two samples were taken at each site in 2004. Macroinfauna cores (6.6-cm inner diameter) were taken to a depth of 5 cm. This method inadequately samples larger, more mobile infauna (e.g., *Nereis diversicolor*) and surface-dwelling epifauna (e.g., amphipods). Cores were placed on ice in the field and fixed with 10% formalin and Rose Bengal in the laboratory. After a minimum of two days, cores were sieved through a 1-mm sieve stacked on top of a 500-µm sieve. Large debris and roots retained on the 1-mm sieve were discarded after visual inspection and removal of large invertebrates. Annelids constituted 97% of macroinfaunal abundances and are the focus of this study. All annelids were sorted and identified to species (some rare species were assigned a nominal species designation).

In 2003 collections, two meiofauna cores (2.2 cm inner diameter) were pooled into a single sample at each site, whereas two samples (each sample consisted of two pooled cores) were taken at each site in 2004. Cores were placed on ice in the field and fixed with 10% formalin and Rose Bengal in the laboratory. After a minimum of two days, cores were sieved through a 500-μm sieve stacked on top of a 63-μm sieve. Meiofauna retained on the 63 μm sieve were extracted from sediments using Ludox centrifugation following Somerfield and Warwick (1996). Meiofauna were identified and enumerated to higher taxonomic status (e.g., nematodes, polychaetes, ostracods). Further, each copepod was examined for sexual maturity. Mature copepods were identified to species, sexed and, if present, egg broods were noted. Demographic data were pooled for all copepods and sex ratio (M/F), percent ovigerous females and percent immature (i.e., copepodites) copepods were calculated for each collection. *Manayunkia aestuarina*, one of the most abundant polychaetes in macroinfaunal samples and the most abundant meiofaunal annelid, was enumerated from macroinfauna and meiofauna samples.

Species diversity (estimated as the number of species, Shannon's value and Pielou's evenness) of copepods and annelids was calculated separately from each sample with the use of PRIMER 5.2.9 software (Clarke and Warwick, 2001). Shannon's value was calculated as $\log_e$.

Univariate Statistical Techniques

We used a before-after, control-impact (BACI) experimental design which pairs experimental units and accounts for variability that may contribute to error in a completely randomized design (Underwood, 1994). Replication of ecosystem-scale experiments is difficult because it is often hard to find similar ecosystems (Carpenter et al., 1995)**;** the matched-pair approach helps ameliorate this difficulty (Stewart-Oaten and Bence, 2001). Although our design entails pseudoreplication, the BACI design is a powerful method for detecting impacts because it incorporates both temporal and spatial variation by observing reference and impact sites over time (Parker and Wiens, 2005). We used a BACI-type ANOVA (based on a level-by-time “parallelism” design) to analyze changes in abundance, copepod demography and species diversity. Level-by-time designs are ineffective if many zeroes are present (Parker and Wiens, 2005), and we analyzed taxa only in habitats where they were abundant. Previous analysis (Johnson et al., 2007) suggested that variance associated with transects for macrofauna populations did not contribute significantly to spatial variation in PIE, and therefore samples for macrofauna and meiofauna from the three transects were pooled for each branch for analysis; n / branch = 3 in 2003 and n / branch = 6 in 2004.

To detect interactions between fertilization and predator reduction, we performed analyses directly on values (abundance, demographic and diversity) instead of deltas (differences between reference and impact sites) (Stewart-Oaten and Bence, 2001). Data were analyzed using GLIMMIX, a SAS macro for fitting generalized linear mixed models (GLMM) using Proc Mixed (SAS v. 9.1.3). GLMMs are extensions of mixed models and can accommodate non-normal errors (Littell *et al.* 1996). GLMMs produce Type III F statistics and P values, which are based on likelihood estimations rather than sums of squares as in ANOVA. The GLIMMIX macro allows one to analyze fixed and random effects and sets the error distribution of the data. All data were $\log_e$-transformed and errors were assumed to have a Poisson distribution (Littell *et al.* 1996). Period, nutrient level, fish level and all possible interactions were set as fixed factors, whereas month within period was defined as a random factor. Only significant period*treatment interactions were of interest because they suggest that change over time occurred due to treatment effects. One assumption using this type of analysis is that although response variables at different sites may differ spatially, those differences track each other over time. This assumption, however, may be violated, reducing confidence in results (Wiens et al., 2004). To bolster confidence and to identify the direction of changes for significant interactions, we visually inspected graphs of data in pre-treatment and treatment periods. While other large-scale impact studies have used an alpha up to 0.20 (e.g., Steinbeck et al., 2005), we chose an alpha of 0.05 to counter the effects of Type I error-rate inflation due to a large number of univariate analyses (~70).

Multivariate Techniques

To detect differences among communities due to treatments, analysis of similarity (ANOSIM) and non-parametric multidimensional scaling (MDS) were conducted with PRIMER 5.2.9 software (Clarke and Warwick, 2001). Copepod and annelid communities were analyzed separately. In all ANOSIMs, creek (nutrient addition) and branch (fish reduction) effects were examined in a 2-way crossed design based on a Bray-Curtis similarity matrix of log (x+1) transformed and non-standardized data. Species were excluded if they comprised less than 1% of the total community. If evidence for a significant treatment affect was detected, MDS plots were generated to visualize trends. Cluster dendrograms were used (but not shown) to verify that sample clusters on plots represented true clustering and were not an artifact of high stress due to dimensional reduction (Clarke and Warwick, 2001). If an outlier was detected, it was removed and the ANOSIM and MDS plots rerun. SIMPER analysis was used to determine species contributing the most dissimilarity to community differences.

Results

Population responses

Macroinfaunal annelid abundance was variable, ranging from ~2000-65000 m^{-2}, across the landscape (Fig. 2). Highest abundances were on creek walls and lowest on the marsh platform. Thirteen major taxa of meiofauna were sampled; nematodes comprised ~80% of the total meiofauna, although copepods and juvenile annelids were also abundant and ubiquitous.

Other common groups included copepod nauplii, ostracods, insect larvae and tanaids. Total meiofaunal abundance ranged from ~300-6000 10 cm^{-2} with lowest values in mudflats and highest at the marsh edge (Fig. 3).

We examined the most abundant (> 5 %) species of annelids and major taxa of meiofauna for treatment effects in all habitats. Most macrofaunal and meiofaunal taxa (including total fauna) were similar in abundance in both creeks and all branches pre-treatment (Johnson et al., 2007) and few showed evidence for divergence post-treatment. Below, we discuss taxa that provide evidence for treatment effects based on BACI results (Tables 1 & 2).

An effect of nutrient addition on population abundance was observed only in meiobenthic ostracods, with a significant increase (period*nutrient interaction, $p = 0.021$; Fig. 3) regardless of fish treatment. Pre-treatment ostracod abundances were relatively low and similar in both creeks and all branches but became more variable and reached much higher values after treatments were initiated. Ostracod increases were notable in the fertilized creek, especially in the creek wall habitat (Fig. 3). Abundance in creek wall diverged between control and nutrient addition creeks in 2004 and differences were consistent in both branches across time. Creek-wall ostracod abundance increased ~2x in the fertilized creek and this abundance difference remained throughout the period of fertilization.

Meiobenthic copepods at the marsh edge provided the strongest evidence for a direct effect of fish reduction. Pre-treatment copepod abundance was similar under tall *S. alterniflora* canopy in both creeks and all branches and was generally lower than abundances in the post-treatment year (Fig. 3). Significant differences between branches emerged post-treatment (period*fish interaction, $p = 0.014$), especially in the creek not receiving nutrient addition; abundances were consistently higher in fish reduction branches. Copepod abundance in this habitat reached the highest observed value in August 2004 in the fish reduction branch in the creek not receiving nutrients and was > 2x that found in the corresponding branch without fish manipulation. This increased copepod abundance as a result of killifish reduction suggests copepods are predator limited.

Significant effects of killifish reduction were also observed at the marsh edge for nematodes and total meiofauna (period*fish interaction, $p = 0.0015$ and $p = 0.0022$, respectively) and in creek wall for copepods (period*fish interaction, $p = 0.0394$). In tall *S. alterniflora*, steep declines in nematode and total meiofauna abundance (nematodes comprised ~85% of the total meiofauna in this habitat) in all branches occurred from June 2004 to August 2004; however, declines in both creeks were less in branches with fish reduction (Fig. 3). Copepods in creek wall similarly experienced large population declines from June to August 2004 with final values becoming very similar among branches within creeks. Our results suggest that killifish reduction moderated these decreases in population size, as would be expected in predator-limited populations. However because the mechanism causing the large apparent changes in density is unclear and unrelated to predation, we consider support for limitation by predation for these taxa to be weak.

Of the macroinfauna taxa, only the subsurface deposit-feeding oligochaete *Cernosvitoviella immota* and total annelids responded to experimental treatments. Significant responses to fish reduction treatment differed with and without nutrient addition for *C. immota* in creek wall habitat (period*nutrient*fish interaction, $p = 0.0006$), but the relationship may be spurious. Sharp abundance increases among all creek branches occurred in June 2004 (after only 4 weeks of fish manipulation, suggesting the effect was not due to fish manipulation) and were not sustained through August 2004 (Fig. 2). Effects of fish reduction were not significant in a

BACI test with June 2004 data removed. Under *S. patens* canopy, abundance of *C. immota* was relatively low throughout 2003 and June 2004. In August 2004, abundance in all branches of both creeks increased by about 3x (Fig. 2). Increases differed among branches within creeks however. In the creek without nutrient addition, *C. immota* abundance increased much more in the branch without fish reduction. With fertilization, August 2004 abundance was similar with and without fish reduction. The effect of killifish reduction therefore differed with and without nutrient addition (period*nutrient*fish interaction was significant, $p = 0.032$). Total annelids in *S. patens* also responded significantly (partly due to the response by *C. immota*), however BACI revealed a significant effect of only fish reduction (period*fish interaction, $p = 0.0249$). Rather than enhancing a population increase, as would be expected if these annelids were limited by killifish predation, fish reduction under *S. patens* canopy inhibited increases in abundance, and may have been caused by an unknown indirect effect associated with a reduction in fish density.

The polychaete *Manayunkia aestuarina* was abundant in macrofauna samples in all habitats, except mudflat. In meiofaunal samples, *M. aestuarina* was found across the gradient examined. The percent of the total *M. aestuarina* population that was meiofaunal in size was 90.7% in creek wall and 85.7% in tall *S. alterniflora*. Neither size class of *M. aestuarina* responded to treatments in any habitat.

Copepod demography

Copepod sex ratio (males/females pooled across species) ranged between 0.1 and 1.0 but did not vary greatly between creeks or among branches in any habitat (data not shown). Percent ovigerous females was variable and ranged from about 2 to 40% (Fig. 4). However, a sharp increase in ovigerous females was observed in August, 2004 in both branches of the fertilized creek. Percent immature copepods averaged about 50% across all collections (Fig. 4).

Generally, values were similar between creeks and among branches in 2003 and little change was noted in 2004, except in creek wall habitat in August when the fraction of immature copepods increased sharply in both branches of the creek receiving nutrients. A nutrient effect (period*nutrient interaction, $p = 0.0004$ for both % immature and % ovigerous copepods) at creek wall was observed, regardless of fish manipulation, suggesting that copepods under conditions of fertilization reproduced more rapidly and exhibited a younger population age structure.

Species diversity responses

Overall, 36 copepod and 17 annelid species were found among the habitats sampled in PIE. The most abundant species were found in all creeks and branches; occurrences of rare species were sporadic. Across all habitats, there was a higher species richness and Shannon diversity for copepods than annelids (mean copepod species number ranged from 5.5-7.8 and mean annelid species number ranged from 2.7-5.9 across the gradient) (Table 3). Species richness and Shannon's diversity decreased similarly for both groups across the inundation/elevation gradient.

Treatment effects on species diversity of annelids and copepods were examined in all habitats with BACI statistics. Diversity (Shannon value, evenness and number of species) was generally similar in all habitats of both creeks and all branches in 2003 (data not shown). Diversity changed little after treatments were initiated. BACI tests revealed two isolated significant results (in habitats without simultaneous treatment effects on population abundance or community structure from the same habitat), and we conclude that infaunal species diversity of these two abundant taxa was not affected by nutrient addition or fish reduction.

Community responses

ANOSIM was conducted on each habitat-specific collection of copepods and annelids separately to determine if community similarities differed among branches or between creeks. Evidence for a treatment effects may best be inferred for a habitat when creeks or branches do not differ before and when differences become evident after treatment initiation. Before treatments were initiated, copepod and annelid communities differed between creeks in the mudflat habitat (see ANOSIM probability values, Tables 4 & 5), suggesting strong natural dissimilarities between the two creeks in this habitat. Therefore, we did not test for community differences after treatment initiation (i.e., for treatment effects) in mudflat. Differences among branches before treatments were initiated also occurred, but were uncommon (Tables 4 & 5). Using the criteria above, we found 10 instances in which ANOSIMs suggested treatment effects (Tables 4 & 5). MDS plots were examined in each of these instances, and some did not show clear separation among treatments, i.e., the annelid community in stunted *S. alterniflora* habitat and copepod community in tall *S. alterniflora* (Fig. 5). Significant ANOSIMs and distinct groupings with MDS occurred in six instances (variation associated with fertilization in annelids in creek wall, tall *S. alterniflora* and *S. patens,* and copepods in creek wall and variation associated with predator reduction in annelids at tall *S. alterniflora* and copepods in creek wall).

Annelid communities differed after treatments were initiated between the two creeks in creek wall, marsh edge and *S. patens* habitats, even though no annelid species individually responded to nutrient addition. ANOSIMs were significant and MDS showed clear separation in August 2004 between the two creeks, suggesting a fertilization effect in these habitats. SIMPER analysis revealed that surface-feeding annelids were associated with community change in 90% of the instances. In addition to changes in indices that indicate increased reproduction in copepods, the copepod community at creek wall differed between the two creeks further suggesting a fertilization effect (significant ANOSIM values, August 2004; $p < 0.05$). SIMPER analysis of copepods suggested a strong differentiation related to two species; in the creek without nutrient addition, *Heterolaophonte* sp. contributed most to average similarity values after *Nannopus palustris* while in the creek with nutrient addition, *Mesochra* sp. contributed most after *N. palustris*.

Fish reduction effects were detected on the annelid community in tall *S. alterniflora* (August 2004, ANOSIM, $p = 0.010$, Fig. 5) and on the copepod community in creek wall, without corresponding changes in abundance. MDS in August 2004 in both taxa suggests that each creek branch could be distinguished from others although groupings due to nutrient addition were more distinct.

Responses of other taxa

Deegan et al. (2007) detail treatment effects on taxa (e.g., killifish and benthic microalgae) that are relevant to explain potential top-down and bottom-up effects on infauna in this experiment. Killifish abundance varied among creeks branches and years in experimental creeks. Abundance was much higher in the pre-treatment year than the first year of treatment in both experimental creeks and differed among creek branches in pre-treatment collections. Fish removal, however, lead to significant reductions in killifish abundance in both the reference and nutrient enrichment creeks (see Deegan et al., 2007, Figure 4). Although benthic microalgal biomass differed among habitats, within habitat biomass was similar among creeks and branches in the pre-treatment and the first post-treatment year in mudflat, tall *S. alterniflora* and *S. patens* habitats (Deegan et al., 2007, Figure 7). A BACI test found no treatment effects until the second year (which is not examined here for invertebrates) when a synergism between fish reduction and nutrient addition was found in creek and marsh edge habitats.

Discussion

Here, we report some results of a long-term, ecosystem-wide experiment designed to examine the effects of multiple factors across a saltmarsh landscape. We fertilized each flooding tide of a creek to mimic the way anthropogenic nutrients are delivered to salt marshes, and achieved annual N loadings of 15-60 g N m^2 y^{-1}. We also significantly reduced the density of *F. heteroclitus* from 65 in reference branches to 30 individuals 30 m^{-2} in treatment branches (Deegan et al., 2007). We were able to detect some early effects (after about 3 mo of manipulation) and draw conclusions regarding responses of two size classes of benthic infauna in a more holistic manner than traditional plot-level experiments.

Nutrient (bottom-up) effects

Benthic macroinfauna responses to increases in nutrient loading have been shown to be highly variable. Some studies suggest strong nutrient-induced increases (Sarda et al., 1995; Nixon and Buckely, 2002) or decreases (Kemp et al., 2005) in abundance or biomass of many taxa, while other studies suggest that increases in abundance occur for only a few taxa (Posey et al., 1999; Posey et al., 2002). In addition to numerical responses, the body size of individual infauna may increase in response to nutrient addition (Posey et al., 2006). There have been fewer studies of nutrient addition effects on meiofauna but changes in community composition are more common than large changes in biomass or abundance (Widbom and Elmgren, 1988; Hillebrand et al., 2002). We found no fertilization effects on infauna at the extremes of the inundation gradient (i.e., mudflat and stunted *S. alterniflora* habitats) (Fig. 6). Under tall *S. alterniflora* and *S. patens* canopy, fertilization caused shifts in macrobenthic annelid community structure (with no change in population size or species diversity). Fertilization at creek wall resulted in increased meiobenthic ostracod abundance, increases in ovigerous female and immature copepods, and simultaneous shifts in copepod and annelid communities (again without changes in total population size or species diversity) (Fig. 6), suggesting effects were strongest here. Shifts in the annelid community were caused mostly by surface-feeding polychaetes, which were much more influenced by fertilization than were subsurface oligochaetes, a finding in opposition to the long-term study of (Sarda et al., 1996) who found that oligochaetes increased with fertilization.

The younger population age structure (demonstrated by increases in the proportion of immature copepods) of the copepod population in the creek wall habitat under the influence of fertilization was probably caused by increased reproductive activity as evidenced by significant increases in the proportion of egg-bearing females. Intuitively, this should stimulate an increase in copepod density; however, copepod densities were not affected by fertilization (Table 2). Total copepod density may have remained unchanged because of a differential response of individual copepod species to fertilization. The most abundant copepod in creek walls (*Nannopus palustris*) did not respond to fertilization but the contribution of *Heterolaophonte* sp. to the community decreased with fertilization while the contribution of *Mesochra* sp. increased with fertilization. Thus, the increase in one species may have offset the decrease in another, leaving total copepod abundance unchanged while altering the copepod community in response to fertilization.

Bottom-up effects of fertilization on infauna are mediated through primary producers. Sediment-dwelling algae associated with creek wall and marsh edge habitats, where most effects on infauna were observed, have a high biomass and may be expected to respond quickly to fertilization (Deegan et al., 2007). Creek wall is dominated by canopy-forming macroalgae, primarily *Enteromorpha* spp., filamentous algae (e.g. *Rhizoclonium* spp.) and associated epiphytic diatoms (Galván et al., 2008). Tall *S. alterniflora* habitats lack macroalgae but non-canopy forming filamentous algae and associated epiphytic diatoms are abundant on the sediment surface. Deegan et al. (2007) examined fertilization effects on sediment algae and found no effects in the first year of treatment manipulation (when our analysis was conducted) in mudflat, marsh edge and *S. patens* habitats, but not did examine responses in creek wall. In the absence of strong responses by sediment algae, we observed few bottom-up effects on infauna in habitats studied by Deegan et al (2007). Galván (unpublished) subsequently examined responses at creek wall (where infaunal responses were strongest) and found that algal biomass increased with nutrient addition and fish removal in the first year of nutrient addition. Taxa that responded to fertilization at creek wall may interact with epiphytic algae associated with filamentous algae. Galván et al. (2008) noted that the harpacticoid, *Heterlaophonte* sp., and some surface deposit feeding annelids took up ^{15}N label in an isotope addition study directly from epiphytes. Copepod reproduction varies with types of microalgae/microbes in its diet (Carli et al., 1995; Pinto et al., 2001) and diatoms are considered an excellent source of nutrition for harpacticoids (Pinto et al., 2001; Caramujo et al., 2005). Ostracods also consume edaphic algae (Goldfinch and Carman, 2000) and infaunal annelids respond positively to increasing algal mat spread (Thiel and Watling, 1998). These observations suggest that nutrient responses by epiphytes (which might respond faster than other algal communities) may influence these taxa. Thus, bottom-up effects on infauna in PIE appear to be generally explained by changes in the amount or the composition of sediment and epiphytic algae.

Predation (top-down) effects

The significance of killifish predation to infauna is poorly understood in salt marshes. Most studies of epibenthic predation use devises designed to exclude all predators of a given size

(Wiltse et al., 1984; Sarda et al., 1992; Foreman et al., 1995; Posey et al., 1995; Posey et al., 2002; Posey et al., 2006), but authors sometimes suggest that predation by *F. heteroclitus* is responsible for resulting infaunal abundance changes because it is such an abundant species (e.g., Sarda et al., 1998). Of the four studies that have isolated the effects of killifish by use of species-specific inclusions, Kneib and Stiven (1982) show that small killifish (< 40 mm) impact polychaetes in sediments under *S. alterniflora* canopy, Walters et al. (1996) found a strong impact of small (< 20 mm) killifish on stem-dwelling copepods while Service et al. (1992) and Cross and Stiven (1999) found that killifish > 40 mm had no effect on macrofauna and meiofauna in sediment populations respectively.

In our study, evidence for direct top down effects by killifish was observed on meiofaunal taxa in the mid range of the tidal inundation gradient (Fig. 6). Copepod densities increased with killifish reduction in tall *S. alterniflora*, suggesting a top-down release from killifish predation. Furthermore and as expected in top-down control, abundances of copepods and killifish were generally inversely correlated among creek branches and years in our experimental creeks. Deegan et al. (2007) reported that killifish density was higher throughout the pre-treatment year than the first year of treatment in these creeks; copepod density was inversely related and was consistently lower in the pre-treatment year only in the tall *S. alterniflora* habitat (Figure 3). We also found weak evidence that copepods at creek wall and nematodes at marsh edge were released from killifish predation, and fish reduction led to a community shift in copepods at creek wall. Meiobenthic copepods are a principal prey of killifish < 40 mm (Kneib, 1986) and may be consumed in high numbers. Walters et al. (1996) found 50% of copepods were consumed by killifish over 3 days from epiphytes on *S. alterniflora* stems. Juvenile killifish may directly influence copepod density in the tall *S. alterniflora* habitat because edaphic algae under *S. alterniflora* does not form a canopy and has little structural complexity that could serve as a refuge from predation for copepods. Creek wall macroalgae may provide a refuge for copepods from killifish predation by its complexity (Coull and Wells, 1983), preventing a predator impact on density but allowing selective predation that may affect community structure.

Surprisingly, we detected no direct effect of killifish reduction on the abundance of annelids, although killifish consume annelids (Kicklighter et al., 2004). The annelid community changed due to fish reduction at the marsh edge, suggesting a modest impact. Johnson (unpublished) conducted an exclusion experiment with grass shrimp and killifish and concluded that predation by grass shrimp on annelids may increase (by a trait-mediated indirect effect) when killifish are reduced in abundance. This increase in grass shrimp predation may compensate for the decreased predation rate by the reduced number of killifish. Although killifish and grass shrimp both probably prey on annelids and copepods, predation effects of killifish and grass shrimp may differ. Gregg and Fleeger (1998) found that grass shrimp are efficient predators on stem-dwelling copepods but that predation is much lower on sediment-dwelling copepods and that a different functional response by grass shrimp is generated when sediment is available to copepods. Perhaps small killifish have higher predation rates on copepods than grass shrimp and grass shrimp have higher predation rates on annelids (shrimp are becoming increasingly recognized as important predators of macroinfauna (Kneib and Stiven, 1982; Posey and Hines, 1991; McTigue and Zimmerman, 1998; Beseres and Feller, 2007). If so, then grass shrimp may not compensate with increased predation on sediment-dwelling copepods when killifish are removed; therefore, copepods increased in abundance when killifish were removed (as a direct effect) while annelids did not.

Possible indirect effects of killifish reduction on annelid abundance (annelids decreased with killifish reduction) were observed under *S. patens* canopy. Indirect effects are often mediated by an intermediate predator (Kneib, 1991). Intermediate consumers such as grass shrimp on the marsh platform may have been responsible for the observed indirect effects of killifish reduction under the *S. patens* canopy, but these effects cannot be isolated without directed experiments (Fleeger et al., 2003). Support for this hypothesis, however, comes from the observation that killifish reduction led to increases in grass shrimp body size (but not abundance) that could be caused by increasing growth rates resulting from increased consumption by grass shrimp (Deegan et al., 2007).

Top-down vs. bottom-up effects

Our work demonstrates that the assumption of independence between factors, an assumption often made in ecological studies (Argawal et al. 2007) may be incorrect. We identified an interaction between fertilization and predator reduction in *S. patens* habitat on annelids associated with an indirect effect of killifish reduction (Table 1). Interactions in salt marshes between fertilization and predator removal have been observed by (Posey et al., 2006) in a mudflat location for haustoriid amphipods, and our related work in PIE suggests that talitrid amphipods at the marsh edge and sediment algae respond to these treatments in a non-additive fashion (Deegan et al., 2007). Other studies (Foreman et al., 1995; Posey et al., 1999; Hillebrand et al., 2002) found no evidence for interactions between nutrient addition and predator reduction on infauna. Trophic cascades mediated by infauna on sediment algae were also not apparent from our study or work by Posey et al. (1995; 2002) suggesting infauna are weak interactors with sediment algae, and that trait-mediated effects associated with top-down factors are functionally similar throughout the inundation gradient. Finally, it is difficult to compare the relative importance of top-down vs. bottom up effects from our study because we did not exclude all epibenthic predators and because indirect effects of killifish reduction on other predators may have occurred, obscuring effects. MDS plots (Fig. 5) show more distinct groupings associated with fertilization than killifish reduction when both effects were significant (e.g., copepods on creek wall) suggesting that fertilization effects on communities were stronger. However, both top-down and bottom up effects were relatively uncommon and similarly mild in our experiments (Fig. 6).

Landscape effects

Our results demonstrate that research programs that focus on one part of the marsh landscape may miss important effects of ecological stressors. In salt marshes, benthic studies examining the anthropogenic activities rarely look across the landscape and generally focus on unvegetated mudflats (Posey et al. 1999). In our experiment, we found no effects of nutrient loading or predator reduction on any taxon in the mudflat habitat and the strongest and most frequent effects were found in the creek wall, a habitat often overlooked and rarely examined. Thus, potentially important effects may go undetected in a sampling program focused strictly on one portion of inundation gradient in salt marshes.

Implications for bioindicators

In terms of abundance, meiofaunal major taxa were more sensitive to our treatment effects than were species of annelids. Interestingly, neither annelid or copepod species diversity responded to treatments, and even though copepods had a higher diversity, community responses of both groups were often similar. Although meiofaunal taxa were more sensitive to treatments, utilizing both macrofauna and meiofauna may enhance benthic monitoring programs because parallel findings may provide strong evidence of an effect (e.g., both groups were significantly affected by fertilization at creek wall) or lack thereof (e.g., treatment effects on meiofaunal and macrofaunal *Manayunkia aestuarina* were size and habitat independent). Regardless of which size class (or both) is utilized, sensitivity – the ability to detect effects – of selected response variables (e.g., abundance) is important in decisions regarding any monitoring program. For meiofauna, copepods reproductive indices proved useful and may be valuable for predicting long-term population effects (Montagna and Harper, 1996). For macroinfauna, we suggest focusing on surface deposit feeders because they proved most sensitive to treatments.

Conclusions

In our experiment examining the effects of whole ecosystem fertilization and predator removal, we found that the most frequent and strongest responses of infauna occurred in the mid region (creek wall and marsh edge, Fig. 6) of the tidal inundation gradient. Although significant effects were found on abundance, reproduction and community structure in some taxa and habitats, the effects were relatively mild (e.g., no effects were found on species diversity of copepods or annelids). Interactions between fertilization and predator reduction were observed in association with indirect predation effects on infauna in one habitat and for benthic microalgae in various habitats (Deegan et al., 2007). These results illustrate the importance of examining effects across the landscape and falsify the assumption of independence among multiple factors (Argawal et al., 2007). More research is needed to determine if trait-mediated effects that contribute to top-down trophic cascades (and other ecological expressions) vary over the landscape, and we will continue to analyze the results of our longer-term manipulations for such effects. We suggest that both macroinfauna and meiofauna provide complementary information for monitoring effects, although meiofauna appear to be more sensitive, at least in the short term.

Limiting study of human-induced stressors to a single habitat may lead to false conclusions about the entire ecosystem.

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Table 1: Summary of p-values for macrofauna from GLMM testing for treatment effects. In this BACI-type design, only Period*Treatment interactions are of interest. MF = mudflat, CW = creek wall, TSA = tall-form *Spartina alterniflora*, SP = *S. patens* and SSA = stunted *S. alterniflora*.

		Macroinfauna						
Habitat	Taxon	Period(B/A)	Nutrients	Fish	Period*Nutrient	Period*Fish	Nutrient*Fish	Period*Nutrient*Fish
MF								
	S. benedicti	0.5437	0.0005	0.0051	0.9066	0.9199	0.4342	0.7196
	Total Oligochaetes	0.6965	0.0771	0.1053	0.7201	0.5570	0.5344	0.4895
	Total Annelids	0.6353	0.0055	0.0009	0.3653	0.3922	0.1983	0.1406
CW								
	M. aestuarina	0.1269	0.3823	0.8643	0.2441	0.8643	0.3158	0.3865
	P. litoralis	0.2352	0.0382	0.2872	0.2335	0.8090	0.7030	0.3589
	C. immota	0.8019	0.7804	0.4900	0.1057	0.8035	0.578	0.0006
	Total Annelids	0.1286	0.3331	0.6060	0.0868	0.4063	0.2700	0.1362
TSA								
	M. aestuarina	0.5778	0.0903	0.1088	0.2091	0.6504	0.6603	0.1306
	P. litoralis	0.251	0.088	0.3450	0.1975	0.9446	0.3636	0.3066
	C. immota	0.2222	0.0602	0.0728	0.0561	0.8557	0.5445	0.5007
	Total Annelids	0.0352	0.0494	0.3175	0.1153	0.5959	0.8395	0.4940
SP								
	M. aestuarina	0.7866	0.3753	0.6228	0.7331	0.1855	0.0485	0.5221
	C. immota	0.2786	0.5564	0.0651	0.6744	0.8709	0.8997	0.0320
	Total Annelids	0.3782	0.045	0.5323	0.4675	0.0249	0.344	0.1631
SSA								
	M. aestuarina	0.442	0.5745	0.0196	0.3930	0.2032	0.0071	0.6703
	P. litoralis	0.3881	0.0808	0.0159	0.7080	0.5704	0.5460	0.4360
	C. immota	0.3647	0.4245	0.7600	0.9765	0.3632	0.4418	0.3769
	Total Annelids	0.3686	0.2208	0.1945	0.6293	0.6794	0.0362	0.1218

Table 2: Summary of p-values for meiofauna from GLMM testing for treatment effects. In this BACI-type design, only Period*Treatment interactions are of interest. MF = mudflat, CW = creek wall, TSA = tall-form *Spartina alterniflora*.

		Meiofauna						
Habitat	Taxon	Period(B/A)	Nutrients	Fish	Period*Nutrient	Period*Fish	Nutrient*Fish	Period*Nutrient*Fish
MF								
	Nematodes	0.2442	0.0380	0.7052	0.3953	0.8678	0.1325	0.9435
	Copepods	0.2863	0.6460	0.9812	0.5824	0.0693	0.2441	0.4508
	Ostracods	0.9219	0.0105	0.2408	0.4145	0.3003	0.8567	0.2295
	M. aestuarina	0.4297	0.0083	0.5569	0.6617	0.5790	0.5002	0.5075
	Total Annelids	0.2152	0.0090	0.1197	0.6807	0.5206	0.3471	0.5358
	Total Meiofauna	0.1865	0.0294	0.6235	0.4753	0.8891	0.2101	0.9768
CW								
	Nematodes	0.2881	0.0706	0.2791	0.4986	0.9835	0.602	0.2340
	Copepods	0.7546	0.0007	0.0106	0.5336	0.0394	0.8296	0.5701
	Ostracods	0.0604	0.269	0.4378	0.0210	0.8588	0.6509	0.8730
	M. aestuarina	0.8932	0.0178	0.0606	0.5187	0.4010	0.5890	0.2070
	Total Annelids	0.7595	0.0208	0.3655	0.5310	0.4408	0.9615	0.2517
	Total Meiofauna	0.3314	0.0334	0.3936	0.4702	0.7257	0.5750	0.2091
TSA								
	Nematodes	0.3927	0.3349	0.2913	0.6256	0.0015	0.9158	0.3191
	Copepods	0.1215	0.1339	0.8998	0.1866	0.0140	0.1235	0.5343
	Ostracods	0.2434	0.515	0.0802	0.6880	0.4169	0.7980	0.1169
	M. aestuarina	0.9994	0.0068	0.0527	0.1860	0.1700	0.5299	0.8628
	Total Annelids	0.9687	0.0566	0.0608	0.2253	0.1359	0.9222	0.7392
	Total Meiofauna	0.3947	0.1760	0.3359	0.8434	0.0022	0.7189	0.4122

Table 3: Average species diversity per sample along the marsh tidal inundation gradient. Values are composites of all samples taken (i.e., all treatments, all time points) for meiofauna and macrofauna. H' = Shannon's diversity index and S = species richness.

	Annelid community		Copepod community	
	H'	S	H'	S
Mudflat	1.28	5.9	1.64	7.8
Creek Wall	1.05	4.9	1.25	7.0
Tall *S. alterniflora*	0.85	4.1	0.93	5.5
S. patens	0.77	2.7	-	-
Stunted *S. alterniflora*	0.65	2.7	-	-

Table 4. Copepod ANOSIM p values. In June and August, 2004, creek included two levels, with and without nutrient addition and fish was reduced in branches within creeks. An * indicates significance of 0.05 or lower. MF = mudflat, CW = creek wall, TSA = tall-form *Spartina alterniflora*. Tests not done in MF in 2004 because differences between creeks were observed in 2003.

	June 2003		July 2003		June 2004		August 2004	
	Creek	Branch	Creek	Branch	Creek	Branch	Creek	Branch
MF	0.180	0.410	0.050*	0.900	-	-	-	-
CW	0.410	0.910	0.600	0.033*	0.100	0.470	0.002*	0.016*
TSA	0.370	0.110	0.490	0.430	0.004*	0.008*	0.003*	0.038*

Table 5. Annelid ANOSIM p values. In June and August, 2004, creek included two levels, with and without nutrient addition and fish was reduced in branches within creeks. An * indicates significance of 0.05 or lower. MF = mudflat, CW = creek wall, TSA = tall-form *Spartina alterniflora*, SP = *Spartina patens,* SSA = stunted *S. alterniflora* = Tests for TSA for August, 2004, and SP and SSA in June, 2004 and August, 2004 were conducted with an outlying data point removed. Tests not done in MF in 2004 because differences between creeks were observed in 2003.

	June 2003		August 2003		June 2004		August 2004	
	Creek	Branch	Creek	Branch	Creek	Branch	Creek	Branch
MF	0.020*	0.130	0.050*	0.720	-	-	-	-
CW	0.400	0.940	0.550	0.820	0.010	0.070	0.020*	0.130
TSA	0.350	0.250	0.080	0.300	0.001*	0.240	0.010*	0.010*
SP	0.100	0.230	0.490	0.260	0.164	0.313	0.039*	0.085
SSA	0.110	0.020*	0.470	0.220	0.036*	0.010*	0.050*	0.045*

Figure Legends

Figure 1. Map of Plum Island Estuary showing location of experimental creeks (MassGIS orthophoto 2002). The upper left insert is a map of Massachusetts, indicating the location of PIE. The upper right insert shows a schematic figure (not to scale) of habitats sampled across the salt marsh inundation gradient. SW = Sweeney Creek, WE = West Creek. MF = mudflat, CW = creek wall, TSA = tall-form *Spartina alterniflora,* SP = *S. patens,* SSA = stunted-form *S. alterniflora*.

Figure 2. Density of total macroinfauna throughout the experimental period in experimental creeks and the oligochaete species *C. immota* in habitats where BACI tests revealed significant treatment effects. Significant effects are listed in the left corner of each panel. Habitat designations as in Fig. 1.

Figure 3. Density of total meiofauna throughout the experimental period in experimental creeks and individual taxa in which BACI tests revealed significant treatment effects. Significant effects are listed in the left corner of each panel. Habitat designations as in Fig. 1.

Figure 4. Percent immature copepods and percent ovigerous female copepods throughout the experimental period in experimental creeks. Significant effects are listed in the left corner of each panel. Habitat designations as in Fig. 1.

Figure 5. MDS plots of copepod and annelid responses in August, 2004 to experimental treatments. Treatment designations: NA = nutrient additions, FR = fish removal, AN = ambient nutrients, AF = ambient fish.

Figure 6. Summary of effects of fertilization and predator removal in PIE across the inundation gradient. NA designates nutrient addition effects and FR designates fish removal effects. Habitat designations as in Fig. 1.

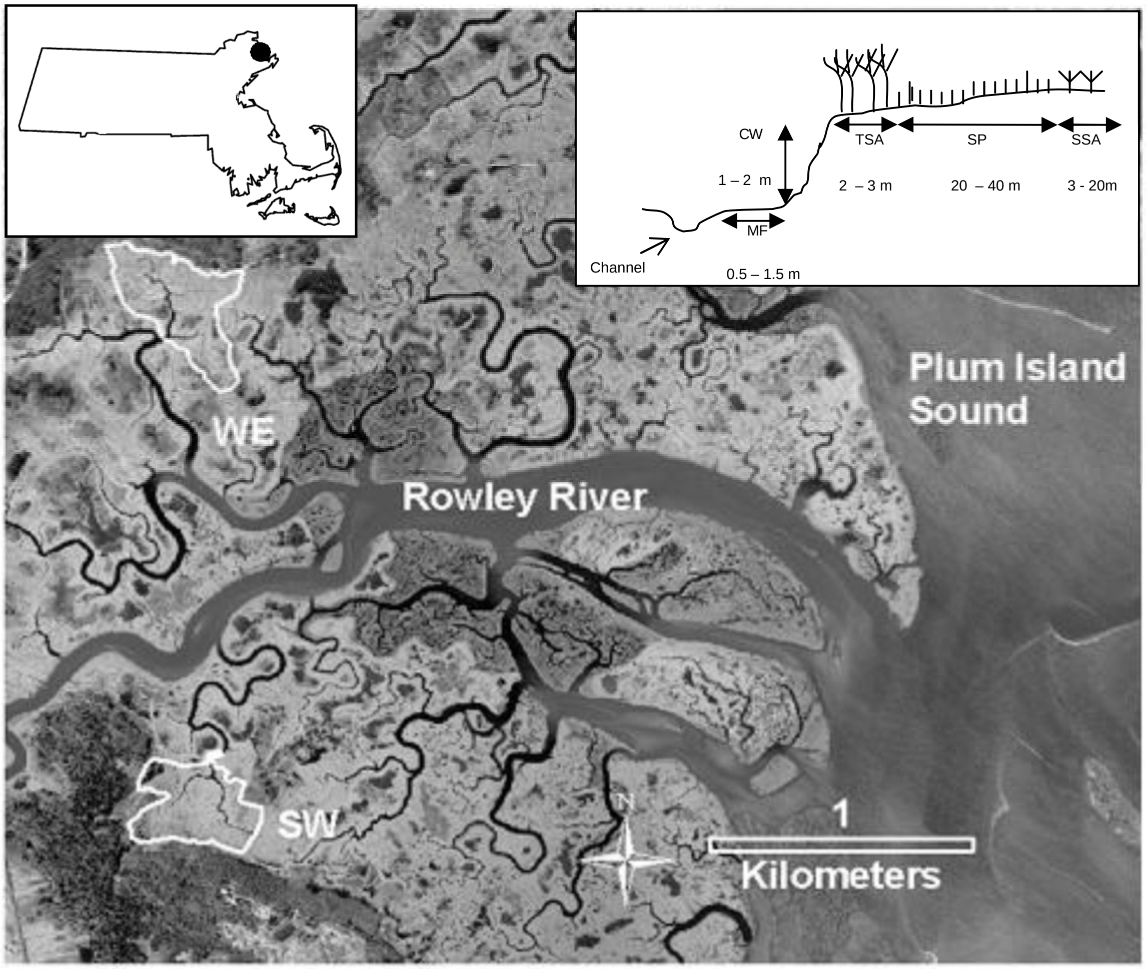

CW
1 – 2 m
TSA
SP
SSA
2 – 3 m
20 – 40 m
3 - 20m
MF
Channel
0.5 – 1.5 m
Plum Island
Sound
WE
Rowley River
SW
N
1
Kilometers

Figure 1

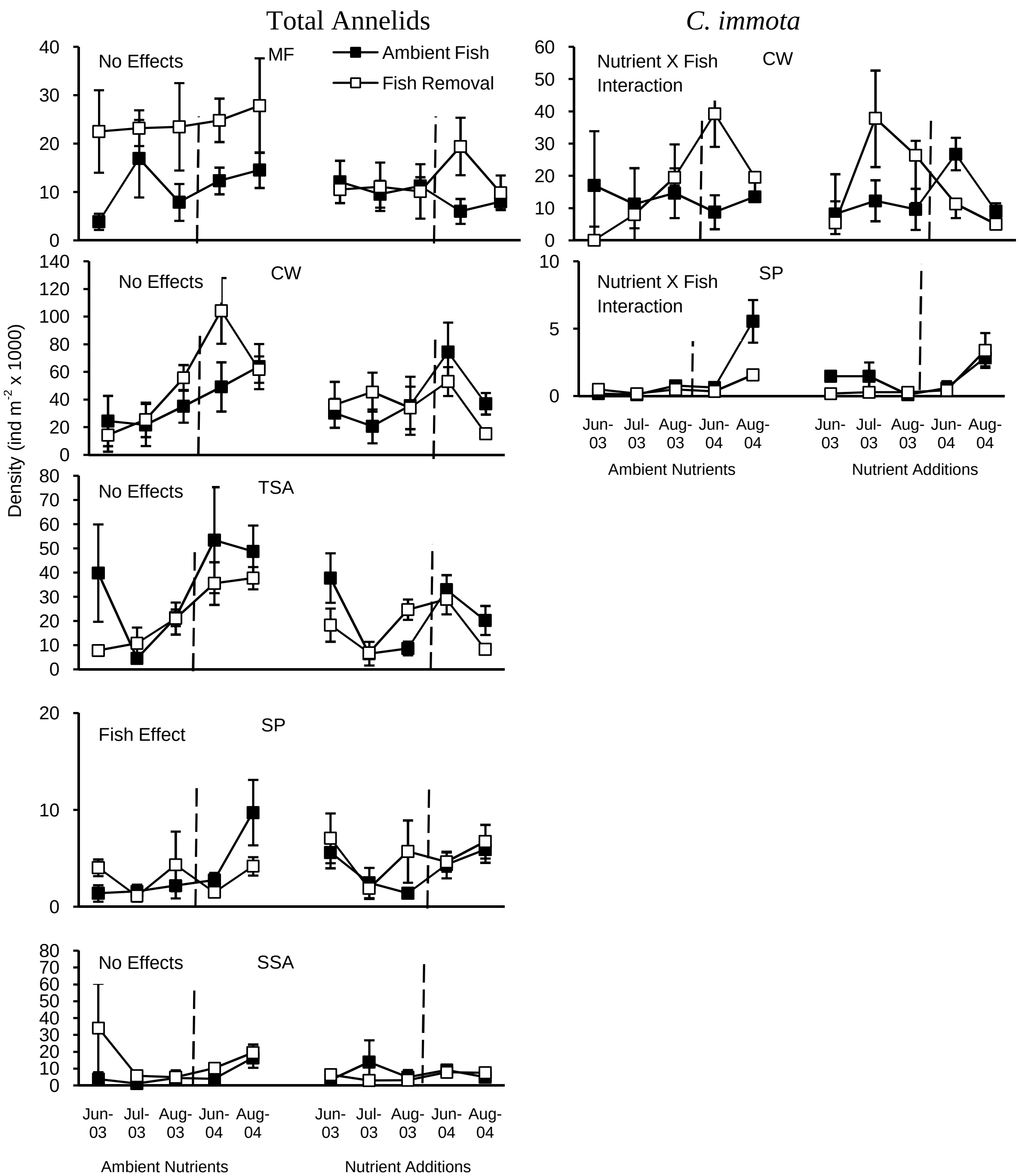
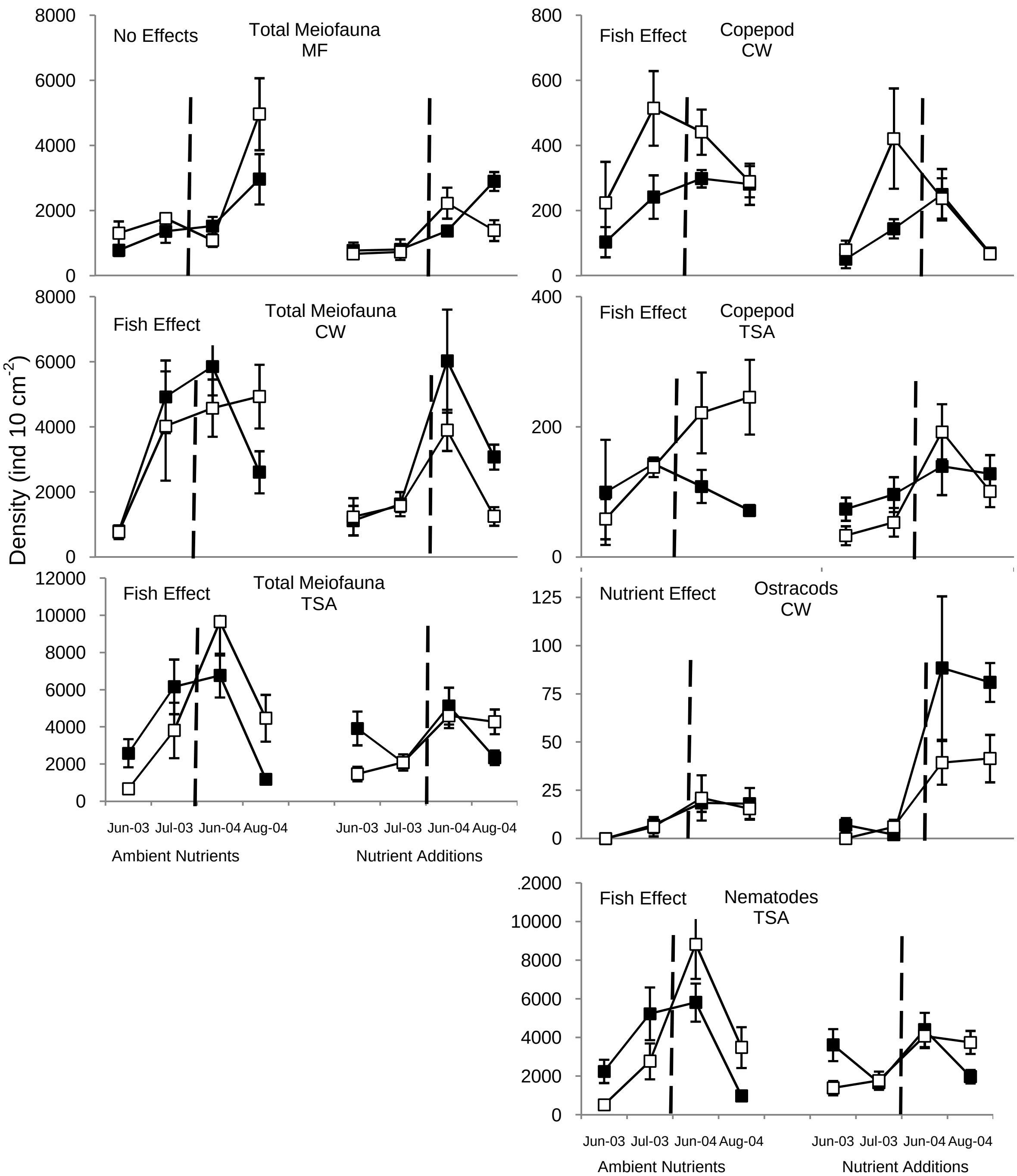

Total Annelids
C. immota
Ambient Fish
Fish Removal
No Effects
MF
Nutrient X Fish Interaction
CW
No Effects
CW
Nutrient X Fish Interaction
SP
No Effects
TSA
Fish Effect
SP
No Effects
SSA
Density (ind m-2 x 1000)
Jun-03
Jul-03
Aug-03
Jun-04
Aug-04
Ambient Nutrients
Nutrient Additions

Figure 2



Density (ind 10 cm-2)
No Effects
Total Meiofauna
MF
Fish Effect
Copepod
CW
Fish Effect
Total Meiofauna
CW
Fish Effect
Copepod
TSA
Fish Effect
Total Meiofauna
TSA
Nutrient Effect
Ostracods
CW
Fish Effect
Nematodes
TSA
Jun-03 Jul-03 Jun-04 Aug-04
Ambient Nutrients
Nutrient Additions

Figure 3

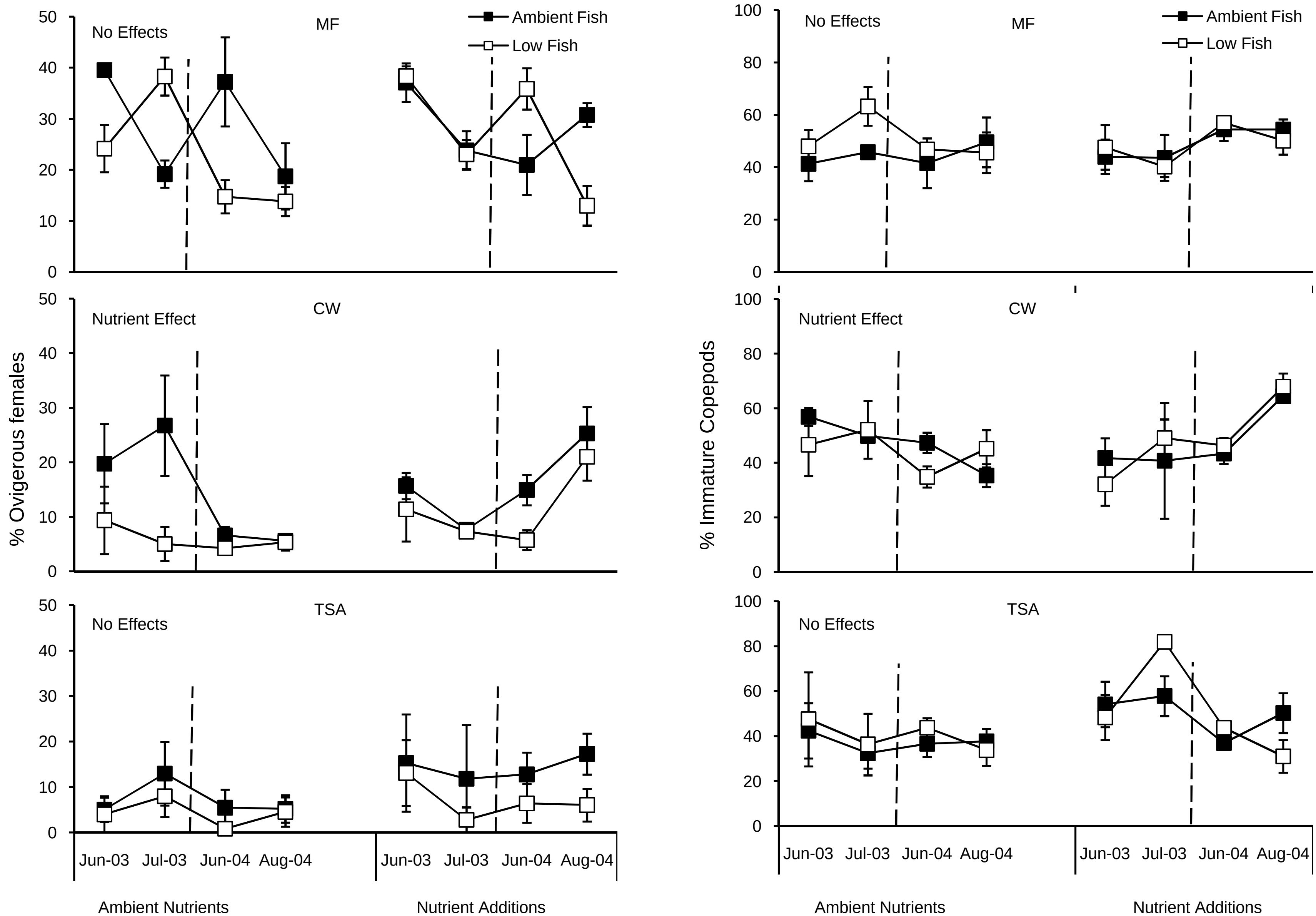

% Ovigerous females
% Immature Copepods
No Effects
MF
Ambient Fish
Low Fish
Nutrient Effect
CW
No Effects
TSA
Jun-03
Jul-03
Jun-04
Aug-04
Ambient Nutrients
Nutrient Additions

Figure 4

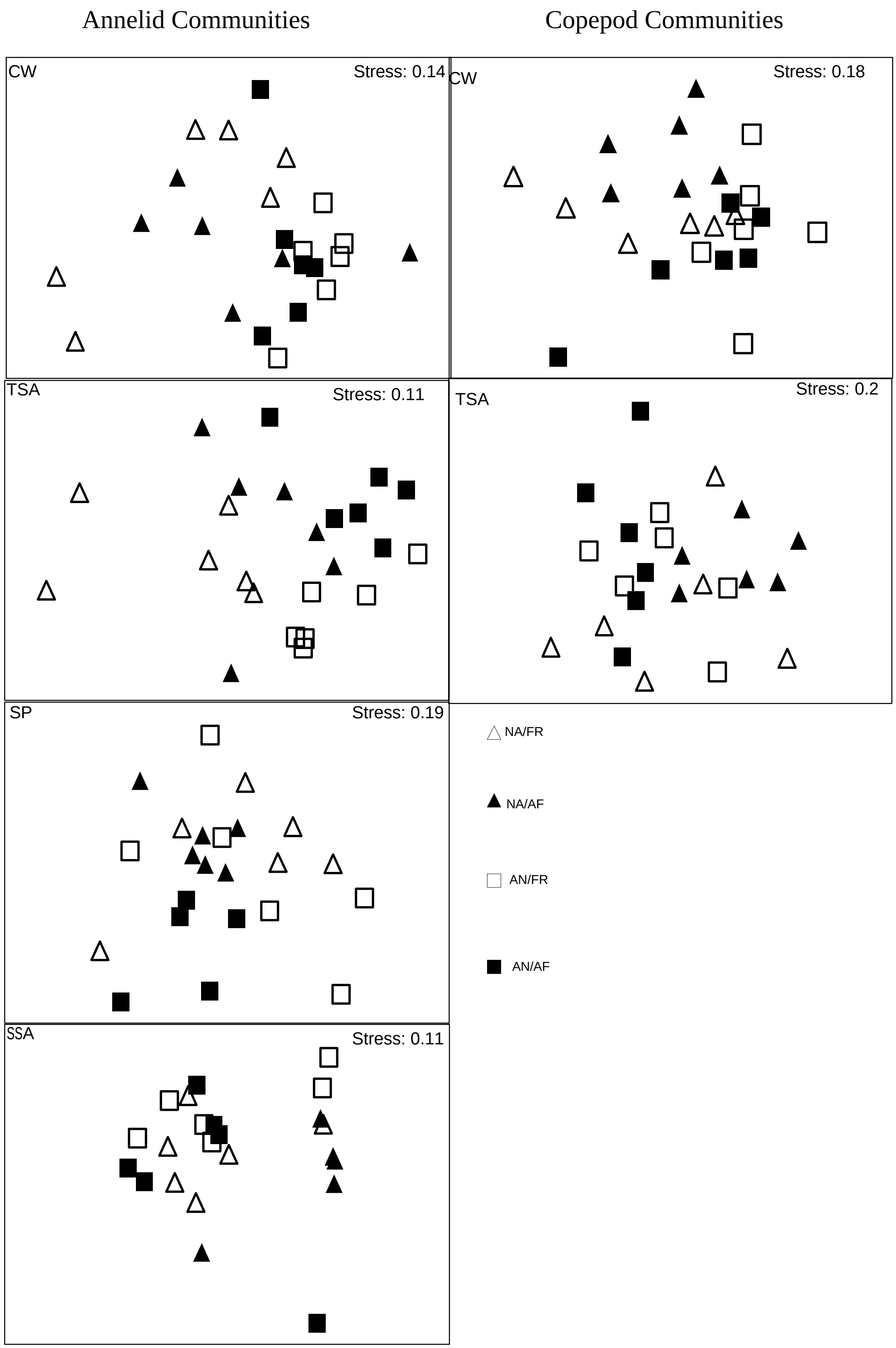

Annelid Communities
Copepod Communities
CW
Stress: 0.14
CW
Stress: 0.18
TSA
Stress: 0.11
TSA
Stress: 0.2
SP
Stress: 0.19
SSA
Stress: 0.11
NA/FR
NA/AF
AN/FR
AN/AF

Figure 5

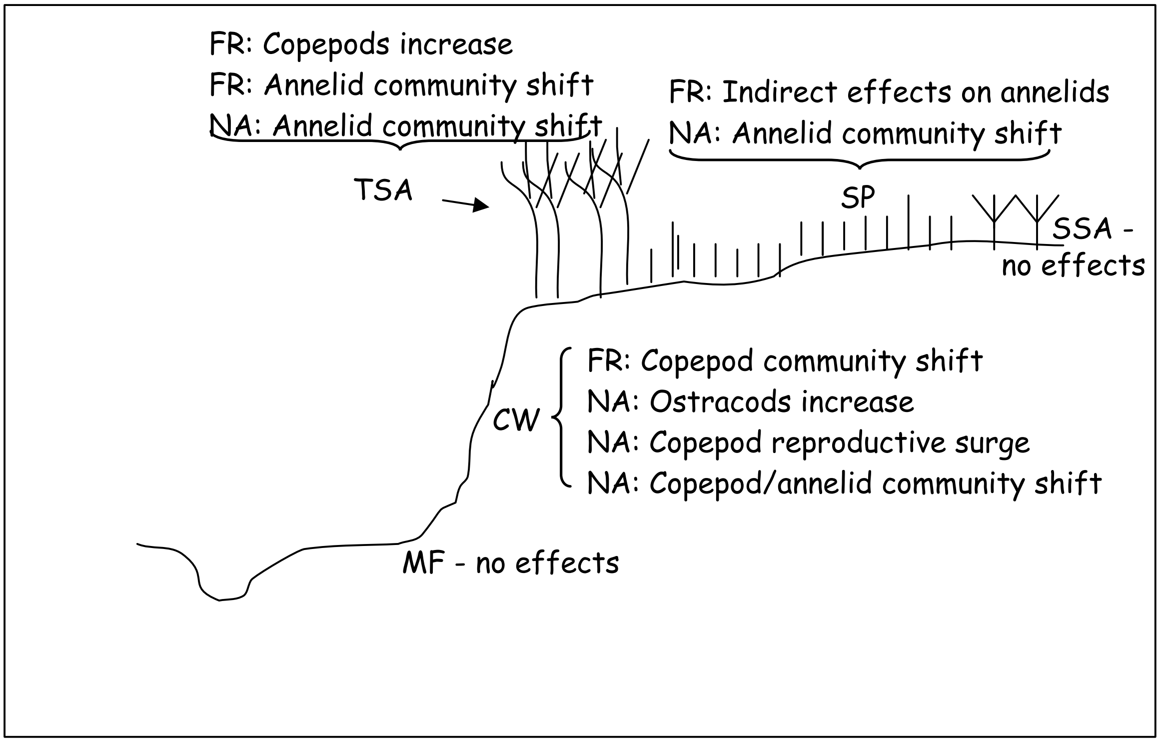

FR: Copepods increase
FR: Annelid community shift
NA: Annelid community shift
FR: Indirect effects on annelids
NA: Annelid community shift
TSA
SP
SSA -
no effects
FR: Copepod community shift
NA: Ostracods increase
NA: Copepod reproductive surge
NA: Copepod/annelid community shift
CW
MF - no effects

Figure 6