Potential effects of an invasive bivalve, *Nuttallia obscurata*, on biogeochemical cycling in the intertidal

by

Kayi Chan

B.Sc. (Hons.), Simon Fraser University, 2008

Thesis Submitted in Partial Fulfillment
of the Requirements for the Degree of
Master of Science

In the
Department of Biological Sciences

Faculty of Science

SIMON FRASER UNIVERSITY

Summer 2012

Approval

Name: **Kayi Chan**

Degree: **Master of Science**

Title of Thesis: **Potential effects of an invasive bivalve, *Nuttallia obscurata*, on biogeochemical cycling in the intertidal**

Examining Committee:

Chair: Margo Moore, Professor

Leah Bendell
Senior Supervisor
Professor

Isabelle Côté
Supervisor
Professor

Mike Hart
Supervisor
Professor

Chris McKindsey
External Examiner
DFO, Maurice Lamontagne Institute

Date Defended: August 27, 2012

Partial Copyright Licence

Abstract

The varnish clam, *Nuttallia obscurata*, is a rapidly spreading invasive species that can reach high densities (i.e. 800 individuals m^{-2}). A three-tiered approach involving a field survey (Tier I), a density manipulation experiment (Tier II) and a microcosm experiment (Tier III) was applied to determine the effects of this invasive bivalve on biogeochemical cycling in the intertidal zone. At natural densities, bivalve distribution was best explained by sediment grain size. High densities of varnish clams did not significantly increase organic matter concentrations; although their ability to deposit feed and bioturbational activities may have prevented accumulations. High densities of varnish clams resulted in significantly higher concentrations of ammonium and percent silt. Nitrogen is a limiting nutrient with ammonium preferentially used by phytoplankton and microphytobenthos. These primary producers form the basis of all marine food webs; thus, changes in the concentration and flux of ammonium may impact ecosystem functioning of the intertidal area.

Keywords: *Nuttallia obscurata*, invasive bivalves, intertidal, nitrogen, biogeochemical cycling

To my friends and family whose love and support have made this thesis possible.

Acknowledgements

I would like to sincerely thank Leah Bendell for her constant guidance, patience and support throughout the course of this project. I am forever grateful for this opportunity and the invaluable experiences it has brought. Thank you to Mike Hart for his insights, assistance in bivalve identification and access to his cold room. Thanks to Isabelle Côté for her thoughtful and detailed advice and comments. I would like to thank the following funding sources: National Sciences and Engineering Research Council of Canada, The Canadian Health Oceans Network, The Glen Geen Graduate Scholarship in Marine Biology and The Coastal Zone Canada (BC) Association Graduate Scholarship in Coastal Studies.

I am genuinely thankful to Anthony Chan, Ben Curtis, Kavindra Pillay, Adeline Piot, Toby St. Clair and Hazel Walling for volunteering their time to help in the field. Thank you to Lindsay Du Gas, Keiko Nabetani, Melissa Orobko and Ummat Somjee for their enthusiasm, dedication and attention to detail in processing numerous samples. The completion of this project would not have been possible without all of you. Thanks to the members of the Bendell lab for all their advice and support. I would especially like to thank my lab-mates Toby St. Clair and Hazel Walling as well as my fellow SFU grad students for making my time on this roller coaster ride, otherwise known as grad school, fabulous.

I would like to thank Niki Cook for allowing me access to her exclusion cages. I am grateful to Norma Rejall who was more than accommodating and made my time on Denman Island absolutely wonderful. Thank you to Jane and Matsuki Masutani for loaning their freezer space out for sediment storage. Numerous thanks to Linda Pinto for all her guidance and support. I am thankful to Marlene Nguyen for all her assistance with the administrative process.

Finally, a special thank you to my family for their unfailing love, support and encouragement throughout the course of this project. Thank you to Tommy Kwok, Bon Lui and Sunny Tse for always trying to remind me of what is important. Thank you to Anthony Chan for always lending your ear.

Table of Contents

List of Tables

List of Figures

An adult varnish clam, *Nuttallia obscurata*, on the left and a juvenile on the right.

1. Introduction

1.1. Background and Rationale

Historically, the distribution dispersal of aquatic species was facilitated by natural means such as ocean currents and distribution was limited by natural barriers such as temperature and salinity (Raaymakers 2002). These distribution regimes have been disrupted by human activities with as of yet unknown consequences. Maritime activities, fisheries, aquaculture, canal developments, escape from aquaria and research facilities are but some of the vectors believed to be responsible for the movement of aquatic species out of their natural range (Naylor et al 2001; Ruiz et al 2000; Raaymakers 2002). The shipping and aquaculture industries have been identified as the two major human-mediated vectors by which exotic aquatic species are being transported into North America (Ruiz et al 2000; Wonham and Carlton 2005). Of particular concern is the movement of larvae via ballast water as they are often deposited into novel habitats free of natural predators (Lafferty and Kuris 1996; Raaymakers 2002). Fortunately, transport does not equate to establishment as many exotics do not survive the journey or are discharged into unsuitable habitat (Mack et al 2000; Raaymakers 2002; Bax et al 2003). However, with expanding commerce, increasing transport speeds, cleaner ballast waters and modified recipient ports susceptible to invasion, the rate of exotic aquatic species establishment is increasing (Ruiz et al 2000; Byers 2002a; Raaymarkers 2002; Bax et al 2003).

An introduced species can either blend into the background flora and fauna or it can go on to become invasive (Bax et al 2003). The high densities reached by invasive aquatic species leads to them dominating the native biota and often result in major and irreversible changes to affected ecosystems (Carlton 1996; Raaymakers 2002; Bax et al 2003; Wallentinus and Nyberg 2007; Williams and Grosholz 2008). In particular, filter feeders and ecosystem engineers have been identified as two functional groups that strongly impact biodiversity and ecosystem functioning (Williams and Grosholz 2008;

Grosholz and Ruiz 2009). The filter feeding activities of bivalves can clear the overlying water column of phytoplankton and inorganic particles; thus, bivalves can reduce turbidity. The increase in the depth of light penetration can have important consequences for vegetation as well as the microphytobenthos community (Strayer et al 1999; Newell et al 2002; Newell 2004).

Ecosystem engineers are defined as organisms that can control resource availability to other organisms by changing the physical state of biotic and abiotic materials (Jones et al 1994). Under this definition, benthic bivalve species are considered ecosystem engineers as their bioturbation activities increase the depth of sediment oxidation, stimulate microflora activities and accelerate decomposition rates, (Jones et al 1994). In addition to bioturbation, the excretion of nitrogenous wastes and biodeposits by bivalves can also alter the rates and processes of nutrient regeneration in coastal sediments (Nakamura and Kerciku 2000; Hasanudin et al 2004; Newell 2004; Wallentinus and Nyberg 2007). The limitation of nutrients is common in all ecosystems and is potentially compensated for by internal recycling (Dame 2011). Thus, any changes to the processes of nutrient regeneration can have impacts at the ecosystem level.

1.1.1. Nutrient Cycling

Fixed nitrogen is a limiting nutrient in marine ecosystems; thus, its cycling is a key process that regulates biological productivity (Kristensen 1988; Feuillet-Girard et al 1997; Falkowski et al 1998; Laverock et al 2011). Benthic bivalve species alter the chemical and physical structure of the sediments they inhabit (Kristensen 1988; Bartoli et al 2001; Lelieveld et al 2004; Nizzoli et al 2007; Laverock et al 2011). Consequently, their invasion of an intertidal area can potentially influence rates of benthic cycling of nitrogen as these areas serve as important sites for nutrient transformations (Nedwell 1993; Lavrentyev et al 2000; Nakamura and Kerciku 2000; Newell et al 2002; Hasanudin et al 2004). While it is not completely understood and quite complex, a brief overview of the nitrogen cycle and the contributions of bivalves to this cycle will be provided here based mainly on the review by Herbert (1999) and schematics by Newell et al (2002).

Although abundant in the atmosphere as nitrogen (N_2) gas, this form of nitrogen is not biologically available and must be fixed. Fixation of nitrogen produces ammonia (NH_3^+), which upon reaction with water, is converted to ammonium (NH_4^+). NH_4^+ in conjunction with nitrate (NO_3^-) make up the major components of the dissolved inorganic nitrogen (DIN) pool in the water column. Although both inorganic forms in the DIN pool are utilized by phytoplankton and microphytobenthos, studies have shown that NH_4^+ is preferentially used (Feuillet-Girard et al 1997; Pietros and Rice 2003). Inorganic nitrogen is incorporated into tissues and continues to be incorporated as it moves up the food chain. In bivalves, any nitrogen that is not incorporated into tissues is excreted as NH_4^+ directly back into the DIN pool of the water column (Newell 2004). In addition, bivalves also excrete faeces and pseudofaeces, collectively known as biodeposits, as part of their excretory processes. These mucus bound particles are relatively resistant to degradation in the water column and have a higher sinking velocity; thus, these particles may survive to become incorporated into sediments (Henriksen et al 1983; Kristensen 1988; Falkowski et al 1998). In the oxic layer, organic nitrogen in the biodeposits undergoes ammonification, releasing NH_4^+. There are three subsequent pathways for this released NH_4^+: 1) diffusion into the DIN pool in the overlying water column; 2) diffusion into the underlying anoxic layer; or 3) nitrification resulting in the production of NO_3^- as a final product. The produced NO_3^- can either diffuse back into the DIN pool or it can diffuse into the underlying anoxic sediment layer. In the anoxic layer, NO_3^- will undergo nitrate reduction, converting NO_3^- to nitrite (NO_2^-) and finally to NH_4^+. The intermediate product, NO_2^-, can undergo denitrification, resulting in the production of N_2 gas. As N_2 gas is not biologically available, it will not stimulate further primary production as it moves out of the sediment layers and back into the atmosphere. Any organic nitrogen that escaped ammonification in the oxic layer can undergo reversible ion exchange, removing NH_4^+ from the sediment particles. Eventually, any remaining organic nitrogen that is not transformed will be buried.

In addition to nitrogen, iron is also limiting in marine systems. Iron is an essential micronutrient that serves as an enzymatic co-factor for a multitude of basic metabolic processes (Barbeau 2006). Despite it being the fourth most abundant element, it has long been known that iron is a limiting nutrient (Cooper 1935). Early experiments showed that growth of *Nitzschia closterium*, a diatom, was accelerated when cultures

were enriched with ferric ammonium citrate, ferric alum or ferrous sulphate (Cooper 1935). Bottle experiments by Martin and Fitzwater (1988) also showed increased chlorophyll *a* (chl *a*) production in proportion to the amount of iron added. Numerous artificial iron experiments since have confirmed that primary productivity is limited by iron supply (Breithbarth et al 2010).

Iron enters the open ocean from the atmosphere (Breithbarth et al 2010). Fe(III) is the most thermodynamically stable form of iron in seawater. However, it tends to hydrolyze and form insoluble oxide and oxyhydroxide complexes or adsorb onto sinking particles, making it extremely limited in the oceans (Geider 1999; Barbeau 2006). Formation of complexes between Fe(III) and organic ligands not only help keep iron in solution, but also make it available for biological uptake (Geider 1999; Archer and Johnson 2000; Poorvin et al 2004; Raiswell 2006). It is suspected that these ligands are bacterial siderophores (Tortell et al 1999; Barbeau et al 2001). The grazing or lysis of bacteria by viruses releases dissolved inorganic iron, which is then available for uptake by phytoplankton (Poorvin et al 2004). Additionally, a small, transient pool of dissolved inorganic iron, Fe(II), which is more biologically available than Fe(III), is also available to phytoplankton via the remineralization of particulate organic matter in the water column (Geider 1999; Barbeau 2006).

Particles that are not remineralized in the water column sink to the sediment surface where oxidation of the organic detrital matter by heterotrophic bacteria releases Fe(II) (Tortell et al 1999; Archer and Johnson 2000). Fe(II) precipitates out in the oxic layer as an iron hydroxide (Sundby et al 1992; Caetano et al 1997; Falkowski et al 1998). Iron oxidation increase particle size and along with the physical disturbance of tidal ebbing, will cause an aggregation of these particles. Thus, a substantial portion of Fe(III) is retained in the sediment (Caetano et al 1997). In the oxic layer, iron hydroxides are free to complex with phosphate; thus, forming a phosphate trap (Sundby et al 1992; Malcolm and Sivyer 1997). Sedimentation and bioturbation move sediment into the anoxic layer causing dissolution of the particulate iron (Sundby et al 1992; Falkowski et al 1998). Diffusion and bioirrigation move the released iron from the porewaters back into the water column (Raiswell 2006).

Organic matter oxidation by bacteria as well as sedimentation leads to the formation of oxic surface layers and anoxic sub-layers (Jickells and Rae 1997). As seen from above, both the nitrogen and iron cycles are dependent upon the redox conditions within the sediments. The tight coupling of nitrification-denitrification processes is due to the close proximity of oxic and anoxic layers in sediments (Laverock 2011). In the oxic layer, particulate iron is not biologically available and acts as a trap for phosphate. Iron only becomes bioavailable upon dissolution in the anoxic layer (Sundby et al 1992; Falkowski et al 1998). High densities of bivalves lead to accumulations of organic matter and over-enrichment of sediments, which stimulates microbial respiration and potentially leads to anoxia (e.g. Bartoli et al 2001; Holmer et al 2003; Hasanudin et al 2004; Giles et al 2006; Nizzoli et al 2007). High concentrations of detrital matter can increase siltation (Karatayev et al 1997; Volkenborn et al 2007). Studies have shown that sediment porosity is correlated to nutrient content with finer materials retaining higher concentrations of nutrients versus looser cobble sediments (Nedwell 1993; Volkenborn et al 2007). Thus, bivalves can potentially impact nutrient cycles via changes in redox conditions within sediments as well as changes in grain size distributions.

Microbes in the water column and within sediments also play a vital role in nutrient cycling in marine systems. As mentioned above, while relatively abundant in the atmosphere, it is only upon interactions with microbes that nitrogen and iron become biologically available. Nitrogen gas in the atmosphere is not biologically available and must be converted into NH_3^+ by nitrogen fixing bacteria before it can be utilized (Herbert 1999; Newell 2002). The most thermodynamically stable form of iron, Fe (III), must be complexed with organic ligands before it can be utilized (Geider 1999; Archer and Johnson 2000; Poorvin et al 2004; Barbeau 2006; Raiswell 2006). It is suspected that these ligands are bacterial siderophores (Tortell et al 1999; Barbeau et al 2001). Predation by suspension feeding or deposit feeding benthic bivalves removes microbes from the water column and sediments. Consequently, benthic bivalves can also influence nutrient cycling indirectly.

1.1.2. Nuttallia obscurata

A recent accidental introduction to the Northeast Pacific is the bivalve *Nuttallia obscurata*. It is a bivalve with compressed valves, which are covered by a brown periostracum that peels like varnish on the exterior and a purple interior surface. Consequently, this species is commonly known as the varnish clam although the official common name is the purple mahogany clam (Gillespie et al 1999, 2001). The body, mantel, foot and siphons of the varnish clam are white, with siphons that are separate for their entire lengths (Gillespie et al 1999). Juvenile varnish clams are generally a light tan colour compared to the dark brown colour of the adults (Gillespie et al 1999). Its unique morphology makes the varnish clam easily identifiable, as no other bivalve species in the Northeast Pacific resembles it (Dudas 2005).

Native to Japan, Korea and China, the varnish clam was first reported in the Northeast Pacific in the early 1990's and it is believed to have been deposited with ballast water into Vancouver Harbour, British Columbia (BC), Canada (Gillespie et al 1999; Dudas 2005). From this point of origin, the varnish clam has spread rapidly at high densities (i.e. up to 800 individuals m^{-2}) and now has a distribution ranging from Raft Cover, BC in the north, to Coos Bay, Oregon, USA to the south (Dudas & Dower 2006; per comm, G Gillespie, Department of Fisheries and Oceans Canada). Varnish clams are generally found in sand and gravel substrates with the highest densities found in the high intertidal, apparently due to their inability to escape from predators in the lower intertidal zone (Gillespie et al 1999; Byers 2002b; Dudas 2005). Peak densities have also been found in areas associated with freshwater runoff (Gillespie et al 1999). Varnish clams are generally found at a depth of 8-10 cm but are capable of burying up to 25 cm (Byers 2002b). Varnish clams were observed to filter feed mostly at night; however, they are also capable of deposit feeding using locomotory and pedal sweep feeding (Gillespie et al 1999). Often found to co-occur with two economically important bivalve species: the native littleneck (*Protothaca staminea*) and the exotic Manila clam (*Tapes philippinarum*); there has been much concern regarding potential competition between the three species. Currently, there have been no conclusive results to suggest that competition is occurring (e.g. Dudas 2005). The ability of the varnish clam to be relatively freshwater tolerant, bury deeper and have alternate feeding modes have been suggested as potential reasons why direct competition may not be occurring between

the varnish clams and the two bivalve species (Gillespie et al 1999). However, the high densities and large population of the varnish clam have the potential to influence sediment chemistry and nutrient fluxes, which will have strong indirect impacts on co-occurring species and the ecosystems that they have invaded (Dudas 2005).

1.2. Hypotheses

The objectives of this study were to examine the potential consequences of the varnish clam invasion on nutrient cycling in intertidal sediments. Previous studies have shown significant accumulations of organic matter in areas with high bivalve densities due to the excretion of biodeposits (e.g. Bartoli et al 2001; Hasanudin et al 2004; Nizzoli et al 2007). Over-enrichment of the sediments can lead to anoxia due to high rates of bacterial respiration (e.g. Holmer et al 2003; Giles et al 2006). As previously mentioned, nitrogen and iron nutrient cycles are heavily dependent upon the redox conditions of the sediments. Changes to the redox conditions can potentially change the recycling rates of these essential nutrients and thus, their availability for biological uptake. Therefore, with high densities of varnish clams, I expect to see:

1) Accumulations of organic matter leading to sediment anoxia

2) Sediment anoxia resulting in the dissolution of particulate iron

3) High NH_4^+ concentrations due to the breakdown of biodeposits and direct excretion

A three-tiered approach was applied to assess the potential effects of the varnish clam on biogeochemcial cycling in the intertidal. A field survey was used to examine the relationship between bivalve densities and sediment geochemistry as well as to establish background reference data (Tier I). A density manipulation experiment was conducted *in situ* to examine the potential influence of varying varnish clam densities on sediment characteristics (Tier II). Finally, laboratory microcosm experiments were performed to determine if varnish clams had any influence on NH_4^+ flux (Tier III). An algae bloom was observed in the midst of the mesocosm experiments (Tier II). Thus, an additional chl *a* analysis was performed on Tier II surficial sediments to attempt to

capture the relationship between sediment nutrient parameters and photopigment concentrations. It is hoped that the outcomes of this research will help inform future management decisions in regards to this invasive species.

2. Methods

2.1. Study Site

Field research was conducted at Fillongley Provincial Park on Denman Island, BC (49º31'59"N, 124º49'0"W) from June to July 2009. Populations of Manila clams and the native Pacific littleneck dominate this site (Gillespie et al 2001). Varnish clams as well as several other bivalve species are also known to be present at this site (Cook 2008).

2.2. Experimental Approach

2.2.1. Tier I: Field Survey

The field survey design was based on Gillespie et al (2001). In the summer of 2009, an 800 m reference line parallel to the tide line was established. From this reference line, five transects were randomly established perpendicular to the shoreline. Subsequently, three tidal heights were randomly selected from each transect and a 0.5x1.0 m quadrat was established at each location resulting in a total of 15 quadrats. Tidal heights were determined using a tide calendar and sampling dates were determined by accessibility of sites. Each quadrat was sampled once.

A PVC pipe (5 cm diameter) was used to take triplicate cores to a depth of at least 10 cm from each quadrat. Porewater studies have shown a concentration gradient of materials associated with depth (e.g. Bendell et al 2010). Thus, each core was spliced every 3 cm down the length of the core to create a sediment depth profile. Splices were placed into small plastic bags, homogenized and subsequently frozen until analyses could be performed. Sediments within quadrats were sieved using a 6 mm sieve down to a depth of approximately 20 cm. A 6 mm sieve was used as Whiteley (2005) found that a 6 mm sieve was more efficient than a 1 mm sieve in terms of

information gain per unit sampling effort and time. All polychaete worms as well as bivalves (>25 mm) were counted and identified down to species whenever possible using field guides. Density values (individuals m^{-2}) were obtained by doubling species count data to scale quadrat area (0.5 m^2) to 1.0 m^2.

2.2.2. Tier II: Density Manipulation Experiments

Exclusion cages were constructed from 1.0x1.0x0.3 m PVC pipe frames and plastic mesh (3 mm), which enclosed an area of 1.0 m^2. Plastic mesh covered all four sides as well as the bottom of the cages. Cages were set-up between 1.7-1.3 m above the tidal datum and were driven down 0.2 m into the sediment, leaving 0.1 m exposed to the tides. Additional cage design and set-up details are as described by Bendell et al (2010). In the summer of 2009, cages were emptied and all sediments within cages were sieved (6 mm) to remove all macrofauna before being returned to the cages. Experimental manipulations consisted of three varnish clam density treatments: low (200 individuals m^{-2}), medium (500 individuals m^{-2}) and high (800 individuals m^{-2}). Control cages were not seeded with varnish clams. Varnish clams, which were harvested two days prior, were purchased from Fanny Bay Oysters (Fanny Bay, BC). Density treatments and controls were replicated three times, resulting in a total of 12 cages. Treatments and controls were randomly assigned to cages. Cages were left for 16 days before sampling began in July 2009. Cages were sampled whenever possible, with one sediment core taken from each cage using the same methodology as the field survey (Tier I). Only seven days worth of samples were analyzed for sediment parameters. Heavy biofouling occurred on all cages; thus, after sampling was complete, cages were cleared of algae as much as possible. Mesocosms were emptied at the end of experimentation to determine final densities of varnish clams.

2.2.3. Tier III: Microcosm Experiments

In the summer of 2010, intertidal sediment and seawater were collected from the same area of Fillongley Provincial Park where field research had been conducted in the previous year. All sediments were first homogenized by wet sieving (2 mm) using seawater. To determine if the NH_4^+ concentrations measured originated from organic matter breakdown or varnish clam excretion, experimental design consisted of two

treatments (sterilized and unsterilized with varnish clams) and their respective controls (no addition of varnish clams). All treatments and controls were replicated three times for a total of 12 microcosms and were randomly assigned to microcosms. Dilute formaldehyde has been shown to be the most effective sterilization technique; however, its ability to react with vital organic nitrogen compounds is not desirable when working with live specimens (Tuominen et al 1994). Instead, sediments and seawater used for sterilized treatments and controls were sterilized using an autoclave as it is the most readily accessible sterilization method available. Sediments were autoclaved twice using the liquid cycle at 121ºC for an hour (Trevors 1996). Seawater was autoclaved once on the liquid cycle at 121ºC for 30 minutes (Harrison and Berges 2005). Prior to use, seawater was allowed to rest for at least 24 hours to allow it to re-equilibrate (Harrison and Berges 2005). Sediments and seawater for the unsterilized treatments were left untreated. All materials were stored in a cold room (5±1ºC) until experimental set-up.

Microcosm experiments began in a cold room at 15ºC as the average temperature for June 2010, when sediments and water were collected, was 14.5ºC (National Climate Data and Information Archive for the Comox A station). Environmental conditions warmed to approximately 20ºC over a period of five days. The environmental conditions were then set at 15ºC with cooling occurring over the final five days of the experiment. Although not intended, these conditions mimic the intense warming events that were observed in the first field season. Consequently, in addition to the original objective of determining the sources of NH_4^+, the effect of warming was also examined.

Plastic Ziploc® containers were used as microcosms and were filled with approximately 500 cm^3 of sediment and 250 cm^3 of seawater. All microcosms were aerated using air stones and pumps to ensure the oxic conditions necessary for nitrification (Newell et al 2002). Microcosms were set-up in the cold room in a 3x4 grid formation and allowed to equilibrate for 24 hours before the start of the experiment. After 24 hours, the first sediment and overlying water samples were taken before the addition of three varnish clams to each of the treatment microcosms. The experiment ran for 22 days in July 2010 with 11 sampling days. On all sample dates, approximately 5 mL of the overlying water was first sampled from the microcosms and frozen until analysis. Subsequently, surficial sediments (i.e. 0-3 cm) were gently scooped up using a

plastic spoon, placed into small plastic bags and frozen until analysis. Overlying waters were replenished approximately every two days and occurred after sampling was completed on sampling days.

2.3. Sediment Analyses

Unless otherwise specified, all sediment samples were defrosted overnight in a fridge (~4ºC). Sediments were homogenized by thoroughly shaking sediments in their plastic bags before subsamples were taken and weighed. All subsamples were dried in a drying oven at 60ºC.

2.3.1. Organic Matter

Organic matter concentration was determined by loss on ignition (Schumacher 2002). Approximately 1 g of wet sediment was dried for at least 48 hours. Dried samples were sieved (2mm) to remove large particles, ashed at 400 ºC for an hour and cooled to room temperature in a glass desiccator before being weighed. Organic matter content was determined from the difference in weight between dried and ashed samples.

2.3.2. Ammonium

Ammonium concentration was determined using the indophenol blue method as described by Keeney and Nelson (1982). Approximately 1 g of wet sediment was mixed with 2*M* of potassium chloride and shaken on a mechanical shaker for an hour. After sediments had settled, the supernatant was filtered through Whatman no.42 filter paper and stored in a fridge (~4ºC) until analysis could be performed. 0.5 mL of the supernatant was combined with EDTA, phenol-nitroprusside and buffer solutions, and finally diluted with double de-ionized water (ddH_2O) for a total volume of 2.5 mL. Samples were placed in a water bath (40ºC) for 30 minutes, cooled to room temperature and read on a spectrophotometer at 636 nm. Ammonium concentrations of samples were determined from a calibration curve created from a set of known standards.

2.3.3. Iron

Iron was extracted from sediments using an aqua regia (AR) digestion (Martin et al 1994). AR solution was made using environmental grade hydrochloric acid and nitric acid (3 HCl: 1 HNO_3). Approximately 0.5 g of dried and sieved (2 mm) sediment subsamples were combined with 10 mL of AR, shaken for two hours on a mechanical shaker at room temperature and stored in a fridge (~4ºC) until analysis. Subsamples were centrifuged (~2500 rpm) for approximately 5 minutes and were subsequently diluted using ddH_2O. Samples were read at 248.3 nm with a slit of 0.2 nm on a Perkin-Elmer SIMAA6000 GFAAS unit.

2.3.4. Grain Size

Wet sieving was used to determine grain size distribution. Approximately 10 g of wet sediment was dried for at least 72 hours. Sediments were separated into three size factions: gravel (>2 mm), coarse sand (>0.25 mm) and fine sand (>0.063 mm). The sieves were stacked together and the sediment sample was washed through the sieves three times using distilled water. Each fraction was then dried for at least 48 hours before being weighed. The silt fraction was determined by the difference in weights between the dried sample and the dried fractions. All fractions were then calculated as percentages of the total dried sample weight.

2.3.5. Chlorophyll a *and Phaeopigment*

Chl *a* was extracted from samples using methods as described by Danovaro and Pusceddu (2010). Surficial sediment splices were defrosted in a cold room (5±1ºC) in the dark overnight. Approximately 2 g of wet sediment was combined with $MgCO_3$ and 90% acetone in glass test tubes in low light conditions. Samples were shaken with a vortex, sonicated and left to extract overnight in the dark at ~4ºC. Test tubes were centrifuged (~3000 rpm) and the supernatant was pipette out into glass cuvettes in low light conditions. Supernatant samples were read on a spectrophotometer at 750 nm and 665 nm. Supernatant samples were read at the same wavelengths again after acidification using 0.1 N HCl. Excess acetone was pipette out of test tubes and test tubes were left at room temperature for several days until all acetone had evaporated. Once sediments were dried, test tubes were re-weighed to determine total weight after

extraction. Chl *a* and phaeopigment concentrations were determined using equations described by Danovaro and Pusceddu (2010).

2.4. Statistical Analyses

Statistical analyses were performed by JMP7 (licensed to Simon Fraser University), SigmaPlot12 or Statistical Analysis Software (SAS). Significance of all tests was accepted at $p<0.05$. All data had obvious outliers removed (3 X SD) (Osborne and Overbay 2004) and were all tested for normality prior to analysis. If normality failed, data were transformed. If assumptions of normality were still not met, then data were ranked and analyzed with non-parametric methods.

2.4.1. Tier I: Field Survey

Data from the field survey (Tier I) were stratified by depth as porewater studies have shown concentration gradients to be associated with depth (e.g. Bendell et al 2010). As many of the quadrats were in rocky areas, most cores were only approximately 10 cm in depth. Consequently, Tier I cores were split into two depths: surficial (0-3 cm) and intermediate (3-9 cm). All quadrats sampled were included in all statistical analyses performed. A Pearson's product-moment correlation analysis was first performed using SAS to assess bivariate relationships among bivalve densities, organic matter and NH_4^+ concentrations and grain size fractions. Tidal height was not included as a variable as it is highly correlated with grain size distribution and is not considered a sediment attribute (Byers 2002b; Dame 2011). Subsequently, these sediment characteristics were used in a maximum r-square improvement procedure to determine the variable(s) that best explained bivalve density at each depth using SAS. A maximum r-square procedure is a modified forward step-wise regression, which switches an included variable with an excluded variable until r-square is maximized. The switches continue until r-square is maximized for all possible models (SAS Institute Inc. 2008).

2.4.2. Tier II: Density Manipulation Experiments

As mesocosms were located in the mid-low intertidal area, it was possible to take an additional depth sample for Tier II sediment cores. Consequently, depths were split as: surficial (0-3 cm), intermediate (3-9 cm) and bottom (9+ cm). Preliminary profile plots were created in JMP7 to determine if there were any interactions between time and density treatments on sediment nutrient concentrations. Mostly parallel lines suggested that there were no interactions (data not shown). A one-way analysis of variance (ANOVA) was then performed on the raw data in Sigmaplot12 to determine if there were significant differences due to density treatments. A common logarithmic transformation was performed on data that failed normality. If treatment was found to have a significant effect, a multiple comparison procedure (Holm-Sidak) was performed. A two-way ANOVA was performed with depth and treatment as factors on grain size fractions. Time was not a factor as unlike nutrient parameters, grain size distributions do not flux over time. If the fraction failed normality, it underwent an arcsine transformation (Ahrens et al 1990).

2.4.3. Sediment Nutrient Correlations with Algal Bloom

To capture the relationship between sediment nutrient concentrations (NH_4^+ and iron), photopigment concentrations, the data was first averaged over time. A linear regression was then plotted with nutrient concentrations as the dependent variable and either chl *a* or phaeopigment concentrations as the independent variable. As phaeopigments degrade more slowly than chl *a*, the ratio of chl *a* to phaeopigments can provide estimations on the relative rate of phytoplankton deposition (Hagy III et al 2005). The rate of deposition was also plotted over time and compared to the changes in temperature as exceedingly high temperatures were noted during the first field season in 2009.

2.4.4. Tier III: Microcosm Experiments

A repeated-measures (RM) ANOVA was performed on the sediment and overlying water NH_4^+ concentrations to determine if concentrations detected were the result of varnish clam excretion or bacterial breakdown of organic matter. A significant interaction between treatment and time occurred for both sediment and overlying water

NH_4^+ concentrations. Thus, on each sampling day, a one-way ANOVA was performed to examine the effects of treatment on NH_4^+ concentrations in the sediments and overlying waters. The diffusive flux co-efficient (K_d) was calculated as sediment NH_4^+ concentration (mg g^{-1}) (NH_4^+ concentration in overlying waters (mg L^{-1}))$^{-1}$. The K_d value was plotted against time to look for trends in the flux of NH_4^+.

3. Results

3.1. Tier I: Field Survey

Manila and Pacific littleneck clams were the dominant bivalves identified within the intertidal region that was surveyed (Appendix A). Distribution was patchy with bivalves found only in nine of the 15 quadrats (4-462 individuals m^{-2}).

Intercorrelations among variables are shown in Table 3.1. At the surficial depth, only the percentage of silt was significantly positively correlated with bivalve density ($p = 0.05$). The percentage of gravel was negatively correlated with the percentages of coarse sand ($p<0.0001$), fine sand ($p<0.0001$), and silt ($p<0.0001$). Organic matter concentrations were positively correlated to the percentage of gravel ($p = 0.05$) and negatively correlated to the percentage of coarse sand ($p = 0.01$) but not significantly correlated to the percentages of fine sand ($p = 0.34$) or silt ($p = 0.26$). Concentrations of NH_4^+ were found to be negatively correlated with the percentage of gravel ($p = 0.0005$) and positively correlated to the percentages of coarse sand ($p = 0.01$), fine sand ($p = 0.0006$) and silt ($p<0.001$). Bivalve densities at the surfical depth were best explained by the percentages of fine sand and silt (Table 3.2).

Table 3.1 Correlations between bivalve density, organic matter (OM), ammonium (NH_4^+) and grain size distributions at the surficial depth (0-3 cm). Pearson correlation coefficients (r) are shown.

	Density	OM	NH_4^+	%G	%CS	%FS	%S
Density	1.00						
OM	-0.05	1.00					
NH_4^+	0.18	-0.14	1.00				
%G	-0.04	0.31*	-0.52*	1.00			
%CS	0.07	-0.41*	0.40*	-0.89*	1.00		
%FS	-0.01	-0.15	0.52*	-0.91*	0.60*	1.00	
%S	0.31*	-0.18	0.63*	-0.85*	0.63*	0.87*	1.00

Note: Grain size distribution fractions in percentages: gravel (%G), coarse sand (%CS), fine sand (%FS) and silt (%S). (*) denotes significance ($p<0.05$).

Table 3.2 Sediment characteristics that best predicted bivalve densities at the surficial depth (0-3 cm) found using a maximum r-square improvement procedure.

Variables Added	r^2 Max Value	p-value	F-statistic	DF
%S	0.10	0.05	4.26	1
%FS + %S	0.41	<0.0001	13.74	2
%CS + %FS + %S	0.42	<0.0001	9.27	3
%G + %CS + %FS	0.42	<0.0001	9.32	3
%G + %CS + %FS + %S	0.43	0.0003	6.85	4
OM + %G + %CS + %FS + %S	0.43	0.0009	5.35	5
OM + NH_4^+ + %G + %CS + %FS + %S	0.43	0.002	4.34	6

Note: Grain size distribution fractions in percentages: gravel (%G), coarse sand (%CS), fine sand (%FS) and silt (%S). Sediment nutrient concentrations denoted as: organic matter (OM) and ammonium (NH_4^+). Model with the maximized r^2 value is highlighted in bold.

Intercorrelations between variables at the intermediate depth can be found in Table 3.3. At the intermediate depth, bivalve densities were significantly negatively correlated to the percentage of gravel ($p = 0.0043$) and positively correlated with the percentages of coarse sand ($p<0.001$) and silt ($p<0.0001$). However, bivalve densities were not significantly correlated with the percentage of fine sand ($p = 0.10$). The percentage of gravel was significantly negatively correlated to the percentages of coarse sand ($p<0.0001$), fine sand ($p<0.0001$) and silt ($p<0.0001$). Organic matter concentrations were not significantly correlated with any of the grain size fractions: gravel ($p = 0.4458$), coarse sand ($p = 0.40$), fine sand ($p = 0.18$) or silt ($p = 0.13$). Concentrations of NH_4^+ were found to be significantly negatively correlated with the percentage of gravel ($p<0.0001$) and positively correlated with percentages of coarse sand ($p = 0.0036$); fine sand ($p<0.0001$) and silt ($p<0.0001$). The concentration of organic matter, percentages of gravel, coarse sand and silt were the best predictors of bivalve density (Table 3.4).

Table 3.3 Correlations between bivalve densities, organic matter (OM), ammonium (NH_4^+) and grain size fractions at the intermediate depth (3-9 cm). Pearson correlation coefficients (r) are shown.

	Density	OM	NH_4^+	%G	%CS	%FS	%S
Density	1.00						
OM	-0.04	1.00					
NH_4^+	0.19	-0.09	1.00				
%G	-0.31*	0.14	-0.57*	1.00			
%CS	0.48*	-0.09	0.32*	-0.91*	1.00		
%FS	0.00	-0.15	0.73*	-0.84*	0.54*	1.00	
%S	0.46*	-0.17	0.55*	-0.78*	0.68*	0.67*	1.00

Note: Grain size distribution fractions in percentages: gravel (%G), coarse sand (%CS), fine sand (%FS) and silt (%S). (*) denotes significance ($p<0.05$).

Table 3.4 Sediment characteristics that best predicted bivalve densities at the intermediate depth (3-9 cm) found using a maximum r–square improvement procedure.

Variable Added	r^2 Max Value	p-value	F Statistic	DF
%CS	0.23	<0.0001	22.46	1
%CS + %FS	0.32	<0.0001	17.75	2
%FS + %S	0.36	<0.0001	21.15	2
%CS + %FS + %S	0.45	<0.0001	20.13	3
%G + %CS + %S	0.45	<0.0001	20.31	3
OM + %G + %CS + %S	0.52	<0.0001	19.74	4
OM + NH_4^+ + %G + %CS + %S	0.56	<0.0001	17.80	5
OM + NH_4^+ + %G + %CS + %FS + %S	0.55	<0.0001	14.64	6

Note: Grain size distribution fractions in percentages: gravel (%G), coarse sand (%CS), fine sand (%FS) and silt (%S). Sediment nutrient concentrations denoted as: organic matter (OM) and ammonium (NH_4^+). Model with the maximized r^2 value is highlighted in bold.

3.2. Tier II: Density Manipulation Experiments

At the end of the three week experiments, mortalities were approximately 30% across all density treatments (Appendix B). A thunderstorm occurred near the start of the experiment (July 6-7 2009) and an algal bloom was observed on July 17, 2009.

There were no significant effects of varying varnish clam densities on organic matter concentrations at any of the three depths: surficial (one-way ANOVA; $p = 0.41$; $F = 0.97$; $DF = 3$), intermediate (Kruskal-Wallis; $p = 0.34$; $H = 3.38$; $DF = 3$) and bottom (Kruskal-Wallis; $p = 0.34$; $H = 3.38$; $DF = 3$). However, while not statistically significant, organic matter concentrations in high varnish clam density treatments appear to be slightly higher than those of the controls and the other two density treatments in sediments at the intermediate depth (Figure 3.1).

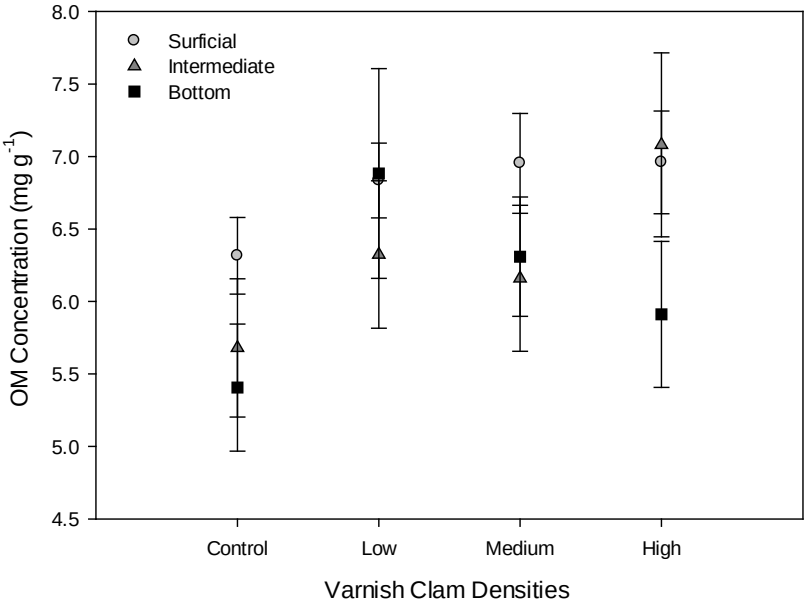


Figure 3.1 Organic matter concentrations in sediments with variable varnish clam densities, stratified by depth.

Treatment had a significant effect of the concentration of NH_4^+ at the intermediate depth (one-way ANOVA; $p<0.001$; $F = 6.63$; $DF = 3$). Concentrations of NH_4^+ were significantly higher in the high density treatments than the control (Holm-Sidak; $p<0.001$; $t = 4.46$) (Figure 3.2). However, neither the low density ($p = 0.07$; $t = 2.13$) nor the medium density treatments ($p = 0.11$; $t = 2.13$) were significantly different from the control. Concentrations of NH_4^+ were significantly different across treatments at the bottom depths as well (one-way ANOVA; $p<0.001$; $F = 9.38$; $DF = 3$). High density treatments had significantly higher concentrations of NH_4^+ than the control (Holm-Sidak; $p<0.001$; $t = 4.52$), low density ($p<0.001$; $t = 4.33$) and medium density treatments ($p<0.001$; $t = 4.14$). There was no significant effect of treatment on NH_4^+ concentrations in the surficial sections (Kruskal-Wallis; $p = 0.96$; $H = 0.28$; $DF = 3$).

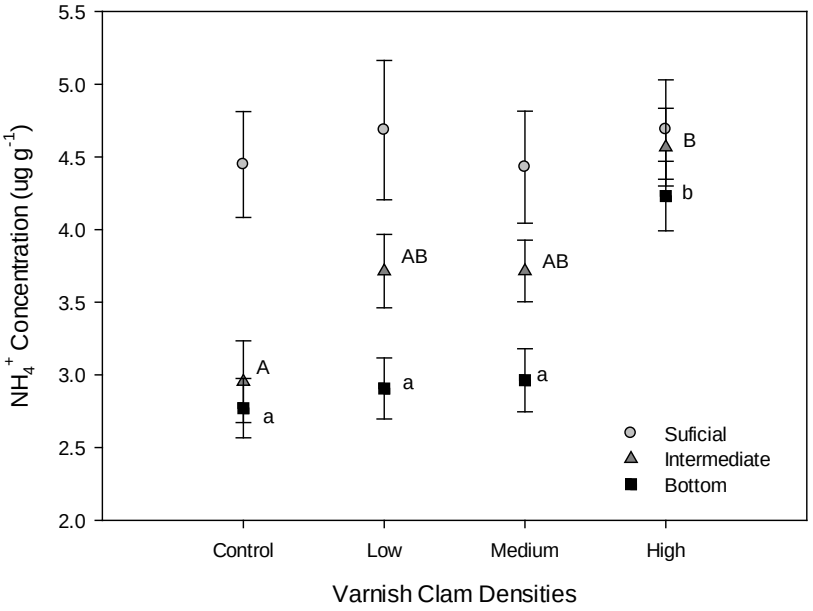


Figure 3.2 ***Ammonium concentrations in sediments with variable varnish clam densities, stratified by depth. Holm-Sidak comparisons at the intermediate depth represented with capitalized letters, lower case letters for the bottom depth. Levels not connected by the same letter are significantly different ($p<0.05$).***

There was no significant effect of varnish clam densities on iron concentrations at any of the depths: surficial (one-way ANOVA; $p = 0.59$; $F = 0.64$; $DF = 3$), intermediate ($p = 0.64$; $F = 0.57$; $DF = 3$) or bottom (Kruskal-Wallis; $p = 0.59$; $H = 1.92$; $DF = 3$) (Figure 3.3). However, when averaged across all densities and plotted against time, there was a distinct pattern that was noted (Figure 3.4). Initially, iron concentrations were significantly higher at the intermediate and bottom depths when compared to the surficial depth (Appendix C). However, after the storm, these levels dropped and were more similar to the concentrations found in the surficial layers (Appendix C).

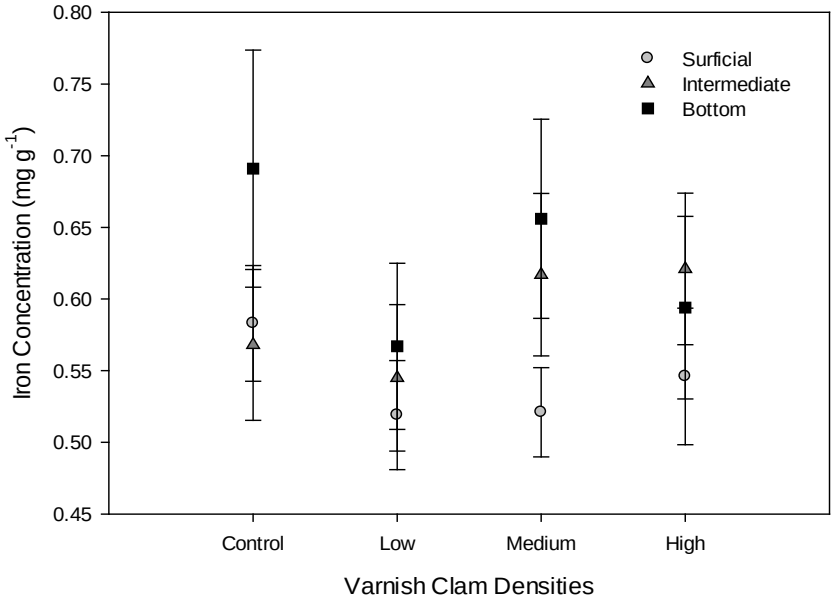


Figure 3.3 ***Iron concentrations in sediments across varying varnish clam densities, stratified by depth.***

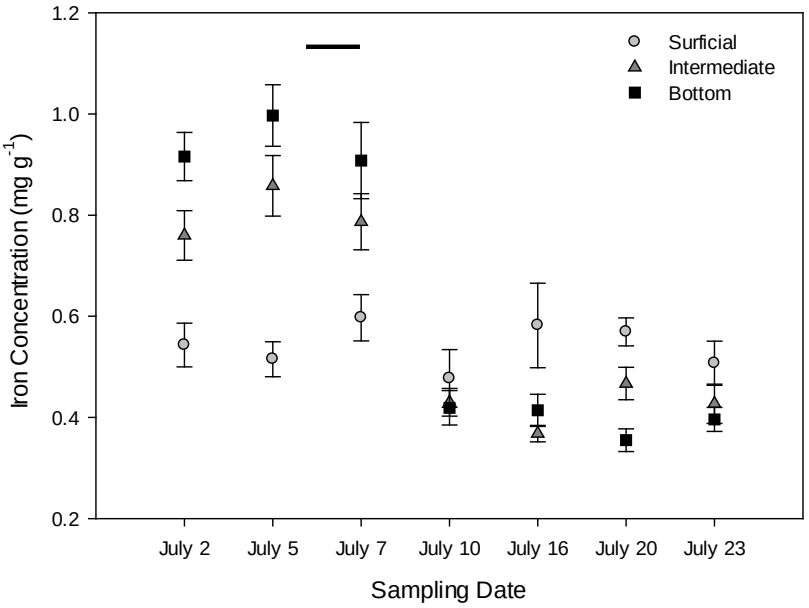


Figure 3.4 ***Iron concentrations in sediments by sampling dates, stratified by depth. Bar represents storming events that occurred from July 6-7 2009.***

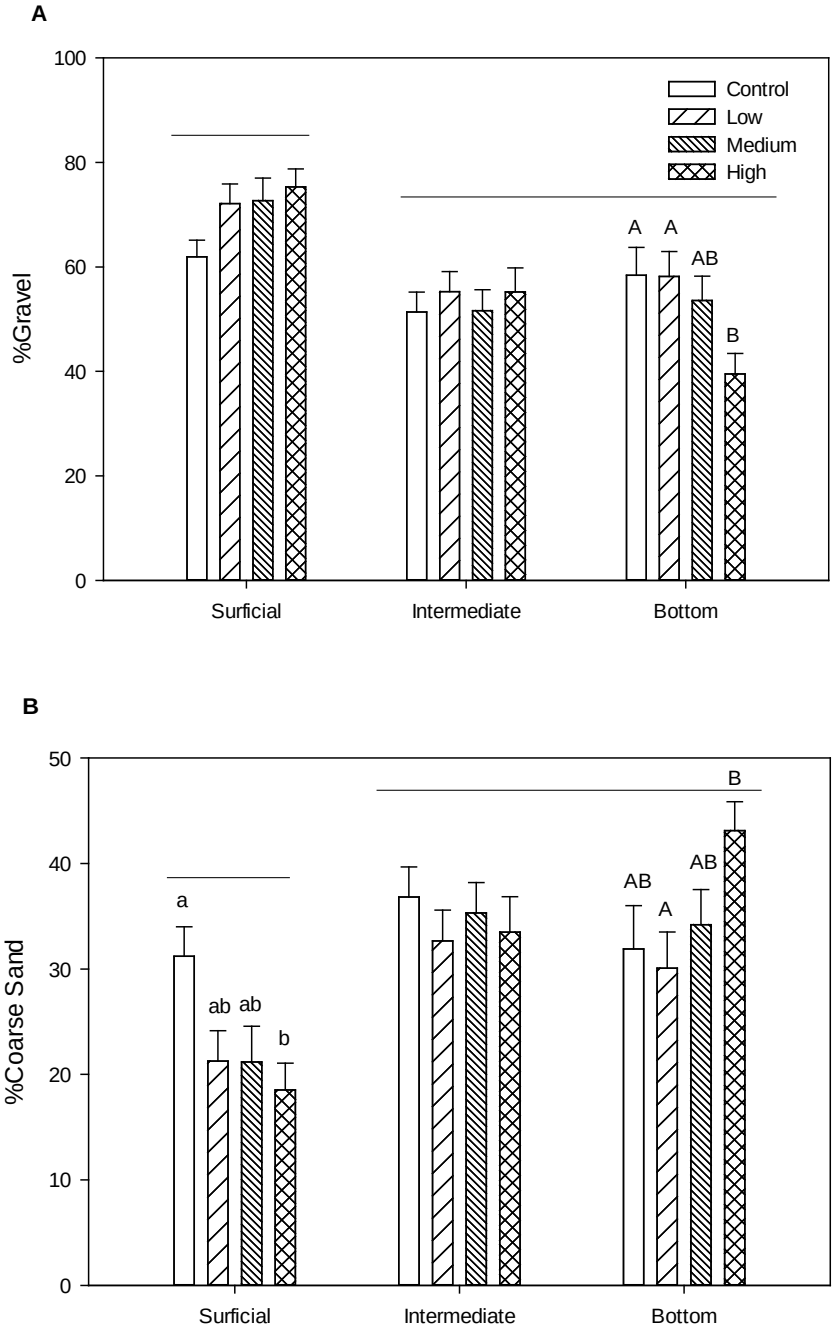
All grain size fractions failed normality even after an arcsine transformation; thus, non-parametric two-way ANOVA on ranks was performed on all fractions. A significant interaction between depth and treatment was detected for all fractions (Table 3.5). Depth alone had a significant effect on all fractions (Table 3.5). There was significantly more gravel in the surficial sediments than in the intermediate (Holm-Sidak; $p<0.001$; $t = 5.95$) and bottom sediments ($p<0.001$; $t = 5.91$). No significant difference was detected in the percentage of gravel between the intermediate and bottom depths ($p = 0.95$; $t = 0.07$) (Figure 3.5a). Significantly less coarse sand was found in surficial sediments when compared to the sediments at the intermediate (Holm-Sidak; $p<0.001$; $t = 5.39$) and bottom depths ($p<0.001$; $t = 5.19$). The amount of course sand did not differ significantly between the intermediate and bottom depths ($p = 0.91$; $t = 0.11$) (Figure 3.5b). There was significantly less fine sand at the surficial depth than at the intermediate (Holm-Sidak; $p<0.001$; $t = 6.23$) and bottom depths ($p<0.001$; $t = 6.73$). No significant differences in the percentage of fine sand were detected between the intermediate and bottom depths ($p = 0.54$; $t = 0.61$) (Figure 3.5c). Significantly more silt was found at the intermediate (Holm-Sidak; $p<0.001$; $t = 7.97$) and bottom depths ($p<0.001$; $t = 9.68$) than at the surficial depth. Intermediate and bottom depths did not differ significantly in the amount of silt ($p = 0.06$; $t = 1.90$) (Figure 3.5d). There was no significant effect of treatment alone for any of the fractions (Table 3.5).

Table 3.5 Non-parametric two-way ANOVA on the effects of depth and treatment on sediment grain size fractions

Fraction	Source of Variation	p-value	F-statistic	DF
%G	Depth*Treatment	0.02	2.70	6
	Depth	<0.001	23.41	2
	Treatment	0.43	0.915	3
%CS	Depth*Treatment	0.01	2.73	6
	Depth	<0.01	18.69	2
	Treatment	0.22	1.47	3
%FS	Depth*Treatment	0.03	2.33	6
	Depth	<0.001	28.09	2
	Treatment	0.26	1.34	3
%S	Depth*Treatment	0.02	2.65	6
	Depth	<0.001	53.55	2
	Treatment	0.31	1.20	3

Note: Grain size fractions in percentages: gravel (%G), coarse sand (%CS), fine sand (%FS) and silt (%S).

Significantly less gravel was found in high density treatments when compared to the control (Holm-Sidak; $p = 0.01$; $t = 3.14$) and low density treatments ($p = 0.01$; $t = 3.02$) within the bottom sediments. There were no significant differences in the percentages of gravel within the surficial or intermediate layers as a result of varnish clam density treatments (Figure 3.5a). Only the high varnish clam density treatment had significantly less coarse sand than the control (Holm-Sidak; $p = 0.03$; $t = 2.83$) within the surficial layer. Significantly higher percentages of coarse sand were found in the high varnish clam density treatments than in the low density treatments (Holm-Sidak; $p = 0.02$; $t = 3.06$) at the bottom depth. There was no significant effect of varying varnish clam density treatments on percent coarse sand within the intermediate layer (Figure 3.5b). Percentages of fine sand were significantly higher in the high varnish clam density treatments than in the control (Holm-Sidak; $p = 0.02$; $t = 3.64$), low density ($p = 0.01$; $t = 3.05$) and medium density treatments ($p = 0.02$; $t = 2.91$) at the bottom depth. Varying varnish clam densities did not have significant effects on the percentages of fine sand in the surifical or intermediate layers (Figure 3.5c). There was significantly more silt in the high varnish clam density treatments than in the controls (Holm-Sidak; $p = 0.01$; $t = 3.02$) and low density treatments ($p = 0.01$; $t = 3.32$) at the bottom depth. No significant differences in the percentages of silt as a result of varying varnish clam densities were detected in the surfical or intermediate layers (Figure 3.5d).


A
100
80
60
40
20
0
%Gravel
Control
Low
Medium
High
A
A
AB
B
Surficial
Intermediate
Bottom
B
50
40
30
20
10
0
%Coarse Sand
a
ab
ab
b
AB
A
AB
B
Surficial
Intermediate
Bottom

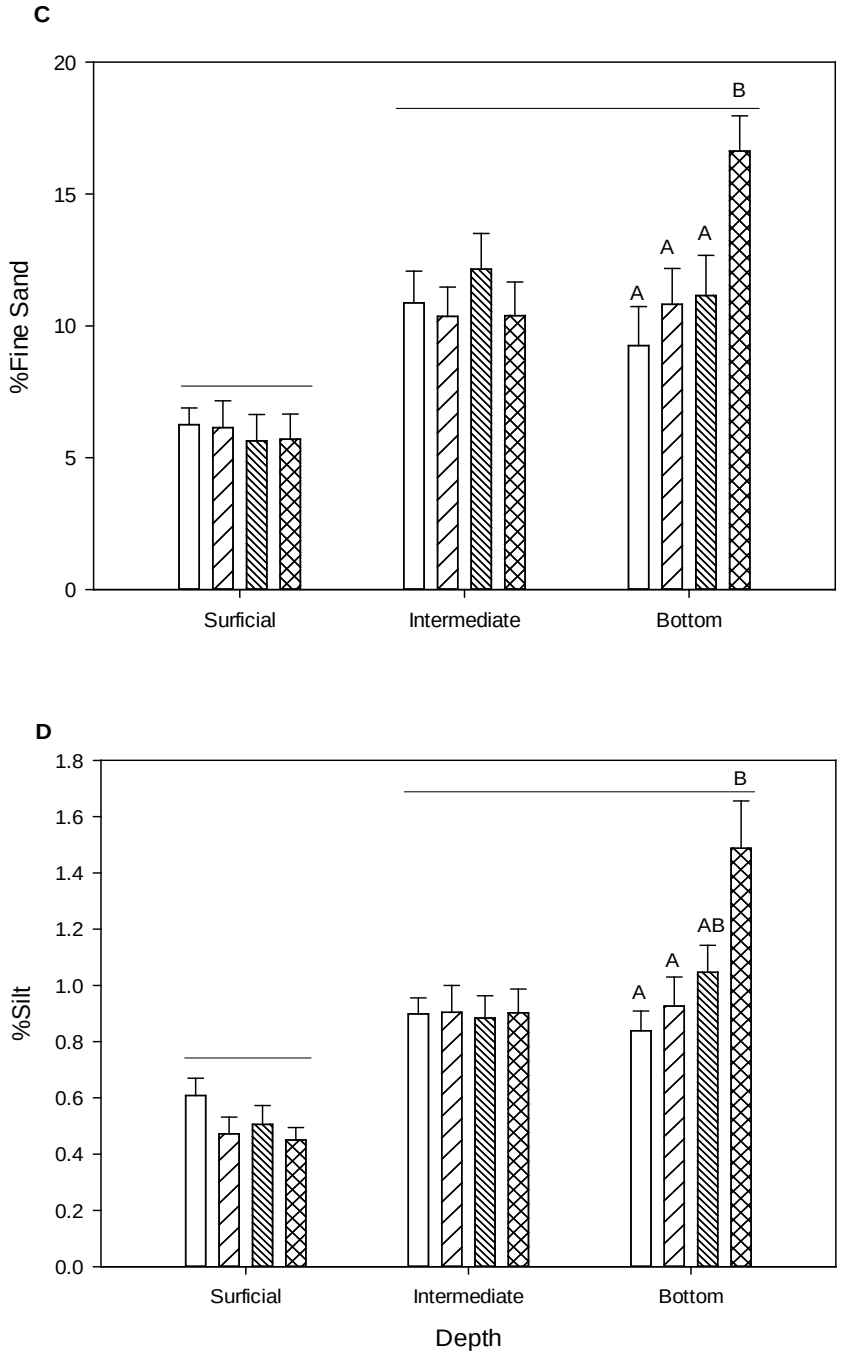


Figure 3.5 ***Percentage of each fraction of the grain size distribution across varying varnish clam density treatments by depth. A) percentage of gravel B) percentage of coarse sand C) percentage of fine sand D) percentage of silt. Levels not connected by the same letter or bar are significantly different ($p<0.05$).***

3.3. Sediment Nutrient Correlations with Algal Bloom

There were no significant relationships detected between photopigments and iron or NH_4^+ concentrations (Table 3.6). There was an increase in fresh phytoplankton that occurred around the same time as when the thunderstorms occurred, which follows a similar pattern to the one noted for iron concentrations (Figure 3.4 and 3.6).

Table 3.6 Linear regressions of photopigments and sediment nutrient (NH_4^+ and iron) concentrations

Pigment	Nutrient	Slope	r^2	p-value	F Statistic
Chl *a*	NH_4^+	- 0.38	0.01	0.80	0.07
Phaeopigment	NH_4^+	0.09	0.02	0.78	0.09
Chl *a*	Iron	- 0.09	0.45	0.10	4.14
Phaeopigment	Iron	0.09	0.02	0.78	0.09

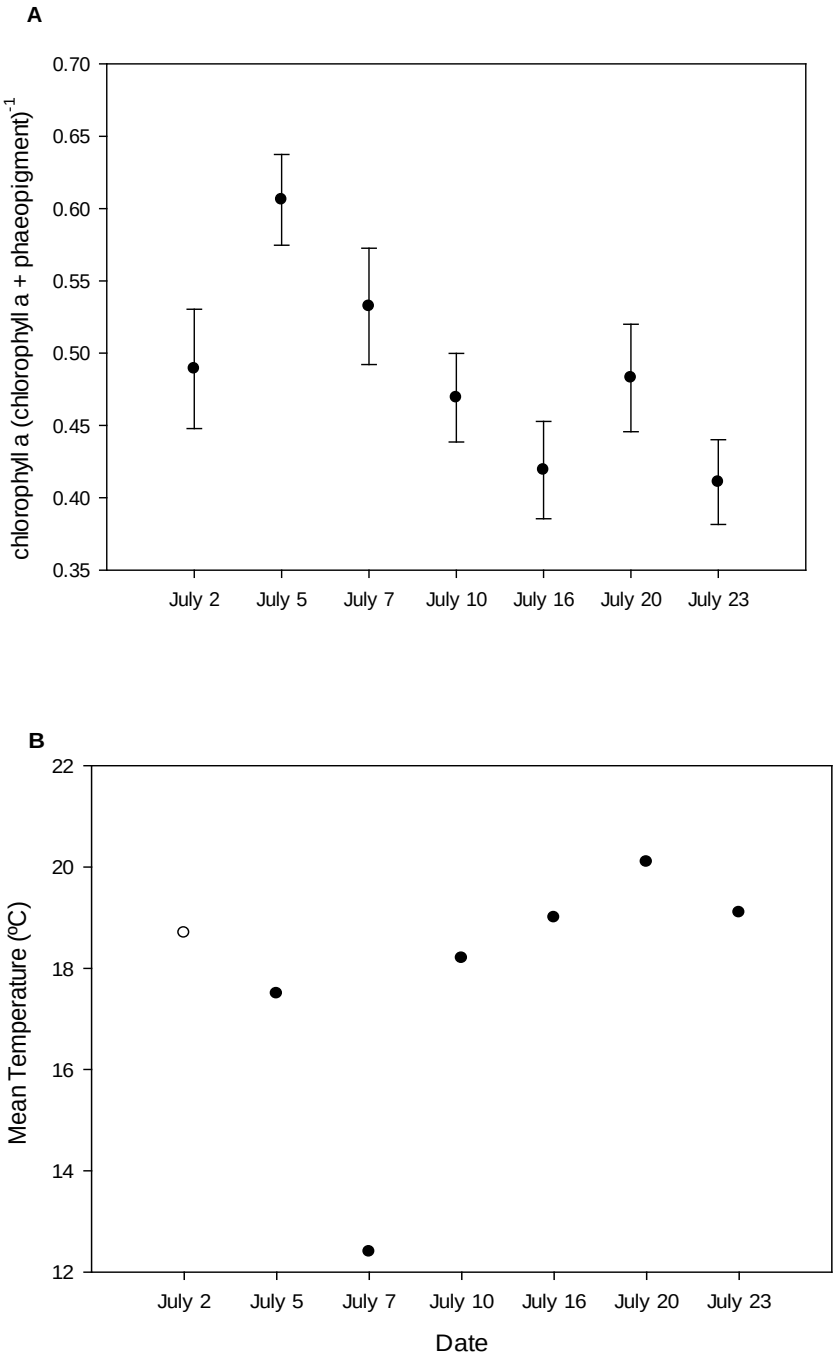


Figure 3.6 ***Bivariate plots across time for A) relative phytoplankton deposition rate estimated from chlorophyll a (chlorophyll a + phaeopigment)$^{-1}$ B) temperature data obtained from the National Climate Data and Information Archive for the Comox A station (49º43'00" N, 124º54'00" W).***

3.4. Tier III: Microcosm Experiments

Both time (RM ANOVA; p = 0.0002; F = 9.38; DF = 2) and treatment (p<0.0001; F = 29.77; DF = 3) had an effect on sediment NH_4^+ concentrations. However, there was also a significant interaction between time and treatment (p<0.0001; F = 5.89; DF = 6) on NH_4^+ concentrations. Subsequent one-way ANOVA analyses on each sampling day found that over the course of the experiment, sediment NH_4^+ concentrations generally were not significantly different between the treatments with varnish clams and the controls without varnish clams (e.g. sterilized treatments with varnish clams did not significantly differ from sterilized controls without varnish clams in the amount of sediment NH_4^+ concentrations on most sampling days) (Figure 3.7; Appendix D). Generally, sterilized treatments and controls had significantly higher sediment NH_4^+ concentrations than the unsterilized treatments and controls (Figure 3.7; Appendix D).

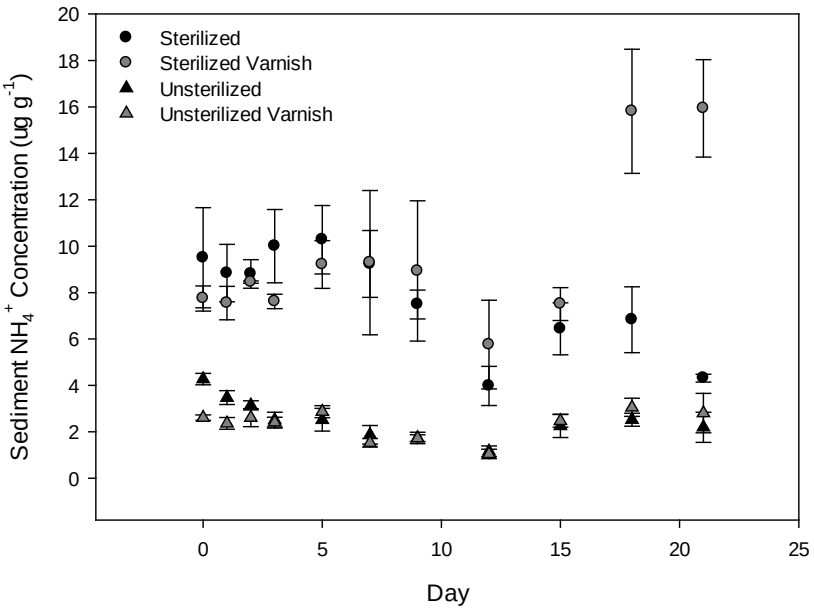


Figure 3.7 Bivariate plot of surficial sediment ammonium concentrations within microcosms across time.

As with sediment, overlying water NH_4^+ concentrations were significantly affected by time (RM ANOVA; p = 0.02; F = 4.05; DF = 2) and treatment (p<0.0001; F = 18.32; DF = 3) with a significant interaction between the two factors (p<0.0001; F = 5.43; DF = 6). Concentrations of NH_4^+ did not differ significantly between the treatments with varnish clams and their respective controls until the last 3 days of the experiment (Figure 3.8; Appendix E). In those last 3 days, sterilized treatments with varnish clams had significantly more NH_4^+ in the overlying waters than in the sterilized treatments with no addition of varnish clams (Appendix E). In general, there were significantly higher concentrations of NH_4^+ in the overlying water in the sterilized treatments and controls than in the unsterilized treatments and controls (Figure 3.8; Appendix E)

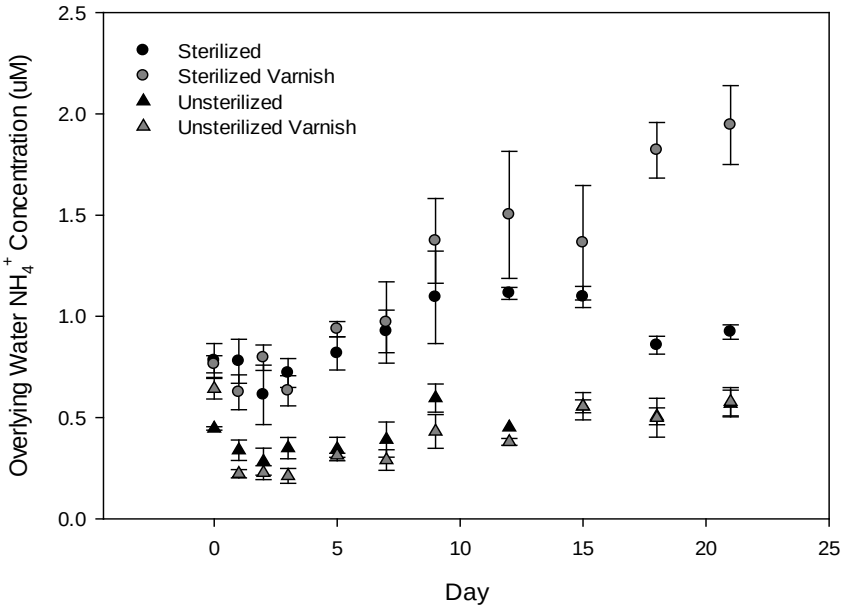


Figure 3.8 Ammonium concentrations in overlying waters of microcosms across time.

All microcosms appeared to follow the same trend as temperatures in the cold room increased (Figure 3.9). The K_d value decreased with increasing temperatures (e.g. more NH_4^+ was found in the water column). As temperatures began to decrease back to 15ºC, less NH_4^+ fluxed out of the sediments.

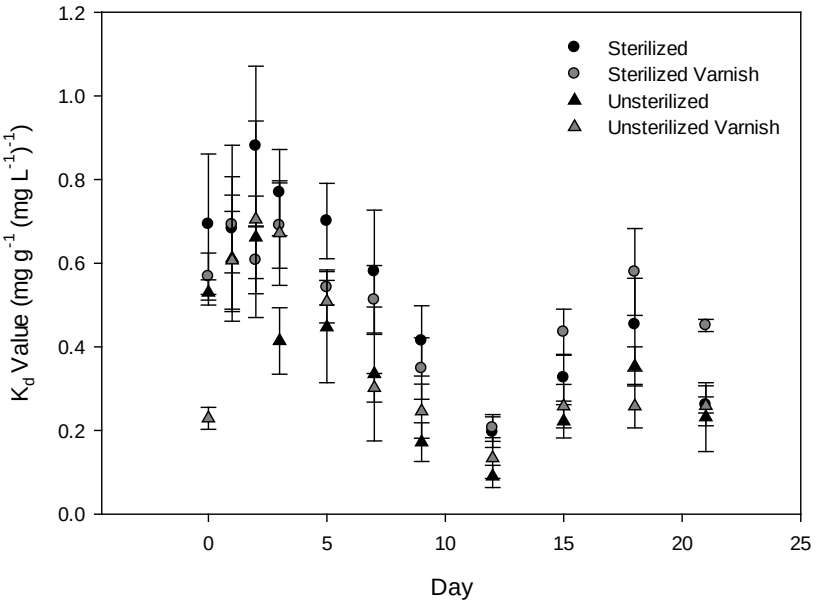


Figure 3.9 Sorption value (K_d), calculated from (sediment ammonium concentrations) (ammonium concentrations in overlying waters)$^{-1}$, of microcosms across time

4. Discussion

4.1. Tier I: Field Survey

The grain size distribution of sediments is important in coastal marine communities as it directly affects the ecology, chemical and physical characteristics of these areas (Mannino and Montagna 1997; Grillo et al 1998). This study demonstrated that bivalve density was best explained by grain size distribution. Numerous studies have shown that the distribution of macroinvertebrates is correlated to sediment composition and salinity gradients (e.g. Schlacher and Wooldridge 1995; Grillo et al 1998; Mannino and Montagna 1999; Byers 2002b; Nanami et al 2005). Studies out of Buzzards Bay, Massachusetts have reported that bivalve distribution is a result of the relationship between feeding type and sediment composition (Sanders 1958; Rhoads and Young 1970). The classic study by Sanders (1958) found that suspension-feeding bivalves were more abundant in sandy flats as strong current activity prevents the accumulation of detrital matter while supplying more potential food to suspension-feeders. Conversely, there were higher abundances of deposit-feeding bivalves in muddy flats as weak currents allowed the accumulation of detritus (Sanders 1958). The present results are consistent with this finding as bivalve densities, which were dominated by Manila and Pacific littleneck clams, were positively correlated to the sand fractions.

In addition to sediment composition, sediment nutrient concentrations have also been found to be correlated to the distribution of bivalves (e.g. Chester et al 1983; Grillo et al 1998; Sousa et al 2008). Numerous studies of mussel and oyster farms have found accumulations of organic matter and high levels of NH_4^+ with high bivalve densities (e.g. Giles et al 2006; Nizzoli et al 2006a; Dame 2011). In the present study, correlation between organic matter concentrations and bivalve densities were detected only at the intermediate depth. Biodeposits often survive degradation in the water column and become incorporated in sediments (Henriksen et al 1983; Kristensen 1988; Falkowski et

al 1998). This may explain why there were no correlations detected between bivalve densities and organic matter in the surficial layer but were detected at the intermediate depth. The densities of the bivalves found ranged between 0-464 individuals m^{-2} with an average of 118 individuals m^{-2}. These values agree with Whiteley (2005) but are an order of magnitude lower than those reported for intensely farmed regions (e.g. Bartoli et al 2001). At these naturally low densities, sediment NH_4^+ concentrations are independent of the number of bivalves present. Rather the physical characteristics of the sediment are more important, which was shown by the positive correlations between NH_4^+ concentrations and finer sedimentary material. It is in agreement with previous studies that have shown a correlation between sediment porosity and nutrient content (Nedwell 1993; Volkenborn et al 2007). As all grain size fractions are correlated, increases in fine materials result in a decrease in larger particulates such as gravel. This was confirmed by the negative correlations between the percentages of gravel with the other grain size fractions.

4.2. Tier II: Density Manipulation Experiments

Numerous studies have shown that high densities of bivalves result in the accumulation of organic matter due to the excretion of biodeposits (e.g. Bartoli et al 2001; Hasanudin et al 2004; Nizzoli et al 2007). No statistically significant differences in the concentration of organic matter were detected as a result of varying varnish clam densities. Varnish clams are not obligatory suspension-feeders; they are also capable of deposit feeding via locomotry and pedal sweep feeding (Gillespie et al 1999). Consequently, they may have reworked the organic matter and prevented accumulations. Bioturbational activities result in deeper penetrations of oxygen and accelerate the rate of decomposition (Jones et al 1994). Thus, microbial decomposition of the organic matter may have been stimulated, resulting in a lack of accumulation.

There were significantly higher concentrations of NH_4^+ in the presence of high densities of varnish clams than in their absence at the intermediate and bottom depths. Numerous studies from shellfish aquaculture have shown that high bivalve densities are correlated to high concentrations of NH_4^+ as a result of high organic matter loading leading to enhanced mineralization rates as well as direct excretion by bivalves (e.g.

Christensen et al 2003; Nizzoli et al 2005, 2006b). The concentrations found here (4.45 ± 0.36 ug g^{-1} ww) were similar to those reported by Cook (2003) who reported median values of 0.006 mg g^{-1} ww for bulk sediments taken from control cages. However, these values are less than those reported for the same area by Bendell et al (2010) (Table 4.1). This is most likely due to cage effects. Air exposure during periods of low tide accelerates the rate of decomposition, which results in increases in NH_4^+ within sediments (Rocha 1998). However, pooling water prevented this from occurring and may have resulted in lower concentrations of NH_4^+. Additionally, Laima et al (2002) found that water logged sediment cores had higher actual nitrification rates than reflooded sediment cores, which may also explain the lower NH_4^+ concentrations.

Table 4.1 Sediment ammonium concentrations from other studies in comparison to sediment ammonium concentrations found from all three experimental tiers.

Site	Description	NH_4^+ Conc.	NH_4^+ **Conc. (µg** g^{-1} dw)	Reference
Tier I	Surficial (0 Density)	1.24 µg g^{-1} ww		
Tier II Control	Surficial	4.45 µg g^{-1} ww		
	Intermediate	2.95 µg g^{-1} ww		
	Bottom	2.77 µg g^{-1} ww		
Tier III	S	7.80 µg g^{-1} ww		
	SV	10.12 µg g^{-1} ww		
	US	2.51 µg g^{-1} ww		
	USV	2.32 µg g^{-1} ww		
Yangtze Estuary LC site: adjacent coastal tidal flat	Surficial sediments	0.57 µg g^{-1} dw	0.57	Hou et al 2003
North Sea Stn 4	Medium sand, 2-3 cm depth	17 nmol g^{-1} dw	0.31	Raaphorst and Malschaert 1996
Laguna Madre Estuary Marker 45	Bulk sediments	4.84 µmol g^{-1} dw	80	Morin and Morse 1999
Fillongley Provincial Park	Bulk sediments	0.006 mg g^{-1} ww	6*	Cook 2003
West coast of BC	Bulk sediments	0.012 mg g^{-1} dw	12	Bendell et al 2010
West coast of BC	Surficial sediments	0.018 mg g^{-1} dw	18	Bendell et al 2010

Note: Tier III treatments denoted as: Sterilized (S), Sterilized with varnish (SV), Unsterilized (US) and Unsterilized with varnish (USV). NH_4^+ concentrations in the left column denote the original concentrations listed in literature. NH_4^+ concentrations on the right are after conversion to a standard unit (µg g^{-1} dw). (*) denotes the exception, units here expressed in µg g^{-1} ww

It was hypothesized that accumulations of organic matter in the presence of high densities of bivalves would result in anoxia and the dissolution of particulate iron into its dissolved form, which is more biologically available (Geider 1999; Barbeau 2006). Thus, concentrations of dissolved iron in the presence of high densities of bivalves would have increased. However, there was no significant effect of density treatments on iron concentrations. Rather, iron concentrations seem to be under the influence of sediment reworking as a result of storming events. The model by Bell et al (1997) showed that episodic storms can rework sediments up to a depth of 8 cm.

The presence of bivalves increases siltation due to increases in detrital matter (Karatayev et al 1997; Volkenborn et al 2007). The results here are in agreement with this finding as there were significantly higher amounts of fine sand and silt in the high density treatments when compared to the controls (Figure 3.5). This increase may have consequences for nutrient concentrations within the sediments. Previous studies have shown that nutrient concentrations are correlated to the amount of silt/clay fractions found within sediments as the finer sediments retain nutrients better when compared to sediments with higher porosity (Nedwell 1993; Volkenborn et al 2007).

4.3. Sediment Nutrient Correlations with Algal Bloom

Nitrogen and iron are both limiting nutrients in marine ecosystems (e.g. Laverock et al 2011; Barbeau 2006). Increased concentrations of nitrogen from eutrophication may lead to algal blooms (e.g. Gray et al 2002). Earlier bottle experiments have shown increased diatom growth with increasing concentrations of iron (Cooper 1935). Consequently, it was hypothesized that the observed algal bloom was the result of increases in one or both of these nutrients. No significant correlations were found between NH_4^+ or iron concentrations and the measures of primary productivity (Table 3.6). However, fresh inputs of phytoplankton appear to coincide with depressed concentrations of iron (Figure 3.6). It is possible that storming events reworked sediments, releasing iron into the water column, which resulted in the algal bloom that was observed.

4.4. Tier III: Microcosm Experiments

It was anticipated that with the sterilization process, that all NH_4^+ concentrations in the sediments and in the water column would be the result of varnish clam excretion rather than the combination of direct excretion and breakdown of organic matter. However, on the whole, sterilized treatments with and without varnish clams did not significantly differ in NH_4^+ concentrations in the sediments or overlying waters. Varnish clam densities may not have been high enough for sufficient accumulations of NH_4^+ to occur (Tier I and II). There is also the potential that the autoclaving process did not completely sterilize sediments. Consequently, populations of nitrifying bacteria may have recovered and utilized the regenerated NH_4^+, resulting in a lack of accumulations. Concentrations of NH_4^+ in sediments and overlying waters did not significantly differ in the presence or absence of varnish clams in unsterilized treatments. This is probably due to the population of microphytobenthos that has been shown to intercept inorganic nutrient diffusion into overlying waters (e.g. Christensen et al 2003; Newell et al 2002).

The concentration of NH_4^+ in the sediments (2.51 ± 0.03 µg g^{-1} ww) was lower than what was found by Cook (2008) (Table 4.1) for sediments from Fillongley Provincial Park. However, this result is not surprising given the amount of processing the sediments went through before being placed into microcosms. Water NH_4^+ concentrations were also lower than those reported from studies of Manila clam aquaculture areas in the Sacca di Goro lagoon, Italy (Nizzoli et al 2005; 2006a, 2006b) (Table 4.2). However, given the low numbers of varnish clams used in the experiment (200 individuals m^{-2}), this was not unexpected.

Table 4.2 Ammonium water concentrations from other studies in comparison to overlying water ammonium concentrations found in microcosm experiments (Tier III).

Site	Description	NH_4^+ Conc. (µM)	Reference
Tier III	S	0.88	
	SV	1.16	
	US	0.47	
	USV	0.41	
Firth of Thames Control site	Bottom water	0.86-1.26	Giles et al 2006
Ria Formosa Stn D	Bottom water	2.3	Falcão and Vale 1998
Sacca di Goro lagoon	Exploited for Manila clam, no clams because of dredge	16.63	Nizzoli et al 2006a
Sacca di Goro lagoon Control site	Licensed for Manila clam framing	14	Nizzoli et al 2006b
Beatrix Bay Control site	250 m away from longline mussel farm, bottom waters	1.9	Christensen et al 2003
Sacca di Goro lagoon Control site	Control but used for Manila farming	5.5	Nizzoli et al 2005
Tagus estuary R1 tidal height 1.4 m	Porewater	8.3	Cabrita and Brotas 2000

Note: Tier III treatments denoted as: Sterilized (S), Sterilized with varnish (SV), Unsterilized (US) and Unsterilized with varnish (USV).

With increasing temperatures, greater flux of NH_4^+ into the water column was observed (e.g. decreasing Kd values) (Figure 3.9). Zhu et al (1999) found that Manila clam excretion rates increased with increasing temperatures. Thus, higher temperatures may have increased metabolic activities of the varnish clams, resulting in higher excretion rates. Increased temperatures may have resulted in NH_4^+ being more readily extracted from sediments, which may also explain the greater flux of NH_4^+ into the water column (Maksymowska-Brossard and Piekarek-Jankowska 2001).

4.5. Integration

High densities of varnish clams did not result in significant accumulations of organic matter potentially due to the alternate feeding modes of the varnish clam (Tier II). It was expected that the resulting anoxia would cause the dissolution of particulate iron. However, no accumulations of organic matter occurred and the anticipated anoxia did not happen; thus, no changes in iron concentrations were noted as a result of varying varnish clam densities. Rather, sediment reworking as a result of thunderstorms seemed to have a much greater influence on iron concentrations (Tier II). Concentrations of NH_4^+ within sediments were higher in the presence of high densities of varnish clams (Tier II). This may have important implications for ecosystem functioning as NH_4^+ is preferentially used by primary producers. However, it appears that at low bivalve concentrations, accumulations of organic matter do not occur and any available NH_4^+ is scavenged quickly by the microphytobenthos that potentially out-compete the microbial components of the system (Tier I and III).

5. Conclusions

Shellfish aquaculture is a multimillion dollar industry off the coast of BC. However, studies have shown that predator netting as well as high bivalve densities results in greater amounts of silt as well as accumulations of organic matter (e.g. Bendell et al. 2010). These activities may facilitate the invasion of varnish clams into these areas as distribution of macroinvertebrates can be linked to sediment composition (e.g. Grillo et al 1998; Byers 2002b; Tier I) and biodeposits are not processed by Manila clams which are strictly suspension-feeders. Additionally, harvesting practices rework sediments, releasing NH_4^+ and potentially iron (e.g. Alstyne et al 2011; Tier II). Thus, these areas may experience enhanced concentrations and recycling of inorganic nitrogen via harvesting practices as well as high densities of varnish clams. Should such increases in nitrogen and iron occur, it could result in eutrophication of the system and increased incidents of algal blooms (e.g. Gray et al 2002).

Other studies have shown significant accumulations of organic matter in the presence of high bivalve densities (e.g. Bartoli et al 2001; Hasanudin et al 2004; Nizzoli et al 2007). The lack of significant effects of high varnish clam densities on organic matter concentrations here suggest that other factors such as deposit-feeding and bioturbational activities may be working here (Tier II). There is the potential that significant bioturbational activities can help mitigate anoxic conditions and changes to redox potentials as the result of accumulating organic matter.

As shown in Tier I and II, if bivalve densities are relatively low (i.e. <500 individuals m^{-2}), there do not appear to be significant impacts on sediment composition or nutrient concentrations. This has potential farming and invasive species management implications. It implies that if managers can maintain low varnish clam densities through perhaps dedicated varnish clam harvesting, some of the negative environmental impacts could be reduced or eliminated. This is in agreement with the findings of Parker et al

(1999) who suggested that the impacts of invasive species were the result of range, abundance, and local impacts.

Much work is still required as the invasion of the varnish clam is most likely not complete. Future studies should include short-term mesocosms without cages to avoid cage effects that may have potentially impacted the results of this study. Given the close association of varnish clams with Manila and Pacific littleneck clams (per obs), studies should be performed to determine changes to sediment characteristics in the presence of all three species as well as direct impacts of the varnish clam invasion on the two economically valuable species. With global climate change, temperatures are expected to increase over the next few decades. As bivalve metabolism is tied to temperature, studies should be performed examining the impact of intense warming periods on varnish clam metabolic activities and sediment parameters.

References

Ahrens WH, Cox DJ, Budhwar G. 1990. Use of the arcsine and square root transformations for subjectively determined percentage data. Weed Sci 38:452-8.

Archer DE, Johnson K. 2000. A model of the iron cycle in the ocean. Global Biogeochem Cycles 14(1):269-79.

Barbeau K. 2006. Photochemistry of organic iron(III) complexing ligands in oceanic systems. Photochem Photobiol 82:1505-16.

Barbeau K, Rue EL, Bruland KW, Butler A. 2001. Photochemical cycling of iron in the surface ocean mediated by microbial iron(III)-binding ligands. Nature 413:409-13.

Bartoli M, Nizzoli D, Viaroli P, Turolla E, Castaldelli G, Fano EA, Rossi R. 2001. Impact of *Tapes philippinarum* farming on nutrient dynamics and benthic respiration in the Sacca di Goro. Hydrobiologia 455:203-12.

Bax N, Williamson A, Aguero M, Gonzalez E, Geeves W. 2003. Marine invasive alien species: A threat to global biodiversity. Mar Policy 27:313-23.

Bell RG, Hume TM, Dolphin TJ, Green MO, Waiters RA. 1997. Characterisation of physical environmental factors on an intertidal sandflat, Mancikau Harbour, New Zealand. J Exp Mar Biol Ecol 216:11-31.

Bendell LI, Duckham C, L'Espérance T, Whiteley JA. 2010. Changes in geochemical foreshore attributes as a consequence of intertidal shellfish aquaculture: A case study. Mar Prog Ser 404:91-108.

Bendell-Young LI. 2006. Contrasting the community structure and select geochemical characteristics of three intertidal regions in relation to shellfish farming. Environ Conserv 33(1):21-7.

Breitbarth E, Achterberg EP, Ardelan MV, Baker AR, Bucciarelli E, Chever F, Croot PL, Duggen S, Gledhill M, Hassellöv M, et al. 2010. Iron biogeochemistry across marine systems - progress from the past decade. Biogeosciences 7:1075-97.

Byers JE. 2002a. Impact of non-indigenous species on natives enhanced by anthropogenic alteration of selection regimes. Oikos 97(3):449-58.

Byers JE. 2002b. Physical habitat attribute mediates biotic resistance to non-indigenous species invasion. Oecologia 130:146-56.

Caetano M, Falcão M, Vale C, Bebianno MJ. 1997. Tidal flushing of ammonium, iron and manganese from inter-tidal sediment pore waters. Mar Chem 58:203-11.

Carlton JT. 1996. Marine bioinvasions: The alteration of marine ecosystems by nonindigenous species. Oceanogr 9(1):36-43.

Chester AJ, Ferguson RL, Thayer GW. 1983. Environmental gradients and benthic macroinvertebrate distributions in a shallow North Carolina estuary. Bull Mar Sci 33(2):282-95.

Christensen PB, Glud RN, Dalsgaard T, Gillespie P. 2003. Impacts of longline mussel farming on oxygen and nitrogen dynamics and biological communities of coastal sediments. Aquaculture 218:567-88.

Cook NA. 2008. Feeding ecology and bioturbation: Determining the ecological role of *Eusipra lewisii* [thesis]. Burnaby (BC): Simon Fraser University.

Cooper LHN. 1935. Iron in the sea and in marine plankton. Proc R Soc London, Ser B 118(810):419-38.

Dame RF. 2011. Physical envrionmental interactions. In: Dame RF, editor. Ecology of marine bivalves: An ecosystem approach. 2nd ed. Boca Raton (FL): CRC Press. p 43-61.

Danovaro R, Pusceddu A. 2010. Photosynthetic pigment concentrations in marine sediments. In: Danovaro R, editor. Methods for the study of deep-sea sediments, their functionality and biodiversity. Boca Raton (FL): CRC Press. p 45-51.

Dudas SE. 2005. Invasion dynamics of a non-indigenous bivalve, *Nuttallia obscurata*, (Reeve 1857), in the Northeast Pacific [dissertation]. Victoria (BC): University of Victoria.

Dudas SE, Dower JF. 2006. Reproductive ecology and dispersal potential of the varnish clam, *Nuttallia obscurata*, a recent invader in the Northeast Pacific. Mar Ecol Progr Ser 320:195-205.

Falkowski PG, Barber RT, Smetacek V. 1998. Biogeochemical controls and feedbacks on ocean primary productivity. Science 281:200-6.

Feuillet-Girard M, Gouleau D, Blanchard G, Joassard L. 1997. Nutrient fluxes on an intertidal mudflat in Marennes-Oléron Bay, and influence of the emersion period. Aquat Living Resour 10:49-58.

Geider RJ. 1999. Complex lessons of iron uptake. Nature 400:815-6.

Giles H, Pilditch CA, Bell OG. 2006. Sedimentation from mussel (*Perna canaliculus*) culture in the Firth of Thames, New Zealand: Impacts on sediment oxygen and nutrient fluxes. Aquaculture 261:125-40.

Gillespie GE, Parker M, Merilees W. 1999. Distribution, abundance, biology and fisheries potential of the exotic varnish clam (*Nuttalia obscurata*) in British Columbia. Ottawa (ONT): Fisheries and Oceans Canada. Canadian Science Advisory Secretariat [CSAS] Research Document 2011/143.

Gillespie GE, Rusch B, Gormican SJ, Marshall R, Munroe D. 2001. Further investigations into the fisheries potential of the exotic varnish clam (*Nuttallia obscurata*) in British Columbia. Ottawa (ONT): Fisheries and Oceans Canada. CSAS Research Document 2001/143.

Gray JS, Shiu-Sun R, Or YY. 2002. Effects of hypoxia and organic enrichment on the coastal marine environment. Mar Ecol Progr Ser 238:249-79.

Grillo MCG, Ventura CRR, da Silva SHG. 1998. Spatial distribution of bivalvia (Mollusca) in the soft-bottoms of Ilha Grande Bay, Rio de Janeiro, Brazil. Rev Bras Oceanogr 46(1):19-31.

Grosholz ED, Ruiz GM. 2009. Multitrophic effects of invasions in marine and estuarine systems. In: Rilov G, Crooks JA, editors. Biological invasions in marine ecosystems. Berlin: Springer. p 305-24.

Hagy III JD, Boynton WR, Jasinski DA. 2005. Modelling phytoplankton deposition to Chesapeake Bay sediments during winter-spring: Interannual variability in relation to river flow. Estuar Coast Shelf Sci 62:25-40.

Harrison PJ, Berges JA. 2005. Marine culture media. In: Andersen RA, editor. Algal culturing techniques. Burlington (MA): Elseviser. 25 p.

Hasanudin U, Fujita M, Kunihiro T, Fujie K, Suzuki T. 2004. The effect of clams (*Tapes philippinarum*) on changes in microbial community structure in tidal flat sediment mesocosms, based on quinone profiles. Ecol Eng 22(3):185-96.

Henriksen K, Rasmussen MB, Jensen A. 1983. Effect of bioturbation on microbial nitrogen transformations in the sediment and fluxes of ammonium and nitrate to the overlying water. Ecol Bull 35:193-205.

Herbert RA. 1999. Nitrogen cycling in coastal marine ecosystems. FEMS Microbiol Rev 23(5):563-90.

Holmer M, Duarte CM, Heilskov A, Olesen B, Terrados J. 2003. Biogeochemical conditions in sediments enriched by organic matter from net-pen fish farms in the Bolinao area, Philippines. Mar Pollut Bull 46:1470-9.

Jickells TD, Rae JE. 1997. Biogeochemistry of intertidal sediments. In: Jickells TD, Rae JE, editors. Biogeochemistry of intertidal sediments. New York (NY): Cambridge University Press. p 1-15.

Jones CG, Lawton JH, Shachak M. 1994. Organisms as ecosystem engineers. Oikos 69(3):373-86.

Karatayev AY, Burlakova LE, Padilla DK. 1997. The effects of *Dreissena polymorpha* (Pallas) invasion on aquatic communities in Eastern Europe. J Shellfish Res 16(1):187-203.

Keeney DR, Nelson DW. 1982. Nitrogen - inorganic forms. In: Page AL, editor. Methods of soil analysis. Part 2: Chemical and microbiological properties. 2nd ed. Madison (WIS): Soil Science Society of America. p 674-682.

Kristensen E. 1988. Benthic fauna and biogeochemical processes in marine sediments: Microbial activities and fluxes. In: Blackburn TH, Søregsen J, editors. Nitrogen cycling in coastal marine environments. SCOPE symposium; 1985 June 3-7; Aarhus, Denmark. Essex (UK): Wiley & Sons. p 275-99.

Lafferty KD, Kuris AM. 1996. Biological control of marine pests. Ecology 77(7):1989-2000.

Laima M, Brossard D, Sauriau P, Girard M, Richard P, Gouleau D, Joassard L. 2002. The influence of long emersion on biota, ammonium fluxes and nitrification in intertidal sediments of Marennes-Oléron Bay, France. Mar Environ Res 53:381-402.

Laverock B, Gilbert JA, Tait K, Osborn AM, Widdicombe S. 2011. Bioturbation: Impact on the marine nitrogen cycle. Biochem Soc Trans 39:315-20.

Lavrentyev PJ, Gardner WS, Yang LY. 2000. Effects of the zebra mussel on nitrogen dynamics and the microbial community at the sediment-water interface. Aquat Microb Ecol 21 (2): 187-94.

Lelieveld SD, Pilditch CA, Green MO. 2004. Effects of deposit feeding bivalve (*Macoma liliana*) density on intertidal sediment stability. N Z J Mar Freshwat Res 38:115-28.

Mack RN, Simberloff D, Lonsdale WM, Evans M, Clout M, Bazzaz FA. 2000. Biotic invasions: Causes, epidemiology, global consequences, and control. Ecol Appl 10:689-710.

Maksymowska-Brossard D, Piekarek-Jankowska H. 2001. Seasonal variability of benthic ammonium release in the surface sediments of the Gulf of Gdańsk (southern Baltic Sea). Oceanologia 43 (1): 113-36.

Malcom JJ, Sivyer DB. 1997. Nutrient cycling in intertidal sediments. In: Jickells TD, Rae JE, editors. Biogeochemistry of intertidal sediments New York (NY): Cambridge University Press. p 84-98.

Mannino A, Montagna PA. 1997. Small-scale spatial variation of macrobenthic community structure. Estuaries 20(1):159-73.

Martin JH, Fitzwater SE. 1988. Iron deficiency limits phytoplankton growth in the north-east Pacific subarctic. Nature 331:341-3.

Martin TD, Creed JT, Brockhoff CA. 1994. Method 200.2: Sample preparation procedure for spectrochemical determination of total recoverable elements. Cincinnati (OH): United States Environmental Protection Agency.

Nakamura Y, Kerciku F. 2000. Effects of filter-feeding bivalves on the distribution of water quality and nutrient cycling in a eutrophic coastal lagoon. J Mar Syst 26(2):209-21.

Nanami A, Saito H, Akita T, Motomatsu K, Kuwahara H. 2005. Spatial distribution and assemblage structure of macrobenthic invertebrates in a brackish lake in relation to environmental variables. Estuar Coast Shelf Sci 63:167-76.

Naylor RL, Williams SL, Strong DR. 2001. Aquaculture - a gateway for exotic species. Science 294:1655-6.

Nedwell DB, Parkes RJ, Upton AC, Assinder DJ. 1993. Seasonal fluxes across the sediment-water interface, and processes within sediments. Phil Trans R Soc Lond A 343:519-29.

Newell RIE. 2004. Ecosystem influences of natural and cultivated populations of suspension feeding bivalve molluscs: A review. J Shellfish R 23(1):51-61.

Newell RIE, Cornwell JC, Owens M. 2002. Influence of simulated bivalve biodeposition and microphytobenthos on nitrogen dynamics: A laboratory study. Limnol Oceanogr 47(5):1367-79.

Newell RIE, Fisher TR, Holyoke RR, Cornwell JC. 2005. Influence of eastern oysters on nitrogen and phosphorous regeneration in Chesapeake Bay, USA. Earth 47:93-120.

Nizzoli D, Bartoli M, Viaroli P. 2006a. Nitrogen and phosphorus budgets during a farming cycle of the manila clam *Ruditapes philippinarum*: An *in situ* experiment. Aquaculture 26:98-108.

Nizzoli D, Bartoli M, Viaroli P. 2007. Oxygen and ammonium dynamics during a farming cycling of the bivalve *Tapes philippinarum*. Hydrobiologia 587:25-36.

Nizzoli D, Welsh D, Bartoli M, Viaroli P. 2005. Impacts of mussel (*Mytilus galloprovincialis*) farming on oxygen consumption and nutrient recycling in a eutrophic coastal lagoon. Hydrobiologia 550(1):183-98.

Nizzoli D, Welsh DT, Fano EA, Viaroli P. 2006b. Impact of clam and mussel farming on benthic metabolism and nitrogen cycling, with emphasis on nitrate reduction pathways. Mar Ecol Progr Ser 315:151-65.

Osborne J, Overbay A. 2004. The power of outliers (and why researchers should always check for them). Pract Assess Res Eval 9(6): 1-10.

Parker IM, Simberloff D, Lonsdale WM, Goodell K, Wonham M, Kareiva PM, Williamson MH, Von Holle B, Moyle PB, Byers JE, et al. 1999. Impact: Toward a framework for understanding the ecological effects of invaders. Biol Invasions 1:3-19.

Pietros JM, Rice MA. 2003. The impacts of aquacultured oysters, *Crassostrea virginica* (Gmelin, 1791) on water column nitrogen and sedimentation: Results of a mesocosm study. Aquaculture 220:407-22.

Poorvin L, Rinta-Kanto JM, Hutchins DA, Wilhelm SW. 2004. Viral release of iron and its bioavailability to marine plankton. Limnol Oceanogr 49(5):1734-41.

Raaymakers S. 2002. The ballast water problem: Global, economical and human health impacts.In: RECSO/IMO joint seminar on tanker ballast water management & technologies [internet]; 2002 December 16-18; Dubai, UAE. London (UK): International Maritime Organization. [cited 2009 Mar 21]; p 1-22.

Raiswell R. 2006. Towards a global highly reactive iron cycle. J of Geochem Explor 88:436-9.

Rhoads DC, Young DK. 1970. The influence of deposit-feeding organisms on sediment stability and community trophic structure. J Mar Res 28(2):150-78.

Rocha C. 1998. Rhythmic ammonium regeneration and flushing in intertidal sediments of the Sado Estuary. Limnol Oceanogr 43(5):823-31.

Ruiz GM, Fofonoff PW, Carlton JT, Wonham MJ, Hines AH. 2000. Invasion of coastal marine communities in North America: Apparent patterns, processes and biases. Annu Rev Ecol Syst 31:481-531.

Sanders HL. 1958. Benthic studies in Buzzards Bay. Limnol Oceanogr 3(3):245-58.

SAS Institute Inc. 2008. SAS/STAT® 9.2 User's Guide. Cary (NC): SAS Institute Inc.

Schlacher TA, Wooldridge TH. 1995. Axial zonation patterns of subtidal macrozoobenthos in the Gamtoos Estuary, South Africa. Estuaries 19(3):680-96.

Schumacher BA. 2002. Methods for the determination of total organic carbon (TOC) in soils and sediments. Las Vegas: United States Environmental Protection Agency.

Sousa R, Rufino M, Gaspar M, Antunes C, Guilhermino L. 2008. Abiotic impacts on spatial and temporal distributions of *Corbicula fluminea* (Müller 1774) in the River Minho Estuary, Portugal. Aquat Conserv: Mar Freshwat Ecosyst 18:98-110.

Strayer DL, Caraco NF, Cole JJ, Findlay S, Pace ML. 1999. Transformation of freshwater ecosystems by bivalves. BioScience 49(1):19-27.

Sundby B, Gobeil C, Silverberg N, Mucci A. 1992. The phosphorous cycle in coastal marine sediments. Limnol Oceanogr 37(6):1129-45.

Tortell PD, Maldonado MT, Granger J, Price NM. 1999. Marine bacteria and biogeochemical cycling of iron in the oceans. FEMS Microbiology Ecology 29:1-11.

Trevors JT. 1996. Sterilization and inhibition of microbial activity in soil. J Microbiol Meth 26:53-9.

Tuominen L, Kairesalo T, Hartikainen H. 1994. Comparison of methods for inhibiting bacterial activity in sediment. Appl Environ Microbiol 60(9):3454-7.

Van Alstyne KL, Flanagan JC, Gifford S. 2011. Recreational clam harvesting affects sediment nutrient remineralization and the growth of the green macroalga *Ulva lactuca*. J Exp Mar Bio Ecol 401:57-62.

Volkenborn N, Hedtkamp SIC, Beusekom JEE, Reise K. 2007. Effects of bioturbation and bioirrigation by lugworms (*Arenicola marina*) on physical and chemical sediment properties and implications for intertidal habitat succession. Estuar Coast Shelf Sci 74(1-2):331-43.

Wallentinus I, Nyberg CD. 2007. Introduced marine organisms as habitat modifiers. Mar Pollut Bull 55:323-32.

Whiteley JA. 2005. Macroinvertebrate community responses to clam aquaculture practices in British Columbia, Canada [thesis]. Burnaby (BC): Simon Fraser University.

Williams SL, Grosholz ED. 2008. The invasive species challenge in estuarine and coastal environments: Marrying management and science. Estuar Coast 31:3-20.

Wonham MJ, Carlton JT. 2005. Trends in marine biological invasions at local and regional scales: The Northeast Pacific Ocean as a model system. Biol Invasions 7:369-92.

Zhu S, Saucier B, Durfey J, Chen S, Dewey B. 1999. Waste excretion characteristics of manila clams (*Tapes philippinarum*) under different temperature conditions. Aquacult Eng 20:231-44.

Appendices

Appendix A

Bivalve species and densities in field survey quadrats (Tier I).

Site	Manila	Littleneck	Oyster	*Mytilus*	Cockle	Softshell	*Macoma*	Butter	Varnish	Total
163 (1.8)	31	46			2		1	1		81
163 (2.0)	87	50		12	1					150
163 (2.7)	208	16	2	3					2	231
314 (1.0)		2			4		1			7
565 (0.7)		20					2			22
565 (2.2)									2	2
568 (1.15)		2			2		1			5
568 (1.6)	1	20	6		2	1		3		33
700 (0.8)		2					1			3

Note: Only sites with bivalves found listed. Sites listed as: transect (tidal height). Total density is the final count found in a 0.5x0.1x0.2 m quadrat. Bivalve species listed are: Manila clam (*Tapes philippinarum*), Pacific littleneck clam (*Protothaca staminea*), Pacific oyster (*Crassostrea gigas*), *Mytilus* mussel, Nuttall's cockle (*Clinocardium nutallii*), softshell clam (*Mya arenaria*), *Macoma* clams, butter clam (*Saxidomus giganteus*) and varnish clam (*Nuttallia obscurata*).

Appendix B

Seeding and final densities of varnish clams in exclusion cages (Tier II)

Treatment	Cage	Seeded Density	Final Density	Est. Biomass (g m^{-2})
Control	4	0	0	0
	8	0	0	0
	9	0	1	3.07
Low	2	200	143	438
	10	200	144	441
	12	200	145	445
Medium	1	500	363	1113
	3	500	326	999
	7	500	391	1199
High	5	800	626	1919
	6	797	596	1827
	11	800	528	1619

Note: Estimated biomass calculated using the average biomass equation for *N. obscurata* found in Bendell-Young (2006). Length used calculated from the average length found for 50 varnish clams used to seed cage 10.

Appendix C

Multiple comparisons (Holm-Sidak) of changes in iron concentrations between depths across time.

Sampling Date	Comparison	p-value	t-statistic
July 2 2009	I vs S	0.005*	3.29
	B vs S	<0.001*	5.66
	B vs I	0.02	2.37
July 5 2009	I vs S	<0.001*	4.45
	B vs S	<0.001*	6.25
	B vs I	0.08	1.84
July 7 2009	I vs S	0.07	2.23
	B vs S	0.003*	3.66
	B vs I	0.17	1.42
July 10 2009	N/A		
July 16 2009	N/A		
July 20 2009	I vs S	0.02	2.55
	B vs S	<0.001*	5.37
	B vs I	0.01*	2.95
July 23 2009	N/A		

Note: Sediment depths are abbreviated as: surficial (S), intermediate (I) and bottom (B). (*) denotes significance ($p<0.02$). No effect of depth was detected for July 10, 16 and 23 2009; hence, the multiple comparison procedure was not available (N/A).

Appendix D

Multiple comparisons (Holm-Sidak) on the effects of sterilized versus unsterilized treatments with and without varnish clams on surfical sediment ammonium concentrations in microcosms.

Sampling Day	Comparison	p-value	t-statistic
0	S vs SV	0.37	0.94
	US vs USV	0.04*	2.93
	S vs US	0.008*	4.50
	SV vs USV	<0.001*	6.48
1	S vs SV	0.44	1.23
	US vs USV	0.32	1.06
	S vs US	0.005*	5.10
	SV vs USV	0.005*	4.92
2	S vs SV	0.60	0.56
	US vs USV	0.63	0.92
	S vs US	<0.001*	9.93
	SV vs USV	<0.001*	9.15
3	S vs SV	0.15	2.04
	US vs USV	0.94	0.08
	S vs US	0.001*	6.40
	SV vs USV	0.009*	4.44
5	S vs SV	0.69	0.81
	US vs USV	0.80	0.26
	S vs US	0.002*	5.84
	SV vs USV	0.004*	4.77
7	S vs SV	0.98	0.02
	US vs USV	0.99	0.14
	S vs US	0.05	3.01
	SV vs USV	0.08	3.18
9	S vs SV	0.95	0.29
	US vs USV	0.98	0.03
	S vs US	0.002*	5.63
	SV vs USV	0.002*	5.89
12	S vs SV	0.46	1.19
	US vs USV	0.94	0.07
	S vs US	0.26	1.89

	SV vs USV	0.08	3.16
15	S vs SV	0.34	1.43
	US vs USV	0.94	0.08
	S vs US	0.43	1.51
	SV vs USV	0.07	3.15
18	S vs SV	0.01*	4.20
	US vs USV	0.47	0.75
	S vs US	0.01*	3.86
	SV vs USV	<0.001*	7.31
21	S vs SV	<0.001*	6.97
	US vs USV	0.72	0.37
	S vs US	0.56	1.27
	SV vs USV	<0.001*	7.87

Note: The two treatments with the addition of varnish clams are abbreviated as: sterilized (SV) and unsterilized (USV). Their respective controls (without varnish clams) are abbreviated as: sterilized (S) and unsterilized (US). (*) denotes significance ($p<0.01$).

Appendix E

Multiple comparisons (Holm-Sidak) on the effects of sterilized and unsterilized treatments with and without varnish clams on ammonium concentrations in the overlying waters of microcosms.

Sampling Day	Comparison	p-value	t-statistic
0	S vs SV	0.81	0.25
	US vs USV	0.12	2.60
	S vs US	0.01*	4.43
	SV vs USV	0.28	1.59
1	S vs SV	0.33	1.46
	US vs USV	0.30	1.10
	S vs US	0.02	4.18
	SV vs USV	0.02	3.82
2	S vs SV	0.33	1.47
	US vs USV	0.67	0.44
	S vs US	0.09	2.65
	SV vs USV	0.01*	4.56
3	S vs SV	0.34	1.03
	US vs USV	0.28	1.60
	S vs US	0.01*	4.32
	SV vs USV	0.006*	4.89
5	S vs SV	0.28	1.58
	US vs USV	0.69	0.41
	S vs US	<0.001*	6.22
	SV vs USV	<0.001*	8.21
7	S vs SV	0.81	0.25
	US vs USV	0.82	0.58
	S vs US	0.05	3.04
	SV vs USV	0.03	3.87
9	S vs SV	0.46	1.20
	US vs USV	0.50	0.71
	S vs US	0.18	2.15
	SV vs USV	0.02	4.06
12	N/A		
15	S vs SV	0.42	1.27
	US vs USV	1.00	0.0002

	S vs US	0.13	2.57
	SV vs USV	0.02	3.84
18	S vs SV	<0.001*	7.65
	US vs USV	0.95	0.06
	S vs US	0.05	2.79
	SV vs USV	<0.001*	10.50
21	S vs SV	0.003*	5.31
	US vs USV	0.92	0.11
	S vs US	0.002*	3.56
	SV vs USV	<0.001*	8.76

Note: The two treatments with varnish clams are abbreviated as: sterilized (SV) and unsterilized (USV). The two controls with no addition of varnish clams are abbreviated as: sterilized (S) and unsterilized (US). (*) denotes significance ($p<0.01$). No effect of treatment was detected for sampling day 12; hence, the multiple comparison procedure was not available (N/A).