THE RESPONSE OF MUDFLAT MICROPHYTOBENTHOS TO BOTTOM-UP AND TOP-DOWN FACTORS IN THE BAY OF FUNDY

BY

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Abstract

Mudflats of the Bay of Fundy are habitats to a variety of organisms ranging from microbes to small invertebrates to mudsnails (*Tritia obsoleta*) and shorebirds. The main source of primary production in mudflats is microphytobenthos. Biotic and abiotic factors affect the abundance of microphytobenthos and ultimately the energy that most of the ecosystem relies on. The effect of bottom-up and top-down factors on mudflat ecosystems have been studied in the past. However, due to the complex overlapping trophic roles in the ecosystem, identifying factors that influence microphytobenthos abundance can be difficult. Previous studies have examined bottom-up and top-down factors, but have either investigated only top-down, or both bottom-up and top-down but without considering all predators known to be important. There is lack of research on the combined effect of known important bottom-up and top-down factors. We investigated the effect of nutrient availability and key predators on microphytobenthos abundance. A manipulative field experiment involving a combination of fertilizer addition and predator exclusion was performed in Grande Anse, in the Bay of Fundy in summer 2020. Fertilizer increased microphytobenthos abundance throughout the experiment, four weeks after application. A seasonal pattern in biofilm abundance was observed with a decrease in abundance form early July to late July and an increase from late July to late August. We found that mudsnails, which were present in low densities in this system, increased microphytobenthos abundance. We suggest bioturbation from mudsnails' grazing stimulated nutrient recycling and increased microphytobenthos production. Mudsnails were more abundant in fertilized sites, as well as where birds could forage as compared to where birds were excluded. We propose that the increased presence of mudsnails where birds could forage could be attributed to bird droppings and surface disturbances, stimulating microphytobenthos production. Apart from mudsnails, invertebrate communities did not affect microphytobenthos abundance, but there were individual group responses to fertilizer and predation. In late August, *Corophium volutator* were more abundant in unfertilized, snail- and bird-excluded sites, and bivalves were less abundant in bird-included sites, regardless of fertilizer treatment. In late July, nematode abundance was higher in fertilized sites than unfertilized. These findings broaden our understanding of ecological factors that affect microphytobenthos abundance, and of mudflat ecology as whole.

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Chapter 1: Introduction

Mudflat ecosystems

Mudflats are environments of high productivity that supports bottom-up and top-down ecological factors across all trophic levels (eg. Posey et al., 2002; Hamilton et al., 2006; Cheverie et al., 2014). They are created via the accumulation of fine-grain sediments that occur in low-energy intertidal areas with high tidal range (Dyer, 1998). The main primary producers on mudflats are microphytobenthos, which are unicellular photosynthetic microorganisms living at or near the sediment surface (Macintyre et al, 1996). Microphytobenthos make up most of what is known as biofilm, a cohesive layer found in the top 2 mm of mudflats and many other locations. They affect the structure of mudflats by stabilizing the sediment surface (Black et al., 2002). Biofilm and its microphytobenthos constituents are the base of mudflat food webs, which can support a diversity of primary consumers as a direct food source (Macintyre et al, 1996). Consumer biomass from grazing on biofilm is dominated by epibenthic and burrowing invertebrates in mudflats (Herman et al., 2000). However, secondary consumers use biofilm as an indirect food source but can also feed directly on it.

The diversity and overlapping trophic roles in mudflat ecosystems make it difficult to understand the bottom-up impacts and top-down controls of biofilm (Wollrab et al., 2012). The Bay of Fundy in New Brunswick, Canada, has mudflats that provide habitat for many organisms including hundreds of thousands migratory shorebirds every year (Hicklin, 1987). Yet the community complexity of such intertidal mudflats is lower than in other well studied mudflat systems, making it easier to investigate the overlap and interaction of trophic roles (Hamilton et al., 2006; Cheverie et al., 2014; Gerwing et al., 2015). Top-down and bottom-up factors typically co-exist (Hunter and Price, 1992; Pace et al., 1999), suggesting that in order to dissect representative ecosystem dynamics, their investigation should be simultaneous.

Biofilm

In addition to microphytobenthos, biofilm consists of detritus, heterotrophic bacteria and sediment (Yallop et al., 2000). Living components secrete biopolymers, forming an extracellular matrix that stabilizes the sediment (Underwood and Paterson, 2003) by increasing hydrodynamic stress resistance and providing protection against erosion of sediments (Black et al., 2002;

Joensuu et al., 2018). Biofilm has important effects on multiple other ecosystem functions, in addition to supporting energy transfers to subsequent trophic levels. Stabilization of sediment by biofilm facilitates invertebrate settlement on mudflats (Dunn et al., 1999). Further, biofilm decreases the re-suspension of particles, limits the release of nutrients from deeper sediments, and traps particles from the overlying water during high tides (O'Meara et al., 2017; Hope et al., 2020). The microphytobenthos within biofilms also help oxygenate surface sediments, which help stimulate denitrifying bacteria (Hope et al., 2020).

Environmental factors and biological impacts from consumers can affect the abundance and community composition of microphytobenthos in biofilm. Microphytobenthos includes unicellular eukaryotes such as diatoms, euglenides, chlorophytes, as well as cyanobacteria (Hope et al., 2020). Community structure and productivity varies with sediment type, nutrient concentrations, light and temperature (Sundbäck and Snoeijs, 1991; Watermann et al., 1999). In temperate regions, diatoms usually dominate the microphytobenthos, with some seasonal shifts to cyanobacteria and chlorophytes (Barranguet et al., 1998). Microphytobenthos biomass has been suggested to be controlled by re-suspension, grazing, light and nutrient availability (Underwood and Kromkamp, 1999; Cartaxana et al., 2016). These organisms have high turnover rates, growth rates, and respond rapidly to nutrient pulses (Yallop et al., 1994; Daggers et al., 2020). Nevertheless, nutrient addition to intertidal flats has shown varying effects on microphytobenthos biomass and community structure, suggesting that microphytobenthos are not always nutrient limited (Rossi, 2006; Tolhurst et al., 2020). Further, effects are sometimes dependent on other biotic factors. Eriksson et al (2017) found that microphytobenthos biomass increased by 90% after fertilization only in areas with high densities of burrowing bivalves. Invertebrates can impose strong grazing pressures on biofilm and cause physical disturbance of biofilm via bioturbation (Kristensen, 2000; Gerwing et al., 2016a). In turn, bioturbation can increase recycling of organic matter and nutrient availability (Connor et al., 1982; Lohrer et al., 2004).

Consumer communities in mudflats

In addition to affecting microphytobenthos, the diversity of consumers in mudflats also shapes physical conditions. Meiofauna consumers in mudflat ecosystems commonly include copepods, ostracods, and nematodes in both mudflats and estuaries (Gerwing et al., 2016a). These taxa are primarily present as infauna, though some are also epibenthic (Boxshall et al., 2016). Burrowing macrofauna commonly include both errant (eg. *Nereididae*, *Nephtyidae*) and sessile (eg. *Spionidae, Capiteillidae*) polychaete worms, bivalves, snails and amphipods (Boxshall et al., 2016; Gerwing et al., 2016a). Burrowing infauna like the amphipod *Corophium voluator*, found on both sides of the North Atlantic, can oxygenate the anaerobic layer and disrupt the stabilization of the sediment via bioturbation (Gerdol and Hughes, 1994). On the other hand, it is argued that bioturbation from burrowing infauna can also stimulate remineralization by bacteria and add mucus layers that enhance the cohesivity of intertidal sediments (Connor et al., 1982; Lohrer et al., 2004). Mudflat invertebrates interact with one another through competition and support secondary consumers as a food source. Juvenile *C.voluator* abundance can be reduced by feeding polychaetes and by intraspecific competition with adult *C.voluator* (Wilson, 1988, Wilson, 1989).

Top predators in mudflats (mudsnails and Semipalmated Sandpiper)

Invertebrates on Bay of Fundy mudflats serve as food sources for other macroinvertebrates such as predatory mudsnails, like *Tritia obsolete* (= *Ilyanassa obsoleta* and *Nassarius obsoletus*) (Coffin et al., 2012), and migratory shorebirds (Quinn and Hamilton 2012). In particular, invertebrates support migratory Semipalmated Sandpipers, *Calidris pusilla*, which uses the Bay of Fundy as a stopover habitat during migration (Hicklin, 1987). *Tritia obsolete*, who are obligate omnivores and *C. pusilla*, can both consume biofilm directly as an alternative food source (Kelaher et al., 2003; Kuwae et al., 2012), which increases the complexity of biotic interaction in these systems. Mudsnails can influence microphytobenthos biomass through both grazing and bioturbation (Coffin et al., 2012; Lohrer et al., 2004). Adult mudsnails are common predators on mudflats in Atlantic Canada, and can reach high densities up to 1000/m^2 (Drolet et al., 2009). High snail densities can reduce microphytobenthos abundance from over grazing (Cheverie et al., 2014) and from excess sediment disturbance (Connor et al., 1982). Microphytobenthos abundance can also be promoted by low snail densities where sediment stirring increases nutrient cycling (Connor et al., 1982). Mudsnails have a strong influence on abundance of other invertebrates on mudflats. Nematodes, *C.volutator*, and polychaetes abundances have been found to negatively correlate with mudsnails (Curtis, 2005; Hamilton et al., 2006; Kelaher et al., 2003).

Semipalmated Sandpipers are abundant visitors to intertidal mudflats in the Bay of Fundy each year in late summer (Hicklin, 1987). They use the region as a migratory stopover en route to their South American wintering habitat. Food quality at stopover locations is essential to support weight gain and successful migration (Warnock, 2010). Semipalmated sandpipers are opportunistic foragers which have recently been found to feed on biofilm directly, in addition to invertebrates like *C.volutator*, ostracods, and polychaete worms (Quinn and Hamilton 2012; Gerwing et al. 2016b). Like other Calidrid Sandpipers (Kuwae et al., 2012), Semipalmated Sandpipers have tongue spines that allow them to graze biofilm from the surface of the mud (D. Hamilton, pers. obs.). Biofilm can make up approximately 45% of Semipalmated Sandpiper diet during stopovers in the Bay of Fundy (Quinn and Hamilton, 2012), and up to 59% of the diets of related species in other locations (Kuwae et al. 2008).

Semipalmated Sandpipers tend to avoid feeding in areas with high mudsnail concentrations (Hamilton et al., 2003), possibly because of competition for shared food resources. Both species consume biofilm and invertebrates like *C.volutator*. Burrowing activities of mudsnails, coupled with direct predation activities, negatively affects *C.volutator*, by reducing their populations through predation and immigration (Coffin et al. 20012) and making them vertically migrate lower in the sediment (Coulthard and Hamilton 2011). By making *C.volutator* less accessible to Semipalmated Sandpipers, an indirect negative interaction between shorebirds and snails occurs.

Abiotic and biotic influences on biofilm

Abundance and taxonomic composition of trophic levels in mudflat ecosystems is influenced by bottom-up and top-down factors. In the context of biofilm, light, temperature, physical disturbance and nutrient availability represent bottom-up factors and influence the amount of biofilm that is present (eg. Thornton et al., 2002; Jesus et al., 2006). Such availability directly impacts the abundance and composition of primary consumers of microphytobenthos (Decho and Fleeger 1988). Conversely, fauna may have high grazing impacts on microphytobenthos abundance, representing the top-down factors. Although likely case-specific and abiotic factor dependent, there is evidence of macrofaunal grazing pressure on microphytobenthos being of similar magnitude to that of microphytobenthos production (Weerman et al. 2011; Savelli et al., 2018; Tolhurst et al., 2020). Top-down factors typically refers to the trophic effects of consumption by predators and grazers on lower trophic levels. However, resultant effects of predator activities, such as bioturbation by organisms like mudsnails, should also be considered. Bioturbation affects biofilm's physical structure and reduces abundance, but sediment disturbances can also promote remineralization of nutrients, and promote primary production (Connor et al., 1982; Lohrer et al., 2004). Nutrient remineralization rates can thereby act as a bottom-up effect on microphytobenthos biomass.

Selective feeding can change prey availability, adding another layer of complexity to mudflat ecosystem dynamics. For example, meiofauna have been shown to feed selectively with a preference for diatoms compared to other microphytobenthos taxa (Azosky et al., 2005; Majdi et al., 2012). It is suggested that size range of microphytobenthos community composition drives selective feeding by meiofaunal copepods (Azosky et al., 2005). Such selective feeding may cause changes in the composition of biofilm, leading to additional effects on communities (Müller-Navarra et al., 2000; Azosky et al., 2005). Further, although Semipalmated Sandpipers are largely opportunistic predators, there is some evidence that they may consume more biofilm in areas where it is of higher quality (Quinn et al. 2017). This suggests that they may exhibit some selective grazing, which means that they could have greater effects on biofilm under particular conditions.

Research objectives

Within Bay of Fundy mudflat communities, the effects of nutrient availability and predator presence on trophic interactions have been investigated in the past (Hamilton et al., 2006; Cheverie et al., 2014). Hamilton et al. (2006) manipulated top-down and bottom-up factors, but considered only shorebird predation in a top-down context. Cheverie et al. (2014) manipulated shorebirds and snails but considered only top-down factors. Thus, there is a lack of information on how microphytobenthos biomass is affected simultaneously by bottom-up and top-down factors, considering multiple predators known to be important on mudflats in the Bay of Fundy.

To investigate these knowledge gaps, we simultaneously manipulated nutrients and predation due to snails and birds on the Grande Anse mudflat in the upper Bay of Fundy, New Brunswick. We collected data on three occasion, with a time scale linked to shorebird presence to evaluate the influence of shorebirds on the ecosystem. We measured chlorophyll-*a* abundance (used as an estimate of microphytobenthos biomass), invertebrate biomass, and organic matter in the sediment, to examine how these changed in response to our manipulative experiments. We tested the hypothesis that microphytobenthos are nutrient limited, and that this limitation imposes a bottom-up effect on the community. We also tested the competing hypothesis that microphytobenthos biomass is more affected by consumers, and compared effects of birds and snails. We predicted that microphytobenthos is affected by both top-down and bottom-up factors, and that snails are an important influencer of microphytobenthos biomass.

Chapter 2.1: Experimental Set up and Field Methods

Study location

We conducted manipulative experiments in the intertidal mudflat of Grand Anse (45^{o}N, -64^{o}W), located in the upper Bay of Fundy, New Brunswick. The Grand Anse mudflat extends about 2 km from shore at low tide. The top aerobic sediment layer of the mudflat is about 10 cm deep, followed by the consolidated layer that is primarily anaerobic. Within the aerobic layer, most of the active biofilm is found in the upper 2 mm.

Experimental design

We arranged 20 experimental sites in two rows of 10, parallel to each other at approximately 300 and 350 m from shore. This arrangement ensured that all sites were exposed to air for approximately the same amount of time during each tide cycle (about 5 minute difference between the rows). Sites within a row were positioned 15-30 m apart. We randomly selected five sites in each row to fertilize, which generated a spilt plot design. Each site consisted of four predator treatments; two controls, a bird exclosure, and a bird and snail exclosure. The treatments were set up in a square formation about 3 m from each other, with the position of each treatment randomized within the site (Figure 2.1).

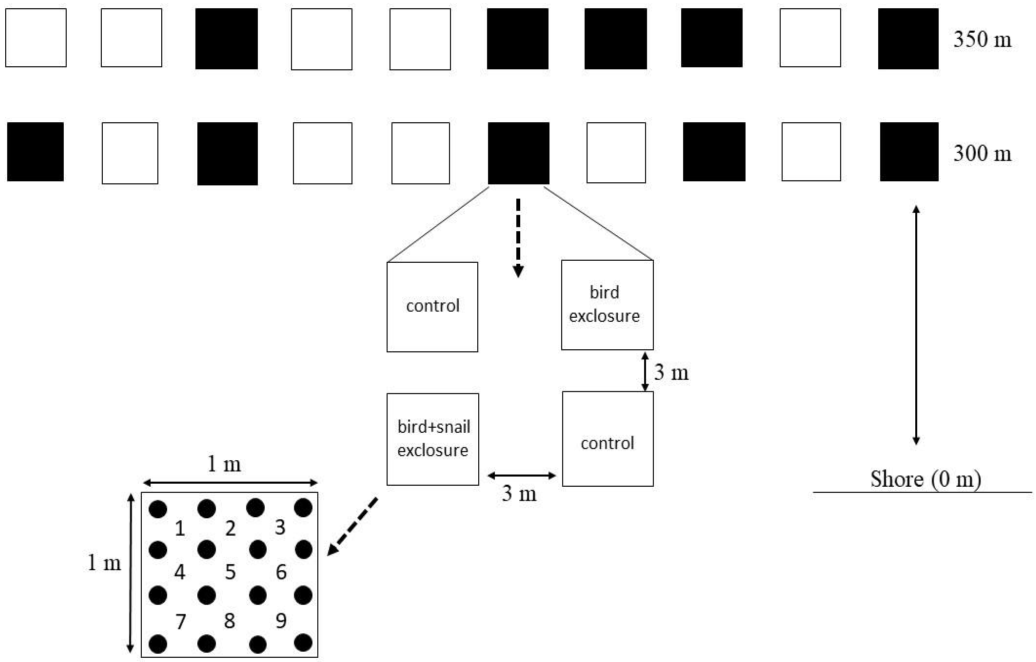


Figure 2.1 Experimental set-up (not drawn to scale). Dark squares indicate fertilized sites, and light squares indicate unfertilized sites (selection was randomized). Each site consisted of four predator treatment plots (two controls, a bird exclosure and a bird and snail exclosure). The positions of the predator treatment plots were chosen randomly at each site and sites were oriented parallel to the shore. In fertilized treatment plots, the dark circles represent the position of the fertilizer bags, resulting in the numbered areas as potential sampling areas, all equidistant from fertilizer bags. Position of the sampling areas were identical in unfertilized treatment plots.

Predator treatments

All treatment plots were 1 m x 1 m. We made the control plots by planting four bamboo stakes into the mud, which allowed organisms to move freely into and out of the plots. We made bird exclosures with bamboo frames and covered the top with clear plastic mesh with 6-mm openings. We positioned the exclosures approximately 15 cm above the sediment surface and placed the mesh only on top to allow mudsnails and other smaller invertebrates to forage, while excluding shorebirds. We attached the top mesh with re-usable cable ties, which allowed us to easily remove the top mesh when sampling. We constructed the bird and snail exclosures with bamboo frames and covered the top and sides of the frames with the same plastic mesh. These frames were also positioned in the mud until they extended 15 cm above the surface, and the mesh on the sides was buried 15-20 cm into the mud to prevent snails from entering the exclosure via burrowing. The top mesh was attached with re-usable cable ties to facilitate opening during sampling. The particular diameter of the openings prevented adult mudsnails from going through the mesh, but still allowed smaller invertebrates to leave and enter the exclosures. In the rare event that exclosures washed away, we replaced them the following day. Our exclosure design was identical to that used by Cheverie et al. (2014), and testing during that study indicated no substantial effect of caging material on light penetration or water flow.

Fertilizer treatment

We used the timed-release fertilizer (18% nitrogen, 6% phosphate and 12% potash) Miracle-Gro© Shake 'n Feed Flowering Trees and Shrubs to add nutrients to our fertilized sites. We placed 56 g of fertilizer in a loose mesh-like bag made from tubular gauze with a diameter of 2.2 cm and a length of 12-15 cm. We inserted 16 fertilizer bags just below the surface of the mud in each fertilized treatment plot, which corresponds to a total enrichment of 162g N/m^2 and 23.5g P/m^2 . We chose this amount of fertilizer because it is close to the median value for nitrogen enrichment (150g/m^2) determined in a review of 47 different experimental studies involving enrichment of soft sediment environments (Douglas et al., 2016). We placed the fertilizer bags with equidistant spacing within the 1 X 1 m area (figure 2.1), with the goal of delivering nutrients evenly across the area. The fertilizer bags were initially placed in the treatment plots the day after the plots were set up. In unfertilized treatment plots we disturbed the mud using a 5.5 cm diameter core in the same equidistant spaces that fertilizer bags were situated in fertilized sites. This was to control for biofilm disturbance that may have been generated by placement of fertilizer. We replaced all fertilizer bags after 4 weeks with new bags containing the same amount of fertilizer to ensure the same dose of fertilizer was available during each of our sampling periods. While we replaced the fertilized bags, no additional disturbance was done to the unfertilized sites.

Sample collection

We collected samples on three occasions, 3-4 July (hereafter termed early July), 30-31 July (late July), and 27-28 August 2020 (late August). The three sampling rounds were timed to coincide with shorebird activities on the mudflat. The first round was approximately one month before birds arrive, the second was around the time they became abundant, and the third was after the peak. During the three rounds, we collected samples from the same three treatments at each site; the bird exclosure, the bird and snail exclosure and one of the control plots, which we selected randomly. The same control plot at each site was used throughout the experiment. We collected samples of biofilm cores and invertebrate cores, and we recorded bird footprints and snail counts at each treatment plot. Each treatment plot had nine areas that we could sample from, based on the center point of the equidistant spacings of the fertilizer bags (Figure 2.1). For each sampling round, we randomly selected two of nine areas from each treatment plot, and we did not re-sample the same area in subsequent sampling rounds to ensure we did not collect previously disturbed biofilm. On the rare occasion that the sampling areas selected had too much water on the surface of the sediment, we randomly selected another area to sample out of the areas not sampled previously. The second control plot in each site was reserved for a separate study not reported here.

Field methods

For biofilm sampling, we placed a 5 cm diameter contact core on the sediment surface and froze the sediment in the contact core by pouring liquid nitrogen into the upper part of the core (Figure 2.2) two to three times. We used a spatula to remove the frozen 2 mm thick biofilm disk from the core and we stored the samples at -20^{o}C in the dark while on the mudflat. In the laboratory the samples were kept in a -80^{o}C freezer. The biofilm samples were crushed 1-2 days after collection and placed in glass scintillation vials. We subsequently freeze dried the samples for a minimum of 5 hours at -50^{o}C and 0.05mBar (FreeZone one-liter benchtop freeze drier, Labconco Corporation, Kansas City, MO). All work on the samples was done in a low light environment.

Figure 2.2. Photo of a biofilm contact core with liquid nitrogen being poured into the upper part of the core, designed to freeze the top 2mm of sediment enclosed in the lower part of the core.

Invertebrate samples were collected using a 5.5 cm diameter corer pushed into the sediment until resistance was felt, indicating that the core had reached the consolidated sediment layer. We placed our hand under the opening at the bottom of the core and pulled the core out, then transferred it to a labeled plastic bag. Within 24 h of collection we sieved the samples through a 250 μm mesh, and the invertebrate retained by the mesh were stored in labeled sample vials in 95% ethanol.

To quantify snail abundance and habitat use by shorebirds, we recorded snail counts and percent bird footprint cover twice weekly in all treatment plots. For each plot we counted the number of adult and juvenile snails present, and removed any adult snails found in the bird and snail exclosures. We did not remove juvenile snails as they were small enough to fit through the clear plastic mesh of the exclosures. Occasionally, when full exclosures had several snails, we tightened the edges of the mesh with re-usable cable ties. We estimated percent bird footprint cover by holding a 1.0 X 1.0 m quadrat (squares of 10 by 10) over the controls and we counted the number of squares that had bird footprints. This estimate provides an index of habitat use by birds (Robar and Hamilton, 2007). In the rare event that bird footprints were seen in bird exclosures, we recorded the number and added additional flagging tape to the corners of the exclosure to further prevent birds form entering.

On all days we collected samples, we used a squeegee to flatten our footsteps behind us after walking around all treatment plots, to ensure our footsteps did not form puddles later on.

Chapter 2.2: Lab Analysis

Invertebrate biomass

We sorted preserved invertebrates and classified them to the lowest taxon possible using a dissecting microscope. We sorted *C.volutator* into 2 mm size classes, and we sorted polychaete worms into 10 mm size classes. Other organisms were not size classed. We organized the sorted invertebrates by fertilizer treatment, predator treatment, size class, and taxon. We dried them for a minimum of 24 h in a 90^{o}C oven (40GC Gravity Convection Oven, Quincy Lab Inc., Chicago, IL) and weighed them. The shelled invertebrates from the phyla *Mollusca* and *Crustacea* were also later ashed to allow us to estimate consumable biomass. These invertebrates were place in crucibles in a muffle furnace (Isotemp Programmable Muffle Furnace 650-750 Series, Fischer-Scientific, Waltman, MA) at 550^{o}C for 5 hours. We re-weighed the shelled invertebrates post-ashing and calculated the mass difference between pre and post-ashing to estimate the consumable biomass of these invertebrates. In order to prevent invertebrates from re-hydrating during the processes, we placed the weight boats/crucibles in a desiccator when they were not in the oven or handled. Invertebrates that were too light for the balance to weigh were estimated to have a minimum mass of 0.00001g, the smallest possible mass to be picked up by the balance used. Meiofauna that were always too light to be weighed in small numbers (copepods, nematodes, ostracods) were pooled, weighted in large groups, and the mass for a single individual was estimated. We multiplied the number of individuals with the mass of the corresponding single prey item to estimate the mass for these invertebrates in each sample.

Chlorophyll-a

To estimate biofilm standing crop we measured chlorophyll-*a* (Chl-*a*) content in all freeze dried biofilm core samples. Chlorophyll-*a* was extracted from ~10-20mg of homogenized, freeze-dried sediment in a 20mL glass scintillation vial with 5mL of extraction solvent, which was a 3:2 mixture of 90% acetone: DMSO (Shoaf and Lium 1976). Extracts were inverted several times to mix and then placed in the dark at room temperature for 30 min, mixed again, and then placed back in the dark for 30 min. Sample Chl-*a* content was then quantified by measuring Chl-*a* fluorescence according to Welschmeyer (1994) using a Trilogy fluorometer (Turner Designs, San Jose, CA) with purified Chl-*a* (Sigma) used as a reference standard. If the fluorometer readings were above calibration limits, samples were diluted with extraction solvent and remeasured. We calculated Chl-*a* abundance from the measured Chl-*a* concentration (µg/L) using the following equation:

$$\frac{\text{Chl } a \text{ (µg)}}{\text{Mass of sediment (g)}} = \frac{\text{Chl a (µg/L)} * \text{Extraction volume (L)}}{\text{Mass of sediment (g)}}$$

Chapter 2.3: Statistical Analyses

Overview

R (version 4.0.2) with R Studio interface (RStudio Team 2018) was used to perform analyses. We excluded 4 sites from our statistical analyses, one fertilized and one unfertilized site per row. We chose to exclude these sites because they were frequently too flooded to sample, had predator exclosures wash away occasionally, or had fertilizer rise to the surface of the sediment. Prior to analyses Shapiro-Wilk normality test, Levene's test of homogeneity of variances, Box's M test for multivariate homogeneity of covariances, and Huyn-Feldt test for sphericity were used to assess parametric assumptions as appropriate for the tests being conducted. We transformed data that did not meet assumptions, and used non-parametric tests if transformed data failed to meet assumptions. Data were plotted using 'ggplot2' package (Wickham, 2016) and 'sciplot' package (Morales, 2020). We tested significance of main effects at α=0.05, and evaluated interactions at α=0.1 to minimize the risk of erroneously interpreting main effects in the presence of an interaction (as in Hamilton et al., 2006).

Chlorophyll-a

We used a multivariate repeated measure analysis of chlorophyll-*a* abundance (R package car, Fox and Weisberg, 2019). Each replicated plot per sites corresponded to a subject in the analysis. Fixed within-subjects factors were sampling round and predator treatments, fertilizer treatment was a fixed between-subjects factor, and site was a random factor nested under fertilizer treatment. We split the analysis by time following a significant interaction between time and predator treatment. Pairwise comparisons were used to identify differences within predator treatments.

Invertebrate biomass

We pooled invertebrates into the following taxonomic groups: *C.volutator*, errant polychaete, sessile polychaete, ostracod, nematode, bivalve, copepod, gastropod. Some taxa were rarely found in samples which resulted us in only including *C.volutator*, errant polychates, ostracods, nematodes and bivalves in analyses. We tested for correlations between Chlorophyll-*a* abundance and grouped invertebrate biomass through separate times and treatments but found no relationships, so Chl-*a* was not included as a covariate in models. Data failed parametric assumptions, so we conducted a nonparametric multivariate repeated measures analysis using the MultRM function in R package MANOVA.RM (Friedrich et al., 2021, permutation = 1000). Biomasses of the different invertebrate taxa were dependent variables and predictors, and the design were as described above for the Chl-*a* analysis. The data set was split by time following an interaction, and reduced models were run. To assess individual taxon responses to our manipulations, non-parametric univariate analyses on the effects of predator and fertilizer treatment within sampling periods where significant multivariate effects were detected were conducted using the function 'f1.ld.f1' from the 'nparLD' package (Noguchi et al., 2012).

Snail abundance

Adult snail counts from the end of July to the end of August were compared among treatments using a generalized linear mixed effects model (R package lme4, Bates et al,. 2015) with a Poisson distribution to account for count data.

Chapter 3: Results

Reliability of predator treatments

Bird exclosures effectively excluded Semipalmated Sandpipers (Figure 3.1). When footprints were detected in exclosures, they were usually around the edges in areas where we were not sampling. Full exclosures were successful in preventing snails from entering, and the few that did enter were removed at least twice weekly during regular visits to the sites.

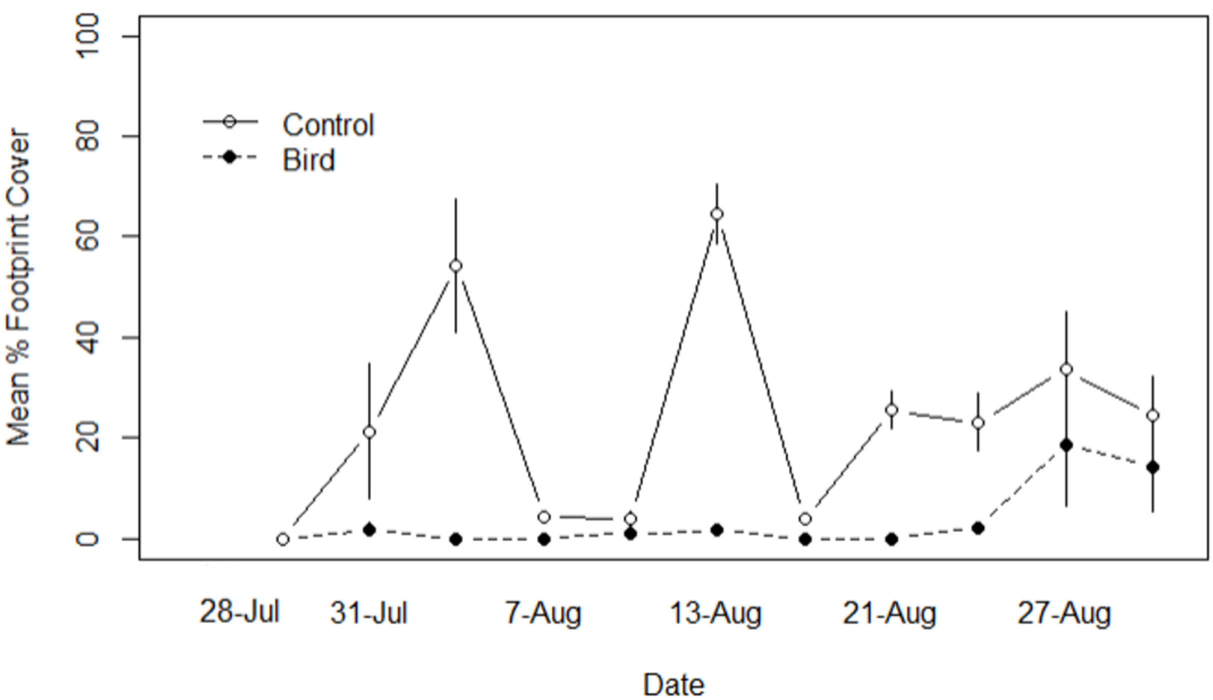


Figure 3.1. Mean percent footprint cover estimated in control and bird exclosures. Error bars show ± 1 standard errors.

Effect of fertilizer and predator treatments on chlorophyll-a abundance

The temporal pattern in chlorophyll-*a* abundance varied with predator treatment (Time x Predator treatment interaction, $F_{4,84} = 5.24$, p=0.0008). During late July and late August, chl-*a* abundance was higher in fertilized sites compared to unfertilized sites (Figure 3.3; Table 3.1). Although not compared statistically because of significant interactions, abundance of chl-*a* declined from early to late July, then increased again by late August (Figure 3.3). Chl-*a* abundance varied between predator treatments in late August (Figure 3.2; Table 3.1). Abundance of chl-*a* was lower in full exclosures than bird exclosures and controls, with no clear difference between bird exclosures and controls (Table 3.2).

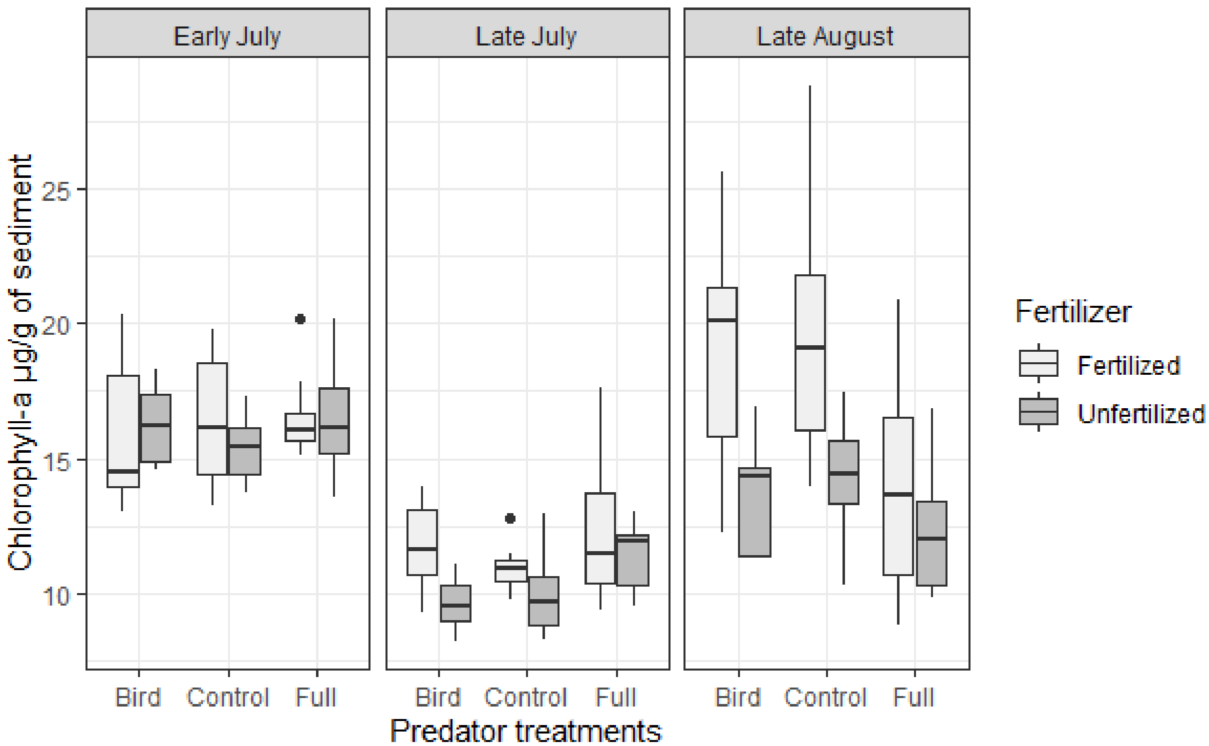


Figure 3.3 Chlorophyll-*a* abundance (µg/g) during early July, late July, and late August sampling rounds in fertilized and unfertilized sites. “Bird” indicates bird exclosures, “Full” indicates full exclosures (restricting snails and birds), and “Control” indicates open plots. Dark grey boxes are unfertilized treatments and light grey boxes are fertilized treatments. Boxes represent 1st and 3rd quartiles around the median line at box centers, and whiskers represent interquartile range. Points represent statistical outliers. Results of statistical tests are provided in Table 3.1 and Table 3.2.

Table 3.1 Results repeated measures analysis on the effect of predator and fertilizer treatments separated by sampling round, following a significant interaction between time and treatment. Significant p-values are indicated with an asterisk (*).

Sampling Round	Factor	DF (Numerator, Denominator)	F-value	p-value
Early July	Fertilizer	1,14	0.12	0.72
	Predator	2,13	0.52	0.61
	Fertilizer x Predator	2,13	0.43	0.65
Late July	Fertilizer	1,14	5.7	0.032*
	Predator	2,13	3.01	0.084
	Fertilizer x Predator	2,13	0.98	0.40
Late August	Fertilizer	1,14	8.67	0.012*
	Predator	2,13	7.07	0.0084*
	Fertilizer x Predator	2,13	1.42	0.28

Table 3.2 Results from pairwise comparisons of chlorophyll-*a* abundance between predator treatments in late August. To control the experiment-wise error rate for multiple comparisons, the critical alpha value was set to 0.017. Statistical significant p-values are denoted with an asterik (*).

Pairwise comparisons	DF (Numerator, Denominator)	F-value	p-value
Bird exclosure – Full exclosure	1,14	12.08	0.0037*
Full exclosure – Control	1,14	11.7	0.0041*
Bird exclosure – Control	1,14	0.4	0.56

Effect of fertilizer on snails

Snails became abundant in our study area at the end of July. The effect of fertilizer on snail abundance varied with treatments (Fertilizer treatment x Predator treatment interaction, X^2= 12.37, p= 0.0021) (Figure 3.2). We found that fertilized sites had higher snail abundance than unfertilized sites in bird exclosures and controls, but not in full exclosures (Figure 3.2, Table 3.3).

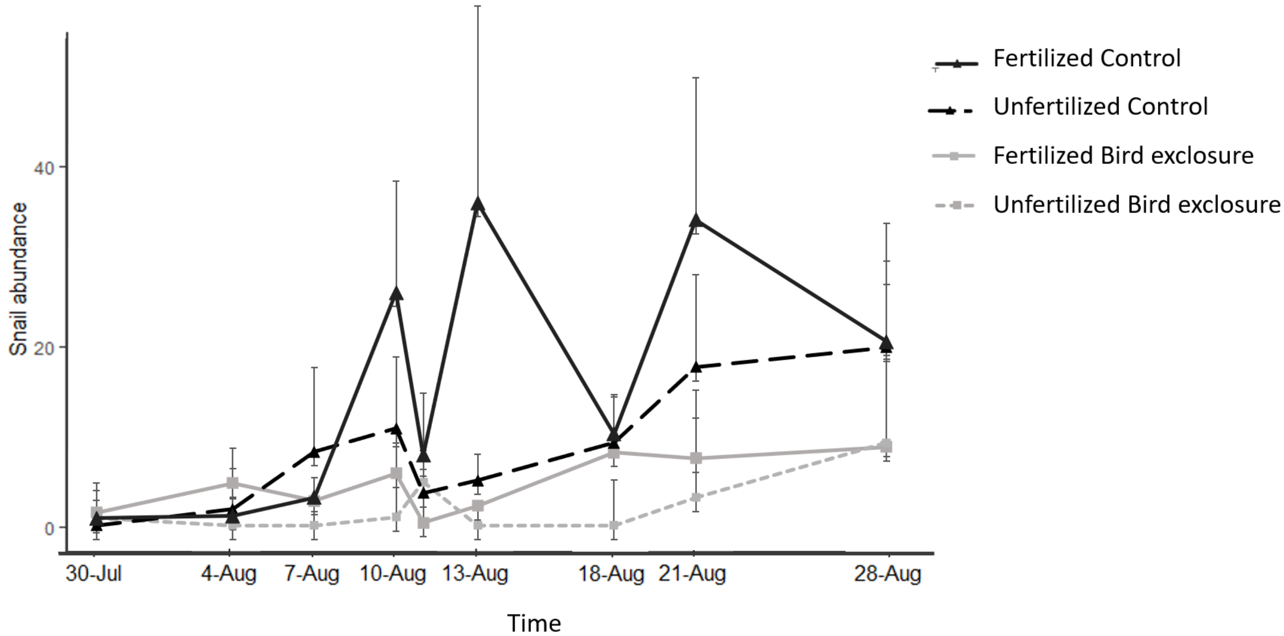


Figure 3.2 Mean of adult snail abundance/m^2, per predator and fertilizer treatments from late July to late August. Controls are denoted by the black lines, bird exclosures are denoted by the grey lines. Fertilized treatments are marked by the solid lines, and unfertilized by the dashed lines. Error bars represent Standard Deviation. Statistically significant differences between treatments are indicated in Table 3.3.

Table 3.3 Results from a generalized linear mixed effects model testing for the effect of fertilizer on snail abundance separately for each predator treatment, from late July to late August. Statistically significant p-values are marked by an asterisk (*).

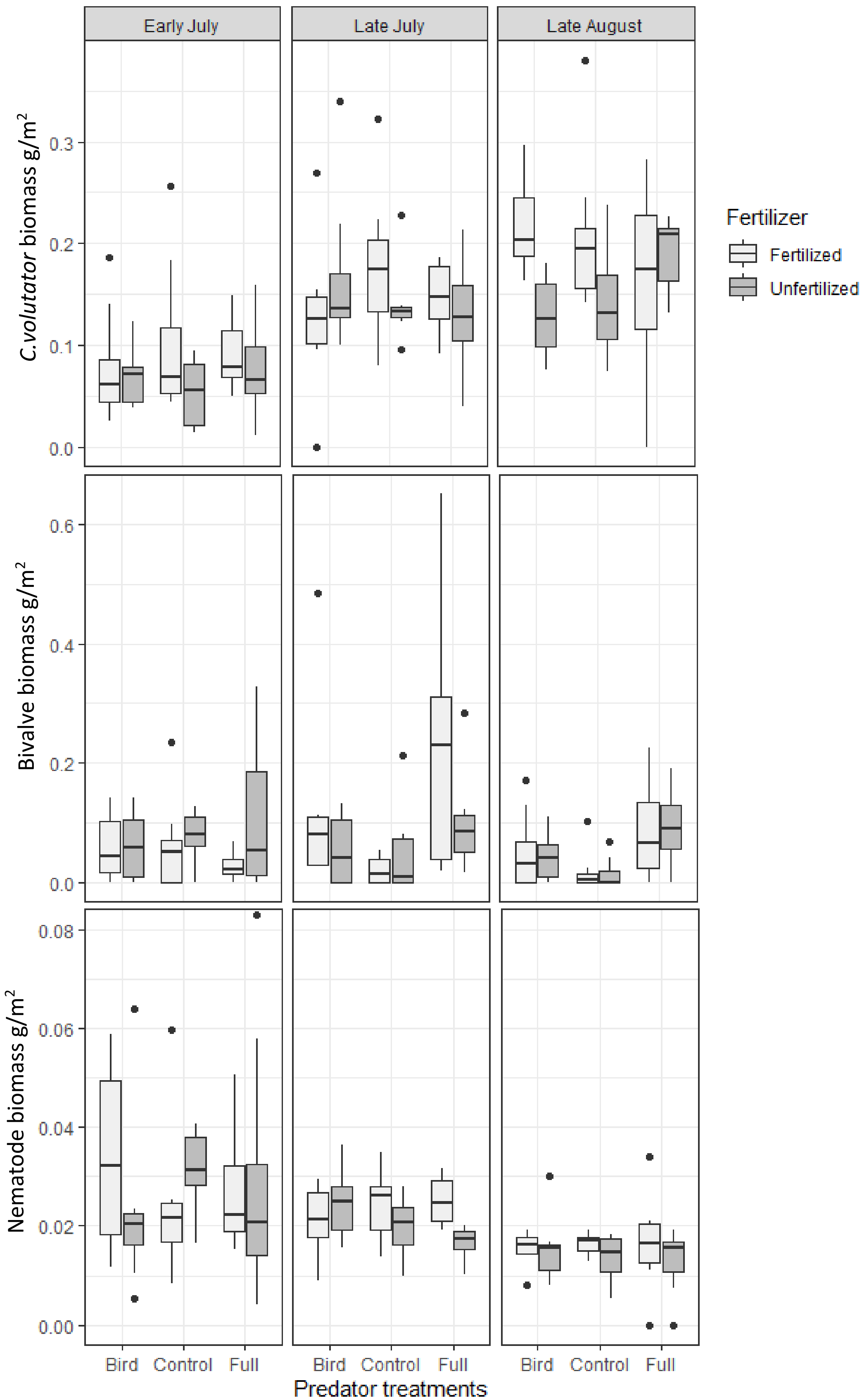
Predator treatment	DF	Chisq -value	p-value
Bird exclosure	1	9.43	0.0025*
Full exclosure	1	1.18	0.28
Control	1	20.93	<0.0001*

Effect of predator and fertilizer treatments on invertebrates

For multivariate analysis, only taxa frequently found in samples were used, which includes *C.volutator*, errant polychaetes, ostracods, nematodes and bivalves. The effect of time on multivariate community biomass varied with fertilizer (Time x Fertilizer treatment interaction, F_{12} =62.91, p= 0.081). In late July, invertebrate community biomass differed among fertilizer levels, and in late August invertebrate community biomass varied among predator treatments (Table 3.4). In an effort to determine which invertebrate taxa were contributing to the multivariate result, we conducted separate non-parametric analyses for all common groups. This analysis was conducted for late July and late August as the multivariate result showed no predator or fertilizer treatment effect in early July (Table 3.4). The effect of fertilizer and predator treatments on overall invertebrate community structure reflect the responses of the abundant taxa *C.volutator*, bivalves and nematodes. Nematode biomass was higher in fertilized full exclosures than unfertilized ones in late July (Figure 3.4; Table 3.6, Table 3.7). Biomass of *C.volutator* varied with a combination of fertilizer and treatment (Fertilizer treatment x Predator treatment interaction, F_2=9.35, p=0.0093). In late August in unfertilized sites, *C.volutator* biomass was higher in full exclosures than in bird exclosures (Figure 3.4, Table 3.6, Table 3.7). In contrast, predator treatments had no apparent effect on *C.volutator* abundance in fertilized sites in late August (Table 3.7). Of the remaining taxa examined, only bivalves responded to any of our experimental manipulations. Bivalve biomass was higher in full exclosures compared to controls in late August (Figure 3.4, Table 3.6, Table 3.7).

Table 3.4 Results from a non-parametric MANOVA for the effect of predator treatments and fertilizer levels on the biomass of common invertebrate taxa separated by sampling round, following a significant interaction between time and fertilizer treatment. Statistically significant p-values are denoted by asterisks (*).

Sampling Round	Factor	DF (Numerator)	Wald-Type Statistic	Parametric bootstrapped p-value
Early July	Fertilizer	6	2.697	0.84
	Predator	12	11.233	0.82
	Fertilizer x Predator	12	17.812	0.59
Late July	Fertilizer	6	22.793	0.045*
	Predator	12	34.734	0.201
	Fertilizer x Predator	12	38.864	0.14
Late August	Fertilizer	6	14.741	0.09
	Predator	12	82.15	0.029*
	Fertilizer x Predator	12	15.767	0.50



Early July
Late July
Late August
C.volutator biomass g/m²
Bivalve biomass g/m²
Nematode biomass g/m²
0.0
0.1
0.2
0.3
0.0
0.2
0.4
0.6
0.00
0.02
0.04
0.06
0.08
Bird
Control
Full
Predator treatments
Fertilizer
Fertilized
Unfertilized

Figure 3.4 Boxplots of *C.voluator*, bivalve and nematode biomass in g/m^2, per predator treatments and fertilizer treatment, across all sampling rounds. For interpretation of the boxplot refer to Figure 3.2. One data point for *C.volutator* included in the statistical analyses were excluded from the graph to improve graph visibility. Statistical comparisons are provided in Table 3.5, 3.6 and 3.7.

Table 3.5 Results of Non-parametric univariate analyses of the effect of predator treatments and fertilizer levels on the biomass of abundant invertebrate communities late July. Statistically significant p-values are denoted with asterisks (*).

Invertebrate	Factor	DF (Numerator)	Wald-Type Statistic	Parametric bootstrapped p-value
C.volutator	Predator	2	1.13	0.42
	Fertilizer	1	0.64	0.56
	Predator x Fertilizer	2	2.04	0.35
Bivalve	Predator	2	18.25	<0.0001*
	Fertilizer	1	0.98	0.32
	Predator x Fertilizer	2	2.0021	0.37
Nematode	Predator	2	0.93	0.065
	Fertilizer	1	3.39	0.20
	Predator x Fertilizer	2	8.21	0.016*
Errant Polychaetes	Predator	2	1.48	0.086
	Fertilizer	1	2.95	0.47
	Predator x Fertilizer	2	0.29	0.86
Ostracod	Predator	2	0.072	0.96
	Fertilizer	1	0.82	0.36
	Predator x Fertilizer	2	2.26	0.32

Table 3.6 Results of non-parametric univariate analyses of the effect of predator treatments and fertilizer levels on the biomass of abundant invertebrates in late August. Statistically significant p-values are denoted with asterisks (*).

Invertebrate	Factor	DF (Numerator)	Wald-Type Statistic	Parametric bootstrapped p-value
C.volutator	Predator	2	1.22	0.54
	Fertilizer	1	5.84	0.015*
	Predator x Fertilizer	2	9.34	0.0093*
Bivalve	Predator	2	12.85	0.0016*
	Fertilizer	1	0.022	0.88
	Predator x Fertilizer	2	0.37	0.82
Nematode	Predator	2	0.04691	0.97
	Fertilizer	1	2.2470	0.13
	Predator x Fertilizer	2	0.3072	0.86
Errant	Predator	2	4.094	0.13
Polychaetes	Fertilizer	1	0.2424	0.62
	Predator x Fertilizer	2	1.5771	0.45
Ostracod	Predator	2	2.8599	0.24
	Fertilizer	1	3.0348	0.081
	Predator x Fertilizer	2	1.8648	0.39

Table 3.7 Results from pairwise comparisons for *C.volutator*, bivalve and nematode biomass based on results from the non-parametric univariate analysis. To control the experiment-wise error rate for multiple comparisons, the critical alpha value was set to 0.017. Statistically significant p-values are denoted with asterisks (*).

Invertebrate and Sampling round	Factor used to split (if applicable)	Factor	DF (Numerator)	Wilcox Test Statistic	p-value
C.volutator Late August	Unfertilized	Bird exclosure and Control	8	16	0.84
		Bird exclosure and Full exclosure	8	1	0.016*
		Control and Full exclosure	8	3	0.039
	Fertilized	Bird exclosure and Control	8	23	0.5
		Bird exclosure and Full exclosure	8	21	0.72
		Control and Full exclosure	8	20	0.84
Bivalve Late August	-	Bird exclosure and Control	16	178.5	0.053
		Bird exclosure and Full exclosure	16	83.0	0.092
		Control and Full exclosure	16	45.0	0.002*
Nematode Late July	-	Fertilizer effect in Bird exclosure	8	11	0.38
		Fertilizer effect in Control	8	28	0.20
		Fertilizer effect in Full exclosure	8	35	0.021

Chapter 3: Discussion

Microphytobenthos is the main source of primary production in mudflats and thus is the ecological foundation of mudflat communities. Effects of various top-down and bottom-up influences on mudflats have been studied in multiple locations, including Europe, New Zealand and Australia (Feuillet-Girard et al., 1997; Murphy et al., 2009; Saint-Beat et al., 2013; Harris et al., 2016; Booty et al., 2020). Similar work and findings on mudflat ecosystem dynamics have been studied in the Bay of Fundy (Hamilton et al, 2006; Cheverie et al, 2014). Many of these studies have not simultaneously manipulated all relevant bottom up and top-down factors that may affect microphytobenthos abundance. To fill this gap in knowledge we manipulated these components simultaneously at Grande Anse, in the Bay of Fundy, Canada, to better understand their effects on microphytobenthos abundance.

Effect of fertilizer and temporal variability

In our study, microphytobenthos biomass increased with nutrient addition, indicating at least some level of nutrient limitation. This corresponds well with other work that has observed high microphytobenthos growth rates and increased biomass in response to nutrient pulses (Yallop et al., 1994; Daggers et al., 2020). Hamilton et al. (2006) found similar results, reporting that fertilizer increased Chl-*a* concentration at a different mudflat in the Bay of Fundy. In addition to responding to nutrient additions, Underwood and Kromkamp (1999) have also described microphytobenthos biomass being controlled by other abiotic factors like light availability and, similarly, substrate resuspension.

We observed a clear seasonal trend in microphytobenthos abundance through the three sampling events. We found that microphytobenthos abundance declined from early to late July, but then increased again by late August. The fertilizer effect persisted despite the mid-summer decline. This drop in biomass is unlikely to be due to high grazing pressures because snail counts were low and juvenile snails mostly dominated the mudflat in July. Both adult snails and Semipalmated Sandpipers were primarily present in August. Similarly, we found no correlation between invertebrate biomass and microphytobenthos abundance across plots, indicating a lack of strong grazing pressure from invertebrate infauna. It is more likely that the temporal trends we observed are due to the limitation of microphytobenthos by abiotic factors such as high temperature and irradiance. Declines in microphytobenthos growth and productivity with the onset of summer have been observed in several other systems and were linked to higher temperatures and greater light intensity (Orvain et al. 2014; Moerdijk-Poortvliet et al. 2018; Savelli et al. 2018). The subsequent rebound of microphytobenthos after this summer decline has also been observed in these studies (Savelli et al. 2018) and in prior work at Grand Anse (Cheverie et al. 2014). These summer declines correspond to known physiological limits for mudflat diatoms (Juneau et al. 2015; Laviale et al. 2015) and suggest a strong seasonal abiotic effect on primary production in mudflat ecosystems (Thornton et al., 2002; Jesus et al., 2006).

Chlorophyll-*a* abundance was consistently more variable in fertilized sites in late August than in previous months, and it was more variable at that time than in unfertilized sites (Figure 3.2). A few mesh bags of fertilizer went missing around a week prior to the August sampling event, which may explain the large variability only in fertilized sites. The variability is also found in full exclosures, where mesh bags would have been displaced at most and not removed from the area. Nevertheless, displacements may have caused uneven distribution of fertilizer and thereby uneven microphytobenthos growth. It is possible that the combination of fertilizer and predators could be producing these variable responses depending on local conditions.

We noted anecdotally a change in sediment density, and presence of hermit crabs over the last month of sampling. Grande Anse sediment and invertebrate composition has historically had high interannual variability (Desplanque and Mossman 2004; MacDonald et al. 2012; Gerwing et al., 2015), but the change to much firmer sediment within a season that we observed is not typical. Yeo and Risk (1979) found that strong temporal changes in mudflat sediment condition can happen after storm events, but there was no large-scale storm conditions during our study. After observing this phenomenon, we collected sediment penetrability measurements (for methods see Gerwing et al., 2016a) within our experimental area and away from it to see if our frequent stepping around the sites led to the mud density change. We found that the change in sediment density was not due to our activities around the sites, as sediment in undisturbed places similarly changed within season. Changes in depth of the aerobic layer can have important consequences on mudflat organisms, such as altering invertebrate activities, including vertical migration within the sediment and bioturbation (Sturdivant et al., 2012). The consequence of the change in sediment property is unclear, and this coupled with some uneven fertilizer distribution during the last month of sampling, could contribute to the variability of microphytobenthos abundance we see in fertilized sites.

Response of the invertebrate community to experimental manipulations

The biomass of abundant invertebrates was uncorrelated with microphytobenthos abundance. We detected a fertilizer effect on the invertebrate community in late July, however this effect did not persist into late August. Hamilton et al. (2006) found similar results where fertilizer addition had short-lived effects on the invertebrate community. In late August, there was significantly lower microphytobenthos biomass in full exclosures, where only the invertebrate community could feed, however this did not translate to higher invertebrate biomass. This may suggest that the invertebrate community is not food-limited in addition to providing limited grazing pressure.

Although the invertebrate community did not respond to increased microphytobenthos biomass in a sustained fashion, some individual invertebrate taxa responded to bottom-up and top-down manipulations at specific points in the experiment. In late August in unfertilized plots, *C.volutator* biomass was generally higher where mudsnails and Semipalmated Sandpipers were excluded. The difference between control plots and full exclosures only approached significance after alpha level corrections were applied, but bird exclosures, where snails but not birds could feed, had less *C.volutator* than full exclosures. This suggests that mudsnails had the stronger effect of the two predators. This is surprising as shorebirds are known to feed heavily on *C.volutator* (Hamilton et al. 2003; Quinn and Hamilton 2012). Hamilton et al. (2006) found reduced *C.volutator* abundance where Semipalmated Sandpipers could feed. Our range of *C.volutator* biomass across our three sample rounds were comparable to the *C.volutator* biomass found in Hamilton et al. (2006) only after shorebird predation. Hence it is possible that our system had low *C.volutator* density, causing shorebirds to use an alternative food source (Macdonald et al., 2012). Mudsnails and *C.volutator* exhibit a density-driven negative relationship, mostly from interference (Cheverie et al., 2014). Therefore, it is likely that in our experiment effects of mudsnails caused the reduction in *C.volutator* more than Semipalmated Sandpiper predation. Additionally, we found that *C.volutator* biomass was higher in full exclosures that had lower microphytobenthos biomass. The high abundance of *C.volutator* could make their grazing pressure on microphytobenthos high enough to reduce biofilm. *C.volutator* have been shown to reduce the abundance of diatoms in European mudflats (Smith et al., 1996; Hagerthey et al., 2002), although there is evidence of the contrary in the Bay of Fundy (Hamilton et al., 2006) and in Europe (Mouritsen et al., 1998). These conflicting findings make it unclear whether *C.volutator* could have reduced microphytobenthos biomass when their abundance was not controlled by predation and snail activity.

In fertilized plots where microphytobenthos biomass was higher, we found no difference in *C.volutator* biomass with and without the presence of predators. The increased availability of microphytobenthos appeared to have prevented the negative effect of predators on *C.volutator* noted in unfertilized plots. *C.volutator,* mudsnails, and Semipalmated Sandpipers can all feed directly on biofilm (Hamilton et al., 2006; Cheverie et al., 2014; Quinn and Hamilton, 2012). When fertilizer increased microphytobenthos biomass, the negative effects of snails on *C.volutator* may have been mitigated through reduced competition for primary production. Although we did not statistically compare the biomass of *C.volutator* across fertilizer levels because it varied with predator treatment, *C.volutator* biomass appeared to be higher in fertilized sites in late August, where higher microphytobenthos biomass was also present. Thus, *C.volutator* could have been food limited at that time, and the two species, mudsnails and *C.volutator*, may be involved in exploitation competition. Findings from Hamilton et al. (2006) suggest that interference competition exists between mudsnails and *C.volutator*, as mudsnails benefited more from fertilizer application than did *C.volutator*. This was not found in our study, as *C.volutator* abundance did not change in fertilized site whether snails were present or not. Findings from Coffin et al. (2012) suggested exploitative competition between mudsnails and *C.volutator* was less likely than interference competition but could not be ruled out as a possible mechanism underlying their results. Our findings suggest that exploitative competition likely drove the mudnsnail and *C.volutator* relationship observed in our study.

We found that bivalve biomasses were lower where Semipalmated Sandpipers and mudsnails could feed compared to where they could not feed in late August. This pattern was also observed in late July, although not statistically analyzed because there was no overall effect of predator treatment on the invertebrate community at that time. Shorebirds do rely on bivalves as a common prey item (Smith et al. 2012; MacKellar 2018). In this case, results suggest that it is shorebirds, rather than mudsnails, that are likely driving the decrease in bivalve biomass via predation. Bivalve biomass was greatest in full exclosures, where microphytobenthos biomass was lowest compared to the other predator treatments in late August. This is different from what Eriksson et al. (2017) found, where areas of high densities of burrowing bivalves increased microphytobenthos biomass. Eriksson et al. (2017) described densities of bivalves of around 2000 per m2, which is well above the densities observed in this study. Our low bivalve density likely explain why such an effect on microphytobenthos abundance was not observed.

When predators were excluded, nematode biomass increased with microphytobenthos biomass only in fertilized sites in late July. Montagna et al. (1995) found that nematodes increased their grazing rates when microphytobenthos productivity was higher. During the end of July, productivity from microphytobenthos was generally lower than during other time periods. The increase in nematode abundance at that time where biofilm was least available suggests that nematodes may have been food limited in such concentrations. We did not observe an increase in nematode biomass in late August, but microphytobenthos abundance was generally higher then, suggesting that nematodes may not have been food limited at such concentrations.

We found that ostracods and errant polychaete worms did not vary with differing microphytobenthos biomass, perhaps suggesting that they were not food limited. We also found that these taxa were not affected by shorebird or snail exclusion. Lack of ostracod response in the system is unsurprising because our ostracod densities were not as high as densities that have been suggested to cause opportunistic feeding by Semipalmated Sandpipers (Macdonald et al., 2012). There was a surprising lack of predatory effect on errant polychaete worms in our study. Numerous studies have demonstrated errant polychaete worms such as Nereididae and Phyllodocidae are preyed on by shorebirds (Commito, 1982; Quinn and Hamilton, 2012; Gerwing et al. 2016b; Bellefontaine, 2020). There is evidence that polychaetes are more important prey for shorebirds when in high densities (Quinn and Hamilton, 2012), and apart from Neredididae, other polychaetes were in low abundance during our study. Hence, they may not have been an attractive prey for birds in this system, leading to a lack of predation effect. Curtis and Hurd (1981) found negative effects of mudsnails on polychaetes. This is consistent with recent studies like Cheverie et al. (2014), which found that Nereididae and especially Phyllodocidae varied with high and low snail densities. Our results do not match these findings. However, we found very few Phyllodocidae in our samples. Negative relationships exist between polychaetes and *C.volutator* (Wilson, 1988; Coffin et al., 2008; Coulthard and Hamlton 2011), but these cannot be assessed in this study because of the lack of response by polychaetes as a whole to treatments.

Relationship of mudsnails and shorebirds with microphytobenthos

In late August, we found that microphytobenthos biomass was higher in treatments where snails could enter. There was no difference between bird exclosures and controls, suggesting that mudsnails, not birds, were responsible for this result. Mudsnails can affect microphytobenthos biomass by grazing and disrupting biofilm via bioturbation. Oxygenation of the sediment via bioturbation can increase nutrient cycling and stimulate microphytobenthos production (Connor et al., 1982). Cheverie et al. (2014) found that primary production was maintained at low snail density but reduced at high snail densities where grazing pressure outweighed microbial stimulation. Mudsnail abundance in our treatments was similar to the low-density treatment in Cheverie et al. (2014). If this bioturbation effect is important, absence of mudsnails in full exclosures may explain the lower microphytobenthos biomass found there. This suggests that bioturbation by low snail densities may boost microphytobenthos production more than invertebrate bioturbation alone, which can be expected in the case of biodiffusive sediment movement by a larger epibenthic invertebrate at low to moderate densities (Lohrer et al. 2004). This increase in microphytobenthos abundance in areas where snails could feed was not observed earlier in the experiment, despite the fact that the late July sampling period followed a month of predator exclusion. However, adult snails were uncommon at the study site until late July, so it is not surprising that a response was not detected before August.

The increase in mudsnails observed in fertilized sites is consistent with previous work. Hamilton et al. (2006) found that snails were more abundant in areas where fertilizer was added. Snails are mobile and capable of moving to areas with elevated primary production (Kelaher et al., 2003; Hamilton et al., 2006). Their ability to be attracted to benthic algae and accumulate could be attributed to their intense olfactory sense and their ability to follow other snail's mucus paths on the mud (Bretz and Dimock, 1983; Coffin et al., 2008). Higher densities of mudsnails in areas of higher microphytobenthos biomass may suggest they are food limited.

Snail abundance was higher in control plots than in bird exclosures. Hamilton et al. (2006) found similar distributions of snails across bird exclosures and control treatments. We considered whether the mesh cover on our bird exclosures may have generated this effect, but ruled it out because Hamilton et al. (2006) used a different exclosure style with no top cover, so we conclude that snails are differentially attracted to areas where birds can feed relative to where they are excluded. It is possible that activities of Semipalmated Sandpipers may have generated this result. The act of walking across the sediment may stimulate biofilm production. Similarly, droppings could be adding dissolved materials and nutrient inputs into the sediment (Schrama et al., 2013), or could introduce bacteria that may contribute to additional nutrient recycling and changes in biogeochemistry (Müller, 1965; Hope et al., 2020). However, there was no difference in microphytobenthos abundance between controls and bird exclosures. For this, we propose that added simulation from droppings and sediment disturbance by shorebirds contributed to an increase resource availability for microphytobenthos, stimulating their productivity. The accumulation of snails in controls may have balanced biofilm production and resulted in no microphytobenthos net gain between controls and bird exclosures.

The relationship between mudsnails and Semipalmated Sandpipers was similar to that seen previously. Hamilton et al. (2003) found that shorebirds avoided areas with high snail density, and we observed that bird footprints were less prevalent in control areas that contained abundant snails. Shorebird activity at our sites (based on footprint cover) was lower than that of Cheverie et al. (2014). The combination of low snail density and low shorebird presence in control plots may have balanced grazing pressures and microphytobenthos stimulation, leading to elevated biofilm presence in these areas. Biofilm is an important component in shorebirds' diet (Kuwae et al., 2008, Quinn and Hamilton 2012). However, with relatively low shorebird presence it is possible that the biofilm foraging pressure was weak.

Conclusions

Microphytobenthos appear to be nutrient limited in the Bay of Fundy. Nutrient availability increased microphytobenthos biomass in both the absence and presence of Semipalmated Sandpipers and low snail densities. Our results suggest that microphytobenthos is strongly limited by bottom-up factors, nevertheless there is a possibility that the presence of high snail densities could override the effect of nutrient availability. Our study was unable to explore this because our snail abundance was low, but Cheverie et al. (2014) found that grazing intensity by high snail densities severely reduced biofilm standing crop. Snail grazing appeared to indirectly increase microphytobenthos production, possibly because bioturbation stimulated nutrient recycling in the sediment (Connor et al., 1982). We know from previous studies that snails have negative associations with organisms like Semipalmated Sandpipers and *C.volutator* (Hamilton et al., 2003; Coffin et al., 2012) and can impose strong grazing pressures on biofilm (Cheverie et al., 2014). Therefore, mudsnails appear to be the main driver of the presence of other organisms and of microphytobenthos abundance, both increasing and decreasing microphytobenthos biomass. Apart from low snail presence possibly increasing primary production, we detected no long-term effect of the invertebrate community on microphytobenthos abundance, which aligns with findings from Hamilton et al. (2006). This suggests that the invertebrate community is likely not food limited.

Microphytobenthos in the Bay of Fundy is the foundation of an important and dynamic coastal ecosystem. Understanding how ecological factors affect biofilm standing crop help us to determine what limits their abundance, which in turn affects the organisms they support. From our findings, it is evident that mudsnail abundance plays a key role in determining ecosystem productivity and interactions, perhaps more so than nutrient availability under natural conditions. The Bay of Fundy mudflats provide critical habitat for Semipalmated Sandpipers, a species experiencing a hemispheric population decline (NABCI Canada 2019). Understanding how biofilm is affected by bottom-up and top-down factors gives us insights into future appropriate conservation efforts, such as the importance of considering mudsnail densities and nutrient availability during mudflat monitoring.

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