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Oyster reef ecosystem services: Macrofauna utilization of restored oyster reefs - Harris Creek, Maryland, USA

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OYSTER REEF ECOSYSTEM SERVICES: MACROFAUNA UTILIZATION OF RESTORED OYSTER REEFS

7/29/2019

Harris Creek, Maryland, USA

A final report to:
NOAA Chesapeake Bay Office

Prepared by:
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HARRIS CREEK, MARYLAND, USA

Award Information

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Project Summary

Oyster reefs provide habitat for a variety of macrofauna species. Our studies focused on the relationship between oyster tissue biomass density and reef-associated macrofauna biomass density. Studies were conducted in 2015-2017 and sites encompassed the majority of the area in which restoration activities were conducted with the Harris Creek Oyster Sanctuary in Maryland. Results presented in this report focus on: 1) interactions between oyster biomass density and season in determining macrofauna biomass, 2) responses of macrofauna to oyster biomass densities below "threshold" levels (0-14.9 g DW m^{-2}) and between threshold and "target" levels (15-49.9 g DW m^{-2}) defined in the success metrics for the Harris Creek restoration effort, 3) the role of tray-scale (0.1 m^2), plot-scale (10 m^2), and reef-scale oyster biomass density in determining associated macrofauna biomass, and 4) larger scale patterns in macrofauna biomass density within the creek. Results of our studies demonstrate that restored reefs in Harris Creek provide habitat for ~50 different macrofauna species. Samples from Harris Creek that had high oyster biomass density (>225 g DW m^{-2}) consistently provided habitat for >5,000 individuals m^{-2} regardless of season. In spring and fall, non-oyster macrofauna abundances reached ~10,000 individuals m^{-2}. Biomass of non-oyster macrofauna on high oyster biomass reefs exceeded 60 g AFDW m^{-2} in all seasons and was sometimes as high as 150 g AFDW m^{-2}. For all sessile and mobile species except small resident fish, there were significant and complex interactions between the effects of oyster biomass density and season on the biomass density of each species. Small resident fish biomass was consistently higher in samples with medium (50-224.9 g DW m^{-2}) and high oyster biomass density than in samples with low oyster biomass density (<50 g DW m^{-2}). They were also significantly higher in fall than in all other seasons. When macrofauna biomass was compared between samples with oyster biomass

densities below the threshold level for restoration ("threshold treatment") and between the threshold and target biomass density for restoration ("target treatment"), only two species showed a significantly higher biomass in the target treatment than in the threshold treatment: 1) biomass of hooked mussels was significantly higher in all seasons, and 2) biomass of *Mya arenaria* was higher only in early summer. Comparison of the effects of oyster biomass density at the tray, plot and reef scales on the biomass of mobile macrofauna species did not find significant effects of plot-scale oyster biomass density for any species. Tray-scale oyster biomass had a significant effect on all species and reef-scale biomass had a significant effect on all groups except small resident fish. Dividing macrofauna biomass density by oyster biomass and plotting the resulting ratio against distance from the mouth of the creek revealed a significant effect of position within the creek on three of the four sessile macrofauna groups, one mobile macrofauna species, and both of the infauna groups. Overall, our studies confirm that oyster reef restoration, especially when high biomass densities of oysters are achieved, leads to increased biomass of macrofauna species but that local oyster biomass density is only one of the factors that significantly influence macrofauna community structure.

Rationale

Recognition that oyster reefs support diverse and abundant benthic communities has provided one of the primary ecological rationales for preserving and restoring these habitats (Coen et al. 2007), and numerous studies have documented enhancements in these metrics on reefs relative to other estuarine habitats (e.g., Coen et al. 1999, Stunz et al. 2010, Rodney and Paynter 2006, Kellogg et al. 2013). Although several studies have characterized macrofaunal communities on restored oyster reefs in Chesapeake Bay (e.g. Rodney and Paynter 2006, Kellogg et al. 2013), these studies have generally focused on comparing reefs or experimental sites with high densities of large adult oysters to sites with very few or no oysters. Little is known about how macrofaunal communities scale with oyster biomass on subtidal oyster reefs restored using hatchery-produced juvenile oysters settled on adult oyster shell (hereafter "spat on shell") or about how these relationships change with season.

The Oyster Metric Workgroup (OMW) has recommended targeted monitoring programs, as well as controlled experiments and modeling studies, as effective ways to evaluate the success of restored oyster reefs. Specifically, the OMW believes that the ability to identify generalizable relationships between easily measured reef characteristics (reef size, oyster abundance/biomass, reef complexity) and the many ecosystem services that oyster reefs provide is crucial to accurate estimation of the ecosystem services provided by the broad range of ongoing oyster reef restoration activities and, in turn, to justifying the expenditure of public funds on these restoration efforts (OMW 2011).

Objectives

Our overarching objective was to quantify the utilization of restored and non-restored oyster reefs in Harris Creek, MD (Fig. 1) as habitat for macrobenthic invertebrate (≥ 1 mm) and small resident finfish communities. Specifically, we sought to answer the following questions:

- What are the dominant macrofauna species found on restored oyster reefs in Harris Creek?
- To what extent does macrofauna community structure change with season?
- Do higher levels of oyster biomass lead to increases in macrofauna biomass and are patterns similar for all species?
- Are patterns in community structure driven primarily local oyster biomass density or do larger scale patterns in oyster biomass alter community structure?
- Is macrofauna biomass influenced by location within the creek?

Project Narrative

Methods

Study Sites

All studies were conducted within the Harris Creek Oyster Sanctuary in the Maryland portion of Chesapeake Bay (Fig. 1). Using a variety of techniques, restoration activities have been implemented on >300 acres of historic oyster bottom (i.e. areas identified as viable oyster habitat at some point in the past) within this sanctuary. Within Harris Creek, we studied five restoration sites and three control sites that were suitable for restoration but were not subject to any restoration activities (hereafter "non-restored"; Fig. 2). To control for the influence of the restoration method employed, we limited our study to sites where juvenile oysters set on oyster shell (i.e. "spat-on-shell") were planted directly on the bottom (i.e. areas with substratum

Fig. 1. Location of Harris Creek Oyster Sanctuary in the Maryland portion of Chesapeake Bay.

conditions suitable for oyster survival and growth without adding hard substrate prior to planting). To control for the influence of oyster age, we selected only sites that were planted in 2012. Prior to site selection, a patent tong survey of potential sites was conducted in 2014 by the Paynter Lab at the University of Maryland. Based upon the resulting data, we delineated eight 1.25-ha study sites for our work. The selected areas provided biomass densities ranging from 2.7 to 98.4 g dry weight (DW) oyster tissue per square meter at the time of initial surveys (Kellogg et al. 2016). The same study sites were used for studies of macrofaunal communities in 2015, 2016 and 2017.

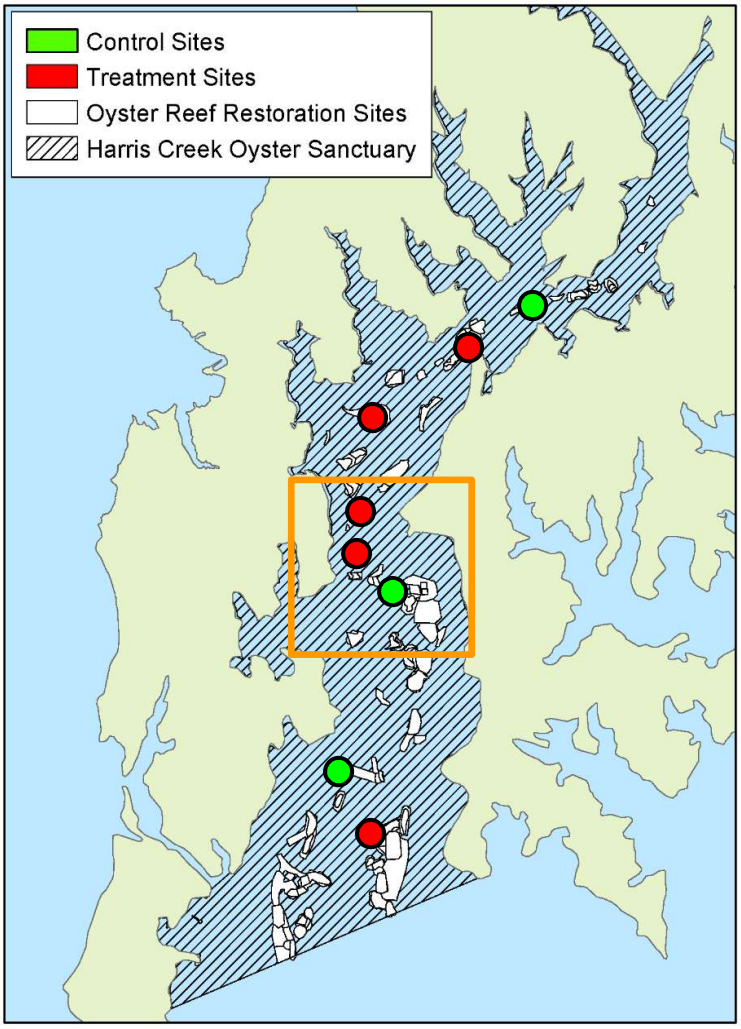

Fig. 2. Location of control (non-restored) and treatment (restored) sites within the Harris Creek Oyster Sanctuary in relation to the larger oyster reef restoration effort (white polygons). Studies of the scale of factors influencing macrofauna community structure were conducted at the three sites within the orange square.

In 2015, we also assessed the role of tray-scale (0.1 m^2), plot-scale (10 m^2), and reef-scale oyster biomass density in determining associated macrofauna biomass. This manipulative experiment was conducted at three of the sites used for the broader scale assessment of macrofaunal community structure in Harris Creek (Fig. 2, sites within orange square). These sites were selected because they were representative of the range of biomass within the creek and their proximity to one another reduced the influence of any upstream-downstream gradients in environmental conditions, larval supply, predation and other factors expected to vary spatially within the creek.

Field sampling

Resident macrofaunal community abundance, diversity, and biomass was determined by sampling attached and mobile macrofauna as well as oysters from each sampling location during each sampling period. In 2015, samples were collected in spring (May),

early summer (June), late summer (late July and August), fall (October) and winter (December). (*Note:* Preliminary data for four of these five sampling periods were provided in the final report for NOAA Award # NA13NMF4570209. To provide a comprehensive overview of seasonal patterns, those data are incorporated into the analyses presented in this report). In 2016, samples were collected in early summer (June), and fall (September and October). In 2017, samples were collected in spring (April), early summer (June), late summer (August), and fall (October).

For the purposes of this project, we define the resident macrofaunal community as all sessile and mobile organisms retained on a 1-mm mesh. In 2015, macrofaunal samples were collected using diver-deployed baskets (0.1 m^2 area x 0.15 m deep, constructed of 1.3-cm vinyl-coated steel wire mesh frame lined fine mesh [≤1mm]). Four baskets were deployed by divers at each site a minimum of one month prior to each sampling period. During deployment, baskets were filled with existing reef material and embedded into the reef matrix. To retrieve baskets, divers covered each basket with a fitted lid lined with ≤1 mm mesh. After samples were collected and returned to the boat, the contents of each basket was bagged to prevent escape of organisms.

Over the course of sampling in 2015, we noted several issues with the use of sample baskets for sample collection which included but were not limited to disturbance of baskets between deployment and retrieval by boat anchors or similar implements, excavation of nests under baskets by organisms including toadfish, and colonization of mesh on baskets by sessile organisms which had to be carefully excluded from samples resulting in increased sample handling time. In 2016, we explored several other sampling options to address these issues and ultimately developed a suction sampling method suited to sampling reefs in Harris Creek. In 2017, all samples were collected using a suction sampler that sieved all material on a 1-mm mesh. Three samples were collected at each site using this technique in 2017.

Sample processing

After all samples were collected, they were transported immediately to VIMS Eastern Shore Laboratory where the contents were thoroughly rinsed through a 4-mm sieve stacked over a 1-mm mesh sieve. Depending on sample contents, larger mesh sizes were added to aid in in sample handling. To ensure we included animals living within the shells of oysters in our samples, all live oysters and oxic oyster shells (<50% black from burial in anoxic sediments) were soaked in freshwater until no additional animals emerged from the shells. Live oysters, oyster shells and mussels were frozen for later analyses. Organisms retained on the 4-mm sieve collected from surrounding substrates at the time of initial processing, fixed in Normalin for at least 48 hours, and then transferred to 70% ethanol to preserve them for laboratory analyses. Material collected from the 1-mm sieve was frozen for later laboratory analyses.

Except as noted below, all macrofauna retained on the 4-mm and 1-mm sieves or attached to shells were identified to the lowest practical taxonomic level (usually

species) and enumerated. If the amount of material retained on the 1-mm sieve was very large, the material was randomly subsampled based on weight. Subsamples always represented at least 20% of the material collected. Prior to final identification, all macrofauna were picked from surrounding substrates and sorted into broad taxonomic categories which were then sorted to the lowest practical taxonomic level and counted prior to biomass analyses. Identifications and counts were made with the aid of a dissecting microscope as needed.

Biomass was determined as dry weight and ash-free dry weight for all faunal groups. Except as noted below, dry weights and ash-free dry weights determined to the nearest 0.001g by direct measurement. Samples were dried in an oven at 60˚C until constant weight was achieved then burned in a muffle furnace at 500˚C. For oysters and for the hooked mussel, *Ischadium recurvum*, seasonal length to biomass relationships were determined using a subset of the animals collected and the resulting equations were used to calculate the biomass of these species based upon length measurements. During time periods when barnacles were especially abundant, they were counted and ≥3 subsets of ≥ 50 animals each were used to estimate biomass.

Statistical analyses:

Patterns in macrofauna community structure were analyzed using ANOVA and linear regression. Two-way ANOVA were used to assess the effects of season and oyster biomass density on macrofauna abundance, biomass and individual size. A three-way ANVOA without interactions and two-way ANOVA with interaction terms were used to assess the influence sample, plot and reef scale factors on macrofauna biomass. Where data failed to meet ANOVA assumptions of normality and equal variance, we attempted to transform data to meet assumptions. If data were resistant to transformation, we assumed ANOVA were robust to these violations.

To assess whether there were significant changes in macrofauna biomass in relation to upstream-downstream location within the creek, we normalized macrofauna biomass to oyster biomass for each sample and regressed the resulting values against distance from the mouth of the creek. For this and all other statistical analyses, the significance level was set at $\alpha = 0.05$.

Results

After review of the oyster biomass collected in samples we defined three biomass categories based on oyster tissue dry weight: low (<50 g DW m^{-2}), medium (50-224.9 g DW m^{-2}), and high (≥ 225 g DW m^{-2}). This division of data provided a sufficient number of samples in each category to assess the effects of both season and oyster biomass on macrofaunal communities in Harris Creek. In spring, all data fell into the low and high biomass categories. To account for the unbalanced nature of this experimental design, ANOVA were run on two subsets of the data to assess all levels and their interactions: 1)

data for low and high biomass treatments for all seasons, 2) data for low, medium, and high biomass treatments for all seasons except spring.

The Oyster Metrics Workgroup (OMW 2011) defined two oyster tissue biomass levels as part of the criteria for restoration success. A reef has achieved "threshold" biomass when it has ≥15 g DW m^{-2} of oyster tissue. A reef has achieved "target" biomass for restoration success when it has ≥50 g DW m^{-2} of oyster tissue. Thus, our "low" oyster biomass category includes samples that meet the threshold biomass for restoration. To assess whether restoration at biomass levels between threshold and target levels had significant effects on macrofauna community structure, we also analyzed patterns in macrofauna biomass within the low biomass category after categorizing samples into those that were below threshold oyster biomass (0-14.9 g DW m^{-2}; hereafter "threshold treatment") and that were above threshold but below target levels of biomass (15-49.9 g DW m^{-2}; hereafter "target treatment).

Below, we present data on macrofauna community structure in terms of abundance and biomass per unit area and mean individual biomass. Because

Table 1. List of all species found in seasonal samples.

Group	Sub-group	Species or Taxonomic Group
Anemone	Anemone	*Diadumene leucolena*
		Diadumene lineata
Bivalve	Clam	*Gemma gemma*
		Macoma balthica
		Macoma mitchelli
		Mulinia lateralis
		Mya arenaria
		Petricolaria pholadiformis
	Mussel	*Ischadium recurvum*
		Mytilopsis leucophaeata
	Oyster	*Crassostrea virginica*
Crustacean	Amphipod	*Apocorophium lacustre*
		Caprella equilibria
		Cymadusa compta
		Gammarus mucronatus
		Melita nitida
		Monoculodes edwardsi
	Barnacle	*Amphibalanus* spp.
	Blue crab	*Callinectes sapidus*
	Grass shrimp	*Palaemonetes intermedius*
		Palaemonetes pugio
		Palaemonetes vulgaris
	Isopod	*Cassidinidea lunifrons*
		Chiridotea almyra
		Cyathura polita
		Erichsonella attenuata
		Probopyrus pandicola
		Synidotea laevidorsalis
	Mud crab	*Eurypanopeus depressus*
	Other crustacean	*Hargeria rapax*
Fish	Fish	*Anguilla rostrata*
		Chasmodes bosquianus
		Gasterosteus aculeatus
		Gobiesox strumosus
		Gobiosoma bosc
		Opsanus tau
Gastropod	Gastropod	*Boonea impressa*
		Corambe obscura
		Ecrobia truncata
		Gyroscala rupicola
		Haminoea solitaria
		Planorbidae
Tunicate	Tunicate	*Molgula manhattensis*
Worm	Other Worm	*Mediomastus* sp.
	Polychaete	*Alitta succinea*
		Mediomastus sp.
		Pectinaria gouldii
		Phillodocidae
		Spionidae
Other	Flatworm	*Stylochus ellipticus*
	Insect	Chironomid

finding relationships between oyster biomass density and macrofauna biomass density was the primary focus of the study, we focused our statistical analyses on these relationships. Because success criteria for the restoration effort are defined in terms of oyster tissue dry weight, we present all results in comparison to this measure of oyster biomass. Separate analyses were carried out for each of the most common and/or high biomass species or taxonomic groups. For sessile species, this included the hooked mussel *Ischadium recurvum*, the sea squirt *Molgula manhattensis*, barnacles of the genus *Amphibalanus*, and sea anemones of the genus *Diadumene*. For mobile species, this included small reef resident fish (primarily *Gobiosoma bosc* but also *Chasmodes bosquianus* and *Gobiesox strumosus*), the mud crab *Eurypanopeus depressus*, the polychaete worm *Alitta succinea*, and amphipods from several genera (dominated by *Melita nitida* with significant numbers of *Gammarus mucronatus*, *Apocorophium lacustre*, and *Cymedusa compta*). We also present data for two infauna groups: clams of the genus *Macoma* and the soft shell clam *Mya arenaria* but caution that the data for these species may, in some cases, be biased by the sampling method used. To understand patterns in total non-oyster macrofauna biomass, we summed the biomass across these groups. Table 1 gives a list of all species found in seasonal samples.

Effects of oyster biomass and season

Oyster Biomass Categories

Analyses of oyster biomass data confirmed that, regardless of season, there were significant differences in biomass between the three primary biomass categories ($p < 0.001$), hereafter referred to as "treatments" (low, medium and high, Fig. 3). Within the high treatment, oyster biomass was significantly higher in spring ($p \leq 0.006$) than in all other seasons. In this same treatment, other seasons had similar biomass except early summer which was significantly lower than the other seasons ($p < 0.001$). Season had no effect on oyster biomass for medium or low treatments.

Total Non-oyster Macrofauna

Both oyster biomass treatment and season had significant effects on total non-oyster biomass but there was a significant interaction between these factors (Fig. 4). In all seasons, total macrofauna biomass was greater for the high treatment than for the low treatment (spring, early summer and winter $p < 0.001$, late summer $p = 0.004$, fall $p = 0.007$). In early summer, the medium treatment was significantly lower than the high treatment ($p < 0.001$) but not significantly different than the low treatment. In late summer and fall, the medium treatment was significantly higher than the low treatment (late summer $p < 0.001$, fall $p = 0.031$) but not significantly different from the high treatment. In winter, total macrofauna biomass in the medium treatment was significantly greater than in the low treatment ($p = 0.021$) and significantly lower than the high treatment ($p = 0.025$).

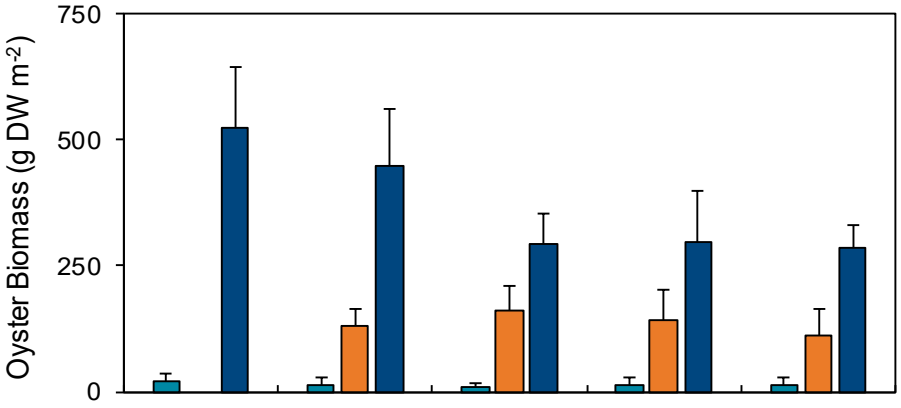

Figure 3. Seasonal oyster biomass density. In spring, none of the samples collected fell in the medium oyster biomass category. Error bars represent one standard deviation. Refer to text for results of statistical analyses of biomass data.

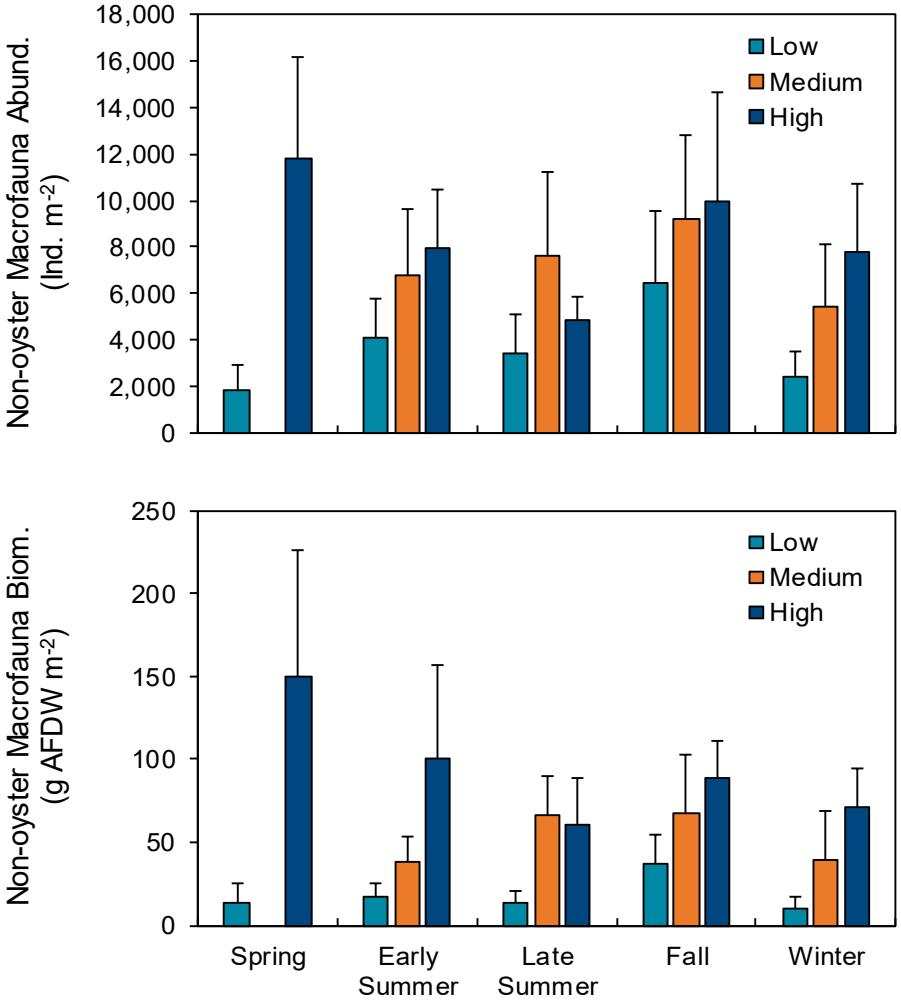

Figure 4. Seasonal non-oyster macrofauna abundance and biomass density. In spring, none of the samples collected fell in the medium oyster biomass category. Error bars represent one standard deviation. Refer to text for results of statistical analyses of biomass data.

Within the low treatment, season did not have a significant effect on total non-oyster macrofauna biomass. Within the medium treatment, fall macrofauna biomass was greater than winter ($p = 0.027$) but no other seasons showed significant differences. Within the high treatment, spring was greater than all other seasons (early summer $p = 0.002$, late summer $p < 0.001$, fall $p = 0.027$, winter $p < 0.001$).

Sessile Macrofauna

Hooked mussels (Ischadium recurvum): Oyster biomass treatment had a significant effect on mussel biomass but the effect varied with season (Fig. 5). Mussel biomass in the high treatment was significantly greater than in the low treatment for all seasons ($p \leq 0.001$) and significantly greater than the medium treatment in early summer ($p < 0.001$), fall ($p = 0.002$), and winter ($p = 0.006$). The medium treatment had significantly greater mussel biomass than the low treatment in late summer ($p < 0.001$) and in fall ($p = 0.037$). For mussels and all other macrofauna species described below, lack of data for the medium treatment in spring precluded analyses of the effect of this treatment during this season.

The effect of season varied within oyster biomass treatments. Within the high treatment, late summer had significantly lower mussel biomass than spring ($p = 0.006$) and early summer ($p = 0.020$). Within the medium biomass treatment, late summer had significantly greater mussel biomass than fall ($p = 0.036$) or winter ($p = 0.002$). Within the low biomass treatment, season did not have a significant effect on mussel biomass.

Sea squirts (Molgula manhattensis): Oyster biomass treatment had a significant effect on sea squirt biomass but the effect varied with season (Fig. 6). Sea squirt biomass in the high treatment was significantly greater than in the low treatment in spring ($p = 0.003$) but significantly less than the low treatment in fall ($p = 0.047$). Sea squirt biomass in the high treatment was significantly less than in the medium treatment in fall ($p = 0.003$). The medium treatment had significantly greater sea squirt biomass than the low treatment in winter ($p = 0.037$).

The effect of season varied within oyster biomass treatments. Within the high treatment, spring had significantly greater sea squirt biomass than early summer ($p = 0.005$) and late summer ($p = 0.005$). Within the medium biomass treatment, fall had significantly greater sea squirt biomass than early summer, late summer, or winter ($p < 0.001$). Within the low biomass treatment, fall had significantly greater sea squirt biomass than all other seasons ($p \leq 0.002$).

Barnacles (Amphibalanus spp.*):* Oyster biomass treatment had a significant effect on barnacle biomass but the effect varied with season (Fig. 7A & 7B). Barnacle biomass in the high treatment was significantly greater than in the low treatment in spring, early summer and late summer ($p < 0.001$) and significantly greater than the medium treatment in early summer ($p < 0.001$) and late summer ($p = 0.004$). The medium and low oyster biomass treatments had similar barnacle biomass regardless of season.

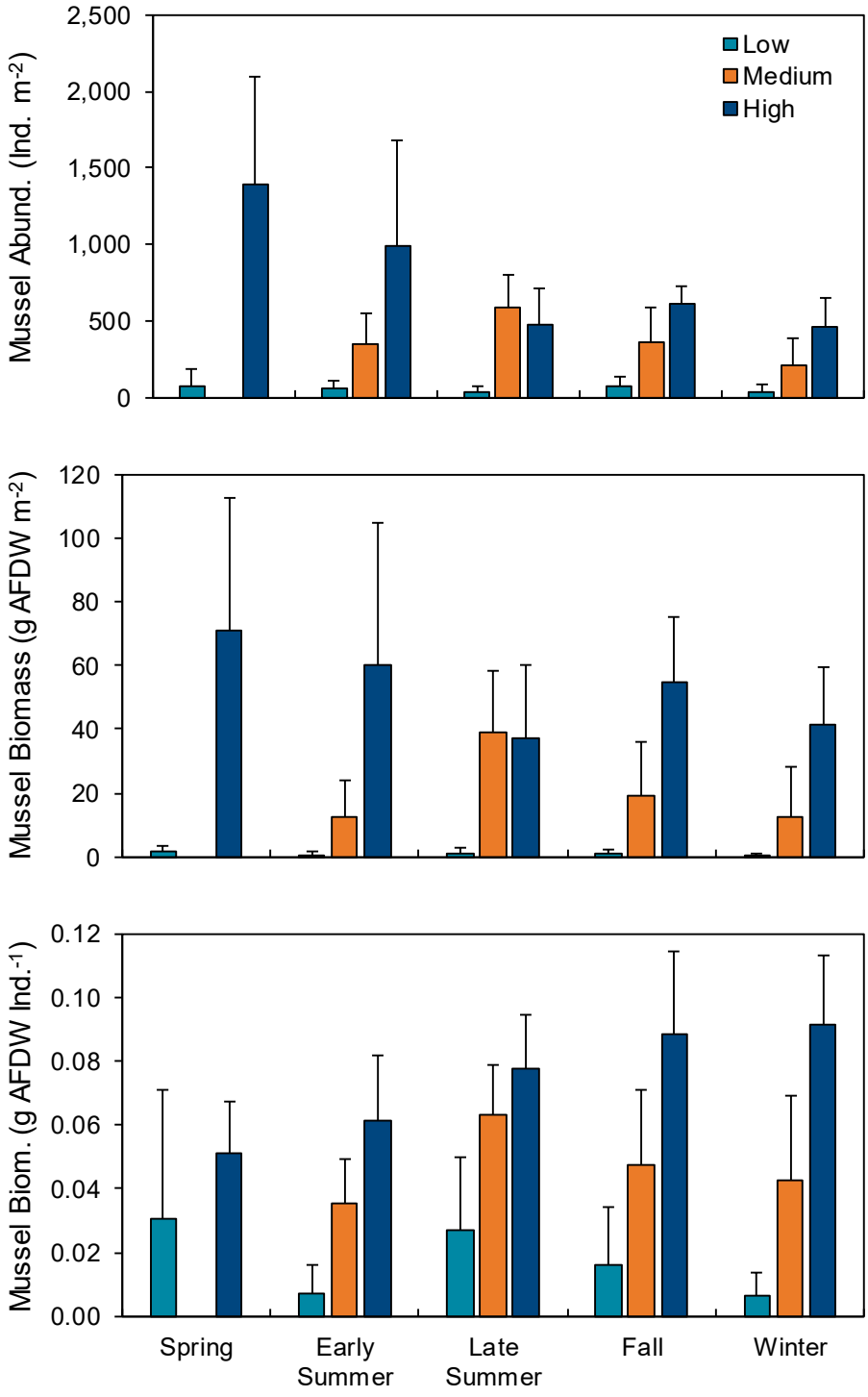

Figure 5. Seasonal mussel abundance, biomass density and individual biomass. In spring, none of the samples collected fell in the medium oyster biomass category. Error bars represent one standard deviation. Refer to text for results of statistical analyses.

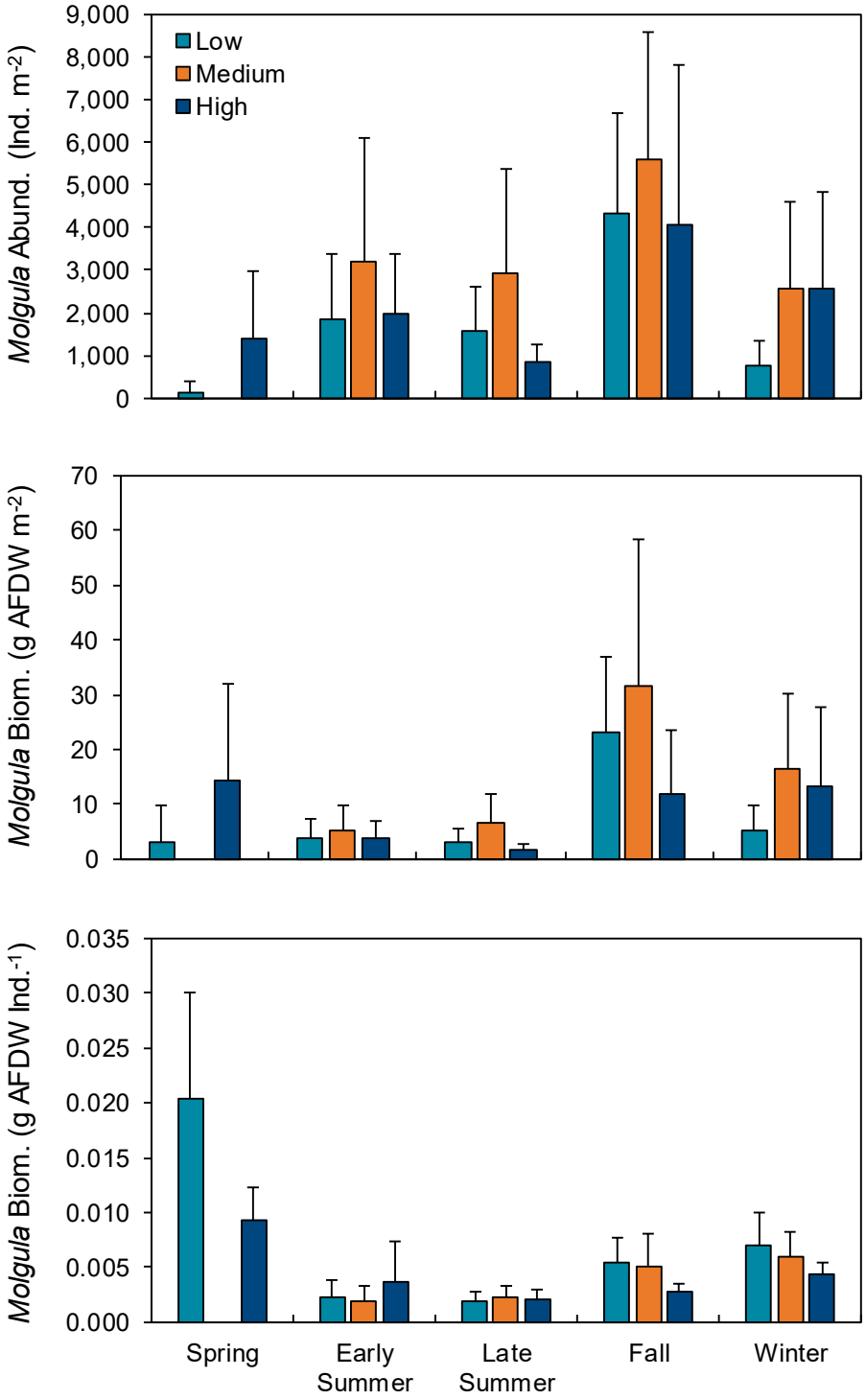

Figure 6. Seasonal sea quirt (*Molgula manhattensis*) abundance, biomass density and individual biomass. In spring, none of the samples collected fell in the medium oyster biomass category. Error bars represent one standard deviation. Refer to text for results of statistical analyses.

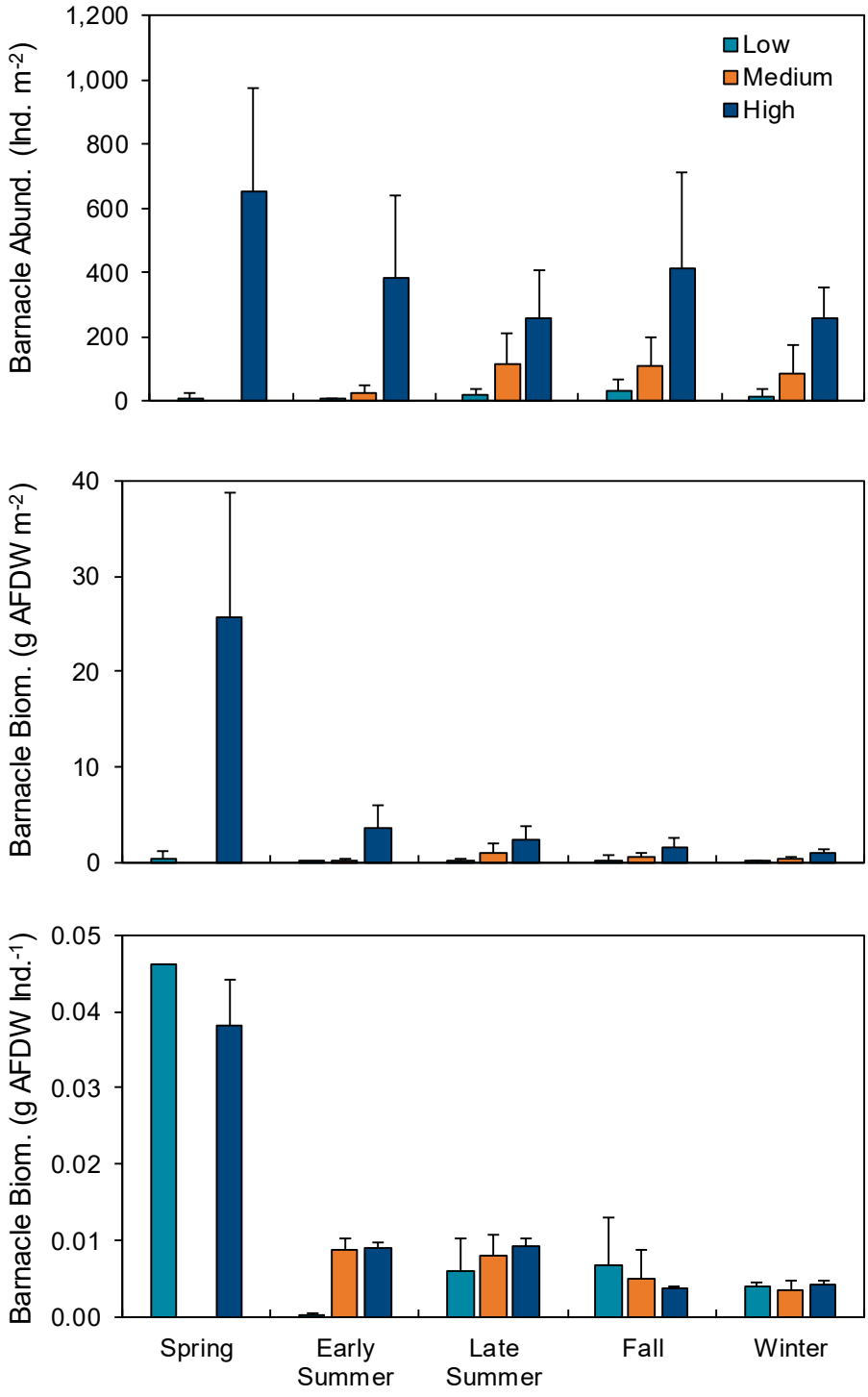

Figure 7A. Seasonal barnacle abundance, biomass density and individual biomass. In spring, none of the samples collected fell in the medium oyster biomass category. Error bars represent one standard deviation. Refer to text for results of statistical analyses.

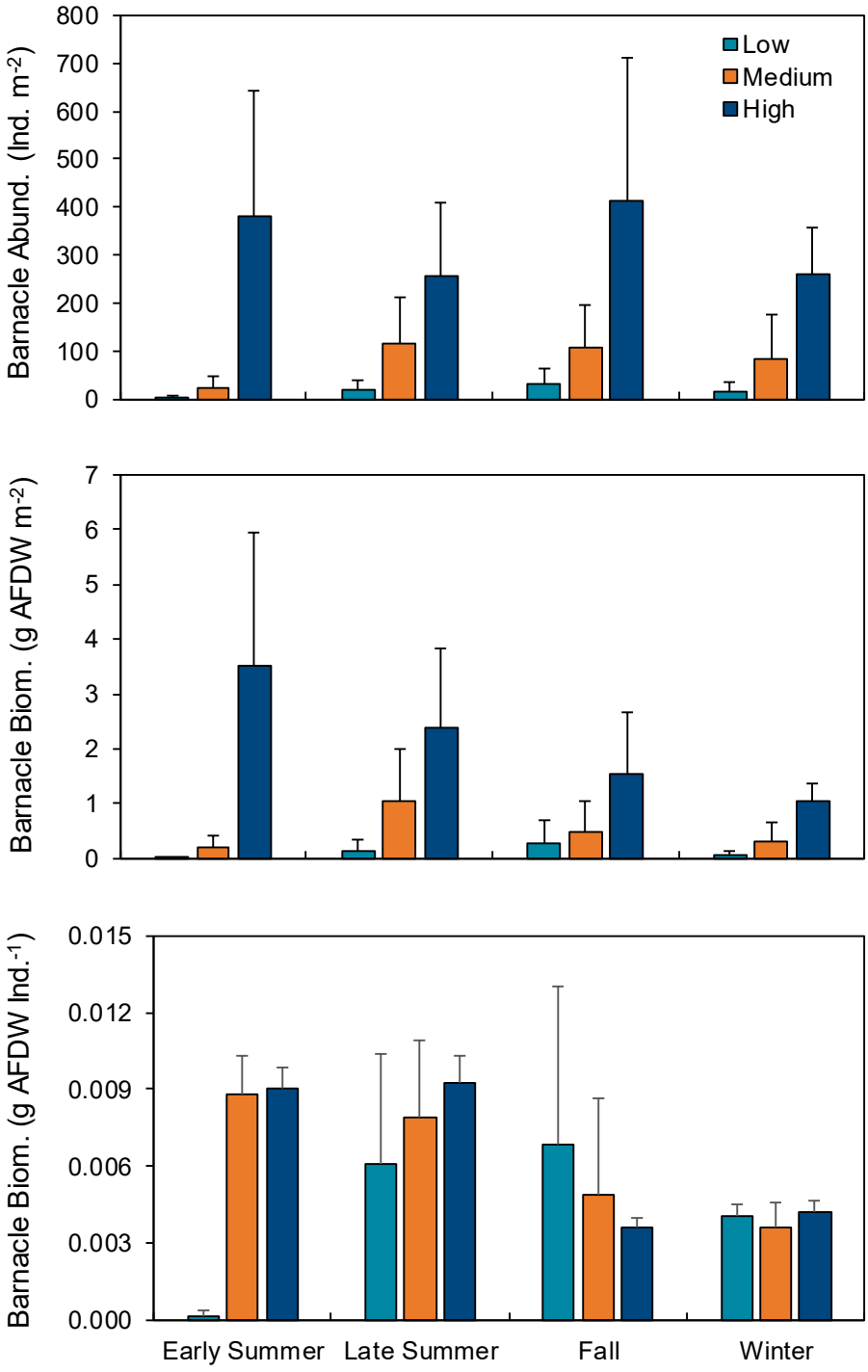

Figure 7B. Seasonal barnacle abundance, biomass density and individual biomass excluding spring. Error bars represent one standard deviation. Refer to text for results of statistical analyses.

The effect of season varied within oyster biomass treatments. Within the high treatment, spring had significantly greater barnacle biomass than all other seasons ($p < 0.001$). Within the medium and low biomass treatments, season did not have a significant effect on barnacle biomass.

Anemones (Diadumene leucolena, Diadumene lineata): Oyster biomass treatment had a significant effect on anemone biomass but the effect varied with season (Fig. 8). Anemone biomass in the high treatment was significantly greater than in the low treatment in spring ($p < 0.001$) but significantly less than the low treatment in winter ($p = 0.0180$). The high treatment had significantly lower anemone biomass than the medium treatment in winter ($p = 0.022$). The medium and low oyster biomass treatments had similar anemone biomass regardless of season.

The effect of season varied within oyster biomass treatments. Within the high treatment, spring had significantly greater anemone biomass than all other seasons ($p \leq 0.005$). Within the medium and low biomass treatments, season did not have a significant effect on anemone biomass.

Mobile Macrofauna

Small resident fish (Gobiosoma bosc, Chasmodes bosquianus, Gobiesox strumosus): Both oyster biomass treatment and season had a significant effect on small resident fish biomass (Fig. 9). Fish biomass in the high and medium treatments was significantly greater than in the low treatment ($p < 0.001$ and $p = 0.049$, respectively). Fish biomass in fall was significantly greater than in all other seasons (spring $p = 0.011$, $p < 0.001$ for other seasons). No other significant differences were found.

Mud crabs (Eurypanopeus depressus): Oyster biomass treatment had a significant effect on mud crab biomass but the effect varied with season (Fig. 10). Mud crab biomass in the high treatment was significantly greater than in the low treatment in spring and early summer ($p < 0.001$). The high biomass treatments was significantly greater than the medium treatment in early summer ($p < 0.001$). The medium and low oyster biomass treatments had similar mud crab biomass regardless of season.

The effect of season varied within oyster biomass treatments. Within the high treatment, early summer had significantly greater mud crab biomass than all other seasons (spring, late summer and winter $p \leq 0.003$; fall $p = 0.029$). Within the medium and low biomass treatments, season did not have a significant effect on mud crab biomass.

Clam worms (Alitta succinea): Oyster biomass treatment had a significant effect on clam worm biomass but the effect varied with season (Fig. 11). Clam worm biomass in the high treatment was significantly greater than in the low treatment in spring ($p <$

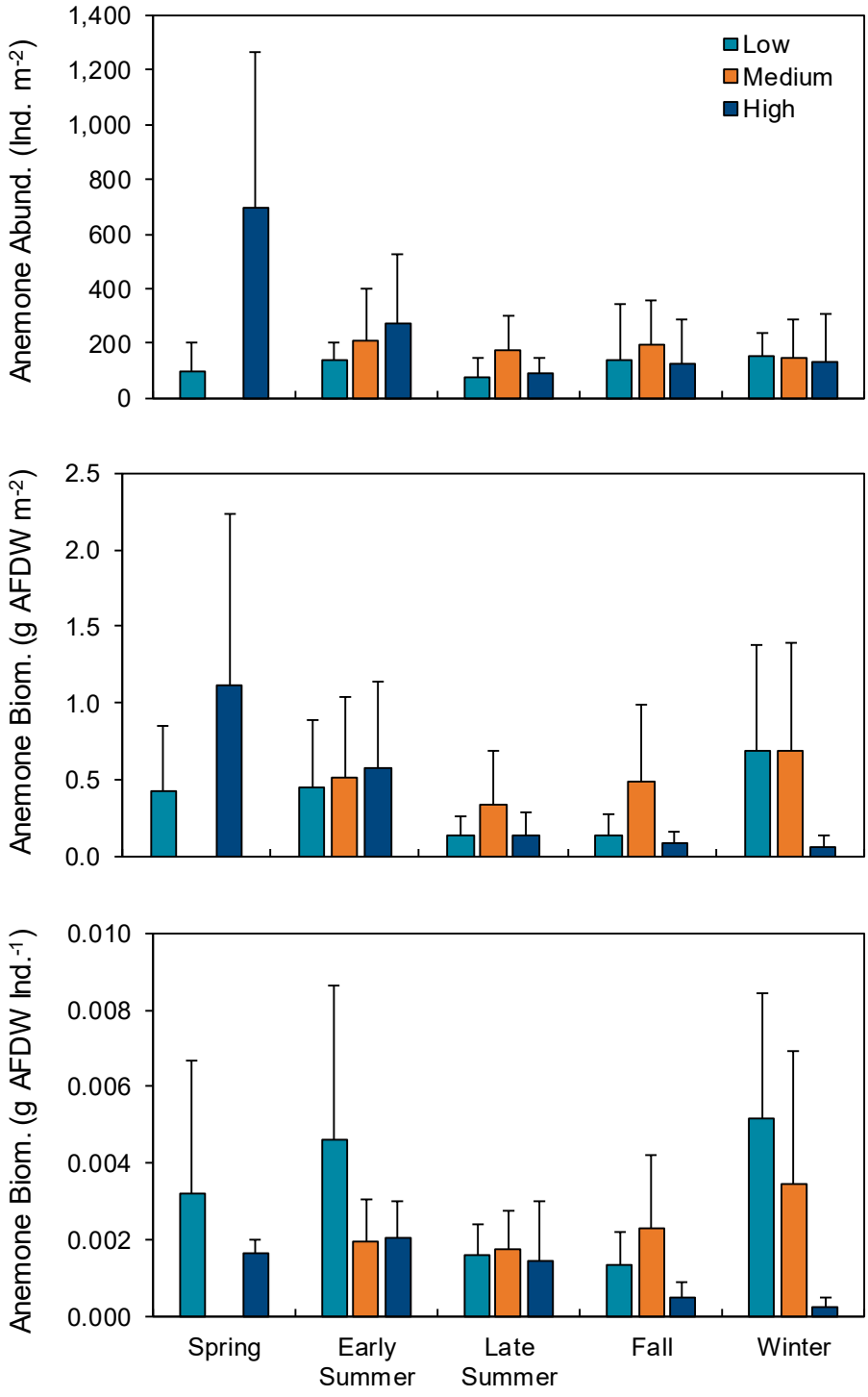

Figure 8. Seasonal sea anemone abundance, biomass density and individual biomass. In spring, none of the samples collected fell in the medium oyster biomass category. Error bars represent one standard deviation. Refer to text for results of statistical analyses.

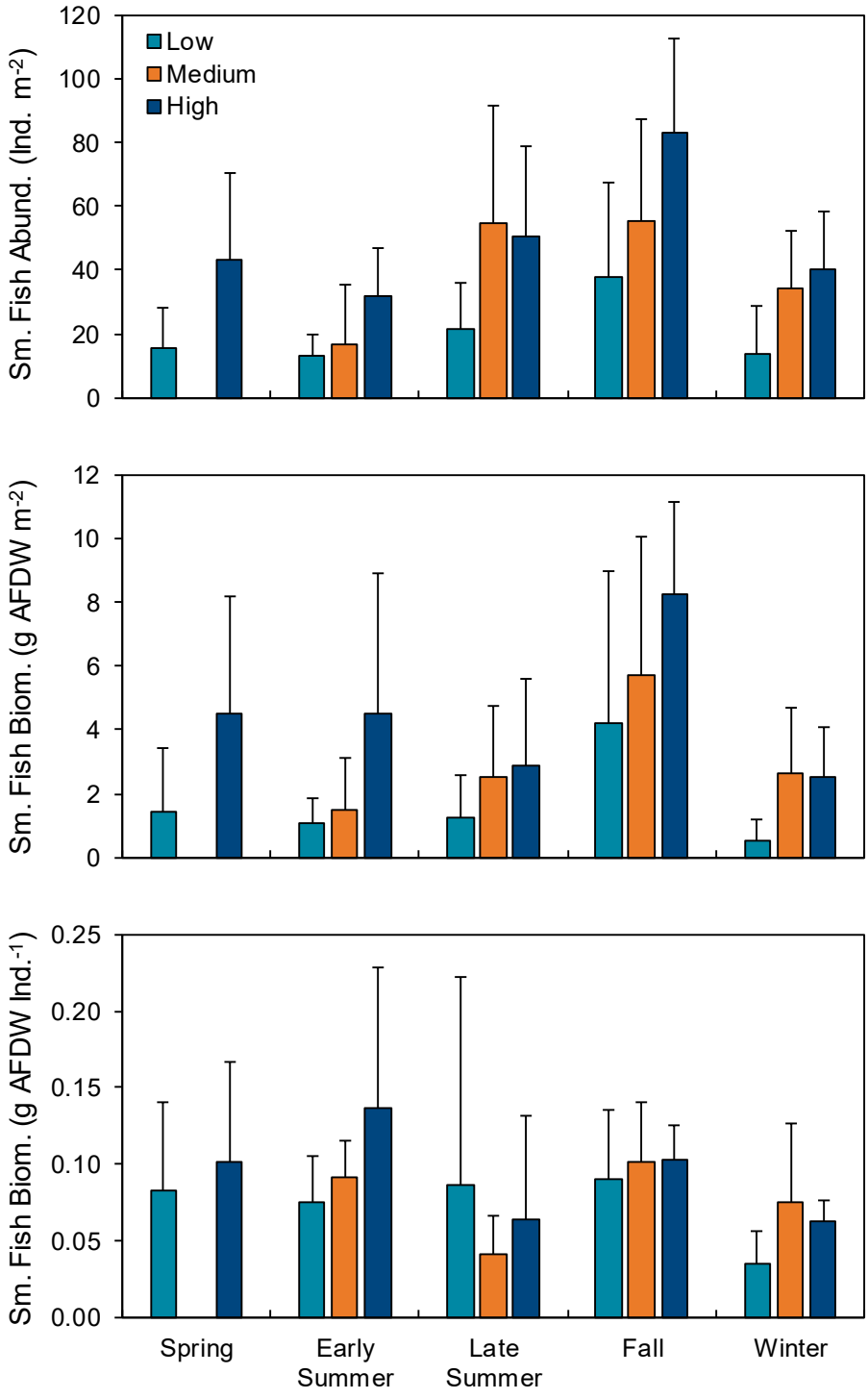

Figure 9. Seasonal small residential fish abundance, biomass density and individual biomass. In spring, none of the samples collected fell in the medium oyster biomass category. Error bars represent one standard deviation. Refer to text for results of statistical analyses.

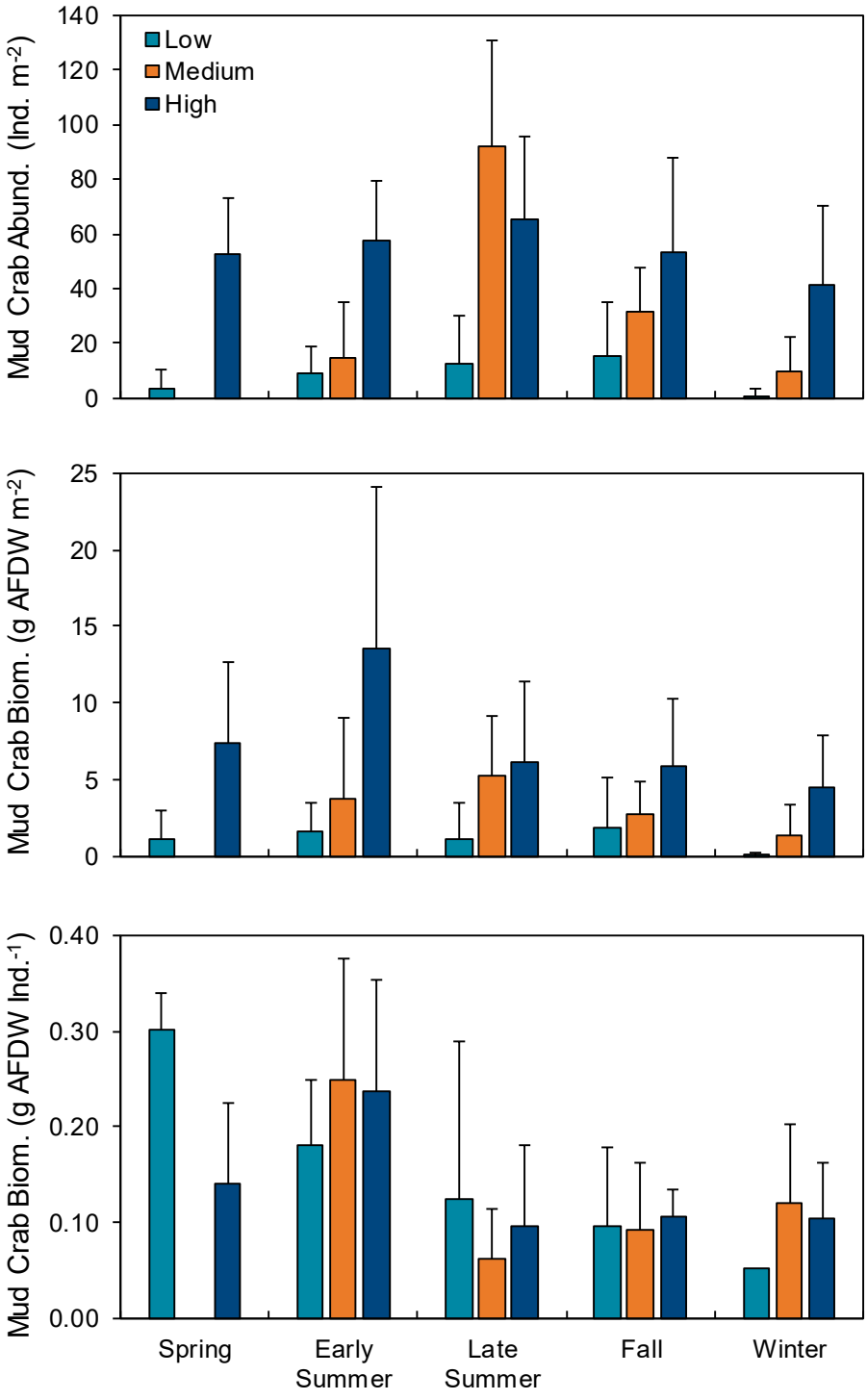

Figure 10. Seasonal mud crab abundance, biomass density and individual biomass. In spring, none of the samples collected fell in the medium oyster biomass category. Error bars represent one standard deviation. Refer to text for results of statistical analyses.

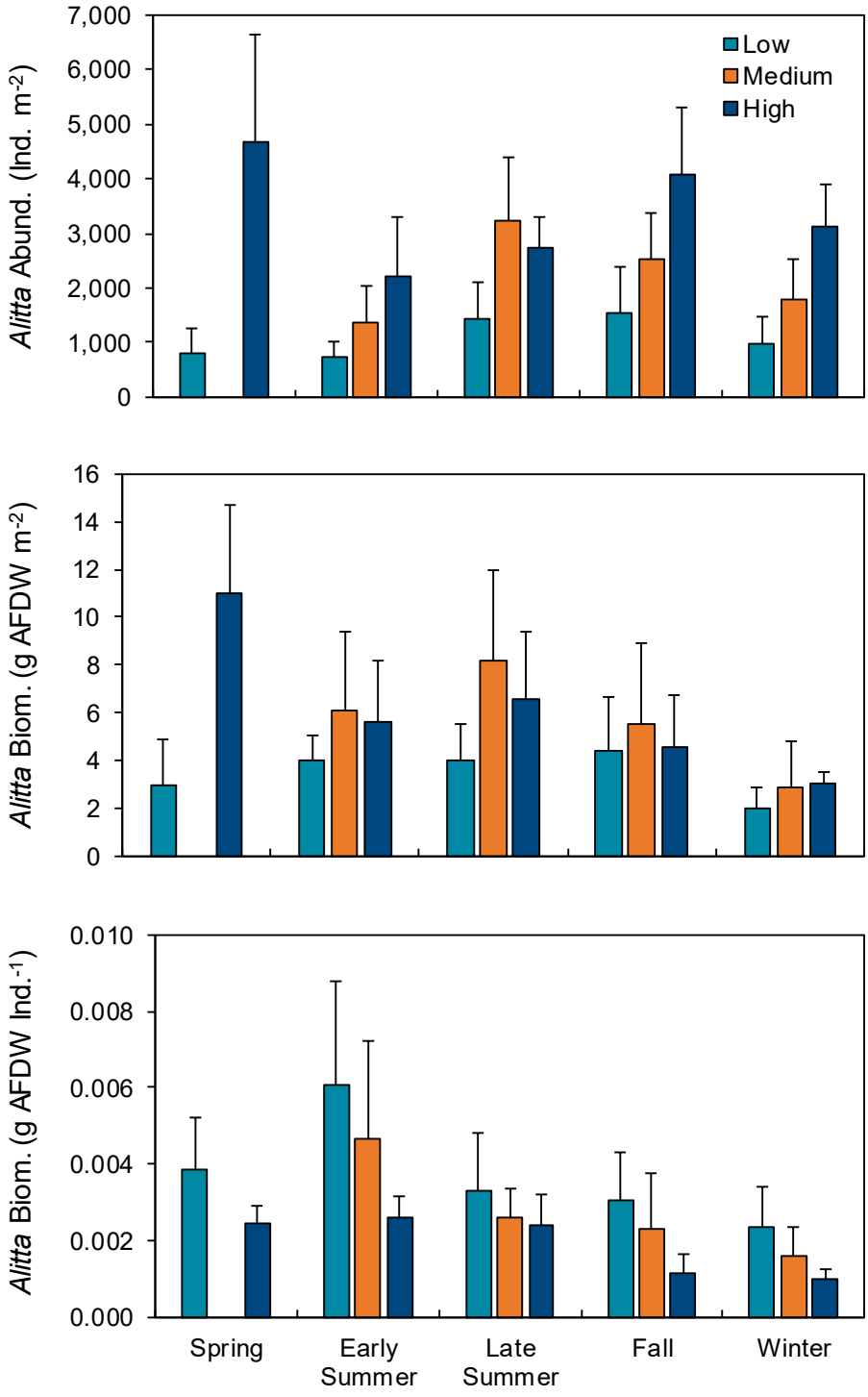

Figure 11. Seasonal clam worm (*Alitta succinea*) abundance, biomass density and individual biomass. In spring, none of the samples collected fell in the medium oyster biomass category. Error bars represent one standard deviation. Refer to text for results of statistical analyses.

0.001). The medium treatment had significantly greater clam worm biomass than the low treatment in late summer ($p = 0.001$).

The effect of season varied within oyster biomass treatments. Within the high treatment, spring had significantly greater clam worm biomass than all other seasons ($p < 0.001$) and late summer had greater biomass than winter ($p = 0.019$). Within the medium biomass treatment, winter had significantly less clam worm biomass than early summer ($p = 0.023$), late summer ($p < 0.001$), and fall ($p = 0.007$) and late summer had significantly more biomass than fall ($p = 0.007$). Within the low biomass treatment, season did not have a significant effect on clam worm biomass.

Amphipods (Melita nitida and six other species*):* Oyster biomass treatment had a significant effect on amphipod biomass but the effect varied with season (Fig. 12). Amphipod biomass in the high treatment was significantly less than in the low treatment in early summer ($p = 0.001$) but significantly greater in winter ($p = 0.017$). The high biomass treatment was significantly greater than the medium treatment in winter ($p = 0.018$). The medium treatment had significantly less amphipod biomass than the low treatment in early summer ($p < 0.001$).

The effect of season varied within oyster biomass treatments. Within the high biomass treatment, the late summer treatment had significantly lower amphipod biomass than spring ($p = 0.041$), early summer ($p = 0.003$), and winter ($p = 0.007$). Also within the high biomass treatment, fall amphipod biomass was lower than early summer ($p = 0.036$) and winter ($p = 0.036$). Within the medium biomass treatment, season had no effect on amphipod biomass. Within the low biomass treatment, spring and early summer had greater amphipod biomass than late summer ($p = 0.028$ and $p < 0.001$, respectively), fall ($p = 0.018$ and $p < 0.001$, respectively), and winter ($p=0.032$ and $p < 0.001$, respectively).

Infauna

Macoma clams (Macoma balthica, Macoma mitchelli, Macoma lateralis): Both oyster biomass treatment and season had a significant effect on *Macoma* biomass (Fig. 13). *Macoma* biomass in the high treatment was significantly less than in the medium treatment ($p = 0.047$). Early summer *Macoma* biomass was significantly greater than late summer, fall and winter ($p < 0.001$). No other significant differences were found.

Soft-shell clams (Mya arenaria): Oyster biomass treatment had no effect on soft-shell clam biomass but season did (Fig. 14). Soft-shell clam biomass in early summer was significantly greater than in late summer, fall and winter ($p < 0.001$).

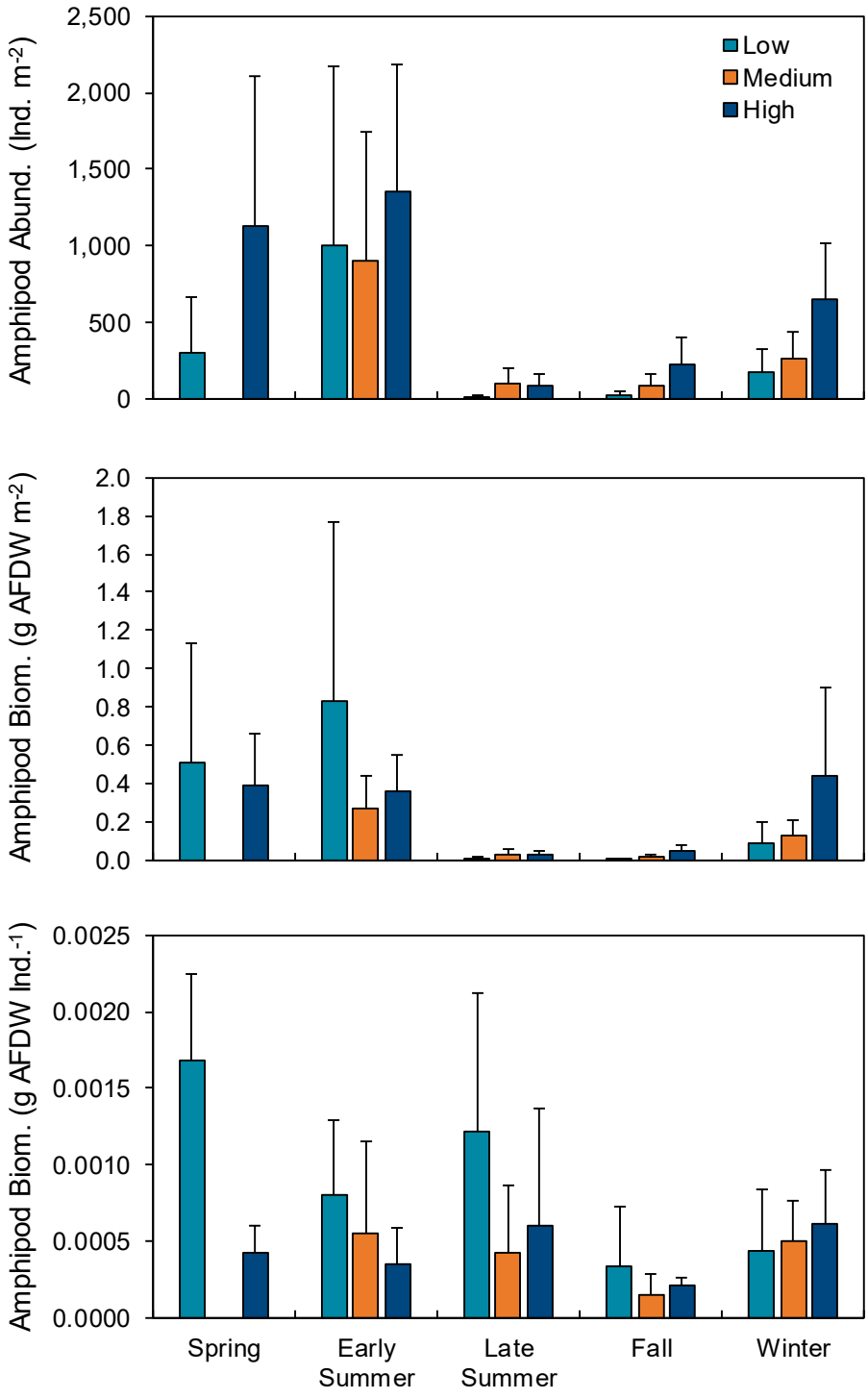

Figure 12. Seasonal amphipod abundance, biomass density and individual biomass. In spring, none of the samples collected fell in the medium oyster biomass category. Error bars represent one standard deviation. Refer to text for results of statistical analyses.

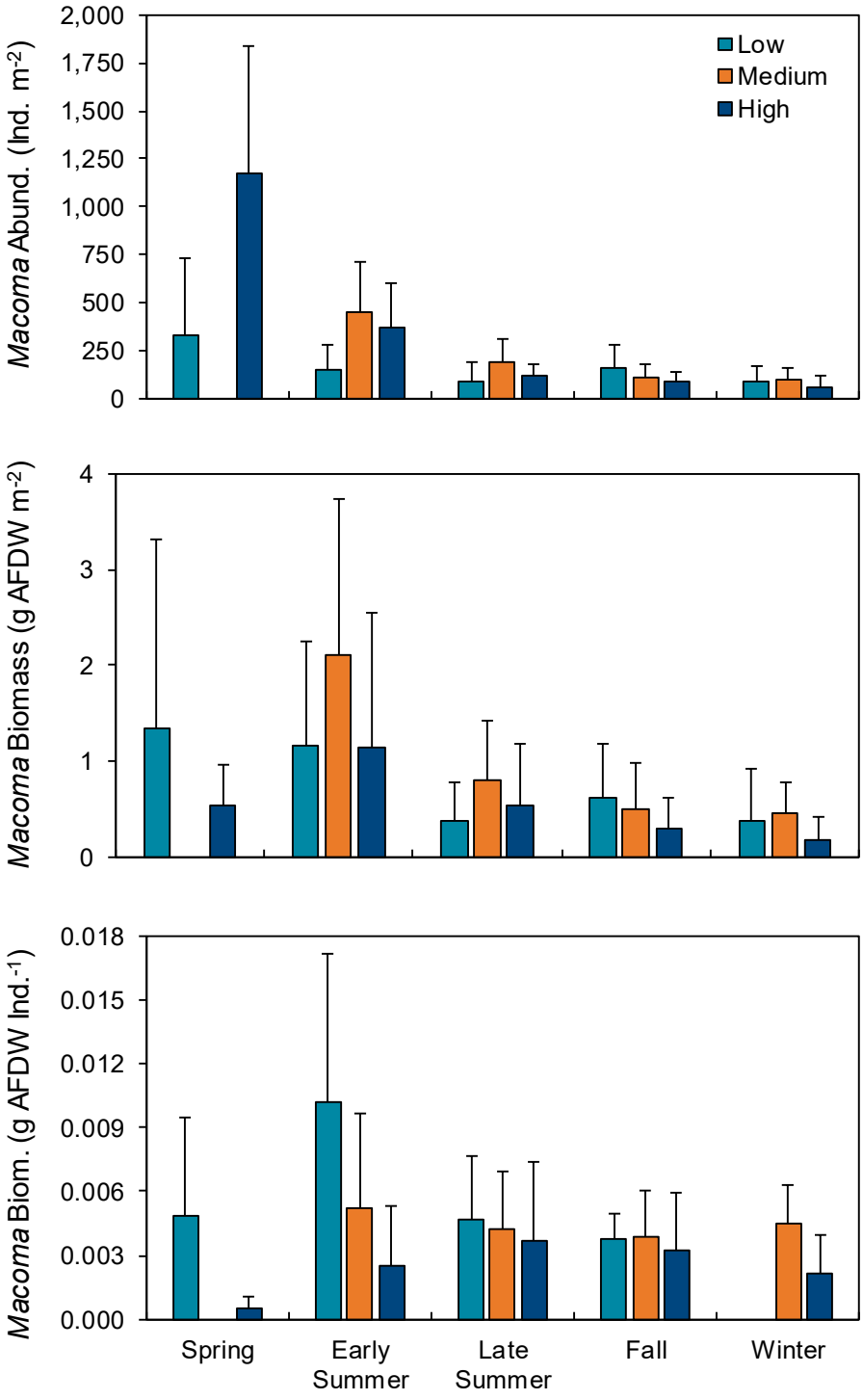

Figure 13. Seasonal *Macoma* spp. abundance, biomass density and individual biomass. In spring, none of the samples collected fell in the medium oyster biomass category. Error bars represent one standard deviation. Refer to text for results of statistical analyses.

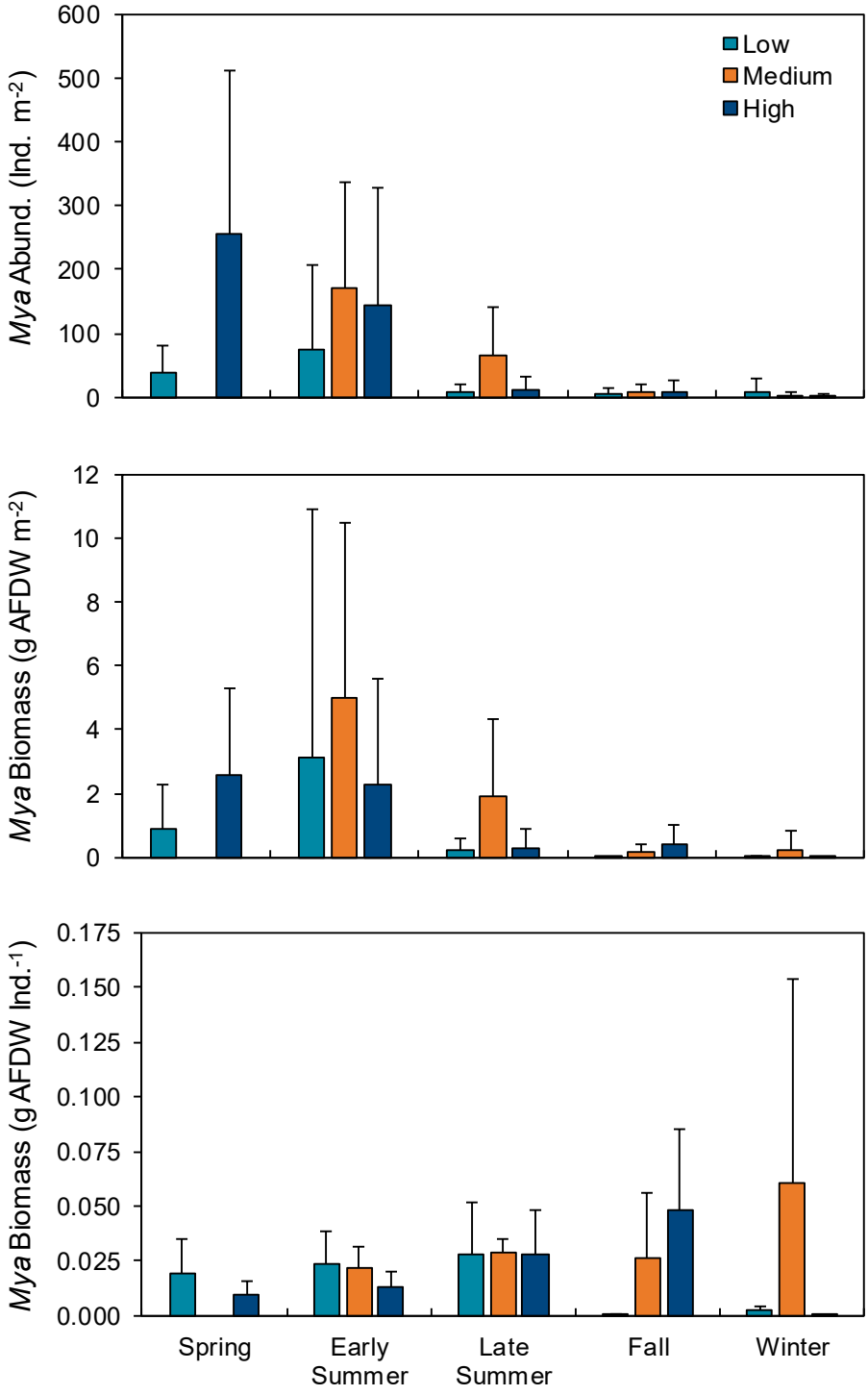

Figure 14. Seasonal *Mya arenaria* abundance, biomass density and individual biomass. In spring, none of the samples collected fell in the medium oyster biomass category. Error bars represent one standard deviation. Refer to text for results of statistical analyses.

Threshold vs. Target Biomass

Using the same data as above, we separated the low biomass category into two secondary biomass treatment levels: "threshold" and "target". To assess whether there were significant differences in the effects of these two biomass categories on macrofauna community structure we ran two-way ANOVA with two levels of biomass (target and threshold) and five levels of season (spring, early summer, late summer, fall and winter). Samples assigned to the threshold biomass category had 0-14.9 g DW m^{-2} and those assigned to the target category had 15-49.9 g DW m^{-2}.

Oysters

As expected, the target oyster biomass treatment had significantly higher oyster biomass than the threshold biomass treatment ($p < 0.001$; Fig. 15). Season did not have a significant effect on oyster biomass.

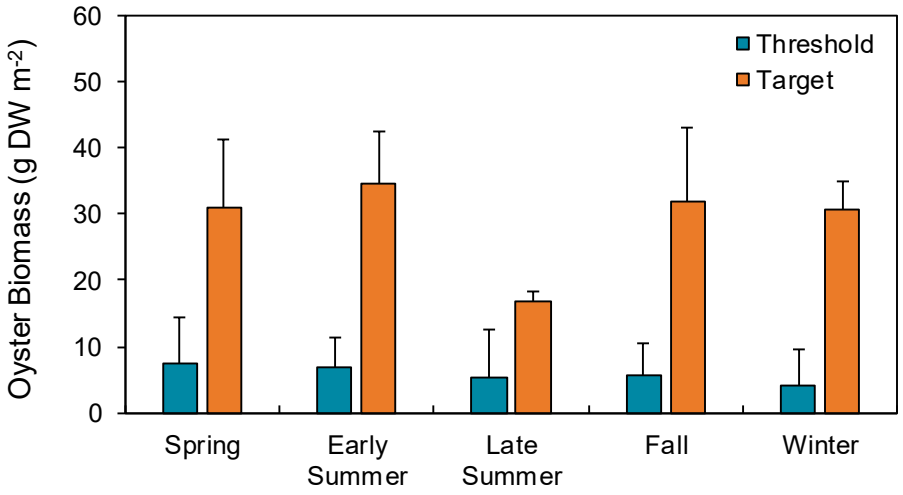

Figure 15. Seasonal oyster biomass density for threshold and target biomass categories within the low biomass category. Error bars represent one standard deviation. Refer to text for results of statistical analyses.

Total Non-oyster Macrofauna

Both oyster biomass treatment and season had a significant effect on total non-oyster macrofauna biomass (Fig. 16). Macrofauna biomass in the target treatment was significantly greater than in the threshold treatment ($p = 0.024$). In fall, total non-oyster macrofauna biomass was significantly greater than in all other seasons (early summer $p = 0.024$, $p < 0.001$ for other seasons). Macrofauna biomass did not differ significantly between spring, early summer, late summer, and winter.

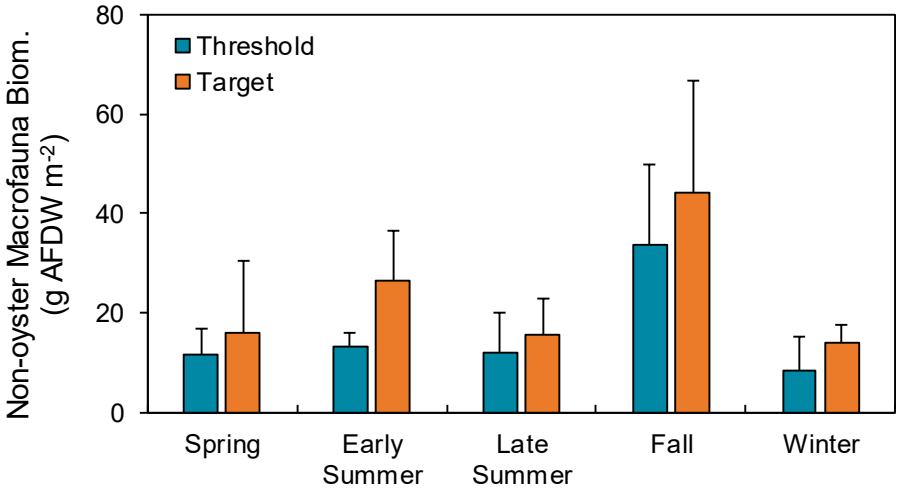

Figure 16. Seasonal non-oyster macrofauna biomass for threshold and target biomass categories within the low biomass category. Error bars represent one standard deviation. Refer to text for results of statistical analyses.

Sessile Macrofauna

Hooked mussels (Ischadium recurvum): Mussel biomass in the target treatment was significantly greater than in the threshold treatment ($p = 0.003$, Fig. 17). Season had no effect on mussel biomass.

Sea squirts (Molgula manhattensis): Oyster biomass had no effect on the biomass of sea squirts. Sea squirt biomass was significantly higher in fall than for all other months ($p < 0.001$, Fig. 17). All other seasons had similar sea squirt biomass.

Barnacles (Amphibalanus spp.*):* Neither oyster biomass treatment nor season had a significant effect on barnacle biomass (Fig. 17).

Anemones (Diadumene leucolena, Diadumene lineata): Neither oyster biomass treatment nor season had a significant effect on barnacle biomass (Fig. 17).

Mobile Macrofauna

Small resident fish (Gobiosoma bosc, Chasmodes bosquianus, Gobiesox strumosus): Oyster biomass had no effect on the biomass of small resident fish (Fig. 17). Fish biomass was significantly higher in fall than for spring ($p = 0.024$), early summer ($p = 0.012$), late summer ($p = 0.028$) and winter ($p = 0.002$). All other seasons had similar fish biomass.

Mud crabs (Eurypanopeus depressus): Neither oyster biomass treatment nor season had a significant effect on mud crab biomass (Fig. 17).

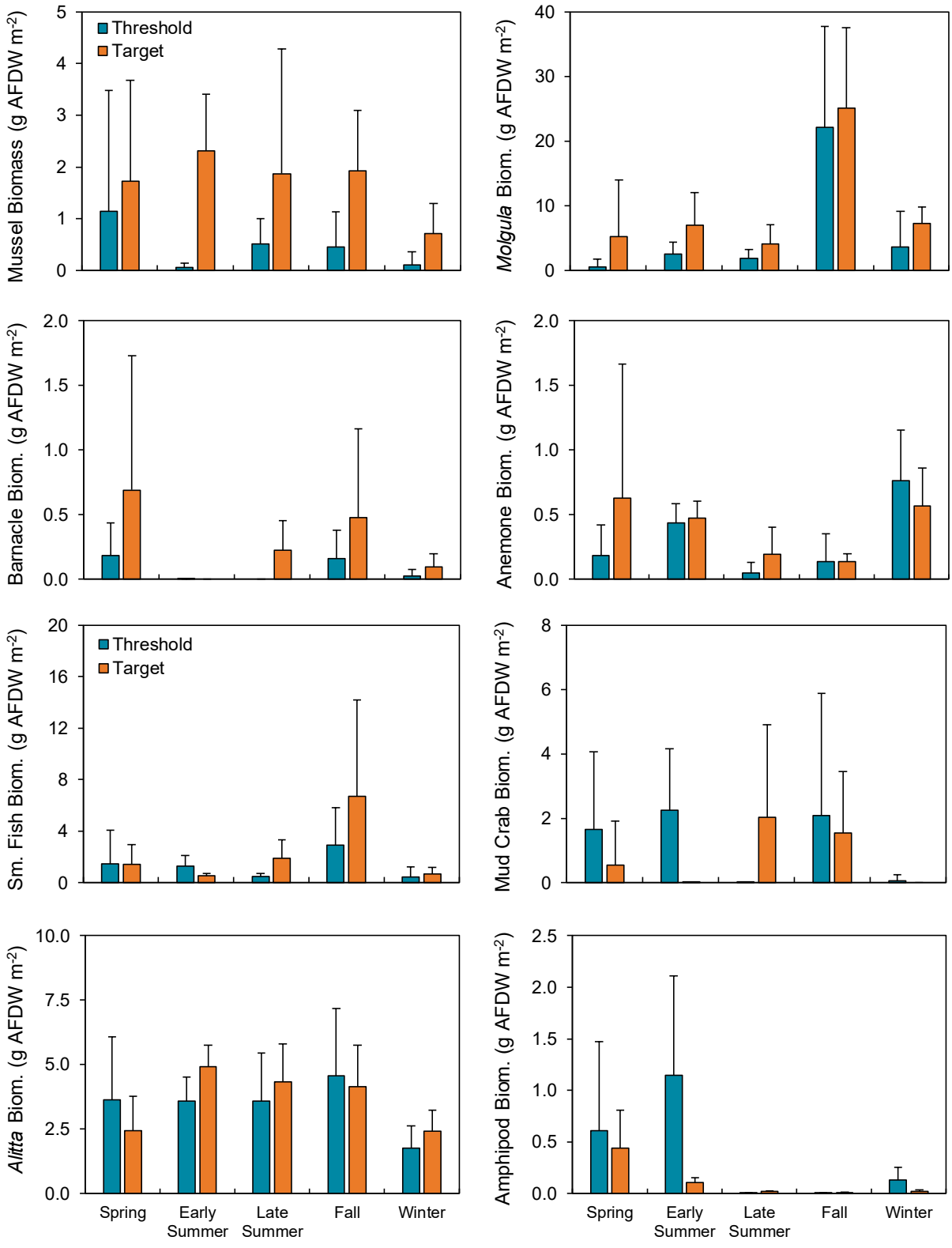

Figure 17. Seasonal macrofauna biomass density of sessile and mobile species for threshold and target biomass categories within the low biomass category. Error bars represent one standard deviation. Refer to text for results of statistical analyses.

Clam worms (Alitta succinea): Oyster biomass had no effect on the biomass of clam worms (Fig. 17). Worm biomass was significantly lower in winter than in early summer ($p = 0.036$) and fall ($p = 0.031$). All other seasons had similar worm biomass.

Amphipods (Melita nitida and six other species*):* Oyster biomass had no effect on the biomass of amphipods (Fig. 17). Although the two-way ANOVA indicated a significant effect of season ($p = 0.016$), post-hoc testing found no significant differences between seasons.

Infauna

Macoma clams (Macoma balthica, Macoma mitchelli, Macoma lateralis): Neither oyster biomass treatment nor season had a significant effect on *Macoma* biomass (Fig. 18).

Soft-shell clams (Mya arenaria): Oyster biomass treatment had a significant effect on soft-shell clam biomass but the effect varied with season (Fig. 18). Soft-shell clam biomass in the target treatment was significantly greater than in the threshold treatment in early summer ($p < 0.001$). In all other seasons, soft-shell clam biomass was similar in threshold and target treatments.

The effect of season varied within oyster biomass treatment. Within the target treatment, early summer had significantly greater soft-shell clam biomass than all other seasons (spring $p = 0.008$, late summer $p = 0.009$, fall $p = 0.011$, winter $p = 0.005$). Within this treatment, all other seasons had similar biomass. Within the threshold biomass treatment, season did not have a significant effect on soft-shell clam biomass.

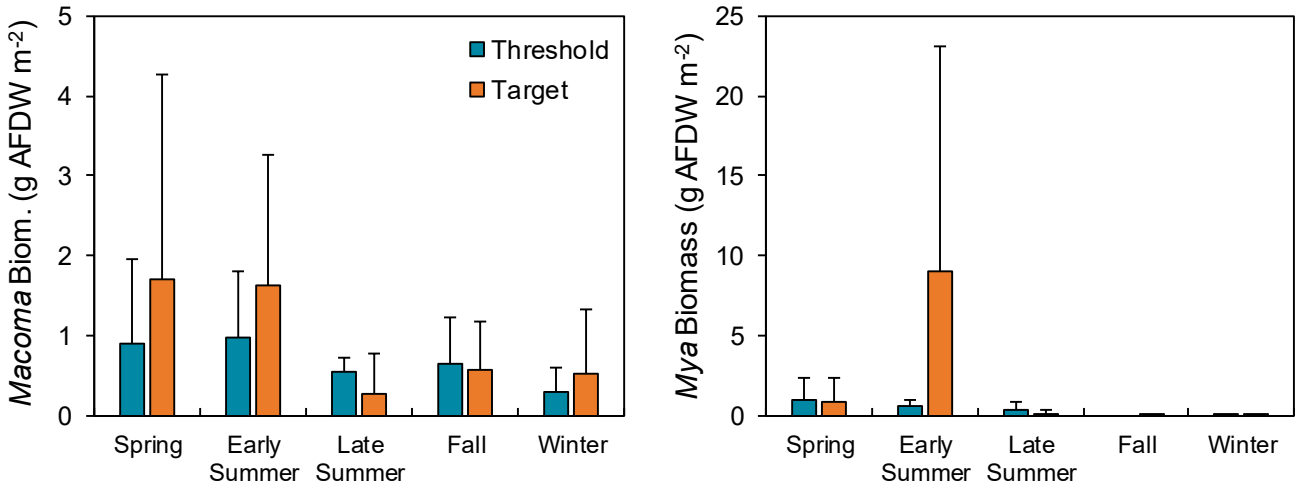

Figure 18. Seasonal macrofauna biomass density of infauna for threshold and target biomass categories within the low biomass category. Error bars represent one standard deviation. Refer to text for results of statistical analyses.

Effects of plot and reef scale oyster biomass:

Oysters

As expected, the low, medium and high oyster biomass treatments within sampling trays differed significantly from one another ($p < 0.001$; Fig. 19) and each tray-scale treatment had similar biomass regardless of the biomass density of the plot or reef within which it was deployed. Because the methods employed in this experiment were most suited to assessment of effects on mobile organisms, we focus on those results below.

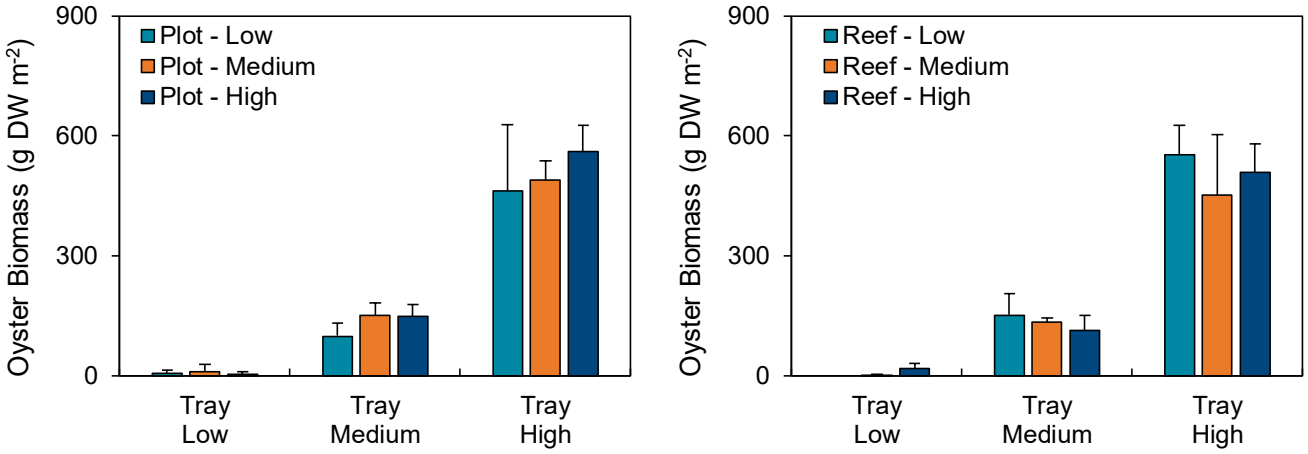

Figure 19. Tray oyster biomass density within plot and reef scale biomass densities. Error bars represent one standard deviation. Refer to text for results of statistical analyses.

Mobile Macrofauna

Because plot level biomass density did not have a significant effect on any species, we ran a reduced complexity model to assess the effects of tray and reef scale oyster biomass on macrofauna community structure. Regardless of species, there was no evidence for interactions between the effects of tray and reef scale biomass.

Small resident fish (Gobiosoma bosc, Chasmodes bosquianus, Gobiesox strumosus): Tray-scale oyster biomass had a significant effect on the biomass of small resident fish ($p = 0.005$) but reef-scale biomass did not (Fig. 20). Fish biomass was significantly lower in the low tray biomass treatment than in the medium ($p = 0.044$) or high tray biomass treatments ($p = 0.004$).

Mud crabs (Eurypanopeus depressus): Both tray-scale ($p < 0.001$) and reef-scale oyster biomass density ($p = 0.003$) had a significant effect on mud crab biomass(Fig. 20). For tray-scale biomass, the high biomass treatment was significantly higher than the low ($p < 0.001$) and medium biomass treatments. For reef-scale biomass, the medium biomass treatment was significantly lower than the high ($p = 0.007$) and low biomass treatments ($p = 0.006$).

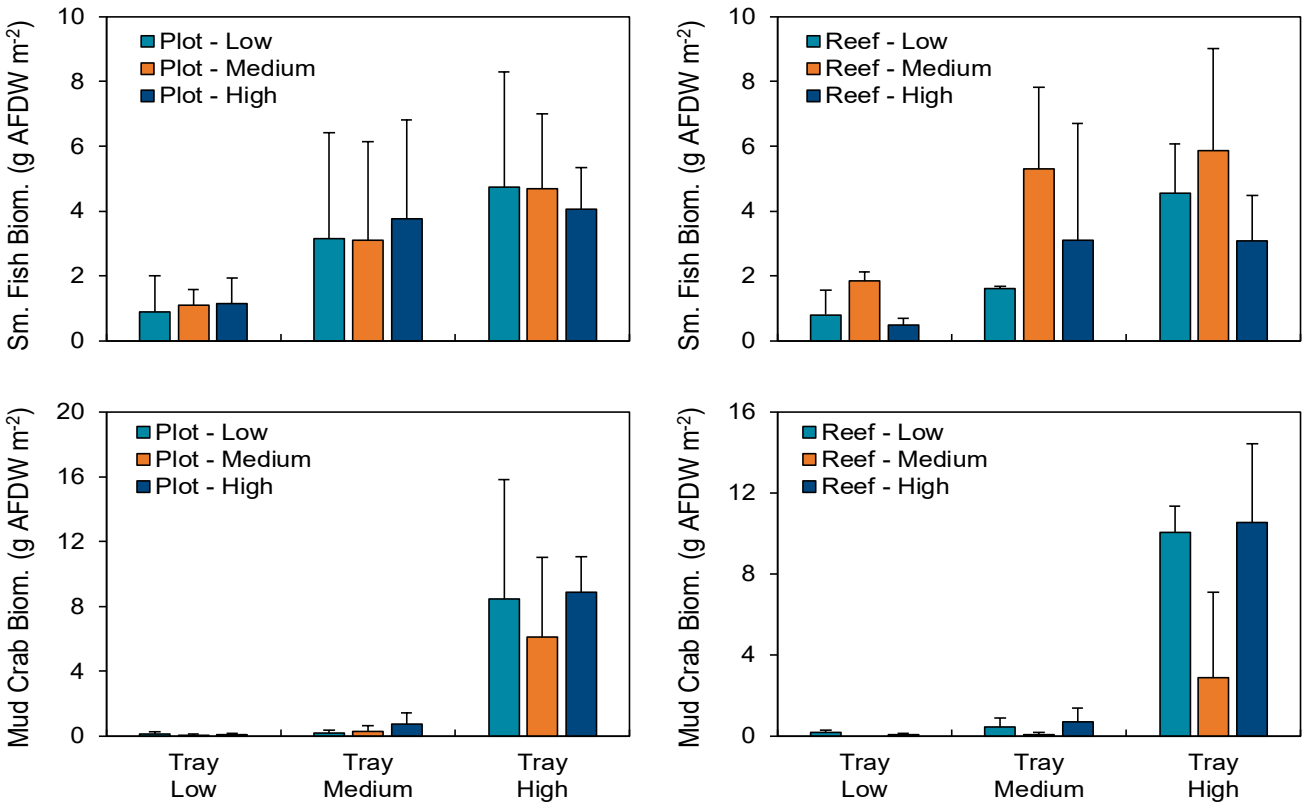

Figure 20. Small resident fish and mud crab biomass density within plot and reef scale biomass densities. Error bars represent one standard deviation. Refer to text for results of statistical

Clam worms (Alitta succinea): Both tray-scale ($p < 0.001$) and reef-scale oyster biomass density ($p < 0.001$) effect on the biomass of clam worms (Fig. 21). For tray-scale biomass, the high biomass treatment was significantly higher than the medium biomass treatment ($p = 0.001$) which was in turn higher than the low biomass treatment ($p < 0.001$). For reef-scale biomass, the medium treatment was significantly lower than both the high ($p < 0.001$) and the low reef biomass treatments ($p = 0.014$).

Amphipods (Melita nitida and six other species*):* Both tray-scale ($p < 0.001$) and reef-scale oyster biomass density ($p = 0.006$) effect on the biomass of amphipods (Fig. 21). For tray-scale biomass, the high biomass treatment was significantly higher than the medium biomass treatment ($p = 0.008$) and the low biomass treatment ($p < 0.001$). For reef-scale biomass, the medium treatment was significantly lower than both the high ($p = 0.017$) and the low reef biomass treatments ($p = 0.009$).

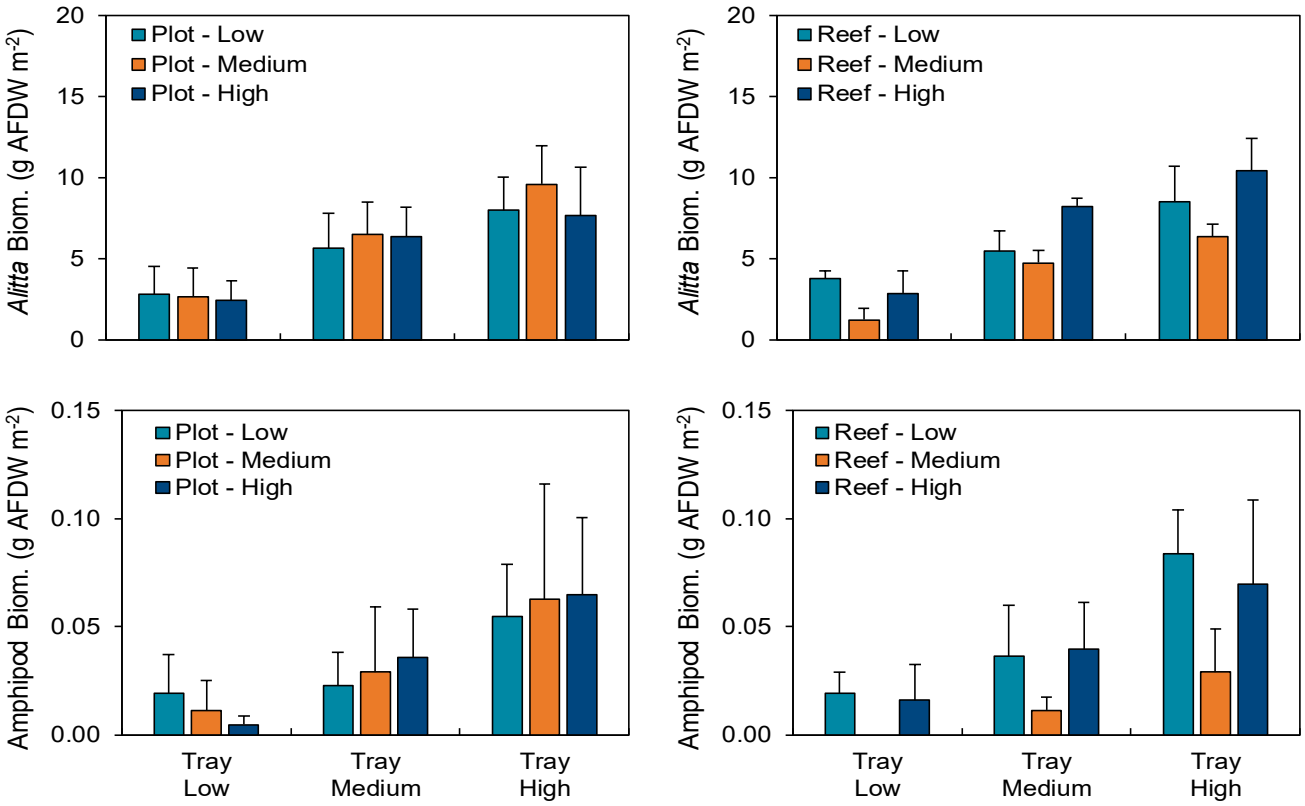

Figure 21. Clam worm (*Alitta succinea*) and amphipod biomass density within plot and reef scale biomass densities. Error bars represent one standard deviation. Refer to text for results of

Effects of distance from mouth of creek

To further investigate larger scale patterns in macrofauna biomass density in Harris Creek, we also examined patterns in relation to distance from the mouth of the creek. To remove the effects of small-scale oyster biomass density, we divided the biomass of each macrofauna species in each sample by the oyster biomass within that same sample to get the ratio of macrofauna biomass to oyster biomass. Using this approach produced high variability at sites with very low oyster biomass density. Below, we present regressions of macrofauna biomass ratios for both the "full" dataset (left-hand panel of each graph) and the "reduced" dataset (right-hand panel of each graph) after removal of low oyster biomass samples (defined as samples with < 50g oyster biomass).

Sessile Macrofauna

Hooked mussels (Ischadium recurvum): For both the full dataset and the reduced dataset, there was a highly significant ($p < 0.001$) inverse relationship between distance from the mouth of the creek and the ratio of mussel biomass to oyster biomass (Fig. 22).

Sea squirts (Molgula manhattensis): For the full dataset, there was not a significant relationship between the distance from the mouth of the creek and the ratio of sea squirt biomass to oyster biomass (Fig. 22). However, when low biomass samples were removed and the reduced dataset was reanalyzed, there was a highly significant ($p < 0.001$) inverse relationship between distance from the mouth of the creek and the ratio of sea squirt biomass to oyster biomass.

Barnacles (Amphibalanus spp.*):* For the full dataset, there was a significant ($p = 0.045$) positive relationship between distance from the mouth of the creek and the ratio of barnacle biomass to oyster biomass (Fig. 22). When low biomass samples were removed from the dataset, there was no longer a significant relationship between distance from the mouth of the creek and the ratio of barnacle biomass to oyster biomass.

Anemones (Diadumene leucolena, Diadumene lineata): For the full dataset, there was not a significant relationship between the distance from the mouth of the creek and the ratio of anemone biomass to oyster biomass (Fig. 22). However, when low biomass samples were removed and the reduced dataset was reanalyzed, there was a highly significant ($p < 0.001$) inverse relationship between distance from the mouth of the creek and the ratio of anemone biomass to oyster biomass.

Mobile Macrofauna

Small resident fish (Gobiosoma bosc, Chasmodes bosquianus, Gobiesox strumosus): For both the full dataset and the reduced dataset, there was not a significant relationship between distance from the mouth of the creek and the ratio of small resident fish biomass to oyster biomass (Fig. 23).

Mud crabs (Eurypanopeus depressus): For both the full dataset and the reduced dataset, there was not a significant relationship between distance from the mouth of the creek and the ratio of mud crab biomass to oyster biomass (Fig. 23).

Clam worms (Alitta succinea): For the full dataset, there was not a significant relationship between the distance from the mouth of the creek and the ratio of clam worm biomass to oyster biomass. However, when low biomass samples were removed and the reduced dataset was reanalyzed, there was a highly significant ($p < 0.001$) inverse relationship between distance from the mouth of the creek and the ratio of clam worm biomass to oyster biomass (Fig. 23).

Amphipods (Melita nitida and six other species*):* For both the full dataset and the reduced dataset, there was not a significant relationship between distance from the mouth of the creek and the ratio of amphipod biomass to oyster biomass (Fig. 23).

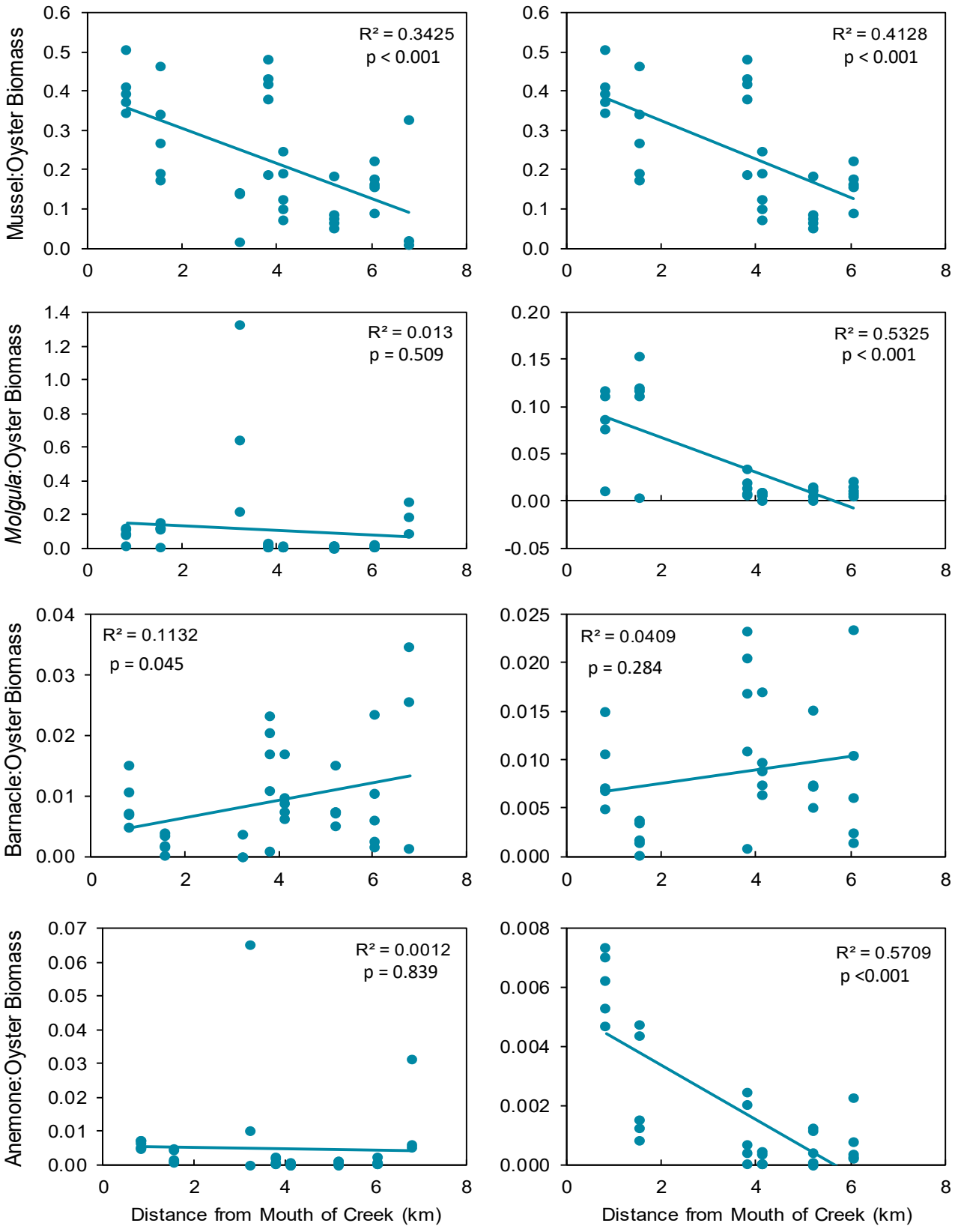

Figure 22. Sessile macrofauna species biomass per unit oyster biomass as a function of distance from the mouth of the creek. Left-hand panels show regressions for full dataset and right-hand panels show regressions for reduced dataset. Refer to text for details of analyses

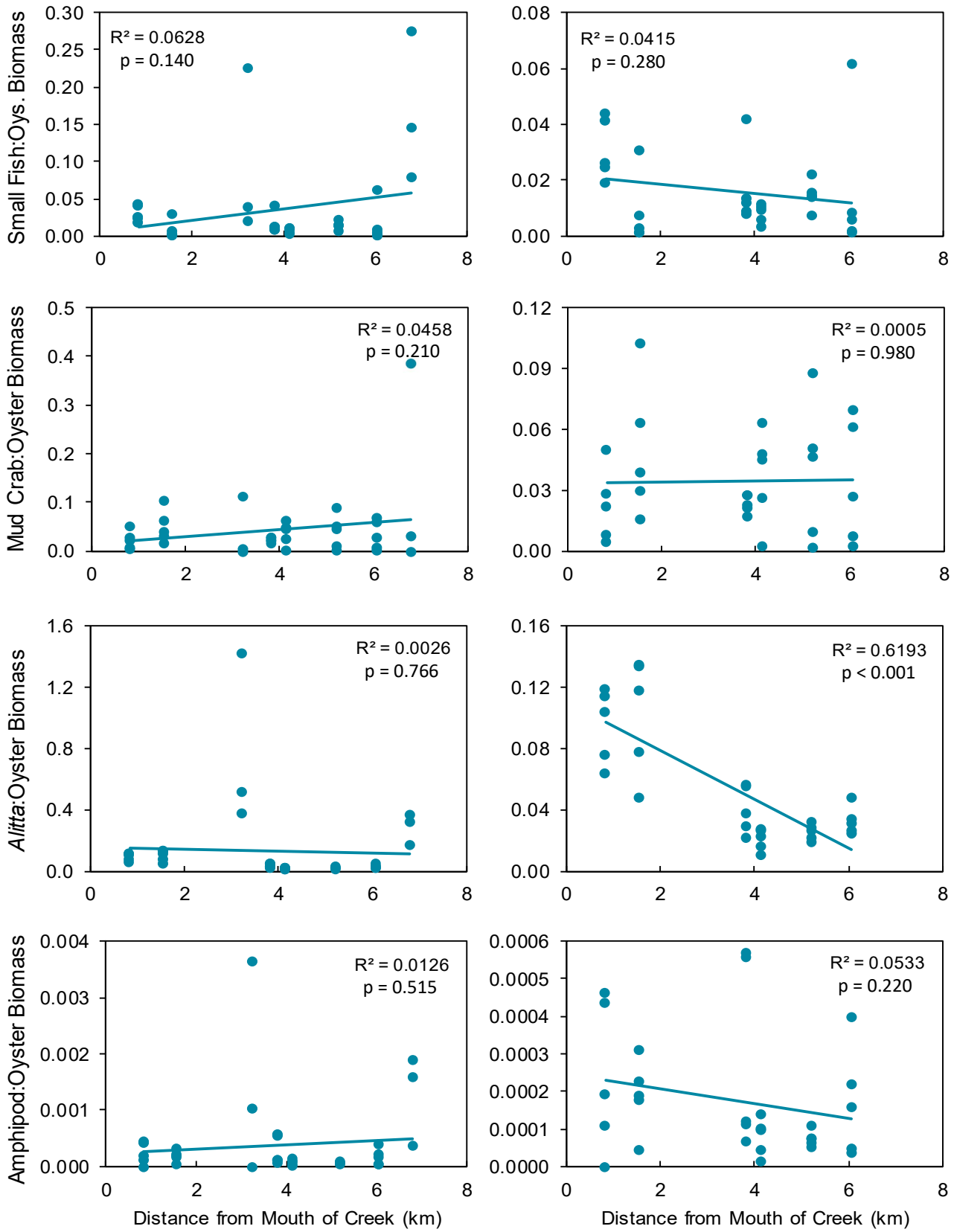

Figure 23. Mobile macrofauna species biomass per unit oyster biomass as a function of distance from the mouth of the creek. Left-hand panels show regressions for full dataset and right-hand panels show regressions for reduced dataset. Refer to text for details of analyses

Infauna

Macoma clams (Macoma balthica, Macoma mitchelli, Macoma lateralis): For the full dataset, there was not a significant relationship between the distance from the mouth of the creek and the ratio of *Macoma* biomass to oyster biomass (Fig. 24). However, when low biomass samples were removed and the reduced dataset was reanalyzed, there was a highly significant ($p < 0.001$) inverse relationship between distance from the mouth of the creek and the ratio of *Macoma* biomass to oyster biomass.

Soft-shell clams (Mya arenaria): For both the full dataset and the reduced dataset, there was a highly significant ($p < 0.001$) inverse relationship between distance from the mouth of the creek and the ratio of *Mya arenaria* biomass to oyster biomass (Fig. 24).

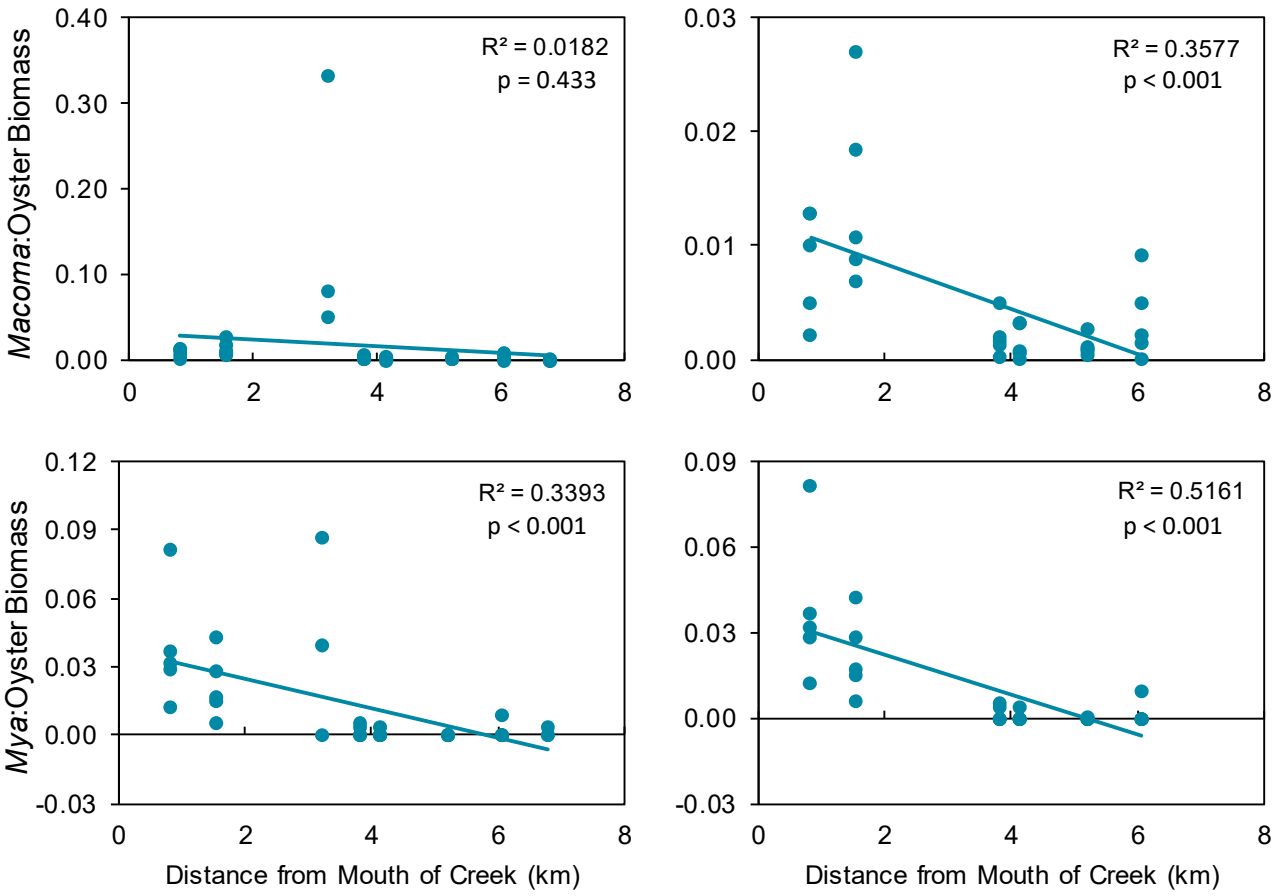

Figure 24. Clam species biomass per unit oyster biomass as a function of distance from the mouth of the creek. Left-hand panels show regressions for full dataset and right-hand panels show regressions for reduced dataset. Refer to text for details of analyses

Conclusions

- Restored reefs in Harris Creek provide habitat for ~50 different macrofauna species.
- Samples from Harris Creek that had high oyster biomass density (>225 g DW m^{-2}) consistently provided habitat for >5,000 individuals m^{-2} regardless of season. In spring and fall, non-oyster macrofauna abundances reached ~10,000 individuals m^{-2}. Biomass of these non-oyster macrofauna exceeded 60 g AFDW m^{-2} in all seasons and was as high as 150 g AFDW m^{-2} in some seasons.
- For all sessile and mobile species except small resident fish, there were significant and complex interactions between the effects of oyster biomass density and season on the biomass density of each species. Small resident fish biomass was consistently higher in samples with medium (50-224.9 g DW) and high oyster biomass density. They were also significantly higher in fall than in all other seasons.
- When macrofauna biomass was compared between samples with oyster biomass densities below the threshold level for restoration (0-14.9 g DW m^{-2}) and between the threshold and target biomass density for restoration (15-49.9 g DW m^{-2}), only two species showed a significant increase in their biomass with increasing oyster biomass. Biomass of hooked mussels was significantly higher in all season but biomass of *Mya arenaria* was higher only in early summer.
- Comparison of the effects of oyster biomass density at the tray scale (0.1 m^2), plot scale (10 m^2) and reef scale on mobile macrofauna species did not find significant effects of plot-scale oyster biomass density for any species. Tray-scale oyster biomass had a significant effect on all species and reef-scale biomass had a significant effect on all groups except small resident fish.
- For medium and high oyster biomass samples, position relative to the mouth of the creek had a significant effect on three of the four sessile macrofauna groups, one of the four mobile macrofauna groups, and both of the infauna groups.

Dissemination of results

Data from or information about this project have been presented at a variety of meetings attended by resource managers, restoration practitioners and researchers and have been incorporated into published and in progress manuscripts including:

Kellogg ML, Cornwell JC, Owens MS (accepted with revisions) Measurement of biogeochemical fluxes in oyster reef environments. Marine Ecology Progress Series

Kellogg, M. L., Brush, M. J., & Cornell, J. C. (2018) An updated model for estimating the TMDL-related benefits of oyster reef restoration Harris Creek, Maryland, USA. Virginia

Institute of Marine Science, College of William and Mary. https://doi.org/10.25773/7a75-ds48

Knoche S, Ihde TF (2019) Estimating Ecological Benefits and Socio-Economic Impacts from Oyster Reef Restoration in the Choptank River Complex, Chesapeake Bay. Final Report to: The National Fish and Wildlife Foundation & The NOAA Chesapeake Bay Office, 62 pp.

Jackson M, Owens MS, Cornwell JC, Kellogg ML (2018) Comparison of methods for determining biogeochemical fluxes from a restored oyster reef. PLOS ONE 13(12): e0209799.

Ricci SW, Bohnenstiehl DR, Eggleston DB, Kellogg ML, Lyon RP (2017) Oyster toadfish (Opsanus tau) boatwhistle call detection and patterns within a large-scale oyster restoration site. PLOS ONE 12(8):e0182757.Kellogg ML and 10 others (2017) Ecosystem services provided by tributary-scale oyster reef restoration in Chesapeake Bay. Coastal and Estuarine Research Federation's Biennial Conference, Providence, RI.

Kellogg ML, Cornwell JC (2017) Benefits of oyster reef restoration in Harris Creek, MD. MARACOOS Workshop, Annapolis, MD.

Kellogg ML, Cornwell JC (2016) Benefits of oyster reef restoration in Harris Creek, MD. Seminar, Horn Point Laboratory, University of Maryland Center for Environmental Science, Cambridge, MD.

Kellogg ML, Cornwell JC, Owens MS, Ross PG, Dreyer JC, Paynter KT, Luckenbach MW (2015) Integrated assessment of ecosystem services provided by tributary-scale oyster reef restoration in Chesapeake Bay. Coastal and Estuarine Research Federation's 23rd Biennial Conference, Portland, Oregon

Kellogg ML, Paynter KT, Cornwell JC, Ross PG, Owens MS, Handschy AV, Dreyer JC, Luckenbach MW (2014) Integrated assessment of oyster reef ecosystem services: Harris Creek, MD. 16th International Conference on Shellfish Restoration, Charleston, SC

Kellogg ML 2015. Measuring the benefits of oyster reef restoration: Quantifying denitrification rates and other ecosystem services. NC State Center for Marine Sciences and Technology, Morehead City, NC.

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