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Invertebrate effects on sediment biogeochemistry and microphytobenthos following estuarine macroalgal blooms

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Invertebrate Effects on Sediment Biogeochemistry and Microphytobenthos Following Estuarine Macroalgal Blooms

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Tables and Figures

CHAPTER FOUR:

Abstract

Eutrophication has led to the proliferation of devastating macroalgal blooms in shallow coastal estuaries. Processes at the sediment surface in shallow marine environments can ultimately affect nutrient cycling and the progress of eutrophication. Through bioturbation and grazing of microphytobenthos (MPB) infaunal invertebrates significantly alter redox dependent chemical and microbially-mediated transformations of nutrients and their subsequent release to or uptake from the overlying water column. This study used a series of experiments to investigate the effects of invertebrates on sediment biogeochemistry and nutrient cycling at early and late stages of eutrophication. Unique aspects of this study were the inclusions of multiple species, trophic levels and the use of technology to better understand the effects of invertebrates on processes. A non-destructive method of remotely sensing the MPB community through the use of reflectance spectra of the sediment surface was developed. Indices, derivatives and continuum removal band depths were valid for non-destructively quantifying MPB biomass at different time points in an ecological microcosm experiment. In another experiment, we evaluated the effects of two species of invertebrates, *Ilyanassa obsoleta* and *Mercenaria mercenaria,* alone and in combination, on the removal of detritus, oxygen consumption and nutrient release following the collapse of a large algal bloom. The effect *I. obsoleta* had on detritus removal was context specific, due to changes in food preferences in different sediment types. *M. mercenaria's* effect on sediment oxygen concentrations was substantially lower in the presence of *I. obsoleta* suggesting the importance of inter-specific interactions. In a third experiment we found that non-lethal predation by mud-crabs substantially affects invertebrates' behavior by reducing their activity. *I. obsoleta* grazed less, resulting in greater MPB biomass and decreased sediment ammonium release. Likewise, higher porewater ammonium

concentrations when *M. mercenaria* was in the presence of a predator indicate lower bioturbation within the sediments. Based on these studies, we conclude that environmental conditions and assemblage diversity ultimately affect the behavior of the organisms, which has potentially important consequences on the progress of eutrophication.

Chapter One

Introduction

1.1 Eutrophication in Shallow Estuaries

Coastal estuaries are highly productive ecosystems with high economic and social importance. They provide a unique environment with abundant natural nutrients from surface runoff and incoming tides. Estuaries serve as nurseries for many species of fish and are home to a wide diversity of invertebrates. Unfortunately, many of these ecosystems are threatened by anthropogenic eutrophication. During eutrophication the supply of organic matter to the system increases (Nixon 1995). The primary cause is allochthonous input of nutrients, particularly nitrogen, from the watershed and airshed. Nutrients enter coastal systems through terrestrial runoff, rivers, groundwater and atmospheric deposition (Nixon 1995). Human nutrient input comes from fertilizers, fossil fuel combustion and human and animal waste. Because nitrogen is typically the limiting nutrient for primary producers in marine systems (Howarth et al. 1988), an increase in the nitrogen load into a shallow estuary can lead to an explosion of opportunistic macroalgae (Valiela 1992, Bricker et al. 1999, Conley 2009).

These "nuisance" macroalgal blooms have detrimental ecosystem effects in shallow estuaries. Bloom collapse and subsequent decay leads to net removal of dissolved oxygen (DO) from the water, which can cause anoxic or hypoxic conditions if the system is overloaded (Bricker et al. 1999, Diaz and Rosenberg 1995). Macroalgal blooms also create shade that has led to losses in submerged aquatic vegetation, particularly the foundation seagrass species *Zostera marina* in Western Atlantic temperate estuaries, which creates crucial habitat for many other species (Hauxwell et al. 2001, McGlathery 2001). Furthermore, algal blooms lead to degradation of sediment quality by increasing organic matter (OM) content, resulting in heightened heterotrophic bacteria activity, lower redox potential and increased sulfide production (Holmer et al. 2009). Sulfide is toxic to seagrasses further reducing their cover (Holmer et al.

2009). As a result of lower DO and declines in seagrass beds, invertebrate and fish populations have also declined (Diaz and Rosenberg 1995). Since large quantities of OM has such drastic effects on ecosystem functioning, understanding this process, and what may affect it, is crucial.

1.2 Diagenetic Processes in Shallow Estuarine Sediments

OM is of central importance in diagenetic reactions however, the dominate pathway of OM breakdown is variable (Aller 1994). Microbes that break down OM mediate decomposition and the release of inorganic nutrients. The pathway of OM decay is dependent on the composition of the microbial community, which varies depending on environmental conditions, including the presence of oxygen or availability of other terminal electron acceptors (TEA). The amount of metabolic energy derived from organic carbon differs depending on the TEA used. For example O_2 yields more energy per mole of carbon than NO_3^- (Aller 1982). Thus, in the presence of oxygen, microbes that use O_2 as an electron acceptor dominate. Under anoxic conditions, anaerobic microbial respiration successively uses nitrate, manganese, iron and sulfate as TEA (Berner, 1981, Aller 1982). Figure 1.1 shows the idealized sequential pathways of organic carbon decomposition based on the theoretical energy yield. Since dissolved oxygen may only penetrate into the top few millimeters (or less) of the sediment, anaerobic respiration is the predominant diagenetic pathway in most marine sediments (Berner, 1981). Because of the high concentrations of sulfate in seawater, anaerobic mineralization in marine ecosystems is typically dominated by sulfate reduction, even though it yields less energy (Berner, 1981).

The growth of macroalgae during eutrophication changes decomposition pathways in estuarine sediments (Hansen and Kristensen 1997). Macroalgal mats float in the water column just above the sediment surface and have a large surface area, resulting in significant shading.

Studies investigating net sediment metabolism under floating algal mats have found that sediments evolve from being net autotrophic to net heterotrophic (McGlathery et al. 2001, Tyler et al. 2003, Corzo et al. 2009). In net autotrophic sediments inorganic nutrients tend to be taken up by the microphytobenthos (MPB) that effectively act as a barrier for nutrient flux between the sediment and the water column (Tyler et al. 2003). In sediment with low autotrophic activity bloom decay leads to significant efflux of inorganic and organic nutrients to the water column (Tyler et al. 2001), where they are subsequently taken up by macroalgae or phytoplankton (Figure 1.2) (McGlathery et al. 2001). Beneath macroalgal mats hydrogen sulfide has been found very close to or at the sediment surface indicating that sulfate reduction has taken over as the dominant diagenetic pathway at the sediment surface (García-Robledo et al. 2008, Corzo et al. 2009, Middelburg and Levin 2009).

In a microcosm study by García-Robledo et al. (2008), a onetime initial spike of dead macroalgae, which mimicked a bloom collapse, was added to the sediment surface. This resulted in an initial reduction in oxygen concentration in both surficial sediments and in the water column, due to increased aerobic respiration during mineralization of the OM. Detritus acted as a physical barrier for dissolved oxygen flux across the sediment water interface, which also lead to a decreased oxygen concentration near the surface. After six days, oxygen levels recovered, indicating that most of the OM had been decomposed. This OM addition ultimately resulted in a release of dissolved organic carbon (DOC), dissolved inorganic carbon (DIC) and dissolved inorganic nitrogen (DIN) to the sediment pore water and the water column.

1.3 Role of Invertebrates in Benthic Processes

Macro-benthic invertebrates may impact the rate of eutrophication by altering the way that nutrients are cycled in shallow estuaries (Gilbert et al. 1998, Ingalls et al. 2000, Mermillod-Blondin et al. 2004, McLenaghan et al. 2011). Bioturbation is defined as reworking of the porewater and sediment by organisms and has been shown to significantly affect the fate of organic matter and nitrogen in a system (i.e. Maught and Graf 1987, Michaud et al. 2005, Norling et al. 2007). Bioturbating animals thus serve an important ecosystem function in soft substrate benthic environments. Invertebrate movement increases porosity and the rate of movement of nutrients and organic matter between the overlying water and the sediment, thereby allowing more nutrients to become available to sediment microbes (Aller & Aller 1992, Gilbert et al. 1998). This physical mixing alters the reduction-oxidation boundary in the sediment. Changes to the redox boundary leave it irregular, which increases the surface area of the top oxidized layer (Figure 1.1b) (Levinton 1995). Most mineralization occurs in bioturbated zones (Aller 1992, Aller 1994, Godbold et al. 2009). Aller (1994) suggested that the redox oscillation caused by invertebrate activity leads to more complete OM decomposition. Below the zone of oxygen penetration sediments have active anaerobic bacteria. Infaunal activity that temporally oxygenates the sediment may lead to more complete and rapid decomposition of OM due to a combination of aerobic and anaerobic microbial activity (Sun et al. 1993, Aller 1994).

Many laboratory studies have investigated and found that single animal species have significant effects on nutrient cycling. Fewer studies have been performed with realistic assemblages of organisms, which have been shown to have different effects on nutrient transformation processes than single animal treatments (Solan et al. 2004, Waldbuser et al. 2004, Mermillod-Blondin et al. 2005, Marie et al. 2010, McLenaghan et al. 2011, Tyler et al. unpub.

data). It has been demonstrated that simply summing the effect on nutrient fluxes of individual species on nutrient fluxes does not necessarily equal the actual flux when organisms are examined in assemblages, with both greater and lesser effects of animals in combination (Emmerson et al. 2001, Waldbuser et al. 2004). More investigation needs to be done to determine solute transport rates and micro-profiles for realistic assemblages of animals. Interspecific interactions may also affect sediment profiles and mineralization processes through indirect effects leading to different overall impacts than single species treatments. For example it has been found that the presence of a predator affects macrofauna behavior: they may burrow deeper or cease feeding activities in order to protect themselves from predation (Doering 1982, Whitlow 2009, Maire et al. 2010). Little work has been done to investigate how changes in behavior may affect biogeochemical cycling of nutrients. It is important to understand how such interactions affect sedimentary and mineralization processes in order to gain a more thorough understanding of these processes in the field. Solan et al. (2004) demonstrated that changes in biodiversity due to the loss of species leads to reduced bioturbation, which may ultimately result in increased OM accumulation at the sediment surface during eutrophication.

1.4 Importance of Non-Destructive Sampling

The use of experimental microcosms has allowed for the exploration of how benthic invertebrates affect sedimentary processes and nutrient cycling (i.e Berg et al. 1998, Levin et. al. 2003). However, algal decomposition is a process, thus in order to understand the process measurements over a period of time are necessary. Conventional methods of measuring parameters such as MPB does not allow for multiple measurements over the course of an experiment. One non-destructive method is the use of micro-electrodes to estimate sediment

micro-scale concentrations of oxygen and sulfide that gives oxygen and sulfide concentrations in the sediment on a micron scale basis. Since microelectrodes are small (10-50 µm tip diameter), the measurement itself is non-destructive. This means that measurements can be taken at multiple time points over the course of an experimental period without disturbing sediment structure or porewater.

Micro-electrode measurements of oxygen are a method of measuring production by MPB. Another is the use of remote sensing technologies, which may allow for quick non-destructive sampling and community determination of micro photosynthetic organisms. Vegetation has a unique reflectance and absorption signal due to structure of the chlorophyll. Figure 1.3 shows a sample MPB spectra curve. Chlorophyll has absorption features in the blue and red region and reflectance features in the green and a strong reflectance feature in the near-infrared (Curran 1989, Gitelston et al. 2003, Smith et al. 2003, Murphy et al. 2005). Because of this unique spectrum, reflectance has been used to quantify biomass in terrestrial (i.e. Gitelson et al. 2003) and aquatic (i.e. Murphy et. al. 2009) environments. Attempting to quantify microalgae biomass on the sediment surface provides a unique set of challenges due to changes in reflectance not just from chlorophyll *a* concentrations, but also from grain size, water content and color (Werdell and Roesler 2003, Murphy et al. 2005, Forster and Jesus 2006). A number of different techniques have been used to overcome these problems, such as use of narrow band ratios, 1^{st} derivatives, 2^{nd} derivatives, and scaled band depths after continuum removal (Murphy et al. 2005).

MPB biomass will inevitably change over time and using non-destructive methods to sample can give insight to sedimentary processes as a whole. Most of the above mentioned studies that seek to quantify MPB biomass have been conducted out in the field, very little has

been done in a laboratory setting or trying to find a model that is applicable to both lab and field sampling. Backer et al. (2009) proved that using reflectance data can adequately describe how invertebrates alter sedimentary characteristics such was water content and grain size. In this study we show the use of similar technology and techniques is a viable method, which can be applied to the investigation of the effects of invertebrates on MBP biomass at different time points in an experimental period.

1.5 Study Site and Macrofauna

West Falmouth Harbor is a shallow estuary (mean water depth 0.6 m) located in the southwestern portion of Cape Cod, MA. Cape Cod is a terminal moraine with unconsolidated sandy soils (Kroger 2003). The harbor is made up of a combination of a drown-river valley and a bar built estuary that collectively spans 197 acres (Howes et al. 2006). Over the past decade or so the harbor has seen a quick degradation in habitat quality as a result of nitrogen loading into the system. Land use shifts in the watershed, such as an increased number of residents with treated lawns, have led to increased nutrient inputs. However, a contaminated plume from the Falmouth wastewater treatment plant, formed by the use of spray irrigation treatment, has significantly increased nitrogen loading, especially to the inner northeastern portion of the Harbor (Jordan et al. 1997, Howes et al. 2006). Because of the proximity to the source of the contaminated plume, the inner portion of the Harbor, Snug's Harbor, receives a substantially higher nutrient load (Figure 1.4). The South Harbor receives external inputs of nutrients primarily from residential development in that area. The Outer Harbor has healthy seagrass cover (Tyler et al. unpub data) and appears less noticeably degraded. In the inner harbor there has been

a significant decline in the eelgrass beds (Howes et al. 2006) that provide important structural habitat for a number of benthic invertebrate populations.

The two species of macrofauna that are the focus of this study are *Mercenaria mercenaria* and *Ilyanassa obsoleta*. Both are abundant in West Falmouth Harbor and found in areas that have been affected by eutrophication. Thus, both species have the potential to significantly affect processes related to OM decomposition and nutrient mineralization. *M. mercenaria* is a burrowing hard-shelled bivalve, which has been shown to enhance seagrass production by increasing light penetration through filter feeding and deposition of nutrients in the sediment (Carroll et al. 2008). *M. mercenaria* can tolerate oxygen levels at less than 1 mg L^{-1} for up to three weeks (Savage 1976) and have been shown to enhance DIN fluxes across the sediment water interface (Doering et al. 1987). *I. obsoleta* is an omnivorous gastropod that consumes other invertebrates' larvae, macroalgae, and is able to efficiently graze on MPB (Connor et al. 1982, Curtis and Hurd 1983, Giannotti and McGlathery 2001, McLenaghan et al 2011). *I. obsoleta* have been shown to limit the distribution of other gastropods and annelids and are strongly attracted to areas with high quantities of detritus (Levinton et al. 1985, Kelaher et al. 2003).

1.6 Scope and Objectives of Study

The overall objective of this study are to investigate the interaction among sediment surfaces processes, MPB, macroalgal detritus and infaunal communities during the early and late stages of eutrophication. These interactions can give insight to nutrient mineralization and

removal in shallow estuaries undergoing or recovering from eutrophication. In this study, a series of microcosm experiments were used to investigate these relationships. My objectives were:

1) To determine the relationship between microphytobenthos and their remotely sensed reflectance signal. I used multiple linear regression models to investigate the relationship of microalgae biomass in microcosms and their reflectance signal in the visible to near-infrared region of the spectra.
2) To investigate the effects of *M. mercenaria* and *I. obsoleta* on sediment surface processes using micro-chemical profiling and flux measurements after a simulated macroalgal bloom event.
3) To understand the effects that the loss of mobile predators' non-consumptive effects, which may occur on the onset of eutrophication, have on invertebrate ability to alter sediment processes and nutrient release.

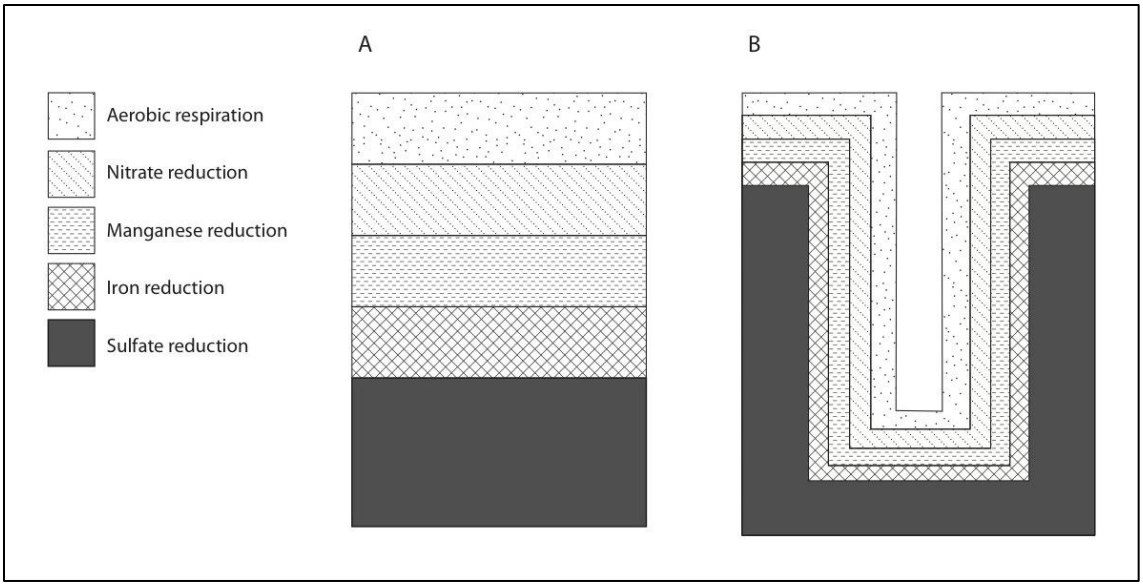

Figure 1.1 Idealized sequential electron acceptor use in marine sediments based on energy yield per mole of carbon. A) Sequential microbial electron acceptor use in physically undisturbed sediments. Oxygen penetration is relatively shallow and sulfate reduction dominates. B) Physical disturbance from infauna, such as the burrow illustrated here, leads to increased oxygen penetration into the sediment and increased surface area for aerobic respiration. Adapted from Aller (1982). Note: oxygen penetration is no more than 2-4 mm in sediment.

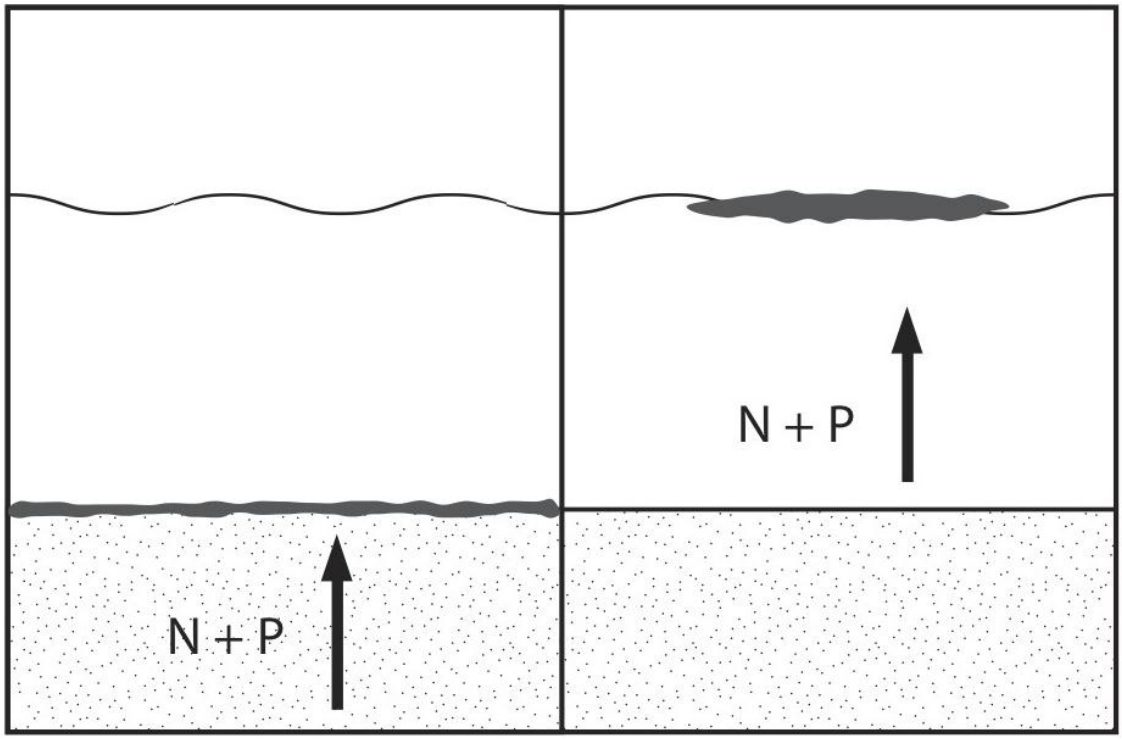

Figure 1.2 Net autotrophic sediments (left). Net heterotrophic under macroalgae mats (right). Without macroalgal mats microphytobenthos act as a cap to the nutrient release from the sediments, taking up mineralized nutrients from the sediment. With dense macroalgal cover, MPB are shaded out and nitrogen (N) and phosphorus (P) are released to the water column where they are available for uptake by macroalgae and phytoplankton.

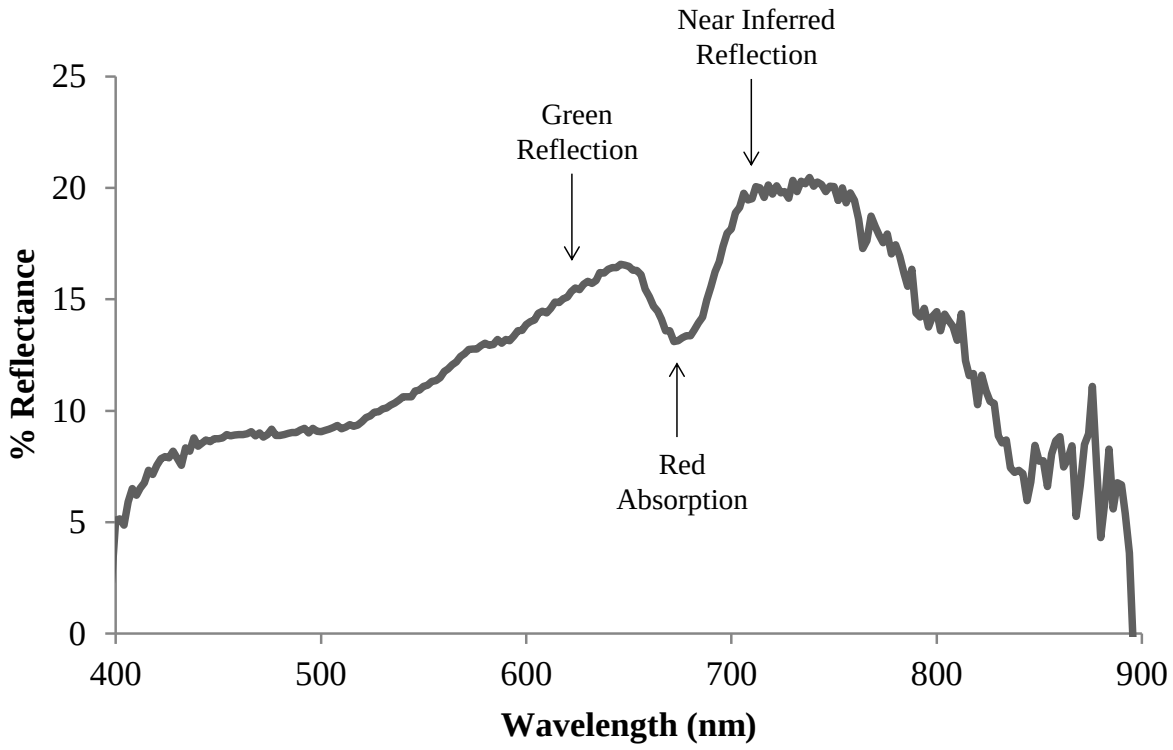

Figure 1.3 Sample spectra of microphytobenthos. Note the areas of absorption and reflectance which are characteristic of chlorophyll and can be used to quantify biomass.

Figure 1.4 West Falmouth Harbor, MA (41°36’N, 70°38’W) and sub basins.

Chapter Two

Spectral Measurements of Microphytobenthos Biomass in Experimental Microcosms

2.1 Introduction

Microphytobenthos (MPB) are a critical part of the ecosystem in shallow estuaries. In the absences of vascular aquatic vegetation MPB are the dominant primary producers and are an important energy source for higher trophic levels (Miller et al. 1996). Even in the presence of other aquatic vegetation MPB are a vital part of the food web (Sullivan and Moncreiff 1990, Currin et al. 1995). MPB are also essential for sediment stabilization because they secrete mucus that binds the sediment (Miller et al. 1996) and for controlling benthic-pelagic fluxes of nutrients, given their ability to act as a "cap" on the sediment surface, decreasing the efflux of nutrients from the sediment to the water column (Tyler et al. 2001, McLenaghan et al. 2011). Quantification of MPB biomass, under varying environmental conditions gives important insight into ecosystem function.

Microcosm experiments are often used to imitate field conditions because they allow the investigator to maintain a controlled environment where single factors can be manipulated to evaluate the response of the system (Benton et al. 2007). Standard methods of measuring MPB in microcosms involve destructive sampling of the sediment to measure chlorophyll *a* or attempt cell counts, which only allows for sampling at the end of the experimental period and eliminates the ability to collect time series data. Using remote sensing techniques that utilize surface reflectance to estimate photopigment concentration, a common proxy for MPB biomass (i.e.Bianchi and Findlay 1991, Pinckney and Zingmark 1993), allows for multiple measurements over time and can give further insight to changes in ecosystem function. For example, after the deposition of a macroalgal bloom, substantial changes in benthic photopigment concentrations appear to occur (i.e. García-Robledo et al. 2008, Larson and Sundbäck 2008), although destructive sampling techniques precluded the ability to evaluate changes over time. Pigment

analysis on a more frequent basis without disturbing the sediment may give further information documenting the ecological relationship between algal detritus and MPB.

Chlorophyll *a* (chl *a*) is used to estimate total MPB biomass, because chl *a* concentrations are highly correlated with MPB production (Pinckney and Zingmark 1993). Photosynthetic activity of chlorophyll results in absorption features in the blue and red regions of the electromagnetic spectrum and reflectance in green and near infrared (NIR) regions (Curran 1989, Gitelston et al. 2003, Smith et al. 2003, Murphy et al. 2005). The absorption feature in the red region, typically centered between 673 nm and 675 nm, is important when quantifying MPB from remote sensing data with high spectral resolution (Méléder et al. 2003, Carrère et al. 2004, Barillé et al. 2007). Phaeopigments, naturally occurring degraded chlorophyll pigments that may be the result of grazing (Bianchi, Dawson and Sawangwont 1988) or heterotrophic microbe activity (Binanchi and Findlay 1991) must also be taken into account when attempting to estimate MPB biomass because they accentuate absorption between 670-680nm (Méléder et al. 2003, Barillé et al. 2007).

Remote sensing techniques have been successfully applied in laboratory settings. For example, monospecific cultures of microalgae have been correctly quantified through the use of reflectance data (Méléder et al. 2003, Barillé et al. 2007). Yet, measurement of MPB in a microcosm may prove to be more difficult than in cultures, because of background effects from sediment water content and grain size. This is seen in the case of coarse-grained sediment, which has a higher albedo than darker fine-grained sediments, resulting in a more pronounced slope between the visible and near infrared (NIR) and generally higher reflectance values (Werdell and Roesler 2003, Murphy et al. 2005, Barillé et al. 2007). Increased water content also leads to less

reflectance in the visible region of the spectra and at longer wavelengths due to absorption (Forster and Jesus 2006).

Single band ratios and normalized ratios in the visible region can effectively quantify biomass. These indices can remove background effects better than reflectance values alone because they look at the ratios rather than absolute values (Carrére et al. 2004, Delalieux et al. 2008). Normalizing a ratio can further remove background effects because each instance is compared to a common baseline. Derivative based approaches can further more enhance absorption and reflection features while removing influences from other factors such as grain size and light variation (Murphy et al. 2005). Murphy et al. (2005) still saw influences from grain size using the first derivative, however it was completely removed in the second derivative. Other studies have found that the red absorption feature incrementally increases with addition of more chl *a* (Wolfe et al. 2006). Because of this, comparing band depth of an absorption feature from a common baseline may be able to be correlated with the amount of pigments in a particular sample.

In this study we demonstrate the efficacy of different approaches, previously proven effective in other environments, to non-destructively estimate MPB in laboratory microcosms. A majority of studies have found indices and derivatives that are correlated to MPB biomass in the field, while fewer have focused on lab settings. Our final approach is a relatively inexpensive method to measure MPB biomass rapidly in multiple samples. Other approaches have used expensive hyper spectral systems that measure wavelengths all the way from UV to long wave infrared. For the purposes of measuring MPB an instrument with a smaller range is sufficient and measurements across instruments have proven to be the same (Foster and Jesus 2006). Single

band ratios, normalized band ratios, 1st derivatives, 2nd derivatives and band depths in the visible region are tested.

2.2Methods

2.2.1 Sampling

Surface sediments (to roughly 10 cm depth) were collected with 9.5 cm (I.D.) by 30 cm (ht) cores and sectioned at depths of 0-2 cm, 2-5 cm, and 5-10 cm below the sediment surface. Larger invertebrates were removed by passing each section separately through a 1 mm mesh sieve. The sediments were homogenized and transported to the Rochester Institute of Technology laboratory in Rochester, NY. These coarse profiles were maintained during microcosm construction in 9.5 cm (I.D) by 30 cm (ht) cores which gave a surface area of 0.071 m^2. After construction, the microcosms were submerged in a Living Stream flowing seawater system for four months with a cycle of 14 hours of light and 10 hours in the dark. Artificial seawater was continuously circulated and salinity was kept between 28-32 ppt and a temperature of 20-22 ºC. To create a range of chlorophyll values, we manipulated light availability and grazing pressure. Four cores had no treatment added to it, four had mesh over the top, four had black plastic covering the top so there was no light directly over the sediment surface and four had grazing snails (*Ilyanassa obsoleta*) added to the cores at a density of 290 individuals per m^2. *I. obsoleta* in high densities (over 60 individuals m^{-2}) have been shown to significantly reduce photopigment concentrations (Connor et al. 1982, McLenaghan et al. 2011). Measured light levels at the sediment surface were approximately 81.33 photons m^{-2} s^{-1}, 68.56 photons m^{-2} s^{-1} and 28.23 photons m^{-2} s^{-1} for no treatment, mesh and plastic respectively. Snails and mesh were

added three weeks before measurements were made and black plastic was added only a week before, allowing for lower concentrations of MPB.

Prior to the collection of reflectance measurements all water was carefully drained from each core, and cores were wrapped in black plastic to reduce reflection from the sides of the cylinders. An Ocean Optic Red Tide USB650 Sptectrometer with a spectral resolution of 2 nm (full-width-half-maximum; FWHM) was used measure reflectance values from 350 to 1000 nm for an average footprint of 37.02± 4.5 cm^2. A white reference measurement, taken from a panel with a near 100 % diffuse reflectance, was used to normalize the specta across illumination and set-up variations. The light source used was a 50W, 4700K/36 º "Flood" bulb from SoLux with a spectrum ranging from UV to IR. Six individual spectra from each core, in the same spot were measured.

Immediately after the spectral measurements were taken, a sediment core was taken for chl *a* and phaeo analysis using a modified 5 cc syringe to a depth of 1 cm within the field of view of the spectrometer. Samples were placed in 15 ml centrifuge tubes and stored in the dark at -80℃ until analysis. Samples were analyzed according to Stickland and Parsons (1972) and chl *a* and phaeo concentrations were calculated according to Lorenzen (1967). Six mL of 90% acetone was added to each tube prior to shaking and sonication in an ice bath for 1 minute on and 1 minute for off for 3 minutes. Extraction proceeded overnight at - 20ºC, after which the sediment was resuspended after shaking and then centrifuged. Absorbance at 665 nm and 750 nm was read on a Shimadzu UV-1800 spectrophotometer before and after acidification with 1 N HCL. Concentrations where determined for chl *a*, phaeo and chl *a* + phaeo in mg m^{-2}. A total of 16 measurements were taken, one per core. The spectra for one core was removed due to a

featureless spectra. Although this is a small sample size for chl *a* assessment, Scheiner (2011) noted a low range (30-103 mg m^{-2}) of concentrations in similar microcosm experiments.

2.2.2 Spectral Analysis

The six spectra taken for each core were averaged and each resulting spectrum was further smoothed by averaging across 10 nm intervals. This was done to reduce noise and to simulate more operational type remote sensing systems (van Aardt and Wynne 2001). The values used for the single band ratio calculations were the reflectances at the center of the absorption features (680 nm, 677 nm, 675 nm, 670 nm) and a band with in a reflectance peak (720 nm and 750 nm). Ratios were also tested by normalizing the reflectance at the absorption feature with reflectances at 720 nm or 750 nm. 1^{st} and 2^{nd} derivatives were evaluated based on visible inspection of the spectra. The values chosen were near inflection points and where a position of a positive or negative peak was in line with an absorption or reflection feature in the raw spectra. These derivatives were deemed useful to describe spectral curve shapes, instead of magnitude (van Aardt and Wynne 2001).

Another set of predictor variables was calculated based on the technique of continuum removal described in Kokaly and Clark (1999). A continuum line is drawn across the absorption feature, between 620 nm and 730 nm (Figure 2.1a). Band depths are found by looking at the difference between the continuum line and spectral curve and then normalized to the center band at 675 nm. Band depths (Figure 2.1b) and normalized band depths (Figure 1c) were used as independent variables. A third regression was run for a combination of all factors, including the indices, derivatives and the continuum removed spectra.

SAS ® 9.2 was used to perform a linear regression using forward step-wise regression (PROC STEPDISC) with chl *a*, pheao and chl *a* + pheao as the dependent variables and independent variable entry set to $\alpha = 0.05$ for all 15 spectra. An analysis was run for indices and derivatives, a separate one for band depths, and normalized band depths, and a combination of all these variables. R^2, adjusted R^2, which takes the number of predictor variables into account, root mean square error (RMSE), and Pearson correlation coefficients were calculated. Variables with a correlation coefficient above 0.75 were considered significantly correlated and the variable with the smaller partial R^2 was removed from the model before the final fit (van Aardt et al. 2006).

2.3 Results

2.3.1. Relationship between amount of spectra shape and pigment concentrations

Chl *a* ranged from 38.6 to 97.6 mg m^{-2} and pheaoranges from 20.5 to 77.1 mg m^{-2}. All values were within the same range of experiments with similar set ups (Scheiner 2011) and are lower than what would be expected in field in the same site where the sediment was collected (Premo unpub data). Although the sediment was collected from the same area at the same time, conditions during the 4 month incubation in the tank lead to variable conditions on the sediment surface. Some cores had a layer of fine material, while others had course sand grains exposed at the sediment surface. Cores noted has having more sand exposed at the sediment surface had comparatively higher reflectance values.

The ratios of chl *a* and phaeopigments were all nearly 1:1 for all sample, meaning that there were equal concentrations of both pigments. The spectral curves, measured in the cores (Figure 2.2) were mostly featureless other than the characteristic red absorption feature centered

between 670 nm and 680 nm. Spectra with greater chl *a* + phaeo concentrations had a steeper slope between 680 nm and 700 nm (Figure 2.3). Finally, the higher the chl *a* + phaeo, the larger the band depths were in the absorption feature between 670 nm and 680 nm.

2.3.2 Model Results

Table 2.1 shows a summary of the model results for each method. The residuals from all models were normal and randomly distributed, which indicated that the dependent-independent relationships were properly modeled. All R^2 values for the models were high (0.7296 to 0.9997). The model with the highest R^2 and lowest RMSE for chl *a* + phaeo was observed when extracting independent variables from the indices and derivatives method. Normalized band ratios for 677 nm and 720 nm contributed the most to explaining the variability in the samples (partial R^2 =0.719). For phaeo alone, the largest independent variable contributing to the model value also involved wavelengths at 677 nm and 720 nm (partial R^2= 0.716). However, for chl *a* the biggest contributor was the normalized band ratios for 680 nm and 720 nm (partial R^2 =0.570). The band depths method yielded the lowest R^2 and highest RMSE for chl *a* + phaeo. Only the band depth at 620 nm was found to be significant. The band depth at 624 nm had the highest partial R^2 for chl *a* (partial R^2 = 0.7102). Again for phaeopigments, 677 nm came up as significant; however, the band depth at 631 nm explained most of the dependent variability (partial R^2 = 0.673). In the case of the combination method chl *a* exhibited the highest R^2, the lowest RSME and highest number of variables (8 variables). Despite the high number of independent variables, the model was still deemed valid because the α level was kept constant, highly correlated variables were removed, and the adjusted R^2 remained high. It was interesting to note that, the band depth at 624 nm contributed most to explaining the variability for the chl *a*

+ phaeo (partial R^2= 0.7296) and chl *a* (partial R^2= 0.7102). Phaeopigments in the combined approach had the highest contribution from the single band ratio 720 nm and 677 nm (partial R^2= 0.7159).

2.4 Discussion

Our study found that the spectra of sediments in microcosm were like areas in the field that appeared un-colonized by MPB and features were similar to sediments with diatoms. The shape of the spectra is similar to what has been found for low field densities of MPB, which would be expected in a laboratory setting (Paterson et al. 1998, Foster and Bruno 2006). It is reasonable to assume that the majority of MPB in our sediment were diatoms because they tend to dominate in MPB communities in similar systems (Paterson et al. 1998, Paterson and Hagerthey 2001). Kromkamp et al.'s (2006) description of the reflectance signal for diatom dominated sediment has a dip centered around 675 nm and broad peak from 550 nm to 610 nm, similar to our spectra.

This study supports work that has shown the utility of both single band and normalized ratios in the red region for estimation of MPB biomass (i.e. Carrère et al. 2004). Our results show that the band with the maximum absorption depth tends to vary between 670 nm and 680 nm. We found a significant relationship using 680 nm for chl *a* and 677 nm, specifically, for phaeo. Varying absorption in the red region is proportional to varying pigment concentrations (Wolfe et al. 2006). Based on a comparison between field measurements and measurements in monocultures lacking phaeopigments, Barillé et al. (2007) suggests that phaeopigments accentuate the absorption at 675 nm. As such, they recommended looking outside the 672 nm and 678 nm range for values to quantify chl *a*. Our results support this because we found

significant influences of phaeopigment concentrations at 677, simultaneously we found indices outside their recommended range to quantify chl *a*. Thus, the model developed proves to be adequate in differentiating between active and degraded pigments.

A number of bands used in our models, in addition to the absorption between 670 nm and 680 nm were useful for estimating MPB biomass. The second derivative at 632 nm significantly contributed to the chl *a* model with the combination of independent variables method (partial R^2 = 0.107). This wavelength corresponds to chlorophyll *c*, a pigment characteristic of diatoms (Paterson 1998, Barillé et al. 2007 and Barillé et al. 2011). In MPB communities containing mostly diatoms, it is suspected that the chl *c* content will increase proportionally with chl *a* content (Méléder et al. 2003). The second derivative at 682 nm also was important in quantifying chl *a* (partial R^2 =0.117). Serôdio et al. (2009) found that the 683 nm second derivative was important because it was attributed to chl *a* fluorescence. In fact, this study recommended the use of fluorescence over reflectance to non-destructively sample MPB biomass, because fluorescence may interfere with the reflectance spectra, however they concluded that smoothing at 10 nm intervals may mask the effects of fluorescence.

Our sediment samples were taken to a depth of 1 cm in order to capture all MPB in the sediment. This depth, however, is likely deeper than what the spectrometer can capture. This would result in an overestimate of MPB biomass, although they are mostly concentrated in the top 2mm of sediment (Hopkins 1963). Jesus et. al (2006) recommend sampling the sediment to a depth of only 750 µm because the remote sensing equipment is limited to measuring only the surface of the sediment, although diatoms and particularly phaeopigments can be somewhat deeper (Hopkins 1963, Méléder et al. 2003). However, for ecological studies involving time series data, relative differences may be most important in measuring MPB concentrations and

ratios of chl *a* to phaeo. If measuring for absolute concentration, the sediment depth recommended should be taking into account, especially when consideration of phaeopigments is important. Chl *a* concentrations measured through remote sensing techniques should be representative of total MPB biomass since diatoms are more highly concentrated at the sediment surface.

In application of these models, it is important to take into account the type of environment under consideration to avoid overextension of the model. A steeper slope at the visible-NIR boundary indicated high chlorophyll values, this is associated with the red edge and is an effective measure of chl *a* content in terrestrial plants (i.e. Gitelson et al. 2003, Smith et al. 2003). However, in marine environments scattering at NIR and a sharper slope between the visible and NIR may be more of a result of sandier sediments (Murphy et al. 2005). Different sediment types may affect the indices and derivatives close to the NIR. For this reason the band depths approach may be valuable as long as photopigment concentrations remain low. Wolfe et al. (2006) found increased band depths at the red absorption feature with increases in chl *a* concentrations, however, Méléder et al. (2003) found that absorption in the red region remained constant above a certain chl *a* content (> 100 mg m^{-2}). Thus, the band depth method should be used with caution, but is a powerful tool.

2.5 Conclusions and Future Work

This study found a number of valid models that can be used in microcosm experiments to compare relative MPB biomass in ecological experiments, especially those seeking to quantify the relative changes in MPB biomass over time. In spite of our small sample size the values used covered a known range of chlorophyll concentration. High correlation coefficients between the

measured and modeled values mean that we found very robust models that are able to predict chlorophyll and phaeopigment concentrations. The work was done in a controlled environment and model inputs were kept constant resulting in accurate and robust models that may be useful in future ecological microcosm studies. Future work will include extending models to field collected data. If similar independent variables are found to be significant between microcosm and field data sets then this would further strengthen the validity of the variables. Florescent data was also measured in a microcosm study which may also prove to be an important method in non-destructive sampling.

Method	Dependant Variabels	R^2	R^2-Adjusted	RMSE	Independent Variables
Indices and Derivatives	Chl *a*	0.8562	0.8171	7.11223	$((R_{750}-R_{680})/R_{750})$, δ667, δδ682
	Pheao	0.9419	0.9186	5.16628	R_{720}/R_{677}, δ624, δ729, δδ632
	Chl *a* + Pheao	0.961	0.9455	7.65222	$((R_{750}-R_{677})/R_{750})$,δ626, δ667, δδ713
Band Depths	Chl *a*	0.8913	0.8617	6.18432	D624, D719, D728
	Pheao	0.8186	0.7883	8.33131	D631, D_n677
	Chl *a* + Pheao	0.7296	0.7088	17.6822	D624
Combination	Chl *a*	0.9999	0.9997	0.27608	D624, D728, δδ632, D719, δ634, D_n674, δ728, δ735
	Pheao	0.9208	0.8992	5.74773	R_{720}/R_{677}, D_n666, D_n627
	Chl *a* + Pheao	0.8302	0.8019	14.58408	D624, δδ693

Table 2.1 Results from regression models in SAS using indices and derivatives method, band depths method and combination of both methods. R indicates an index, δ and δδ first and second derivatives, respectively. D indicates a band depth and D_n indicated a normalized band depth.

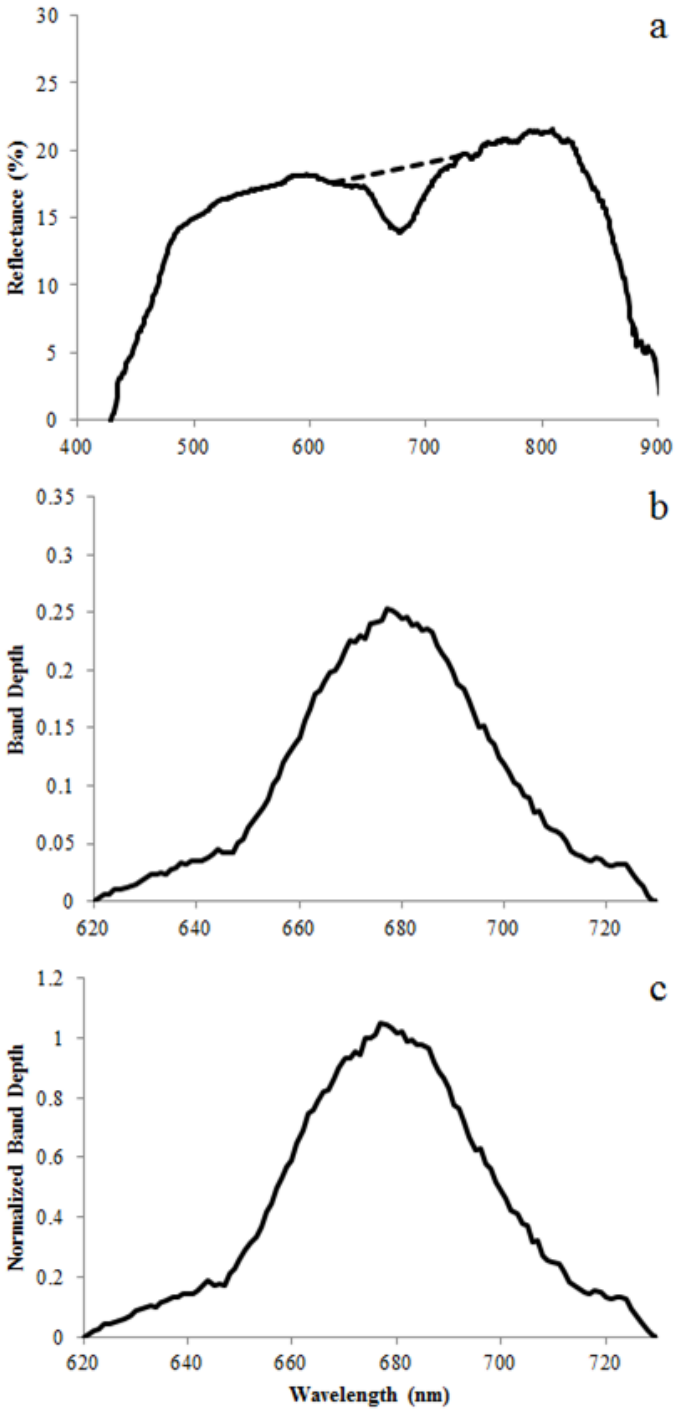

Figure 2.1 An example of the continuum removal process. First the continuum line [---] is established from 620 nm to 730 nm (a). Band depths are calculated from the continuum line to the spectra (b) and then normalized against the center band 675 nm (c) (Kokaly and Clark 1999).

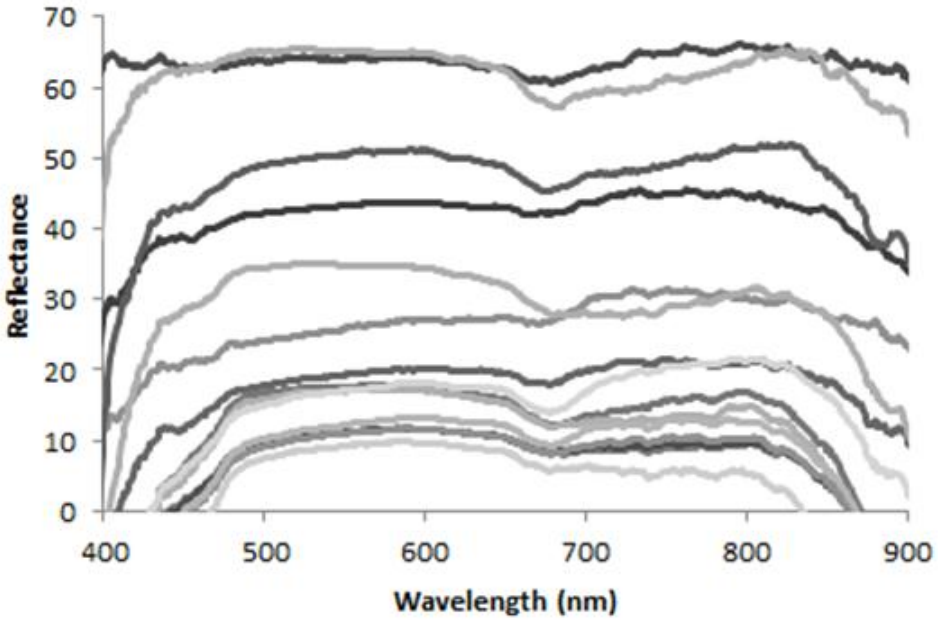

Figure 2.2 Spectra for all core samples. Note the distinct dip between 670 and 680 nm, attributed to chlorophyll absorption of red energy. Higher reflectance values are a result of sediment grain size.

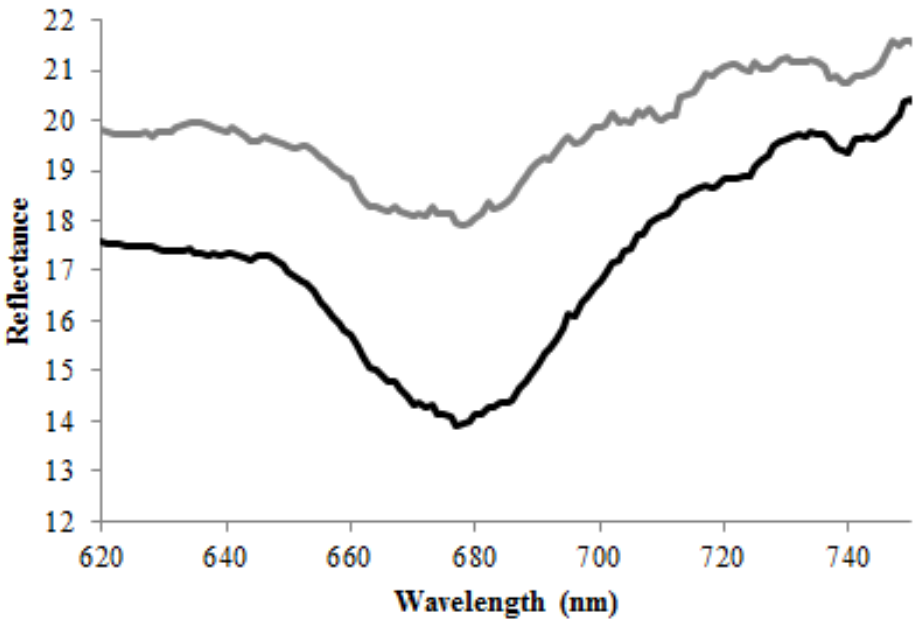

Figure 2.3 An example of the absorption feature with high chl a + phaeo levels (black line) and low chl a + phaeo levels (grey line). Note not only the magnitude differences, but also the slope (derivative) and peak absorption feature differences

Chapter Three

Effects of macro-benthic invertebrates on benthic fluxes and sediment biogeochemistry after the collapse of a macroalgal bloom

3.1 Introduction

Many temperate coastal estuaries today are threatened by eutrophication due to excess inputs of nitrogen (Howarth et al. 1988). Increases in nitrogen loads lead to explosions in opportunistic macroalgae population and when blooms collapse organic matter (OM) accumulates on the sediment surface leading to increased benthic oxygen consumption (BOC) (Valiela 1992, Bricker et al. 1998). As a result of increased oxygen demand dissolved oxygen levels drop, often devastating invertebrate and fish populations (Diaz and Rosenberg 1995).

Benthic invertebrates in shallow systems have the ability to alter sediment characteristics and nutrient cycling (Michaud et al. 2005, Kristensen and Mikkelsen. 2003, Norling et al. 2007). One way that they may affect these processes is through bioturbation, which is the physical mixing of sediment particles, OM and porewater (PW). In undisturbed, non bioturbated, sediments oxygen penetration is shallow, which limits the surface area for aerobic mineralization of OM, thus anaerobic mineralization is dominated by sulfate reduction, the predominate pathway (Middleburg and Levin 2009). In bioturbated sediments, oxygen penetration depth can oscillate and there is an increased surface area where aerobic microbes can exist, resulting in suppressed anaerobic activity (Aller 1982). Aerobic mineralization of OM is thought to be more efficient and complete pathway than anaerobic pathways (Aller 1982, Hansen and Blackburn 1991). Changes in the redox boundary may ultimately affect the fate of nutrients. For example, Gilbert et al. (1998) found that reworking of the sediment led to higher denitrification rates, where nitrogen is removed from the system. Mermillod-Blondin et al. (2004) also found that increased oxygen penetration was important because it lowered the amount of sulfate reducing bacteria in the presence of bioturbating invertebrates.

Bioturbating macrobenthic invertebrates also play a key role in redistributing of OM. Detritus gathered at the sediment surface not only leads to higher BOC, but also decreased diffusion of solutes between the sediment-water interface. Burrowing activities lead to increased contact of detritus with sediment particles, which may allow for increased contact of microbes with OM (Nordstöm et al. 2006). Several species of burrowing polychetes substantially alter the distribution of OM by displaying a trait termed "gardening," where they remove detritus or live algae from the surface and bring it down into burrows. (Kristensen and Mikkelsen 2003, Nordstöm et al. 2006, McLenghan et al. 2011).

Microphytobenthos (MPB) are important in controlling nutrient fluxes because the trophic status of the benthos controls whether it is sink or sources of nutrients (Tyler et al. 2003, Englesen et al. 2008). Macroalgal mats float in the water column just above the sediment surface and have a large surface area, resulting in significant shading. Studies investigating net sediment metabolism under floating algal mats have found that sediments change from being net autotrophic to net heterotrophic (Tyler et al. 2003, Corzo et al. 2009). Following the deposition of a collapsed bloom, where light can once again reach the sediment surface, MPB biomass can increase due to large amounts of nutrient mineralization in the sediment (García-Robledo et al. 2008). Grazing or disturbance to MPB by bioturbators leads to increases in nutrient fluxes because the "cap" on the sediment is gone, which may sustain nutrient levels in the water column (McLenaghan et al. 2011).

This study investigates how benthic invertebrates affect MPB, oxygen penetration, fate of detritus and nutrient fluxes after the collapse of a large macroalgal bloom. We investigated *Mercenaria mercenaria* and *Ilyanassa obsoleta* because they are common along the east coast of North America and are likely to be found in areas where there has been a bloom collapse. *M.*

mercenaria are filter feeding bivalves that can tolerate hypoxic conditions for up to three weeks (Savage 1976). *I. obsoleta* is an omnivorous gastropod that efficiently graze on MPB and are attracted to patches of detritus (Kelaher et al. 2003). We measured the effects of invertebrates on decomposition in two different sediment types, coarse-grained sediment composed mostly of sand and fine-grained sediment with a higher mud and OM content.

3.2 Methods

3.2.1 Sediment Collection and Experimental Set-Up

Sediment, invertebrates and *Ulv*a sp. were collected from West Falmouth Harbor located in the southwestern portion of Cape Cod, MA. West Falmouth Harbor is a shallow estuary with a mean water depth of 0.6 m (Howes et al. 2006). Over the past ten years a contaminated ground water plume has made its way into the inner portion of the harbor leading to large macroalgal blooms and degradation of the habitat (Jordan et al. 1997, Howes et al. 2006). Sediment for both the coarse-grained experiment and fine-grained experiment were collected from the inner harbor. Substrate in the inner harbor is extremely heterogeneous and variable compared to other basins in the harbor (Scheiner 2011).

The coarse-grained sediment collection site (Figure 3.1) was estimated to have 7% gravel, 79% sand and 14% mud and the fine-grained site 0.5% gravel, 53% sand and 46% mud (Scheiner 2011). Sediment, invertebrates and *Ulva* for the coarse-grained experiment were collected in June of 2011 and for the fine-grained experiment in August of 2011. Surface sediments to about 10 cm was collected using a 9.5 cm (I.D.) x 30 cm (ht) cores and sectioned at depths of 0-2 cm, 2-5 cm and 5-10 cm. Large invertebrates and gravel were removed by passing each section through a 1 mm mesh sieve. Microcosms were constructed in rectangular Snap ‘N

Lock polypropolyene containers, which had a sediment surface area of 244 cm^2. Sediment depth profiles were maintained during construction. Microcosms were maintained in a LS-700 "Living Stream" system, with the dimensions of 84"L x 24"W x 22"D and holding capacity of 140 gallons, by Frigid Units, Inc. (Toledo, OH) Water was maintained at 28-32 ppt and at 20-22ºC. Mechanical bubbles were installed in each microcosm to allow water circulation. For the coarse-grained experiment microcosms were acclimated for three weeks and for the fine-grained experiment microcosm were allowed to acclimate for four months. Acclimation for coarse-grained sediment needs considerably less time, minimum optimum acclimation time is three weeks, to restore sediment and PW profiles. Restoring PW profiles takes longer in fine-grained sediments because there is lower porosity in fine-grained sediments, which results in lower permeability (Tyler et al. unpub data).

For the coarse-grained sediments treatments included a control, *M. mercenaria*, *I. obsoleta* and a combination of both species (n=3) (Table 3.1) For the fine-grained experiment there were two treatments, a control and *I. obsolelta* (n=3). Densities were chosen based on realistic field densities for this harbor (McLenaghan 2009, Scheiner 2011, Yarrington unpub data). For the coarse-grained experiment invertebrates were added to the cores on Day 0, which allowed for *M. mercenaria* to bury before the addition of *Ulva* detritus on day 4. For the fine-grained experiment *Ulva* detritus was added on day 0 and *I. obsoleta* on day 1. These differences in detritus addition dates are for two reasons. One was to make sure *M. mercenaria* in the coarse-grained sediment would bury themselves, which they may not due in low oxygen conditions as the result of the detritus. And second was due to an expected lag time for OM mineralization in fine-grained sediment, due to the already high OM content (about 12% OM content for the fine-grained sediment compared to ~3% for the coarse-grained measured in the field by Scheiner

(2011). *Ulva* was frozen at -80º C and manually shredded to roughly 1 cm pieces before addition. 20 g ww of *Ulva* detritus was added to each microcosm. This biomass is consistent with 100% coverage of live *Ulva* in the field (Yarrington unpub. data). A timeline of the sampling days is shown in Table 3.2. On day 6 and 20, micro-electrode measurements were taken. On days 7, 14 and 21 sediment-water-column oxygen and nutrient flux samples were measured and on day 22, at the end of the experimental period, pore water ammonium and sulfide (coarse-grained only), chlorophyll *a* (chl *a*), OM, and mass of remaining detritus samples were measured.

3.2.3 Micro-electrode measurements

For the coarse-grained experiment glass oxygen (O_2) micro-electrodes (OX-50 from UNISENSE in Aahrus, Denmark) with a diameter of 25 µm were used to measure micro-scale O_2 concentrations using a manual micromanipulator. In the fine-grained experiment, O_2 and sulfide (S^{2-}) micro-electrode 50 µm (H_2S-50 from UNISENSE in Aahrus, Denmark) were used. The oxygen probe was connected to a pico-ammeter (PA2000 from UNISENSE on Aahrus, Denmark) and calibrated by measuring the O_2 concentration in the water column with a Hach HQ40d meter (LDO101 probe) and the reading at the bottom of the profile, which was assumed to be completely oxygen-depleted. The S^{2-} probe, which measures the partial pressure of hydrogen sulfide (H_2S), was calibrated according to Levin et al. (2003). A stock solution for calibration was prepared by anoxically dissolving Na_2S in N-flushed DI water to make a solution with a S^{2-} concentration of 100 mM which was later confirmed using the method from Cline (1969). On each day a calibration chamber containing with N-flushed DI water had the stock solution incrementally added so that the concentration of total sulfide was known. The instrument reading, pH, salinity and temperature were read five times during the calibration so

the concentration of each sulfide species could be determined (Unisense 2010). H_2S concentration in the sediment was measured on a 10 micron scale and O_2 on a 5 micron scale using the micromanipulator.

In the coarse-grained experiment a total of two microcosms from each treatment had oxygen micro-profiles measurements made on day 6 and day 20. Each microcosm measured had two profiles taken in the light and two in the dark. For the fine-grained experiment on day 6 two light O_2 and light H_2S measurements were made in each microcosm. On day 20 two H_2S and two O_2 profiles were made in the light and dark in each microcosm. Since the depth where the probes crossed the sediment water interface could not be determined visually due to the small tip size, the sediment water interface was determined via inspection of the slope of the O_2 curve. The first H_2S reading was determined to be at the depth where oxygen was depleted. Using the PROFILE program developed and explained in Berg (1998), O_2 production and consumption were calculated along with the calculated O_2 flux, calculated O_2 concentration at the sediment surface and O_2 extinction depth. Figure 3.2 is a sample oxygen profile showing the slope of the curve in the water column, through the detritus layer and into the sediment. In the sediment, the calculated production and consumption rates are shown.

3.2.4 Oxygen and Nutrient Flux Measurements

Oxygen and nutrient fluxes were measured according to Tyler et al. (2001). Prior the start of the flux roughly two-thirds of the water was gently siphoned off and replaced with fresh artificial seawater. Microcosms were sealed with air tight Snap ‘N Lock lids, so that there was no gas exchange, and two rotating Teflon® coated magnets were used to mix the water column at 60 rpm. Measurements were taken at 5 time points, over a 6 to 8 hour period, the first three in the

dark and the final two in the light. Oxygen measurements were made with a Hach HQ40d meter (LDO101 probe) and water samples were taken with a 60 cc syringe for ammonium (NH_4^+), nitrate (NO_3^-), total nitrogen (TN), and phosphate (PO_4^{3-}). Water was then replaced with a known fill volume. If oxygen levels dropped below 4 mg L^{-1} during the incubation ~50% of the water was emptied and replaced and the chamber resealed. In this case, samples were taken before and after refill.

All water samples were immediately filtered with a Supor TM 0.45µm membrane filter and frozen at – 20ºC. NO_3^- (as NO_3^-+NO_2^-), PO_4^{3-} and TN were analyzed on a Lachat Quikchem 8500 autoanalyzer with the cadmium reduction, molybdate complex and in-line digestion methods, respectively (Lachat Instruments 2003). Ammonium was analyzed according to Solorzano (1969) using the phenol-hypochlorite method. Dissolved inorganic nitrogen (DIN) was calculated by adding NO_3^-+NO_2^- and NH_4^+ concentrations. Dissolved organic nitrogen (DON) concentrations were calculated by subtracting NO_3^-+NO_2^- and NH_4^+ from TN. Hourly (BOC) and nutrient flux rates were calculated based on changes in concentration over time (Tyler et al. 2001). A 14 hour light, 10 hour dark period was used to estimate daily rates.

3.2.5 Porewater, Chlorophyll a*, and Organic Matter*

Porewater (PW) NH_4^+ and S^{2-} samples were taken according to Berg and McGlathery (2001). A 2 mm diameter stainless steel probe was used to extract 2.5 ml of water at depths of 1.5 cm, 3, cm, 5 cm, and 7 cm. PW was immediately filtered with a 0.45µm membrane filter. NH_4^+ samples were frozen at - 20ºC until analyzed according to Solorzano (1969). S^{2-} samples were analyzed according to Cline (1969). After PW profiles were taken remaining intact detritus

resting on the surface was collected. Using a spork, detritus was gently gathered, towel dried, and weighed.

Chl *a* samples, which were used as a proxy for microalgal biomass, were collected with a modified 5 cc syringe to the depth of a one cm. Sediment was immediately placed in the dark and frozen at -80ºC. Chl *a* was analyzed within 30 days according Strickland and Parsons (1972) as descried in Chapter Two. Chl *a* and phaeopigment, which are degraded chl *a* pigments, concentrations were calculated according to Lorenzen (1967). A modified 60 cc syringe was used to collect two 10 cm deep cores for OM samples from each microcosm. Sediment was sectioned at 0-1 cm, 1-2 cm, 2-5cm, and 5-10 cm. OM content of each section was determined by the loss on ignition procedure described in Heiri et al. (2001). Samples were dried at 60º C for at least 48 hours. Between 5-6 grams were weighed out on previously tarred tins and placed in a muffle furnace at 550ºC for six hours. After samples were weighed % OM was determined based on the mass difference.

3.2.6 Data Analysis

All statistical analyses were conducted on SAS® 9.2. For the oxygen and nutrient fluxes a two-way ANOVA was used for each day with light or dark fluxes for each day. For data not normally distributed Friedman's two-way analysis of variance was used. Daily BOC and nutrient fluxes were analyzed with a one-way ANOVA for each day. When significant effects were found, a Tukey test (HSD) with $\alpha=0.05$ was used. PW, chl *a* and OM were tested for significance between treatments with a one-way ANOVA or a Kruskal Wallis test for non-normally distributed data. O_2 parameters from the PROFILE output were analyzed for significant differences between treatments for each day with either a one-way ANOVA or Kruskal Wallis

test. Significance for O_2 parameters were also tested for between days with a one-way ANOVA or Kruskal Wallis test.

3.3 Results

3.3.1 Oxygen Fluxes and Sediment Profiles

In the coarse-grained sediment the control treatment had a significantly lower daily BOC than the combination treatment only for day 7 and day 14 (Table 3.3, Figure 3.2a). Hourly flux rates between the light and dark were statistically similar (range = -1.1 to -4.8 mmol m^{-2} d^{-1}, -0.19 to -4.1 mmol m^{-2} d^{-1}, 1.3 to -12.5 mmol m^{-2} d^{-1} for days 7, 14 and 21 respectively), although on day 21, *M. mercenaria* BOC in the dark was significantly lower than the other treatments and significantly greater in the light. This resulted in significantly lower GPP for *M. mercenaria* on day 21 (Figure 3.3a). In contrast, *I. obsoleta* significantly increased hourly BOC on all days in the fine-grained sediment however, daily BOC was only statistically significant on day 7 and 21 (Table 3.3, Figure 3.2b). BOC for day 21 was statistically significant in the dark compared to the light. Despite the light/dark differences for day 21, there were no significant differences in GPP for fine-grained sediment (Table 3.3, Figure 3.3b).

Figure 3.4 is a sample O_2 profile showing the slope of the curve in the water column, through the detritus layer and into the sediment. In the sediment the calculated production and consumption rates are shown. In the coarse-grained sediment *M. mercenaria* significantly increased O_2 extinction depth on day 20 in the light compared to all other treatments (Table 3.4, Figure 3.5a*). M. mercenaria* also increased O_2 surface concentrations in the light on day 20 relative to *I.obsoleta* (Figure 3.6a). In the fine-grained sediment *I. obsoleta* increased O_2 penetration on day 20 and on the contrary, it had no effect on concentration at the sediment

surface (Figure 3.5b). For both coarse-grained and fine-grained sediment between day 6 and day 20 there was a significant increase in both oxygen concentration at the sediment surface and an increase in O_2 penetration (Table 3.5).There was no effect between days or treatments on calculated oxygen flux at the sediment surface.

Sample sulfide profiles for the fine-grained sediment are shown in Figure 3.7. Visual inspection indicates that concentrations increased closer to the sediment surface creating a "shoulder" in the profile, which existed in all control treatments for both day 6 and day 20. This shoulder was suppressed in 40% of the profiles for *I. obsoleta* on day 6 and for all measured profiles, except for one, on day 20.

3.3.2. Nutrient Fluxes

For the coarse-grained sediments there were few differences in nutrient fluxes between treatments. The only significant difference was dark/light fluxes on day 14 for DIN, NH_4^+, NO_3^- and PO_4^{3-} (Tables 3.6, 3.7, 3.8 and 3.10 respectively) where DIN, NO_3^-, NH_4^+ and PO_4^{3-}had greater nutrient uptake in the light compared to the dark. NH_4^+ fluxes were small over the course of the coarse-grained experiment (range: -0.11 to 0.30 mmol m^{-2} d^{-1}) compared to NO_3^- fluxes (range: -7.31 to -0.06 mmol m^{-2} d^{-1}) thus NO_3^- (Figure 3.10) was the largest contributor to DIN flux. DON fluxes for days 7 and 14 were relatively small (-10 to 8.8 mmol m^{-2} d^{-1}) however, on day 21 DON flux became large and extremely variable (Figure 3.11a). The range for day 21 was between -45 to 278 mmol m^{-2} d^{-1}. There were no significant differences between treatments or dark/light flux for DON (Table 3.9).

The fine-grained sediment hourly NH_4^+ release was significantly greater for *I. obsoleta* for all days, however there were no significant differences between the light and dark (range for

day: 7: 21.6 to 230 µmol m^{-2} per d^{-1}, 14: 19 to 179 µmol m^{-2} d^{-1}, 21: 62 to 178 µmol m^{-2} d^{-1}) (Table 3.7). The daily results show that there was a significantly greater efflux on day 7 and 14 for *I. obsoleta,* yet not for day 21 (Figure 3.9b). On day 7 in the light *I. obsoleta* significantly increased DON efflux relative to the control (Table 3.9). On day 21 PO_4^{3-} uptake was significantly higher for the *I. obsoleta* treatment and in the dark (Table 3.10, Figure 3.12b). In contrast to the coarse-grained sediments, NH4+ contributed more to DIN because it had a comparatively larger efflux (0.55 to 4.4 mmol per m^2 per day). This resulted in *I.obsoleta* releasing more DIN, both daily and hourly, on day 14 (Table 3.6) despite NO_3^- having no statistically significant differences. DIN release was also greater in the light than the dark on day 21.

3.2.4 Sediment Characteristics

There were no significant differences between either NH_4^+ of S^{2-} porewater for any depth (Table 3.11). For NH_4^+ the range of concentrations for each depth was; 1.5 cm: 0-4.9 µM, 3 cm: 0-5.1 µM, 5 cm: 0.1-6.6 µM, 7 cm: 0.5-7.8 µM. For S^{2-} ranges for each depth were; 1.5cm: 0-0.64 mmol, 3 cm: 0-0.85 mmol, 0.2-.8mmol 7 cm: 0.1-1.0 mmol. OM also showed no significant difference between any treatments for either experiment (Table 3.12). For the coarse-grained experiment %OM content in the top centimeter of the sediment ranged from 0.4% to 1.3% and for fine-grained it ranged from 8.4% to 9.9%. In the coarse-grained sediments there was substantial detritus lost in both treatments containing *I. obsoleta* (Table 3.12, Figure 3.14a). On day 14, by visual inspection almost all (>90 % lost) detritus had disappeared and by day 22 when sampling took place nearly all was gone (97%-100% lost). For the coarse-grained there was no significant differences in either chl *a* or phaeopigments (Table 3.12, Figure 3.14b). The sediment

surface color in all microcosms was a patchy black-brown, which was not observed in the fine-grained sediment. In the fine-grained experiment there was no difference in detritus lost (Figure 3.14c), but there was a significant decrease in chl *a* content in the *I. obsoleta* treatments (Figure.14d). There were no significant differences in phaeopigments.

3.4 Discussion

3.4.1 Macrofauna Effects After a Bloom Collapse

These experiments give insight into to how invertebrates in different assemblages and different estuarine environments affect processes in a system immediately following OM accumulation on the sediment surface. In the coarse-grained experiment few substantial differences in nutrient fluxes were found as a result of invertebrate treatments, which contrasts with findings from Doering et al. (1987) who measured a 57% increase in DIN fluxes in the presence of *M. mercenaria* and McLenghan et al. (2011) who observed increased NH_4^+ efflux due to *I. obsoleta*. The most likely explanation for our results has to do with the added component of detritus, which resulted in extreme variability in nutrient fluxes and masked any effects that invertebrates may have had. Some variability may be attributed to the condition of the detritus before it was added, for example some pieces may have been more degraded than others.

Despite the high variability in other fluxes, there were some significant differences in terms of BOC. The combination treatment had increased BOC for day 7 and day 14 compared to the control, most likely because of increased invertebrate respiration due to a greater animal density. On day 21, the substantial increase in BOC indicates that the detritus became more incorporated with the sediment leading to increased aerobic activity. It is likely that the BOC of

the detritus over-rode the effects of the invertebrates when sediment microbes had greater access to the OM.

On day 21, PO_4^{3-}, DON and O_2 fluxes as well as porewater and chl *a* concentrations in the coarse-grained sediment were extremely variable. Some of this variability may have to do with the patchy distribution of detritus across the sediment surface, which may have led to various micro-environments. For example, the patchy discoloration of the sediment indicates that there were some areas within a microcosm with higher MPB, or higher O_2, than others. This would lead to varying oxygen production, and nutrient uptake/release. For treatments with *I. obsoleta*, where all the detritus vanished, variability may have been maintained by *I. obsoleta* creating a heterogeneous environment through burrowing and grazing activities.

Although there were few effects of invertebrates on nutrient flux rates, there were differences between days. The DON and DIN flux results were surprising for this experiment. It was expected that initially there would be a large DON release and subsequent decrease in release as the excess OM became mineralized resulting in a large DIN flux. In microcosm experiments, García-Robledo et al. (2008) saw a large initial increase in nutrient release followed by subsequent decrease and Lomstein et al. (2006) found large fluxes of NH_4^+ in the dark. However, in the coarse-grained experiment DON fluxes remained small on days 7 and 14 and compared to the flux rates on day 21, which were quite higher than other studies have measured in natural sediments (Nowicki and Nixon 1985, Tyler and McGlathery 2003) and extremely variable. The high and variable DON flux on day 21 suggests that this is when some of the detritus became incorporated into the sediment, which is supported by the BOC.

There was a much more consistent effect on BOC and nutrient fluxes due to the presence of *I. obsoleta* in the fine-grained sediment. The higher BOC associated with *I. obsoleta* suggests

that the differences were mostly due to *I. obsoleta* respiration rather than MPB production, except on day 21 when BOC in the light was significantly less than in the dark. This suggests that at this point, the MPB community was active. These results contrast with García-Robledo et al. (2009) who saw an increase in MPB three days after the addition of detritus. Differences between our results, for both sediment types, and their findings may have to do with how well the detritus was incorporated into the sediment or differences in plant material. Godbold et al. (2009) found that algal composition was an important factor when considering decomposition rates.

In the fine-grained sediments, NH_4^+ and PO_4^{3-} fluxes appear to be less influenced by MPB, but rather by *I. obsoleta* activity. One explanation for increased NH_4^+ release is from their excretion. In ongoing work by Yarrington et al. (unpublished) a large NH_4^+ release was observed due to *I. obsoleta* excretion. The greater PO_4^{3-} uptake on day 21 for *I. obsoleta* treatment may be a result of changes in reducing/oxidizing environments caused by *I. obsoleta* activity. PO_4^{3-} effluxes are ultimately controlled by redox conditions in the sediment. If the sediment becomes a reducing environment, PO_4^{3-} is released into the water column as opposed to an oxic environment where PO_4^{3-} is taken up by the sediment (Rozan et al. 2002). Micro-oxygen profile data supports that *I. obsoleta* increases oxygen concentration and suppresses sulfide which would also lead to the binding of PO_4^{3-}. The suppression of sulfide, could be from *I. obsoleta* bioturbation, which temporarily oxygenates the sediment. This is what Aller (1994) referred to as redox oscillation, which may lead to more effective OM breakdown due to the work of both aerobic and anaerobic microbes. Thus, in an environment with fine-grained sediment it appears that nutrient dynamics may be more heavily regulated by *I. obsoleta* metabolic and bioturbation activity rather than MPB.

The O_2 micro-electrode profiles indicate that initial O_2 levels were low at the sediment surface, likely because of the lack of diffusion from the water column, through the detrital mat, to the sediment surface. As a result, *M. mercenaria* may either close up to survive the low O_2 conditions or move closer to the sediment surface (Weissberger et al. 2009), leaving the lower sediment layers anoxic. As oxygen levels recovered and feeding activities resumed, movement in the sediment and siphon extension could lead to higher O_2 concentrations at the sediment surface and increased O_2 penetration, which is indicated by the significant effect of *M. mercenaria* on O_2 concentration and penetration at the sediment surface. This increase in O_2 concentration should allow for more aerobic bacteria activity, resulting in more complete breakdown of OM. The lack of a light-dark difference in BOC, and resulting low GPP for the *M. mercenaria* treatment compared to other treatments, suggests a small role in O_2 dynamics by MPB and illuminates the important role of the bivalves activity in controlling BOC. On the other hand, the lack of similar results in the combination treatment suggests an interspecific interaction. *M. mercearia* has been observed to burrow deeper and retract siphons in response to predators (Smee and Weissburg 2006ab, Doering 1982). Tactile stimulation from *I. obsoleta* may have invoked a similar response leading to reduced *M. mercenaria* influence at the sediment surface.

3.4.2 Context Specific Behavior of Ilyanassa obsoleta

The most startling difference between the two sediment types was how the behavior of *I. obsoleta* changed. In the coarse-grained sediment nearly all the detritus disappeared in cores containing *I. obsoleta,* while chl *a* concentrations where high but remained the same; however, in the fine-grained sediment there were no difference between *I. obsoleta* treatment and control yet there was less chl *a* (Figure 3.13). OM results show no differences at the sediment surface

between treatments with or without *I. obsoleta*, thus we conclude that the mud-snails are consuming the detritus rather than incorporating it into the sediment. This change in food preference could be for a number of different reasons, including changes in behavior at different times of the year, changes in food source preference due to sediment type or nutritional value of the detritus versus MPB. In contrast, Giannotti and McGlathery (2001) found that *I. obsoleta* grazing on live *Ulva lactuca* did not change based on the sediment type and that grazing rates on macroalgae were higher when the algae had a greater N content. In our experiment the *Ulva* detritus was collected at different times in the summer. Earlier in the summer the *Ulva* would have had a higher N and C content making it a better food source for the mud-snails. Microalgae from the organic-rich muddy sediments most likely had a higher N content due to the high nutrient content in these fine-grained sediments.

3.4.3 Future Implications and Conclusions

Godbold et al. (2009) noted that immediately following algal deposition, effects of invertebrates may be masked due to detritus, which may be the case in our experiments because there were little differences observed in nutrient fluxes. Regardless, our findings indicate what may happen due to the presence of invertebrates after the deposition of a large bloom. Increased oxygen penetration and concentration at the sediment surface due to the effects from *M. mercenaria* indicate that once OM has been incorporated into the sediment, there may be more efficient decomposition and nutrient regeneration. Ingalls et al. (2000) used a sub-surface deposit feeding bivalve to investigate its ability to degrade chl *a* from algae and found that because they increase the porosity of the sediment, chl *a* was more efficiently degraded in suboxic conditions. This seems to be a likely pathway for decomposing algae in our bivalve treatment. Similarly in

the *I. obsoleta* treatment where sulfide levels appear depressed there may be more efficient degradation. Kristensen et al. (1991) found that in the presence of inhibitory metabolites, such as sulfide, anaerobic decomposition is inhibited. Suppressed sulfide and increased oxygen penetration allows for increased break down of OM.

Macrofauna behavior is dependent on many environmental factors including the presence of other invertebrates and different sediment types. These experiments show that benthic invertebrates affect oxygen penetration, DIN release, phosphate release, and sedimentary conditions after the collapse of a macroalgae bloom. Although full decomposition of the detritus layer was not the end result in either substrate, oxygen and sulfide profile data suggest that the presence of invertebrates may increase the oxic zone where OM can be more efficiently mineralized. *M. mercenaries'* ability to withstand the onset of hypoxia will ultimately lead to increased OM degradation unless in the presence of an interacting species. *I. obseoltas* effects on OM removal are context specific, thus so are their effects on sediment biogeochemistry.

Coarse Grained Sediment		
Treatment	Density (ind per m^2)	Biomass (g)
Con	---	---
Mer	240	56.1 ± 0.5
Ily	320	19.6 ± 3.0
Both	560	76.4 ± 2.8
Mer	240	56.4 ± 0.3
Ily	320	20.0 ± 2.5
Fine Grained Sediment		
Treatment	Density (ind per m^2)	Biomass (g)
Con	---	---
Ily	320	22.0 ± 0.8

Table 3.1 Overview of treatments for both experiments using coarse-grained and fine-grained sediments. Treatments are control (Con) *Mercenaria mercenaria* (Mer), *Ilyanassa obsoleta* (Ily), and a combination of the two species (Both). Density refers the number of individuals (ind) per meter squared and biomass is the average biomass of each species per microcosm.

Experimental Time Line: Coarse-Grained Sediment	
Day 0	Invertebrate Addition
Day 4	Detritus Addition
Day 6	Oxygen Profiles
Day 7	Nutrient and Oxygen Flux
Day14	Nutrient and Oxygen Flux
Day 20	Oxygen Profiles
Day 21	Nutrient and Oxygen Flux
Day 22	Porewater, Chl *a* and OM sampling
Fine-Grained Sediment	
Day 0	Detritus Addition
Day 1	Invertebrate Addition
Day 6	Oxygen and Sulfide Profiles - Light Only
Day 7	Nutrient and Oxygen Flux
Day14	Nutrient and Oxygen Flux
Day 20	Oxygen and Sulfide Profiles
Day 21	Nutrient and Oxygen Flux
Day 22	Porewater, Chl *a* and OM

Table 3.2 The experimental time line for the coarse-grained and fine-grained experiments. Chl *a* represents chlorophyll *a* concentration, which is a proxy for measuring benthic microalgae, and OM stands for organic matter.

Coarse-Grained Sediment				Fine-Grained Sediment			
Two-Way ANOVA	**df**	**F**	**P**	**Two-Way ANOVA**	**df**	**F**	**P**
BOC (hourly)				**BOC (hourly)**			
Day 7				**Day 7**			
Treatment	**3**	**6.62**	**0.004**	Treatment	**1**	**11.64**	**0.009**
Dark/Light	1	0.30	0.593	Dark/Light	1	0.45	0.522
Treatment x Dark/Light	3	2.20	0.128	Treatment x Dark/Light	1	0.10	0.761
Day 14				**Day 14**			
Treatment	**3**	**7.13**	**0.003**	Treatment	**1**	**7.66**	**0.024**
Dark/Light	1	2.02	0.174	Dark/Light	1	0.30	0.598
Treatment x Dark/Light	3	1.30	0.308	Treatment x Dark/Light	1	5.31	0.050
[a]**Day 21**				**Day 21**			
Treatment	3	6.45	0.092	Treatment	**1**	**13.43**	**0.006**
Dark/Light	1	0.26	6.133	Dark/Light	**1**	**11.27**	**0.010**
Treatment x Dark/Light	**3**	**8.60**	**0.035**	Treatment x Dark/Light	1	1.00	0.346
One-Way ANOVA	**df**	**F**	**P**	**One-Way ANOVA**	**df**	**F**	**P**
BOC (daily) & GPP				**BOC (daily) & GPP**			
Day 7				**Day 7**			
Daily	**3**	**4.40**	**0.042**	Dailly	**1**	**16.86**	**0.015**
GPP	3	3.85	0.057	GPP	1	2.97	0.160
Day 14				**Day 14**			
Daily	**3**	**5.25**	**0.027**	Daily	1	6.27	0.067
GPP	3	2.17	0.169	GPP	1	0.22	0.665
Day 21				**Day 21**			
Daily	3	0.20	0.891	Daily	**1**	**9.30**	**0.045**
[b]GPP	**3**	**4.64**	**0.037**	[b]GPP	1	0.29	0.622

[a]Friedman's Two-Way ANOVA

[b]Log Transformation

Table 3.3 Results of two-way ANOVA for hourly benthic oxygen consumption (BOC) rates and one-way ANOVA for daily BOC rates for coarse-grained and fine-grained experiments. Bold values indicates significance ($p<0.05$).

Coarse Grained Sediment			
	df	**F/χ^2**	**P**
Day 6 Light			
[a]O_2 Extinction	3	3.68	0.298
O_2 Flux	3	3.13	0.066
O_2 Concentration at Surface	3	1.84	0.193
Day 6 Dark			
O_2 Extinction	3	2.67	0.128
O_2 Flux	3	3.70	0.070
O_2 Concentration at Surface	3	3.55	0.076
Day 20 Light			
[b]O_2 Extinction	**3**	**5.33**	**0.015**
[a]O_2 Flux	3	0.41	0.937
O_2 Concentration at Surface	**3**	**6.37**	**0.008**
Day 20 Dark			
bO_2 Extinction	3	0.09	0.966
O_2 Flux	3	0.62	0.614
O_2 Concentration at Surface	**3**	**38.77**	**<0.001**
Fine Grained Sediment			
	df	**F/χ^2**	**P**
Day 6 Light			
[a]O_2 Extinction	1	0.01	0.934
[a]O_2 Flux	1	0.00	1.000
[a]O_2 Concentration at Surface	1	0.25	0.618
Day 20 Light			
O_2 Extinction	**1**	**5.79**	**0.037**
[a]O_2 Flux	1	2.08	0.150
O_2 Concentration at Surface	1	0.51	0.490
Day 20 Dark			
O_2 Extinction	1	2.79	0.128
O_2 Flux	1	2.69	0.132
O_2 Concentration at Surface	1	0.42	0.532

[a]Kruskal Wallis Test

[b]Log Transformation

Table 3.4 One-way ANOVA or Kruskal Wallis test results for significant differences between treatments with oxygen (O_2) profile measured on day 6 and day 20 in both the light and the dark. Parameters tested include O_2 extinction depth, calculated O_2 flux of the sediment and calculated O_2 concentrations at the sediment surface. Bold values are significant ($p<0.05$).

Coarse Grained Sediment			
	df	**F/χ^2**	**P**
Light			
[a]O_2 Extinction	**1**	**5.33**	**0.021**
[a]O_2 Flux	1	1.00	0.317
O_2 Concentration at Surface	**1**	**21.46**	**0.002**
Dark			
O_2 Extinction	1	4.08	0.090
O_2 Flux	1	2.49	0.190
[b]O_2 Concentration at Surface	**1**	**14.86**	**0.018**
Fine Grained Sediment			
	df	**F/χ^2**	**P**
Light			
[a]O_2 Extinction	**1**	**5.56**	**0.018**
[a]O_2 Flux	1	1.47	0.226
[a]O_2 Concentration at Surface	**1**	**4.76**	**0.029**

[a]Kruskal Wallis Test

[b]Log Transformation

Table 3.5 One-way ANOVA or Kruskal Wallis test results for significant differences between O_2 profile measurements on day 6 and day 20. Parameters tested include O_2 extinction depth, calculated O_2 flux and calculated O_2 concentrations at the sediment surface. Bold values indicate significance ($p<0.05$).

Coarse-Grained Sediment				Fine-Grained Sediment			
Two-Way ANOVA	**df**	**F / χ^2**	**P**	**Two-Way ANOVA**	**df**	**F / χ^2**	**P**
DIN (hourly)				**DIN (hourly)**			
[a]**Day 7**				**Day 7**			
Treatment	3	6.61	0.086	Treatment	1	0.40	0.544
Dark/Light	1	0.18	0.674	Dark/Light	1	1.15	0.314
Treatment x Dark/Light	3	1.80	0.615	Treatment x Dark/Light	1	0.64	0.447
Day 14				**Day 14**			
Treatment	3	1.13	0.365	Treatment	**1**	**5.28**	**0.049**
Dark/Light	**1**	**9.93**	**0.006**	Dark/Light	1	0.15	0.706
Treatment x Dark/Light	3	1.27	0.318	Treatment x Dark/Light	1	0.21	0.655
Day 21				**Day 21**			
Treatment	3	0.03	0.994	Treatment	1	0.96	0.356
Dark/Light	1	3.21	0.095	Dark/Light	**1**	**6.18**	**0.038**
Treatment x Dark/Light	3	0.43	0.737	Treatment x Dark/Light	1	0.75	0.412
One-Way ANOVA	**df**	**F / χ^2**	**P**	**One-Way ANOVA**	**df**	**F / χ^2**	**P**
DIN (daily)				**DIN (daily)**			
Day 7	3	0.26	0.850	**Day 7**	1	0.60	0.480
Day14	3	1.61	0.263	**Day14**	**1**	**8.51**	**0.043**
Day 21	3	0.20	0.891	**Day 21**	1	1.07	0.359

[a]Friedman's Two-Way ANOVA

Table 3.6 Results of two-way ANOVA/Friedman's analysis of variance for hourly dissolved inorganic nitrogen (DIN) flux rates and one-way ANOVA for daily DIN rates for coarse-grained and fine-grained experiments. Bold values indicate significance ($p<0.05$).

Coarse-Grained Sediment				Fine-Grained Sediment			
Two-Way ANOVA	**df**	**F / χ^2**	**P**	**Two-Way ANOVA**	**df**	**F / χ^2**	**P**
NH_4^+ (hourly)				**NH_4^+ (hourly)**			
[a]Day 7				**[b]Day 7**			
Treatment	3	2.12	0.547	Treatment	**1**	**24.45**	**0.001**
Dark/Light	1	1.84	0.175	Dark/Light	1	0.10	0.755
Treatment x Dark/Light	3	0.42	0.936	Treatment x Dark/Light	1	0.92	0.365
[b]Day 14				**Day 14**			
Treatment	3	3.17	0.053	Treatment	**1**	**14.81**	**0.005**
Dark/Light	**1**	**6.60**	**0.021**	Dark/Light	1	1.50	0.256
Treatment x Dark/Light	**3**	**3.46**	**0.041**	Treatment x Dark/Light	1	1.00	0.347
[a]Day 21				**Day 21**			
Treatment	3	4.82	0.185	Treatment	**1**	**10.68**	**0.0114**
Dark/Light	1	1.92	0.166	Dark/Light	1	0.31	0.5937
Treatment x Dark/Light	3	0.08	0.994	Treatment x Dark/Light	1	0.17	0.6919
One-Way ANOVA	**df**	**F / χ^2**	**P**	**One-Way ANOVA**	**df**	**F / χ^2**	**P**
NH_4^+ (daily)				**NH_4^+ (daily)**			
Day 7	3	0.78	0.535	**Day7**	**1**	**10.39**	**0.032**
Day14	3	2.69	0.117	**Day14**	**1**	**8.01**	**0.047**
Day21	3	0.43	0.740	**Day21**	1	6.66	0.061

[a]Friedman's Two-Way ANOVA

[b]Log Transformation

Table 3.7 Results of two-way ANOVA or Friedman's analysis of variance for hourly ammonium (NH_4^+) flux rates and one-way ANOVA for daily NH_4^+ rates for coarse-grained and fine-grained experiments. Bold values indicate significance ($p<0.05$).

Coarse-Grained Sediment				Fine-Grained Sediment			
Two-Way ANOVA	**df**	**F / χ^2**	**P**	**Two-Way ANOVA**	**df**	**F / χ^2**	**P**
NO_3^- (hourly)				**NO_3^- (hourly)**			
[a]Day 7				**Day 7**			
Treatment	3	6.45	0.092	Treatment	1	2.01	0.193
Dark/Light	1	0.01	0.925	Dark/Light	1	1.70	0.229
Treatment x Dark/Light	3	2.48	0.479	Treatment x Dark/Light	1	0.49	0.503
Day 14				**Day 14**			
Treatment	3	0.39	0.763	Treatment	1	0.12	0.742
Dark/Light	**1**	**5.74**	**0.029**	Dark/Light	1	1.38	0.273
Treatment x Dark/Light	3	1.09	0.383	Treatment x Dark/Light	1	0.07	0.793
Day 21				**Day 21**			
Treatment	3	0.03	0.993	Treatment	1	0.83	0.388
Dark/Light	1	2.74	0.120	Dark/Light	1	2.60	0.146
Treatment x Dark/Light	3	0.38	0.767	Treatment x Dark/Light	1	0.41	0.538
One-Way ANOVA	**df**	**F / χ^2**	**P**	**One-Way ANOVA**	**df**	**F / χ^2**	**P**
NO_3^- (daily)				**NO_3^- (daily)**			
Day 7	3	0.56	0.656	**Day 7**	1	3.70	0.127
Day 14	2	0.37	0.774	**Day 14**	1	0.12	0.747
Day 21	3	0.06	0.981	**Day 21**	1	0.71	0.448

[a]Friedman's Two-Way ANOVA

Table 3.8 Results of two-way ANOVA or Friedman's analysis of variance for hourly nitrate (NO_3^-) flux rates and one-way ANOVA for daily NO_3^- rates for coarse-grained and fine-grained experiments. Bold values indicate significance ($p<0.05$).

Coarse-Grained Sediment				Fine-Grained Sediment			
Two-Way ANOVA	**df**	**F / χ^2**	**P**	**Two-Way ANOVA**	**df**	**F / χ^2**	**P**
DON (hourly)				**DON (hourly)**			
Day 7				**Day 7**			
Treatment	3	0.37	0.777	Treatment	1	0.54	0.482
Dark/Light	1	1.21	0.288	Dark/Light	1	0.34	0.574
Treatment x Dark/Light	3	0.35	0.788	Treatment x Dark/Light	**1**	**15.70**	**0.004**
Day 14				**Day 14**			
Treatment	3	0.11	0.952	Treatment	1	0.02	0.896
Dark/Light	1	2.06	0.171	Dark/Light	1	2.32	0.166
Treatment x Dark/Light	3	1.07	0.388	Treatment x Dark/Light	1	0.01	0.900
[C]**Day 21**				**Day 21**			
Treatment	3	3.08	0.062	Treatment	1	0.25	0.632
Dark/Light	1	1.37	0.261	Dark/Light	1	0.23	0.648
Treatment x Dark/Light	3	3.27	0.053	Treatment x Dark/Light	1	0.63	0.451
One-Way ANOVA	**df**	**F / χ^2**	**P**	**One-Way ANOVA**	**df**	**F / χ^2**	**P**
DON (daily)				**DON (daily)**			
Day 7	3	0.32	0.813	**Day 7**	1	6.73	0.060
Day 14	3	0.14	0.934	**Day 14**	1	0.02	0.901
Day 21	3	2.09	0.190	**Day 21**	1	0.27	0.631

[a]Friedman's Two-Way ANOVA

[C]Square Root Transformaiton

Table 3.9 Results of two-way ANOVA for hourly dissolved organic nitrogen (DON) flux rates and one-way ANOVA for daily DON rates for coarse-grained and fine-grained experiments. Bold values indicate significance ($p<0.05$).

Coarse-Grained Sediment				Fine-Grained Sediment			
Two-Way ANOVA	**df**	**F / χ^2**	**P**	**Two-Way ANOVA**	**df**	**F / χ^2**	**P**
PO_4^{3-} (hourly)				**PO_4^{3-} (hourly)**			
[a]Day 7				**[a]Day 7**			
Treatment	3	0.45	0.721	Treatment	1	0.44	0.524
Dark/Light	1	3.07	0.099	Dark/Light	1	0.02	0.903
Treatment x Dark/Light	3	0.06	0.980	Treatment x Dark/Light	1	2.20	0.176
Day 14				**Day 14**			
Treatment	3	0.04	0.988	Treatment	1	0.04	0.838
Dark/Light	**1**	**8.49**	**0.010**	Dark/Light	1	1.65	0.234
Treatment x Dark/Light	3	1.54	0.242	Treatment x Dark/Light	1	0.80	0.394
Day 21				**Day 21**			
Treatment	3	0.34	0.797	Treatment	**1**	**13.43**	**0.006**
Dark/Light	1	1.52	0.238	Dark/Light	**1**	**11.27**	**0.010**
Treatment x Dark/Light	3	0.84	0.494	Treatment x Dark/Light	1	1.00	0.346
One-Way ANOVA	**df**	**F / χ^2**	**P**	**One-Way ANOVA**	**df**	**F / χ^2**	**P**
PO_4^{3-} (daily)				**PO_4^{3-} (daily)**			
Day7	3	0.26	0.852	**Day7**	1	0.23	0.658
Day 14	3	0.10	0.959	**Day 14**	1	0.00	0.963
Day 21	3	0.20	0.893	**Day 21**	**1**	**8.30**	**0.045**

[a]Friedman's Two-Way ANOVA

Table 3.10 Results of two-way ANOVA or Friedman's analysis of variance for hourly phosphate (PO_4^{3-}) flux rates and one-way ANOVA for daily PO_4^{3-} rates for coarse-grained and fine-grained experiments. Bold values indicate significance ($p<0.05$).

Coarse Grained Sediment			
	df	**F**	**P**
NH_4^+ Porewater			
[c]1.5 cm	3	0.19	0.900
[c]3 cm	3	1.35	0.324
5 cm	3	2.23	0.162
[c]7 cm	3	0.84	0.508
S^- Porewater			
[c]1.5 cm	3	1.16	0.382
[c]3 cm	3	0.51	0.689
5 cm	3	1.64	0.256
7 cm	3	2.29	0.165

[c]Square Root Transformation

Table 3.11 One-way ANOVA results for pore water ammonium (NH_4^+) and sulfide (S^{2-}) from the coarse grain sediment experiment. There were no significant differences between treatments.

Coarse Grained Sediment			
	df	**F**	**P**
OM			
0-1 cm	3	1.09	0.406
1-2 cm	3	2.02	0.189
2-5 cm	3	1.95	0.211
5-10 cm	3	2.94	0.121
Final Chlorophhyll			
Chl a	3	0.23	0.875
Pheaopigments	3	0.52	0.681
[a]Chl a: Pheaopigments	3	2.28	0.516
[a]**Remaining Detritus**	**3**	**8.78**	**0.030**
Fine Grained Sediment			
	df	**F**	**P**
OM			
0-1 cm	1	0.62	0.475
[a]1-2 cm	1	1.19	0.275
[a]2-5 cm	1	1.19	0.275
5-10 cm	1	0.33	0.599
Final Chlorophhyll			
Chl a	**1**	**18.15**	**0.013**
Pheaopigments	1	0.84	0.412
[b]Chl a: Pheaopigments	1	0.21	0.211
Remaining Detritus	1	2.33	0.202

[a]Kruskal Wallis Test

[b]Log Transformation

Table 3.12 One-way ANOVA or Kruskal Wallis test results for sediment and detritus samples taken on day 22 for the coarse-grained sediment and fine-grained sediments. Tested variables are organic matter (OM), chlorophyll *a* (chl *a*) concentrations, phaeopigment concentrations, ratio of chl *a* to phaeopigments, and remaining detritus weight. Bold values are significant ($p<0.05$).

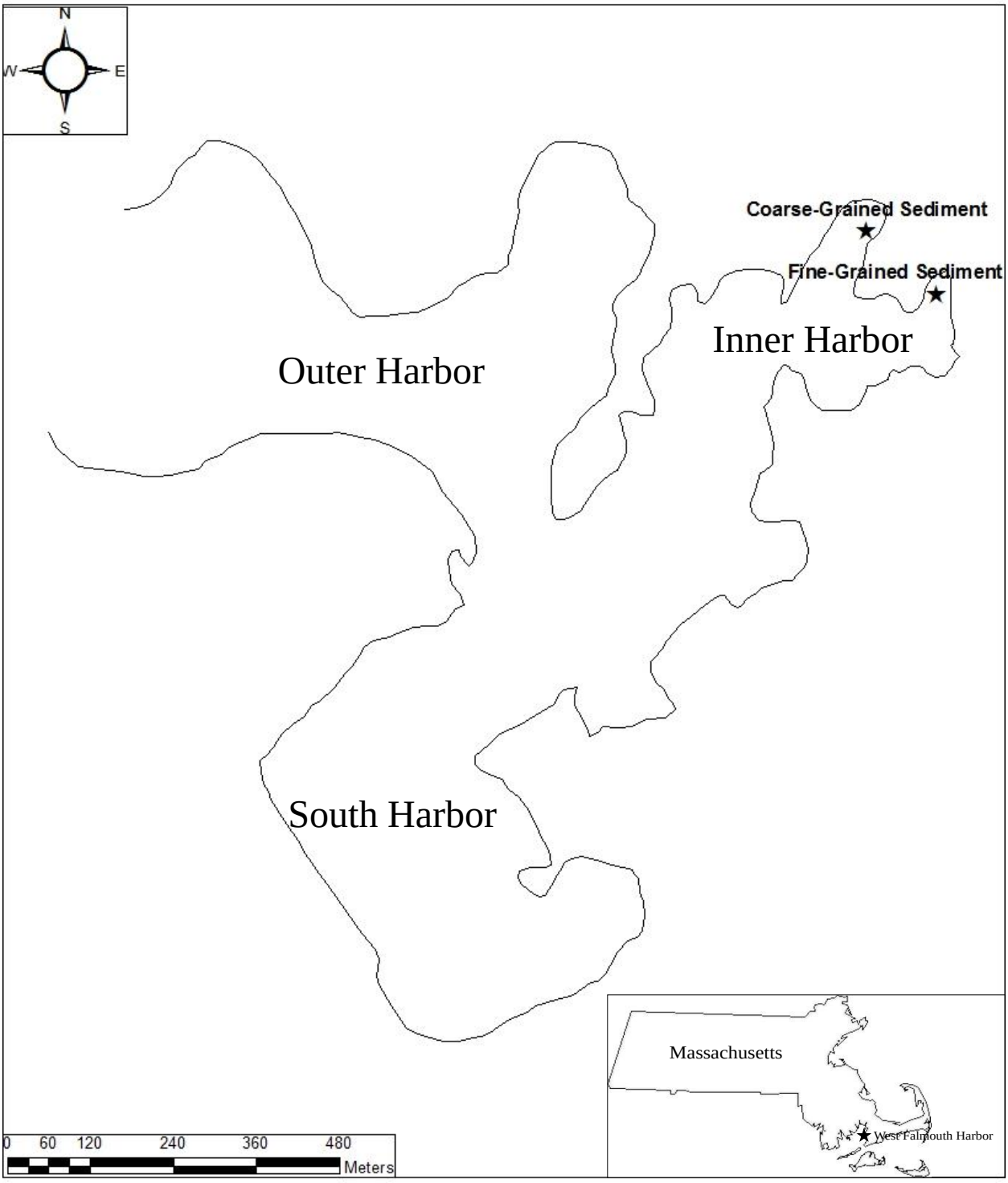

Figure 3.1 Sediment sampling locations for each sediment type in West Falmouth Harbor, MA.

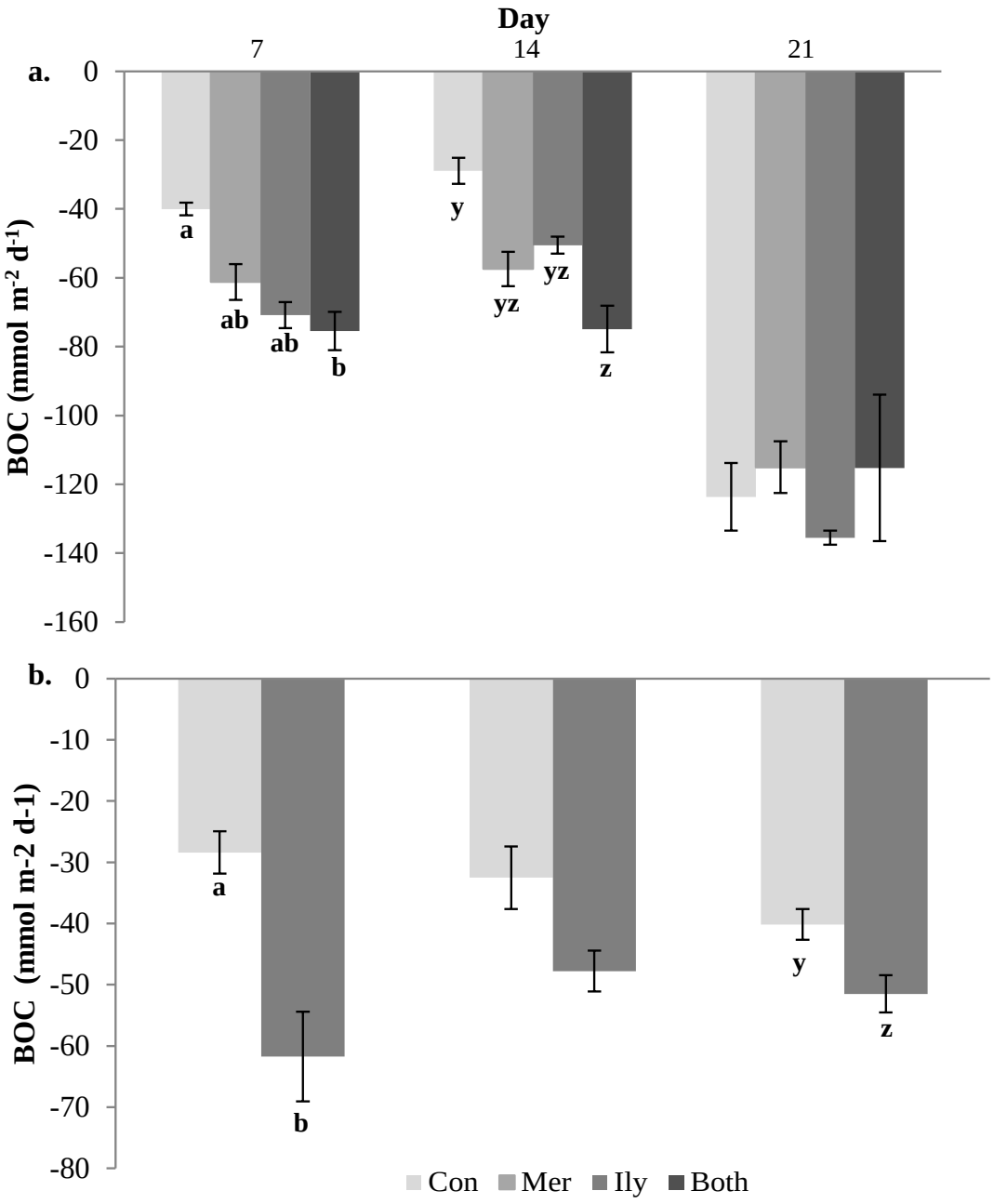

Figure 3.2 Average daily benthic oxygen consumption (BOC) for the (a) coarse-grained and (b) fine-grained sediments (+/- standard error). Treatments were control (Con), *Mercenaria mercenaria* (Mer), *Ilyanassa obsoleta* (Ily) and a combination (Both). Negative fluxes represent sediment uptake. Similar letters indicate statistically similar values within each day ($p<0.05$).

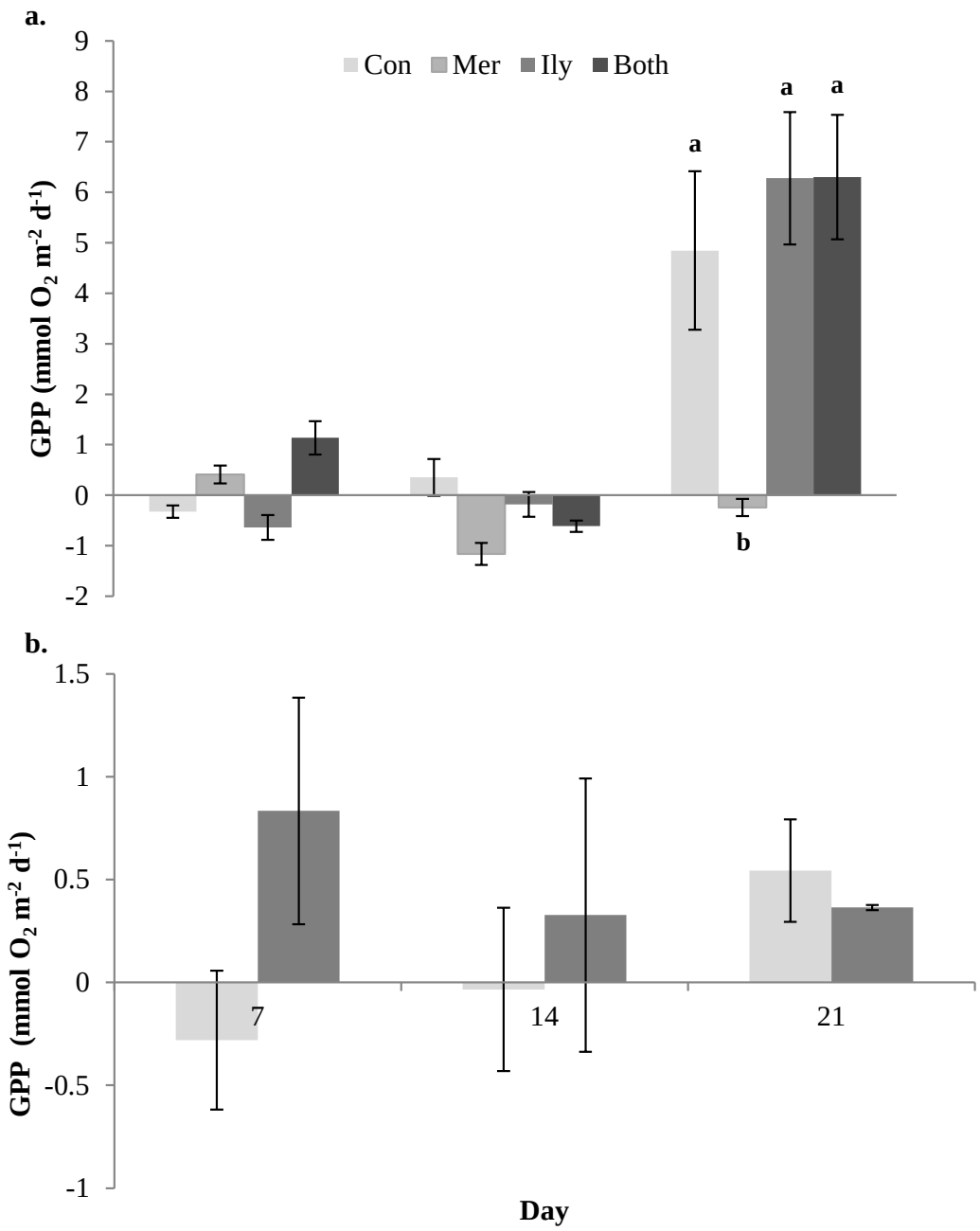

Figure 3.3 Average gross primary production (GPP) for the (a) coarse-grained and (b) fine-grained sediments (+/- standard error). Treatments were control (Con), *Mercenaria mercenaria* (Mer), *Ilyanassa obsoleta* (Ily) and both species (Both). Similar letters indicate statistically similar values within each day ($p<0.05$). Positive values represent production.

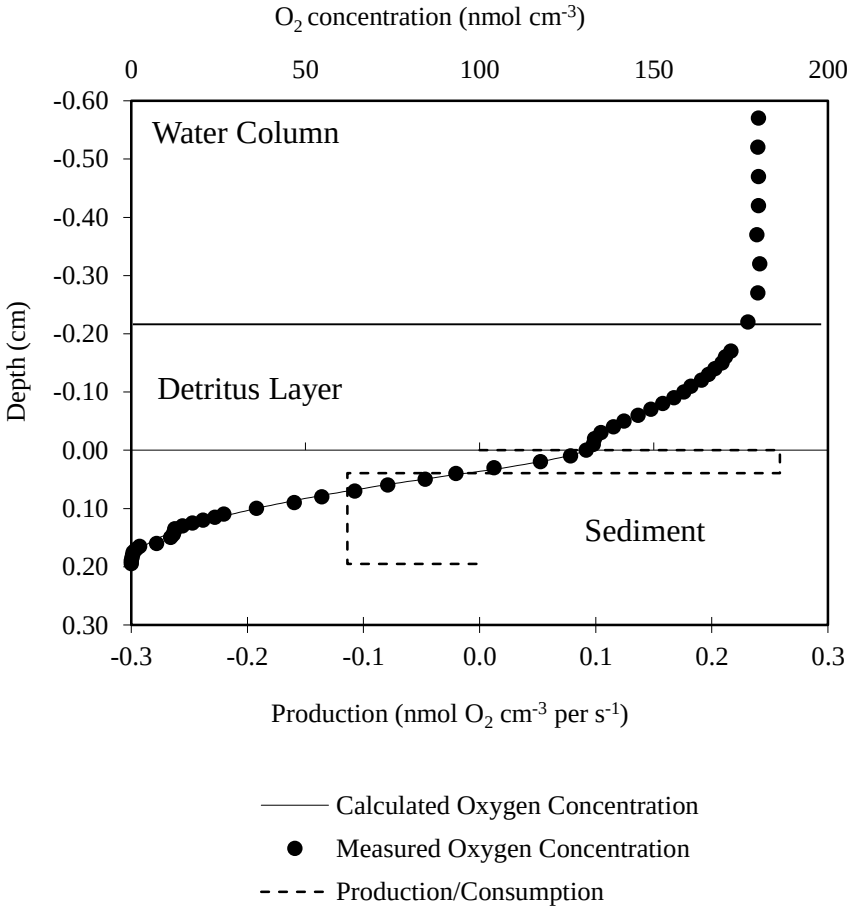

Figure 3.4 Sample of oxygen out from the PROFILE program. Positive values for the production/consumption line represent production and negative represent consumption.

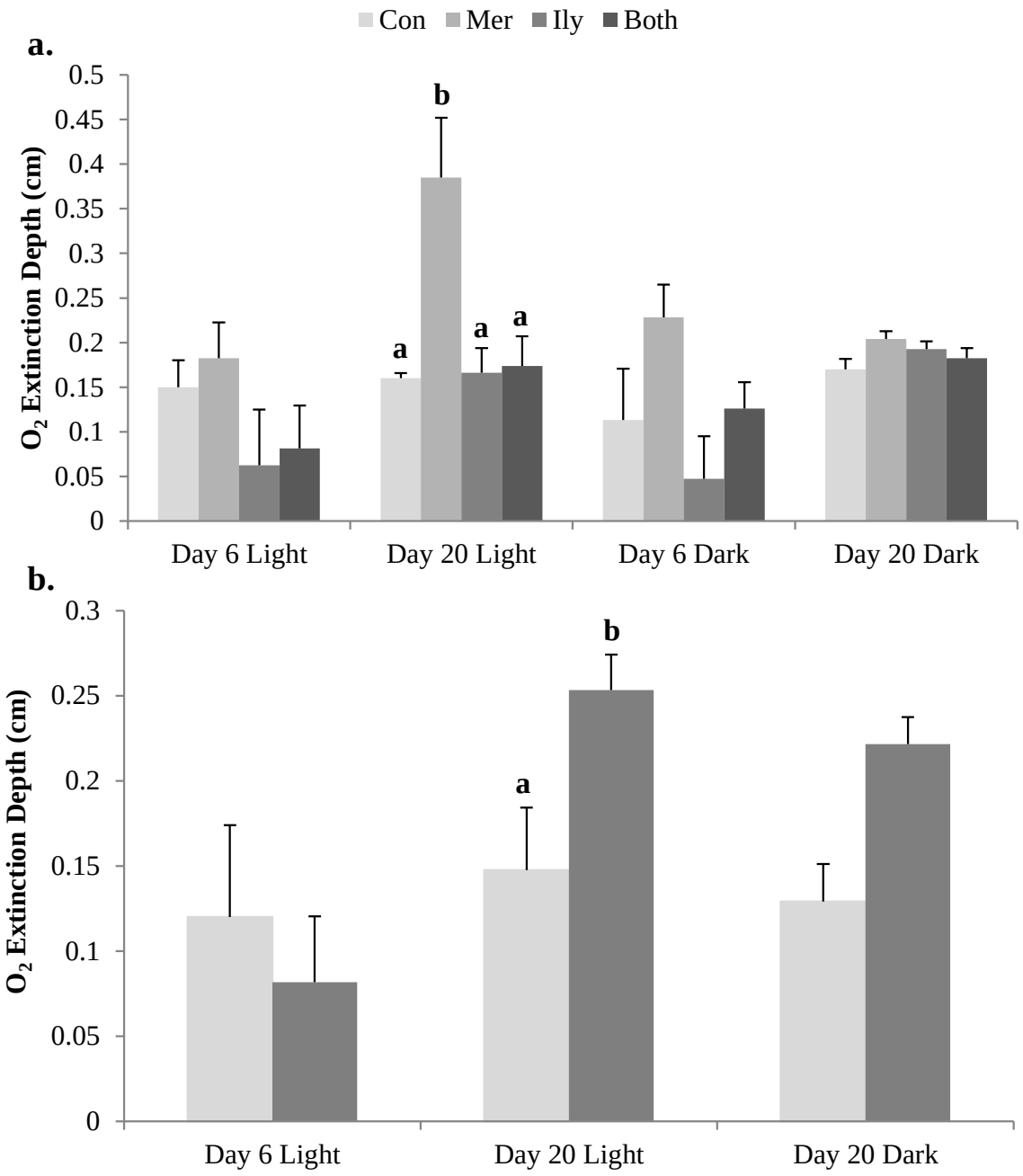

Figure 3.5 Average oxygen (O_2) extinction depths for the (a) coarse-grained and (b) fine-grained sediments (+ standard error). Treatments were control (Con), *Mercenaria mercenaria* (Mer), *Ilyanassa obsoleta* (Ily) and both species (Both). Different letters represent significant differences within each combination of day and dark/light ($p<0.05$)

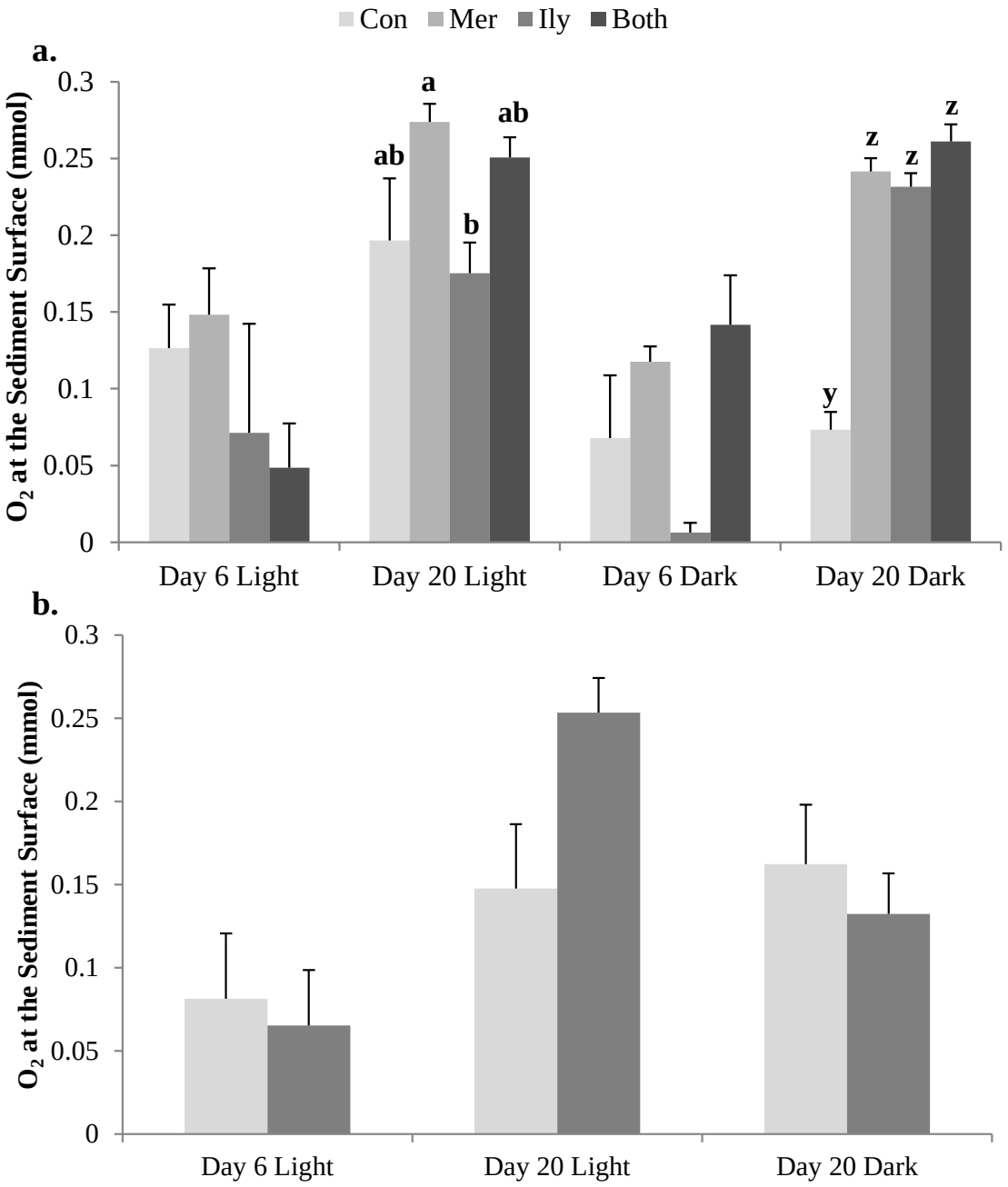

Figure 3.6 Average calculated oxygen (O_2) concentration at the sediment surface for the (a) coarse-grained and (b) fine-grained sediments (+ standard error). Treatments were control (Con), *Mercenaria mercenaria* (Mer), *Ilyanassa obsoleta* (Ily) and both species (Both). One-way ANOVAs were run for each day. Letters represent significant treatments within a day ($p<0.05$) for one-way ANOVA.

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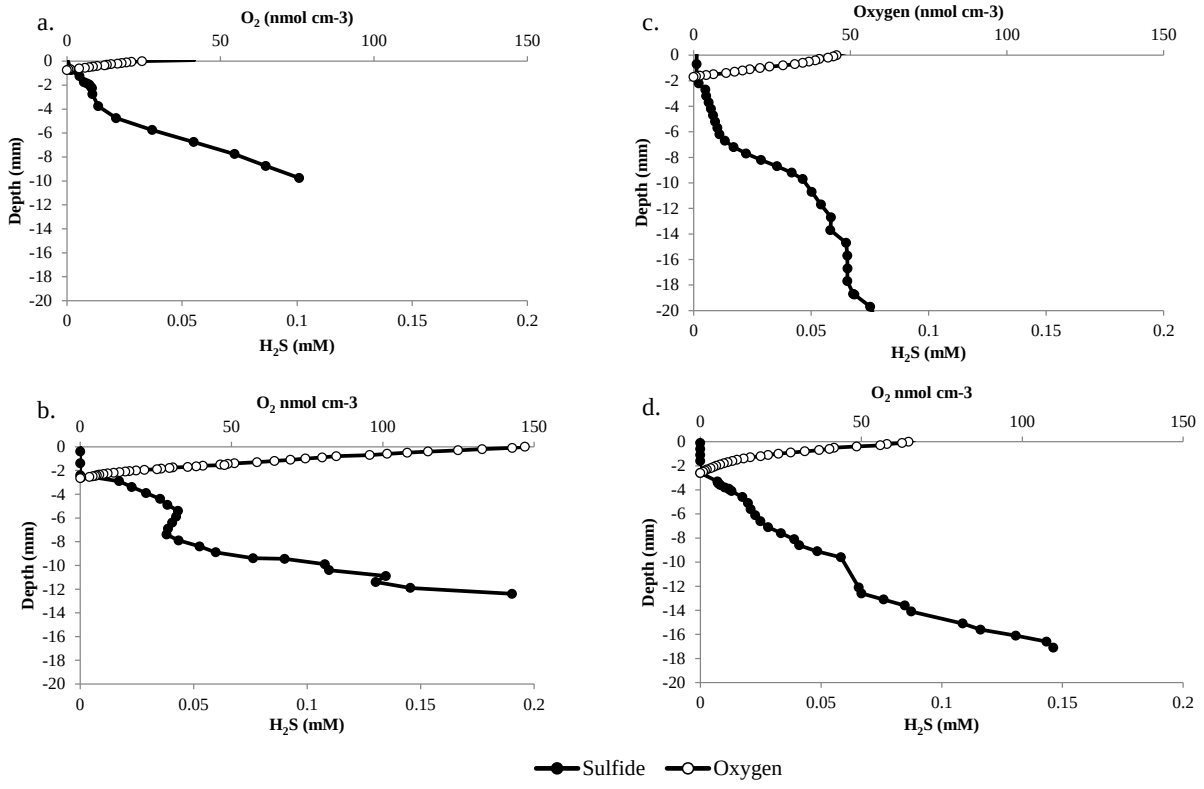

Figure 3.7 Measured oxygen (O_2) and hydrogen sulfide (H_2S) concentrations for fine-grained sediment. On top are (a) control and (b) *Ilyanassa obsoleta* profiles taken on Day 6. On bottom are (c) control and (d) *Ilyanassa obsoleta* profiles taken on day 20.

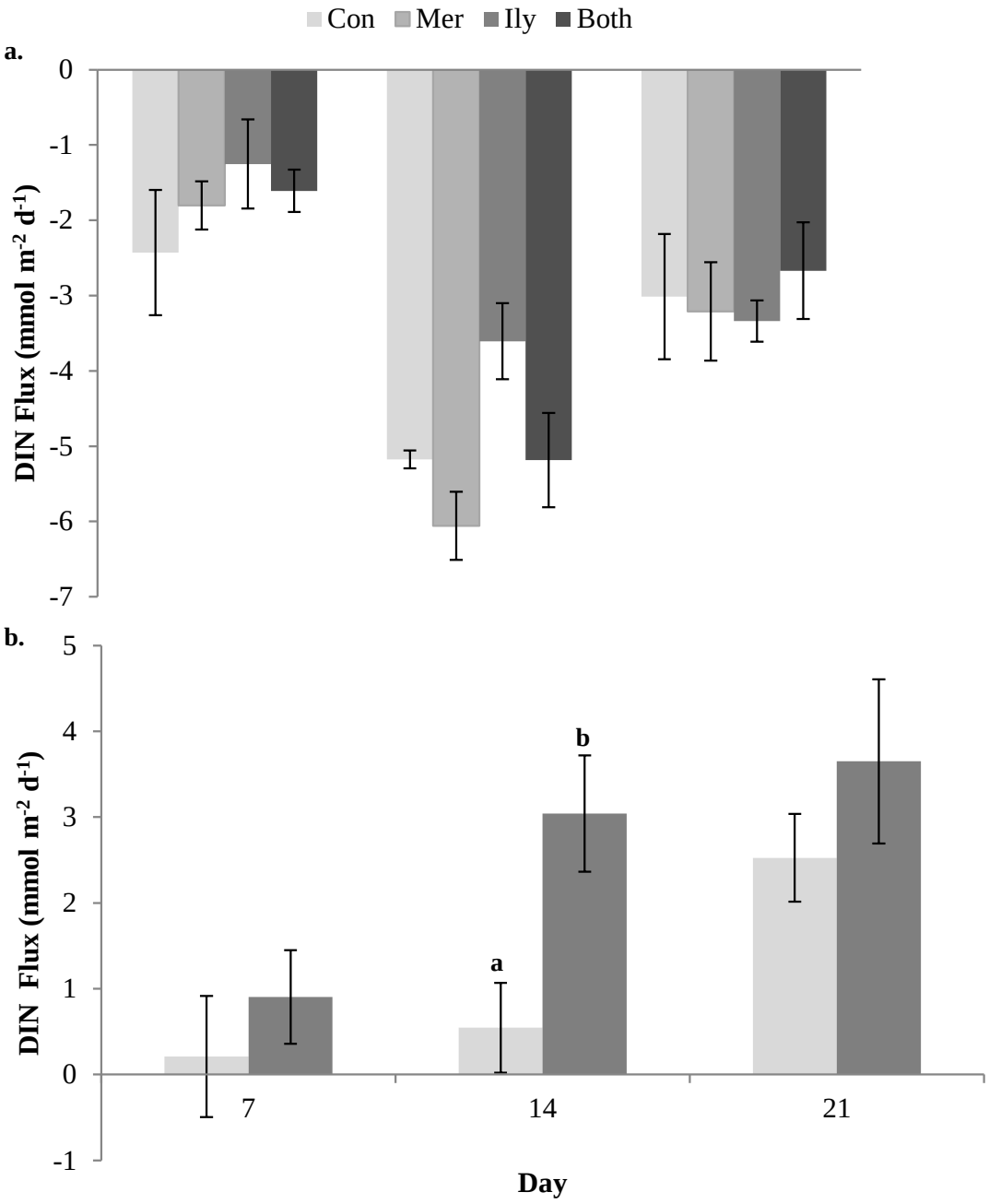

Figure 3.8 Average dissolved inorganic nitrogen (DIN) daily flux for the (a) coarse-grained and (b) fine-grained sediments (+/- standard error). Treatments were control (Con), *Mercenaria mercenaria* (Mer), *Ilyanassa obsoleta* (Ily) and both species (Both). Similar letters indicate statistically similar values within each day ($p<0.05$). There were no significant differences for the coarse-grained sediment. Negative values indicated sediment uptake and positive values indicate sediment release.

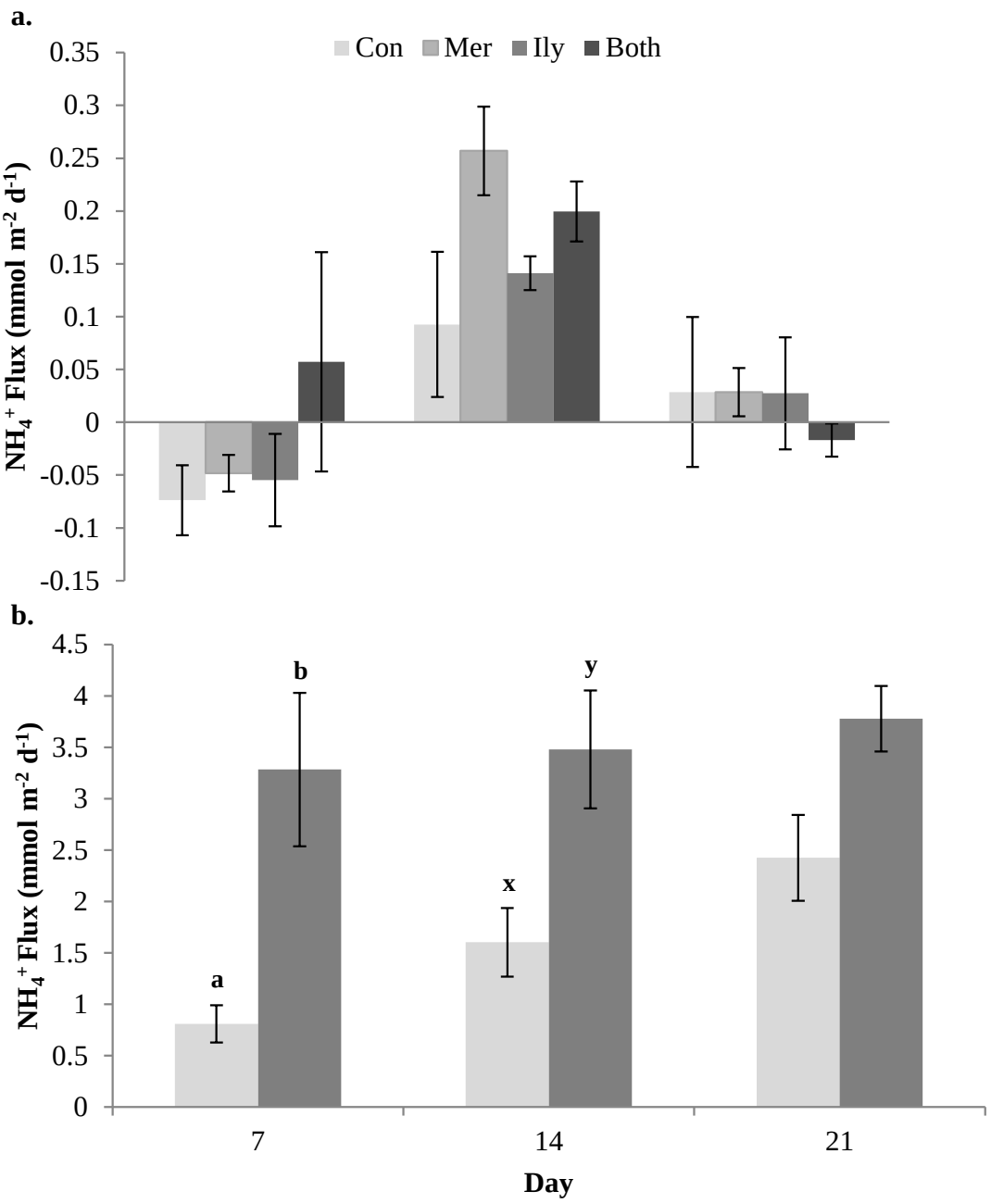

Figure 3.9 Average ammonium (NH_4^+) daily flux for the (a) coarse-grained and (b) fine-grained sediments (+/- standard error). Treatments were control (Con), *Mercenaria mercenaria* (Mer), *Ilyanassa obsoleta* (Ily) and both species (Both). Similar letters indicate statistically similar values with each day ($p<0.05$). There were no significant differences for the coarse-grained sediment between treatments for any day. Negative values indicated sediment uptake and positive values indicate sediment release.

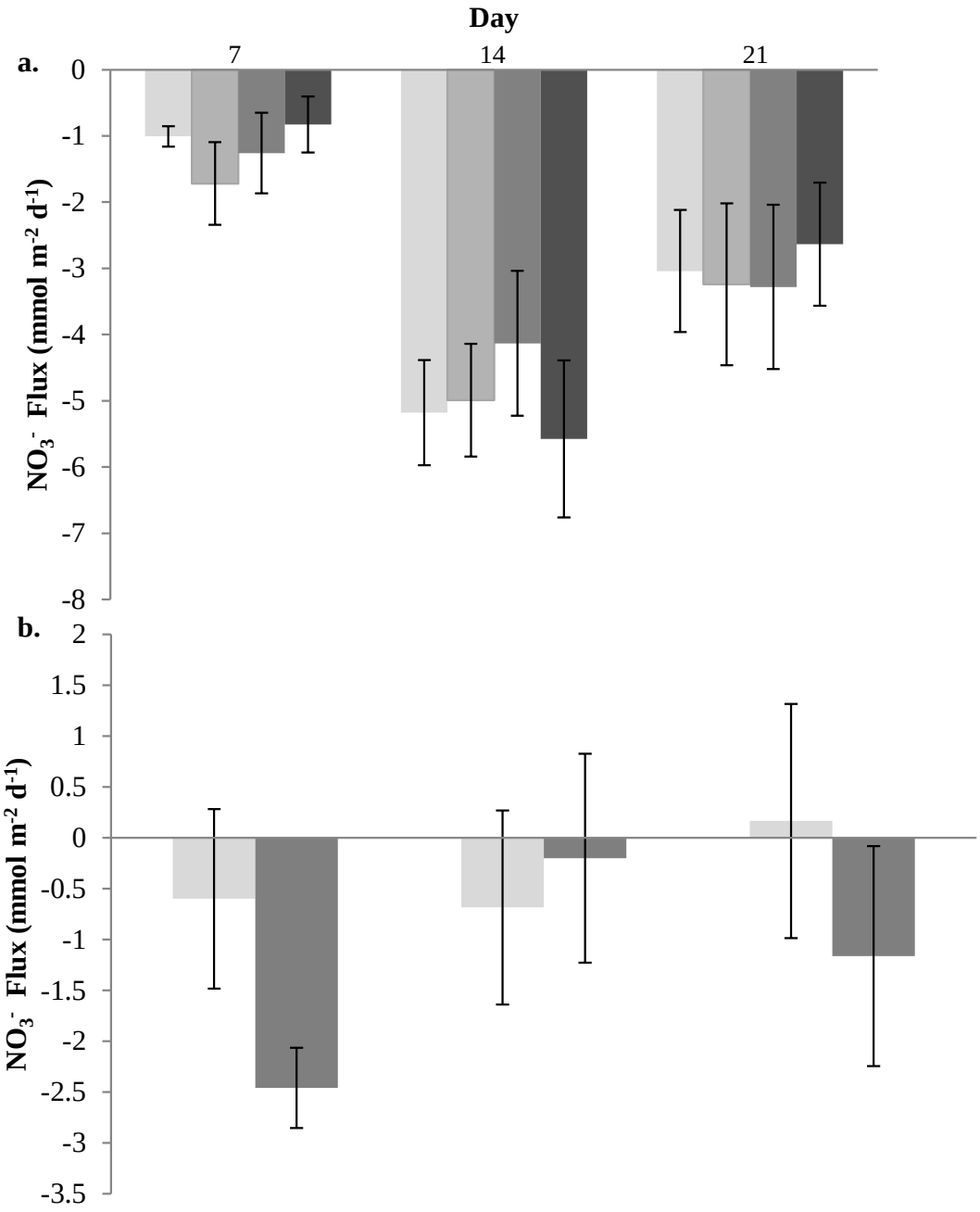

Figure 3.10 Average nitrate (NO_3^-) daily flux for the (a) coarse-grained and (b) fine-grained sediments, (+/- standard error). Treatments were control (Con), *Mercenaria mercenaria* (Mer), *Ilyanassa obsoleta* (Ily) and both species (Both). There were no significant differences for the coarse-grained or fine-grained sediment between treatments for any day ($p<0.05$). Negative values indicated sediment uptake and positive values indicate sediment release.

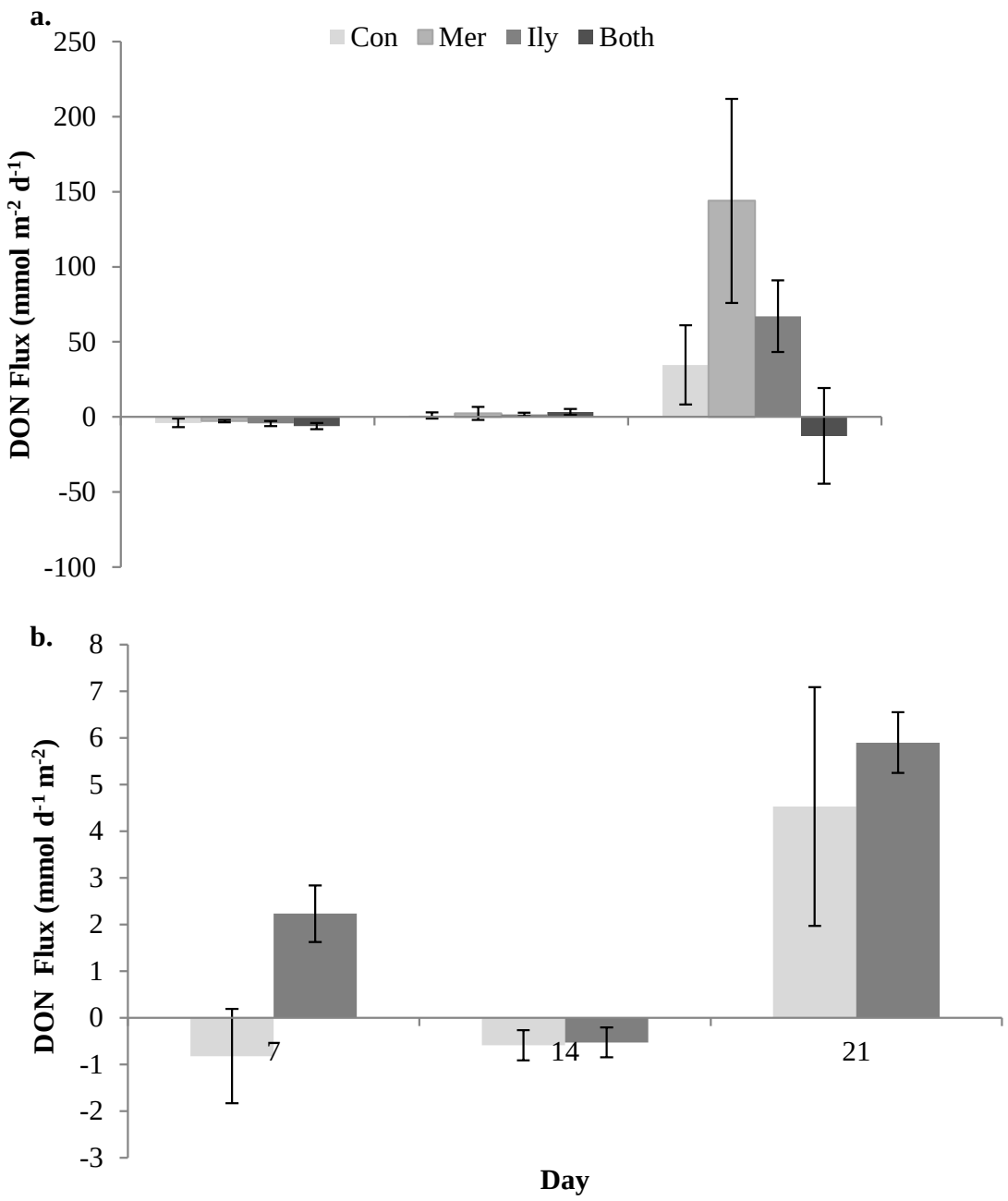

Figure 3.11 Average dissolved organic nitrogen (DON) daily flux for the (a) coarse-grained and (b) fine-grained sediments (+/- standard error). Treatments were control (Con), *Mercenaria mercenaria* (Mer), *Ilyanassa obsoleta* (Ily) and both species (Both). There were no significant differences between days or between treatments ($p<0.05$).

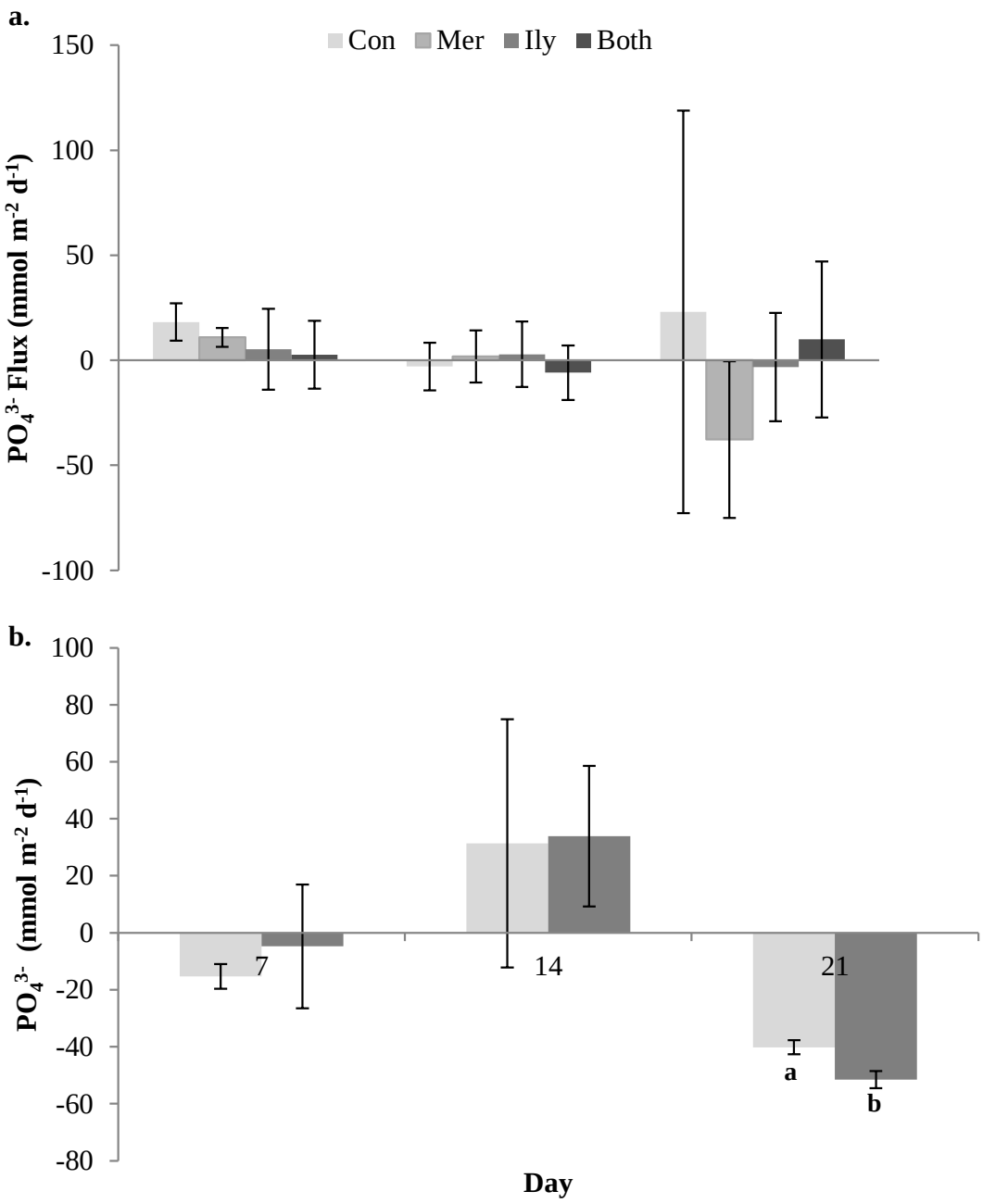

Figure 3.12 Average phosphorous (PO_4^{3-}) daily flux for the (a) coarse-grained and (b) fine-grained sediments (+/- standard error). Treatments were control (Con), *Mercenaria mercenaria* (Mer), *Ilyanassa obsoleta* (Ily) and both species (Both). Similar letters indicate statistically similar values within each day ($p<0.05$). There were no significant differences for the coarse-grained sediment. Negative values indicated sediment uptake and positive values indicate sediment release.

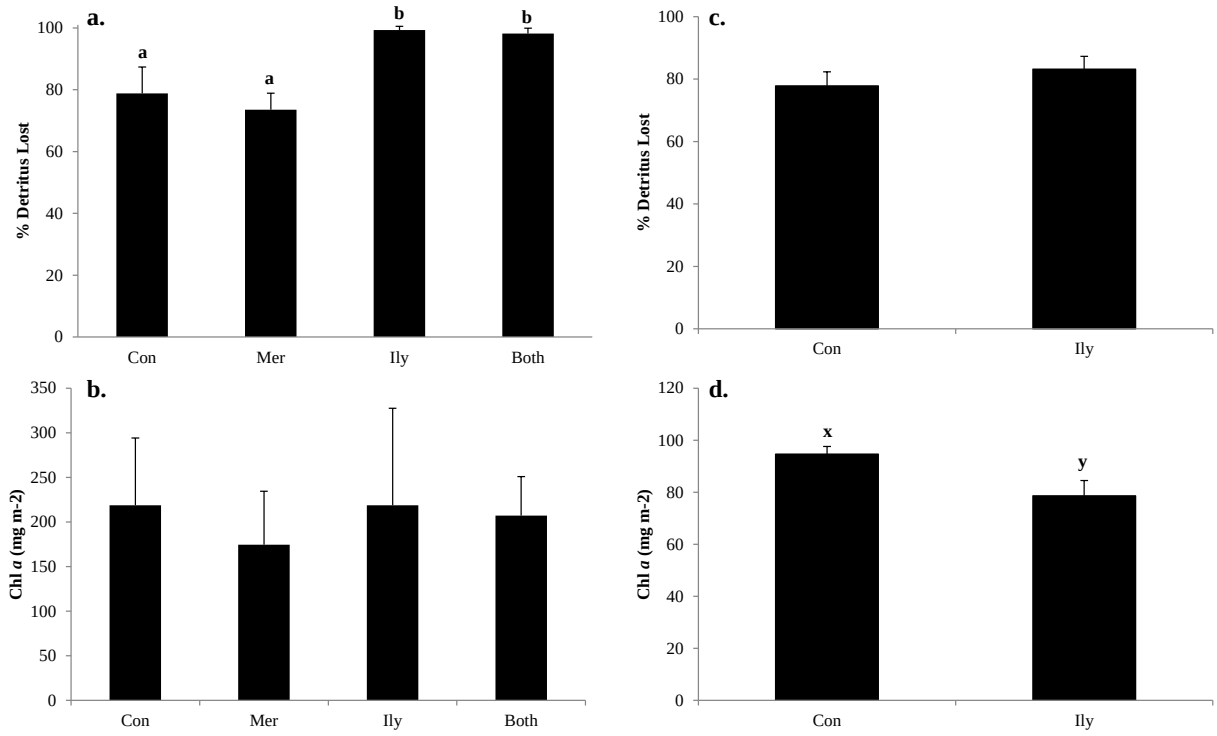

Figure 3.13 Coarse-grained sediment remaining detritus and chlorophyll *a* (chl *a*) concentration (a and b respectively) and fine grain experiment remaining detritus and chlorophyll a (chl *a*) concentration (c and d respectively). Treatments include control (Con) *Mercenaria mercenaria* (Mer) *Ilyanassa obsoleta* (Ily) and combination treatment (Both). Different letters represent significant differences ($p<0.05$). What is clear is that I. obsoleta removes detritus in the coarse-grained sediment and reduces chl *a* concentration in the fine-grained sediments.

Chapter Four

Non-consumptive effects of predators alter the ability of invertebrates to modify sediment biogeochemistry and benthic microalgal abundance

Introduction 4.1

Eutrophication is a major threat to many shallow estuarine ecosystems. Anthropogenic increase in nutrient supply results in an explosion of opportunistic macroalgal species leading to organic matter (OM) accumulation (Bricker et al. 1999, Conley et al. 2009). Subsequent decay of OM results in lower dissolved oxygen due in heightened aerobic activity (Valiela 1992, Bricker et al. 1999, Conley et al. 2009). Loss of predators, who are unable to survive the lowered oxygen levels, change prey populations and ultimately ecosystem functions (Solan et al. 2004, Lotze et al. 2006, Diaz and Rosenberg 2008).

Predators exert consumptive and non-consumptive controls on prey populations. Consumptive effects are the direct lethal effects of predation, while non-consumptive effects refer to behavioral and morphological changes that occur to avoid predation (Lima 1998, Peckarsky et al. 2008). A number of marine studies have discussed the effects of predation on phenotypic and behavioral change (i.e. Doering 1982, Leonard 1999, O'Connor 2008). Consequences of non-consumptive predation on benthic invertebrates include increased burrowing depths (Doering 1982, Whitlow 2009), increased shell thickness (Leonard 1999), reduced size (Nakaoka 2000), reduced feeding (Smee and Weissburg 2006ab, Marie et al. 2010) and increased siphon length (Whitlow 2009).

While the investigation of non-consumptive effects of predators on prey behavior and morphology has been extensive, relatively few studies have explored how changes in behavior may affect sediment biogeochemistry and nutrient mineralization. Marie et al. (2010) found that bioturbation depth increases when *Macoma balthica*, a deposit feeding bivalve, is in the presence of a predatory shrimp. This bioturbation activity can significantly alter conditions in the sediment because bioturbating organisms play an important role as ecosystem engineers (Levinton 1995),

and their activity leads to redistribution of OM and porewater (PW) and alterations in the oxic/anoxic boundary with important implications for OM degradation and nutrient cycling (Kristensen et al. 1991, Sun et al. 1993, Aller 1994, Sun and Dai 2005). This study showed that in a predator's absence there is decreased bioturbation and alterations in OM degradation processes. Similarly, O'Connor et al. (2008) speculated that loss of predators would lead to changes in sediment OM content because the presence of predatory crabs suppressed settlement of oyster larvae, which produce pseudo-feces. They suggest that loss of predators may allow for more settlement, ultimately leading to increased sediment OM.

This study investigates how the eastern mud snail *Ilyanassa obsoleta* and the hard shelled clam *Mercenaria mercenaria* change nutrient release and sediment characteristics in the presence of the predatory crab, *Panopeus herbstii*. *Mercenaria mercenaria* react to the presence of a predators by increasing their burrow depth (Doering 1982) and reduced feeding time (Smee and Weissburg 2006a,b). *I. obsoleta* show increased "alarm" when presented with the scent of a predator and turn away from the offensive odor (Rahman et al. 2000). We hypothesized responses to chemical cues would include increased burrowing and avoidance behavior resulting in increased sediment oxygen penetration and release of inorganic nutrients from the sediment. On the other hand, we also hypothesized that reduced feeding would leave microphytobenthos intact, resulting in a reduced flux of sediment derived nutrients to the water column.

Methods 4.2

4.2.1 Sediment Collection and Microcosm Construction

Sediment, invertebrates and *Ulv*a sp. were collected from West Falmouth Harbor located in the southwestern portion of Cape Cod, MA during August of 2011. West Falmouth Harbor is a

shallow estuary with a mean water depth of 0.6 m (Howes et al. 2006). Development has increased in the watershed surrounding the south embayment of the harbor, potentially decreasing habitat quality. The mean grain size of the harbor in this region is 1.0 mm with an average composition of 0.1% gravel, 90.3% sand and 9.6% mud (Scheiner 2011).

Surface sediments to about 10 cm depth were collected using a 9.5 cm (I.D.) x 30 cm (ht) polycarbonate tube and sectioned at depths of 0-2 cm, 2-5 cm and 5-10 cm. Sediment from each section was homogenized separately and large invertebrates and gravel removed using a 1 mm sieve. Microcosms were reconstructed in cores with the dimensions of 9.5 cm (I.D.) x 30 cm (ht) giving a surface area of 0.071 m^2. Sediment depth profiles were maintained during final construction. Each microcosm had approximately 1 L of headspace. Microcosms were maintained in a "Living Stream" flowing seawater system with artificial seawater (28-32 ppt, 18-22ºC and 10 hours dark 14 hours light cycle). Mechanical bubbles were introduced in each microcosm to allow for water circulation. The microcosms were allowed to acclimate for one month.

4.2.2 Experimental Set-Up

The mud crab, *P. herbstii*, was chosen as the predator species because it is common in West Falmouth Harbor and preys on both *M. mercenaria* and *I. obsoleta. P. herbstii* preys on *M. mercenaria* by either crushing or slowly chipping away at the shell (Wheston and Eversol 1981). They have also been observed to consume *I. obsoleta* by reaching into the shell to access the snail and pick away at the operculum (pers. obs.). Five treatments (control, *M. mercenaria*, *I. obsoleta*, *M. mercenaria* and *P. herbstii* effluent *and I. obsoleta* with *P. herbstii* effluent) with

four replicates each were set-up, with densities based on field observations (McLenaghan 2009 and Scheiner 2011) (Table 4.1).

The day before animal additions, 100 g dry weight per m^2 of dried ground *Ulva* sp. was added to each microcosm to simulate OM levels after a bloom collapse based on standing stocks from nearby Waquoit Bay (Hauxwell et al. 1998). Twenty-four hours later *M. mercenaria* and *I. obsoleta* were added to their designated cores. *M. mercenaria* was observed to make sure that they buried and were replaced with more active individuals when they did not.

Animals were allowed to acclimate in microcosms for one week prior to implementing crab treatment. A week following invertebrate additions, all microcosms were raised so that the head space was no longer connected to the surrounding water but were sufficiently submerged to allow temperatures within the microcosm to be maintained. A series of siphon drip systems was set up to allow crab effluent into the designated cores, but not to nearby cores. A schematic of this is shown in Figure 4.1. A drip was set up so that seawater would flow from a reservoir into two 8.3 l containers, one containing no animals and another containing 5 *P. herbstii* (10-12 mm carapace width). *P. herbstii* individuals were kept in separate screened containers within the larger one to prevent fighting and mortality. Flow from the containers was through Tygon tubing controlled with a gang valve so that flow was at a rate of 1 ml per minute, allowing for total headspace water volume exchanged approximately every 17 hours. Excess water spilled over the top of the microcosms and a siphon system was used to maintain the overall water level. Smee and Weissburg (2006b) found that *M. mercenaria* only responded to chemical cues from blue crabs that had been fed as well as damaged conspecifics. Thus each *P. herbstii* was fed twice a week with approximately 1 g ww frozen *M. mercenaria* tissue. Two water samples were taken

from the containers with and without *P. herbstti* to test for nutrient concentrations during acclimation period (Table 4.2).

4.2.3 Micro-electrode measurements

Twenty days after the addition of *P. herbstii* effluent micro-electrode measurements were taken glass oxygen (O_2) micro-electrodes (OX-50, UNISENSE, Aahrus, Denmark) with a diameter of 25 µm were used to measure micro-scale O_2 concentrations using a manual micromanipulator. The oxygen probe was connected to a pico-ammeter (PA2000, UNISENSE, Aarhus, Denmark) calibrated by measuring the O_2 concentration in the water column with a Hach HQ40d meter (LDO101 probe) and the reading at the bottom of the profile, which was assumed to be completely oxygen-depleted. Three profiles were measured in the light and dark for each treatment. Because the depth at which the probe crossed the sediment-water interface could not be determined visually due to the small tip size, this boundary was determined by looking at the slope of the resulting oxygen profile. Using the PROFILE model developed and explained in Berg (1998), production and consumption boundaries were calculated based on the slope of the line, as well as calculated oxygen flux, calculated O_2 concentration at the sediment surface and O_2 extinction depth.

4.2.4 Oxygen and Nutrient Flux Measurements

Oxygen and nutrient flux measurements were taken 21 days after the addition *P. herbstii* effluent according to the methods of Tyler et al. (2001). Prior the start of the flux, two-thirds of the overlying water was gently siphoned off and replaced with fresh artificial seawater. Replacement water for *P. herbstii* treatments was comprised of 50% effluent containing water

and 50% fresh seawater. The microcosm were sealed with a gas tight polycarbonate lid with a butyl rubber o-ring so that there was no gas exchange. One rotating Teflon® coated magnet was used to mix the water column at approximately 60 rpm. Measurements were taken every 1-2 hours for a total of 5 time points, the first three in the dark and the remaining two in the light. Oxygen measurements were made with a Hach HQ40d meter (LDO101 probe) and water samples were taken with a 60 cc syringe for ammonium (NH_4^+), nitrate (NO_3^-), total nitrogen (TN), and phosphate (PO_4^{3-}) analyses. Water was then replaced with a known fill volume.

All water samples were immediately filtered with a Supor TM 0.45µm membrane filter and frozen at – 20ºC. $NO_3^- + NO_2^-$, PO_4^{3-} and TN were analyzed on a Lachat Quikchem 8500 autoanalyzer with the cadmium reduction, molybdate complex and in-line digestion methods respectively (Lachat 2003). Ammonium was analyzed according to Solorzano (1969) using the phenol-hypochlorite method. Dissolved organic nitrogen (DON) concentrations were calculated by subtracting NO_3^-+ NO_2^- and NH_4^+ from TN. Dissolved inorganic nitrogen (DIN) was calculated by adding $NO_3^- + NO_2^-$ and NH_4^+ concentrations. Hourly benthic oxygen consumption (BOC) and nutrient flux rates were calculated based on changes in concentration over time (Tyler et al. 2001). A 14 hour light, 10 hour dark period was used.

4.2.5 Porewater, Chlorophyll a*, and Organic Matter*

Porewater (PW) NH_4^+ samples were taken according to Berg and McGlathery (2001). A 2 mm stainless steel probe was used to extract 2.5 ml of water at depths of 2 cm, 5 cm, 7 cm, and 10 cm. PW was immediately filtered with a 0.45 µm membrane filter. NH_4^+ samples were frozen at - 20ºC until analyzed according Solorzano (1969). Chl *a* samples, which are used as a proxy for microphytobenthos (MPB) biomass, were taken with a modified 5 cc syringe to the depth of a

one cm. Sediment was immediately placed in 15 ml centrifuge tubes, darkened and frozen at -80ºC, until analysis within 30 days according Strickland and Parsons (1972). Chl *a* and phaeopigment, which are degraded chl *a* pigments, concentrations were calculated according to Lorenzen (1967). A modified 60 cc syringe was used to collect one OM samples from each microcosm. Sediment was sectioned off at depths of 0-1 cm, 1-2 cm, 2-5cm and 5-10 cm. OM content was determined by loss on ignition procedure described in Heirie et al. (2001).

4.2.6 Data Analysis

All statistical analyses were conducted on SAS® 9.2. Hourly oxygen and nutrient fluxes were analyzed using a two-way ANOVA with light/dark fluxes and individually for control + all *M. mercenaria* treatment and control + all *I. obsoleta* treatment as fixed factors. The two sets of invertebrate treatments were run separately because we were primarily interested in effect of effluent rather than differences between the two invertebrates. For data not normally distributed, Friedman's two-way analysis of variance was used. When significant difference was found ($p<0.05$) a Tukey (HSD) was run. Hourly light/dark measurements were analyzed separately from the daily rates to observe the effect of treatments on MPB activity. Daily oxygen and nutrient fluxes were analyzed with a one-way ANOVA for control + all *M. mercenaria* treatment and control + all *I. obsoleta* treatments. When significant effects were found a contrast analysis was run. Porewater, chl *a* and OM were tested for significance between treatments with a one-way ANOVA or a Kruskal Wallis test for non-normally distributed data. O_2 output parameters from the PROFILE model were analyzed for significant difference between treatments with either a one-way ANOVA or Kruskal-Wallis test.

4.3 Results

4.3.1 Benthic Oxygen Consumption and Micro-Oxygen Profiles

As anticipated, treatments with invertebrates had significantly higher daily benthic oxygen consumption (BOC) relative to controls (Table 4.3, Figure 4.2 and Figure 4.3). Dark BOC was significantly greater than light BOC in all treatments, but there were no significant interactions between treatments and dark/light flux rates (Table 4.4). Figure 4.4 shows representative samples of O_2 profiles measured in the light for the various invertebrate treatments. O_2 penetration depth (range: 1-3.4mm) and concentration at the sediment surface (0.19-0.30 mmol) showed no significant differences between treatments in the dark or the light. The effects of *M. mercenaria* were evident in the calculated oxygen flux at the sediment surface in the light and in the dark, where *I. obsoleta* only had effects in the dark (Table 4.5). Calculated O_2 uptake (Figure 4.5a) remained high for the *M. mercenaria* treatment and was significantly higher for *M. mercenaria* and effluent in the dark, as opposed to *M. mercenaria* alone. In the dark *I. obsoleta* significantly increased sediment oxygen uptake however there were no significant effects in the light (figure 4.5b).

4.3.2 Nutrient Fluxes

The most substantial difference in nutrient fluxes was observed for *I. obsoleta* NH_4^+ (Table 4.3 and 4.4). There was a significant treatment effect on daily and hourly fluxes as well as increased NH_4^+ release in the dark (0.9 to 7.6 µmol m^{-2} h^{-1}) compared to the light (0 to 3.0 µmol m^{-2} h^{-1}), however, there was no effect of an interaction between treatment and dark/light fluxes. Figure 4.6 shows daily NH_4^+ release. *I. obsoleta* is significantly higher than the than the control and the treatment with effluent ($p< 0.001$, $p=0.001$ respectively) and the effluent treatment is

significantly higher than the control (p=0.002). There were no significant effects of *I. obsoleta* on either hourly light/dark fluxes or daily fluxes of NO_3^- (-92 to 32 µmol m^{-2} h^{-1}) or PO_4^{3-} (-0.9 to 1.0 µmol m^{-2} h^{-1}).

Hourly nutrient fluxes for *M. mercenaria* treatments compared to control had substantial differences between the dark and the light (Table 4.4). For *M. mercenaria* there was increased NH_4^+ release in the dark (0.9 to 3.1 µmol m^{-2} h^{-1}) than in the light (0 to 2.0 µmol m^{-2} h^{-1}) however, there were no significant differences between the two treatments and control for hourly or for daily fluxes. In the light there was also increased NO_3^- uptake (-146 to 21.4 µmol m^{-2} h^{-1}) compared to dark (-41 to 116 µmol m^{-2} h^{-1}), however there were no differences between treatments. PO_4^{3-} flux rates in the light (-0.8 to 0.3 µmol m-^{2} h^{-1}) were greater than in the dark (-0.5 to 0.7 µmol m^{-2} h^{-1}), but again no significant daily or treatment differences were observed (Table 4.4).

4.3.3 Porewater, Chlorophyll a, and Organic Matter

M. mercenaria had lower PW NH_4^+ concentrations than *M. mercenaria* and effluent and control, yet it was only significantly lower at 2 cm (Figure 4.7). Although not statistically significant there was a pattern in PW NH_4^+ of *M. mercenaria* had the lowest concentrations followed by *M. mercenaria* and effluent and then control with the highest. NH_4^+ seems to be suppressed by *I. obsoleta* in the effluent treatment, however there were no significant differences for *I. obsoleta* PW profiles. *M. mercenaria* significantly decreased the ratio of chl *a* to phaeopigments, although phaeopigment concentrations did not significantly increase nor chl *a* significantly decrease due to the presence of *M. mercenaria* (Table 4.7, Figure 4.8). For *I. obsoleta,* chl *a* was significantly higher compared to the control and *I. obsoleta* and effluent was

significantly greater than *I. obsoleta* alone (Table 4.7, Figure 4.9). *I. obsoleta* also significantly increased OM in the presence of *P. herbstii* effluent relative to the control and just *I. obsoleta* treatments (Table 4.8, Figure 4.10). There were no other significant differences in OM although *M. mercenaria* with effluent was higher than *M. mercenaria* and control treatments.

4.4 Discussion

This study showed that not only do single species treatments affect sediment characteristics and the release of nutrients, but that non-lethal predation alters how organisms affect their environment. *M. mercenaria* and *I. obsoleta* both had different effects on biogeochemistry in estuarine sediments depending on the presence or absence of *P. herbstii* effluent.

4.4.1 Macrofauna and Non-Consumptive Predation Effects

The effects of non-lethal predation were much more obvious with *I. obsoleta*, but there were some subtle differences between the *M. mercenaria* treatments. Calculated flux measurements from the PROFILE program give the estimated flux of only the sediment at that particular spot, whereas total microcosm BOC incorporates the sediment as well as animal respiration. All animal treatments in this study significantly increased total microcosm BOC, most likely due to animal respiration. *I. obsoleta* increase in calculated oxygen uptake of the sediment in the dark may have to do with increase the porosity of the sediment due to movement, which allows for greater diffusion and aerobic microbes. Biodeposits from the mud-snail may also stimulate microbial activity (Aller 1982). These affects appear to be masked by MPB in the light. *M. mercenaria* also increased the calculated flux in just the sediment which may be a result

of increased oxygen diffusion into the sediment resulting in increased aerobic bacteria activity. Oxygen flux data suggest that the *M. mercenaria* and effluent are less active at the sediment surface. The increased sediment flux in the effluent treatment maybe a result of increased burrow depths and siphon retraction. The fact that it is only observed in the dark suggests that when there is light, microalgae override the different treatment effects. Increased vertical movement in the sediment has been observed when *M. mercenaria* are in the presence of a predatory starfish (Doering 1982) and less feeding due to non-lethal predation is evident due to smaller sizes in the presence of a predator (Nakaoka 2000). PW NH_4^+ supports this hypothesis that the *M. mercenaria* are less active because there was a significantly lower NH_4^+ concentration in the *M. mercenaria* treatment alone. The increased NH_4^+ at the sediment surface for the effluent treatments may be a result of increased NH_4^+ in the effluent itself (Table 4.2), however, it does support the above conclusion that may have to do with decreased *M. mercenaria* activity at the sediment surface (i.e. greater burrowing depths or less feeding).

While *P. herbstii* effluent did not significantly affect photopigment concentrations, *M. mercenaria* activity resulted in a higher chl *a* to phaeopigment ratio. This is in contrast to McLenaghan et al.'s (2011) findings that benthic invertebrates increase turn-over resulting in a lower chl *a* : phaeopigment ration. On the contrary, Sun and Dai (2005) found that macrofauna increase phaeophytin ***a*****,** which is a component of phaeopigments, in sediments relative to chl *a*. Oscillation of the oxic/anoxic boundary by invertebrates leads to more rapid chl *a* breakdown compared to phaeophytin *a*, which degrades more slowly (Sun et al. 1993, Sun and Dai 2005). This redox oscillation would explain the differences in ratios for the *M. mercenaria* treatment compared to the control. Increased ratios may in part be due to biodeposits from the bivalve (Bianchi 1988, Ingalls et al. 2000).

Similarly, *I. obsoleta* significantly increased phaeopigment concentrations, most likely for the aforementioned reasons. Yet, unlike the *M. mercenaria* treatments there was a significant effect of effluent on *I. obsoletas* ability to control MPB biomass. Despite the higher levels of NH_4^+ in *P. herbstii* effluent we do not think that it is responsible for the increased MPB biomass. TN concentrations were greater in the other tank and the *M. mercenaria* treatments had no significant differences between their MPB biomass and the effluent for the *M. mercenaria* and *I. obsoleta* came from the same tank. *I. obsoleta* in low densities (< 80 individuals m^{-2}) have been known to stimulate microalgae growth, through either fertilization effects or increased nutrient regeneration, and inhibition of growth at higher densities due to increased grazing pressures (Conner et al. 1982). This study found that *I. obsoleta* stimulates MPB growth compared to the control and even more so in the effluent treatment. OM data further supports the conclusion that they increase MPB relative to other treatments because there is a significantly higher OM content just at the sediment surface. Fear of predation led to decreased grazing activity allowing for substantial growth compared to the other snail treatment.

Increased NH_4^+ release in the *I. obsoleta* treatment compared to the one with effluent is most likely due to the result of two processes, one involving MPB activity and two due to differences in metabolic rates. In the light NH_4^+ release was substantially less than the dark, indicating that MPB are actively taking up NH_4^+. Because the effluent treatment had higher MPB there was a decrease in overall NH_4^+ efflux. Metabolic rates may also be affecting release because in ongoing work by Yarrington et al. (unpublished) found that *I. obsoleta* have significant amounts of NH_4^+ in their excreta. The *I. obsoleta* alone treatment had actively grazing snails compared to the effluent treatment as evident by the lowered chl *a*, which should result in increased metabolic rates and increased excreta.

4.4.2 Ecosystem Consequences in Eutrophic Systems

This study found that interspecific interaction between predators and prey can indirectly affect nutrient dynamics in a shallow estuary. Turner and Montgomery (2003) suggest that mobile predators create a "behavioral landscape" as they pass through an area because prey exhibits behaviors that are reversible, for example reduced feeding activity. Reduced feeding for bivalves in the presence of predators happens during the onset of exposure and immediately reverses when the predators are removed (Smee and Weissburg 2006ab, Marie et al. 2010). Bivalves also seem to react more strongly to tactile stimulation or vibrations from movements of a predator. *Macoma balthica* was shown to cease feeding and increase bioturbation activity when in the same physical location as a predatory shrimp, even if the shrimp was not actively foraging, possibly due to their ability to sense their movement. There were little effects due to chemical cues alone (Marie et al. 2010). Smee and Weissburg (2006ab) showed that feeding decreased in close proximity to a predator, however not to an empty crab carapace, just upstream of the bivalve's location. This implies that *M. mercenaria* may respond more strongly to a predator's movement, although we did observe decreased activity with effluent. In the field this "behavioral landscape" theory could mean more efficient degradation of OM because repeated siphon retraction would increase porosity, which allows increased nutrients to sediment microbes (Aller and Aller 1992). Loss of predators indicates that there would be increased feeding, which would allow for decreased movement of nutrients between the sediment water interface and decreased sediment oxygen uptake, implying that there would be a greater accumulation of OM at the sediment surface.

I. obsoleta are extremely sensitive to the scent of predators (Rahman et al. 2000). Our experiment implies that when unable to escape an area with a high density of predators, they are

more likely to hide and become less active. *I. obsoleta* have previously been shown to facilitate the growth of macroalgae through grazing on MPB which release sediment-derived nutrients (McLenghan et al. 2011) and snail excreta alone has been shown to promote macroalgae biomass (Yarrington et al. unpub). The presence of crab effluent clearly affected the behavior of *I. obsoleta*. Loss of predators in areas with *I. obsoleta* would lead to increased macroalgal growth and greater release of nutrients to the water column. Thus, the loss of predators during eutrophication may be a positive feedback that enhances sediment nutrient release and leads to further proliferation of algal blooms.

Macrofauna			
Treatment	**Replicates**	**Density (ind m^{-2})**	**Biomass (g)**
Con	4	---	---
Mer	4	215	14±0.5
Ily	4	290	7.2±0.5
Mer and Effluent	4	215	13.8±0.3
Ily and Effluent	4	290	7.1±0.7

Table 4.1 Macrofauna size and density for each treatment. Density is in units of individuals (ind) per m^{-2}. Biomass is average per microcosm (+/- standard deviation). Treatments were: control (Con), *Mercenaria mercenaria* (Mer), *Ilyanassa obsoleta* (Ily), *M. mercenaria* and effluent (Mer and Effluent), and *I. obsoleta* and effluent (Ily and Effluent).

Average Nutrient Concentration				
	TN	**NO_3^-**	**NH_4^+**	**PO_4^{3-}**
Tank w/ *P.herbstii*	164 ± 21	126	0.63 ± 0.20	0.80
Tank w/o *P. herbstii*	264	146	0.46 ± 0 .01	0.98
Initial Average Concentration w/ *P.herbstii*	134 ± 29	116 ± 20	0.73 ± 0.29	0.89 ± 0.11
Initial Average Concentration w/o *P.herbstii*	129 ± 26	110 ± 19	0.51 ± 0.10	0.89 ± 0.11

Table 4.2 Nutrient concentrations in µM from tanks with and without *Panopeus herbstii*. Tank values are average concentrations for two days during the acclimation period (+/- standard deviation). Initial average concentrations are the first measurement (time point 0) from the headspace during fluxes (+/- standard deviation).

***Mercenaria mercenaria* Daily Fluxes**			
	df	**F**	**P**
BOC	**2**	**9.39**	**0.008**
GPP	2	0.64	0.552
DIN	2	0.31	0.739
NH_4^+	2	2.91	0.112
NO_3^-	2	0.65	0.547
DON	2	0.33	0.728
PO_4^{3-}	2	1.72	0.239
***Ilyanassa obsoleta* Daily Fluxes**			
	df	**F**	**P**
BOC	**2**	**12.09**	**0.004**
GPP	2	0.06	0.939
DIN	2	0.19	0.829
NH_4^+	**2**	**39.05**	**<0.001**
NO_3^-	2	0.17	0.8428
DON	2	0.24	0.789
PO_4^{3-}	2	0.54	0.601

Table 4.3 Results from one-way ANOVA for daily benthic oxygen consumption (BOC), gross primary production (GPP), dissolved inorganic nitrogen (DIN), ammonium (NH_4^+), nitrate (NO_3^-), dissolved organic nutrients (DON), and phosphate (PO_4^{3-}) daily fluxes. Top panel are results from testing for significance between control, *Mercenaria mercenaria* and *M. mercenaria* + *Panopeus herbstii* effluent treatments. Bottom panel are results from testing between control, *Ilyanassa obsoleta*, and *I. obsoleta* + *P. herbstii* effluent treatments. Bold values are significant ($p<0.05$).

Mercenaria mercenaria Hourly Fluxes				*Ilyanassa obsoleta* Hourly Fluxes			
	df	**F**	**P**		**df**	**F**	**P**
BOC				**[b]BOC**			
Treatment	**2**	**4.34**	**0.031**	Treatment	**2**	**4.88**	**0.022**
Dark/Light	**1**	**79.08**	**<0.001**	Dark/Light	**1**	**101.74**	**<0.001**
Treatment x Darl/Light	2	0.92	0.420	Treatment x Darl/Light	2	0.38	0.688
DIN				**DIN**			
Treatment	2	0.33	0.7239	Treatment	2	0.26	0.772
Dark/Light	1	2.03	0.1734	Dark/Light	1	1.36	0.261
Treatment x Darl/Light	2	0.32	0.7301	Treatment x Darl/Light	2	0.88	0.433
NH_4^+				**[b]NH_4^+**			
Treatment	2	2.98	0.079	Treatment	**2**	**14.95**	**<0.001**
Dark/Light	**1**	**7.68**	**0.014**	Dark/Light	**1**	**16.76**	**<0.001**
Treatment x Darl/Light	2	0.65	0.536	Treatment x Darl/Light	2	0.60	0.561
NO_3^-				**NO_3^-**			
Treatment	2	1.91	0.180	Treatment	2	0.26	0.773
Dark/Light	**1**	**4.78**	**0.044**	Dark/Light	1	1.46	0.244
Treatment x Darl/Light	2	0.44	0.652	Treatment x Darl/Light	2	0.88	0.432
DON				**[e]DON**			
Treatment	2	0.11	0.893	Treatment	2	0.03	0.969
Dark/Light	1	1.93	0.185	Dark/Light	1	2.79	0.115
Treatment x Darl/Light	2	1.09	0.362	Treatment x Dark/Light	2	2.33	0.129
PO_4^{3-}				**PO_4^{3-}**			
Treatment	2	0.65	0.534	Treatment	2	0.31	0.739
Dark/Light	**1**	**5.28**	**0.035**	Dark/Light	1	0.47	0.503
Treatment x Darl/Light	2	0.06	0.941	Treatment x Dark/Light	2	1.91	0.330

[b]Square Root Transformation

[e]Cube Root Transformation

Table 4.4 Results from two-way ANOVA for benthic oxygen consumption (BOC), dissolved inorganic nitrogen (DIN), ammonium (NH_4^+), nitrate (NO_3^-), dissolved organic nutrients (DON), and phosphate (PO_4^{3-}) hourly fluxes in the light and dark. Left panel treatments are control, *Mercenaria mercenaria*, and *M. mercenaria +Panopeus herbstii* effluent,and right panel treatments are control, *Ilyanassa obsoleta*, and *I. obsoleta +Panopeus herbstii* effluent. Measurements in the dark and light and interaction of treatment and dark/light were tested significance was tested for significance. Bold values are significant ($p<0.05$).

	df	F/χ^2	P
Light			
O_2 Concentration at the Sediment	2	1.15	0.377
O_2 Flux	**2**	**17.19**	**0.003**
O_2 Extinction Depth	2	0.83	0.480
Dark			
O_2 Concentration at the Sediment	2	0.16	0.855
O_2 Flux	**2**	**29.51**	**0.004**
O_2 Extinction Depth	2	3.96	0.113
Ilyanassa obsoleta			
	df	F/χ^2	**P**
Light			
O_2 Concentration at the Sediment	2	1.33	0.334
O_2 Flux	2	2.18	0.195
O_2 Extinction Depth	2	1.37	0.324
Dark			
O_2 Concentration at the Sediment	2	2.85	0.149
O_2 Flux	**2**	**55.72**	**<0.001**
O_2 Extinction Depth	2	0.06	0.938

Table 4.5 One-way ANOVA or Kruskal Wallis test results from oxygen (O_2) modeled parameters calculated from micro-electrode profiles using PROFILE model. Top shows significance between control, *Mercenaria mercenaria* and *M. mercenaria* + *Panopeus herbstii* effluent treatments and bottom show control, *Ilyanassa obsoleta* and *I. obsoleta* + *P. herbstii* effluent treatments. Bold values are significant ($p<0.05$).

***Mercenaria mercenaria* PW**			
	df	**F**	**P**
Depth			
[b]2 cm	**2**	**8.79**	**0.010**
5 cm	2	23.23	0.094
7 cm	2	0.67	0.538
10 cm	2	2.10	0.185
***Ilyanassa obsoleta* PW**			
	df	**F**	**P**
Depth			
2 cm	2	1.34	0.321
5 cm	2	1.16	0.366
7 cm	2	0.32	0.727
10 cm	2	0.20	0.826

[b]Log Transformation

Table 4.6 One-way ANOVA for porewater (PW) ammonium (NH_4^+) at various depths. Top panel shows significance between control, *Mercenaria mercenaria* and *M. mercenaria* + *Panopeus herbstii* effluent treatments and bottom panel shows results for control, *Ilyanassa obsoleta* and *I. obsoleta* + *P. herbstii* effluent treatments. Bold values are significant ($p<0.05$).

Mercenaria mercenaria			
	df	**F/χ^2**	**P**
[a]**Chl *a***	2	5.56	0.062
[a]**Pheaopigments**	2	5.14	0.076
Chl *a* : Pheaopigments	**2**	**5.64**	**0.030**
Ilyanassa obsoleta			
	df	**F/χ^2**	**P**
Chl *a*	**2**	**18.33**	**0.001**
Pheaopigments	**2**	**7.76**	**0.013**
Chl *a* : Pheaopigments	2	0.99	0.413

[a]Kruskal Wallis Test

Table 4.7 One-way ANOVA or Kurskal Wallis test results from chlorophyll *a* (chl *a*), phaeopigments and ratio of chl *a* to phaeopigments. Top shows significance between control, *Mercenaria mercenaria* and *M. mercenaria* + *Panopeus herbstii* effluent treatments and bottom shows results for control, *Ilyanassa obsoleta* and *I. obsoleta* + *P. herbstii* effluent treatments. Bold values are significant ($p<0.05$).

Mercenaria mercenaria **OM**			
	df	**F**	**P**
Depth			
[b]0 to 1 cm	2	2.22	0.179
1 to 2 cm	2	0.10	0.907
2 to 5 cm	2	0.63	0.562
5 to 10 cm	2	0.85	0.473
***Ilyanassa obsoleta* OM**			
	df	**F**	**P**
Depth			
[b]0 to 1 cm	**2**	**9.90**	**0.013**
1 to 2 cm	2	0.32	0.738
2 to 5 cm	2	0.10	0.909
5 to 10 cm	2	0.53	0.615

[b]Log Transformation

Table 4.8 One-way ANOVA for organic matter (OM) at various depths. Top panel shows significance between control, *Mercenaria mercenaria* and *M. mercenaria* + *Panopeus herbstii* effluent treatments and bottom panel shows results for control, *Ilyanassa obsoleta* and *I. obsoleta* + *P. herbstii* effluent treatments. Bold values are significant ($p<0.05$).

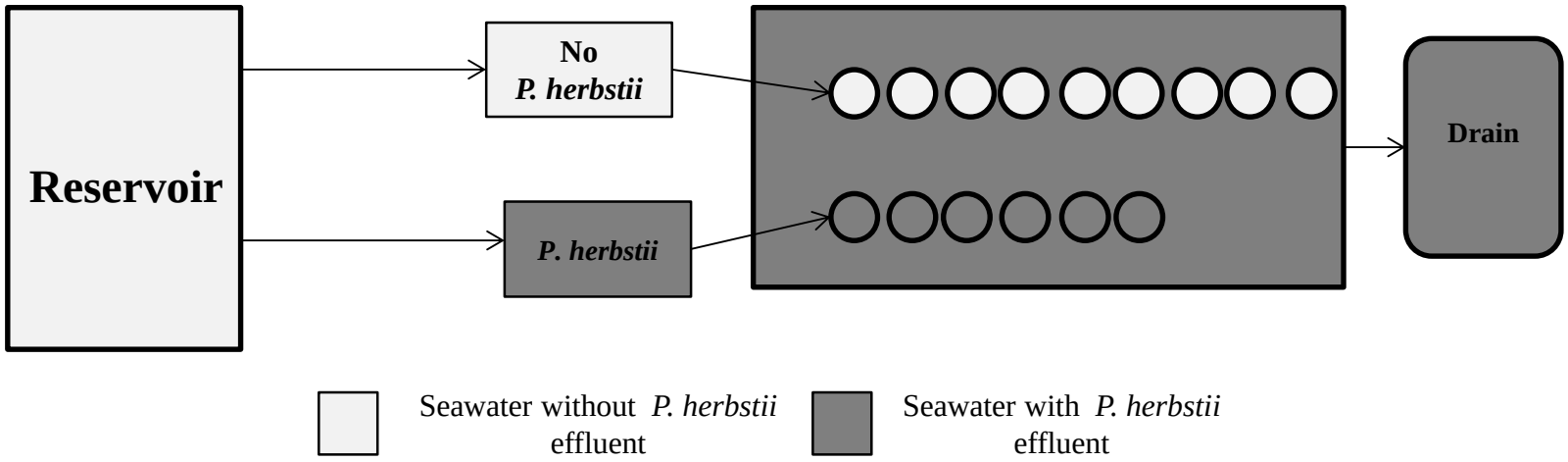

Figure 4.1 Schematic diagram of tank set-up with siphon drip systems. Seawater flowed from the reservoir into designated tanks with or without *P. herbstii*, then into specific cores. Water overflowed from microcosms into a tank whose water level was maintained by a siphon connected to the drain. Note not to scale.

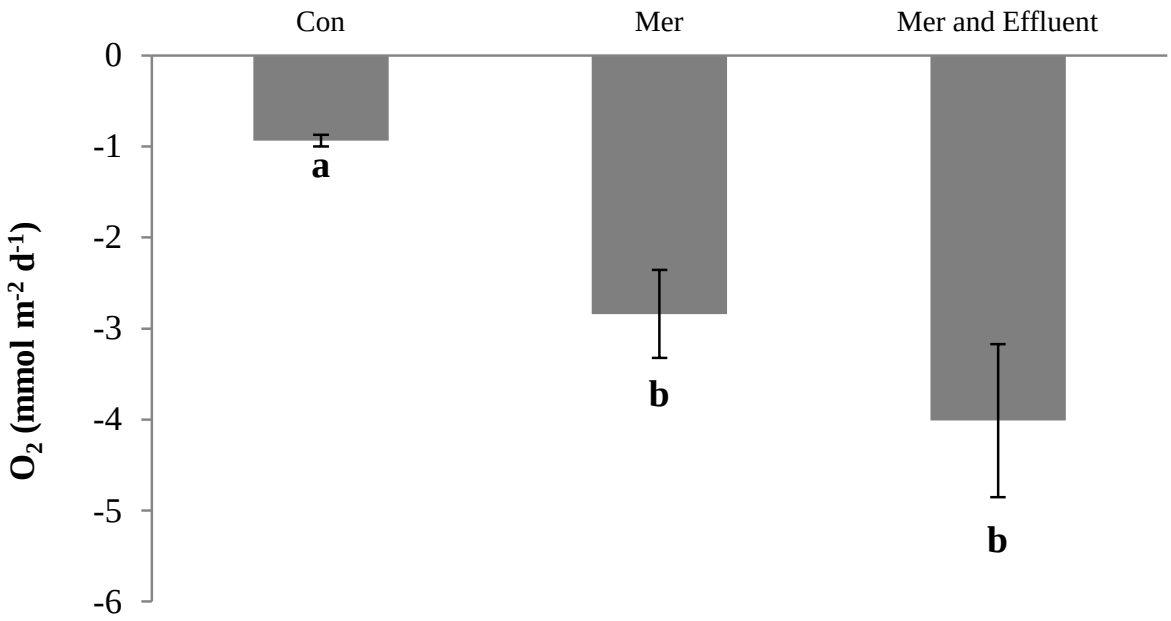

Figure 4.2 Average daily benthic oxygen consumption for control (Con), *Mercenaria mercenaria* (Mer) and *M. mercenaria* + *Panopeus herbstii* (Mer and Effluent) (+/- standard error). Different letters indicate significance ($p<0.05$) between treatments. Negative values represent uptake by the sediment

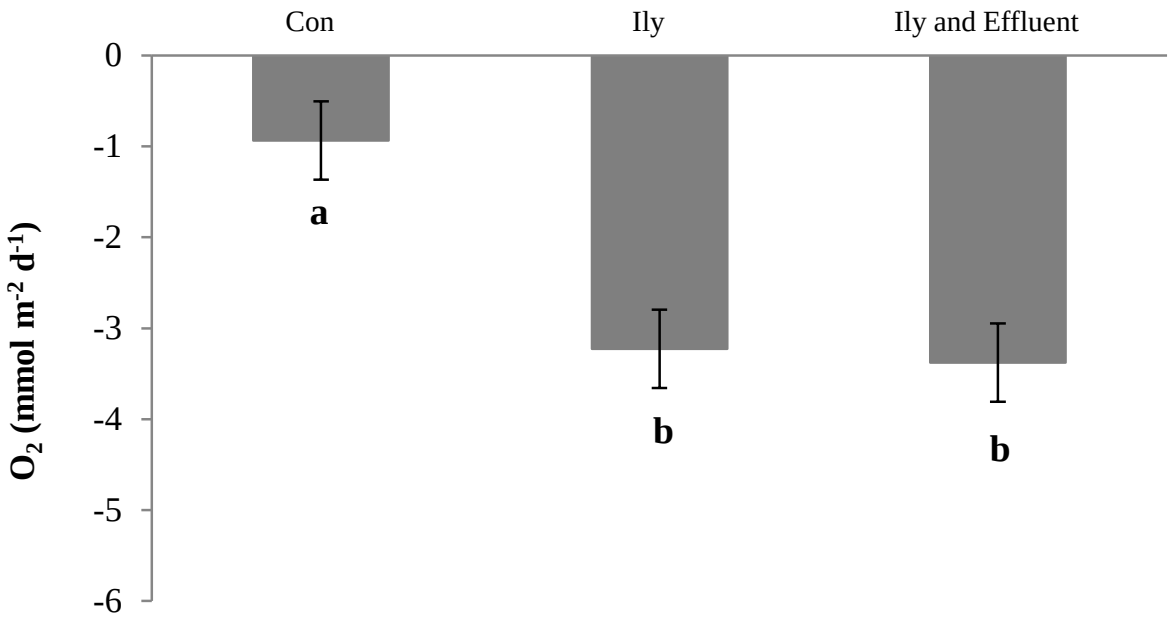

Figure 4.3 Average daily benthic oxygen consumption for control (Con), *Ilyanassa obsoleta* (Ily) and *I. obsoleta* + *Panopeus herbstii* (Ily and Effluent) (+/- standard error). Different letters indicate significance ($p<0.05$) between treatments. Negative values represent uptake by the sediment.

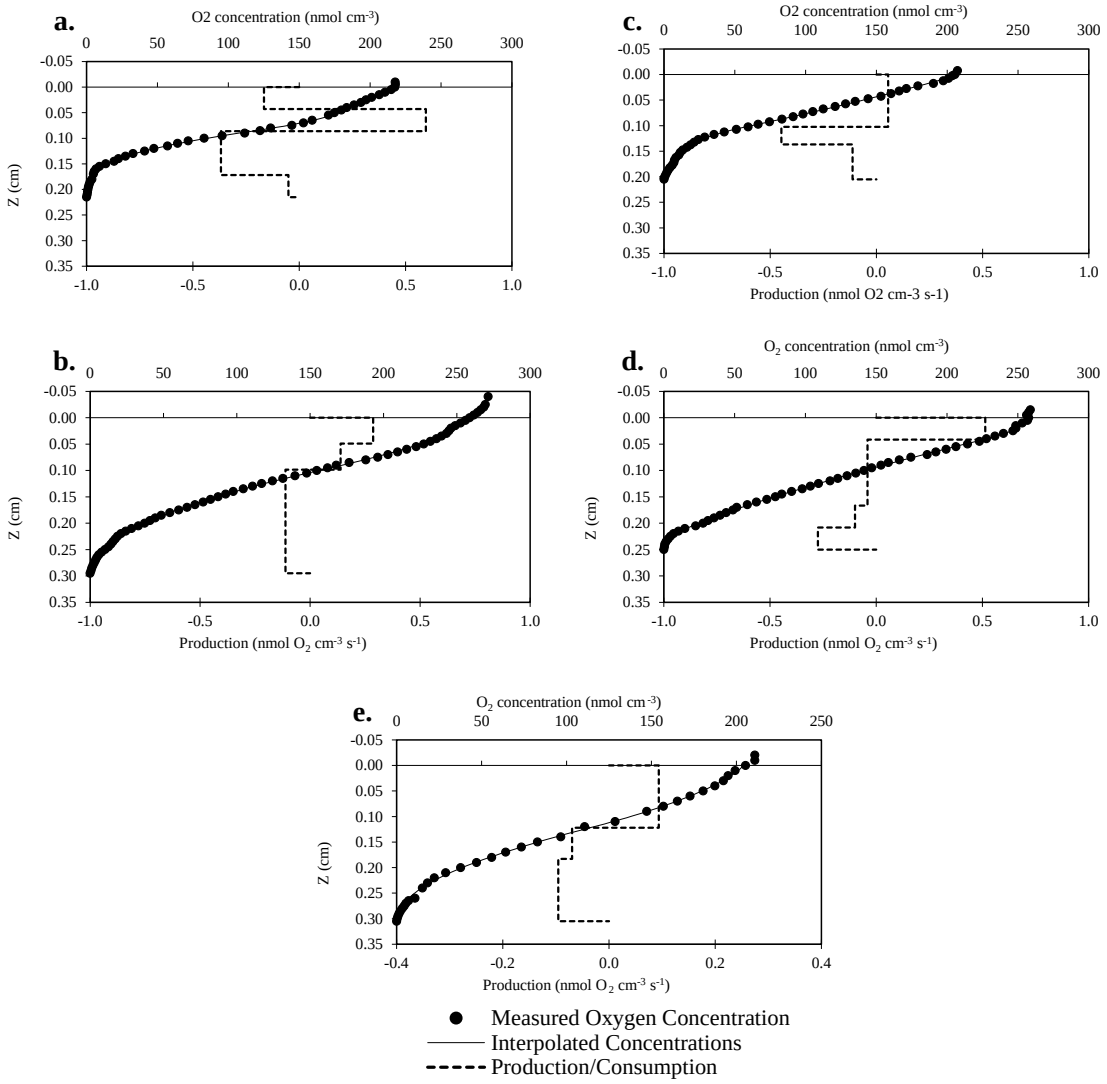

Figure 4.4 Sample micro-oxygen profile measurements for (a) *Mercenaria mercenaria*, (b) *Ilyanassa obsoleta*, (c*) Mercenaria mercenaria + Panopeus herbstii* effluent, (d) *Ilyanassa obsoleta + P. herbstii* effluent and (e) control. Positive production/consumption values represent oxygen production within sediment and negative values represent oxygen consumption.

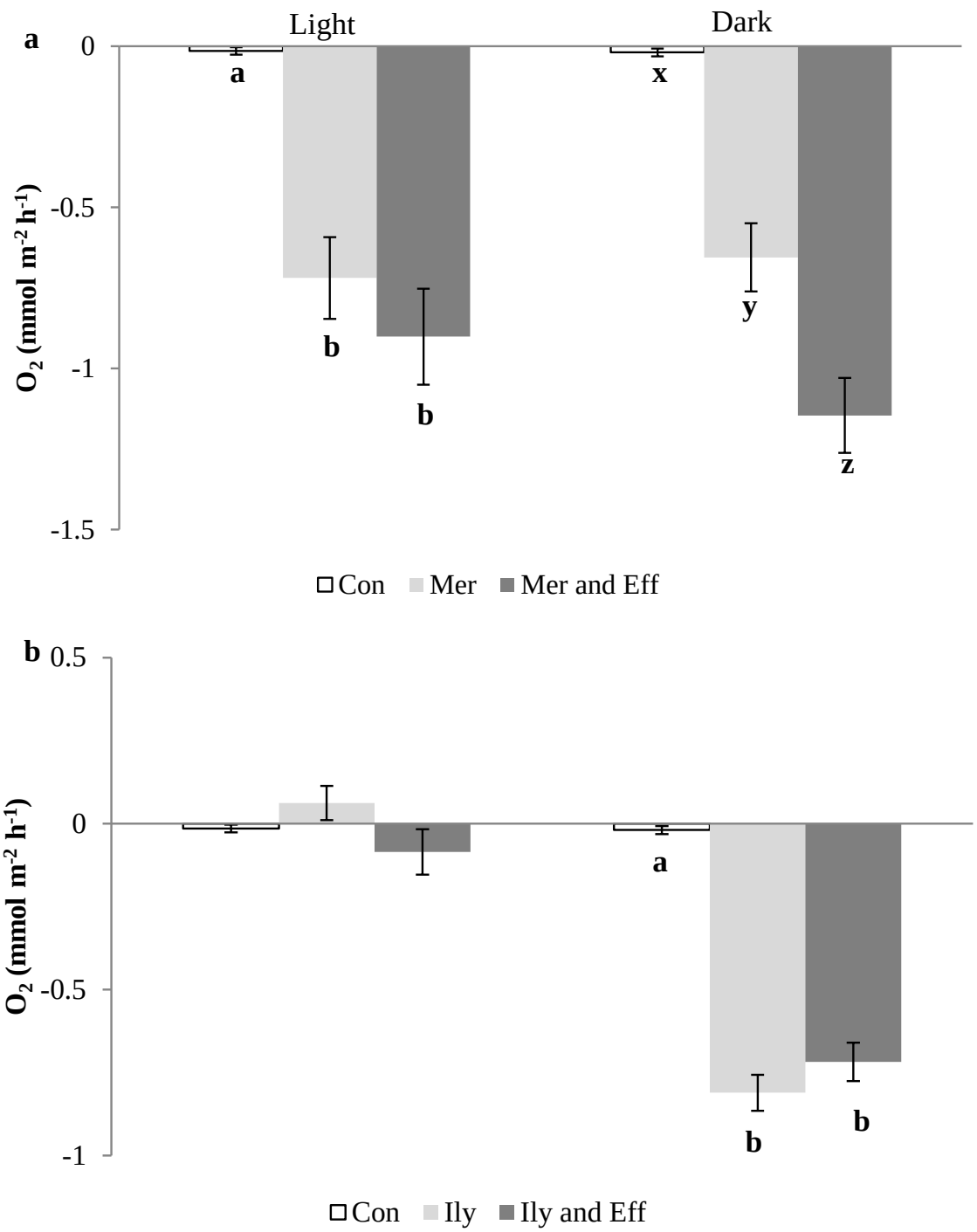

Figure 4.5 Average calculated oxygen flux at the sediment surface based on micro-electrode oxygen (O_2) measurements. On top (a) are control (Con) *Mercenaria mercenaria* (Mer) *M. mercenaria* and *Panopeus herbstii* effluent (Mer and Eff). (+/- standard error). On bottom (b) are control (con) *Ilyanassa obsoleta* (Ily) and *I. obsoleta* and *Panopeus herbstii* effluent (Ily and Eff). (+/- standard error). Negative values represent uptake by the sediment. Different letters represent significant differences within the light and dark ($p<0.05$).

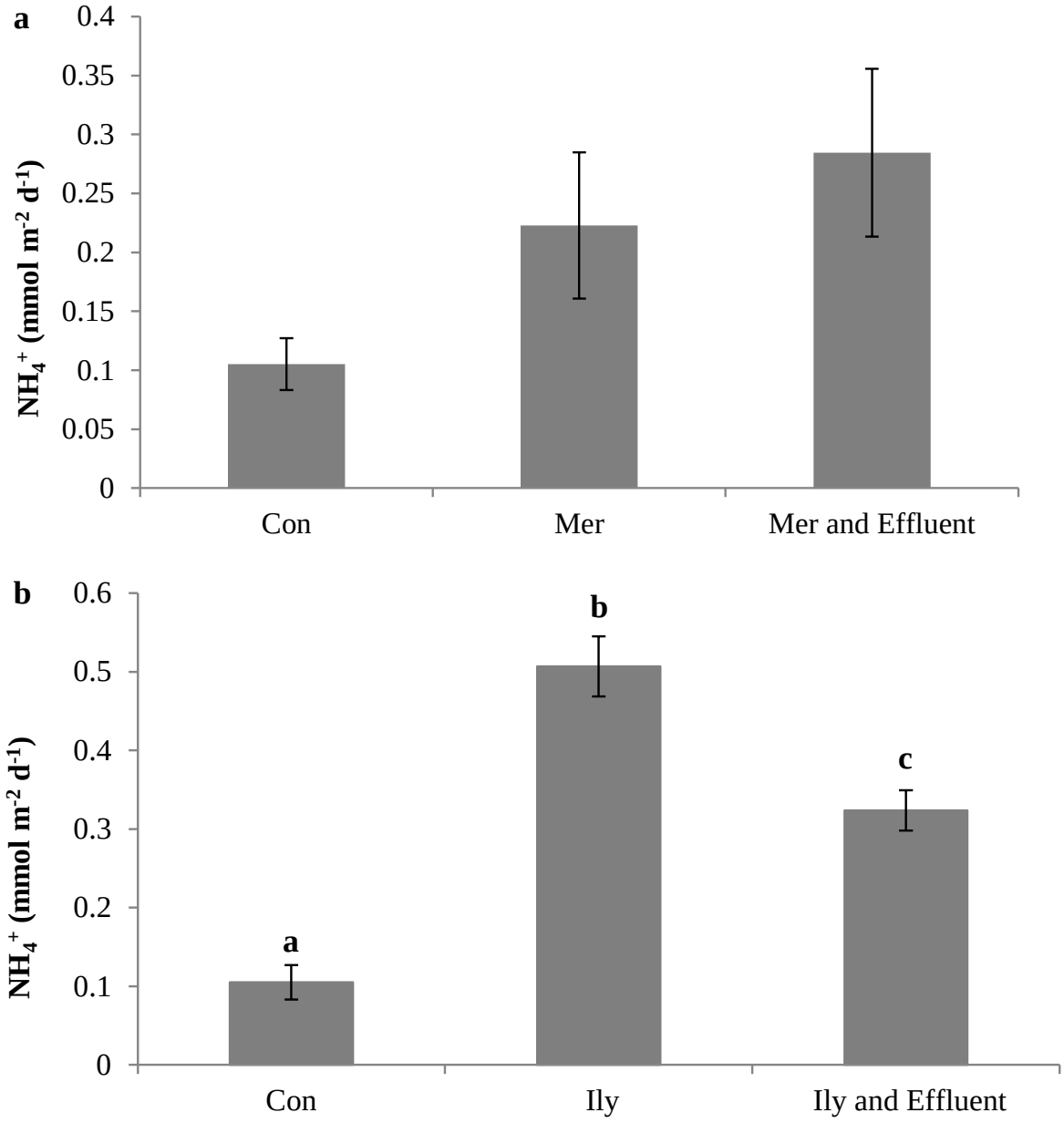

Figure 4.6 Average daily ammonium (NH_4^+) flux for control (Con), (a) *Mercenaria mercenaria* (Mer) *M. mercenaria* and *Panopeus herbstii* effluent (Mer and Eff) (b) *Ilyanassa obsoleta* (Ily) and *I. obsoleta* + *Panopeus herbstii* (Ily and Effluent) (+/- standard error). Different letters indicated significance ($P<0.05$). Positive values represent release from the sediment.

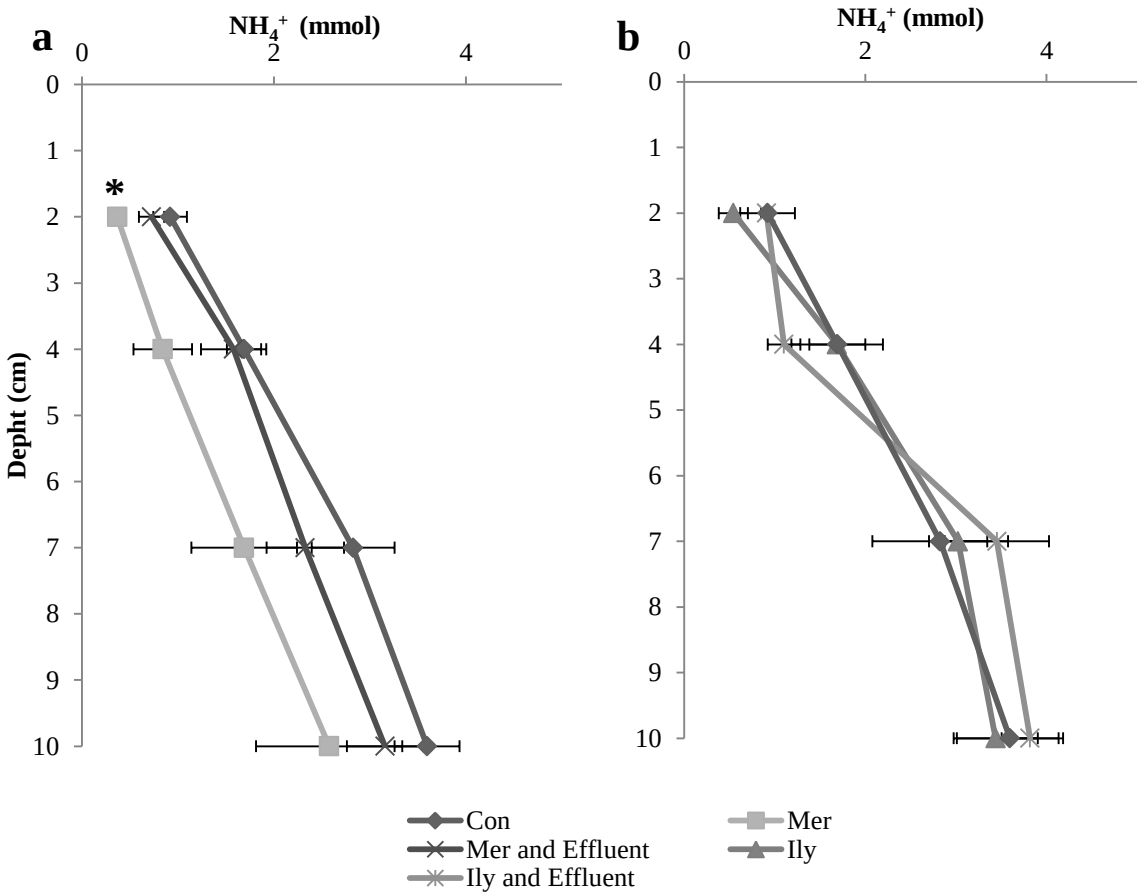

Figure 4.7 Average porewater (PW) ammonium (+/- standard error). The * represents significantly different treatments ($p<0.05$). Treatments for (a) are control (Con), *Mercenaria mercenaria* (Mer), *M. mercenaria* + *Panopeus herbstii* effluent (Mer and Effluent). Treatments for (b) are Con *Ilyanassa obsoleta* (Ily) and *I. obsoleta* + *P. herbstii* (Ily and Effluent).

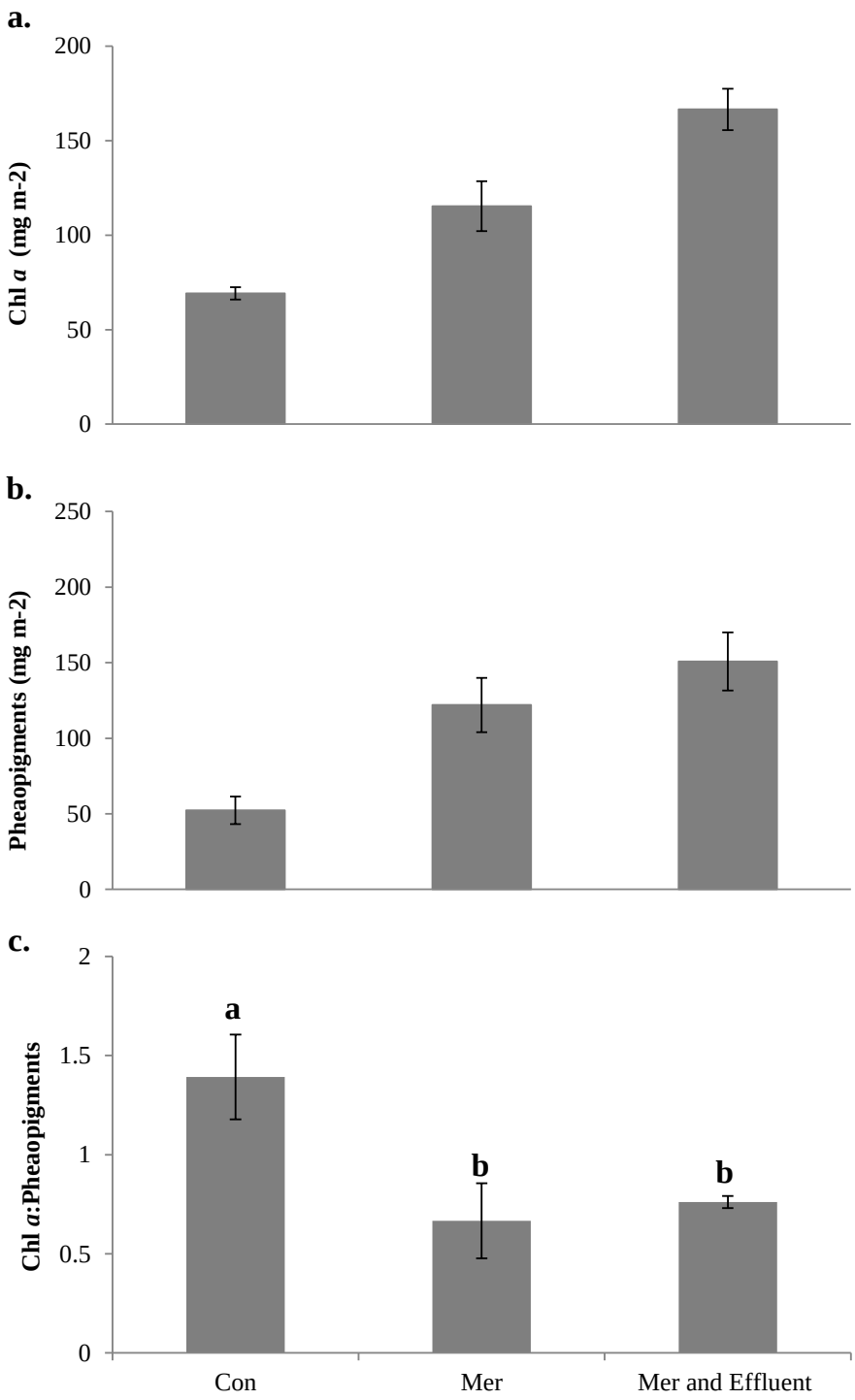

Figure 4.8 Average (a) chlorophyll *a* (chl *a*), (b) phaeopigments, and (c) ratio of chl *a* to phaeopigments (+/- standard error). Con represents control, Mer represent *Mercenaria mercenaria* treatment and Mer and Effluent is *M. mercenaria* + *Panopeus herbstii* effluent treatment. Different letter represent significant difference ($p<0.05$).

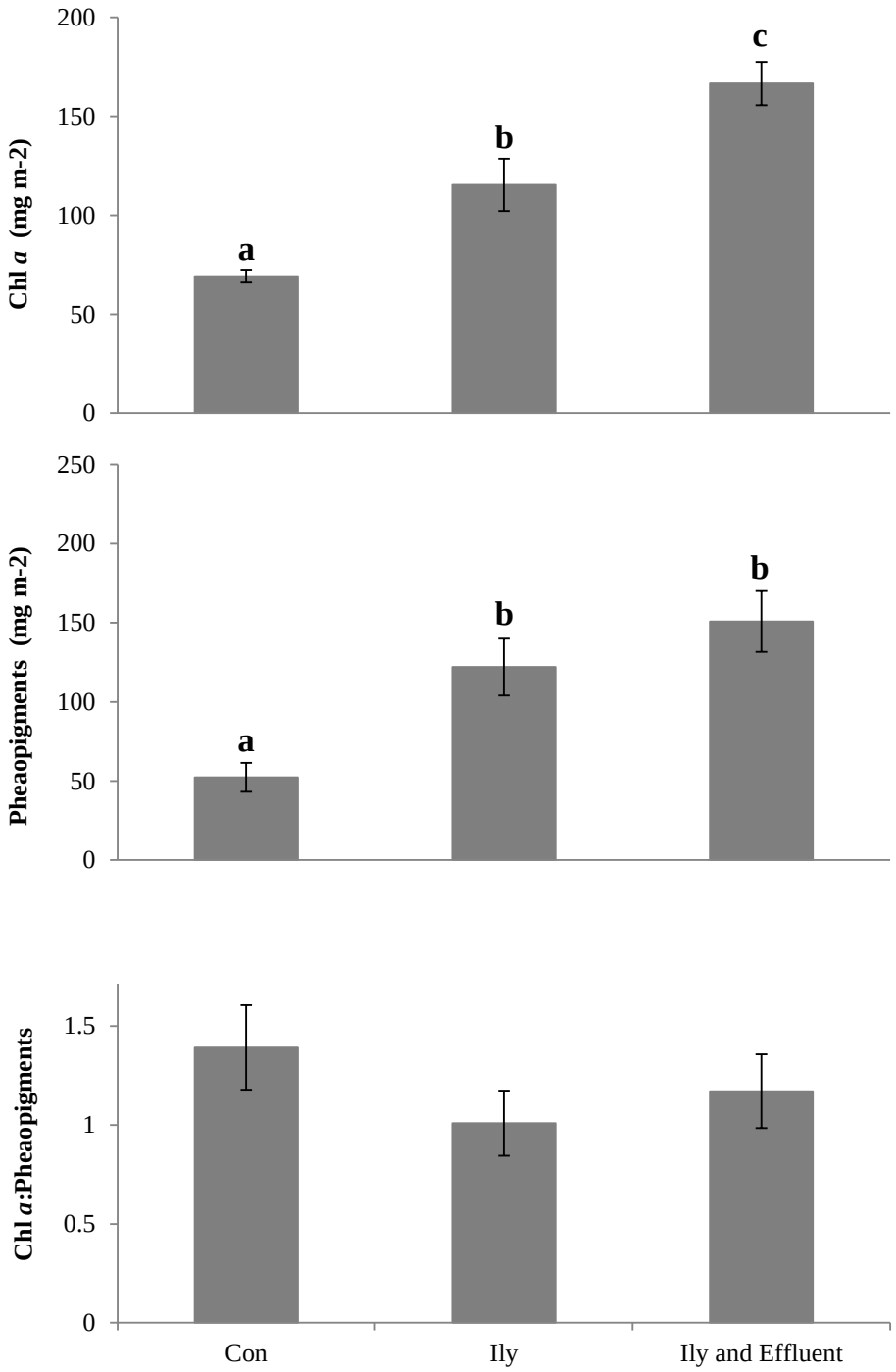

Figure 4.9 Average (a) chlorophyll *a* (chl *a*), (b) phaeopigments, and (c) ratio of chl *a* to phaeopigments (+/- standard error). Con represents control, Ily represent *Ilyanassa obsoleta* treatment and Ily and Effluent is *I. obsoleta* + *Panopeus herbstii* effluent treatment. Different letter represent significant difference ($p<0.05$).

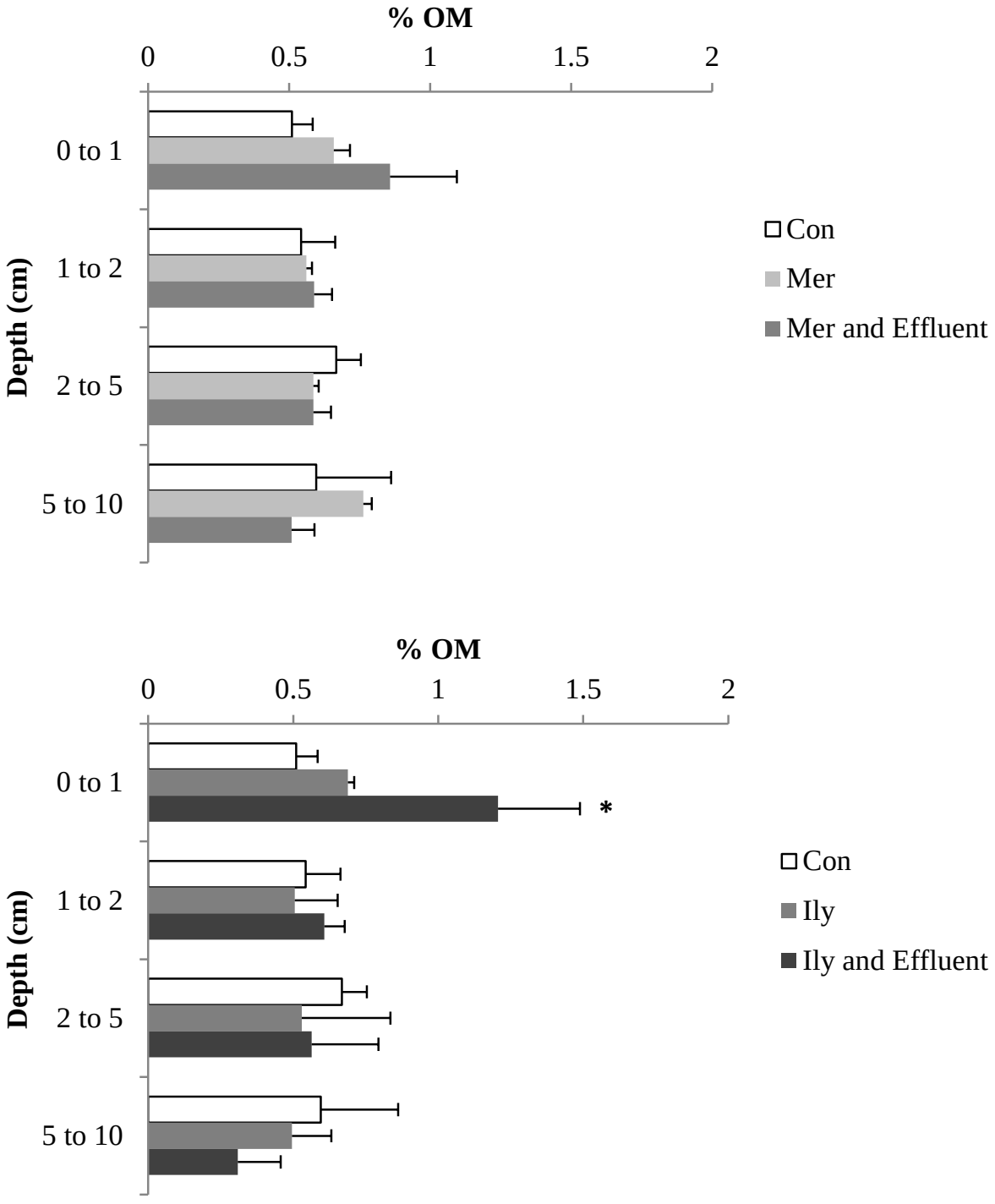

Figure 4.10 Average dry weight percent organic matter (%OM) (+ standard error). One-way ANOVAs were ran for each depth and * represents significant treatment ($p<0.05$). Treatments are control (Con),(a) *Mercenaria mercenaria* (Mer), *M. mercenaria + Panopeus herbstii* effluent (b) *Ilyanassa obsoleta* (Ily) and *I. obsoleta + P. herbstii* effluent (Ily and Effluent).

Chapter Five

Conclusions

5. Conclusions

Macrofauna behavior changes depending on the surrounding environmental conditions, including sediment type, food availability and interspecific interactions. Variable behavior of invertebrates results in both direct and indirect effects on microphytobenthic (MPB) biomass and organic matter (OM) distribution, ultimately resulting in changes in sediment biogeochemistry and water column nutrient availability.

In the second chapter we investigated a non-destructive method to investigate changes in MPB in ecological microcosm experiments. We found a number of robust models that can effectively estimate chlorophyll *a* and phaeopigment concentrations. However, it should be noted that change in environmental conditions (i.e. changes in sediment) can render these models ineffective. For this reason it is recommended that band depth be utilized, as long as concentrations remain low (<100 mg m^{-2}), because they may be less affected by changes in sediment type. Because of the importance of MPB in shallow marine ecosystem, the ability to obtain time series data for MPB biomass simply and inexpensively will aid substantially in our ability to explain nutrient cycling and should be considered viable for future investigations.

In our subsequent investigations, we found that the studied macrofauna had substantial effects on important ecosystem processes, including benthic oxygen consumption, nutrient release to the water column and microalgal biomass during both the early stages of eutrophication when higher trophic levels may be lost (Chapter 4) and the later stages of eutrophication when only highly resistant organisms remain (Chapter 3). Our study focused on species that are ubiquitous in temperate Western Atlantic estuaries and that are relatively resistant to the detrimental effects of eutrophication.

We found that the eutrophication-resistant snail *I. obsoleta's* food preference was strongly context-dependent, which may have been driven by changes in the availability and

especially nutrient content of the potential food sources. These changes in behavior have important consequences for the removal of macroalgal detritus following a substantial macroalgal bloom and subsequently for sediment biogeochemistry and OM content during the latter stages of eutrophication. This suggests that the effects of eutrophication may proceed at different rates under different environmental conditions, even in the same estuary.

We also found that biological interactions can alter the impact of *I. obsoleta,* as this species' behavior was extremely sensitive to crab effluent, resulting in lower grazing rates and decreased ammonium release from the sediment to the water column. These results indicate that the loss of predators in environments with these grazing mud snails may actually promote the progression of eutrophication, since there is excess release of plant-available nitrogen.

The effects of our second species of interest, *Mercenaria mercenaria*, were somewhat less striking than the effects of *I. obsoleta*, however the observed changes in porewater chemistry have important consequences for nitrogen removal from marine sediments. For example, in Chapter Three we illustrated that *M. mercenaria* alone increased oxygen penetration, thus detritus may be more efficiently broken down in the presence of the bivalve leading to a more rapid release of inorganic nutrients or increased MPB biomass. With the mud-snail present these effects were not seen, suggesting that interspecific interactions have important consequences for the actual combined effects of these species. Further work should be done to see the long-term effects of *M. mercenaria* on decomposition. Likewise, the lower porewater ammonium with *M. mercenaria* in the absence of predation stress has important implications for nitrogen removal in estuaries. Reversible effects, such as reduced feeding, in the presence of non-lethal predation may also increase porosity in the sediment and promote more rapid nutrient mineralization.

Our results have enhanced our understanding of the role of environmental and species diversity on the ability of fauna to regulate MPB and nutrient release. In particular, we have highlighted the importance of including interspecific interactions and small-scale variation in environmental context in our understanding of ecosystem function in shallow estuaries. We have shown that changes in benthic community structure may ultimately affect estuarine susceptibility to and potential for recovery from eutrophication. Each species studied plays an integral role, both alone and in combination with other invertebrates, and loss of species may change biogeochemical cycling of nutrients. Thus, it is important to try and maintain benthic community health, by removing stressors such as the input of excess nutrients.

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