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Eva Dusek Jennings

Toxic or not? Modeling *Pseudo-nitzschia* Consumption and
Domoic Acid Cycling in Washington's Intertidal Bivalves

Eva Dusek Jennings

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Reading Committee:
Charles A. Simenstad, Chair
André E. Punt
Micaela S. Parker

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University of Washington

Abstract

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Eva Dusek Jennings

Chair of the Supervisory Committee:

Research Professor Charles A. Simenstad

The cosmopolitan diatom genus *Pseudo-nitzschia* includes a number of species that are capable of producing the neurotoxin domoic acid (DA). When blooms of toxin-producing *Pseudo-nitzschia* are advected over intertidal shellfish beds, suspension-feeding clams, including mussels and oysters, feed on the diatom and bio-accumulate DA in their soft tissues. This results in a potential threat for humans, marine mammals, and sea birds that consume DA-tainted clams. On the outer coast of Washington State, DA has been responsible for numerous harvest closures for razor clams (*Siliqua patula*). In Puget Sound, however, only three harvest closures have occurred because of DA. This disparity raises the question: Why has *Pseudo-nitzschia* only caused three harvest closures in Puget Sound, while the coastal razor clam fishery has been

plagued by frequent closures? In this dissertation, I hypothesized that: 1) clam competition in Puget Sound reduces *Pseudo-nitzschia* concentrations in seawater, thus DA toxicity in all clams; and, 2) the physiology of Puget Sound clams reduces DA uptake or retention relative to Washington outer coast razor clams. I explore several factors that affect DA concentrations in clams after a *Pseudo-nitzschia* bloom has already been established, using integrated oceanographic and food web models. Model inputs include: clam assemblage composition and abundance, beach slope profiles, tidal advection, suspension-feeding rates, DA assimilation efficiency, and depuration rates. These parameters were obtained through field studies and laboratory experiments. Comparison of sand/cobble beaches in Puget Sound to the Washington outer coast indicates that Puget Sound beaches are steeper, have a greater variety of suspension-feeding clam species, and have higher clam standing stock than outer coast beaches. Comparison of clam physiology between Washington outer coast and Puget Sound species indicates that outer coast razor clams feed on *Pseudo-nitzschia* and depurate DA at a slower rate than Puget Sound species. Model results reveal several factors that determine clam DA concentrations: tidal height of clams, beach gradient, and clam standing stock, especially at low tidal elevations. In addition, DA uptake and depuration rates play a major role in regulating DA concentrations in clams, thus affecting harvest closure regimes on the outer Washington coast and in Puget Sound.

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DEDICATION

To Chad, Jarmila, Vaclav and Amelia

Introduction

Over the last three decades, harmful algal blooms have received increasing worldwide attention as a potential threat to human health (Horner et al. 1997, Van Dolah 2000). The threat comes in the form of marine algal toxins, which are produced by certain species of phytoplankton. Under favorable conditions, phytoplankton can proliferate rapidly, forming dense aggregations of cells, called phytoplankton blooms. When blooms of toxin-producing cells move onshore and are consumed by suspension-feeding benthic shellfish, the toxins can move up the food chain to humans.

The marine diatom genus *Pseudo-nitzschia* includes a number of species that are capable of producing the potent neurotoxin domoic acid (DA), which is mostly retained within the phytoplankton cells (e.g., Hasle 2002). When suspension-feeding clams, including oysters and mussels, feed on this toxin-containing phytoplankton, they bioaccumulate DA in their tissues. Human consumption of clams containing elevated levels ($>20 \text{ mg} \cdot \text{kg}^{-1}$) of DA can lead to gastrointestinal and neurologic symptoms, including short term memory loss that can persist for years after the incident, and even death (Todd 1993).

Within Washington State, *Pseudo-nitzschia* is commonly found both along the outer coast, as well as within Washington's large estuarine complex, Puget Sound. Concerns exist about human exposure to DA through shellfish, as well as the economic costs of shellfish harvest closures resulting from DA. Within the United States, Washington State is the leading producer of commercially harvested clams (Puget Sound Action Team 2003). Along the outer Washington

coast, numerous shellfish harvest closures have already occurred as a result of DA contaminated razor clams (*Siliqua patula*) and Dungeness crab (*Metacarcinus magister*). In contrast, to date Puget Sound has only had three shellfish harvest closures due to DA. Two questions arise as a result of this difference: Why has *Pseudo-nitzschia* only caused three relatively short harvest closures in Puget Sound, while the coastal razor clam fishery has been plagued by frequent closures? And what is the risk in Puget Sound of more frequent harvest closures because of *Pseudo-nitzschia*? These questions form the basis for my dissertation research.

In this dissertation, I explore the effect that differing clam assemblages on the outer Washington coast and in Puget Sound have on DA concentrations in clam tissues. My hypotheses are: 1) competition between intertidal suspension-feeding clams in Puget Sound reduces *Pseudo-nitzschia* concentrations in the seawater, thus reducing DA concentrations in all clams; and, 2) the physiology of Puget Sound clams reduces DA uptake or retention compared to razor clams on the Washington outer coast. The ultimate objective of my dissertation research was to develop a mathematical model to predict DA concentrations in Puget Sound intertidal clams after feeding on a *Pseudo-nitzschia* bloom, for comparison to DA concentrations in outer Washington coast clams. The model allows exploration into the effects of clam assemblage composition and abundance, bivalve physiology, and physical beach characteristics on *Pseudo-nitzschia* availability and domoic acid concentrations in bivalve tissues. The importance of this research is two-fold. Firstly, creating of a model to predict DA concentrations in edible clams will help us understand some of the dynamics around clam toxicity in Puget Sound and why it differs from the Washington outer coast. Secondly, understanding differences between DA uptake potential in Puget Sound and the outer Washington coast will help shellfish managers prepare for future

DA impacts to clam harvest and public safety in Puget Sound. If *Pseudo-nitzschia* blooms become common in Puget Sound, understanding the dynamics of toxin uptake and purging in clams will be vital for mitigating impacts to shellfisheries.

In the remaining sections of this introduction, I describe: the discovery of DA production by *Pseudo-nitzschia*; toxicity of DA; DA impacts in Washington State; details and context of this study; and the study locations. At the end of the introduction, I provide a chapter list with a brief description of each chapter.

The research presented in this dissertation was completed as part of consortium research on *Pseudo-nitzschia* and its impacts along the outer Washington coast of the United States, and within Puget Sound, by the Pacific Northwest Center for Human Health and Ocean Studies (PNW H2O) at the University of Washington. The PNW H2O research consortium addresses questions about several aspects of *Pseudo-nitzschia* dynamics and transmission of DA in Washington State, including: What environmental factors lead to development of toxic blooms? Why do certain types of shellfish retain toxin? What are the biological processes of DA toxicity? Which human populations are at greatest risk of exposure? And how do human dietary and consumption behaviors contribute to exposure? Thus, this project was completed in the context of a larger effort to collect data and understand the rising occurrence of *Pseudo-nitzschia* and DA impacts in Washington State.

DISCOVERY OF DOMOIC ACID PRODUCTION BY *PSEUDO-NITZSCHIA*

Prior to 1987, scientists were unaware of DA production by *Pseudo-nitzschia*. In 1958, the toxin had been isolated from the red seaweed *Chondria armata* in Japan (Daigo 1958). It was known to be an effective insecticide (Maeda et al. 1984), and was also used within Japan as an antidote for parasitic worms (Ramsdell 2007).

In 1987, scientists first became aware that DA can also be produced by certain phytoplankton species, and that clams can serve as vectors of the toxin to humans. Following consumption of commercially harvested blue mussels (*Mytilus edulis*), over 100 people fell ill in Prince Edward Island, Canada (Wright 1989), and three elderly victims died as a result of the poisoning (Perl et al. 1990). The source of DA was traced to a bloom of the diatom *Nitzschia pungens*, now known as *Pseudo-nitzschia multiseries* (Rao et al. 1988, Bates et al. 1989, Hasle 1994, 1995). Since the discovery of DA production by *Pseudo-nitzschia*, research and monitoring have reduced the incidence of DA poisoning in humans (Smith et al. 1990, Villac et al. 1993, Van Dolah 2000).

Members of the genus *Pseudo-nitzschia* are cosmopolitan, and are found in all waters of the world (Hasle 1965, Hasle et al. 1996, Hasle 2002). Of the several dozen species that have been identified, at least nine species of *Pseudo-nitzschia* and one species of *Nitzschia* have been shown to produce DA (Bates 1998, Orsini 2002). Production of DA varies by species, and can also vary among individuals of the same species that are found in different geographic regions (Bates 1998). Additionally, production of DA seems to be related to a number of physical and chemical factors, including water chemistry, light availability and nutrient availability (e.g. Bates

et al. 1991, Pan et al. 1996a, Bates 1998, Pan et al. 1998, Maldonado et al. 2002, Lundholm et al. 2004).

DOMOIC ACID TOXICITY

Domoic acid is an analogue of glutamate, which is an excitatory neurotransmitter that activates glutamate receptors in the brain. Within the brain, DA specifically affects the hippocampus, causing brain lesions (reviewed in Pulido 2008). In affected humans, it has caused a series of effects, beginning with gastrointestinal symptoms (nausea, vomiting, diarrhea and severe abdominal cramps); followed by loss of short-term memory, seizures, stroke-like paralysis, and even death (reviewed in Todd 1993). Sea lions, whales and seabirds have also been poisoned and killed following consumption of DA-tainted anchovies (Sierra Beltran et al. 1997, Scholin et al. 2000).

DOMOIC ACID IMPACTS IN WASHINGTON STATE

Following the discovery of DA production by *Pseudo-nitzschia*, monitoring programs were put in place in many parts of the world, including the West Coast of the United States. The Washington State Department of Health's (WaDOH) regulatory limit for DA in shellfish tissue is $20 \text{ mg} \cdot \text{kg}^{-1}$. When this limit is exceeded in clams or crabs, the affected beach is closed for shellfish collection. High concentrations of DA were first detected in Dungeness crab and in their prey, the razor clam, along the Washington and Oregon coasts in late October and November 1991 (Wekell et al. 1994, Horner et al. 1997). Since 1991, DA has caused many razor clam harvest closures along the outer Washington coast (Horner et al. 1997, Wekell JC 2002). Once DA has accumulated in razor clam tissues, depuration (purging) of the toxin is a long-term

process, taking on the order of months to a year to complete (Wekell et al. 1994, Adams et al. 2000, Trainer & Bill 2004).

Shellfish harvest closures on the Washington outer coast have negative impacts for several sectors of society, including commercial and tribal harvesters, and coastal communities who rely on tourism income from razor clam harvesters. To quantify the hardship to coastal communities, Dyson and Huppert (2010) calculated that overall visitor expenditures associated with recreational clamming on the outer Washington coast in the 2007-2008 season were \$24.4 million. A full season of harvest closures on all of Washington's outer coast razor clam beaches would result in loss of support for 339 full-time jobs.

Although the effects of *Pseudo-nitzschia* have been more conspicuous on the Washington outer coast, several species of *Pseudo-nitzschia* have also been observed in the inland waters of Puget Sound (Trainer et al. 1998, Stehr et al. 2002, Bill et al. 2006). Prior to 2003, impacts to shellfish harvest by *Pseudo-nitzschia* blooms in Puget Sound were of limited consequence. In 1994, DA was detected in Hood Canal, where the bay mussel (*Mytilus trossulus*) reached toxicity levels of $10 \text{ mg} \cdot \text{kg}^{-1}$, but this was not high enough to trigger a harvest closure (Horner et al. 1996). In 1997, bay mussels in Penn Cove contained detectable levels of DA but again not at high enough levels to trigger action from WaDOH (Trainer et al. 1998). Puget Sound experienced its first harvest closure resulting from a *Pseudo-nitzschia* bloom in September 2003, when bay mussels at Fort Flagler State Park attained high levels of DA (Bill et al. 2006). Two subsequent beach closures occurred in the fall of 2005: in September, Pacific oysters (*Crassostrea gigas*) in Sequim Bay became toxic, and in October, bay mussels and Manila clams (*Ruditapes*

philippinarum) in Penn Cove became toxic (Trainer et al. 2007). To date, several other species in Puget Sound have attained concentrations of DA above the regulatory limit of $20 \text{ mg} \cdot \text{kg}^{-1}$, including: littleneck clams (*Leukoma staminea*) and geoduck clams (*Panopea generosa*) (Bill et al. 2006, Trainer et al. 2007).

Concern that Puget Sound might be on a trajectory to regular *Pseudo-nitzschia* blooms and shellfish harvest closures has inspired comparative studies of oceanographic and ecological dynamics between Puget Sound and the Washington coast. It is likely that multiple factors are responsible for the difference in harvest closure history between the two locations. One possible factor is the difference in complexity of the intertidal suspension-feeding benthic macroinvertebrate communities in the two locations. In Puget Sound, sand/cobble beaches are frequently populated with a variety of suspension-feeding clams, while sandy beaches along the Washington coast are predominantly home to one species of suspension-feeding clam, the razor clam (Kozloff 1983). Through competition for planktonic food resources, the highly complex suspension-feeding benthic macroinvertebrate community in Puget Sound may spread the available quantity of DA amongst many species, some of which are not commonly harvested. Another possible factor relates to depuration rates of DA by harvestable clams. A comparison of DA depuration rates between razor clams and blue mussels, a close relative to the bay mussels found in Puget Sound, indicates that blue mussels depurate the toxin from their tissues on a timescale of hours to days (Novaczek et al. 1992, Wohlgeschaffen et al. 1992, Krogstad et al. 2009), while razor clams take months to complete the same process. If Puget Sound clams depurate DA at rates more similar to blue mussels than to razor clams, it may account for part of the reason why harvest closures have not been as frequent or persistent in Puget Sound.

DETAILS AND CONTEXT OF THIS STUDY

The motivation behind this research was to explore clam- and beach-centric factors that may be responsible for the difference in DA harvest closures between the Washington outer coast and Puget Sound. I hypothesized that harvestable Puget Sound clams exposed to a toxin-producing *Pseudo-nitzschia* bloom would accumulate less DA than coastal razor clams, and that they would purge it faster than razor clams as well. I also hypothesized that competition amongst intertidal suspension-feeding benthic macroinvertebrates for *Pseudo-nitzschia* would reduce the availability of this phytoplankton to harvested clams, thus reducing the concentration of DA in harvested species. These questions were ultimately addressed with a model that tracked *Pseudo-nitzschia* concentrations in the water column over time, and resultant DA concentrations in different clam species over time.

The model that is described in this research (Chapter 5) required several data inputs, each of which comprises a chapter of the dissertation. I started by characterizing the intertidal suspension-feeding benthic macroinvertebrate community in Lofall, with detailed information about species assemblage, abundance and size, and how these change by tidal height (Chapter 1). These data formed the basis for exploring whether competition may exist between benthic intertidal suspension-feeders for *Pseudo-nitzschia*.

Next, I needed an estimate of phytoplankton flux into the intertidal. For this estimate, I used data on particle velocity from an Acoustic Doppler Current Profiler (ADCP) deployed in the shallow subtidal in Lofall, along with sampling experiments to profile the water column over a tidal cycle (Chapter 2). The ADCP provided net directional water movement and the rate of movement in

the cross-shore and long-shore directions, which I used to infer the rate and directionality of *Pseudo-nitzschia* bloom movement over the intertidal zone.

Estimates of clam and barnacle suspension-feeding rate on *Pseudo-nitzschia* did not exist for Puget Sound or Washington outer coast species, so I conducted laboratory experiments to obtain those parameters (Chapter 3). Clearance (feeding) rate studies on *Pseudo-nitzschia* had only been published for a handful of species prior to this dissertation, including: blue mussels (Wohlgemuth et al. 1992), deep sea Atlantic scallops *Placopecten magellanicus* (Wohlgemuth et al. 1992), and copepods (Olson et al. 2006).

Existing studies of DA accumulation and toxin duration in clams were similarly sparse, including: blue mussels (Novaczek et al. 1992); California mussels *Mytilus californianus* (Whyte et al. 1995); bay scallops *Argopecten irradians* (Scarratt 1991); sea scallops *Placopecten magellanicus* (Douglas et al. 1997); and razor clams (Drum et al. 1993, Horner et al. 1993). None of the clams commonly found in Puget Sound intertidal areas had been studied for DA uptake and depuration, so I completed laboratory experiments to determine these values as well (Chapter 4).

STUDY LOCATIONS

The Lofall region of Hood Canal (Fig. 1) was selected as the study location for this research for several reasons. Firstly, water movement dynamics in the Lofall region of Hood Canal are relatively representative of sill regions in Puget Sound's five basins. Secondly, the clam assemblage at Lofall is representative of clam assemblages in Puget Sound's quiet bays and salt

marshes, where the substratum is cobble or gravel mixed with sand or mud (Kozloff 1983, Dethier 2006). Thirdly, working in this area is advantageous because of the extensive bathymetric and oceanographic data in existence for this region of Hood Canal. These data provided necessary information for obtaining a first-order estimate of water advection into the intertidal zone where harvestable shellfish are located. Lastly, although beach closures for DA have not yet been necessary in Hood Canal, *Pseudo-nitzschia* is found in the Canal, particularly during spring and fall months (G. Armbrust, UW; pers comm). It may only be a matter of time before DA is found at elevated concentrations in Hood Canal shellfish.

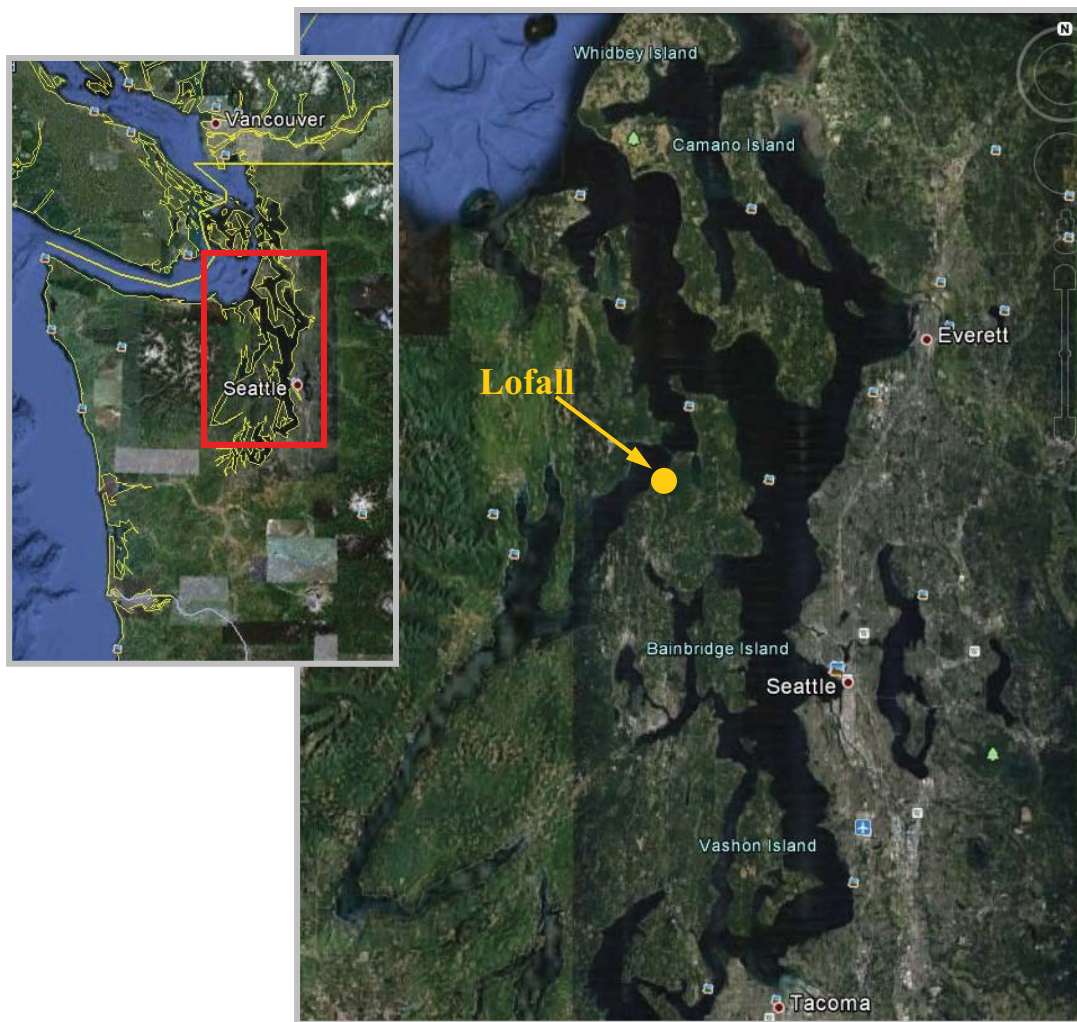


Figure 1. Map of western Washington State, with inset showing the Lofall region in the northern part of Hood Canal.

Lofall is within the sill region of the Hood Canal basin; as a result, the water column is fairly well mixed over the sill due to turbulence (Gregg & Pratt 2010). The three beaches that I sampled in Lofall were within 2.5km of each other, and were all mixed sand/ gravel beaches inhabited by a variety of suspension-feeding clams. Beach slope profiles differed among the three locations: one beach had a steep fore-beach with a low tide terrace; another beach was steep throughout the whole intertidal range; and the third beach was a shallow delta. Tidal range in Lofall varies by season, but ranges from about +4m above Mean Lower Low Water (MLLW; NOAA tidal datum) to -1m above MLLW during the most dramatic (spring) tidal cycles, and +3.8m above MLLW to -0.3m above MLLW during the least dramatic (neap) tidal cycles. Water temperature varies by depth and season, ranging from 12-18 °C during the summer, with warmer temperatures at the surface; and from 7-9 °C during the winter, with colder temperatures at the surface. Salinity also varies by depth, ranging between approximately 28 - 31 practical salinity units (psu).

In addition to Lofall, I selected one beach on the outer Washington coast, Twin Harbors, for a comparative study between outer coast razor clams, and the diverse Puget Sound clam assemblage. Twin Harbors beach is situated between two outer coast bays: Grays Harbor and Willapa Bay (Fig. 2). The beach has a long, shallow slope, with fine sand inhabited by razor clams. Ocean surf pounds the beach, with waves exceeding 7m during winter storms. Water temperatures range from 7 - 12 °C, and salinity is 32 – 33 psu.



Figure 2. Twin Harbors Beach on the outer Washington coast.

CHAPTER LIST & DESCRIPTIONS

The Doctoral dissertation is divided into five chapters representing distinct research goals. Chapters one through four provide necessary data to parameterize the model, which is described in chapter five.

Chapter I: Intertidal Zonation of Suspension-Feeding Epi- and Infauna at Lofall, Hood Canal. This chapter presents data from field studies to survey the assemblage composition and

intertidal zonation of nearshore benthic suspension-feeding macro-invertebrates in Lofall, Hood Canal. Beach slope profiles are also presented for the sampled beaches. Data were collected in August and September 2005, and were important for determining suspension-feeding species present in Hood Canal and for recording the beach slope profiles at the three study sites.

Chapter II: Phytoplankton Advection into the Intertidal Zone of a Temperate Fjord.

Chapter two presents data from two field experiments, conducted in August and December 2005, to obtain an estimate of phytoplankton advection from mid-channel neritic waters to shallow subtidal/intertidal waters at Lofall, Hood Canal. Sample data included CTD casts, water samples, and particle velocity data. These data were necessary to understand how water moves from the deeper portions of Hood Canal into the intertidal, and to estimate flux of a *Pseudo-nitzschia* bloom from the main channel into the intertidal.

Chapter III: Clearance Rate Measurements on Thirteen Intertidal Clams and Acorn Barnacles Fed on the Toxin-Producing Diatom *Pseudo-nitzschia*. This chapter presents clearance rate studies on *Pseudo-nitzschia* by thirteen species of intertidal bivalves: twelve from Puget Sound, plus razor clams from the Washington outer coast; and on Puget Sound acorn barnacles. Laboratory experiments were performed between June and October 2006, with follow-up experiments in 2008 and 2009. Data were critical for understanding how much domoic acid each species has the potential to consume and thereby accumulate in its soft tissues.

Chapter IV: Domoic Acid Uptake and Depuration by Nine Intertidal Clams Experimentally Fed on Toxin-Producing *Pseudo-nitzschia multiseries*. Chapter four

describes laboratory experiments to determine domoic acid uptake and depuration rates by nine species of intertidal bivalves. Experiments were performed in 2008 and 2009, and provide crucial information for the model about the purging rate of domoic acid from shellfish tissue.

Chapter V: Modeling *Pseudo-nitzschia* Consumption and Domoic Acid Cycling in Puget Sound and Washington Outer Coast Clams. This chapter presents a model that predicts how much toxin-producing *Pseudo-nitzschia* is consumed by intertidal suspension-feeding bivalves in Lofall and on the Washington outer coast, as well as how much domoic acid each bivalve can accumulate over a tidal cycle.

Synthesis. This section synthesizes findings from the dissertation chapters, and explores implications of the research findings for Puget Sound.

Chapter I:

Intertidal Zonation of Suspension-Feeding Epi- and Infauna at Lofall, Hood Canal

ABSTRACT

Washington State is the top producer of commercially harvested clams, including oysters and mussels, in the United States of America. As such, it is important to ensure that harvested clams are safe for human consumption. One threat to clam harvests and consumers in Puget Sound is in the form of toxins produced by the genus *Pseudo-nitzschia*, which is found in local waters. When toxin-producing *Pseudo-nitzschia* species proliferate into dense blooms and are consumed by suspension-feeding organisms such as clams, the toxin, domoic acid (DA), can be retained in clam tissues. However, competition between intertidal suspension-feeding benthic macroinvertebrates (SFBMs) for available phytoplankton, including *Pseudo-nitzschia*, may modulate the accumulation of DA in harvestable clams on a beach. Information about SFBM assemblages on Puget Sound intertidal beaches was collected to support a model that will assess whether competition between suspension-feeders for *Pseudo-nitzschia* may reduce the likelihood of toxicity in Puget Sound clams. In this study, I examined three beaches in the Lofall region of Hood Canal, Washington State, to assess beach gradients and SFBM assemblages. The three beaches are within 2.5 km of each other, but display a range of beach slopes, from relatively steep to very shallow, and a variety of substrates, from primarily cobble and small rocks, to mostly sand. At each beach, I sampled three to four habitat types at different tidal elevations. Samples of SFBM species were collected for assessment of species composition, size and

abundance. Data were organized into a list of species, with average sizes and quantities in and below a 1-m² surface area within each habitat type. These data will form the basis for a separate study, which analyzes competition for *Pseudo-nitzschia* in a Puget Sound suspension-feeding community.

INTRODUCTION

The threat of toxic algal blooms appears to be on the rise (Van Dolah 2000) and has the potential to cause severe economic impacts to shellfish industries and local communities in affected regions (e.g., Hoagland et al. 2002, Dyson & Huppert 2010). When toxic algal blooms intercept intertidal areas, suspension-feeding benthic macroinvertebrates (SFBMs) feeding on the phytoplankton can accumulate toxins in their tissues. One such algal toxin is domoic acid (DA), produced by certain species of the diatom genus *Pseudo-nitzschia*. Toxin production by *Pseudo-nitzschia* was only discovered in 1987, when over 100 people fell ill and three died after consuming blue mussels (*Mytilus edulis*) from Prince Edward Island, Canada (Bates et al. 1989, Perl et al. 1990). Although testing now occurs worldwide to prevent DA poisoning in humans, high frequencies of shellfish harvest closures have the potential to cause severe economic impacts in the proximate regions (e.g., Dyson & Huppert 2010).

Clams, including oysters and mussels, are extensively harvested in Washington State, underscoring the potential for severe impacts from DA, and the importance of ensuring that harvested clams are safe for human consumption. Washington State is the top producer of commercially-harvested clams in the United States of America (Puget Sound Action Team,

2003). Much of the shellfish production occurs within Washington's largest inland estuarine complex, Puget Sound.

Pseudo-nitzschia is a cosmopolitan genus, and is also prevalent within Puget Sound (WaDOH 2011). Since testing for DA began in Washington State in 1991, only three beach harvest closures have occurred in Puget Sound because of DA, all of which have occurred since 2003 (Bill et al. 2006, Trainer et al. 2007). While the incidence of toxic *Pseudo-nitzschia* blooms in Puget Sound may be on the rise, there may also be some mechanisms which make Puget Sound less likely to have elevated toxin concentrations in shellfish. Competition between SFBMs for available phytoplankton, including *Pseudo-nitzschia*, may modulate the accumulation of DA in harvested clam species.

Competition for phytoplankton can occur between intertidal SFBMs on a beach (Peterson & Black 1987). Knowing species composition and abundance of SFBMs at a beach, and correlating it with the rates at which they feed, allows examination as to whether competition may be occurring at a site. As a first step, beach surveys are required for SFBM species composition, abundance, and size; and for physical beach characteristics, such as beach slope, which play a role in determining how much phytoplankton is available to clams on the beach. While many beach surveys have been completed within Puget Sound, data are not reported with specific information about sizes of organisms that were surveyed; this information is critical to determining the level of competition for *Pseudo-nitzschia* that may occur on a beach.

Puget Sound has over 4,000 kilometers of shoreline, with a variety of shoreline environments that have led to rich assemblages of SFBMs (Kozloff 1983), including clam populations. Mixed sand, gravel, and cobble beaches are the most common shoreline type in Puget Sound (Shipman 2008). The complexity of intertidal communities on mixed sand/ gravel beaches in Puget Sound varies by proximity to the mouth of the estuary, with higher levels of complexity in northern and central Puget Sound compared to southern portions of the Sound (Dethier & Schoch 2005). In addition, there can also be dramatic differences in macroinvertebrate assemblages over narrow spatial scales, so that beaches in close proximity to each other, but with different substrates, may have different species assemblages (Carefoot 1977, Kozloff 1983, Dethier & Schoch 2005).

Sand and cobble beaches of Puget Sound are habitat for a broad assemblage of suspension-feeding clams that are harvested commercially and recreationally, and other taxa of unharvested suspension-feeders. Popularly harvested clam species include: Pacific oyster *Crassostrea gigas*, bay mussel *Mytilus trossulus*, Manila clam *Ruditapes philippinarum*, native littleneck *Leukoma staminea*, butter clam *Saxidomus giganteus*, and geoduck *Panopea generosa* (Dethier 2006). Harvest of these and other species occurs commercially on shellfish farms, as well as recreationally and tribally on public and private beaches. Unharvested suspension-feeding organisms also present on Puget Sound sand/ cobble beaches include: acorn barnacle (*Balanus glandula*), brittle star (*Amphipholis pugetana*), and tube dwelling polychaetes of the genus *Spiochaetopterus* (Kozloff 1983).

Knowing the tidal height where organisms live is important to determining their potential to accumulate toxins such as DA. As the tide rises, the lower portions of the intertidal zone are

inundated earlier and for a longer period of time than at higher tidal elevations. Clams and other SFBMs on Puget Sound beaches display classic intertidal vertical zonation, where each species is found within a specific vertical range of the intertidal zone (Kozloff 1983). The vertical distribution of species in the intertidal zone corresponds to tolerance of physical stresses such as thermal and desiccation stress, competitive interactions, avoidance of predators, and succession after disturbance (Bertness et al. 2001). In addition to tidal height, substrate type is also an important determinant of species presence. Beaches with finer sand will attract organisms that can adapt to shifting of habitat during storms and waves, whereas beaches with coarser gravel will be home to species that are not susceptible to shell breakage (Bertness et al. 2001). Thus, organisms that are restricted to the lower intertidal zone – such as horse clams (*Tresus capax*) and butter clams in Hood Canal – have a longer period of time for feeding and purging toxins from tissues than organisms that reside higher in the tidal frame, such as acorn barnacles, bay mussels and Pacific oysters. Beach slope is also important for determining toxin accumulation dynamics. The slope of a beach determines how much water inundates the intertidal zone during high tide. Beaches with a steep slope will hold less water over them during high tide than beaches with a shallow slope, resulting in lower access to phytoplankton for species at steep beaches compared to shallow beaches.

Objectives

This chapter presents information on intertidal SFBM assemblages in terms of species composition, abundance and organism size, and beach slopes at three mixed sediment (sand/cobble) beaches at Lofall, in Hood Canal, Washington State. These data are important inputs for a model (see Chapter V: Modeling *Pseudo-nitzschia* Consumption and Domoic Acid

Cycling in Puget Sound and Washington Outer Coast Clams), to assess suspension-feeding capacity along a beach, and the possibility of competition between SFBMs for toxin-producing *Pseudo-nitzschia*, which might limit toxin accumulation in harvested clams. Clam suspension-feeding capacity is determined by species and size, so calculating suspension-feeding capacity on a beach-wide basis requires detailed information on species abundance by size class: data that were not available elsewhere. Data on non-bivalve suspension-feeding organisms were also collected to determine whether other organisms have high enough concentrations to significantly contribute to suspension-feeding capacity at a beach. The choice of three study sites in Lofall, Hood Canal, was meant to provide scenarios from Puget Sound, with which to parameterize the modeling described in Chapter 5.

MATERIALS & METHODS

Study Sites

To gain a representative view of the intertidal suspension-feeding assemblage composition in Lofall, I surveyed three beaches that are located within approximately 2.5 km of each other (Fig. 1): (1) Thomas Residence (hereafter referred to as Beach 1), which has a steep fore-shore and a low tide terrace; (2) Kitsap Memorial State Park (hereafter referred to as Beach 2), which is characterized by a steeper gradient than the other two beaches; and, (3) Edgewater Beach Community Club (hereafter referred to as Beach 3), which is a delta for a small stream, and has a very gradual beach slope profile.

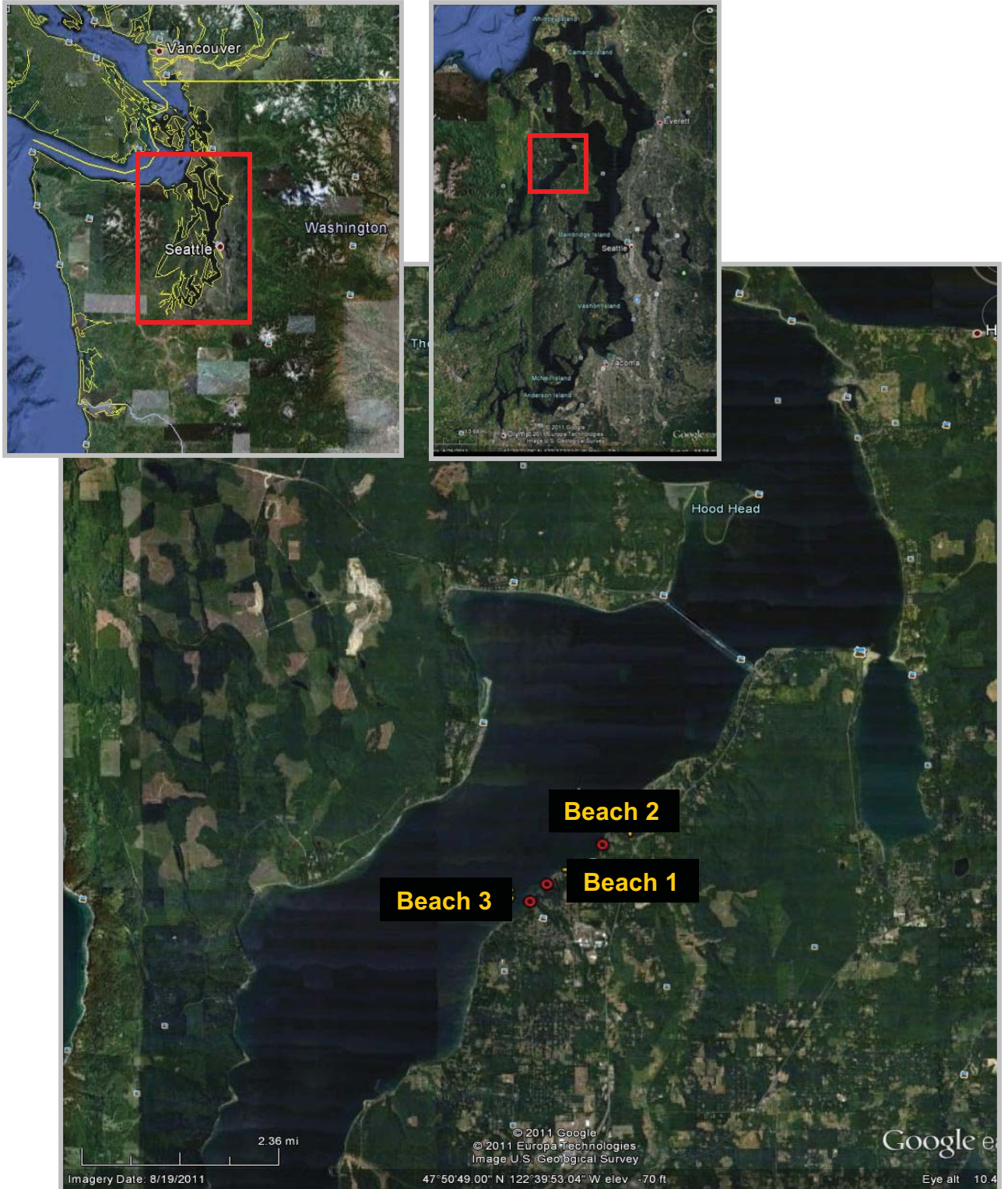


Figure 1. Lofall region of Hood Canal in Washington State, USA, showing study sites: Beach 1 -- Thomas Residence; Beach 2 -- Kitsap Memorial State Park; and Beach 3 -- Edgewater Beach Community Club.

Beach Slope Profiles

A laser level and survey rod were used to measure elevations at 5-m intervals along a measured transect from the edge of the land into the water. At the end of the survey, three additional pieces of information were collected: 1) location of the water's edge along the transect; 2) difference in elevation between the location of the water's edge and the starting point; and 3) time at which these measurements were recorded. These three pieces of information were used to ground-truth relative differences in elevation to absolute elevations using the following methodology:

- 1) using NOAA's published information on predicted and observed tides for Seattle, Washington (NOAA Tides and Currents: <http://tidesandcurrents.noaa.gov>), to determine Δ_{tide} , the difference between observed and predicted tidal heights for the date and time that the beach slope profile was surveyed;
- 2) obtain predicted tidal heights for Lofall for the date and time of the survey using a water circulation model for Puget Sound, *PSTides*;
- 3) add Δ_{tide} to the predicted tidal height at the time of the survey and obtain the vertical elevation relative to mean lower low water (MLLW) at the location on the transect coinciding with the water's edge; and,
- 4) reconcile the relative differences in elevation to the one known tidal height.

Nearshore Benthic Macroinvertebrate Surveys

Both epi- and infaunal intertidal SFBMs were sampled at the three study sites during the lowest tides in August and September, 2005. Sampling at each beach was stratified along three or four 50-m transects at different tidal elevations parallel to the shoreline (Fig. 2). Strata were selected based on qualitative beach characteristics (e.g., sediment composition, dominant epibiota,

epibenthic or emergent vegetation) rather than absolute tidal height, to allow for consistency of comparison in bivalves between the three beaches. The selected beach strata were, from high to low tidal elevation: 1) oyster/ mussel zone; 2) sandy area above dense surface coverage by algae of the genus *Ulva* (at Beach 1 only: no oyster/ mussel bed was present at this beach); 3) areas densely covered by *Ulva* spp.; 4) sandy area between *Ulva* spp. and eelgrass (*Zostera marina*) (at Beach 3 only: the distance between the *Ulva* and eelgrass transects at this site was large, so an extra transect was characterized); and, 5) eelgrass. Six to ten quadrats were randomly placed along each transect. Small (0.1-m²) quadrats were used in areas where SFBMs were relatively homogeneously distributed, such as along some transects covered by oysters, mussels or eelgrass. Medium (0.4-m²) quadrats were used to capture variability in more heterogeneously-distributed macrofauna. I also used large (4-m²) quadrats to assess the presence of large clam shows (siphons) that were widely spaced between patches of eelgrass at Beach 3. In addition, I subsampled sediment under the eelgrass so that abundance of small suspension-feeders living between eelgrass rhizomes could be assessed without destroying large amounts of eelgrass habitat.

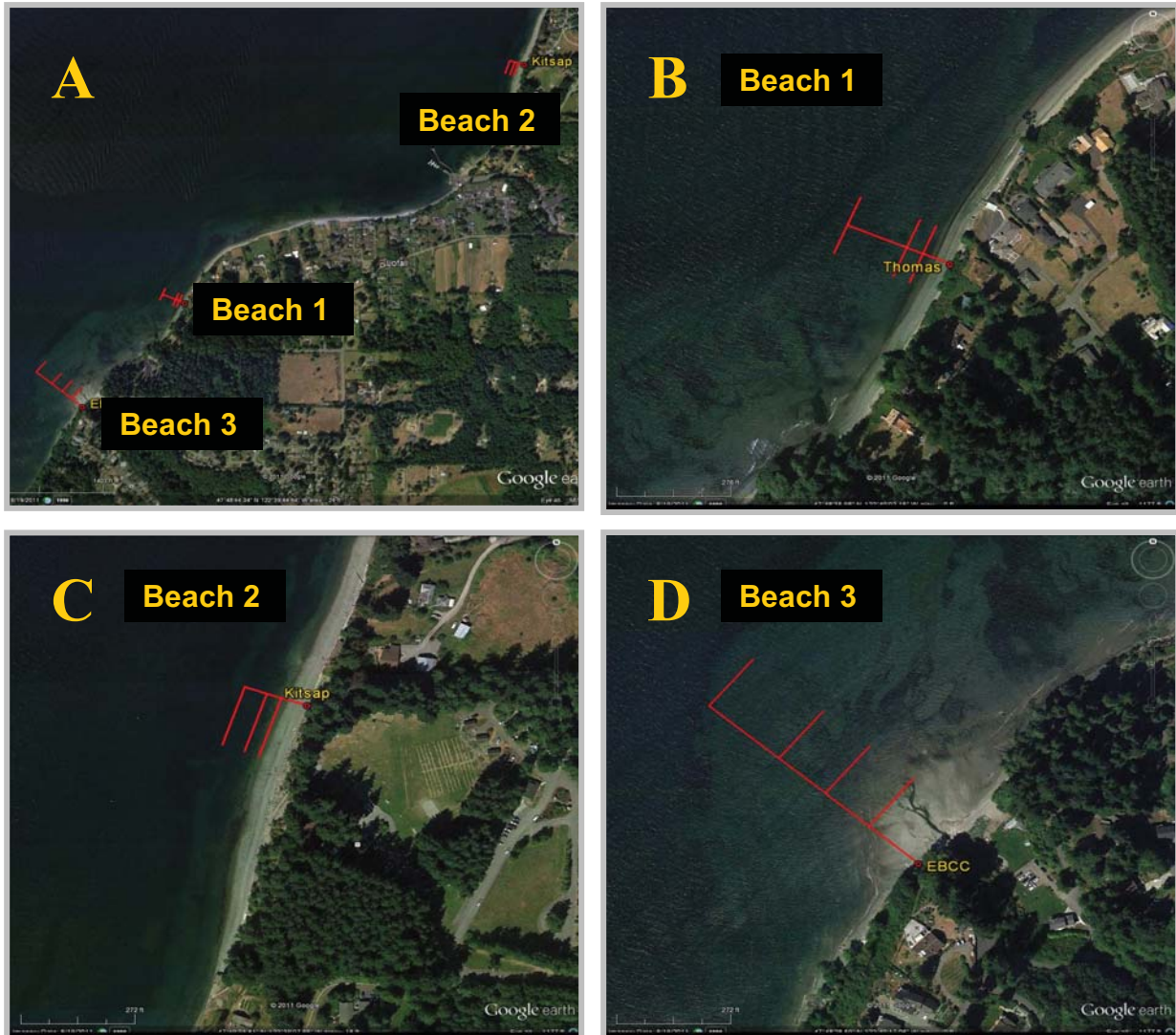


Figure 2. Transect locations at each site. Lines parallel to shore indicate locations of sampling transects. A) Lofall area map showing three sampling beaches with transect locations. B) Transect locations at Beach 1: from shore towards water, transects are: upper sand; dense *Ulva*; eelgrass. C) Transect locations at Beach 2: oyster/ mussel bed; dense *Ulva*; eelgrass. D) Transect locations at Beach 3: oyster/ mussel bed; dense *Ulva*; lower sand; eelgrass.

For transects over oysters, mussels, sandy areas, or *Ulva*, each quadrat was examined from the surface, photographed using a digital camera, and species abundance and size were recorded for SFBM epifauna except barnacles (see Barnacles section below). Following assessment of epifauna, each quadrat was excavated to 15cm (in cobble areas) or 45cm (in sandy areas) to identify infaunal clams and other SFBMs. Clams were identified to the species level and their

shell length was measured (Fig. 3). Abundance of other SFBM taxa were recorded either as individual organisms, or as number of worm tubes for polychaetes, since it was generally not possible to find the polychaetes following excavation. Organisms that could not be identified in the field were preserved with buffered formalin for later identification in the laboratory.

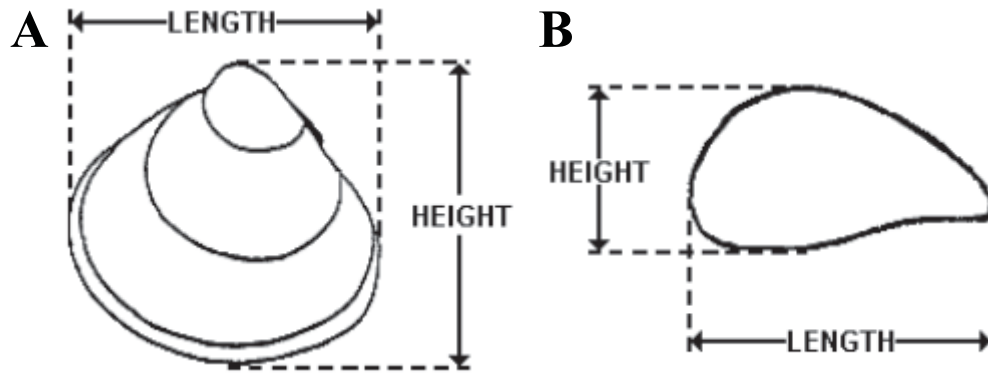


Figure 3. Length measurement directions for siphonate clams (A) and mussels/ oysters (B).

Three sampling schemes were used to assess different populations of SFBMs in the transects over eelgrass. Small (0.1-m^2) quadrats were used to assess abundance of epifauna, and eelgrass blade counts, as a relative measure of eelgrass density. One core subsample (10cm diameter) was collected to 15cm depth from the upper left corner of each 0.1-m^2 quadrat. These core subsamples were used to assess infauna such as small clams and tube worms. Core subsamples were washed over 2-mm wire mesh sieves, and retained SFBMs were collected into plastic jars and preserved with formalin for lab identification. At Beach 3, siphons of large clams (horse clam and geoduck) were observed along the eelgrass transect, but the density was too low to capture their presence with the small quadrats. Thus, large 4-m^2 quadrats were used at Beach 3 to assess abundance of large clam siphons. All siphons from unexcavated large clams were assigned a shell length of 15 cm.

Analysis of Species Composition

Data analyses were performed using the open source statistical programming language R. Clam data were binned by size class into 1-cm interval bins: for example, all organisms between 2.0 and 2.9cm, inclusive, were in one size class, with the midpoint of the size class set at 2.5cm. Non-bivalve suspension-feeding organisms, such as polychaetes and brittle stars, were counted only as number of individuals. All organism data were standardized to 1-m^2 . The mean density, ± 1 s.d., of individuals $\cdot \text{m}^{-2}$ in each species and size category was calculated for each transect.

Barnacle densities were assessed from digital photographs of each quadrat by overlaying a grid of 100 points over the photographs and determining the percentage of points overlaying live barnacles. Foster et al. (1991) found that this method results in underestimates of organismal coverage because barnacles can attach to all surfaces of cobbles, but are only estimated on the upper surfaces in photographs. Thus, it should be recognized that these barnacle percent cover estimates are conservative.

Total suspension-feeding clam standing stock, as $\text{g tissue dry weight} \cdot \text{m}^{-2}$, was calculated for each transect to allow comparisons of suspension-feeding potential by clams. Clam length to tissue dry weight relationships were obtained from Chapter III: Clearance Rate Measurements on Thirteen Intertidal Clams and Acorn Barnacles Fed on the Toxin-Producing Diatom *Pseudo-nitzschia*.

RESULTS

Beach Slope Profiles & Substrate Characteristics

Beach 1 has a steep upper beach with a low tide terrace, Beach 2 is steep throughout, and Beach 3 is a stream delta with a very gradual slope (Fig. 4).

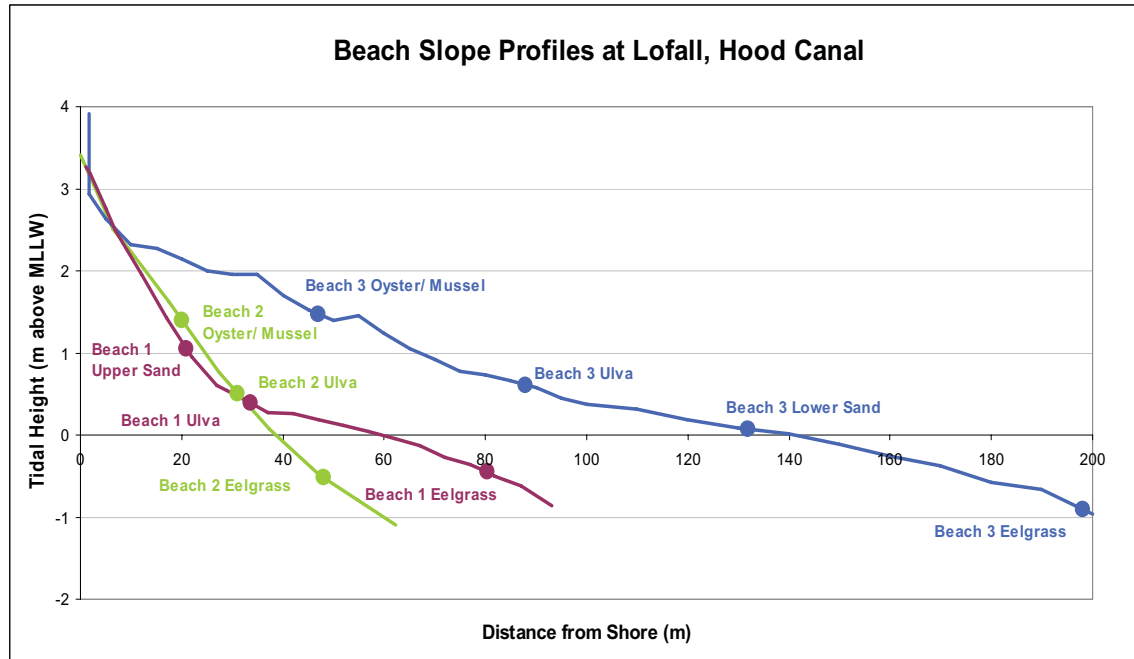


Figure 4. Beach slope profiles for three sites at Lofall, Hood Canal, Washington. Purple line represents Beach 1; green line represents Beach 2; and blue line represents Beach 3. Transect locations along each beach are shown as labeled dots.

Over the same tidal height range at each beach (+3.2m above MLLW to -1.0m below MLLW), a different length of beach (in the direction perpendicular to shore) was available as suspension-feeder habitat: 95m at Beach 1, 60m at Beach 2, and 200m at Beach 3. Each beach slope was approximated using a system of two linear equations, thus generating piece-wise equations for each beach slope:

Beach 1:

$$\boldsymbol{\eta} = \begin{cases} \boldsymbol{x} \leq 35.83 | -0.096 \cdot \boldsymbol{x} + 4.00 \\ \boldsymbol{x} \geq 35.83 | -0.019 \cdot \boldsymbol{x} + 1.25 \end{cases} \quad \text{(Eqn 1)}$$

Beach 2:

$$\boldsymbol{\eta} = \begin{cases} \boldsymbol{x} \leq 39.65 | -0.094 \cdot \boldsymbol{x} + 4.00 \\ \boldsymbol{x} \geq 39.65 | -0.046 \cdot \boldsymbol{x} + 2.12 \end{cases} \quad \text{(Eqn 2)}$$

Beach 3:

$$\boldsymbol{\eta} = \begin{cases} \boldsymbol{x} \leq 125.14 | -0.026 \cdot \boldsymbol{x} + 4.00 \\ \boldsymbol{x} \geq 125.14 | -0.013 \cdot \boldsymbol{x} + 2.44 \end{cases} \quad \text{(Eqn 3)}$$

where η is tidal height (m above MLLW) and x is shore distance in the x-direction (m). The fit of each system of equations is shown in Figure 5.

Beach Slope Profiles for Lofall Beaches

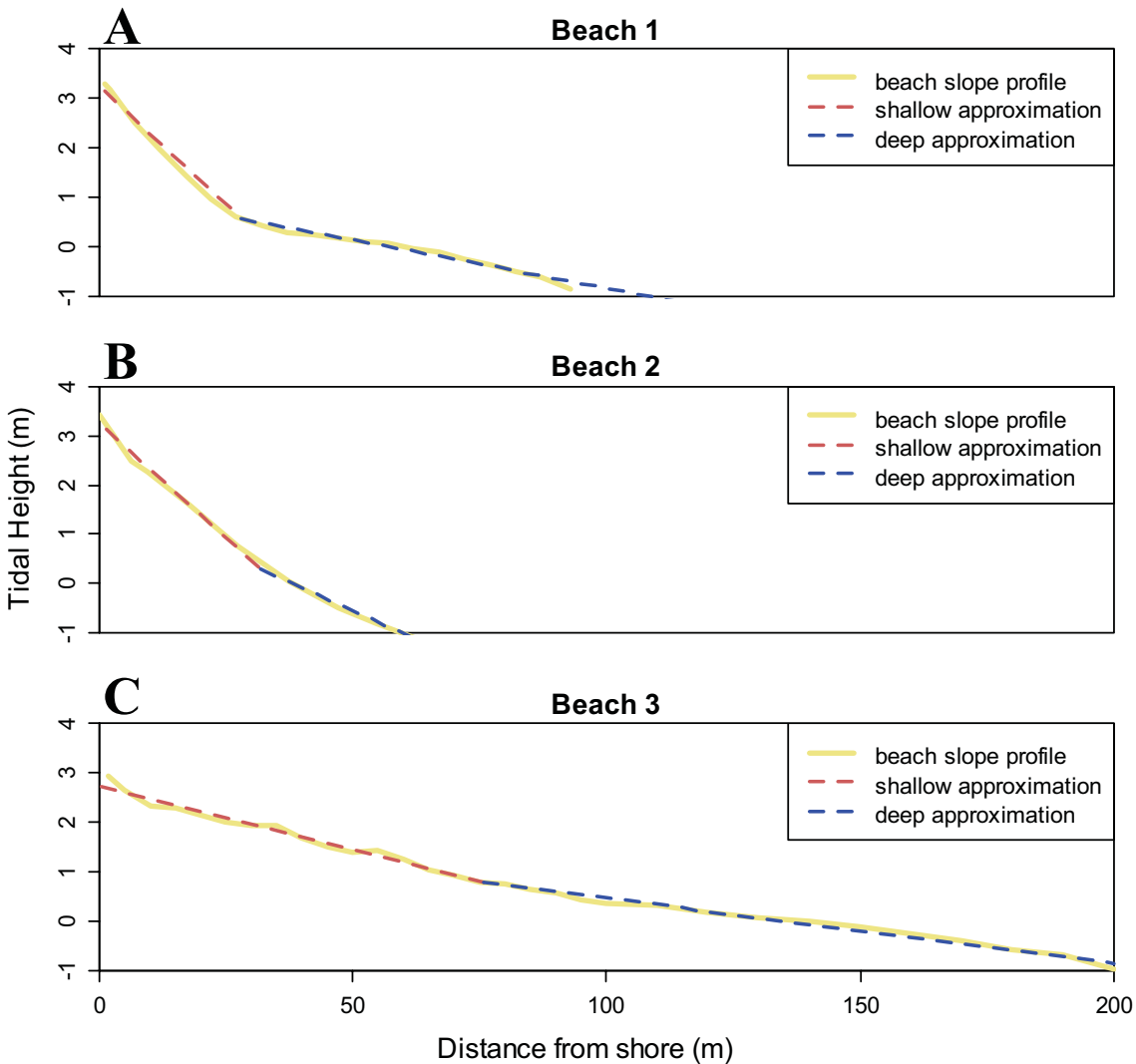


Figure 5. Beach slope profile data for Lofall Beaches 1,2 and 3 (A,B,C) shown in tan, with the fit of shallow (maroon) and deep (blue) linear approximations superimposed.

Substrate varied by beach and by tidal height, such that lower tidal heights at each beach were comprised of more sand than higher tidal heights. At Beach 1, the upper sand transect was mostly sand mixed with silt, pebbles and small cobble; the *Ulva* transect was coarse to medium grained sand; and, the eelgrass transect was sand. At Beach 2, the oyster/ mussel transect was small to large cobble with large-grained sand between the stones; the *Ulva* transect was cobble and small rocks with coarse sand in between the cobble; and, the eelgrass transect was mostly

sand, with some small to large cobbles. At Beach 3, the oyster/ mussel transect was coarse sand mixed with small to medium sized cobble; the *Ulva* transect was coarse to medium grained sand; the lower sand transect was coarse sand; and, the eelgrass transect was medium-grained sand mixed with some silt. Substrate was only characterized qualitatively; approximate percentages of cobble/ pebbles and sand/ silt along each transect are provided in Table 5.

Table 1. Approximate proportions of cobble/ pebbles and sand/ silt substrate along each sampled transect at each beach.

	Beach 1	Beach 2	Beach 3
Oyster/ Mussel	--	cobble/ pebbles: 75% sand/ silt: 25%	sand/ silt: 75% cobble/ pebbles: 25%
Upper Sand	sand/ silt: 75% cobble/ pebbles: 25%	--	--
Ulva	sand/ silt: 100%	cobble/ pebbles: 75% sand/ silt: 25%	sand/ silt: 100%
Lower Sand	--	--	sand/ silt: 100%
Eelgrass	sand/ silt: 100%	sand/ silt: 75% cobble/ pebbles: 25%	sand/ silt: 100%

Intertidal Suspension-Feeding Bivalves

Commonly harvested clams found in the surveys include two varieties of littleneck clam (native littleneck and thin-shell littleneck *Leukoma tenerrima*), Manila clam, soft-shell clam (*Mya arenaria*), two species of horse clam (*Tresus capax* and *T. nuttallii*), butter clam, cockle (*Clinocardium nuttallii*), bay mussel, and Pacific oyster (Fig. 7). The suspension-feeding clam biomass at Beach 1 was predominantly butter clam, littleneck clam and horse clam; while at Beach 2 it was mostly Pacific oyster; and, a combination of mussel, butter clam and horse clam

at Beach 3. Beach 1 showed the most consistency between transects in relative assemblage structure, while Beach 3 showed the most variability between transects in relative assemblage structure (Table 2).

Estimates of suspension-feeding clam standing stock, measured as $\text{g tissue dry weight} \cdot \text{m}^{-2}$, indicated the highest standing stock at Beach 1 upper sand, followed by Beach 2 oyster /mussel bed, and then Beach 1 *Ulva* (Fig. 7). The lowest suspension-feeding clam standing stock was found along the three eelgrass transects: Beach 1 had no suspension-feeding clams, while Beaches 2 and 3 had very low of standing stock (Fig. 7).

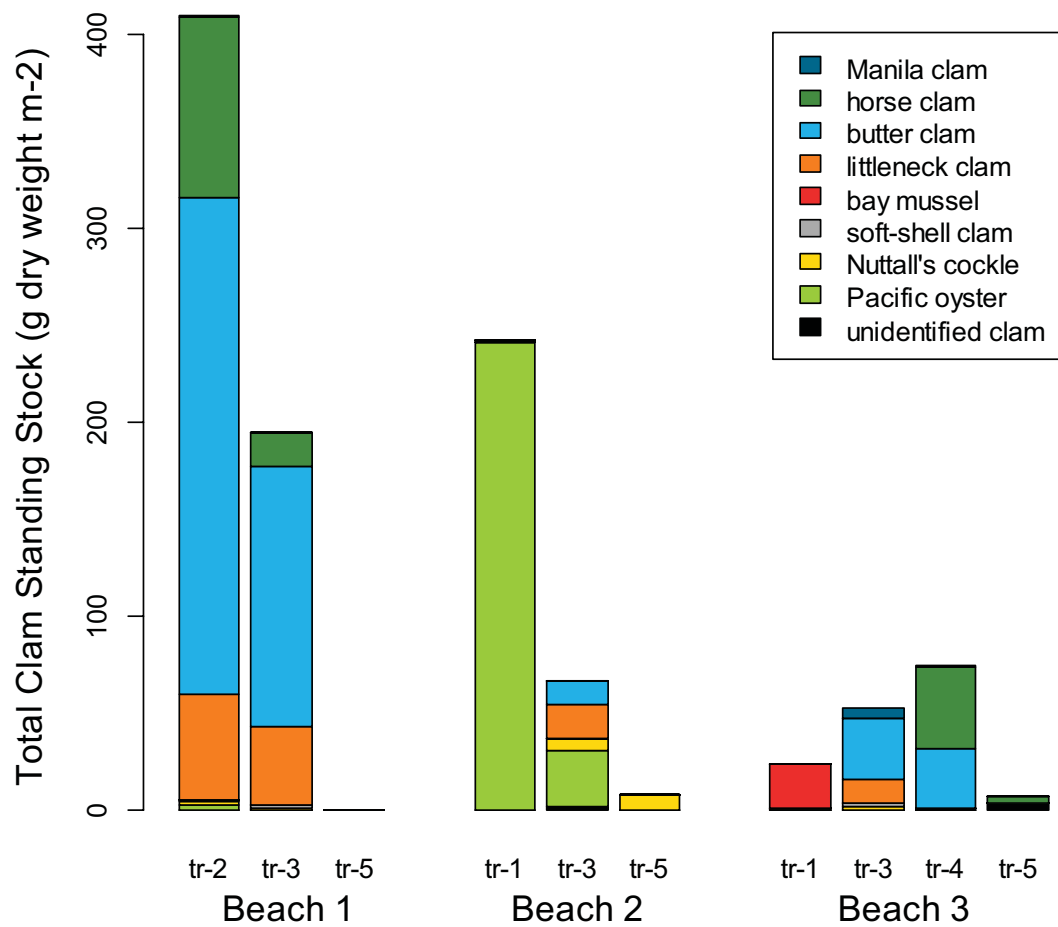


Figure 7. Suspension-feeding clam standing stock as tissue dry weight ($\text{g} \cdot \text{m}^{-2}$) by species along each transect.

Table 2. Relative assemblage structure of suspension-feeding clams as a percentage of standing stock at each beach and along each transect.

	unid. clam	Pacific oyster	Nuttall's cockle	soft-shell clam	bay mussel	littleneck clam	butter clam	horse clam	Manila clam
Beach 1									
upper sand		1%				13%	63%	23%	
<i>Ulva</i>				1%		21%	69%	9%	
eelgrass									
Beach 2									
mussel/ oyster		100%			1%				
<i>Ulva</i>	6%	50%	8%			20%	15%		
eelgrass	3%		96%			1%	1%		
Beach 3									
mussel/ oyster		4%			96%				
<i>Ulva</i>			3%	3%		25%	59%		10%
lower sand			1%				42%	57%	1%
eelgrass	45%						1%	55%	

Inter-beach comparison of relative species standing stock at each strata indicated substantial inter-beach variability in the oyster/ mussel transects and the eelgrass transects, and some similarity in the *Ulva* transects (Fig. 8). The *Ulva* transects at Beaches 1 and 3 were most similar in relative species abundance: both beaches had the majority of their clam standing stock as butter clam, followed by littleneck clam. Beach 2's *Ulva* transect had the majority of its standing stock as Pacific oyster, followed by littleneck clam and butter clam.

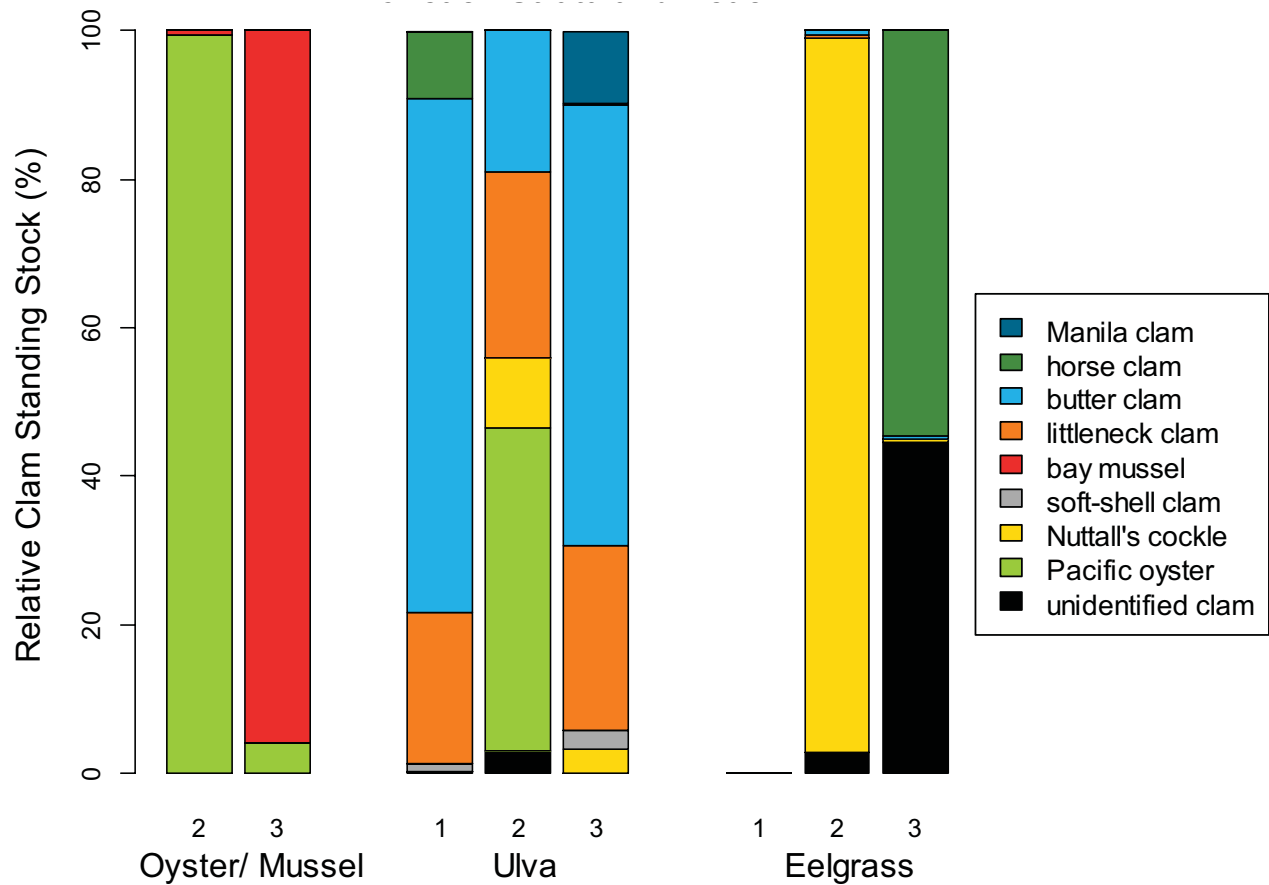


Figure 8. Relative standing stock species composition of suspension-feeding clams at each tidal strata, with each bar representing a beach: Beach 1 (labeled “1”), Beach 2 (“2”) or Beach 3 (“3”).

Although there was substantial overlap in the bivalve species found among the three sites, the densities and sizes of bivalves differed from site to site (Figs. 9-14). Along each transect, high variability was observed among quadrats in terms of species composition, density and organism size (see error bars in Figs. 9-14).

Oyster/ mussel transects (~ +1.5m above MLLW). Pacific oysters dominated the transect at Beach 2, while Beach 3 was dominated by bay mussels (Fig. 9). Beach 1 lacked an oyster or mussel bed, although occasional oysters were encountered in the upper sand transect at Beach 1.

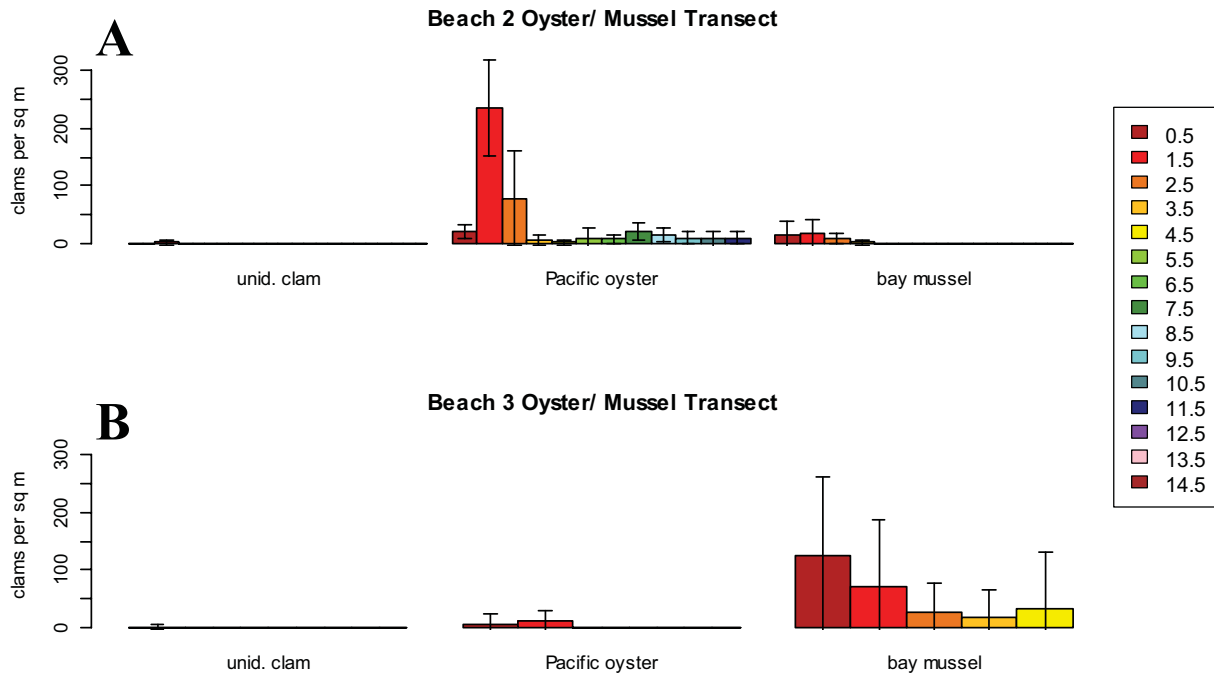


Figure 9. Average densities (number \cdot m⁻²) and sizes (cm, shown in different colors) of suspension-feeding clams along transects of oyster/ mussel beds (tidal height \sim +1.5m above MLLW) at Beach 2 (A) and Beach 3 (B).

Upper sand transect (\sim +1.0m above MLLW). The Beach 1 upper sand transect was the most diverse of the Beach 1 transects, and had the densest population of bivalves found on the three Lofall beaches (Fig. 7). Seven genera of suspension-feeding clams were represented along this transect, with littleneck clam and butter clam the most abundant (Fig. 10). Clam sizes ranged from 1 to 11cm.

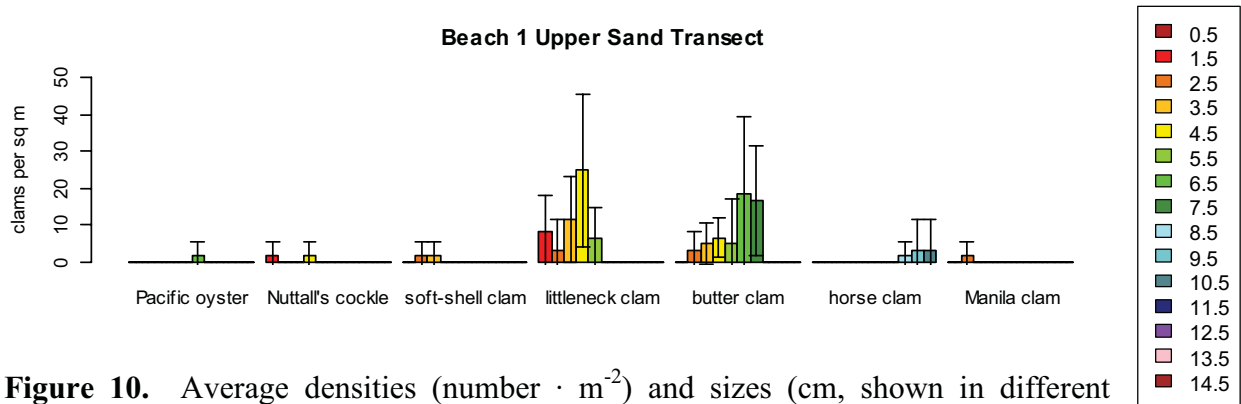


Figure 10. Average densities (number \cdot m⁻²) and sizes (cm, shown in different colors) of suspension-feeding clams along transects of sandy beach above dense beds of *Ulva* spp. (tidal height \sim +1m above MLLW) at Beach 1.

Ulva transects (\sim +0.5m above MLLW). *Ulva* transects all had a covering of sea lettuce (*Ulva* spp.) during low tide, although the percent cover varied from site to site, with *Ulva* density highest at Beach 1 and lowest at Beach 3. These transects were among the most diverse in terms of clam species: a total of six or more clam genera were found along each *Ulva* transect (Fig. 11). Each *Ulva* transect had littleneck clams and butter clams in a range of sizes and at relatively high densities. In addition, Beach 2 also had a large number of Pacific oysters attached to the mostly cobble substrate. Clams sizes ranged from 0 to 7cm at Beaches 2 and 3, with a few larger oysters at Beach 2 (10-11cm); clams at Beach 1 tended to be slightly larger, ranging from 1 to 10cm, with a few large horse clams (13-14cm).

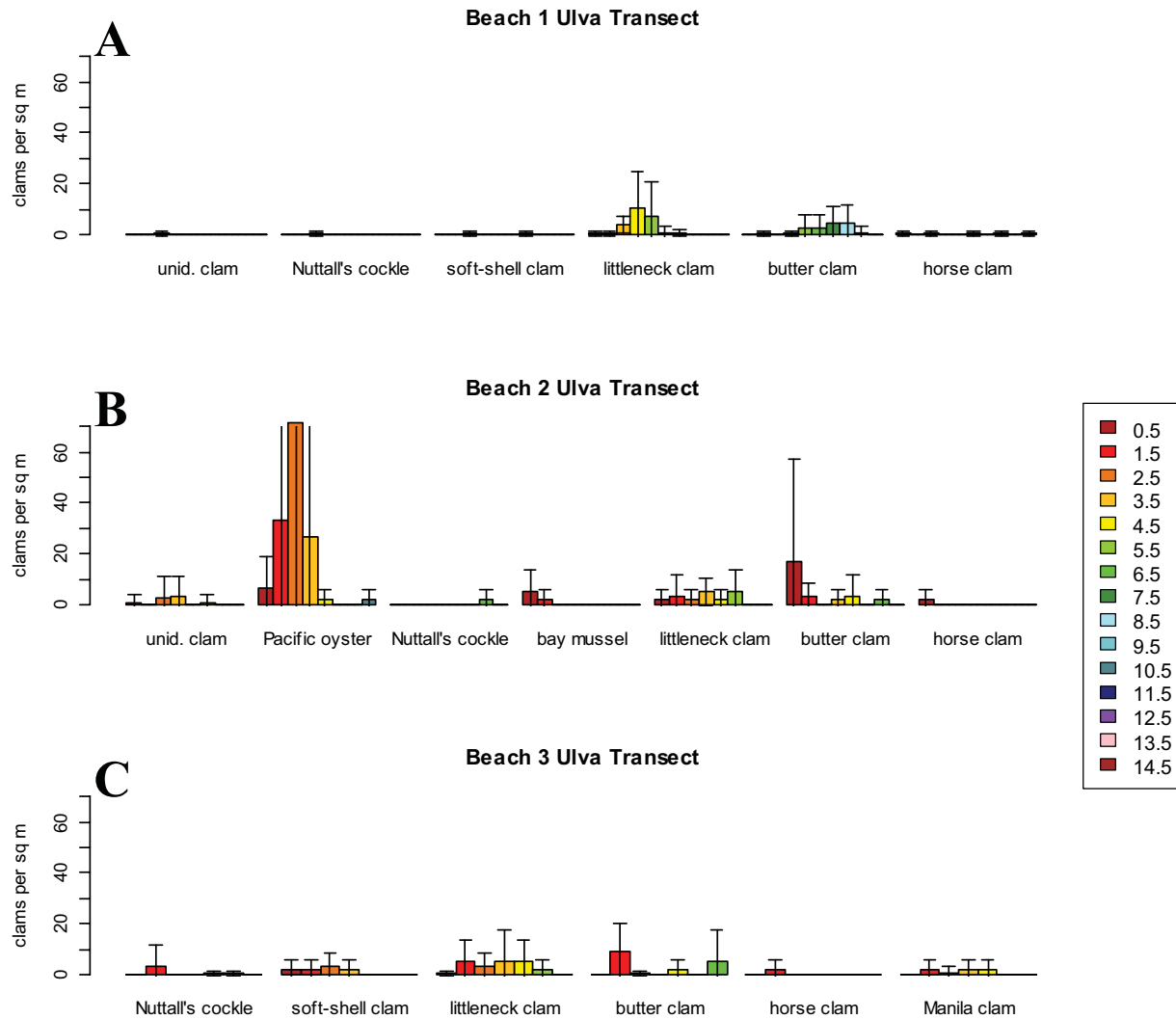


Figure 11. Average densities (number \cdot m⁻²) and sizes (cm, shown in different colors) of suspension-feeding clams along transects through dense beds of *Ulva* (tidal height \sim +0.5m above MLLW) at Beach 1 (A), Beach 2 (B) and Beach 3 (C).

Lower sand transect (\sim 0m, or \sim MLLW). The lower sand transect at Beach 3 had low clam density, with four genera present (Fig. 12). Clams along this transect had a bi-modal size distribution, and were either small (1-4cm) or large (9-13cm).

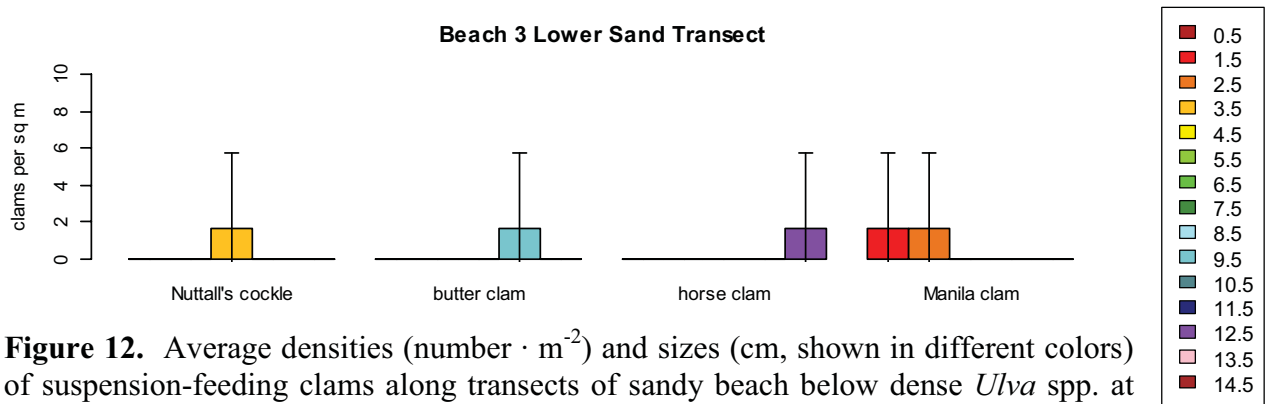


Figure 12. Average densities (number \cdot m⁻²) and sizes (cm, shown in different colors) of suspension-feeding clams along transects of sandy beach below dense *Ulva* spp. at Beach 3 (tidal height \sim 0m).

Eelgrass transects ($\sim -0.5\text{m}$ to -1.0m below *MLLW*). Infaunal clams found along eelgrass transects were generally small (length $<2\text{cm}$; Fig. 13). The exception was a few large (11-15cm) horse clam and/ or geoduck found at Beach 3, whose abundance of 0.05 to 0.1 \cdot m⁻² was too low to appear in Figure 13. Beach 1 had no suspension-feeding clams along the eelgrass transect, while Beaches 2 and 3 each had representation from three or more clam genera.

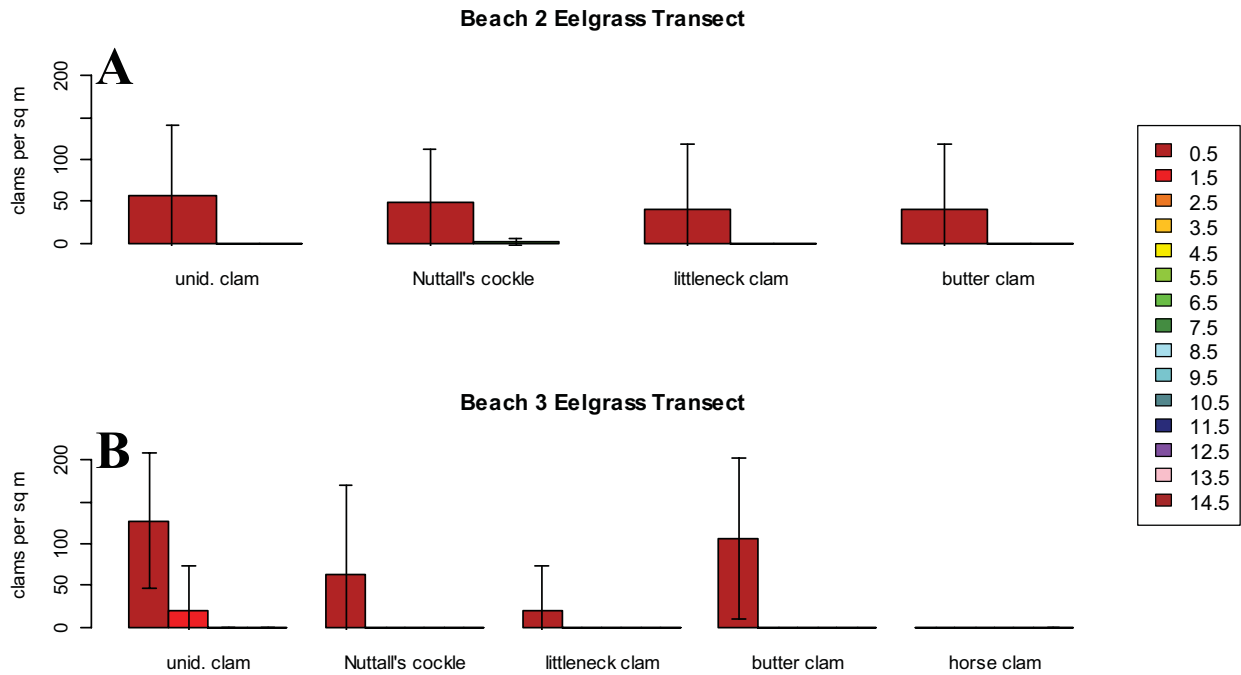


Figure 13. Average densities (number \cdot m⁻²) and sizes (cm, shown in different colors) of suspension-feeding clams along transects through beds of eelgrass (*Zostera* spp.) (tidal height \sim -0.5 m to -1.0 m below MLLW) at Beach 2 (A) and Beach 3 (B).

Eelgrass blade density was highest at Beach 1 (mean= $43 \cdot 0.1\text{m}^{-2} \pm 11$), followed by Beach 3 (mean= $33.5 \cdot 0.1\text{m}^{-2} \pm 35$) and Beach 2 (mean= $25 \cdot 0.1\text{m}^{-2} \pm 35$). The relationship between number of suspension-feeding clams per small (0.1-m^2) quadrat and eelgrass blade count pooled over all sites was slightly negative (clams = $28.05 - \text{blades} \cdot 0.08$), but not significant (p-value= 0.58 ; Fig. 14).

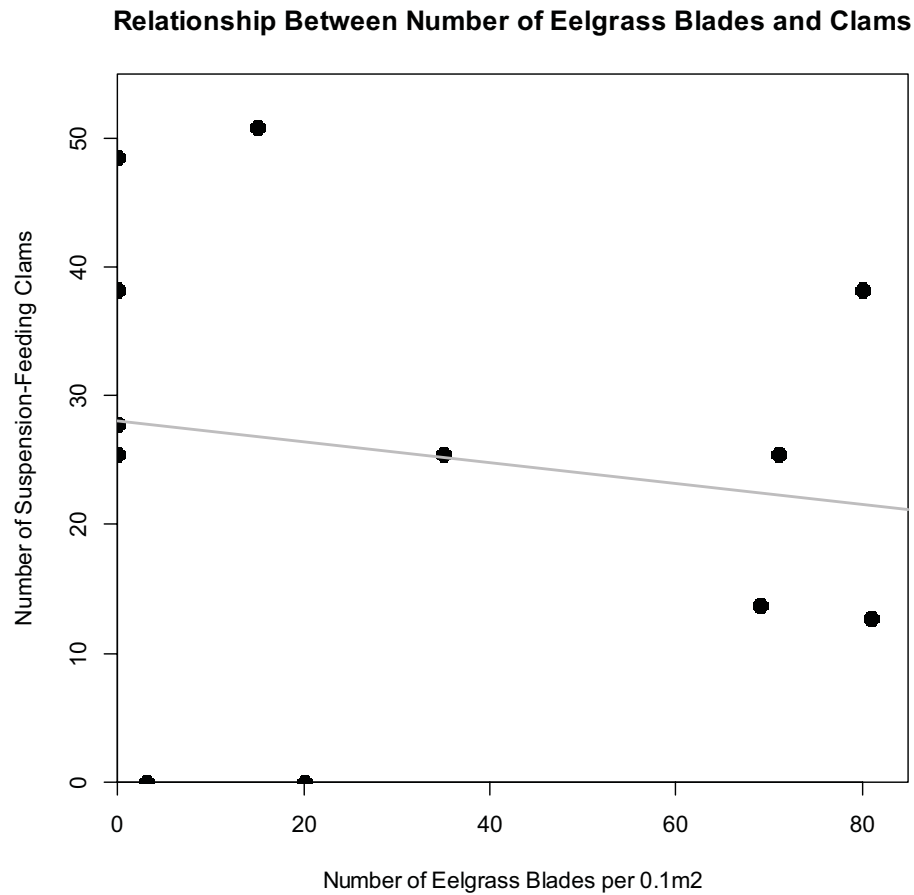


Figure 14. Relationship between number of eelgrass blades per small (0.1-m²) quadrat, and number of suspension-feeding clams in that quadrat. Data are pooled over all sites. Linear model for the relationship is shown in gray.

Variation in Bivalve Distribution as a Function of Beach Structure

Correlations between suspension-feeding clam standing stock in a 1-m² quadrat, measured as tissue dry weight $\text{g} \cdot \text{m}^{-2}$, and approximate proportion of sand in the quadrat indicated a slight positive relationship between infaunal clam standing stock and proportion of sand as substrate (biomass = $29.1 + \text{sand} \cdot 57.7$; p-value: 0.46), and a strong negative relationship between epifaunal clam biomass and proportion of sand as substrate (biomass = $173.3 - \text{sand} \cdot 185.6$; p-value: $\ll 0.001$; Fig. 6).

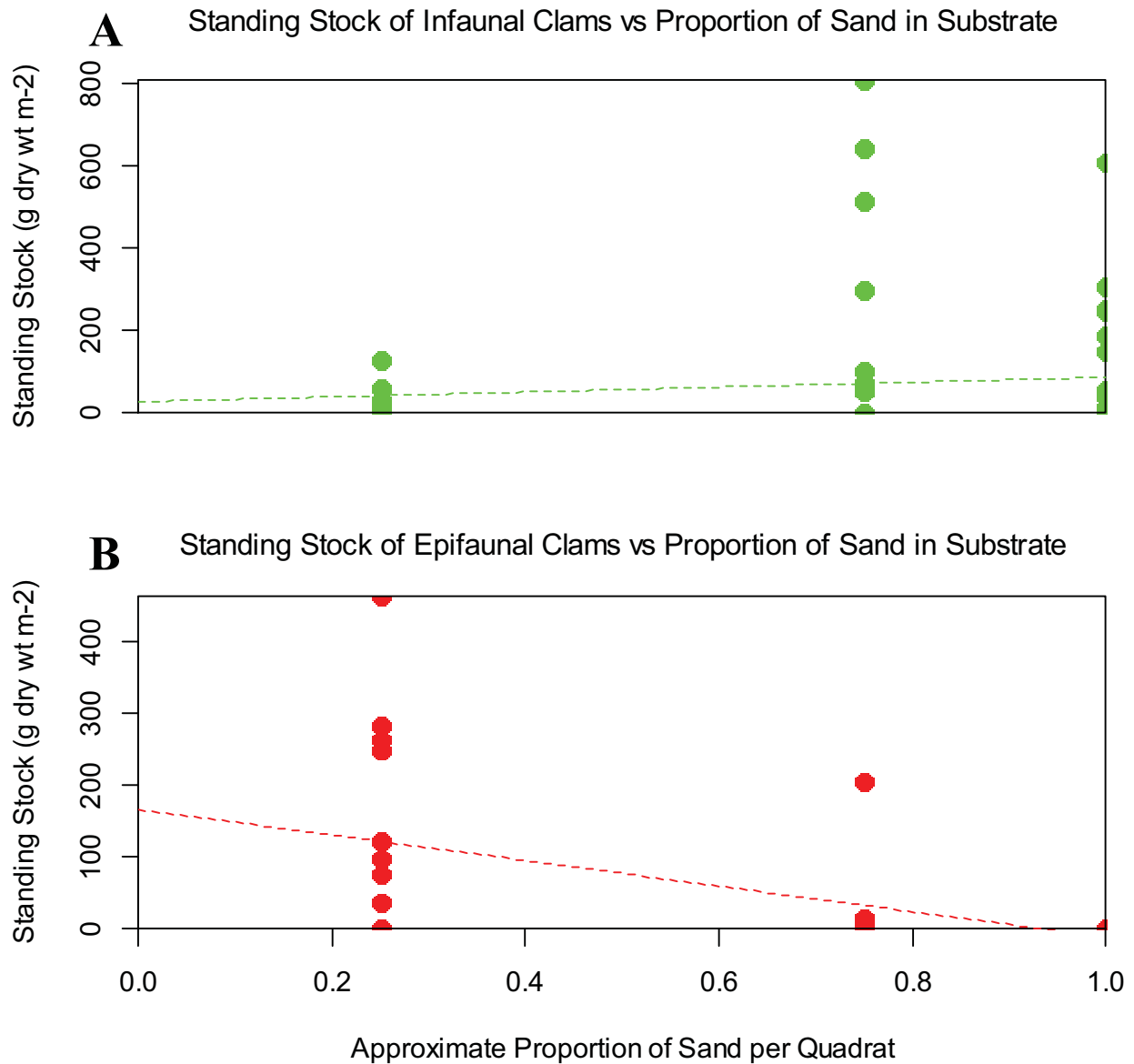


Figure 6. Relationship between suspension-feeding clam standing stock and approximate proportion of sand as substrate for infaunal clams (A) and epifaunal clams (B). Data are shown for each 0.1-m² quadrat, and are pooled over all sites.

Non-Bivalve Suspension-Feeding Organisms

Non-bivalve SFBMs found along the transects included: acorn barnacle; brittle stars; tube worms of the genus *Spiochaetopterus*, as well as other unidentified tube worms; and bryozoans.

Barnacle Density. Three transects had barnacles present: oyster/ mussel transects at Beaches 2 and 3, and the *Ulva* transect at Beach 2. The oyster/ mussel transect at Beach 2 had the highest percent cover of barnacles (mean=35% \pm 11%), followed by relatively equal cover between the oyster/ mussel transect at Beach 3 (mean=22% \pm 14%) and the *Ulva* transect at Beach 2 (mean=22% \pm 16%). Variability between quadrats was too great to determine significant differences in barnacle density between the three transects.

Brittle Star, Bryozoan and Polychaete Density. Brittle stars and bryozoans were only found along the *Ulva* transect at Beach 2 (Fig. 15A,B). Tube worms were more common, such that tubes (usually empty) were found in high abundance in all eelgrass sites (Fig. 15C,D). In particular, tubes of *Spiochaetopterus* were often observed intertwined with eelgrass rhizomes.

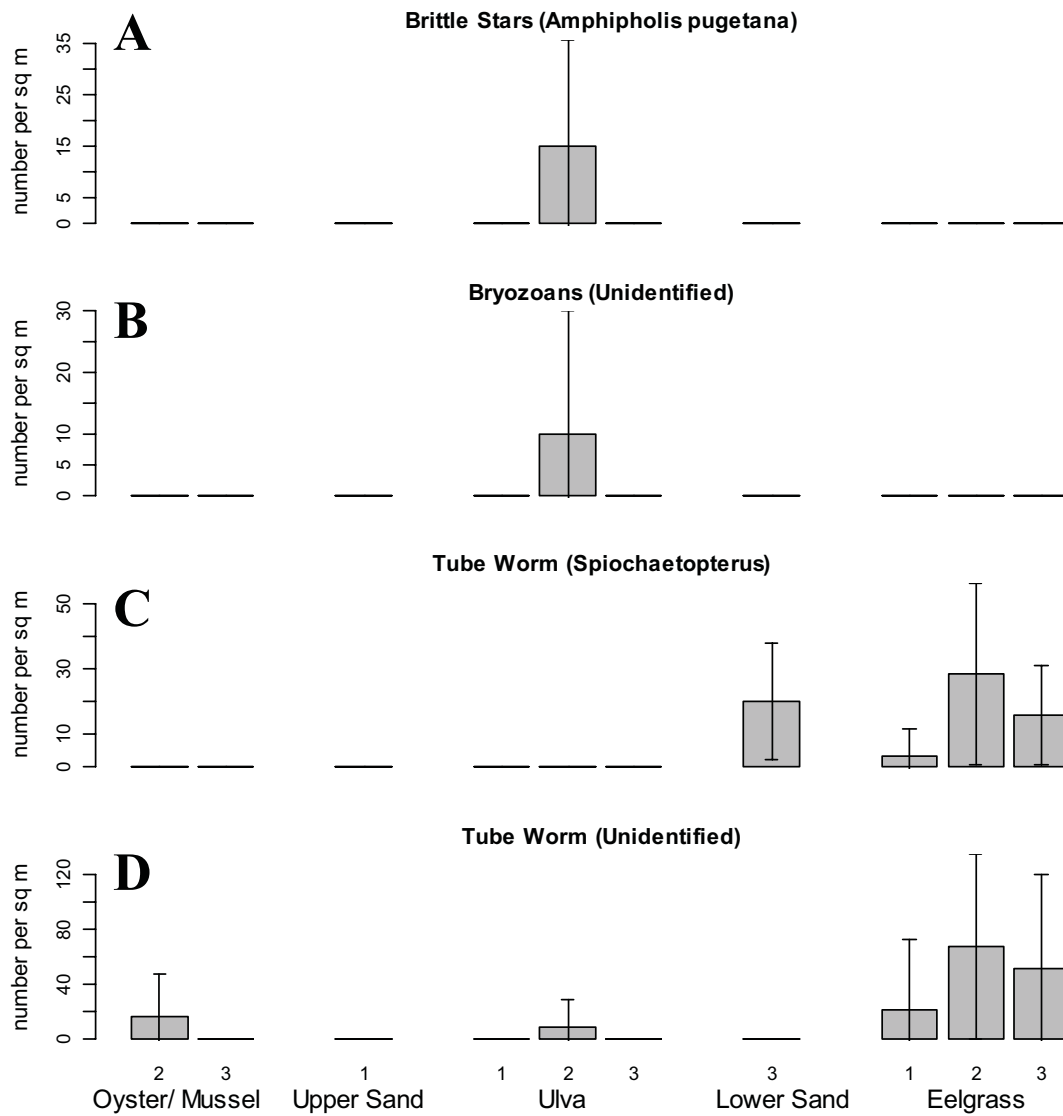


Figure 15. Density (number \cdot m⁻²) of non-clam SFBMs at each tidal strata, with each bar representing a beach: Beach 1 (labeled “1”), Beach 2 (“2”) or Beach 3 (“3”). Tube worm plots (C,D) indicate presence of worm tubes, which were frequently empty once excavated.

DISCUSSION

The primary purpose of this study was to explore several beaches in one geographic location and assess the suspension-feeding benthic macroinvertebrate assemblage composition on the sand/cobble beaches. These data will form the basis for a model to determine whether competition

among SFBMs for toxin-producing *Pseudo-nitzschia* may reduce toxin accumulation in harvested clams.

Of primary importance was assessing which SFBMs are most abundant, and which may be most important to a beach's suspension-feeding capacity. The species abundance (standing stock) and composition data suggest that clams and barnacles are the most important suspension-feeders in the Lofall study sites, along with polychaetes at lower tidal heights. Although a fair number of tubes or fragments of tubes from annelid polychaetes were found along eelgrass transects, these numbers likely represent inflated estimates of suspension-feeding polychaetes. Some of the retrieved tubes, especially of the genus *Spiochaetopterus*, were likely fragments of the same tube, and some of the polychaete tubes may have been empty before the survey. Additionally, some unidentified tubes may have belonged to polychaetes that are not suspension-feeders.

At least some of the variability among sites in terms of SFBM abundance and species composition may be accounted for by difference in substrate. This was particularly true for epifaunal clam species. In this study, the proportion of sand in the substrate was inversely related to standing stock of epifaunal clams. The oyster/ mussel transect at Beach 2 had a much lower proportion of sand (25%) than Beach 3 (75%), and also had a much higher suspension-feeding clam biomass. Sandy substrate is not suitable for epifaunal clams, as sands can smother or bury epifaunal organisms (Bertness et al. 2001).

Substrate composition along the *Ulva* transects may explain some of the differences in SFBM species assemblage composition. The *Ulva* transects at Beaches 1 and 3 were both made up

almost entirely of sand, while at Beach 2, the proportion of sand was only 25%. Indeed, this was evident in the species composition at the three sites: Beaches 1 and 3 both had a high proportion of butter clam and littleneck clam in the *Ulva* transects, while Beach 2 had the majority of its standing stock as Pacific oyster.

On a site-wide basis, clam standing stock along all Beach 3 transects was relatively low. One possible reason for this is that the substrate at Beach 3 is sandier than at the other two sites. During strong storms, sands can shift and smother epifaunal clams or dislodge or bury infaunal clams, resulting in lower abundance (Bertness et al. 2001).

Of all transects and strata, eelgrass transects generally had the lowest bivalve abundance. This seems to support studies that show negative effects of eelgrass on clam growth (Allen & Williams 2003, Tsai et al. 2010), although within eelgrass beds, I did not find a significant correlation between blade density and suspension-feeding clam abundance.

Future Work

Three sites within Lofall, Hood Canal were assessed in this study. One avenue of future work would be to examine how species abundance and composition at the Lofall sites relates to the rest of Puget Sound.

In this study, I assessed SFBM abundance and species composition along three to four transects parallel to shore at each site. In Chapter V: Modeling *Pseudo-nitzschia* Consumption and Domoic Acid Cycling in Puget Sound and Washington Outer Coast Clams, I use linear

interpolation between transects to obtain a beach-wide profile of suspension-feeding clam assemblage. Future work could assess the suspension-feeding assemblages that occur in between the transects that were sampled, to determine whether there is a smooth linear transition in species composition and abundance between transects, or whether dramatic boundaries define species composition and abundance. If dramatic boundaries in species composition and abundance exist, they could have a dramatic effect on total suspension-feeding capacity for a beach. These data could be collected both in a field study with sampled transects perpendicular to shore, and then correlated to data on shoreline habitat.

Chapter II:

Phytoplankton Advection into the Intertidal Zone of a Temperate Fjord

ABSTRACT

In coastal waters, blooms of toxin-producing phytoplankton, such as the cosmopolitan diatom genus *Pseudo-nitzschia*, can tidally advect over intertidal shellfish beds, becoming available to suspension-feeding clams, including mussels and oysters, that feed on the phytoplankton and bio-accumulate toxin in their soft tissues. Understanding phytoplankton flux into the intertidal may prove valuable for predicting availability of toxin-producing phytoplankton to intertidal clams living at various tidal heights. Although several models of water circulation exist for Puget Sound, Washington, these models lack the spatial resolution to predict phytoplankton advection into intertidal areas. In order to estimate the concentrations of *Pseudo-nitzschia* diatoms that might become available over a tidal cycle to suspension-feeding benthic macroinvertebrates at different intertidal elevations, I combined several types of water column profile data for a nearshore-intertidal beach region in Hood Canal, a fjord-like extension of Puget Sound, Washington USA. In collaboration with a group of oceanographers and fishery scientists, I collected water column profile data from six stations along a sampling transect oriented from nearshore to intertidal on August 8, 2005 (9hr period, from shortly after lower high water to lower low water to shortly before higher high water) and December 13-14, 2005 (24hr period, from higher low water through a full tidal cycle and back to higher low water). I acquired: vertical profiles of conductivity, temperature, depth and fluorescence; water samples

from the surface and from the depth of chlorophyll maximum, which were analyzed for total suspended solids and chlorophyll pigments; particle velocity throughout the water column from an Acoustic Doppler Current Profiler (ADCP) that was deployed at one of the sampling stations; daily average wind velocities; and Light Detection And Ranging (LiDAR) bathymetry data. Synthesis of these data enabled determination of phytoplankton flux over the intertidal, and the path that a bloom takes over a tidal cycle. The results reveal that tidal current speed in the long-shore direction is approximately ten times as strong as in the cross-shore direction, and that the rate of tidal advection in the cross-shore direction is highly variable, but can be approximated as tidal ebb and flow. I also were able to estimate the percent time that a bloom is available to submerged clams as a function of bloom length in the long-shore direction. This study begins to constrain estimates of phytoplankton availability to intertidal clams as the tide rises and falls at Lofall, in the temperate fjord of Hood Canal.

INTRODUCTION

In recent decades, reports of harmful algal blooms (HABs) have increased in frequency throughout many parts of the world (e.g. Smayda 1990, Hallegraeff 1993, Anderson 1995). HABs occur when certain species of microscopic algae proliferate into dense concentrations (“bloom”) with detrimental effects to plants, animals or the environment. Some HABs produce algal toxins that can cause illness or death in humans. When blooms of these toxin-producing species are advected into the intertidal, they become available to suspension-feeding clams, including mussels and oysters, which feed on the phytoplankton and accumulate toxin in their tissues. Clams frequently serve as the vector for the majority of marine algal toxins (Van Dolah

2000). To mitigate the frequency of toxin transfer to humans, monitoring programs exist in many parts of the world to test harvestable clams and crabs, and close areas to harvest when shellfish become toxic.

In Washington State, the two most common toxin-producing phytoplankton genera are: diatoms in the genus *Pseudo-nitzschia*, which behave as passive particles in the water column; and dinoflagellates in the genus *Alexandrium*, which are motile plankton with vertical swimming behavior. This study focuses on *Pseudo-nitzschia*, and delivery of *Pseudo-nitzschia* to suspension-feeding clams for consumption.

Species of *Pseudo-nitzschia* are found in all waters of the world, including the coastal waters of Washington state and the inland waters of Washington's largest estuary, Puget Sound (Horner & Postel 1993, Hasle 2002, Stehr et al. 2002). Under certain conditions, including nutrient limitation which occurs during algal blooms, some species of *Pseudo-nitzschia* can produce the neurotoxin domoic acid (DA) (Pan et al. 1996a, 1996b). *Pseudo-nitzschia* and DA have caused numerous shellfish harvest closures on the outer Washington coast over the last two decades (Horner et al. 1997, Wekell et al. 2002), but only three harvest closures in Puget Sound, all since 2003 (Bill et al. 2006, Trainer et al. 2007). The infrequency to date of DA toxicity in Puget Sound clams raises the question of whether there are aspects of Puget Sound's hydrology or clam assemblage which reduce the likelihood of clam toxicity. One such aspect is competition amongst intertidal suspension-feeding benthic macroinvertebrates for available phytoplankton such as *Pseudo-nitzschia*. As water travels over the intertidal during flood tide, suspension-feeders have the opportunity to decrease the phytoplankton concentration in the water that passes

over them. Such decreases in phytoplankton concentration during flood tide were documented in Shark Bay, Western Australia, and are hypothesized to cause decreased growth in clams at higher tidal elevations (Peterson & Black 1987, 1991). Knowing how much phytoplankton from neritic mid-channel waters becomes available to suspension-feeders at various tidal heights in the intertidal is essential to determining whether competition for food resources may reduce the likelihood of DA accumulation in clam tissues.

Within large estuaries such as Puget Sound, algal blooms that form in neritic mid-channel waters can be advected over intertidal clam beds by tidal currents, winds, and waves. Although several models exist for predicting water movement within Puget Sound, the grids in these models are relatively coarse, limiting the ability to understand water movement over intertidal areas. Two aspects of water delivery dynamics are particularly relevant to toxin accumulation in intertidal bivalves: 1) the path taken by the bloom across different clam habitats, including the rate of movement and previous consumers encountered; and, 2) the degree of vertical mixing that occurs over the intertidal. The path that the bloom takes over the intertidal and the speed with which it travels are important because the suspension-feeders encountered by the bloom determine the amount of decline in *Pseudo-nitzschia* concentration. Suspension-feeding species assemblages and suspension-feeding capacity vary according to tidal height, resulting in different rates of bloom concentration decline. The degree of vertical mixing of the water column determines how much of the phytoplankton in the water directly above clams is available for consumption. A vertically well-mixed water column allows clams to feed from phytoplankton originating at all depths, whereas a stratified water column only allows clams access to phytoplankton near the seafloor. Understanding these phytoplankton delivery dynamics will provide basic information

for studies to explore whether competition can limit availability of toxin-containing phytoplankton to some intertidal clams.

The nearshore region around Lofall, in northeastern Hood Canal (Fig. 1) was selected for this case study on phytoplankton advection. Lofall is reasonably representative of water flow dynamics in channel-type areas of Puget Sound. It falls in the sill region of Hood Canal (Gregg & Pratt 2010), so water is relatively well-mixed due to turbulence over the sill. Hood Canal's bathymetry has been extensively analyzed, and Lofall was the deployment site for an Acoustic Doppler Current Profiler (ADCP) in 2005 that measured particle direction and velocity. The intertidal clam assemblage at Lofall is also representative of clam assemblages in Puget Sound's quiet bays and salt marshes, where the substratum is cobble or gravel mixed with sand or mud (Kozloff 1983, Dethier 2006). Hood Canal was one of the first sites within Puget Sound to experience a *Pseudo-nitzschia* bloom, although shellfish did not accumulate enough DA to warrant a harvest closure (Horner et al. 1996). Finally, Hood Canal is the site of frequent water-related problems such as hypoxia, making it of interest to oceanographers and the public.

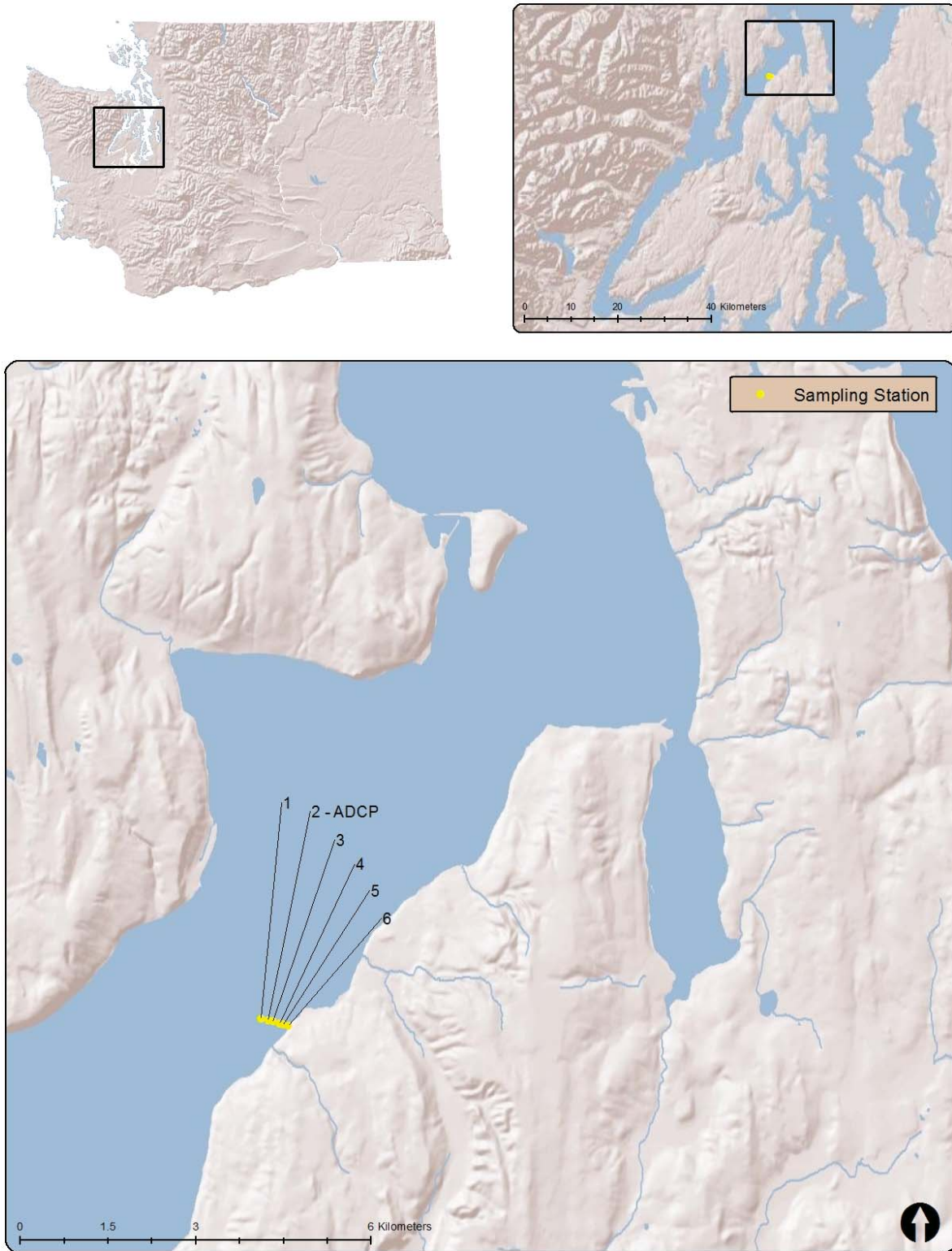


Figure 1. Northern Hood Canal with inset of Lofall region showing sampling locations

Scope of Paper

Between August and December 2005, I worked with a collaborative group of oceanographers and fishery scientists to conduct both continuous data collection and two intensive sampling series to evaluate the advection of phytoplankton from neritic mid-channel waters into the intertidal zone in Lofall, Hood Canal. The aim of this study was to estimate availability of *Pseudo-nitzschia* diatoms to intertidal bivalves during a tidal cycle. The research presented here is part of a broader study to investigate competition between intertidal suspension-feeders for available *Pseudo-nitzschia*, and the effects of competition on bivalve toxicity.

The specific questions that I investigate in this paper are: 1) the extent to which mid-channel neritic phytoplankton are advected into the intertidal zone at Lofall, Hood Canal, to become available for consumption by suspension feeding clams; 2) water column mixing near and within the intertidal; and 3) the source of water parcels intersecting intertidal clam communities. To address these questions, I used several types of oceanographic sensors, water sampling methods, and available data to document: continuous particle velocity data, which were collected over several months; subtidal and intertidal bathymetry data; water column profiles and water samples collected on two discrete sampling dates; and environmental conditions present on the sampling dates. Based on the continuous particle velocity data, I was able to determine the relative strength of tidal currents in the long-shore and cross-shore directions. Through the water column profiling experiments, I identified a large bloom in December 2005, and performed a back-of-the-envelope calculation to explore bloom size and the direction from which it came before intercepting the intertidal zone. I also discuss the environmental conditions that were present, and the depth and concentration profile of the bloom.

MATERIALS & METHODS

Several types of data were collected at Lofall. Additionally, available bathymetry and wind data are included in the analyses.

Continuous Particle Velocity Data

Between September 26th and December 14th 2005, an Acoustic Doppler Current Profiler (ADCP; Workhorse™, Teledyne RD Instruments) was deployed on the seafloor in the shallow subtidal approximately 13.7m deep (MLLW) just offshore of Lofall. The ADCP collected data on particle velocity throughout the water column at 20-min intervals in 0.35-m depth bins (with the exception of a 0.86-m bin at the seafloor, to allow space for correlation of the four ADCP beams).

The purpose of the ADCP was to obtain net directional movement of particles, and the rate of particle movement. Although other data were available from the ADCP, this paper focuses only on the former. ADCP data were parsed and analyzed using Matlab® (The Mathworks) to run programs adapted from code generously provided by Andrew Stevens, PhD, USGS Santa Cruz. Parsed data were rotated west from magnetic north to true north based on a declination at Lofall of +17.51°. Data recorded by the ADCP of reflection above the water's surface were cropped, and any necessary corrections to pressure and time were applied to the data. Axes were then rotated so that particle velocities could be calculated in the principle direction of currents (parallel to shore), and also perpendicular to the principle direction of currents (perpendicular to shore). Compass headings of principle current direction were identified by comparing depth-averaged data at each time point to a range of values ($\pm 10^\circ$) around the mean over all time

points. Root mean squared variance was then minimized to find the best value for the principle current direction.

Water Column Profiles

On December 13-14, 2005, we conducted a 24-hour experiment (0930 – 0930) from 0.5-hr after higher low water (HLW, starting tidal height $\sim 2.3\text{m}$) to 0.5-hr before HLW the next day (tidal height $\sim 2.4\text{m}$) (Fig 2). Six sampling stations (labeled 1 through 6 on Fig 1) were selected along a 490-m transect approximately perpendicular to the shoreline, from subtidal neritic waters (Station 1) to the intertidal zone (Station 6).

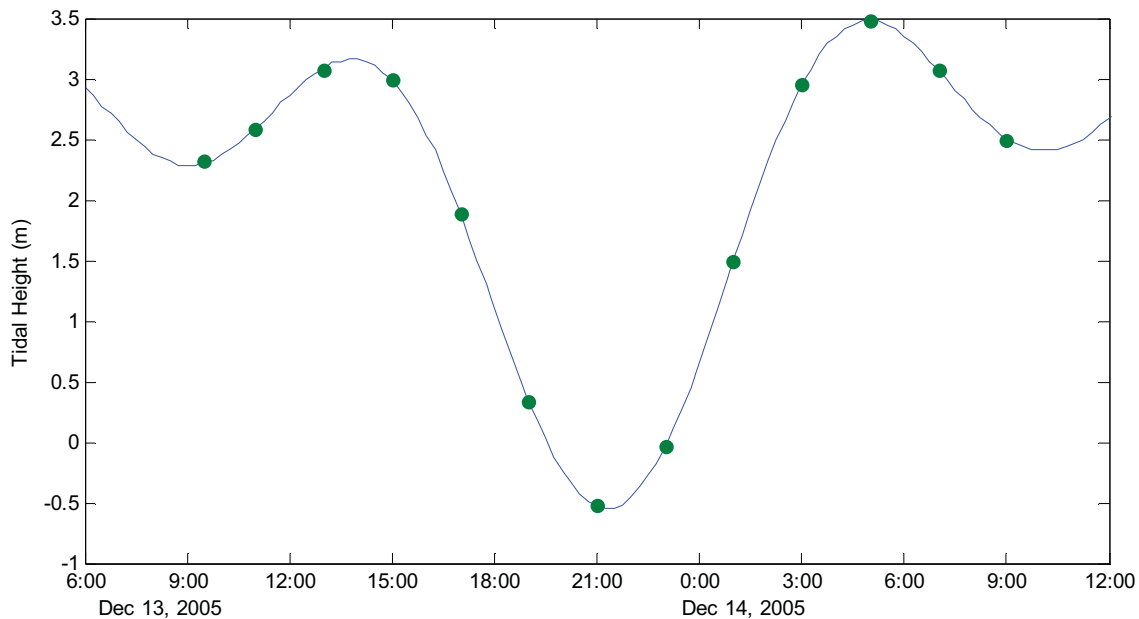


Figure 2. Tidal cycle on Dec 13-14, 2005 at Lofall, Hood Canal. Filled circles indicate time points when water column profiles were completed and water samples were collected.

Every two hours over the course of the experiment, Conductivity-Temperature-Depth (CTD) and fluorescence profiles were obtained using a sensor package (Sea-Bird Electronics with Wet Labs™ Fluorometer) deployed by winch from the boats. We determined the depth of maximum

chlorophyll at each station based on the fluorescence profile, and then collected water samples in Niskin bottles at the surface and at the depth of the chlorophyll maximum. All water samples were analyzed for Total Suspended Solids (TSS) and water samples from Stations 2 and 5 (or Station 4 when Station 5 was not accessible) were also analyzed for chlorophyll *a* (chl *a*).

We sampled from a 65.5 ft research vessel and a small boat. The research vessel was anchored at Station 2 near the ADCP, while the small boat visited each of the other stations (Stations 1 and 3-6). During the daytime (0930 – 1900 on Dec 13, and 0800-0930 on Dec 14), the Station 2 sampling and water column profiling was conducted off the research vessel. At night, all sampling and water column profiling was conducted from the small boat. During the December sampling experiment, the fluorometer on the research vessel was not calibrated properly, so Station 2 fluorescence data are not available between 0930 to 1900 on December 13th, and between 0800 and 0930 on December 14th.

Data from a trial run of the water column profiling, taken on August 8, 2005, are included in the analyses. On this day, we conducted a nine hour experiment (0930 – 1830) from the time period starting three hours after lower high water (LHW, starting tidal height ~1.6m) and proceeding through lower low water (LLW, tidal height ~0.2m) to higher high water (HHW, tidal height ~3.1m) (Fig. 3). Water column sampling was identical to above, except that all water column profiling and sampling was conducted from a small boat, chl *a* analysis was completed on deep water samples from a range of stations rather than from Stations 2 and 5, and no ADCP data are available.

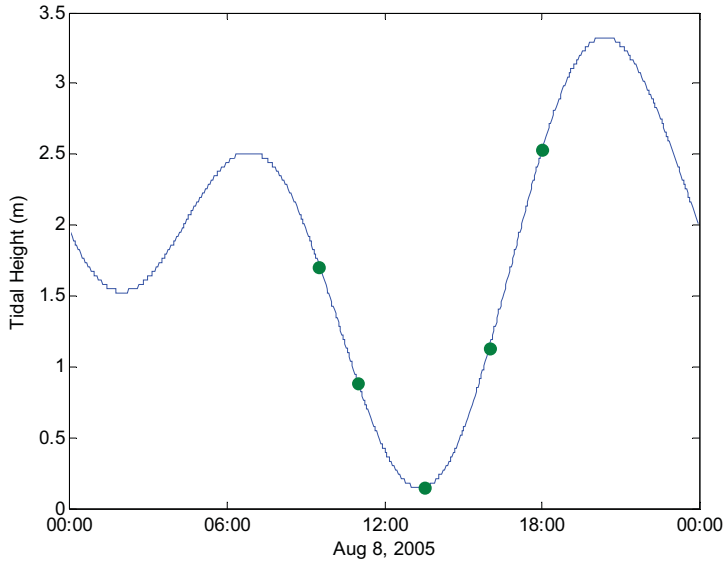


Figure 3. Tidal cycle on Aug, 8 2005 at Lofall, Hood Canal. Filled circles indicate time points when water column profiles were completed and water samples were collected.

Water Sample Analysis

Chlorophyll samples were required to correlate fluorescence measurements from the fluorometer to actual chl *a*. A single sample (125mL) per station was taken during the August experiment; whereas during the December experiment, samples were all taken in triplicate and the volume increased to 250mL. Following collection, water samples were immediately filtered through GF/F filters, wrapped in aluminum foil and frozen for later extraction. Chlorophyll samples were extracted in acetone and measured for chl *a* corrected with acidification using a TD-700 fluorometer from Turner Biosystems (Parsons et al. 1984).

Water samples were processed for total suspended solids (TSS) by filtering seawater (130mL in August, 290mL in December) onto pre-weighed filter papers, drying in an oven at 58°C, and cooling in a desiccator. Dried and desiccated samples were reweighed, and the weight difference was recorded as total suspended solids.

CTD & Fluorescence Analysis

CTD and fluorescence data were processed using the program SBE Data Processing to align data from all sensors (some data were collected automatically, other data required water to be pumped past a sensor, which results in a slight time delay of data recording), derive additional data types from raw data, then bin data into 0.5-m depth bins.

Fluorescence data were ground-truthed with chl *a* data from the same location and depth, to obtain corrected values of fluorescence. Fluorescence data and corresponding chl *a* data were plotted and a linear regression was fitted to the data. Each fluorescence reading was then corrected for actual chl *a* based on the slope of the regression.

Bathymetry

Subtidal and intertidal bathymetry data for this region of Hood Canal were obtained from the Scanning Hydrographic Operational Airborne Lidar Survey (SHOALS) bathymetric light detection and ranging (LiDAR) survey over Hood Canal, completed in September-October 2003 by the Joint Airborne Laser Bathymetry Technical Center of Expertise and distributed through the Puget Sound LiDAR Consortium. These LiDAR data were parsed by David Finlayson, University of Washington (UW) School of Oceanography, and processed in ArcGIS by Mary Ramirez, UW School of Aquatic and Fishery Sciences.

Bathymetric data were used to calculate depth-averaged tidal advection rate in the cross-shore direction, as a function of time over the December 13-14th tidal cycle. The depth-averaged advection rate was calculated above the ADCP, for a cross-section of the intertidal and shallow

subtidal. The trapezoidal area of water that represents gain or loss of tidally advected water over a 15-minute interval was calculated, then divided by the depth of water at the ADCP. The result was the depth-averaged cross-shore tidal advection rate at the ADCP, in $\text{m} \cdot \text{s}^{-1}$.

Wind

I obtained available mean wind velocity, maximum wind velocity, maximum gust velocity, and wind direction data for Bremerton National Airport, approximately 36km S/SW of Lofall. Hourly data were collected by the Bremerton National Airport, and averaged and archived by Weather Underground (www.wunderground.com). Long term wind data were used to determine how representative the August 8th and December 13-14th sampling dates were to summer and fall wind conditions in Lofall.

RESULTS

Wind

Between September 26th and December 14th, mean wind speed at Bremerton National Airport ranged from 0 to $6.3 \text{ m} \cdot \text{s}^{-1}$ (14mph), with maximum speeds up to $10.3 \text{ m} \cdot \text{s}^{-1}$ (23mph) and gusts up to $14.8 \text{ m} \cdot \text{s}^{-1}$ (33mph) (Fig. 4). Approximately one third of the time, winds were out of the N/NE (mean direction = 35 °NE), while the remaining two thirds of the time, winds were out of the S/SW (mean direction = 194 °SW).

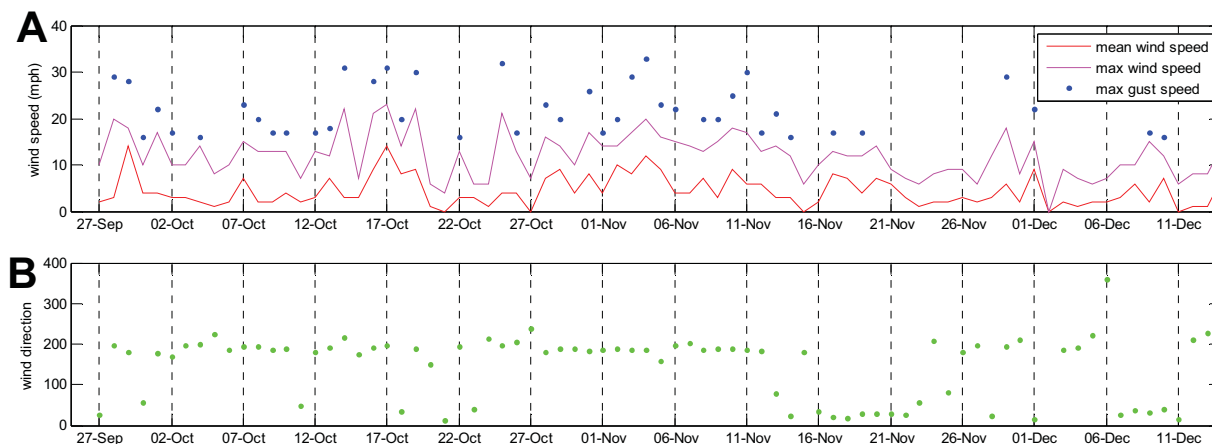


Figure 4. Wind speed (A) and direction (B) at Bremerton National Airport, Washington, between Sep. 27 and Dec. 14, 2005. Wind data courtesy of Weather Underground (www.wunderground.com).

During the sampling experiment on December 13-14th, mean wind speed ranged between 0.4 and 3.1 $\text{m} \cdot \text{s}^{-1}$ (1-7mph), with maximum speeds up to 6.3 $\text{m} \cdot \text{s}^{-1}$ (8-14mph). Wind was out of the SW (228 °SW) on December 13th, shifting to 30 °NE on December 14th. The wind patterns observed during the sampling experiment were within a normal range for fall weather.

During the sampling experiment on August 8th, mean wind speed was 0.9 $\text{m} \cdot \text{s}^{-1}$ (2mph), with maximum speeds up to 4.5 $\text{m} \cdot \text{s}^{-1}$ (10mph), and a wind direction of 213 °SW. This pattern was common for the first half of August (Fig. 5).

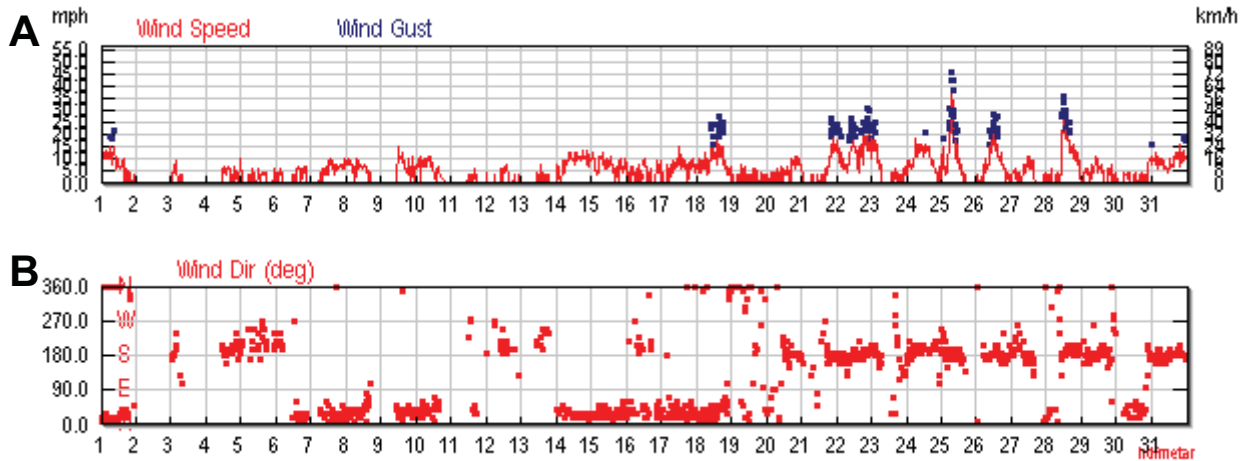


Figure 5. Wind speed (A) and direction (B) in August, 2005 at Bremerton National Airport, Washington. Figure from Weather Underground (www.wunderground.com).

Continuous Particle Velocity Data

The ADCP was deployed between September 26th and December 14th. On December 13th, just prior to the sampling experiment, the ADCP battery pack was replaced and data were downloaded, before returning the ADCP to the seafloor. Thus, data should be viewed as two separate deployments of the ADCP: one for the date range September 26th – December 12th, and another for December 13th – 14th.

The principle direction of currents was parallel to shore in both deployments of the ADCP. The depth-averaged principle direction of currents between September 26th and December 12th was from the NE (bearing 217.7 °SW) or SW (bearing 37.7 °NE), while the depth averaged principle direction of currents for December 13-14th was from the NE (bearing 234.2 °SW) or SW (bearing 54.2 °NE) (Fig. 6). Sub-sampling of the September 26th – December 12th data into 24-hour periods and subsequent root-mean-squared analysis on the 5453 individual segments resulted in a range for principle current direction between 34 and 50 °NE (214 and 230 °SW), and a mode of

38 °NE (217.7 °SW) (Fig. 7). Thus, the principle current direction of the December 13-14th data differs significantly from the principle current direction of the September 26th – December 12th data.

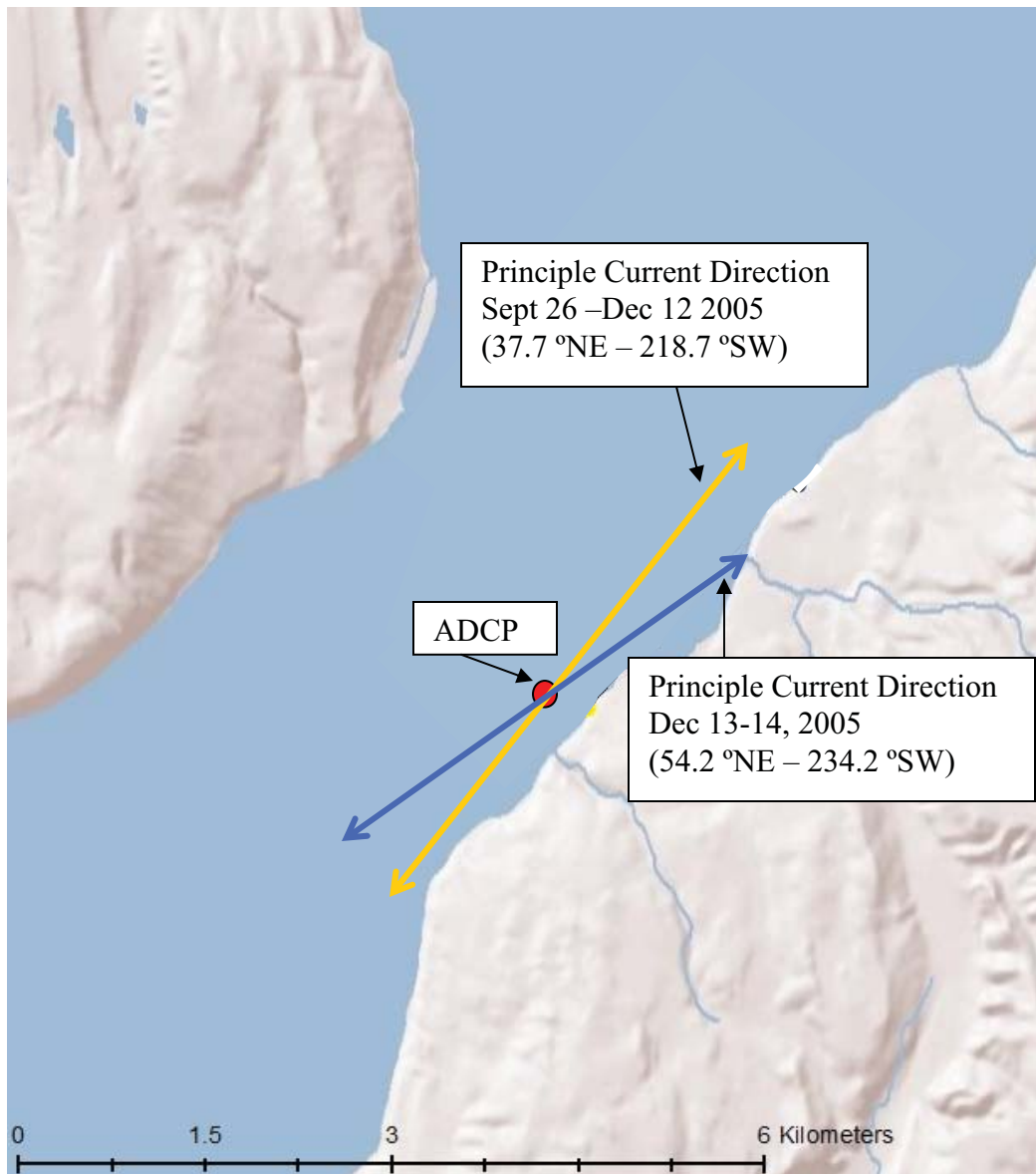


Figure 6. Principle current direction at Lofall for September 26-December 12, 2005 (yellow), and for December 13-14, 2005 (blue), obtained from the ADCP data. Location of ADCP shown with filled red circle.

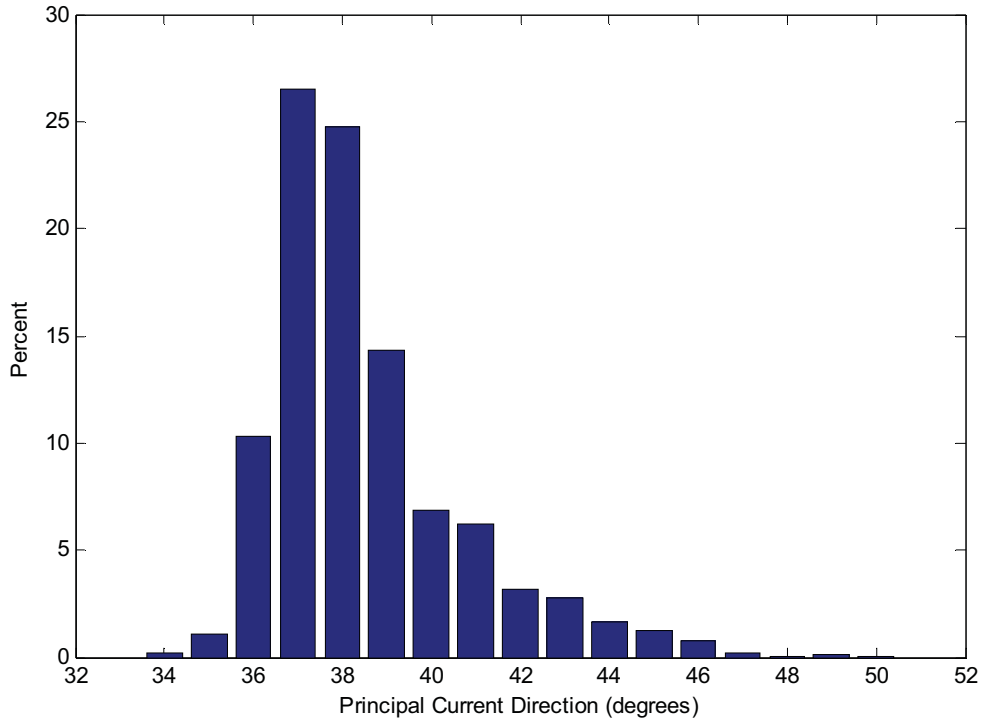


Figure 7. Histogram of principle current direction calculated from 24-hour subsamples of the September 26th – December 12th ADCP data. The x-axis is principle current direction in degrees NE; adding 180 degrees to this value provides the two compass bearings along which currents principally travel.

The rate of water movement perpendicular to the principle direction (perpendicular to shore) measured by the ADCP ranged from $-0.07 \text{ m} \cdot \text{s}^{-1}$ to $0.09 \text{ m} \cdot \text{s}^{-1}$, with substantial variability over time (Fig. 8). In comparison, the calculated rate of water movement into the intertidal in this region was an order of magnitude lower, from -0.006 to 0.006 .

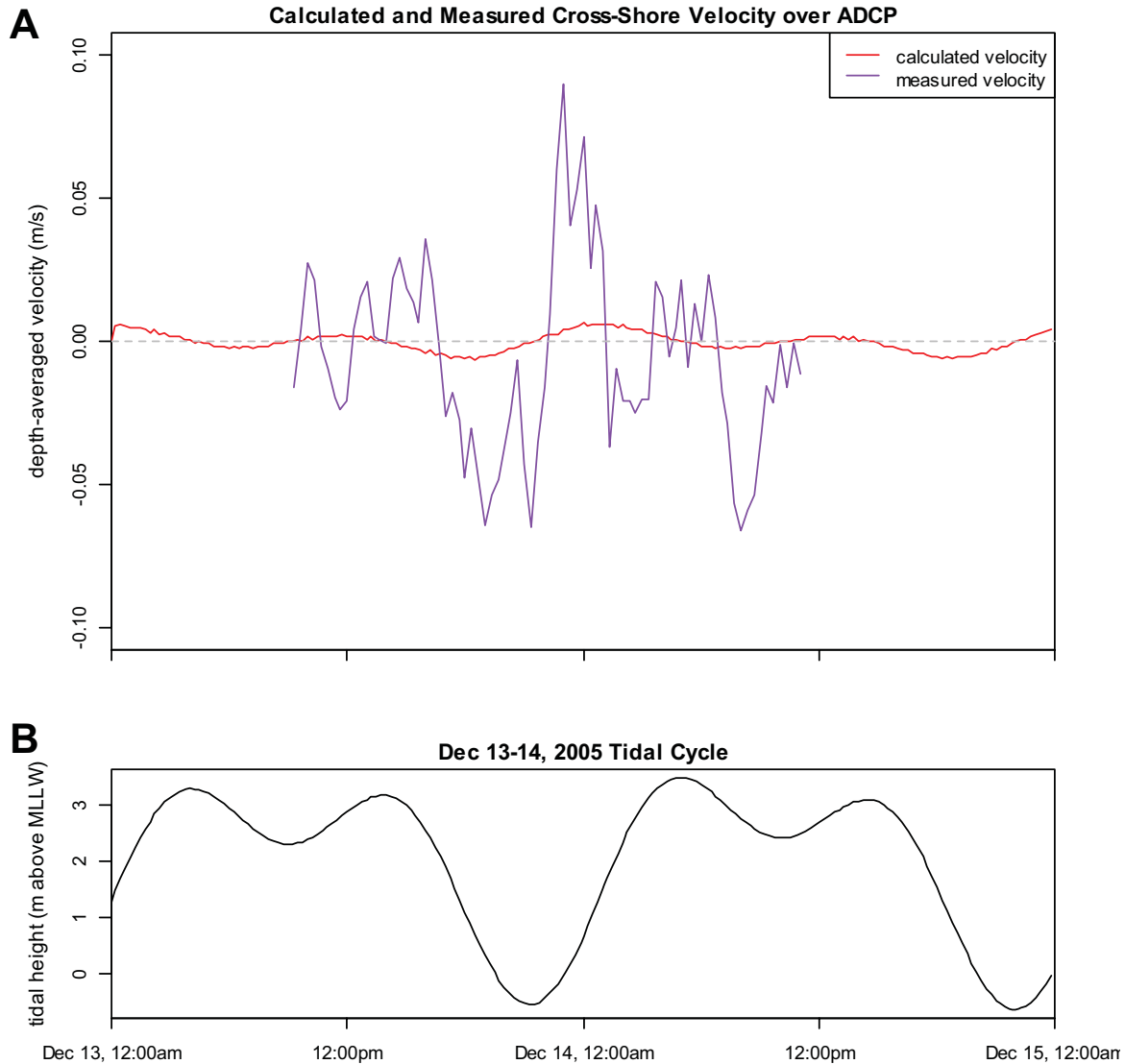


Figure 8. Calculated (red line) and measured (purple line) depth-averaged cross-shore velocity ($\text{m} \cdot \text{s}^{-1}$) over the ADCP, caused by tidal flood and ebb (A) and the December 13-14th tidal cycle (B).

Water Column Profiles

On August 8th, the water column was profiled from +1.6m above MLLW (3hr after LHW) to +0.2m (LLW) to +3.1m (HHW). Salinity and temperature profiles indicate a weakly stratified system (Fig. 9A,B). Warmer, fresher water on the surface generally overlaid cooler denser water in the deeper parts of the sampling transect, with possible seiche (sloshing across the

channel) or mixing. Salinities ranged from 28 to 31 practical salinity units (psu), and temperatures ranged from 12.5 to 18°C. Depth profiles of salinity indicate some vertical mixing of the water column over the course of the nine hour experiment (Fig 9A, 10). Water column profiles for fluorescence show some spots of higher phytoplankton concentration near Station 1 (closest to mid-channel) as the tide receded to LLW (Fig. 9C). As the tide began to flood the intertidal, Station 1 showed an area of high fluorescence ($>6 \text{ ug} \cdot \text{L}^{-1} \text{ chl } a$), which then centered on Station 3 at HHW. This area of high fluorescence may have been a single phytoplankton bloom, or two discrete patches of high phytoplankton concentration.

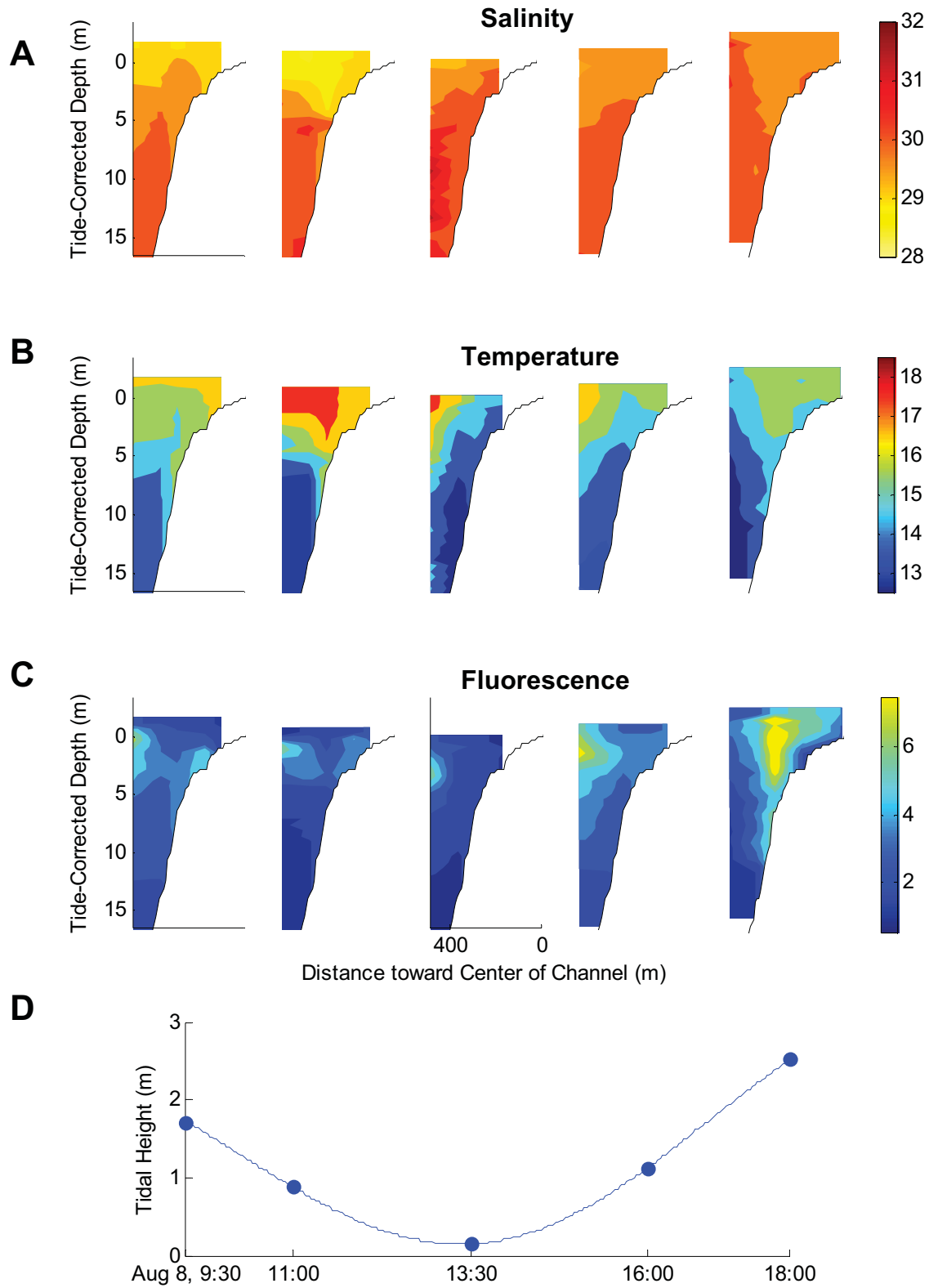


Figure 9. Salinity (A), temperature (B), fluorescence (C), and the tidal cycle (D) on Aug 8, 2005. Points on the tidal cycle plot represent sampling times.

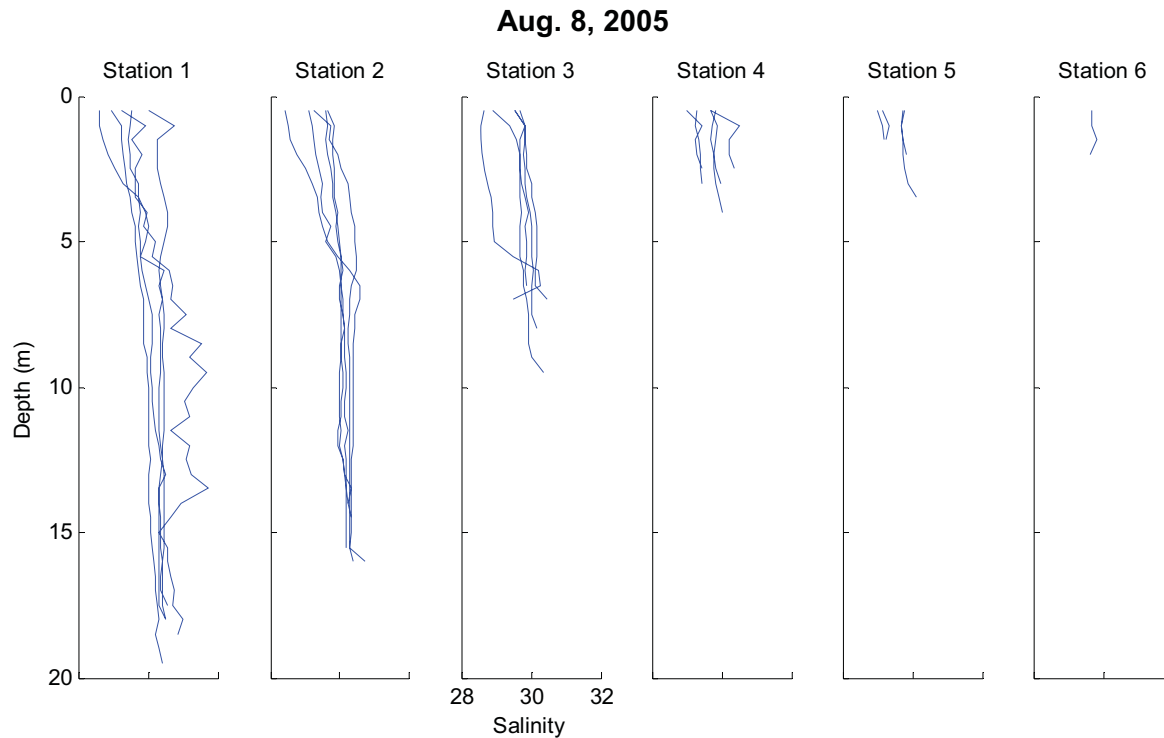


Figure 10. Salinity versus depth profiles on August 8, 2005. Each blue line represents one CTD cast. Salinity profiles show some degree of vertical mixing over time.

On December 13-14th, the water column was profiled from +2.3m above MLLW (0.5hr after HLW) to +3.2m (LHW) to -0.5m (LLW) to +3.5m (HHW) to +2.4m (HLW). Water column profiles show a thin layer of fresher water (21-28psu) on the surface, and more saline water (30psu) in deeper parts of the sampling transect (Figs. 11A, 12), indicating a highly stratified water column. As the tide receded from LHW to LLW and then halfway to HHW, however, the water column became less vertically stratified, indicating possible seiching or mixing (Fig. 11A,B). Water temperature ranged from 7.5 to 9.25°C, but because of the low air temperature in December, the surface water was cooler than the deeper water. During the four hours leading up to LLW and the four hours following LLW, substantial fluorescence $>7 \text{ ug} \cdot \text{L}^{-1}$ chl *a* was detected at a number of stations at a depth of about 2m, indicating the likely presence of a phytoplankton bloom.

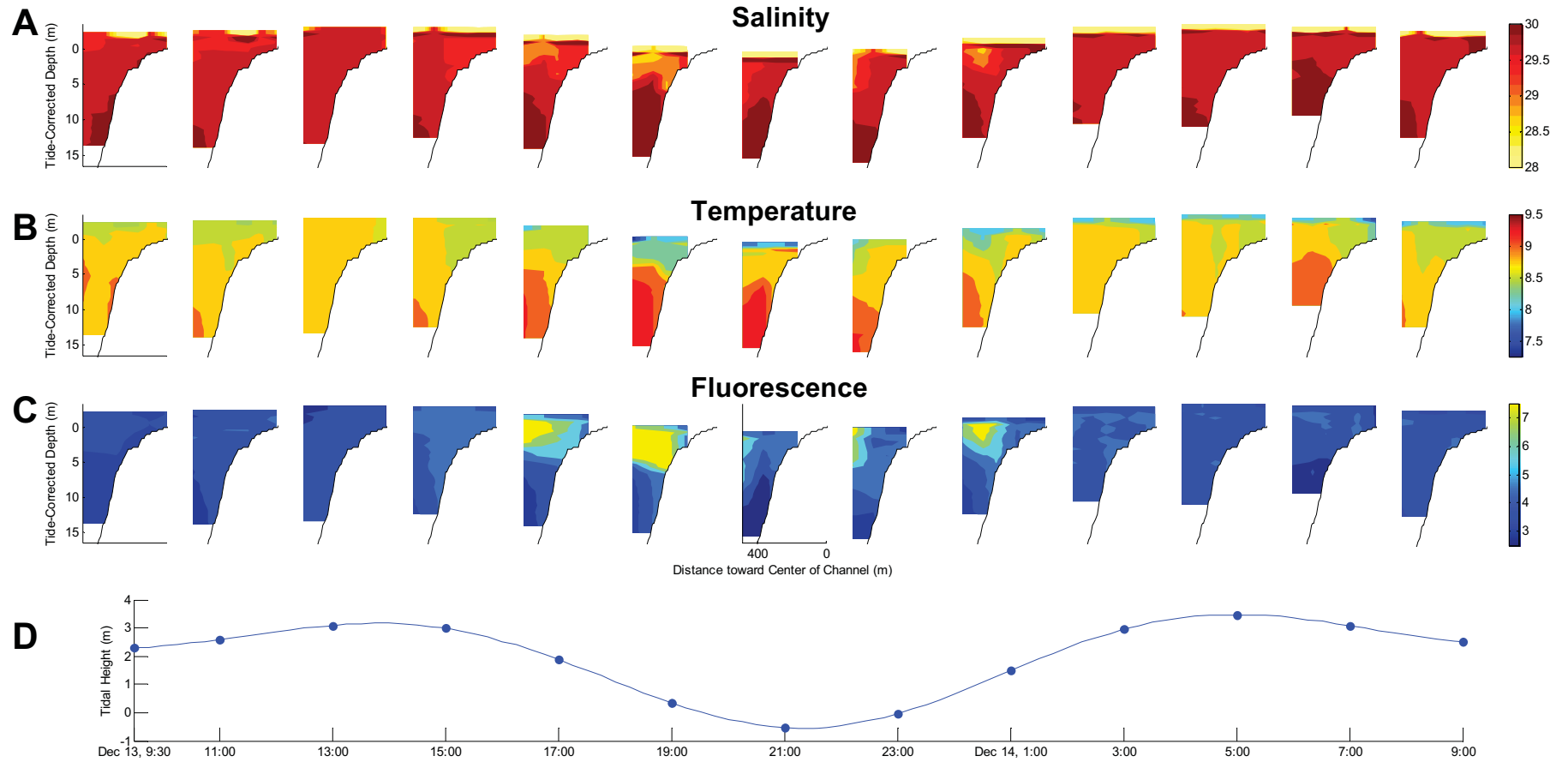


Figure 11. Salinity (A), temperature (B), fluorescence (C), and the tidal cycle (D, points are sampling times) on Dec 13-14, 2005.

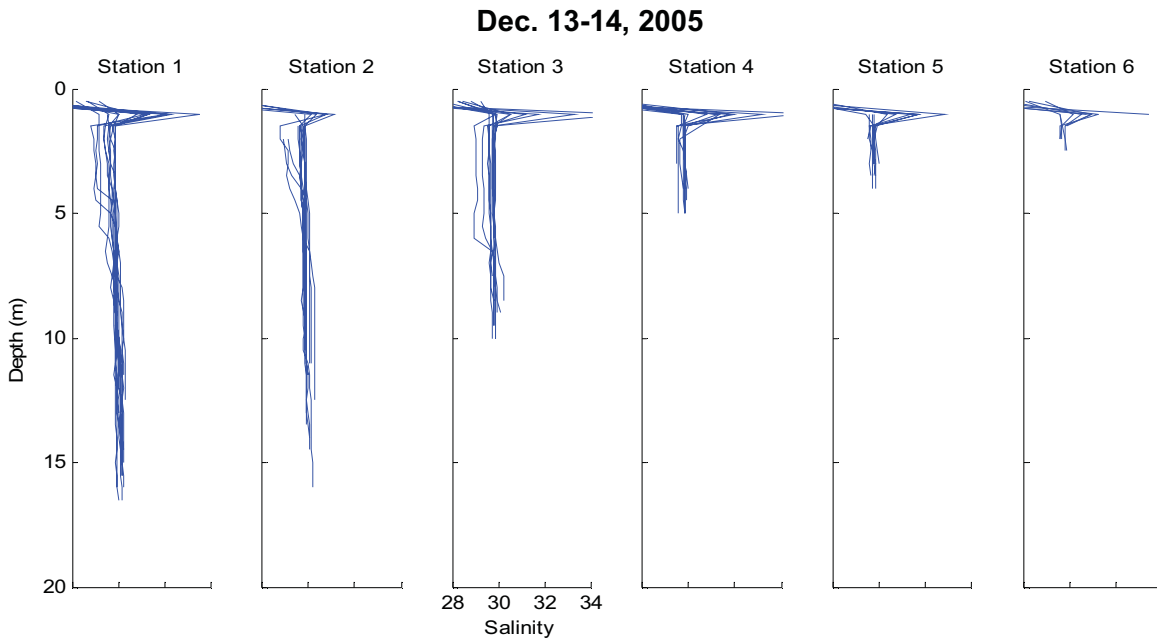


Figure 12. Salinity versus depth profiles on Dec. 13-14, 2005. Each blue line represents one CTD cast. Salinity profiles indicate a highly stratified water column, with small amounts of vertical mixing.

Estimate of Phytoplankton Bloom Dimension and Transport

I estimated the size of the phytoplankton bloom observed in the December 13-14th sampling experiment by integrating the longshore water velocity at a depth of 2m over the time interval of the bloom. Based on this calculation, the phytoplankton bloom must have been at least 6km long and over 250m wide. Despite its large size, this bloom only intersected the sampling transect for eight hours that day: four hours as the tide receded, and four hours as the tide flooded.

TSS and Chlorophyll *a*

Water samples analyzed for TSS showed a mean value of $0.064 \text{ mg} \cdot \text{mL}^{-1}$ in August (standard deviation: $0.004 \text{ mg} \cdot \text{mL}^{-1}$; range between 0.046 and $0.071 \text{ mg} \cdot \text{mL}^{-1}$) (Fig. 13A), and a mean value of $0.027 (\pm 0.003 \text{ s.d.}) \text{ mg} \cdot \text{mL}^{-1}$ in December (range 0.005 - $0.034 \text{ mg} \cdot \text{mL}^{-1}$) (Fig. 14A).

Chlorophyll *a* values in water samples were generally higher in December (mean: $4.45 \pm 1.53 \mu\text{g} \cdot \text{L}^{-1}$) (Fig. 14B) than in August (mean: $3.62 \pm 2.63 \mu\text{g} \cdot \text{L}^{-1}$) (Fig. 13B). Several samples during each sampling experiment showed chl *a* concentrations above $7 \mu\text{g} \cdot \text{L}^{-1}$. These elevated chl *a* concentrations correlated well with high fluorescence values obtained from the fluorometer. No correlation was observed between TSS and chlorophyll *a*.

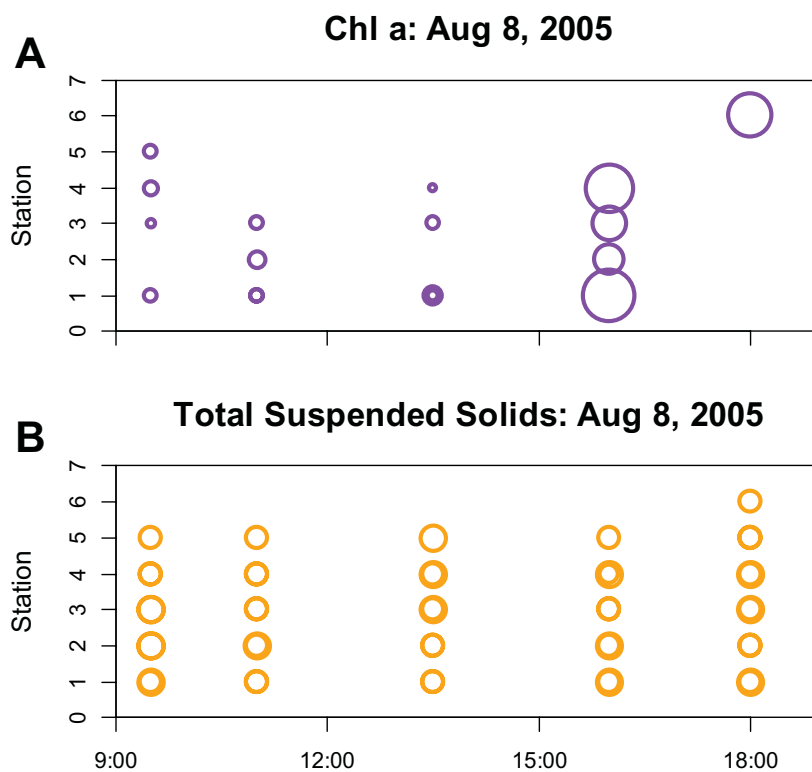


Figure 13. Aug 8, 2005 chlorophyll *a* (A) and total suspended solids (B) shown over time at each station. Circle diameter represents relative magnitude. Data collected at two depths (surface and the depth of maximum chlorophyll) at the same station are shown as concentric circles.

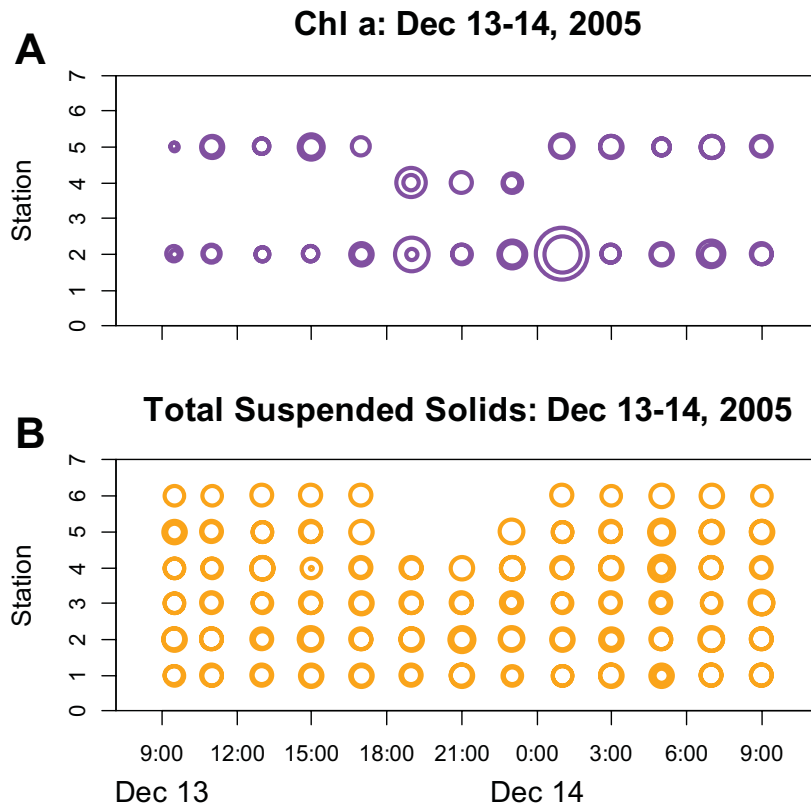


Figure 14. Dec 13-14, 2005 chlorophyll *a* (A) and total suspended solids (B) shown over time at each station. Circle diameter represents relative magnitude. Data collected at two depths (surface and the depth of maximum chlorophyll) at the same station are shown as concentric circles.

Particle Transport over the Intertidal Zone

Progressive vector diagrams over three hour intervals for tracer particles located at a depth of 2m showed fast particle advection toward the NE during ebb tide, and slightly slower advection toward the SW during flood tide (Fig. 15). From the September 26th – December 12th data, it appears that, if a tracer particle were to continually experience similar forces of currents that are found above the ADCP, it would travel in a direction generally parallel to shore for up to 6 km NE in three hours, or 3 km SW, depending on the direction of tidal currents (Fig. 15A). Of the 613 progressive vector lines from the September 26th – December 12th ADCP data, only 59

(9.6%) intersected the intertidal zone, usually to the S or SW of the sampling transect (Fig. 15B). Progressive vector diagrams based on data from December 13-14th also show fast advection of water; however, the direction of movement is oriented slightly more perpendicular to shore than for the September 26th – December 12th diagrams (Fig. 15C).

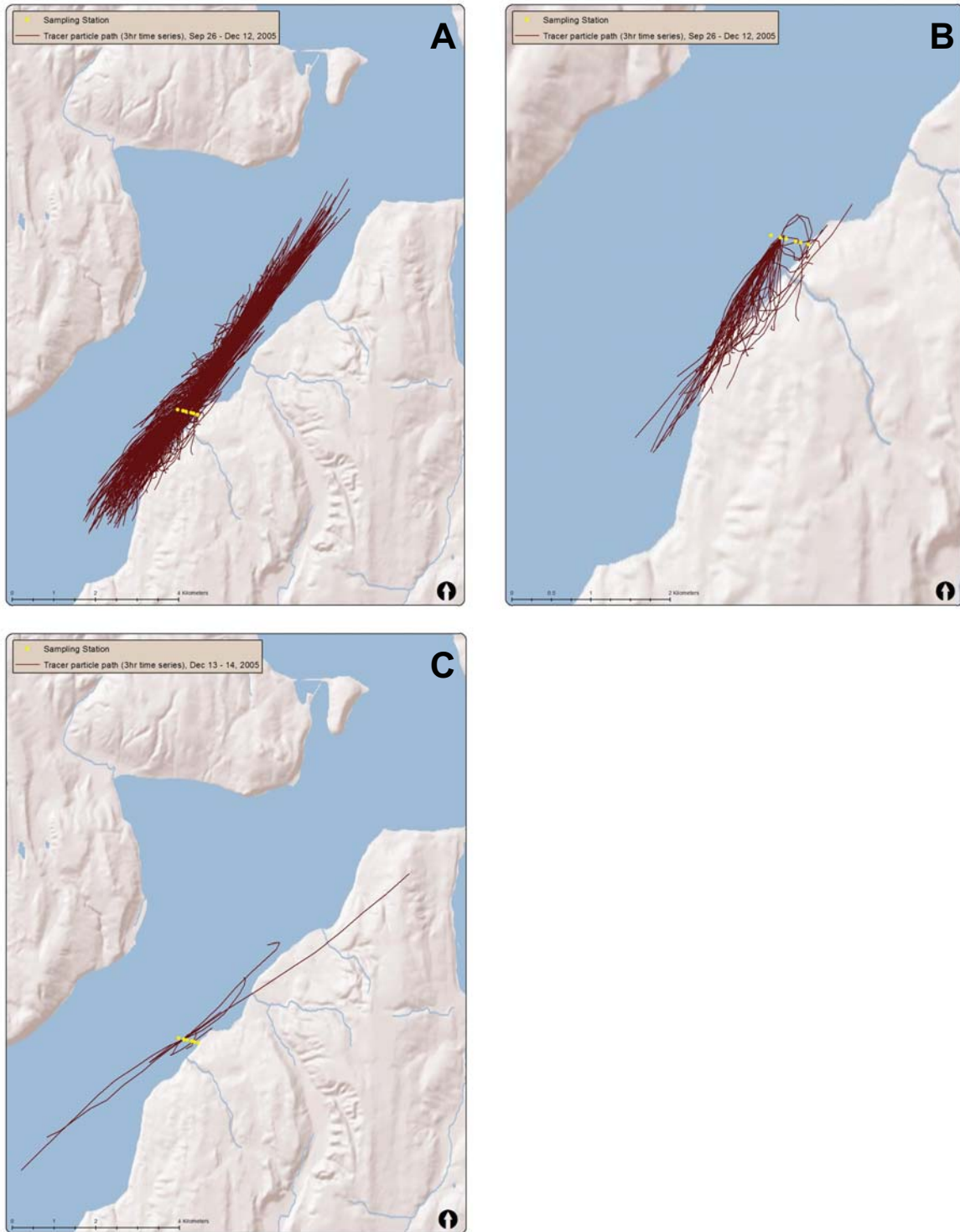


Figure 15. Progressive vector diagrams showing tracer particle paths over 3-hour time intervals based on ADCP data at 2m depth. Figure 15A shows all tracer particle paths for the Sep 26 – Dec 12, 2005 timeframe, while Figure 15B shows only the paths that intercept land. Figure 15C shows tracer particle paths for the Dec 13-14, 2005 timeframe.

DISCUSSION

In this study, I set out to explore three questions: 1.) the extent to which mid-channel neritic phytoplankton are advected into the intertidal zone to become available for consumption by suspension feeding clams; 2) water column mixing near and within the intertidal; and 3) the source of water parcels intersecting intertidal clam beds. Using a three-month time series of ADCP data, and two one-day intensive water column profile surveys, I was able to explore each of these questions at the study site (Lofall, Hood Canal, WA). Specifically, I found that currents generally run parallel to shore at Lofall, at rates between 0.2 and $0.6 \text{ m} \cdot \text{s}^{-1}$ during flood and ebb tides. In contrast, currents ran perpendicular to shore at a mean rate of $0.03 \text{ m} \cdot \text{s}^{-1}$, or one order of magnitude less than parallel to shore. This means that phytoplankton travel a long distance in the longshore direction, passing over many benthic suspension-feeders on their path to the intertidal. This further supports the conclusion that water reaching the intertidal has traveled mostly in a long-shore direction, with only a slight component of its travel path directed cross-shore, as tidal rise and fall. Phytoplankton blooms must therefore be many kilometers long, to be available to intertidal clams at Lofall for an extended period of time.

The water column was weakly stratified over the sampling transect on August 8th, and highly stratified on December 13-14th, although evidence of seiching or mixing was present during both sampling events. Mean wind speed was $<1 \text{ m} \cdot \text{s}^{-1}$ on August 8th and December 13th, although the mean wind speed increased to $3.1 \text{ m} \cdot \text{s}^{-1}$ on December 14th. Low wind speeds do not have a large effect on mixing the water column (Stewart 2008). Although the wind speeds increased on December 14th, no evidence of water column mixing was seen in the latter salinity profiles from the December sampling experiment. The degree of vertical mixing is important to clams, as it

indicates which part of the water column is accessible to clams, and thus how much phytoplankton may be available for consumption. A well-mixed water column will provide better access to phytoplankton for benthic suspension-feeders.

The progressive vector diagrams show that particles starting at Station 2 near the offshore end of the sampling transect will travel up to 3 km to the SW or 6km to the NE in a span of three hours. Approximately 10% of the time, water parcels originating at Station 2 over the ADCP travel S/SW at approximately 185 to 210° before intercepting shore within about one to two hours, at a location approximately 0.5 to 2km SW of the sampling transect. This indicates that a nearshore bloom may return to approximately the same location in the intertidal at high tide over several tidal cycles. This was supported by the December 13-14th fluorescence data, which showed a bloom of phytoplankton moving away from the intertidal at low tide, and then returning to the intertidal during tidal flood.

Using the December 13-14th progressive vector diagrams and tidal cycle, I were able to plot a relationship between the proportion of time that a bloom covers submerged clams in Lofall, and bloom length in the long-shore direction. This exercise indicates that only very large blooms (>10km in long-shore length) will be available for consumption by submerged intertidal clams over a tidal cycle (Fig. 16). These dynamics may be very different for a bay with less dramatic long-shore currents.

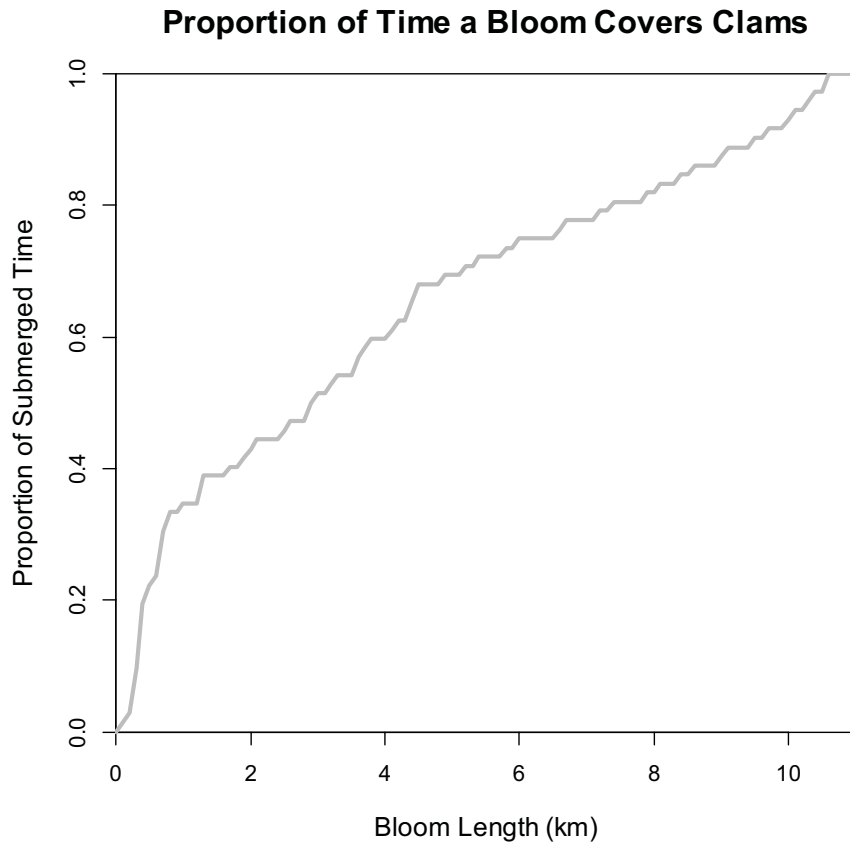


Figure 16. Proportion of time that a bloom covers clams as a function of bloom length. Bloom length is assumed to be in the long-shore direction, and the proportion of time is for time that clams are submerged. Relationship is based on ADCP data from December 13-14, 2005, and progressive vector diagrams that were generated from these data.

Uncertainty

One source of uncertainty that I encountered was in comparing the principle direction of currents for September 26th – December 12th and December 13-14th. It seems unusual that the principle direction from December 13-14th was not replicated in any of the subsampled data packets from September 26th – December 12th, particularly considering the relatively mild wind speeds present during the sampling experiment. While it is possible that some external conditions caused such a difference, the more likely explanation is that the compass on the ADCP was not properly calibrated, or was affected by nearby metal. The ADCP was mounted within a metal cage,

requiring that the compass be oriented in a particular position to avoid interference from the metal cage. During servicing of the ADCP unit on December 13th, the compass position may have shifted, affecting subsequent compass readings. Of the two ADCP data sets, the compass readings from the September 26th – December 12th dataset seem more likely, as they show currents running parallel to shore, while the December 13-14th data show currents oriented in a slightly more cross-shore direction (Fig. 6).

While the progressive vector diagrams are informative, it is also important not to overemphasize their results. The fundamental assumption of progressive vector diagrams is that the conditions and forces present at the starting point (in this case, the ADCP) continue to be present in the same magnitudes and directions, even as tracer particles move far away from the starting point. With the high current velocity ($0.2 - 0.6 \text{ m} \cdot \text{s}^{-1}$) and a relatively long time intervals between consecutive particle velocity measurements (20 min), tracer particles in the progressive vector diagrams quickly move far from the ADCP. Thus, the assumption that the particles continue to experience similar forces as they did above the ADCP becomes increasingly less probable.

Results from water column profiling with CTD casts indicated the presence of possible phytoplankton blooms during both sampling experiments. During the December 13-14th experiment, this phytoplankton bloom was present around the time of low tide. It would have been very interesting to see if that bloom returned to the same location around low tide on the following tidal cycle. Similarly, following the mass of high fluorescence (presumed phytoplankton bloom) on August 8th for a longer sampling time period would have been

provided interesting information about the return of water parcels to the same points in the intertidal, and mixing of the water column.

Future Directions

This study confirmed that currents in Lofall, Hood Canal run predominantly parallel to shore, with a small component of the currents moving in a cross-shore direction. In this study, I observed large variability in cross-shore currents measured by the ADCP (Fig. 8). This variability was so great that cross-shore currents were up to an order of magnitude greater than the cross-shore advection that we calculated. Using numerical methods, it would be interesting to take the integrals of the measured cross-shore currents during each flood and ebb, and compare them to the integrals of the calculated cross-shore advection. The pairs of integrals may compare much more closely than the time-series data.

Using the ADCP data, I was able to approximate the path that water travels into the intertidal. These predictions of phytoplankton bloom advection into the intertidal are quite general, and would be improved with multiple ADCPs at various locations in the intertidal, in conjunction with a well-resolved model for the study area. Another way to explore the path that surface water travels into the intertidal would be to release tracer particles with nearly neutral buoyancy (such as floating oranges) into the intertidal, and note their path at various time points after release. This method has the drawback of only capturing patterns of water movement at the surface, but not at the depth of maximum phytoplankton concentration (2m in these experiments). Conducting these types of studies in several parts of Puget Sound with different

bathymetry and tidal and wind forcing would also allow regional comparisons in terms of variation in the mechanisms and scales of phytoplankton bloom availability to intertidal clams.

Chapter III:

Clearance Rate Measurements on Thirteen Intertidal Clams and Acorn Barnacles Fed on the Toxin-Producing Diatom *Pseudo-nitzschia*

ABSTRACT

Along the Washington outer coast, domoic acid (DA), a neurotoxin produced by the diatom *Pseudo-nitzschia*, has caused frequent harvest closures for razor clam *Siliqua patula*, the dominant suspension-feeder on Washington's sandy, open coastal beaches. In comparison, the inland estuary of Puget Sound has only had three shellfish harvest closures for DA. I measured clearance rates of outer coast razor clam, twelve intertidal Puget Sound clam species, including mussel and oyster, and acorn barnacle *Balanus glandula*, feeding on naturally occurring phytoplankton augmented with a toxin-producing strain of *Pseudo-nitzschia multiseries*. Clearance rates were measured as a first step in trying to model and understand consumption and retention of DA by clams in both regions. I analyzed clearance rate data using non-linear mixed effects models to obtain allometric relationships between clearance rate and shell length, as well as between clearance rate and tissue dry weight. For their respective sizes, bay mussel *Mytilus trossulus* and Pacific oyster *Crassostrea gigas* had the highest clearance rates, followed by Nuttall's cockle *Clinocardium nuttallii*, Manila clam *Ruditapes philippinarum* and butter clam *Saxidomus giganteus*. Nearly all Puget Sound clams consumed *P. multiseries* at higher rates than outer coast razor clams. Acorn barnacles also consumed *P. multiseries*: a fully covered

patch of barnacles that is 7.5cm x 7.5cm in size will feed at approximately the same rate as an 11-cm long razor clam. These clearance rate estimates provide more definitive information regarding how much toxin-producing *Pseudo-nitzschia* might be consumed by different clam species and by acorn barnacles over a tidal cycle.

INTRODUCTION

Washington State is the top producer of commercially harvested bivalves in the United States of America (Puget Sound Action Team, 2003). Razor clams *Siliqua patula* dominate the suspension-feeding benthic macroinvertebrate (SFBM) assemblage along sandy beaches on the outer Washington coast, (Kozloff 1983). In Washington, these clams are found only on the outer coast, where they attract large numbers of recreational and tribal harvesters. In contrast to the single-clam dominance on the outer coast, Puget Sound, Washington's largest inland estuarine complex, has a broad assemblage of harvested and unharvested clams, including mussels and oysters, that co-occur on intertidal mixed-sediment (sand and cobble) beaches. Popularly harvested species from Puget Sound include: Pacific oyster *Crassostrea gigas*, bay mussel *Mytilus trossulus*, Manila clam *Ruditapes philippinarum*, native littleneck *Leukoma staminea*, and geoduck *Panopea generosa*. Harvest of these and other species occurs commercially on shellfish farms, as well as recreationally and tribally on public and private beaches.

Contamination of shellfish with domoic acid (DA), a neurotoxin produced by certain species and strains of the cosmopolitan diatom *Pseudo-nitzschia* (e.g. Hasle et al. 1996, Hasle 2002), threatens the local shellfish harvest on Washington State beaches nearly every year. When blooms of toxin-producing *Pseudo-nitzschia* are advected into the nearshore and consumed by

filter-feeding clams, DA can bioaccumulate to high levels in clam tissue. Consuming clams contaminated with DA can cause amnesic shellfish poisoning, including memory loss, and death in humans (reviewed in Todd 1993), and illness and mortality in marine mammals and other vertebrates such as sea birds (Sierra Beltran et al. 1997, Scholin et al. 2000). The Washington State Department of Health has set a regulatory limit of $20\text{mg}\cdot\text{kg}^{-1}$ in shellfish tissue to protect people from exposure to DA. If this limit is exceeded in clams at a beach, the beach is closed for shellfish collection.

Several toxin-producing *Pseudo-nitzschia* species have caused frequent disruptions to razor clam harvests along Washington's outer coast (Horner et al. 1996, Stehr et al. 2002). High concentrations of DA were first detected in Washington and Oregon razor clams in late October and November 1991 (Wekell et al. 1994, Horner et al. 1997). Researchers have since discovered that depuration (purging) of the toxin takes up to a year to complete once DA accumulates in razor clam tissues (Drum et al. 1993, Horner et al. 1993, Wekell et al. 1994, Adams et al. 2000). The long depuration time combined with coastal *Pseudo-nitzschia* bloom dynamics has impacted many fall and spring recreational razor clam harvests since the initial closure in 1991 (Horner et al. 1997, Wekell et al. 2002).

Pseudo-nitzschia is also common in the inland waters of Puget Sound (Horner & Postel 1993, Horner et al. 1996, Horner et al. 1997, Trainer et al. 1998, Stehr et al. 2002), but in contrast to the outer Washington coast, it has only caused three shellfish harvest closures, all since 2003 (Bill et al. 2006, Trainer et al. 2007). Concerns that Puget Sound may be on a trajectory to more frequent *Pseudo-nitzschia* blooms and shellfish harvest closures have led to comparative studies

of oceanographic and ecological dynamics between Puget Sound and the Washington coast. Of particular interest is the question: Why has *Pseudo-nitzschia* only caused three harvest closures in Puget Sound, while the coastal razor clam fishery has been plagued by frequent closures since 1991? One hypothesis that can be tested through experimental data collection and modeling is that competition for phytoplankton such as *Pseudo-nitzschia* among Puget Sound's variety of intertidal SFBMs might limit accumulation of DA in harvestable clams. Supporting this hypothesis is the observation that low levels of DA contamination have been observed in Puget Sound clams for more than a decade (reviewed in Trainer et al. 2007). On the outer coast, where razor clams dominate the suspension-feeding community, toxin accumulation can only occur in these harvested organisms. Understanding the dynamics of toxin uptake by Washington's intertidal clams can lead to better predictions of toxicity in different clam species, as well as strategies to mitigate the negative impacts to shellfisheries.

To date, published studies of *Pseudo-nitzschia* consumption and DA uptake in SFBMs have focused on highly harvested clam species (e.g. Douglas et al. 1997, Liu et al. 2008, Mafra et al. 2010b, Thessen et al. 2010). Data on *Pseudo-nitzschia* consumption by less commonly harvested clams or other SFBMs is lacking; this prevents the possibility of modeling consumption dynamics by whole communities of intertidal SFBMs, where co-occurring species can be important competitors for available food resources (Peterson & Andre 1980).

Thus, the purpose of this research was to obtain data on consumption of toxin-producing *Pseudo-nitzschia* by thirteen species of intertidal clams and acorn barnacle *Balanus glandula* from Washington State, under conditions that are representative of feeding during a rising tide. For

this reason, emphasis was placed on collecting data for as many species and size classes as possible. I also tested animals in groups, and did not eliminate experiments where some individuals occasionally closed valves and/ or withdrew siphons. These data do not necessarily represent the maximal clearance rates attained by suspension-feeders, but rather reflect clearance rates that might occur as the tide rises and communities of SFBMs are inundated by a source of toxin-producing *Pseudo-nitzschia*.

MATERIALS & METHODS

Clearance rates on a toxin-producing strain of *Pseudo-nitzschia multiseriis* were measured for 12 species of clams commonly found in Puget Sound, acorn barnacle, and for razor clams from the Washington outer coast (Table 1). Clearance rate experiments were first performed in 2006, with several follow-up experiments conducted in 2008 and 2009 under different food regimes and slightly different temperature regimes (Table 2). I divided clams into size classes by species, and performed one to three clearance rate experiments for each size class over a course of two to four hours. Six experiments for acorn barnacles were also performed, where the density of barnacles—determined by square centimeter coverage on cobble-sized rocks—varied in each experiment. I estimated clearance rates in each experiment by measuring the decline in phytoplankton concentration over four or five time points throughout the experiment. For clam species, I used non-linear mixed effects models to obtain allometric relationships between clearance rate and shell length, and between clearance rate and tissue dry weight. In barnacles, I used a linear mixed effects model to obtain clearance rate per cm^2 area of barnacle coverage.

Table 1: Taxa, size and location of collection for clams and barnacles in clearance rate studies on *Pseudo-nitzschia multiseriis*. Size range indicates shell lengths of clams used in the experiments, or cm² of barnacles on cobble-sized rocks. Only one size is listed for *Protothaca tenerrima*, as only one individual of this species could be obtained.

Latin Name	Common Name	Size Range Used in Experiments	Collection Location
<i>Balanus glandula</i>	acorn barnacle	13.2cm ² – 20cm ²	San Juan Island, Puget Sound
<i>Clinocardium nuttallii</i>	Nuttall's cockle	37mm - 90mm	San Juan Island, Puget Sound
<i>Crassostrea gigas</i>	Pacific oyster	49mm - 131mm	San Juan Island, Puget Sound †
<i>Macoma inquinata</i>	pointed Macoma	21mm - 47mm	San Juan Island, Puget Sound
<i>Macoma nasuta</i>	bent-nose Macoma	20mm - 51mm	San Juan Island, Puget Sound
<i>Mya arenaria</i>	soft-shell clam	28mm - 94mm	Cultus Bay, Puget Sound
<i>Mytilus trossulus</i>	bay mussel	19mm - 82mm	San Juan Island, Puget Sound
<i>Nuttallia obscurata</i>	purple varnish clam	31mm - 63mm	Cultus Bay, Puget Sound †
<i>Protothaca staminea</i>	littleneck clam	34mm - 71mm	San Juan Island, Puget Sound
<i>Protothaca tenerrima</i>	thin-shell littleneck	65mm	Lofall, Hood Canal, Puget Sound
<i>Saxidomus giganteus</i>	butter clam	47mm - 106mm	Cultus Bay, Puget Sound
<i>Siliqua patula</i>	razor clam	49mm - 132mm	Grays Harbor, Washington coast
<i>Tresus capax</i>	horse clam	102mm - 146mm	Cultus Bay, Puget Sound
<i>Ruditapes philippinarum</i>	Manila clam	32mm - 62mm	Cultus Bay, Puget Sound †

† Species not indigenous to Puget Sound

Animal Collection and Maintenance.

Clams representing a range of sizes were collected from sand and cobble beaches in Northern Puget Sound (primarily from Cultus Bay, Whidbey Island, and from San Juan Island) and in Grays Harbor on the oceanic Washington coast (Fig. 1). Medium-sized pieces of cobble (~ 4cm diameter) with attached barnacles at medium density were also collected from San Juan Island. Collected organisms were transported in water-filled coolers to the University of Washington - Friday Harbor Laboratories on San Juan Island, Washington. This study included coastal razor clams and most of the commonly harvested clam species from Puget Sound (Table 1), with the exception of geoduck, which could not be obtained in harvestable size classes for this study. Individual clams were measured and marked with a unique identification code consisting of three stripes of colored fingernail polish. Each piece of cobble was also marked, and the square centimeter coverage by barnacles was measured. Clams and barnacles were maintained in flow-through tanks of natural (unfiltered) seawater until the experiment date (between two weeks and three months after collection). Water temperature in the flow-through tanks varied from ~11°C at night to ~16°C during the day in the 2006 experiments (which were carried out during the summer), and between ~8°C and ~11°C in the 2008 and 2009 experiments (which were carried out during the spring). In the flow-through tanks, infaunal clams were allowed to burrow in sand; mussels, oysters and barnacles were positioned so that their openings faced the flow. Burrowing clams were transferred to glass bowls filled with sand several days prior to the clearance rate experiments; these bowls could then be moved into the experimental tanks without disturbing their occupants. Mussels and oysters were scrubbed clean of epibionts such as sponges, anemones and bryozoans. A small number of barnacles that were epibionts on mussels

and oysters were difficult to remove; to prevent these barnacles from feeding during the experiments, their thecal plates were sealed with wax.

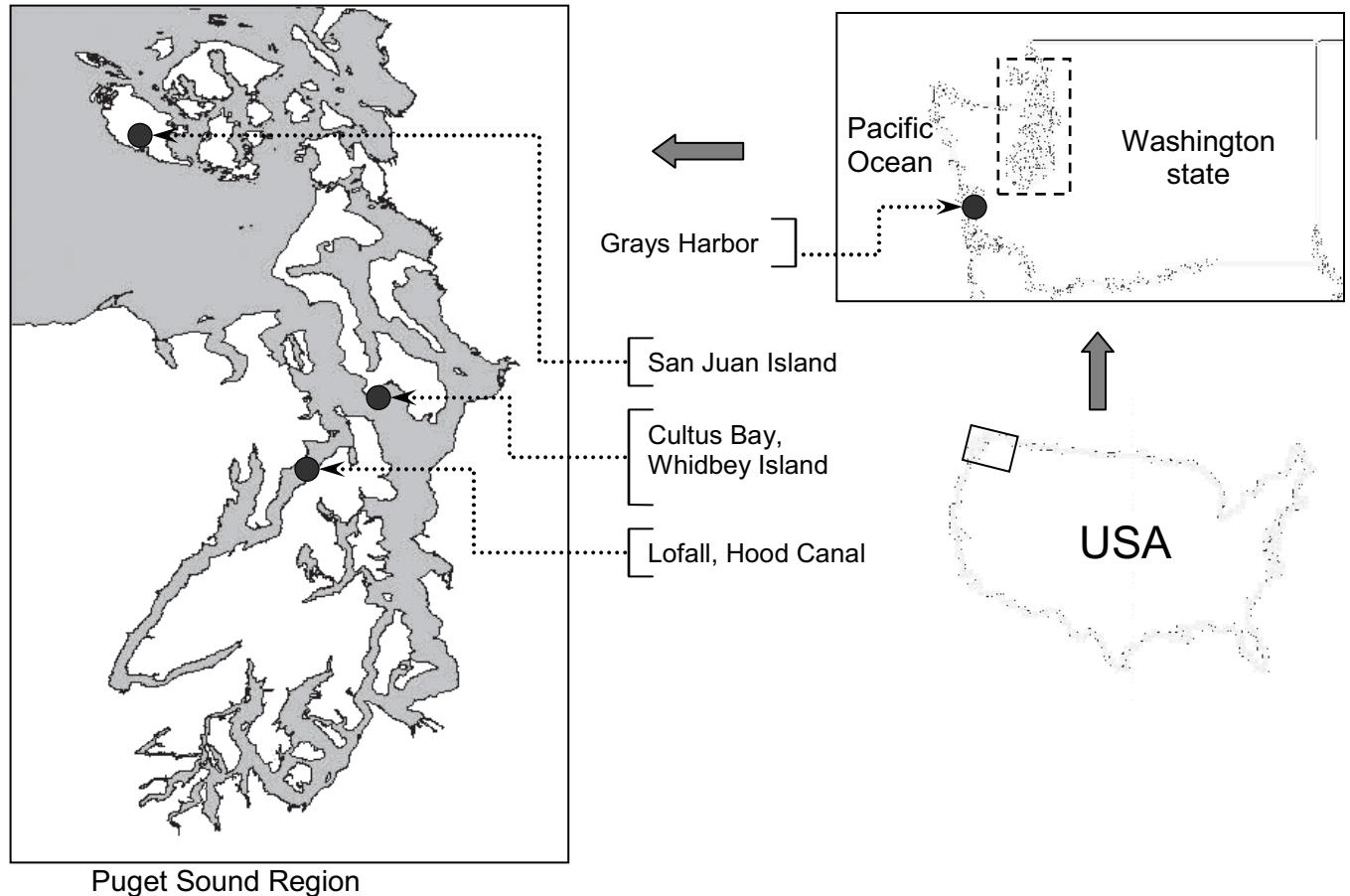


Figure 1: Washington state and the greater Puget Sound area. Locations of clam collection shown with filled black circles.

Algal Culture.

I obtained pure cultures of *Pseudo-nitzschia multiseriis* strains known to produce DA (CLN-47 in 2006 and CLN-17 in 2008 and 2009, from Stephen Bates and Claude Leger). To grow *P. multiseriis* in large volumes, natural seawater was filtered through a 10- μ m cloth filter to remove zooplankton and larger particles, then combined with f/2 solution (manufactured by Fritz Industries, Inc) (Guillard 1975). Silica (in the form of sodium metasilicate: Na_2SiO_3) was added

to the flasks, to create f/2+Si growth medium. The growth medium was then autoclaved in 3- to 10-liter glass flasks. Cooled medium was decanted into sterilized 18-liter plastic carboys. Carboys were then inoculated with *P. multiseriis* culture and maintained at 13°C under 24-hr fluorescent light with moderate aeration and daily agitation, which served to balance pH (Lund et al. 1958). Cultures were harvested in the late-log or early stationary phase when cell density had reached approximately 1.3×10^5 cells·ml⁻¹. Cell densities were determined using a Palmer Maloney slide to count at least 400 algal cells in two or three subsamples (Lund et al. 1958).

Pseudo-nitzschia multiseriis is a chain-forming diatom. Under the culturing conditions, *P. multiseriis* formed short chains or remained as single cells. I measured chain lengths in eight samples (1,197 cells total) from 2006 and four samples (680 cells total) from 2008 using a Sedgewick-Rafter counting slide under an inverted microscope to count all *P. multiseriis* cells or chains in a sample. In the 2006 experiments, average chain length was 1.89 cells, median chain length was two cells, and the mode chain length was one cell. In the 2008 experiments, average chain length was 1.02 cells, and median and mode chain lengths were one cell.

Experimental Procedure.

Clams of the same species and similar size were placed together into glass or plastic aquaria ranging in volume from 2.5 to 85 L (Table 2). One or two pieces of barnacle-covered cobble were placed into 1L glass aquaria. Air-stone bubblers (1-4) and submersible water pumps (for large tanks) provided oxygenation and water circulation. The majority of experiments (those completed in 2006) were conducted at water temperatures between 12.0 °C and 13.2 °C.

Experiments conducted in 2008 ranged in temperature between 9.5 °C and 11 °C, and those completed in 2009 ranged between 8.2 °C and 10.7 °C.

Prior to each experiment, infaunal clams in their sand-filled bowls were transferred into aquaria filled with natural seawater. Cockles – which consistently migrated out of their sand-filled bowls – and epifaunal species (mussels, oysters and barnacles) were placed directly onto the bottom of aquaria. Following a 1-hr acclimation period, an algal suspension of *Pseudo-nitzschia multiseries* was added to each aquarium. Enough algal suspension was added to raise the concentration of *P. multiseries* cells in the well-mixed aquarium to 1.0×10^6 cells·l⁻¹ in 2006, 1.5 to 3.5×10^6 cells·l⁻¹ in 2008, and 2.0 to 3.0×10^6 cells·l⁻¹ in 2009, concentrations that are within the range observed in field samples during Puget Sound *Pseudo-nitzschia* blooms (Bill et al. 2006, Trainer et al. 2007). Algal concentrations varied between the years, because 2008 and 2009 data were collected as part of a DA uptake and depuration experiment, and used opportunistically in this study. Data collection for clearance rate estimates began after a 30 min (2006) or 5 min (2008 & 2009) period of exposure to *P. multiseries*, so that any non-feeding organisms would be stimulated to open their valves or extend cirri and begin feeding before clearance rate measurements were made.

In the 2006 experiments, multiple aquaria were tested simultaneously with a *Pseudo-nitzschia multiseries* control (same concentration of *P. multiseries* but no added suspension feeders) to ensure that significant loss of algal cells from settling was not occurring over the course of the experiments.

During each clearance rate experiment, suspension-feeders were monitored at approximately hourly intervals to ensure that siphons were extended, valves were gaping, and/or cirri were extended. However, valve gape was not measured, so it is not possible to ascertain that clams were filtering at their maximal rate (Riisgard et al. 2003). In clam species that primarily suspension-feed (all clam species except the two *Macomas*, which are primarily detritivores that suspension-feed opportunistically), I observed that valves were gaping and/or siphons were extended at least 80% of the time during the experiments.

Sample Collection and Analysis.

Two types of water samples were collected during the experiments: 20-ml samples for chlorophyll analysis, and 5-ml preserved samples for phytoplankton counts. Chlorophyll samples (collected in triplicate) were used to measure decline of all phytoplankton in the aquaria. Following collection, these samples were immediately filtered through GF/C filters, which were then wrapped in aluminum foil and frozen for later extraction. Chlorophyll samples were extracted in acetone and measured for chl *a* corrected with acidification using a TD-700 fluorometer from Turner Biosystems (Parsons et al. 1984).

I used preserved water samples (one per time point) to measure decline of only *Pseudo-nitzschia multiseries* cells over time. These samples were collected into 7-ml glass scintillation vials, preserved with 100 μ l of Lugol's solution, and kept in cool and dark conditions until analysis. Preserved water samples were gently agitated, and a 1-ml subsample was placed onto a Sedgwick-Rafter counting slide and counted under an inverted microscope to determine *P.*

multiseries concentration. At least 30 fields or 300 cells (whichever came first) were counted for each preserved sample.

In all years, the experiments were conducted in natural seawater, so very low levels of other phytoplankton taxa were present in addition to the inoculated dominant algae. In addition, during the 2006 experiments, an unknown flagellate phytoplankton from the natural phytoplankton community in Puget Sound was accidentally introduced into the algal cultures at the beginning of the study. Although this was not originally intended, it provides an interesting point of comparison for the study. Since phytoplankton blooms can either be dominated by a single algal species or be a mixed assemblage of co-dominant species, comparing the 2006 data (mixed assemblage) to the 2008 and 2009 data (unialgal dominance of *Pseudo-nitzschia multiseries*) gives examples of two scenarios that can be encountered in natural environments.

As a result of the co-dominance of two algae in the 2006 experiments, I used preserved water samples to determine clearance rates on *Pseudo-nitzschia multiseries* in these experiments. In 2008 and 2009, when the algal cultures were pure, I used chlorophyll samples to determine clearance rates on *P. multiseries*. Throughout all of the experiments, the background chlorophyll of the natural seawater used in the experiments was very low, thus it is possible to be confident that measuring chlorophyll decline provides valid estimates of clearance rates on *P. multiseries*.

Clearance Rate Equations.

Clearance rate, defined by Coughlan (1969) as the volume of water “cleared” of phytoplankton per unit time by a consumer, is calculated by:

$$m = \frac{V}{n} \cdot \left[\frac{\ln C_0 - \ln C_t}{t} \right] \quad \text{Eqn. 1}$$

where m is the clearance rate of a single animal, V is the volume of water used in the experiment, C_0 and C_t are the initial concentration of phytoplankton and the concentration after time t , and n is the number of animals in the tank.

In the control experiments with no animals, the rate at which particles settle out of suspension (either sticking to walls of the container or settling at the bottom) can be calculated using the equation:

$$s = \frac{\ln C_0 - \ln C_t}{t} \quad \text{Eqn. 2}$$

where s is the rate at which phytoplankton settle out of suspension every hour (Coughlan 1969). I used this equation to verify that no significant settling of phytoplankton cells occurred over the duration of the experiments.

In clams, the relationship between clearance rate (m) and shell length (L) is of the form:

$$m = aL^b \quad \text{Eqn. 3}$$

where a and b are species-specific parameters. The allometric relationship is appropriate in clams, because clearance rate should be proportional to gill area (e.g. Hughes 1969, Meyhofer

1985), which, in turn, is proportional to shell length squared (e.g. Foster-Smith 1975, Riisgard & Seerup 2003). Tissue dry weight (W) can be used instead of shell length in a similar allometric equation:

$$m = cW^d \quad \text{Eqn. 4}$$

where c and d are species-specific parameters.

Data Analysis.

All data analyses were conducted using the open source statistical programming language R. In analyzing clearance rate data, I first used linear regression to plot decline in \ln -transformed phytoplankton concentrations against time, to verify that clearance rates were constant over time (indicated by a straight line on the semi-log plot). I then used non-linear mixed effects models, with replicate experiment as the random effect, to analyze all phytoplankton decline data together for each species. I could have analyzed data for each species using a two-step process, where I first obtained regression slopes (m) for semi-log plots of phytoplankton decline over time per a rearrangement of Eqn. 1:

$$\ln C_t = \ln C_0 - \frac{n}{V} \cdot m \cdot t \quad \text{Eqn. 5}$$

then plotted the regression slopes against \ln -transformed bivalve size data (shell length or tissue dry weight) to obtain the slope (b or d from Eqn's. 3 & 4) and intercept (a or c from Eqn's. 3 & 4). However, this two-step process does not allow error in the first slope to be translated to the final estimates of allometric parameters, resulting in underestimated variance around the parameters. By using non-linear mixed effects models, the two-step process is combined into one step, which provides accurate errors for the model parameters (Zuur et al. 2009).

Clearance rate models for clams were of the form:

$$\ln C_t = \ln C_0 - \frac{n \cdot t}{V} \cdot aL^b \quad \text{Eqn. 6}$$

or

$$\ln C_t = \ln C_0 - \frac{n \cdot t}{V} \cdot cW^d \quad \text{Eqn. 7}$$

Each of these models is a simple algebraic combination of Eqn. 5 for calculating the regression slope for \ln -transformed phytoplankton decline over time, and Eqn.'s 3 and 4 for calculating clearance rate based on shell length or tissue dry weight. These types of non-linear mixed effects models are appropriate for analyzing clearance rate data because they include fixed terms of explicit interest (in this case, the allometric variables a and b for clearance rate vs. shell length, or c and d for clearance rate vs. tissue dry weight) and random terms that are not of explicit interest (in this case, $\ln C_0$ the \ln -transformed initial concentrations of *Pseudo-nitzschia multiseries* in the experimental tanks, which varied slightly from experiment to experiment).

At least two factors differed between the 2006 clam clearance rate experiments and the 2008 and 2009 experiments. First, temperature in 2006 was higher (12 – 13.2°C) than in 2008 (9.5 – 11°C) or 2009 (8.2 – 10.7°C). Second, the algal culture fed to the clams in 2006 was a mixture of *Pseudo-nitzschia multiseries* and a smaller flagellate, whereas the algae fed to the clams in the 2008 and 2009 experiments was a monoculture of *P. multiseries*. As a result of these differences, I analyzed only the 2006 data using non-linear mixed effects models. The data from

the 2008 and 2009 experiments weren't available for a sufficiently wide range of size classes to perform a non-linear mixed effects model, so they are provided for comparative purposes only. One bivalve species, thin-shell littleneck (*Protothaca tenerrima*), was only available in one size, hence clearance rate was simply calculated using Eqn. 1.

Early examination of barnacle clearance rate data indicated that barnacles changed their feeding rate over time. During the first hour of the experiments, I observed a rapid decline in *Pseudonitzschia multiseries* concentration as well as high percentages of barnacles displaying feeding behavior (cirri extended); in the latter two hours of the experiment, decline in *P. multiseries* concentration was much smaller, and substantially lower percentages of barnacles displayed feeding behavior. Hence, analysis of decline in *P. multiseries* concentration **Eqn. 8** le clearance rate experiments was calculated first for the first hour of each experiment, and then for the time interval between one and three hours. These data were analyzed using a linear mixed effects model with a random intercept, and a common slope (m) was obtained for clearance rate per cm^2 of barnacles. The model was of the form:

$$\ln C \cdot \frac{V}{d} = \beta - m \cdot t$$

where C is *P. multiseries* concentration at time t , V is volume of water in the experiment, d is barnacle density (cm^2), β represents a random intercept, and m is clearance rate ($\text{L} \cdot \text{h}^{-1} \cdot \text{cm}^{-2}$).

RESULTS

No significant settling of *Pseudo-nitzschia multiseriis* cells occurred during the control experiments: $s = 0.023 \text{ h}^{-1}$ (± 0.023 ; p-value = 0.31).

I was able to develop allometric equations for clearance rate by length or tissue dry weight for the clam species tested in these experiments (Table 3), and clearance rate calculations for barnacles as a function of cm^2 coverage. The clams with the highest clearance rate for their size range (length or tissue dry weight) at temperatures between 12.0 and 13.2 °C were: bay mussel and Pacific oyster, followed by Nuttall's cockle, Manila clam and butter clam (Figs. 2 & 3). The slowest clearance rates for their size are recorded for the two *Macoma* clams, which are primarily detritivores that use suspension-feeding opportunistically. Of the clam species that primarily suspension-feed, razor clam displayed the lowest clearance rate by shell length and tissue dry weight (Figs. 2 & 3). Clearance rate for the thin-shell littleneck with a shell length of 65mm was: $m = 0.93 \text{ l}\cdot\text{h}^{-1}$. This was slightly higher than for a razor clam of the same size ($m = 0.70 \text{ l}\cdot\text{h}^{-1}$), and slightly lower than for a soft-shell clam of the same size ($m = 1.32 \text{ l}\cdot\text{h}^{-1}$). Tissue dry weight was not measured for the thin-shell littleneck.

Table 3

Top portion: Regression parameters for clam clearance rate related to shell length per the allometric equation $m = aL^b$, where m is clearance rate ($l \cdot h^{-1}$), L is shell length (mm), and a and b (\pm standard deviation) are species-specific parameters. The last column provides p-values to summarize likelihood ratio tests comparing best fit models to models where the b -exponent was fixed at $b = 2$.

Bottom portion: Regression parameters for clam clearance rate related to tissue dry weight per the allometric equation $m = cW^d$, where m is clearance rate ($l \cdot h^{-1}$), W is tissue dry weight (g), and c and d (\pm standard deviation) are species-specific parameters. Last column is same as above, except the d -exponent was fixed at $d = 2/3$. The likelihood ratio test could not be completed for razor clam because the model where d was fixed to $2/3$ did not converge.

Clearance Rate (l/hr) vs Shell Length (mm)

Species	# of exp'ts	size range (shell length, mm)	a (\pm sd)	b (\pm sd)	p-value (for b=2)
bay mussel	6	19 - 82	0.006 (\pm 0.0042)	1.72 (\pm 0.17)	0.11
Pacific oyster	8	49 - 131	0.011 (\pm 0.012)	1.56 (\pm 0.25)	0.11
Nuttall's cockle	7	37 - 90	0.0081 (\pm 0.0083)	1.49 (\pm 0.25)	0.15
Manila clam	8	32 - 62	0.078 (\pm 0.144)	0.89 (\pm 0.35)	0.04
butter clam	9	47 - 106	0.0035 (\pm 0.0044)	1.63 (\pm 0.3)	0.23
purple varnish clam	5	31 - 63	0.0088 (\pm 0.0117)	1.37 (\pm 0.42)	0.05
littleneck clam	6	34 - 71	0.29 (\pm 0.22)	0.45 (\pm 0.31)	0.00
horse clam	8	102 - 146	0.00032 (\pm 0.0011)	2.03 (\pm 0.72)	0.97
soft-shell clam	7	28 - 76	0.0044 (\pm 0.0091)	1.46 (\pm 0.54)	0.24
razor clam	7	49 - 128	0.00045 (\pm 0.00076)	1.76 (\pm 0.37)	0.48
bent-nose Macoma	7	20 - 51	0.052 (\pm 0.085)	0.40 (\pm 0.48)	0.02
pointed Macoma	6	27 - 47	0.00004 (\pm 0.00006)	2.43 (\pm 1.02)	0.25

Clearance Rate (l/hr) vs Tissue Dry Weight (g)

Species	# of exp'ts	size range (tissue dry wt, g)	c (\pm sd)	d (\pm sd)	p-value (for d=2/3)
bay mussel	5	0.03 - 2.79	5.98 (\pm 0.38)	0.60 (\pm 0.04)	0.10
Pacific oyster	8	0.95 - 7.18	6.03 (\pm 0.71)	0.72 (\pm 0.08)	0.49
Nuttall's cockle	7	0.69 - 14.11	2.25 (\pm 0.28)	0.42 (\pm 0.07)	0.01
Manila clam	7	0.61 - 4.03	1.97 (\pm 0.29)	0.21 (\pm 0.21)	0.02
butter clam	8	1.63 - 23.45	1.58 (\pm 0.24)	0.45 (\pm 0.08)	0.02
purple varnish clam	4	0.33 - 3.24	1.53 (\pm 0.19)	0.41 (\pm 0.14)	0.03
littleneck clam	5	0.41 - 5.75	1.64 (\pm 0.15)	0.15 (\pm 0.05)	0.00
horse clam	6	13.07 - 37.22	0.29 (\pm 0.30)	0.89 (\pm 0.31)	0.42
soft-shell clam	6	0.19 - 3.00	0.80 (\pm 0.13)	0.44 (\pm 0.13)	0.09
razor clam	7	0.59 - 13.31	0.50 (\pm 0.12)	0.58 (\pm 0.12)	--
bent-nose Macoma	7	0.07 - 0.96	0.26 (\pm 0.09)	0.16 (\pm 0.17)	0.03
pointed Macoma	5	0.14 - 0.62	0.68 (\pm 0.17)	0.93 (\pm 0.20)	0.16

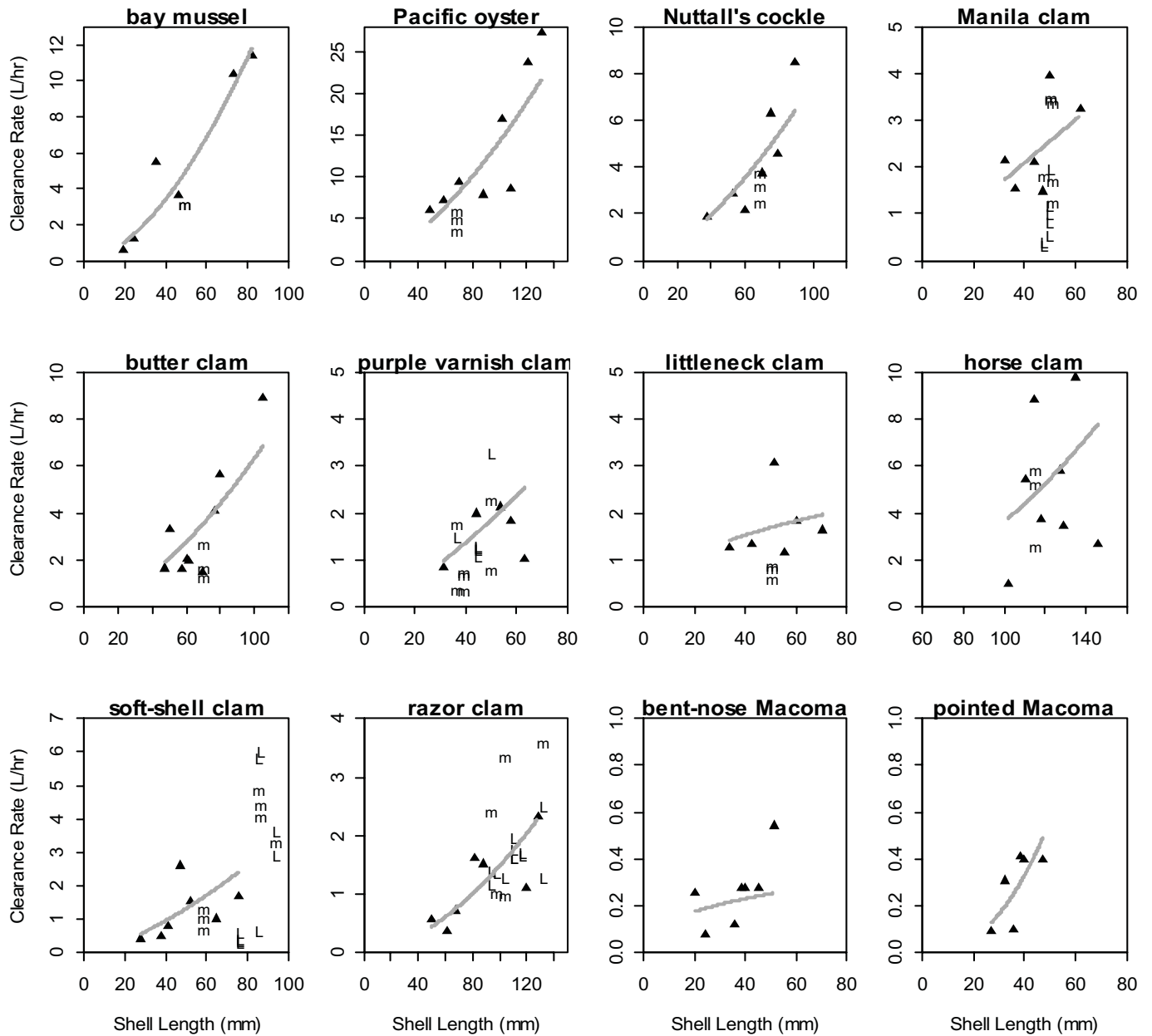


Figure 2: Clearance rate versus shell length regressions for 12 clam species. Solid triangles indicate clearance rate experiments that were conducted between 12 and 13.5°C, “M” symbols indicate experiments conducted between 10 and 12 °C, and “L” symbols indicate experiments conducted between 8.5 and 10 °C. Grey regression lines are for data from the highest temperature range only (12 to 13.5°C). Note the different scale on each plot.

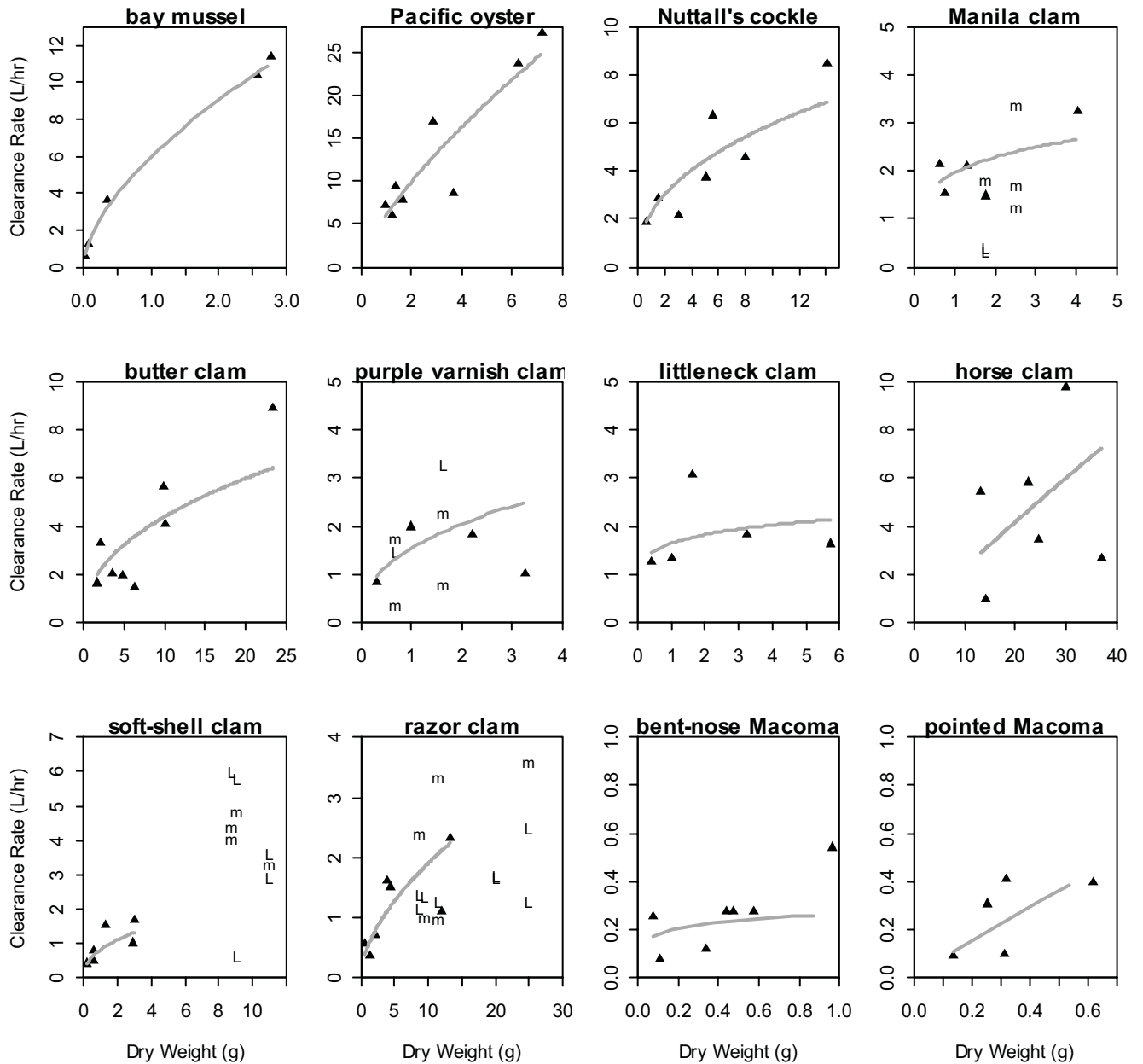


Figure 3: Clearance rate versus tissue dry weight regressions for 12 clam species. Solid triangles indicate clearance rate experiments that were conducted between 12 and 13.5°C, “m” symbols indicate experiments conducted between 10 and 12 °C, and “L” symbols indicate experiments conducted between 8.5 and 10 °C. Grey regression lines are for data from the highest temperature range only (12 to 13.5°C). Note the different scale on each plot. Also, not all data points from Figure 2 have corresponding data in Figure 3; tissue dry weight data were not available for all species and all experiments.

Allometric equation exponents b and d ranged from $b = 0.40$ to 2.43 for the relationship between clearance rate and shell length, and from $d = 0.15$ to 0.93 for the relationship between clearance rate and tissue dry weight (Table 3; Figs. 4 & 5).

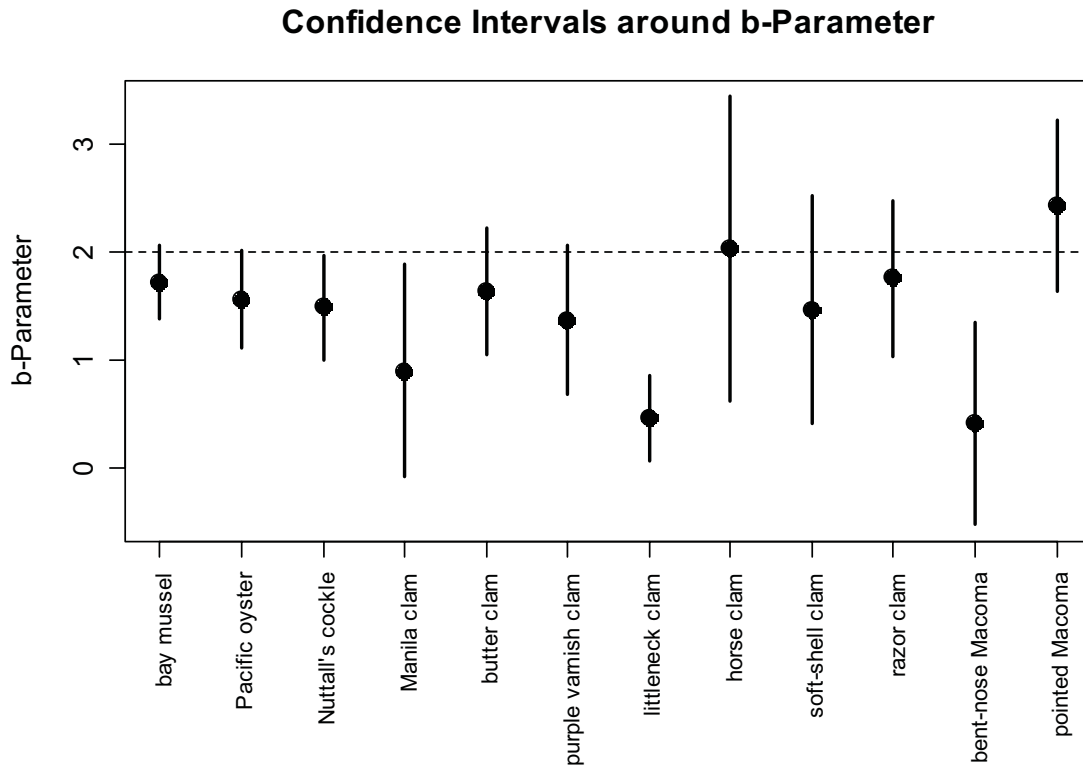


Figure 4: Exponent from the allometric equation $CR = aL^b$, shown for each species with 95% confidence intervals. Dashed horizontal line indicates the theoretically expected value of $b = 2$.

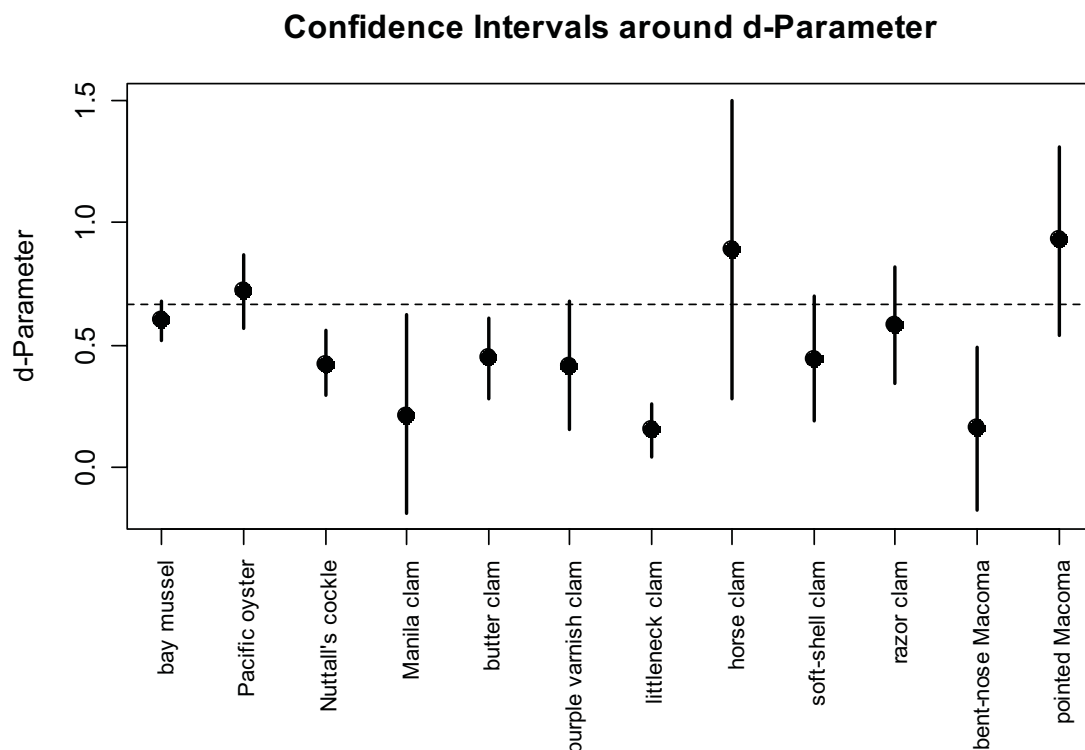


Figure 5: Exponent from the allometric equation $CR = cW^d$, shown for each species with 95% confidence intervals. Dashed horizontal line indicates the theoretically expected value of $d = 2/3$.

Clearance rates obtained in the 2008 and 2009 experiments, were similar to or slightly lower than those obtained in 2006. The 2008 and 2009 experiments were conducted at slightly lower temperatures than the 2006 experiments, and used a monospecific culture of *Pseudo-nitzschia multiseries* rather than a mixed culture (Figs. 2 & 3).

Observations of feeding behavior (2-6 observations per experiment; mean = 3.8) were averaged over all experiments for each clam species to obtain an estimate of percent observed feeding behavior. For each suspension-feeding clam species in these experiments, this metric was between 96 – 100%, indicating that clams had siphons extended and/ or valves gaping in nearly all observations. In the two *Macoma* species, which are primarily detritivores, clams displayed

extended siphons and/ or gaping valves in 49% of observations for pointed Macoma, and 59% of observations for bent-nose Macoma.

For barnacles, the mean clearance rate per square centimeter of barnacle surface area during the first hour of the feeding experiments (heavy feeding) was: $m = 0.031 \text{ l}\cdot\text{h}^{-1} \pm 0.0056$. The mean clearance rate per square centimeter of barnacles during the time interval between one hour and three hours (reduced feeding) was $m = 0.0064 \text{ l}\cdot\text{h}^{-1} \pm 0.0024$. In Puget Sound, average dry meat mass of acorn barnacles $\cdot \text{cm}^{-2}$ in an area totally covered with barnacles is 0.021g (M. Dethier, unpublished data). Thus, clearance rate $\cdot \text{g}^{-1}$ tissue dry weight is $\sim 1.48 \text{ l} \cdot \text{h}^{-1} \cdot \text{g}^{-1}$ for acorn barnacles.

DISCUSSION

Intertidal clams and acorn barnacles from sand/ cobble beaches in Puget Sound and sandy beaches on the outer Washington coast were exposed to conditions that mimicked a toxin-producing *Pseudo-nitzschia* bloom in Puget Sound, to measure clearance rates on *P. multiseriis*. These data will be used in a model to predict filtration capacity for an assemblage of SFBMs, as tidal advection brings toxin-producing *Pseudo-nitzschia* to the intertidal. For this reason, I was most interested in the *average* rates at which Washington clams consume *P. multiseriis* over several hours, during which time not all individuals would necessarily exhibit constant feeding. Through the experiments, I also wanted to compare the clearance rates of Puget Sound clam species to outer coast razor clams. All Puget Sound clams, with the exception of the detritivorous pointed Macoma and bent-nose Macoma, fed on *P. multiseriis* at rates of about 1.5

to 12 times those of razor clams with the same shell length, and rates of about 1.2 to 16 times those of razor clams with the same tissue dry weight (Table 3 & Figs. 2 & 3).

Indirect Method for Measuring Clearance Rate

The indirect clearance method used in this study has been proven reliable in many clearance rate studies (reviewed in Riisgard 2001); however, a disadvantage of the method is that the food concentration is constantly declining over the course of the experiment (Winter 1978, Riisgard 2001). A requirement for using the indirect clearance rate equation is that, even as food concentration declines over time, clearance rate remains relatively constant (Coughlan 1969), as evidenced by good data fit to linear regressions for semi-log plots of phytoplankton decline vs. time. This was indeed the case in these experiments, where the mean R-value for all linear regressions was $R=0.91$. For this study, where I tried to simulate conditions associated with tidal inundation, I also point out that the depletion of *P. multiseriis* cells in the experiments would not necessarily be atypical of a bloom being transported over an intertidal area where there is a high density of benthic suspension feeders (Banas et al. 2007).

Another requirement of the indirect clearance rate method is that particle retention by suspension-feeders must either be 100% efficient, or a known and constant percentage in order for clearance rate equations to be accurate (Coughlan 1969). During the feeding rate experiments on clams, pseudofeces were produced in most tanks, indicating that not all food particles were retained by the clams. However, pseudofeces remained intact enough for collection at the close of each experiment, indicating that phytoplankton cells trapped within pseudofeces were not recirculated into the water.

Effectiveness of the Study Design

Clam feeding rates have been shown to vary with a number of parameters, including: temperature (e.g. Denis et al. 1999, Jihong et al. 2004); algal concentration (e.g. Foster-Smith 1975, Denis et al. 1999); food quality, including ratio of organic particles to sediment (e.g. Newell & Jordan 1983, Stenton-Dozey & Brown 1992); and size or shape of available food particles (e.g. Newell et al. 1989, Defosse & Hawkins 1997). Throughout the 2006 experiments, which were used to estimate relationships between clearance rate and bivalve size, external parameters such as temperature, type of food particles, and initial concentration of *P. multiseriis* were kept constant, so as to reduce variability in clearance rates. Even so, for some species (e.g. Manila clam, purple varnish clam, horse clam, soft-shell clam), there was substantial scatter of data points around the line that modeled the relationship between clearance rate and clam size (Figs. 2 & 3). In some species, this scatter was more pronounced for the relationship with clam length than with tissue dry weight or vice versa, indicating variability in the “meatiness” of the clams.

For this study, I used temperatures and algal concentrations representative of natural conditions during a toxin-producing bloom of *Pseudo-nitzschia* in Puget Sound. To date, three such blooms have resulted in DA concentrations above $20\text{mg}\cdot\text{kg}^{-1}$ in clam tissue, triggering beach harvest closures in Puget Sound. The concentrations of *Pseudo-nitzschia* during these blooms ranged from $2.0 \times 10^4 \text{ cells}\cdot\text{l}^{-1}$ to $2.3 \times 10^7 \text{ cells}\cdot\text{l}^{-1}$, with water temperatures between $11.3 \text{ }^\circ\text{C}$ and $15.0 \text{ }^\circ\text{C}$ (Bill et al. 2006, Trainer et al. 2007). All three of the blooms were nearly monospecific for one of the highly toxic *Pseudo-nitzschia* species (Bill et al. 2006, Trainer et al. 2007).

Temperatures in this study ranged from 8.2 °C to 13.2 °C, with the allometric equations for clearance rate versus clam size based on data collected over a temperature range of 12 °C to 13.2 °C. These temperatures fall within the lower range of water temperatures associated with maximum particulate DA in the Puget Sound blooms that caused harvest closures. Concentrations of *Pseudo-nitzschia multiseriis* in these experiments were also within the range of phytoplankton concentrations reported for all three Puget Sound blooms. The algal culture fed to bivalves in 2006 was a mixed phytoplankton assemblage rather than a monoculture of *P. multiseriis*, which would have been more representative of the highly toxic Puget Sound blooms to date. However, *Pseudo-nitzschia* blooms are not always mono-specific and may include multiple species of *Pseudo-nitzschia* co-dominating the bloom (e.g. see Table 2, Schnetzer et al. 2007), or may include other non-*Pseudo-nitzschia* species (e.g., Olson 2006).

Effects of Temperature on Clearance Rate

It has long been known that temperature affects clearance rate in clams (Loosanoff 1958). Within the temperature tolerance of a species, there is debate among clam physiologists as to whether the clearance rate increases gradually with temperature (e.g. Walne 1972, Haure et al. 1998), or whether the clearance rate increases, to an optimum point, beyond which it decreases (e.g. Schulte 1975, Bougrier et al. 1995). The temperature range in these experiments (8.2 – 13.2 °C) is well within the range of temperatures that intertidal organisms in Puget Sound experience on an annual cycle, and is approximately the temperature range that coastal razor clams experience over an annual cycle (NOAA 2010). In these experiments, it appears that clearance rates for some species (Pacific oyster, Nuttall's cockle, Manila clam, butter clam, littleneck

clam) may have been slightly reduced at lower temperatures (Fig. 2). However, algal culture also varied in these experiments, preventing further analysis of either variable.

Effects of Phytoplankton Concentration and Composition on Clearance Rates

There is significant debate as to whether clams alter clearance rates in response to variability in their diets. One group of scientists presents theory and evidence suggesting that filtration rate is adjusted according to food quantity and quality, and that maximum filtration rate is only attained under optimal food conditions (Sprung & Rose 1988, Bayne et al. 1993, Arifin & Bendell-Young 1997, Bayne 1998). In this way, clams can reduce energy expenditure during sub-optimal feeding conditions, provided that the physiological cost of such a control system is low. Another group of researchers shows evidence that filter-feeding is an automated process based on the capacity of the gills, and that clams filter at their maximum capacity whenever food concentrations are maintained above a critical “trigger” level (Jorgensen 1996, Riisgard et al. 2003). Furthermore, the extent to which clam valves are open determines the amount of space that the gills have to expand, and because gill size correlates well with filtration rate (Meyhofer 1985, Riisgard 2001), if valves are fully open, filtering rate is at its maximum.

In this study, I was not able to support or refute either theory: the experimental methodology that I used is neither able to verify that measured clearance rates are the maximal rates for these species, nor whether clearance rates changed based on food type and concentration. While the 2008 and 2009 data for clearance rate appeared to be slightly lower than the 2006 data, I do not have sufficient data points to quantify this trend, or to discern whether it was caused by the effects of food type and concentration or by temperature.

Expectations of Allometric Equations for Clearance Rate

The rate at which suspension-feeding clams filter water is proportional to the surface area of their gills (Hughes 1969, Vahl 1973, Foster-Smith 1975, Mohlenberg & Riisgard 1979, Meyhofer 1985). While many studies present clearance rate as a function of tissue dry weight, shell length (L) may be a better correlate with clearance rate because shell length is closely associated with gill area: neither gill area nor shell length change seasonally as clams go through periods of weight gain and loss due to spawning in spring, rapid growth in summer and starvation in winter (Riisgard 2001). A number of authors generally agree that in the allometric equation relating clearance rate to clam length, $m = aL^b$, the b exponent should be ~ 2 , since gill area is proportional to shell length squared (e.g. Foster-Smith 1975, Riisgard & Seerup 2003). Using similar logic, several authors suggest that the allometric equation relating clearance rate to clam tissue dry weight, $m = cW^d$, should have a d exponent $\sim 2/3$, because clam weight (W) is proportional to length cubed, or, reversed, L is proportional to $W^{1/3}$, thus $m = c(W^{1/3})^2 = cW^{2/3}$ (e.g. Jones et al. 1992, Riisgard 2001). However, others suggest that the d exponent should be higher (e.g. between 0.66 and 0.82; Jorgensen 1976, Winter 1978, Gerdes 1983), indicating a proportionality somewhere between surface area ($d = 2/3$) and body weight ($d = 1$).

There is some divergence in published findings or predictions about the exponents in the allometric equations relating clearance rate to clam size for the taxa tested in this study (Table 4). I used likelihood ratio tests to compare best fit models to ones where the b exponent was fixed at 2 or the d exponent at $2/3$, and obtained p-values for the comparisons (Table 3). Several of the b exponents in this study were close to 2, including those for bay mussel ($b = 1.72$; p-value =

0.11), horse clam ($b = 2.03$; p-value = 0.97) and razor clam ($b = 1.76$; p-value = 0.48). Others were closer to 1.5 (Pacific oyster, p-value = 0.06; Nuttall's cockle, p-value = 0.15; butter clam, p-value = 0.23; purple varnish clam, p-value = 0.05; soft-shell clam, p-value = 0.24). The b exponents for Manila clam ($b = 0.89$, p-value = 0.04) and littleneck clam ($b = 0.45$, p-value = 0.00) were lower than expected for suspension-feeding species. The two detritivorous *Macoma* species had b exponents of 0.4 (bent-nose *Macoma*; p-value = 0.02) and 2.69 (pointed *Macoma*; p-value = 0.44). The discrepancy between b exponents from best fit models, and the anticipated value of 2, may be a result of a difference in experimental purpose and design. While many experiments are aimed at obtaining the maximal filtration or clearance rate, these experiments were instead designed to mimic tidal inundation of clams during a toxin-producing *Pseudo-nitzschia* bloom, where not all clams necessarily feed at their maximum rates for the duration of time that they are submerged. Thus, the b exponent of 2 may be most likely during maximal feeding, but a lower exponent may occur as an average under natural intertidal conditions.

Table 4: Clearance (CR), filtration (FR) or pumping (P) rates in suspension-feeding clams from this and other studies. Other studies present maximal clearance rates, while this study presents average clearance rates over several hours. Clearance rates ($l \cdot hr^{-1}$) as a function of size (W , g dry body weight) or shell length (L , mm) are presented as allometric equations $FR = cW^d$ or $FR = aL^b$

Species & Reference	Clearance, Filtration or Pumping Rate (l/hr)	Temp (°C)	tissue dry weight (g)	shell length (mm)
Blue mussel				
Kiorboe & Mohlenberg (1981) ‡	CR = $0.0012L^{2.14}$	17		17 - 63
Mohlenberg & Riisgard (1979)	FR = $7.45W^{0.66}$	10-13	0.011 - 1.36	
bay mussel				
Dusek Jennings, this study	CR = $0.006L^{1.72}$	12-13.2		19 - 82
Dusek Jennings, this study	CR = $5.98W^{0.60}$	12-13.2	0.03 - 2.79	
Pacific oyster				
Gerdes (1983)	FR = $0.19W^{0.73}$	20	0.005 - 0.81	
Akashige et al (2005) *	FR = $2.15W^{0.96}$	12.5	0.26 - 7.20	
Dusek Jennings, this study	CR = $6.03W^{0.72}$	12-13.2	0.95 - 7.18	
Nuttall's cockle				
Meyhofer (1985)	P = $3.06W^{0.80}$	12.75	2.47 - 4.46	50 - 73
Dusek Jennings, this study	CR = $2.25W^{0.42}$	12-13.2	0.69 - 14.11	37 - 90
Manila clam				
Nakamura (2001) †	CR = $1.61W^{0.84}$	24	0.069 - 0.66	
Dusek Jennings, this study	CR = $1.97W^{0.21}$	12-13.2	0.61 - 4.03	
soft-shell clam				
Riisgard & Seerup (2003)	FR = $0.0007L^{2.47}$	11	0.20 - 2.23	21 - 45
Dusek Jennings, this study	CR = $0.0044L^{1.46}$	12-13.2	0.19 - 3.00	28 - 76
bent-nose Macoma				
Meyhofer (1985)	P = $0.083W^{0.86}$	12.75	0.241 - 1.37	35 - 49
Dusek Jennings, this study	CR = $0.26W^{0.16}$	12-13.2	0.07 - 0.96	20 - 51

‡ Obtained from Riisgard (2001), who provides the corrected equation according to Kiorboe pers. comm.

* Akashige et al's (2005) equation incorporates a variable for temperature, which is based on their study over a range of 9.7 to 25.7°C. We adapted it by substituting 12.75 °C for the temperature variable.

† Clearance rate data from Nakamura (2001) were \ln -transformed and analyzed using linear regression in order to obtain parameters for the allometric equation.

In the allometric equations relating clearance rate to tissue dry weight, I found the d exponent was closest to $2/3$ in bay mussel ($d = 0.60$; p-value = 0.10), Pacific oyster ($d = 0.72$; p-value = 0.49) and razor clam ($d = 0.58$; p-value not available). Two species had d exponents close to 1.0 (horse clam, $d = 0.89$, p-value = 0.42; pointed Macoma $d = 0.93$, p-value = 0.16), and the majority had d exponents ranging between 0.21 and 0.44. Littleneck clam ($d = 0.15$, p-value = 0.00) and the detritivorous bent-nose Macoma ($d = 0.16$, p-value = 0.03) had the lowest d -values. The likelihood ratio test could not be completed for allometric equations relating clearance rate to tissue dry weight for razor clam, because the model with the d exponent fixed at $2/3$ did not converge.

In comparing results of clearance rate versus shell length to clearance rate versus tissue dry weight, I noticed some discrepancies in the two sets of results. For example, while bay mussel, horse clam and razor clam have b exponents close to the expected value of 2 for clearance rate as a function of shell length, it is bay mussel, Pacific oyster and razor clam that have d exponents close to the expected value of $2/3$ for clearance rate as a function of tissue dry weight. There are two possible reasons for the discrepancy: 1) not all experiments that were analyzed for clearance rate versus shell length could be analyzed for clearance rate versus tissue dry weight; a number of clams from the clearance rate experiments were subsequently used in another study on DA uptake and retention, hence tissue dry weights were not obtained for these clams (particularly true for horse clam and littleneck clam, where tissue dry weights were unavailable for two experiments per species). And, 2) differences within some species may have existed between individuals in their condition index (CI), calculated as $CI = W/L^3$; i.e., some clams were meatier than others. For two species, bay mussel and purple varnish clam, several individuals spawned

either during or just after the clearance rate experiments. Although experiments where spawning had occurred were excluded from the study and were subsequently repeated, the tissue dry weight of recently spawned clams would be lighter than that of other individuals of the same species that continued to retain their gametes.

Differential Clearance Rates in Barnacles

At *Pseudo-nitzschia multiseriis* concentrations between 1.0 and 3.5×10^6 cells·l⁻¹, all suspension-feeders except acorn barnacles fed at a constant rate over the 2.5 to 4 hour study; barnacles fed at a higher rate for the first hour, followed by a period of slower feeding during the remaining two hours. Because of the pattern observed in barnacles, I divided barnacle clearance rate analysis into two time-periods: early feeding (first hour) and later feeding (remaining two hours). There are two reasons why this feeding behavior may have been observed in acorn barnacles. The first is that the barnacles may have reached satiation and stopped feeding in response to fullness or the need to handle prey items (e.g. Dill & Gillett 1991). The second possibility is that some threshold concentration was reached in the aquaria, below which it was no longer metabolically optimal for barnacles to continue feeding. Wu and Levings (1978) calculated energy budgets for acorn barnacle and found that over two-thirds of acquired energy was lost in respiration. It follows that active feeding when food abundance is low might not be metabolically efficient. I suspect that either option is possible, as I did observe that feeding tapered off coincident with each tank reaching approximately the same *P. multiseriis* concentration.

Comparison of Clam Clearance Rates to Other Puget Sound Taxa: Barnacles and Polychaetes

Several non-bivalve suspension-feeding taxa are found at relatively high densities on Puget Sound sand/gravel beaches, including acorn barnacles and polychaetes (Chapter I: Intertidal Zonation of Suspension-Feeding Epi- and Infauna at Lofall, Hood Canal). These suspension-feeders have varied methods of obtaining food that is suspended in the water. The suspension-feeding polychaete genus *Spiochaetopterus*, which is common in Puget Sound, feeds by creating a mucus net that is held in flowing water to trap passing particles (Barnes 1964). Although filtration rates have not been measured for *Spiochaetopterus*, the polychaete *Nereis diversicolor* has a similar suspension-feeding mechanism, and has been shown to filter particles out of water at a rate of 0.05 to $0.57 \text{ l} \cdot \text{hr}^{-1}$, depending on size (4 to 63mg tissue dry weight) (Riisgard 1991). This translates to a clearance rate $\cdot \text{g}^{-1}$ tissue dry weight of $\sim 9.0 \text{ l} \cdot \text{hr}^{-1} \cdot \text{g}^{-1}$, where one g corresponds to 16-250 polychaetes. In contrast, acorn barnacles feed at a clearance rate $\cdot \text{g}^{-1}$ tissue dry weight of $\sim 1.48 \text{ l} \cdot \text{h}^{-1} \cdot \text{g}^{-1}$, where one g corresponds to a 47 cm^2 patch of barnacles.

In these experiments, suspension-feeding clams cleared *Pseudo-nitzschia* from seawater at rates ranging from 0.35 to $27.3 \text{ l} \cdot \text{hr}^{-1}$, depending on species and size. The average clearance rate $\cdot \text{g}^{-1}$ tissue dry weight calculated over all Puget Sound suspension-feeding clams was $\sim 1.15 \text{ l} \cdot \text{hr}^{-1} \cdot \text{g}^{-1}$ for infaunal clams, and $\sim 7.45 \text{ l} \cdot \text{hr}^{-1} \cdot \text{g}^{-1}$ for epifaunal clams. One g corresponds to 0.14-39 epifaunal Puget Sound clams, and 0.03-5 infaunal Puget Sound clams. Comparing clams to barnacles and polychaetes in terms of clearance rate $\cdot \text{g}^{-1}$ tissue dry weight, it is apparent that polychaetes feed at higher rates than both epi- and infaunal clams for their weight, and for their weight, acorn barnacles feed at higher rates than infaunal, but not epifaunal, Puget Sound clams.

Potential for Toxin Accumulation in Puget Sound Clams

As I have shown in this chapter, all Puget Sound clams that I studied consume *Pseudo-nitzschia multiseries* when the phytoplankton is present at concentrations typically found during Puget Sound blooms of *Pseudo-nitzschia*. Specifically, I showed that bay mussel and Pacific oyster consume *P. multiseries* at the fastest rates for their size, followed by Nuttall's cockle and Manila clam. The actual concentration of DA toxin that accumulates in clam tissues, however, is dependent on three further aspects of clam biology: 1) amount of time that the clams are submerged and able to feed; 2) efficiency with which ingested DA is assimilated into soft tissues; and, 3) the rate at which DA is depurated from soft tissues. In combination with this study on clearance rates of Puget Sound clams, these additional data will be necessary to answer an important question: When a sentinel species, such as blue mussel, is tested for DA concentration, what are the DA concentrations in other harvestable clams species?

Currently, the Washington State Department of Health sampling protocol for DA in Puget Sound clams entails collection and analysis of bay mussels from 30 sites on a bi-weekly basis (F. Cox, WaDOH, pers.comm. 6/10/12). I have shown that bay mussels feed on *Pseudo-nitzschia multiseries* at a high rate, making them favorable as sentinel organisms. However, closely related blue mussels have been shown to depurate DA on the order of hours to days (Novaczek et al. 1992, Wohlgeschaffen et al. 1992, Krogstad et al. 2009), leaving the possibility that other species on the beach will contain elevated concentrations of toxin over longer periods. Based on clearance rate measurements, other potential sentinel species could be: Pacific oyster, Nuttall's

cockle, Manila clam or butter clam. Of course, depuration rates for these species will require investigation to assess their suitability as sentinel organisms in Puget Sound.

Chapter IV:**Domoic Acid Uptake and Depuration by Nine Intertidal Clams
Experimentally Fed on Toxin-Producing *Pseudo-nitzschia multiseries*****ABSTRACT**

Domoic acid (DA), a neurotoxin produced by the diatom *Pseudo-nitzschia*, has caused frequent harvest closures for razor clam *Siliqua patula* along the outer coast of Washington State (USA). In comparison, shellfish harvest closures for DA have occurred only three times in Washington's inland estuarine complex: Puget Sound. As a first step toward modeling consumption and retention of DA by clams in both regions, I conducted clearance (feeding) and depuration experiments for outer coast razor clam and eight species of intertidal Puget Sound clams, including oysters and mussels, fed on toxin-producing *Pseudo-nitzschia multiseries*. Using an exponential decay model for depuration rate, I found that rates ranged between 0.03 and 0.86 · day⁻¹, with the highest rates in Pacific oyster *Crassostrea gigas* followed by butter clam *Saxidomus giganteus*, and the lowest rates in razor clam followed by purple varnish clam *Nuttallia obscurata*. Other tested species include: littleneck clam *Leukoma staminea*, Nuttall's cockle *Clinocardium nuttallii*, horse clam *Tresus capax*, soft-shell clam *Mya arenaria*, and Manila clam *Ruditapes philippinarum*. In four species, I also obtained estimates of the efficiency with which ingested DA is assimilated into tissues (AE): razor clam AE=11%, soft-

shell clam AE=59%, purple varnish clam AE~100%, and Manila clam AE~100%. Through model sensitivity analysis, I tested the effects of inter-individual variability in clearance and depuration rates on model fit, and found that allowing for inter-individual variability in clearance rates produced model output that accounted for a greater proportion of the clam DA data in soft-shell clam, razor clam and purple varnish clam, than simulations that allowed for inter-individual variability in depuration rate. In Manila clam the opposite was true: simulations allowing inter-individual variability in depuration rate accounted for a slightly greater proportion of the data than simulations allowing variability in clearance rate. This implies that intra-species differences in feeding on *Pseudo-nitzschia* may be driving the variability in DA concentrations that is seen within some species. Additionally, the present study suggests that purple varnish clam, which assimilated all consumed DA and depurated it slowly, may be a good sentinel organism for testing beaches for DA toxicity.

INTRODUCTION

In the closing months of 1987, residents of Prince Edward Island, Canada were startled by an outbreak of food-borne illness that occurred following consumption of commercially-harvested blue mussels (*Mytilus edulis*) (Addison & Stewart 1989). Over 100 people displayed acute intoxication (abdominal cramps and vomiting within the first few hours, and memory loss that could persist indefinitely), and three elderly victims died as a result of the poisoning (Perl et al. 1990). None of the familiar shellfish toxins were found to be responsible for the outbreak. In mid-December 1987, the toxin was identified as domoic acid (DA) (Wright et al. 1989), and later it was discovered that the source of the toxin was a bloom of the phytoplankton *Nitzschia pungens* (Rao et al. 1988, Bates et al. 1989), now known as *Pseudo-nitzschia multiseriata* (Hasle 1994, 1995). The discovery of toxin-production by *Pseudo-nitzschia* species led to research and monitoring efforts which have reduced the incidence of DA poisoning in humans (e.g. Smith et al. 1990, Villac et al. 1993, Wekell et al. 1994). Although monitoring has been crucial in preventing human exposure to DA, scientific efforts to understand shellfish toxicity dynamics have primarily been from a monitoring and reactive effort rather than a predictive one.

Understanding the processes that make shellfish toxic to humans poses a challenge that includes examining how ecological and physiological attributes of diverse shellfish influence their ability to take up and retain toxins. When toxin-producing phytoplankton such as *Pseudo-nitzschia* bloom and are advected over intertidal areas, suspension-feeding clams, including mussels and oysters, consume the phytoplankton and can accumulate toxin in their tissues. Not all ingested *Pseudo-nitzschia* result in DA accumulation, however. Selective rejection of *Pseudo-nitzschia* cells in the form of pseudofeces may allow some clam species to reduce DA burden in tissues

(Mafra et al. 2010b). Poor absorption can also decrease tissue DA concentrations: relatively low assimilation efficiencies (AEs; amount of toxin assimilated into tissues as a percentage of net consumption) have been reported for sea scallop, AE=51% (Douglas et al. 1997); blue mussel, AE=39%; and eastern oyster, AE=42% (Mafra et al. 2010b). Depuration dynamics play a role in DA concentrations too. Razor clams, for example, take months to a year to fully depurate elevated concentrations of DA from their tissues (Wekell et al. 1994, Adams et al. 2000, Trainer & Bill 2004), while blue mussels are capable of depurating the toxin within hours to days (Novaczek et al. 1992, Wohlgeschaffen et al. 1992, Krogstad et al. 2009 in press). Despite some physiological processes that may limit DA accumulation in clams, high in situ DA concentrations have been reported in a number of species besides blue mussel, including razor clam *Siliqua patula* (Wekell et al. 1994), king scallop *Pecten maximus* (James et al. 2005) and sea scallop *Placopecten magellanicus* (Douglas et al. 1997).

In Washington State, USA, where DA is emerging as a health issue on the outer coast, the Washington Department of Health regulatory limit for DA in shellfish tissue is $20 \text{ mg} \cdot \text{kg}^{-1}$. When this limit is exceeded, the affected beach is closed for shellfish collection. High concentrations of DA were first detected in razor clams, and their predators Dungeness crab (*Metacarcinus* [*Cancer*] *magister*), along the outer Washington and Oregon coasts in late October and November 1991 (Wekell et al. 1994, Horner et al. 1997). Since this initial closure, many fall and spring razor clam harvests have been impacted, owing to the long depuration time of razor clams, combined with frequent coastal *Pseudo-nitzschia* blooms (Horner et al. 1997, Wekell JC 2002). These beach closures have caused significant economic hardship to coastal

communities who rely on income from recreational razor clam harvesters, and to native tribes for whom razor clams are an important subsistence fishery (Dyson & Huppert 2010).

Although *Pseudo-nitzschia* is more conspicuous along the outer Washington coast, several species of the diatom have also been observed in the inland waters of Puget Sound (Trainer et al. 1998, Stehr et al. 2002, Bill et al. 2006). Prior to 2003, the presence of *Pseudo-nitzschia* in Puget Sound was of limited consequence to shellfisheries. However, Puget Sound experienced its first harvest closure resulting from a *Pseudo-nitzschia* bloom in September 2003, when bay mussels (*Mytilus trossulus*, a close relative to blue mussel) at Fort Flagler State Park attained high levels of DA toxin (Bill et al. 2006). Two subsequent beach closures occurred during fall 2005: in September, Pacific oysters (*Crassostrea gigas*) in Sequim Bay became toxic; and in October, bay mussels and Manila clams (*Ruditapes philippinarum*) in Penn Cove became toxic (Trainer et al. 2007). To date, several other clam species in Puget Sound have attained DA concentrations above the regulatory limit of $20\text{mg} \cdot \text{kg}^{-1}$, including littleneck clams (*Leukoma staminea*) and geoduck clams (*Panopea generosa*) (Bill et al. 2006, Trainer et al. 2007).

Of particular interest to Washington State's policymakers is the question: why has *Pseudo-nitzschia* only led to three relatively short harvest closures in Puget Sound, while the razor clam fishery on the outer coast has been plagued by frequent closures since 1991? The answer may be, in part, because of the clam species present in the two locations, and their differing physiologies. Suspension-feeding clam assemblages on Washington's sandy outer coast beaches are dominated by razor clams, whereas Puget Sound's sand and coarse sediment (mixed sand and gravel) beaches have a wide variety of clam species (not including razor clams) (Kozloff 1983).

Despite the growing threat of *Pseudo-nitzschia* in Washington State, little is known about clearance rates, assimilation efficiencies, and depuration rates as they pertain to DA accumulation in Washington's many clam species. It is known that razor clams depurate DA very slowly, while blue mussels, a close relative to Puget Sound's bay mussels, have poor DA absorption and depurate the toxin quickly. If Puget Sound's clam species display physiologies more similar to blue mussel than to razor clam, it may provide insight into the low frequency of DA harvest closures in Puget Sound.

In this paper I present part of a larger study to model DA flux in a Puget Sound intertidal suspension-feeding benthic macroinvertebrate assemblage intercepted by a *Pseudo-nitzschia* bloom. The goal of this particular manuscript is to assess DA assimilation and depuration in some of Washington's harvestable clam species. I present experimentally-derived estimates of clearance rate and DA depuration rate for nine clam species commonly found in Washington State (eight species from Puget Sound, plus razor clams from the outer coast), along with modeling results on DA assimilation efficiency and possible causes of inter-individual variability in DA concentration for four of the species.

MATERIALS & METHODS

All experiments were conducted at the University of Washington's Friday Harbor Laboratories on San Juan Island, Washington in the springs of 2008 and 2009. Experiments were divided into two phases: a feeding phase, where clams received a diet of DA-producing *Pseudo-nitzschia multiseriis* and their clearance rate was measured; and a depuration phase immediately following

the feeding phase, where clams were allowed to purge toxin from their tissues in unfiltered seawater and were periodically sampled for DA.

Cultures of *Pseudo-nitzschia multiseriis*

Clams were fed a toxin-producing strain of *Pseudo-nitzschia multiseriis* (CLNN-17 from Stephen Bates and Claude Leger) as a source of DA. This phytoplankter is a known producer of DA, is cosmopolitan in range, and is common as a component of phytoplankton assemblages in Puget Sound.

To grow *Pseudo-nitzschia multiseriis* in large volumes, natural seawater was filtered through a 10- μm cloth filter to remove zooplankton and larger particles, and combined with silica in the form of sodium metasilicate: Na_2SiO_3 . The seawater mixture was autoclaved in 3- to 10-L glass flasks, and following autoclaving, f/2 solution (manufactured by Fritz Industries, Inc) was added to the flasks to create f/2+Si growth medium (Guillard 1975). The cooled medium was decanted into sterilized 18-liter plastic carboys. Carboys were then inoculated with pure cultures of *P. multiseriis* and maintained at 13°C under 24-hr fluorescent light with moderate aeration, which served to balance pH (Lund et al. 1958). The cultures were used for the feeding experiments after they had been in stationary growth phase for at least three days to increase the probability of a relatively high DA content in the cells. At the time of use, cell densities in the cultures ranged between $6.12 - 14.5 \times 10^7$ cells \cdot L⁻¹ in 2008, and $1.64 - 7.52 \times 10^7$ cells \cdot L⁻¹ in 2009, determined using a Palmer Maloney slide to count at least 400 algal cells in two or three subsamples (Lund et al. 1958). Samples of the algal cultures for DA analysis were collected just prior to each experiment: 10mL of culture were filtered onto Millipore HA 0.45 μm filters, placed

in microcentrifuge tubes and frozen at -20°C for particulate DA analysis; and 5mL of the remaining filtrate were collected into plastic-capped centrifuge tubes and frozen at -20°C for dissolved DA analysis.

One concern in conducting clam feeding experiments on a chain-forming species such as *Pseudo-nitzschia* is that suspension-feeders may selectively reject longer chains of the phytoplankter. Under the culturing conditions, *P. multiseriis* cells generally did not form chains, but remained as single cells. Chain lengths were measured in four samples (680 cells total) from 2008 using a Sedgewick-Rafter counting slide under an inverted microscope to count all *P. multiseriis* cells or chains in a sample. Average chain length was 1.02 cells, and median and mode chain length was one cell. Qualitative inspection of cell cultures in 2009 indicated similar results.

Clam Feeding

Nine species of commonly harvested clams from Washington State beaches were tested: eight species were collected from mixed sand/gravel beaches in northern Puget Sound (Whidbey Island, San Juan Island and Orcas Island), and razor clams were collected from a sandy beach (Copalis Beach) on the outer coast (Table 1). For each species, individuals of similar size were collected for the experiments (Table 1). Following collection, clams were transported in water-filled, aerated coolers to Friday Harbor Labs, where individuals were measured and marked with a unique identification code. Clams were allowed to acclimate in flow-through aquaria of natural (unfiltered) seawater until the experiment date (27-47 days in 2008; 3 days for razor clams and 21 days for other species in 2009). In the acclimation aquaria, clams were separated

by species and placed into species-specific containers. Burrowing clams were placed into containers of sand, while Nuttall's cockles (*Clinocardium nuttallii*) and Pacific oysters were placed onto plastic trays without substrate. Water temperature in the acclimation aquaria varied between 8°C and 11°C.

Table 1. Clam species, collection location and size classes for feeding and depuration phases of the experiment. The last two columns are aquarium volume and number of clams used for clearance rate measurements that were conducted during the feeding phase of the experiment.

Common Name	Latin Name	Shell Length (mm)	Collection Location	Aquarium Vol (L)	# Clams
2008					
butter clam	<i>Saxidomus giganteus</i>	67-75	Cultus Bay, Whidbey Island	36.3-45.8	13-14
horse clam	<i>Tresus capax</i>	109-122	Cultus Bay, Whidbey Island	32.6-49.8	8-10
littleneck clam	<i>Protothaca staminea</i>	49-53	Cultus Bay, Whidbey Island	9.3-18.3	14
Nuttall's cockle	<i>Clinocardium nuttallii</i>	67-71	English Camp, San Juan Island	28-38.7	14
Pacific oyster	<i>Crassostrea gigas</i>	64-80	Argyle Lagoon, San Juan Island	41.5-65.3	13
2009					
Manila clam	<i>Ruditapes philippinarum</i>	46-55	Cultus Bay, Whidbey Island	83.3-84.7	33-38
purple varnish clam	<i>Nuttallia obscurata</i>	42-47	Crescent Beach, Orcas Island	26.1-26.2	33-38
razor clam	<i>Siliqua patula</i>	100-120	Copalis Beach, Washington outer coast	80-82.2	34-42
soft-shell clam	<i>Mya arenaria</i>	72-82	Cultus Bay, Whidbey Island	38.7-39.8	27-32

Five Puget Sound clam species were tested in 2008: butter clam (*Saxidomus giganteus*), horse clam (*Tresus capax*), littleneck clam, Nuttall's cockle and Pacific oyster. The experiments for each species were based on ten to 14 individuals. All species, along with their sand-filled containers or plastic trays, were placed together into one large (~550L) aquarium and allowed to acclimate for two hours. The aquarium was equipped with air-stone bubblers and submersible water pumps to provide oxygenation and water circulation; water temperature ranged between

9.5°C and 11°C. Over a 15-day period, *Pseudo-nitzschia multiseriis* culture was added to the aquarium twice daily (every 12 hours). At each feeding, 8L of toxin-producing *P. multiseriis* culture were gently poured into the aquarium. Prior to the first feeding of the day, visible parts of the bivalves were inspected for signs of poor health (tops of shells gaping widely; droopy siphons on horse clams restricting water intake), and suspect individuals were removed. The aquarium was cleaned every two to three days: water was removed and aquarium walls were washed before the aquarium was again refilled with unfiltered natural seawater. It was not necessary to remove clams and their containers from the aquarium during the cleaning.

For three of the 30 feedings in 2008, clams in their containers were removed from the large aquarium and transferred to individual aquaria according to species so species-specific clearance rates on *Pseudo-nitzschia multiseriis* could be obtained (methodology described below). Each aquarium was equipped with air-stone bubblers for water circulation and aeration, and water temperatures were 10-11°C. Aquarium volume varied by species, as did number of individuals in each aquarium (Table 1).

Four other Puget Sound clam species were included in the 2008 experiments: soft-shell clam *Mya arenaria*, purple varnish clam *Nuttallia obscurata*, Manila clam and bay mussel. The data for these species did not fit the assumed default depuration model, and the data were not rich enough to explore more complex depuration models. Consequently, three of the four species (all but bay mussel, whose close cousin, blue mussel, had already been tested by other authors) were re-tested in 2009 to obtain data over more time points and to assess inter-individual variability in tissue DA concentrations. Based on the 2009 findings, it appears that inter-individual variability

in DA measurements was high at the low tissue DA concentrations attained in 2008; so analyses presented in this paper are based on the 2009 data set for the re-tested species.

In addition to the three Puget Sound species that were retested in 2009 (soft-shell clam, purple varnish clam, and Manila clam), Washington outer coast razor clams were also tested in 2009. For this round of experiments, clams were placed into separate aquaria by species. Aquarium volume and number of clams varied in each aquarium (Table 1). Air-stone bubblers and submersible water pumps (for large aquaria) provided oxygenation and water circulation in the aquaria, and water temperatures were 8-10°C. Clams were fed twice daily over a three-day period, for a total of six feedings. During four of these feedings, clearance rate measurements were carried out as described below. Once a day, visible parts of clams were inspected, and individuals suspected to be in poor condition were removed. Aquaria were also cleaned on a daily basis by flushing with unfiltered seawater for about four hours while the clams remained in place; aquarium walls were also wiped with a sponge to remove any remaining phytoplankton cells clinging to the walls.

At the conclusion of both feeding experiments, clams were placed into flow-through aquaria of natural seawater and allowed to depurate DA for up to 15 days. At designated time-points, clams were lethally sampled for DA concentration in their tissues. Sample collection methodology and timing is described below.

Clearance Rate Measurements

Clams were allowed to acclimate in individual aquaria for one hour before an algal suspension of *Pseudo-nitzschia multiseriis* was added to each aquarium in 2008. The acclimation period prior to the first clearance rate measurement in 2009 was also at least one hour; however, clams remained in their individual tanks for the duration of the 2009 experiment, so no acclimation period was necessary prior to subsequent clearance rate measurements. The initial concentration of *P. multiseriis* during clearance rate measurements in 2008 ranged between 1070 – 2470 cells · mL⁻¹, while in 2009 this range was 700 – 3300 cells · mL⁻¹. These concentrations are within the range observed in field samples during Puget Sound *Pseudo-nitzschia* blooms (Bill et al. 2006, Trainer et al. 2007). Following addition of algal suspension to the aquaria, clams were given a five-minute period of exposure to *P. multiseriis* before data collection began, to stimulate valve opening and the initiation of filtering activity.

Throughout each clearance rate measurement, three to five water samples were collected over 2.5 to four hours following addition of *Pseudo-nitzschia multiseriis* to the aquaria. Water samples were also collected immediately before algal culture was added to aquaria, for the purpose of measuring the quantity of phytoplankton present in the natural seawater. Two types of water samples were collected at each sampling time step: 20-mL samples for chlorophyll *a* (chl *a*) analysis, and 5-mL preserved samples for phytoplankton counts. Chl *a* samples were collected in triplicate for the purpose of measuring decline of all phytoplankton in the aquaria. Samples were immediately filtered through GF/C filters that were then wrapped in aluminum foil and frozen for later extraction. Filters were extracted in acetone and measured for chl *a* corrected with acidification using a TD-700 fluorometer from Turner Biosystems (Parsons et al.

1984). Preserved water samples were collected for the purpose of measuring *Pseudo-nitzschia multiseries* chain lengths, and determining concentrations of *P. multiseries* in the algal cultures. Samples were collected into 7-mL glass scintillation vials, preserved with 100 μ L of Lugol's iodine solution, and kept in cool and dark conditions until analysis. Prior to use, samples were gently agitated, and a 1-mL subsample was placed onto a Sedgwick-Rafter counting slide and examined under an inverted microscope. At least 30 fields or 300 cells (whichever came first) were counted to determine *P. multiseries* concentration.

Clearance rate measurements were conducted in natural seawater, so very low levels of other phytoplankton taxa were present in addition to the inoculated dominant *Pseudo-nitzschia*. The background chl *a* of the natural seawater used in the experiments was low: $\leq 0.4 \text{ ug} \cdot \text{L}^{-1}$ in 2008; $\leq 2.3 \text{ ug} \cdot \text{L}^{-1}$ in 2009. Following addition of *P. multiseries* to the aquaria, chl *a* rose to $>4.5 \text{ ug} \cdot \text{L}^{-1}$ in 2008 and $>11.3 \text{ ug} \cdot \text{L}^{-1}$ in 2009, so it is possible to be confident that measuring chl *a* decline provides valid estimates of clam clearance rates on *P. multiseries*.

As defined by Coughlan (1969), clearance rate was calculated as the volume of water “cleared” of phytoplankton per unit time by a consumer,

$$CR = \frac{V}{n} \cdot \left[\frac{\ln C_0 - \ln C_t}{t} \right] \quad \text{Eqn. 1}$$

where *CR* is the clearance rate of a single animal, *V* is the volume of water used in the experiment, *C*₀ and *C*_{*t*} are the initial concentration of phytoplankton and the concentration after time *t*, and *n* is the number of animals. *CR* measures the average clearance rate for one clam in the aquarium because there were ten or more bivalves in each of the experiments.

For each clam species, decline in phytoplankton concentration was measured in three (2008) or four (2009) clearance rate measurement experiments, after which linear mixed effects models were applied to the data for each species to obtain a single clearance rate value (nine models total, one per species). Linear mixed effects models, in which the replicate clearance rate experiments were treated as a random effect, were used to remove concerns due to pseudo-replication and hence appropriately estimate the uncertainty of the clearance rate estimate (Zuur et al. 2009). In the three or four experiments per species, there was variability in several aspects of the experiment: initial *Pseudo-nitzschia multiseriis* concentration, aquarium size, and number of clams. Within each species, the variability between experiments in aquarium size and number of clams was small; and although clams have been shown to reduce their pumping rate in response to very low (e.g., Riisgard et al. 2003) or very high (e.g., Foster-Smith 1975) concentrations of phytoplankton, the range of initial *P. multiseriis* concentrations was not expected to trigger reductions in clearance rate. The impacts of aquarium size, number of clams, and initial phytoplankton concentration are captured by a normally-distributed random effect for replicate. The linear mixed model is derived from Eqn 1:

$$\frac{V_i}{n_i} \cdot \ln C_{i,t} = \alpha + b_i - CR \cdot t + \varepsilon_{i,t} \quad \text{Eqn. 2}$$

where i refers to each replicate clearance rate experiment, α and b_i determine the y-axis intercept for each replicate experiment (the intercept for the population of replicates is α), CR is the common slope, and $\varepsilon_{i,t}$ is normally-distributed residual error.

Sampling of Clams for DA

At each sampling time point, clams were removed from the water and placed onto clean towels for 30 to 60 minutes to drain seawater retained within the shells. Clams were then placed into

sealed freezer bags and frozen until DA analysis could be completed. Three clams of each of several species were sampled prior to the start of the feeding phase of the experiments to assess initial DA concentration in their tissues, although no DA was expected because no *Pseudo-nitzschia* blooms had been reported in the regions from which the clams had been recently collected.

The first clam DA samples were collected 14 hours after the last feeding, to allow clams to consume available *Pseudo-nitzschia* and purge or digest any whole phytoplankton cells remaining on their gills. This first DA sampling represents the time point $t=0$ in the depuration rate models.

During the 2008 experiments, subsequent DA samples were collected 2, 5, and 9 days after the initial DA sample. For each sample, two to three clams per species were analyzed together to obtain an average DA concentration.

In 2009, DA samples were collected 1, 2, 4, 8 and 15 days after the initial DA sample for Puget Sound species; and 2, 4 and 9 days after the initial DA sample for outer coast razor clam. In addition, several clams of each species were removed halfway through the feeding experiment (12 hours after the third feeding), and held in clean natural seawater, allowing them to purge their gills for 2 hours before being sampled for DA. The 2009 experiments were structured to assess inter-individual variability in DA uptake and depuration in four species, so each clam was analyzed for DA individually.

DA Depuration Analysis

Within two weeks of the experiments, frozen clams were thawed and dissected to remove all soft tissue, which was then blended to a fine homogenate. Clam homogenate was analyzed for DA using enzyme-linked immunosorbent assay (ELISA) test kits from Biosense Laboratories, and following the Biosense methodology (Kleivdal et al. 2007). Water samples for particulate and dissolved DA were also analyzed within two weeks of the experiment using ELISA test kits and following the Biosense protocol.

The rate of DA depuration from clam tissue was calculated for each species using a one-compartment exponential decay model,

$$DA_t = DA_0 e^{-rt} \quad \text{Eqn. 3}$$

where DA_t is domoic acid concentration after t days, DA_0 is domoic acid concentration at the beginning of the depuration portion of the experiment, r is the depuration rate, and t is days elapsed. DA_0 and r were estimated using linear regression after log-transformation.

Modeling

All data analyses were conducted using the open source statistical programming language R (The R Project for Statistical Computing).

Species specific models were created using the 2009 data to explore the reasons behind observed variability in clam DA concentrations, and to calculate the DA assimilation efficiency: the percentage of ingested DA (contained within *Pseudo-nitzschia* cells) that is actually incorporated into clam tissue. The data inputs were: clearance rate, DA depuration rate, DA concentrations in

individual clams, and DA content in *P. multiseriis* cells that were fed to the clams. Using measured clearance rates, the models tracked how much *P. multiseriis* was filtered by clams in each aquarium, and recorded decline of the phytoplankton as well as maximum possible accumulation of DA in clam tissue (assuming all filtered *P. multiseriis* resulted in DA within clam tissue). The models, which have a 15-minute time-step, were based on the following assumptions: 1) clearance rate remains constant throughout the experiment; 2) DA depuration occurs concurrently with consumption of DA-containing *P. multiseriis*; and 3) DA depuration occurs at a constant rate regardless of the type and concentration of food surrounding the clams. The models were fitted to existing DA concentration data for each species, assuming a normal distribution for the likelihood function. Steps in the models were:

- 1) tracking phytoplankton concentration in the aquarium,

$$[Pn]_j = [Pn]_{j-1} \cdot \frac{V - CR \cdot n \cdot t}{V} \quad \text{Eqn. 4}$$

where $[Pn]_j$ is the *P. multiseriis* concentration in the aquarium at time step j , V is the volume (liters) of water in the aquarium, CR is clearance rate by one clam, n is the number of clams in the aquarium, and t is length (in hours) of one time step;

- 2) tracking DA accumulation per clam,

$$[DA]_j = [DA]_{j-1} + AE \cdot CR \cdot t \cdot \frac{[Pn]_j + [Pn]_{j-1}}{2} \cdot c[DA] \cdot \frac{1}{W} \quad \text{Eqn. 5}$$

where $[DA]_j$ is the DA concentration in one clam at time step j , AE is assimilation efficiency, $c[DA]$ is the concentration of DA in one cell of *P. multiseriis*, which changes for every feeding, and W is clam weight. Various values between 0 and 1 were tested for AE before estimating this parameter using maximum likelihood estimation; and

3) tracking DA depuration per clam,

$$[DA]_{j'} = [DA]_j \cdot e^{-r \cdot \frac{t}{24}}$$

Eqn. 6

where j' refers to the end of time step j .

The species-specific models were used to explore the effect on clam DA concentrations of inter-individual variability around clearance and depuration rates. In one scenario, clearance rate was increased for some individuals by 50% and decreased for others by 50%, such that the mean clearance rate for all clams remained constant. This created a predicted high and low clam DA concentration at each time point. In another scenario, the depuration rate was similarly increased by 50% for some individuals, and decreased by 50% for others, such that the mean depuration rate remained constant. The proportion of clam DA data points that fell between the high and low predictions created by varying clearance rate was then compared to the proportion of clam DA data points that fell between the high and low predictions created by varying depuration rate, to determine which type of inter-individual variability accounted for a larger proportion of the DA data.

To better understand the interplay of DA accumulation and depuration dynamics for each species in this study, a bloom of *Pseudo-nitzschia multiseries* that maintained a constant concentration of 1500 cells · L⁻¹ and toxicity of 7 pg DA · cell⁻¹ was also modeled. Clams were fed the bloom for 12 days, after which time the bloom ended and the clams depurated DA for 50 days. The model was designed with the assumptions that each species has a constant clearance and depuration rate, regardless of tissue DA concentration; that the measured depuration rates also apply at high

DA concentrations; and that AE is 100% for all species where AE was not estimated using maximum likelihood estimation.

RESULTS

Phytoplankton

Pseudo-nitzschia multiseriis concentration at the beginning of each feeding ranged between 885 and 2550 cells · mL⁻¹ over the 30 feedings in 2008, and between 700 and 3300 cells · mL⁻¹ over the six feedings in 2009. Particulate DA per liter was 0.3 to 14.6 µg · L⁻¹ in 2008, and 20.5 to 54.0 µg · L⁻¹ in 2009. By dividing the particulate DA per liter by the number of *P. multiseriis* cells per liter, values of DA per *P. multiseriis* cell are obtained: 0.3 to 11.9 picograms (pg) · cell⁻¹ in 2008, and 9.3 to 71.3 pg · cell⁻¹ in 2009.

Clam Clearance and Depuration Rates

Clearance rates (± one standard deviation) ranged from 0.4 L · hr⁻¹ (±0.02) in soft-shell clam to 5.2 L · hr⁻¹ (± 0.5) in Pacific oyster (Fig 1). The maximum DA concentration in clam tissue at the beginning of the depuration phase (t=0), was observed to be 4.2 mg · kg⁻¹ in 2008 (Pacific oyster), and 34.4 mg · kg⁻¹ in 2009 (purple varnish clam). Only two species (purple varnish clam and Manila clam) attained DA concentrations above the 20 mg · kg⁻¹ threshold set by the Washington State Department of Health. The exponential depuration rates from linear regressions (Eqn. 3) were lowest in razor clam (0.03 day⁻¹ ±0.09) and purple varnish clam (0.11 day⁻¹ ±0.05), and highest in horse clam (0.78 day⁻¹ ±0.03), butter clam (0.84 day⁻¹ ±0.06) and Pacific oyster (0.86 day⁻¹ ±0.07) (Figs. 2 & 3). Estimates of inter-individual variability (standard

deviations) in DA concentration over all time points for 2009 experiments were: Manila clam: $sd=1.5\text{mg} \cdot \text{kg}^{-1}$; soft-shell clam: $sd=1.7\text{ mg} \cdot \text{kg}^{-1}$; razor clam: $sd=2.1\text{mg} \cdot \text{kg}^{-1}$; and purple varnish clam: $sd=3.4\text{ mg} \cdot \text{kg}^{-1}$.

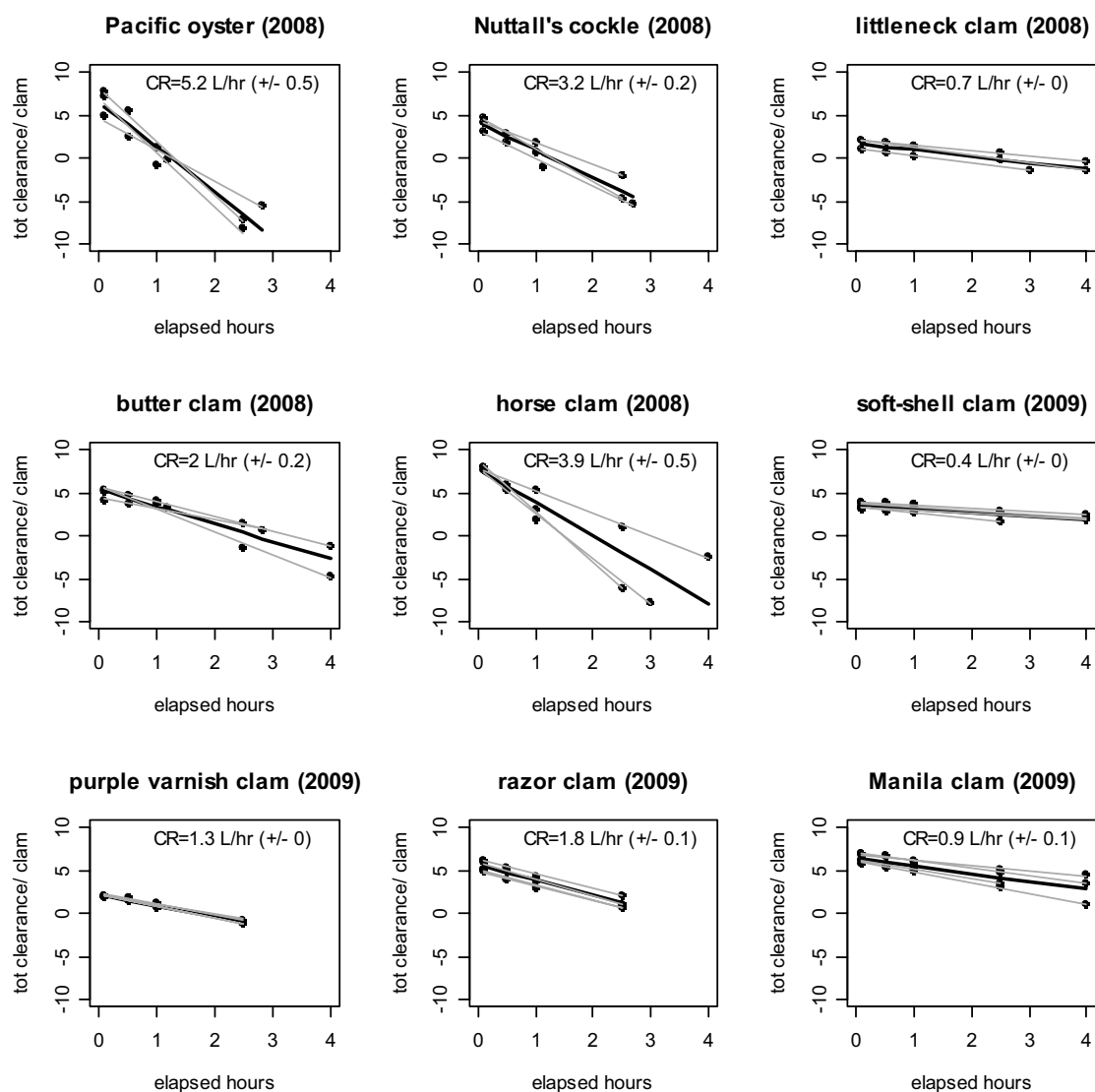


Figure 1. Clearance rate on *Pseudo-nitzschia multiseriis* per clam for nine species, calculated over three feedings in 2008, and four feedings in 2009. The y-axis is defined as total clearance per clam = aquarium volume/ number of clams $\cdot \ln(\text{chl } a \text{ concentration})$. Thick lines represent clearance rates obtained from linear mixed effects models fitted to all data simultaneously (slope reported within each plot as clearance rate (CR) \pm standard deviation), while thin grey lines represent linear regressions fitted to data from each feeding separately. Black dots represent data for groups of clams in each experiment.

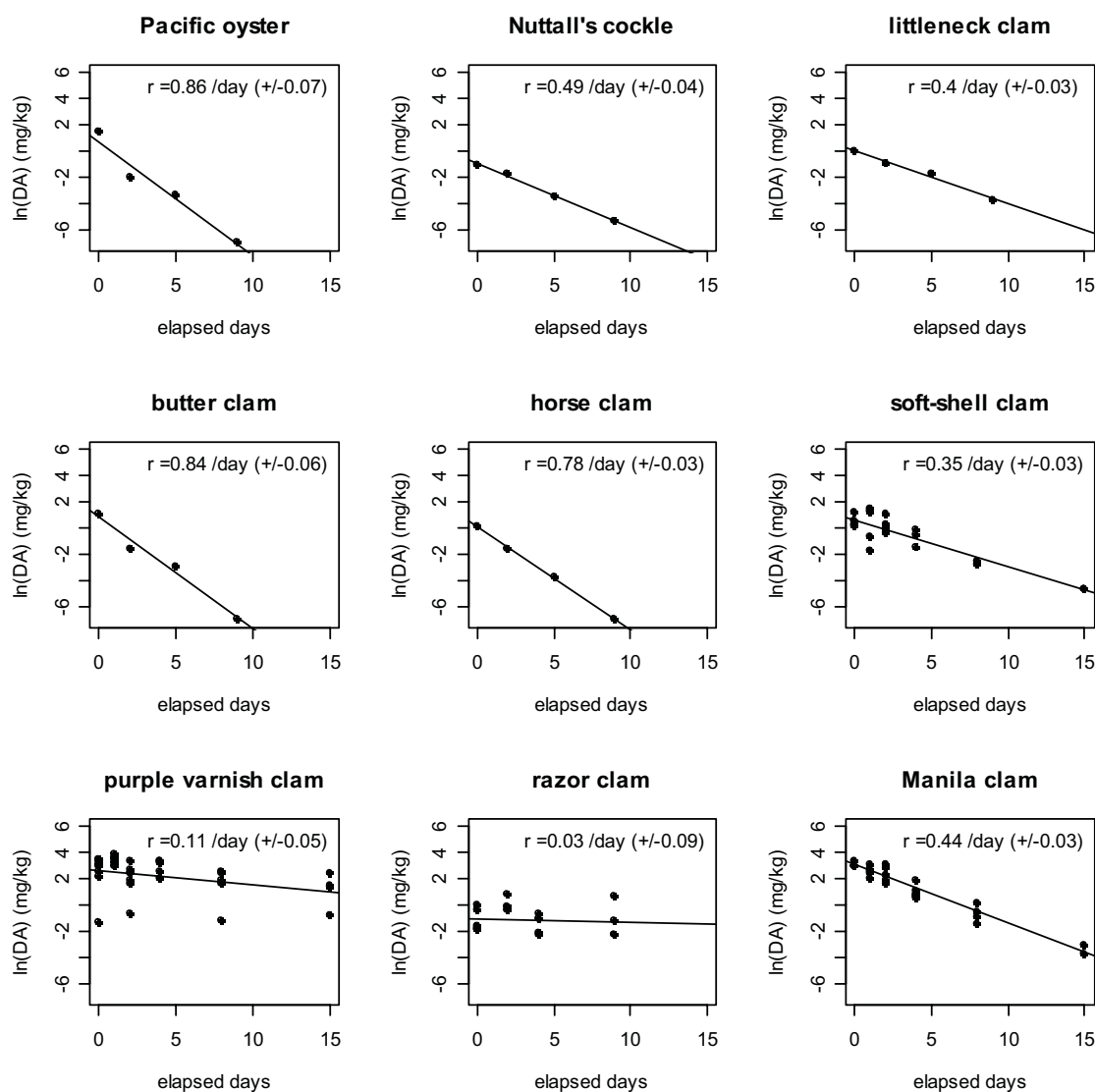


Figure 2. Exponential DA depuration rate per day (r) for each clam species. Slope of the regression line is provided in text, along with standard deviation. Species in the first five plots were analyzed for DA as composite samples of 2-3 clams per sample, while species in the last four plots were analyzed for DA as individual clams. On the x-axis, 0 corresponds to the beginning of the depuration portion of the experiment, when clams were no longer being fed toxin-producing *Pseudo-nitzschia multiseries*.

Domoic Acid Depuration Rate for Exponential Depuration Model

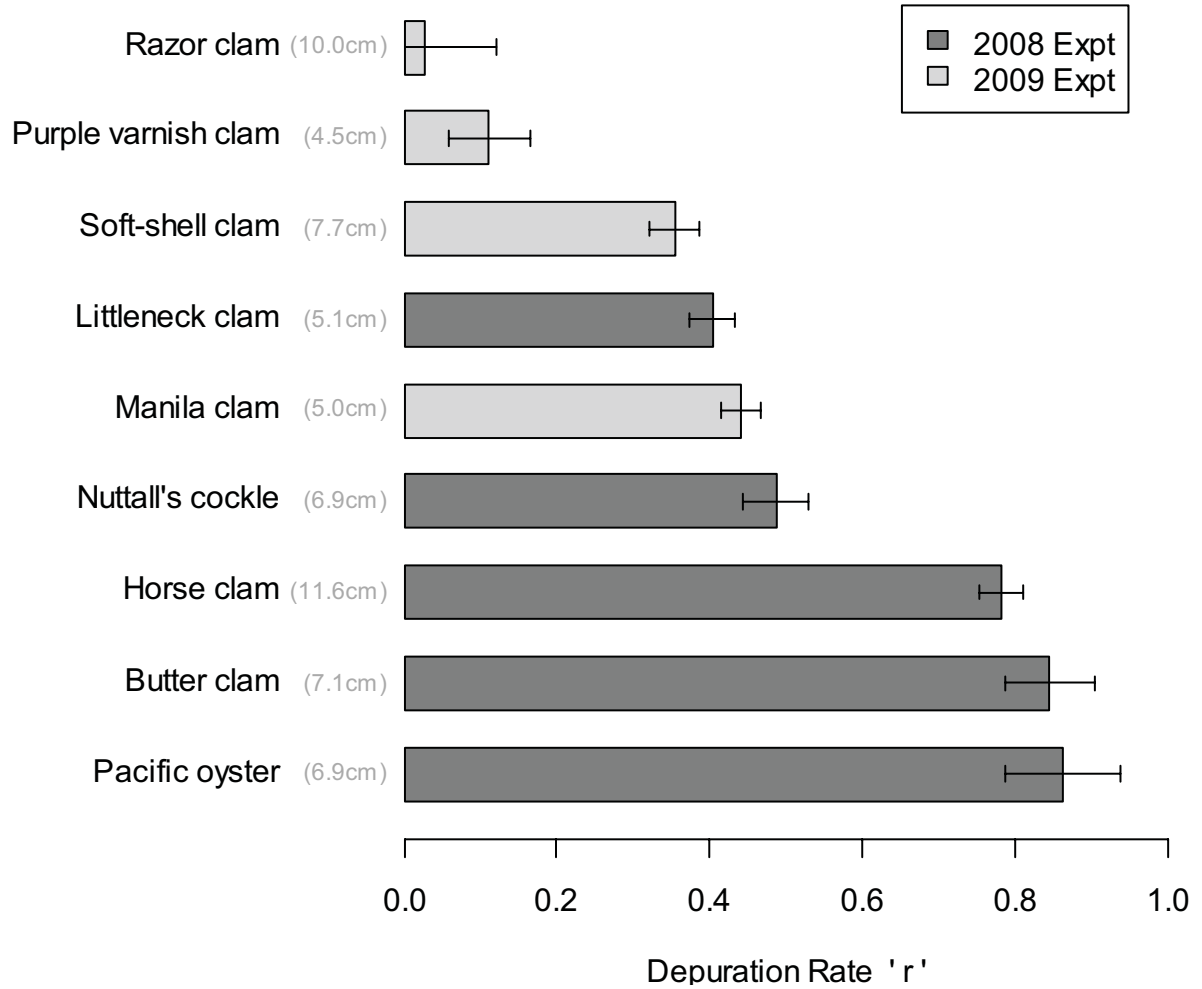


Figure 3. Domoic acid depuration rates for each clam species (shell length in parenthesis). Error bars indicate standard error around the slope estimate (which equals depuration rate, r) from regression analysis.

DA Uptake & Depuration Model

The maximum likelihood estimates of AE from the 2009 experiments were: AE=59% in soft-shell clam, AE=11% in razor clam, and AE~100% in purple varnish clam and Manila clam (Fig. 4). Once AE was included in the models, simulations that allowed for inter-individual variability in clearance rate (range of +/- 50% of average clearance rate; dashed lines in Fig. 4), resulted in a higher proportion of data points falling between the dashed lines for all species except Manila clam, than simulations that allowed for inter-individual variability in depuration rate (range of +/- 50% of average depuration rate) (Table 2).

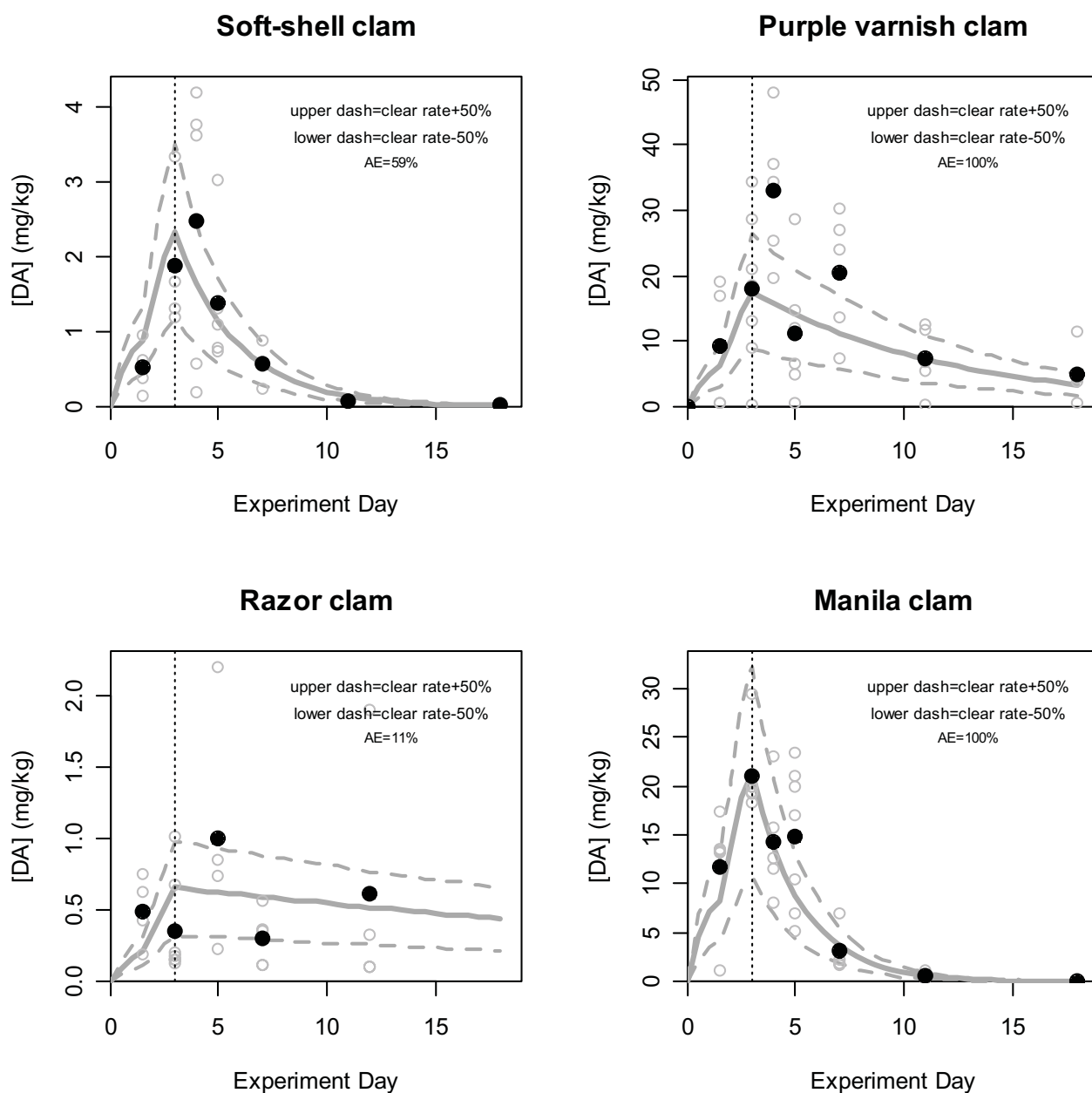


Figure 4. Modeled results from the domoic acid uptake and depuration experiments. Actual DA concentrations in clam tissue are shown in hollow grey circles, while filled black circles show average DA concentration in all shellfish from that time point. On the x-axis, Experiment Day=0 marks the beginning of the feeding phase of the experiment, and the dotted vertical line at Experiment Day=3 marks the end of the feeding phase and the beginning of the depuration phase. Solid grey lines indicate modeled clam DA concentrations if all clams feed at the measured average clearance rate. Dashed grey lines show modeled clam DA concentrations if some clams feed at the average clearance rate + 50%, and an equal number feed at the average clearance rate - 50%.

Table 2. Proportion of clam DA data falling between the upper and lower model predictions generated by allowing inter-individual variability in clearance rate ($\pm 50\%$) or inter-individual variability in depuration rate ($\pm 50\%$).

	inter-individual variability in clearance rate ($\pm 50\%$)	inter-individual variability in depuration rate ($\pm 50\%$)
razor clam	0.33	0.04
purple varnish clam	0.39	0.24
soft-shell clam	0.59	0.48
Manila clam	0.57	0.69

Scenario Bloom to Predict DA Concentrations over Time

Given a constant *Pseudo-nitzschia multiseries* concentration and toxicity, the modeled clam DA concentration curves for most species suggest a species-specific asymptote for toxicity, where DA uptake equals DA depuration (Fig. 5). This asymptote shifts if the *P. multiseries* concentration or toxicity changes, but the relative magnitudes of the curves for each species do not change. Profiles are only presented for the four most dynamic species; the remaining five species all had curves similar in shape but lower in magnitude than the profile shown for Manila clam. The highest DA concentrations are predicted in purple varnish clams, and the duration of toxicity ($> 20 \text{ mg} \cdot \text{kg}^{-1}$) is longest in this clam as well. Razor clams do not accumulate enough DA to reach the Washington State Department of Health safety threshold ($20 \text{ mg} \cdot \text{kg}^{-1}$) at the *P. multiseries* concentration and toxicity that was modeled.

Modeled [DA] in Clams During and After a Hypothetical *Pseudo-nitzschia* Bloom

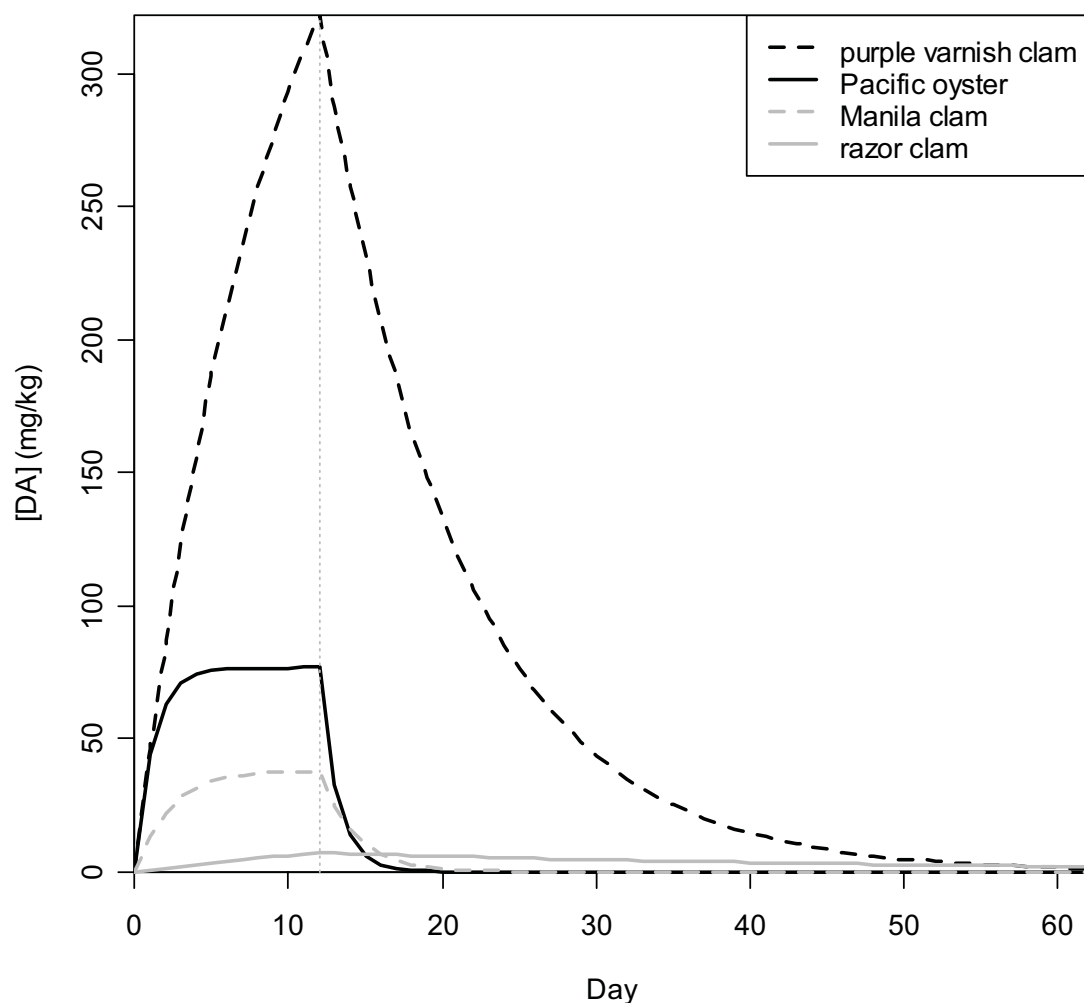


Figure 5. Modeled results for a hypothetical 12-day bloom and 50-day post-bloom depuration phase for four clam species. On the x-axis, Day 0 marks the beginning of a bloom that provides clams with *Pseudo-nitzschia* at a constant concentration of $1500 \text{ cells} \cdot \text{L}^{-1}$ and a constant cellular DA of $7 \text{ pg} \cdot \text{cell}^{-1}$. Day 12 (vertical dotted line) marks the end of the bloom and the beginning of the depuration phase, where *Pseudo-nitzschia* concentration is $0 \text{ cells} \cdot \text{L}^{-1}$. All other species discussed in this paper had DA concentration profiles that were similar to Manila clam in shape, but fell below the curve depicted for Manila clam.

DISCUSSION

In this study, I measured the rates at which seven species of clams depurate low concentrations of DA ($<5 \text{ mg} \cdot \text{kg}^{-1}$), and the rate at which two species of clams depurate elevated concentrations

of DA ($>20\text{mg} \cdot \text{kg}^{-1}$). Clearance rates were also measured during the experiments, and assimilation efficiency was estimated for four species using maximum likelihood estimation. Of the nine species that were tested, Pacific oyster cleared *Pseudo-nitzschia multiseriata* at the fastest rate ($5.2 \text{ L} \cdot \text{hr}^{-1} \pm 0.5$; assimilation efficiency assumed to be 100%), but also depurated DA toxin at the fastest rate ($0.86 \text{ day}^{-1} \pm 0.07$). In contrast, razor clam cleared the diatom from seawater at a substantially slower rate ($1.8 \text{ L} \cdot \text{hr}^{-1} \pm 0.1$; assimilation efficiency = 11%), and depurated DA at the slowest rate ($0.03 \text{ day}^{-1} \pm 0.09$). The combined effects on clam DA concentrations of clearance, assimilation and depuration rates indicate that purple varnish clam should attain the highest toxicity and maintain it for the longest duration of time (Fig. 5).

One-Compartment Depuration Rate Models

A simple exponential decay model, also called a one-compartment model, was used for the depuration rate calculations. This type of model has been used effectively to model DA depuration in blue mussel (Novaczek et al. 1992, Silvert & Rao 1992, Mafra et al. 2010a), Mediterranean mussel (*Mytilus galloprovincialis*) (Blanco et al. 2002), and Atlantic oyster (*Crassostrea virginica*) (Mafra et al. 2010a). The term “one-compartment” refers to tissue compartments and, as its name indicates, this model assumes that toxin depuration from clam tissue occurs at a uniform rate. This is a simplified way to view depuration. Douglas et al. (1997) describe DA transfer between tissues in sea scallop (*Placopecten magellanicus*) whereby toxin enters through the digestive gland and gradually transfers to other tissues through circulation in the hemolymph. Although several authors have used two-compartment models to characterize phycotoxin depuration dynamics in clams (e.g., Silvert & Cembella 1995, Blanco et al. 2002), the data in this paper fit the one-compartment model well, and may be more

appropriate because DA concentrations in this study are based on whole animal homogenates rather than on individual tissues (Silvert & Cembella 1995).

Possible Causes of Reduced DA Assimilation Efficiency

AE was estimated for soft-shell clam, purple varnish clam, razor clam and Manila clam using maximum likelihood. It appears that razor clams only assimilated a small amount (11%) of the DA that they ingested, while soft-shell clams assimilated 59% of ingested DA. However, the experimental methodology did not allow identification of the physiological processes that result in reduced DA assimilation in these species. Other authors have reported AEs less than 100% for various clam species. Douglas et al. (1997) found that sea scallop fed on toxin-producing *Pseudo-nitzschia multiseries* for 22 days incorporated only 51% of the DA available to them. Wohlgeschaffen et al. (1992) reported very low assimilation efficiency in the blue mussel (1-5%), speculating that DA was lost into solution during digestion. Mafra et al. (2010a) found that eastern oyster (*Crassostrea virginica*) and blue mussel fed selectively when non-diatom phytoplankton were present, rejecting *P. multiseries* as pseudofeces, and thereby accumulating much lower DA levels than predicted from clearance rates.

Rejection as pseudofeces was at least partially responsible for loss of ingested DA in razor clam and soft-shell clam: pseudofeces were observed in the aquaria for these two species following feeding on *Pseudo-nitzschia multiseries*. It is unlikely that pseudofeces formation was a result of selective feeding *per se*, as non-*Pseudo-nitzschia* phytoplankton only made up a small percentage (<20%) of phytoplankton biomass available to the clams. Rather, it seems more plausible that pseudofeces formation occurred either in response to high phytoplankton

concentrations, or in response to chemical or physical properties of *Pseudo-nitzschia* that make it less desirable for consumption. Mafra et al. (2009) showed this type of rejection in pseudofeces as one way in which juvenile eastern oysters limit DA concentration in their tissues.

In these experiments, where the algal assemblage was nearly monospecific for *Pseudo-nitzschia multiseriis*, razor clams did not attain DA concentrations as high as expected based on *in situ* clam DA concentration data from the Washington outer coast (WDFW 2011). On the outer Washington coast, *Pseudo-nitzschia* tend to make up a much smaller fraction (5-10%) of the algal assemblage, even during blooms (Olson 2006). If particle rejection mechanisms utilized by razor clams are enhanced under conditions where *Pseudo-nitzschia* overwhelmingly dominate the phytoplankton assemblage, it could result in low AE estimates generated in the laboratory. Physiological responses, in the form of clearance rate reductions, have been shown in eastern oyster fed on monospecific assemblages of *P. multiseriis* (Mafra et al. 2009). Perhaps razor clam particle rejection mechanisms are also more active when *Pseudo-nitzschia* dominate the available phytoplankton.

Management Effects of Variability in Clam DA Concentrations

The amount of inter-individual variability in DA concentration varies by bivalve species. Manila clam and purple varnish clam attained the highest DA concentrations; however, purple varnish clam showed much higher variability between individuals in DA concentration than did Manila clam. Knowledge of species-specific levels of variability could be useful in determining sampling regimes for DA in field populations of suspension-feeding clams. Other species with high levels of inter-individual variability in DA concentration include blue mussel (Gilgan et al.

1990), sea scallop (Douglas et al. 1997), Mediterranean mussel (Blanco et al. 2002), and razor clam (Wekell et al. 2002). In a study on the effects of tidal height and beach location on DA content in razor clams along the Washington coast, Wekell et al. (2002) found high levels of variability within transects, and a coefficient of variation among all samples of 122%. The authors pointed out that harvest closures based on composite samples of six razor clams may result in erroneous false positives, although false negatives could also result. This may also be true in Puget Sound for composite samples of blue mussel or other clams with high inter-individual variability in DA concentrations. Choosing sentinel clam species with inherently lower inter-individual variability in DA content may help reduce error around beach closure decision analysis.

Inter-Individual Variability in Clearance Rate

Allowing for inter-individual variability in clam clearance rates within the model accounted for a greater proportion of clam DA data for three of four species, than model scenarios that allowed for inter-individual variability in depuration rate (Fig. 4). Over the course of multi-day experiments, it is possible that not all clams will be open and feeding at all times. In fact, some purple varnish clams in these experiments contained DA concentrations in excess of $25\text{mg} \cdot \text{kg}^{-1}$ following 3 days of *P. multiseriis* consumption, while others showed DA concentrations of $0\text{mg} \cdot \text{kg}^{-1}$. Model scenarios which added inter-individual variability in depuration rate to the model did improve fit with data points later in the time series, but did not address variability in DA concentrations from the middle of the feeding phase or the first two days of the depuration phase. To explore whether cessation of feeding in response to low phytoplankton concentrations could account for variability in clam DA concentrations at each time point, a scenario was also

modeled whereby clams feed and depurate until the *P. multiseriis* concentration in the seawater dips below a low threshold, at which point clams either reduce or cease feeding and depuration. Although this situation may occur in natural environments, this particular modeling scenario did not account for the variability in measured clam DA concentrations.

Experiment Limitations

It was only possible to attain low modeled concentrations of DA in clam tissue during the feeding phase of the experiments. A few of the phytoplankton cultures in 2009 attained high concentrations of DA per cell ($>50 \text{ pg} \cdot \text{cell}^{-1}$); however, the majority of cultures from 2008 had much lower cellular DA ($<5 \text{ pg} \cdot \text{cell}^{-1}$). It was not possible to analyze phytoplankton cultures in real-time for DA content; thus I did not know exactly when to commence the experiment to maximize cellular DA content in the cultures.

When clam DA concentration is high, depuration rates may differ from those obtained under low DA concentrations, both in magnitude as well as in data fit to an exponential decay model. In modeling DA flux through blue mussels on Prince Edward Island between 1988 and 1990, Silvert and Subba Rao (1992) found that their model showed good fit to blue mussel DA data at all times except when DA content was very high ($\sim 200 - 300 \text{ mg} \cdot \text{kg}^{-1}$). The authors surmise that DA depuration in the blue mussel occurs at a slower rate once high concentrations of toxin have accumulated. While the impact of DA concentration on depuration rate may be species-specific, the depuration rate results presented in this paper can only be confidently applied to clams containing low concentrations of DA.

The effect of clam size may also be an important factor in determining DA assimilation efficiency and depuration rate. Mafra et al. (2010a) reported significant effects of body size on DA uptake and depuration for eastern oyster, but not for blue mussel. In their study, both DA uptake and depuration occurred at faster rates in smaller oysters than in larger individuals. The experiments of this paper were limited by only being able to test depuration rate and assimilation efficiency for one size interval for each clam species.

Conclusion

Exponential DA depuration rates modeled in this study ranged between 0.03 and $0.86 \cdot \text{day}^{-1}$, with the highest rates in Pacific oyster followed by butter clam, and the lowest rates in razor clam followed by purple varnish clam. DA assimilation efficiency was very low in razor clam: $AE=11\%$; higher in soft-shell clam: $AE=59\%$; and highest in purple varnish clam and Manila clam: $AE\sim 100\%$. In a modeled scenario to compare nine species in terms of DA toxicity over time, purple varnish clam showed the highest DA concentrations and the longest duration of toxicity. With its low DA depuration rate and high DA assimilation efficiency, purple varnish clam may be a good sentinel organism for testing beaches for DA toxicity. High inter-individual variability in DA concentration was observed in purple varnish clam and other species, indicating the benefits of testing several individuals separately, rather than as a composite sample, to determine DA toxicity in clams. Model sensitivity analysis indicated that variability in DA concentrations in this study were more likely linked to inter-individual variability in clearance rates than inter-individual variability in depuration rate. This implies that inter-individual differences in feeding on *Pseudo-nitzschia* may be driving the variability in DA

concentrations that has been reported in some species following toxin-producing blooms of *Pseudo-nitzschia*.

Chapter V:

Modeling *Pseudo-nitzschia* Consumption and Domoic Acid Cycling in Puget Sound and Washington Outer Coast Clams

ABSTRACT

The cosmopolitan diatom genus *Pseudo-nitzschia* includes a number of species that are capable of producing the neurotoxin domoic acid (DA). When blooms of toxin-producing *Pseudo-nitzschia* are advected over intertidal shellfish beds, suspension-feeding clams, including mussels and oysters, feed on the diatom and bio-accumulate DA in their soft tissues. This results in a potential threat for humans, marine mammals, and sea birds that consume DA-tainted clams. On the outer coast of Washington State, DA has been responsible for numerous harvest closures for razor clams (*Siliqua patula*). In Puget Sound, however, only three harvest closures have occurred because of DA. This disparity raises the question: Why has *Pseudo-nitzschia* only caused three harvest closures in Puget Sound, while the coastal razor clam fishery has been plagued by frequent closures? In this chapter, I hypothesized that: 1) clam competition in Puget Sound reduces *Pseudo-nitzschia* availability and thus DA toxicity in all clams; and, 2) the physiology of Puget Sound clams reduces DA uptake or retention relative to Washington outer coast razor clams. I explore some of the factors that affect DA concentrations in clams after a *Pseudo-nitzschia* bloom has already been established using integrated oceanographic and food web models. Inputs to my model include: clam assemblage composition and abundance, beach slope profiles, tidal advection, suspension-feeding rates, domoic acid assimilation efficiency, and

depuration rates. Model results indicate several physical factors that are important in dictating clam DA concentrations, including: tidal height of clams, slope of the beach, clam abundance, and density of clams at low tidal elevations. In addition, domoic acid uptake and depuration rates play a major role in regulating DA concentrations in clams, and thus affecting harvest closure regimes on the outer Washington coast and in Puget Sound.

INTRODUCTION

The cosmopolitan diatom genus *Pseudo-nitzschia* has a number of species that are capable of producing the neurotoxin domoic acid (DA) (e.g., Hasle et al. 1996, Hasle 2002). Filter-feeding clams, including mussels and oysters, can ingest *Pseudo-nitzschia* and take up DA toxin into their tissues when a bloom (rapid proliferation) of *Pseudo-nitzschia* intersects nearshore shellfish beds. While DA doesn't seem to harm adult clams, it acts as a neurotoxin in humans, causing gastrointestinal problems, memory loss, and even death (reviewed in Todd 1993). DA can also cause illness and mortality in marine mammals and sea birds (Sierra Beltran et al. 1997, Scholin et al. 2000). To protect people from exposure to DA, most state and international governments have set the regulatory action level for DA at $20\text{mg} \cdot \text{kg}^{-1}$ in clam tissue. If this limit is exceeded in clams at a beach, the beach is closed for shellfish collection until toxin concentrations fall below $20\text{mg} \cdot \text{kg}^{-1}$.

Toxin-producing *Pseudo-nitzschia* have caused frequent, sometimes long, closures for razor clam (*Siliqua patula*) harvest along Washington's outer coast beaches (Horner et al. 1996, Stehr et al. 2002). *Pseudo-nitzschia* is also common in the inland waters of Puget Sound (Horner & Postel 1993, Horner et al. 1996, Horner et al. 1997, Trainer et al. 1998, Stehr et al. 2002), but in

contrast to the outer Washington coast, it has only caused three clam harvest closures, all since 2003 (Bill et al. 2006, Trainer et al. 2007). The difference between harvest closure frequency on the outer Washington coast and in Puget Sound raises the question: Why has *Pseudo-nitzschia* only caused three harvest closures in Puget Sound, while the coastal razor clam fishery has been plagued by frequent closures? Several hypotheses that could contribute to the explanation are:

- 1.) although *Pseudo-nitzschia* is present in Puget Sound, oceanographic conditions do not favor blooms of *Pseudo-nitzschia*;
- 2.) the species of *Pseudo-nitzschia* found in Puget Sound differ from those found on the Washington outer coast, and the Puget Sound species are lower in toxicity;
- 3.) when *Pseudo-nitzschia* blooms occur in Puget Sound, pelagic suspension-feeders grazes them down before they intercept clam beds;
- 4.) there is greater benthic suspension-feeding capacity in Puget Sound than on the outer Washington coast; thus Puget Sound suspension-feeding benthic macroinvertebrates (SFBMs) compete for available phytoplankton, resulting in lower *Pseudo-nitzschia* concentrations in seawater, and lower DA toxicity in harvestable clams; and
- 5.) Puget Sound clams differ from Washington outer coast razor clams in DA uptake and depuration physiology, such that Puget Sound clams take up less DA and/ or are more efficient at depurating (purging) DA from their tissues.

In this chapter, I use a numerical model to examine the final two of these alternative hypotheses by exploring factors that influence clam DA concentrations in Washington State, USA. Previous chapters collected data for the model parameters, which include: SFBM assemblage and density, beach gradient, phytoplankton delivery into the intertidal zone, clearance (feeding) rates on *Pseudo-nitzschia*, DA assimilation efficiency (AE) rates, and DA depuration rates (Fig. 1). This

chapter models DA exposure, uptake and retention in intertidal suspension-feeding clams in Puget Sound and the Washington outer coast. On sand/ cobble beaches in the two locations, clams dominate the SFBM assemblages (see Chapter I: Intertidal Zonation of Suspension-Feeding Epi- and Infauna at Lofall, Hood Canal), and are the most important contributors to beach suspension-feeding capacity (see Chapter III: Clearance Rate Measurements on Thirteen Intertidal Clams and Acorn Barnacles Fed on the Toxin-Producing Diatom *Pseudo-nitzschia*). Intertidal clams are also the primary vector for DA exposure in humans.

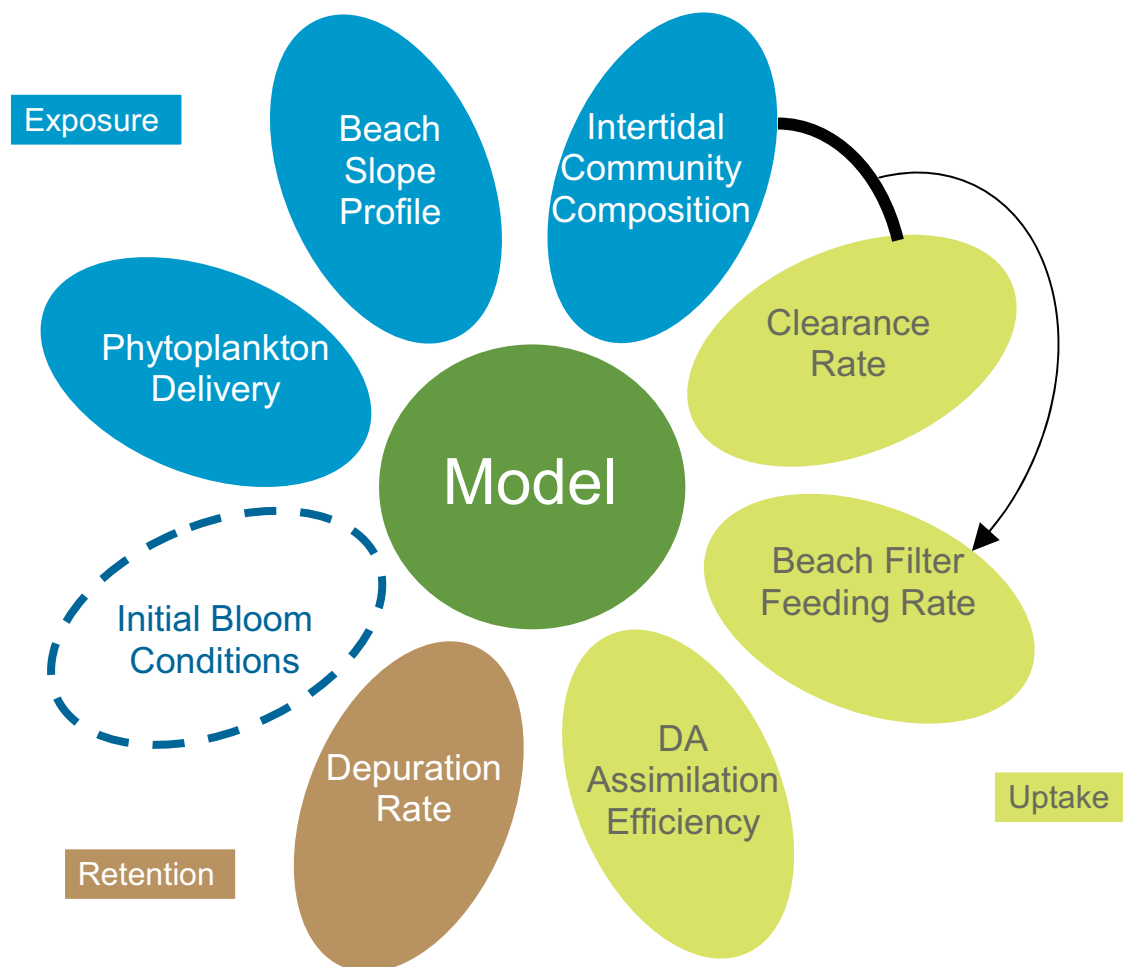


Figure 1. Conceptual model of data inputs that were used as model parameters. Filled blue ovals represent data related to exposure of clams to *Pseudo-nitzschia*, light green filled ovals represent data related to DA uptake by clams, and the brown filled oval represents data related to clam retention of DA. The blue dashed outline oval represents a parameter obtained from literature values. Beach filter-feeding rate is calculated in this chapter as a combination of intertidal community composition and clearance rate.

In order for clams to become toxic on a beach, a series of biological and oceanographic events must take place. Toxin-producing *Pseudo-nitzschia* species must be present, and the right oceanographic conditions must exist for a bloom to occur. The bloom must then be tidally advected over intertidal shellfish beds, and clams must consume high enough quantities of DA to

become toxic. While some work has been conducted to understand the oceanographic aspects of *Pseudo-nitzschia* bloom formation and toxin production, and explore DA uptake or depuration by individual clam species, no studies have been performed to comprehensively investigate aspects of beach habitat and SFBM assemblage characteristics that affect DA concentrations in clams. In addition, these dynamics have not been compared between Puget Sound and the outer Washington coast, to determine whether differences exist between the two locations to cause the disparity in harvest closure history.

Both species composition and density of SFBMs differ between the outer Washington coast and Puget Sound. Razor clams dominate the SFBM assemblage along sandy beaches on the outer coast (Kozloff 1983). They thrive on surf-pounded, low gradient beaches with fine-grained sand, and are not found in the calmer estuarine waters of Puget Sound (Lassuy & Simons 1989). Density of adult (>7.6cm) razor clams on Washington outer coast beaches in 2011 ranged between 0.51 and 1.85 clams · m⁻² (WDFW 2011), which is relatively low density compared to Puget Sound (Chapter I: Intertidal Zonation of Suspension-Feeding Epi- and Infauna at Lofall, Hood Canal).

In contrast to the single-clam dominance on Washington's outer coast, Puget Sound's mixed sediment (sand and cobble) beaches have a broad assemblage of SFBM species. The various Puget Sound species reside at different tidal heights (Kozloff 1983). The mid-upper intertidal (approximately +1.5m above mean lower low water, MLLW) is habitat for epiphytes: Pacific oyster *Crassostrea gigas*, bay mussel *Mytilus trossulus*, and acorn barnacle *Balanus glandula*; as well as to the introduced infaunal purple varnish clam *Nuttallia obscurata*. A large variety of

species burrow in sandy gravel at mid- to low-intertidal elevations (approximately -1.0m to +1.0m above MLLW), including: Manila clam *Ruditapes philippinarum*, native littleneck *Leukoma staminea*, soft-shell clam *Mya arenaria*, Nuttall's cockle *Clinocardium nuttallii*, butter clam *Saxidomus giganteus*, horse clam *Tresus capax* and *T. nuttallii*, geoduck *Panopea generosa*, and a number of suspension-feeding polychaetes, including the genus *Spiochaetopterus*. Although acorn barnacles and polychaetes are both found at appreciable densities on Puget Sound beaches (see Chapter I: Intertidal Zonation of Suspension-Feeding Epi- and Infauna at Lofall, Hood Canal), their total effect on beach-wide suspension-feeding capacity is substantially lower than suspension-feeding clams (see also Chapter III: Clearance Rate Measurements on Thirteen Intertidal Clams and Acorn Barnacles Fed on the Toxin-Producing Diatom *Pseudo-nitzschia*). As a result, this chapter models only clam suspension-feeding capacity and its impacts on DA levels in harvestable clams.

In Puget Sound, the hypothesis that the diverse assemblage of SFBMs compete for available *Pseudo-nitzschia*, thereby reducing DA toxin concentrations in all harvested clams, is supported by the fact that low levels of DA contamination have been observed in Puget Sound clams for more than a decade (reviewed in Trainer et al. 2007). Additionally, clam depletion of phytoplankton has been shown to occur in shallow estuaries (Banas et al. 2007), and clams at low tidal elevations can limit food to clams at higher tidal elevations (Peterson & Black 1987).

Differing DA depuration rate physiologies in Puget Sound and outer Washington coast clams may also play a large role in decreasing the likelihood of DA harvest closures in Puget Sound. Razor clams on Washington's outer coast have been shown to depurate DA very slowly from

their tissues, taking up to a year to complete the process (Drum et al. 1993, Horner et al. 1993, Wekell et al. 1994, Adams et al. 2000). In contrast, the depuration rates for Puget Sound clam species are on the order of four to twenty times that of coastal razor clams (Chapter 4: Domoic Acid Uptake and Depuration by Nine Intertidal Clams Experimentally Fed on Toxin-Producing *Pseudo-nitzschia multiseriata*). With such marked differences in depuration rate, it is conceivable that this factor may be partially responsible for the differences in harvest closure regimes between the Washington outer coast and Puget Sound.

In this chapter, I hypothesized that: 1) clam competition in Puget Sound reduces *Pseudo-nitzschia* concentrations and thus DA toxicity in all clams; and, 2) the physiology of Puget Sound clams reduces DA uptake or retention relative to Washington outer coast razor clams. Construction of a model to test these two hypotheses required many pieces of information which were not available in published studies. In order to parameterize the model, I completed field and laboratory studies to obtain the necessary data. The approaches, methods, and results involved in acquiring these data are presented in the prior four chapters of this dissertation:

Chapter I: Intertidal Zonation of Suspension-Feeding Epi- and Infauna at Lofall, Hood Canal, presents surveys of SFBM assemblage composition and standing stock at three Lofall, Hood Canal sites with different beach topographies, along with beach slope profiles for the beaches.

Chapter II: Phytoplankton Advection into the Intertidal Zone of a Temperate Fjord, presents field data on water advection into the intertidal zone at Lofall, which is important for choosing appropriate assumptions about the availability of *Pseudo-nitzschia* to intertidal suspension-feeders.

Chapter III: Clearance Rate Measurements on Thirteen Intertidal Clams and Acorn Barnacles Fed on the Toxin-Producing Diatom *Pseudo-nitzschia*, presents laboratory feeding experiments during which Puget Sound bivalves, acorn barnacles, and Washington outer coast razor clams were fed *Pseudo-nitzschia* and their clearance rates were measured.

Chapter IV: Domoic Acid Uptake and Depuration by Nine Intertidal Clams Experimentally Fed on Toxin-Producing *Pseudo-nitzschia multiseriata*, provides DA depuration rates for all modeled species, with the exception of bay mussel. Since bay mussel depuration data were not available, depuration rates for blue mussels were substituted (Mafra et al. 2010a). It would be reasonable for these species to have similar physiology, as they are closely related, and can only be distinguished genetically. In Chapter IV, I also modeled the DA AE rates for four species.

In the current dissertation chapter, I relate data on clam biology and on beach characteristics and communities into two linked models: (1) a model that tracks *Pseudo-nitzschia* concentrations at different tidal heights as a function of time; and, (2) another model that tracks DA concentrations in clams at different tidal heights over time.

MATERIALS & METHODS

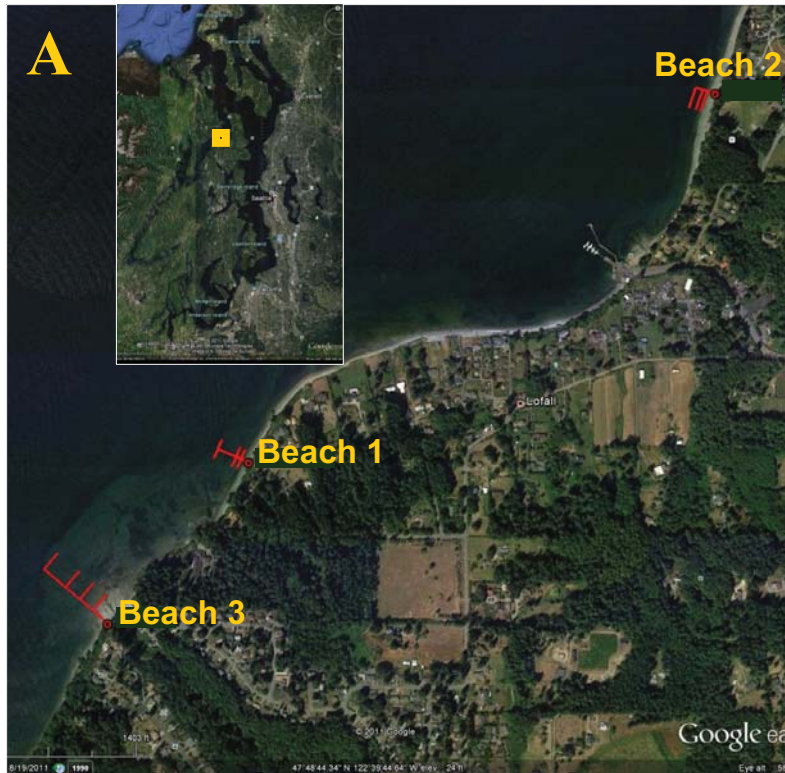
Study Location

The Lofall region of Hood Canal, a fjord arm of Puget Sound (Fig. 2A) was selected as the study location for this research and as a model case study for several reasons. Firstly, water movement dynamics in the Lofall region of Hood Canal are relatively representative of sill regions in Puget Sound's five basins. Secondly, the clam assemblage at Lofall is representative of clam

assemblages in Puget Sound's quiet bays and salt marshes, where the substratum is cobble or gravel mixed with sand or mud (Kozloff 1983, Dethier 2006). Thirdly, working in this area is advantageous because of the extensive bathymetric and oceanographic data in existence for this region of Hood Canal. These data provided necessary information for obtaining a first-order estimate of water advection into the intertidal zone where harvestable shellfish are located. Lastly, one of the first recorded *Pseudo-nitzschia* blooms in Puget Sound occurred in Hood Canal, although shellfish did not attain high enough concentrations of DA to cause a beach closure (Horner et al. 1996). *Pseudo-nitzschia* is found in the Canal, particularly during spring and fall months (G. Armbrust, UW; pers comm), and it may only be a matter of time before DA is found at elevated concentrations in Hood Canal clams.

Three beaches in Lofall were modeled in order to capture natural variability in beach geomorphology and SFBM assemblages: Beach 1 is part of a private residence owned by the Thomas family, and has a low tide terrace that is common on Puget Sound sand/ cobble beaches (Finlayson 2006); Beach 2 is Kitsap Memorial State Park, with a steep, mostly cobble beach, and Beach 3 is a sandy delta that is cooperatively owned by members of the Edgewater Beach Community Club. As a contrast to the Puget Sound (Lofall, Hood Canal) conditions, I used data from Twin Harbors on the Washington outer coast (Fig. 2B). Data on razor clam standing stock and size composition, along with beach slope profile approximations, were obtained for Twin Harbors from the Washington Department of Fish and Wildlife (WDFW 2011).

Hood Canal



Washington outer coast



Figure 2. Model scenario locations in (A) Lofall, Hood Canal, with Puget Sound inset; and (B) Twin Harbors on the Washington outer coast.

Model Overview

Addressing the question “Why have there been fewer harvest closures for DA in Puget Sound than on the Washington outer coast” required examination of three aspects of clam interaction with DA: 1) exposure of clams to DA; 2) uptake of DA by clams, and, 3) retention of DA within clam tissue (Fig. 1). The exposure of a clam to DA-producing *Pseudo-nitzschia* is a function of the physical environment of the clam, including: tidal elevation of the clam, steepness profile of the beach (this determines the quantity of water that passes over the clam during a tidal cycle), rate of phytoplankton delivery to the clam’s tidal elevation, and abundance and species composition of neighboring suspension-feeding clams (this determines the level of competition

for available phytoplankton that has been advected over the intertidal zone). Uptake of DA differs between clam species, and is a function of feeding rate on *Pseudo-nitzschia* and DA AE. Retention of DA within clam tissue is determined by the species-specific rate at which DA is depurated (purged) from clam tissues.

Thus, the content of DA in a single clam can be calculated as a combination of DA uptake and DA loss, where DA uptake is a function of exposure to *Pseudo-nitzschia*, toxicity of the *Pseudo-nitzschia* cells, clam feeding rate, and DA assimilation efficiency. Loss of DA from clam tissue is calculated as the depuration rate multiplied by DA contained within clam tissue. Finally, the actual concentration of DA in a clam is calculated as milligrams (mg) of DA within tissue divided by clam wet weight (kg).

I explored clam competition for *Pseudo-nitzschia* and species-specific differences in DA uptake and loss using a system of two linked models. The first model simulates a *Pseudo-nitzschia* bloom as it moves back and forth over the intertidal zone with the tide rises and falls. This model uses a Lagrangian perspective to track phytoplankton concentrations in a row of adjacent three-dimensional, rectangular columns, starting at the water's landward edge and going toward deeper water (Figs. 3 & 4). The number of columns is such that all columns sit over the intertidal region during high tide. Each column's height (in the z-direction) extends from surface to the ocean floor. These columns are fixed in volume (5m^3) and move with the bloom as it is tidally advected over the intertidal zone with the flood tide and then ebbs away from intertidal zone with the change in tide. The output of this model is *Pseudo-nitzschia* concentration over time at particular tidal heights.

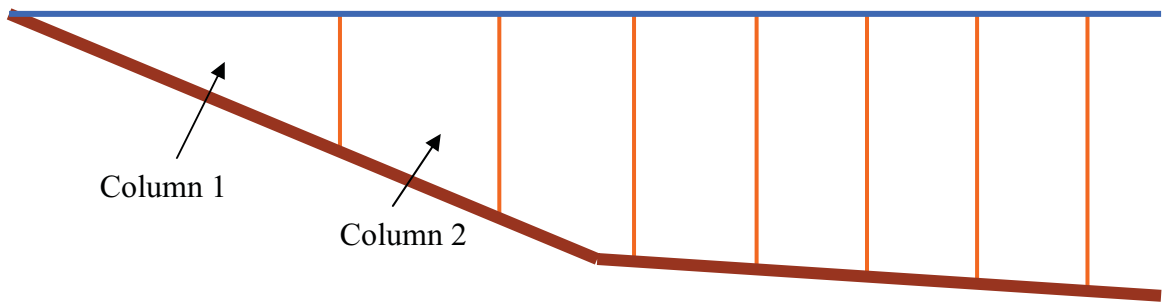


Figure 3. Cross-section of shore showing three-dimensional columns of water for the bloom model. Each column extends from the surface to the sea floor, and columns move with the tide, following the same volume (5m^3) of water through time. Column numbers increase from shore towards deeper water.

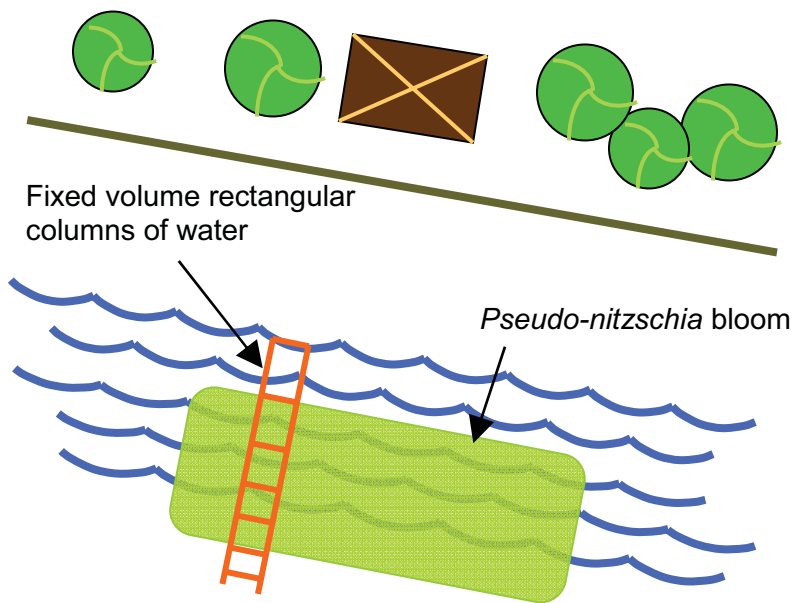


Figure 4. Plan view of rectangular columns of water for tracking *Pseudo-nitzschia* concentration over time.

The second model tracks accumulation and depuration of DA in intertidal clams at particular tidal heights using an Eulerian perspective. This model takes the output from the bloom model: *Pseudo-nitzschia* concentrations over time at particular tidal heights, and allows submerged

clams to feed on available *Pseudo-nitzschia* and depurate DA. The final output of this model is DA concentration in each species of clam over time at particular tidal heights.

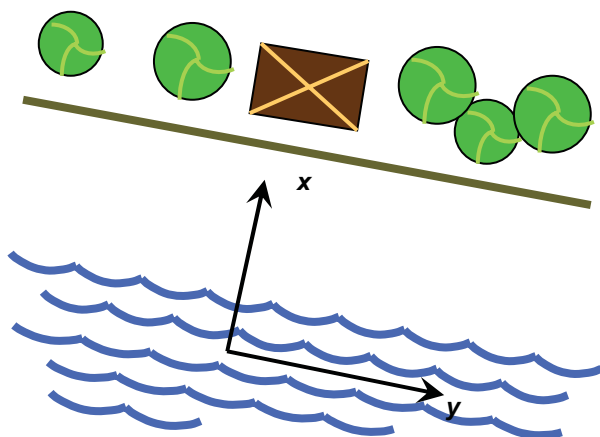


Figure 5. Plan view of model region, defining x and y directions. Z-direction (not shown) is the height of water above the ocean floor

Model Definitions. Defined x and y directions are shown in Fig. 5.

x-direction: perpendicular to shore

y-direction: parallel to shore

z-direction (not shown): height of water above the ocean floor at any point

Data Inputs

Numerous data and parameters were necessary for these models, including phytoplankton delivery data, tidal height calculation parameters, beach slope profile data, SFBM species composition and standing stock, species-specific clearance rate parameters, species-specific DA depuration rate parameters, and species-specific DA AE parameters (Fig. 1). In addition,

historical data from the 2005 Sequim Bay *Pseudo-nitzschia* bloom were used as a scenario for *Pseudo-nitzschia* cell concentration and particulate DA per cell (Trainer et al. 2007).

Phytoplankton Delivery Data. The rate of phytoplankton delivery to the intertidal zone determines how much *Pseudo-nitzschia* in neritic nearshore waters will be available to intertidal clams for consumption. Tidal currents in the Lofall region run predominantly parallel to shore, with only a small component of tidal energy directed perpendicular to shore (Chapter II: Phytoplankton Advection into the Intertidal Zone of a Temperate Fjord). The ratio in current speed between long-shore and cross-shore directions is approximately ten to one.

Tidal Height Parameters. In the model, the rate of water movement from the neritic mid-channel waters of Hood Canal into the intertidal at Lofall is calculated from beach slope profiles and equations that predict tidal rise and fall. Together, these determine the volume of water that advects to or from the intertidal over a period of time, allowing calculation of depth-averaged water velocity in the cross-shore direction at each time point. This serves as a simplification of the highly variable cross-shore component of tidal currents (see Chapter II: Phytoplankton Advection into the Intertidal Zone of a Temperate Fjord).

Tidal height oscillations determine which tidal heights are submerged at each point in time, thus the duration of time that clams at each tidal height can feed on *Pseudo-nitzschia* or depurate DA. Tidal height predictions for Lofall, Hood Canal were made using the six strongest tidal harmonic constituents (M2, S2, K1, O1, P1, N2) for Seattle (Mofjeld et al. 2002). Harmonic constituents

were only available for a few locations in Puget Sound, and Seattle was selected because the tidal range was most similar to Lofall.

The equation for tidal height is the sum of six cosines with different amplitude, frequency and phase lag:

$$\eta = \sum_i A_i \cos(\omega_i t - \phi_i) \quad (\text{Eqn 1})$$

where:

A is amplitude, in meters (depends on location)

ω is the frequency, calculated as:

$$\omega = \frac{2\pi}{\text{period (hrs)}} \cdot \frac{1\text{hr}}{3600\text{sec}} \quad (\text{Eqn 2})$$

ϕ is phase lag, in radians relative to Greenwich Mean time (GMT).

Tidal oscillations were modeled using the six harmonic parameters (Table 1), and the resulting model's tidal range was compared to the tidal range for Lofall. Because the tidal elevation of 0m corresponds to the NOAA Mean Lower Low Water (MLLW) tidal datum, rather than to mean tidal elevation, the tidal oscillation model was shifted up until modeled high and low tide levels fit the predicted high and low tide levels for Lofall. This resulted in adding 2.05m to the sum of cosines.

For the Washington outer coast, I obtained the appropriate tidal harmonic parameters for La Push, Washington (NOAA Tides & Currents Webpage)(Table 1), and added 1.4m to the sum of cosines so that the tidal oscillation model matched predicted high and low tide levels for La Push.

Table 1. Six most important tidal harmonic constituents for Seattle, Washington and La Push, Washington. A is amplitude in meters, and Φ is phase lag, in radians relative to Greenwich Mean time.

constituent	period	Seattle, Washington		La Push, Washington	
		A	Φ	A	Φ
M2	12.42	1.07	0.199	0.932	4.1
S2	12	0.258	0.661	0.262	4.581
K1	23.93	0.831	4.84	0.432	4.175
O1	25.82	0.458	4.458	0.259	3.899
P1	24.07	0.252	4.791	0.133	4.131
N2	12.66	0.212	5.938	0.192	3.676

Beach Slope Profile Data. A beach's steepness determines how much water is required to fill the intertidal during high tide. Steep gradient beaches require much less water to pass over them during flood tide than shallow gradient beaches, thus lower quantities of phytoplankton are available to clams living on steep beaches. Beach slope profile data from Chapter I: Intertidal Zonation of Suspension-Feeding Epi- and Infauna at Lofall, Hood Canal, were used for Beaches 1, 2 and 3. For each of these beaches, I approximated the beach slope profile using two linear equations, thus generating piece-wise equations for beach slope:

Beach 1:

$$\eta = \begin{cases} x \leq 35.83 & | -0.096 \cdot x + 4.00 \\ x \geq 35.83 & | -0.019 \cdot x + 1.25 \end{cases} \quad \text{(Eqn 3)}$$

Beach 2:

$$\eta = \begin{cases} \mathbf{x} \leq 39.65 | -0.094 \cdot \mathbf{x} + 4.00 \\ \mathbf{x} \geq 39.65 | -0.046 \cdot \mathbf{x} + 2.12 \end{cases} \quad \text{(Eqn 4)}$$

Beach 3:

$$\eta = \begin{cases} \mathbf{x} \leq 125.14 | -0.026 \cdot \mathbf{x} + 4.00 \\ \mathbf{x} \geq 125.14 | -0.013 \cdot \mathbf{x} + 2.44 \end{cases} \quad \text{(Eqn 5)}$$

where η is tidal height (m) and x is shore distance in the x-direction (m). The fit of the system of two linear equations for each beach slope profile is shown in Figure 6A-C.

Beach Slope Profiles for Lofall & WA Outer Coast

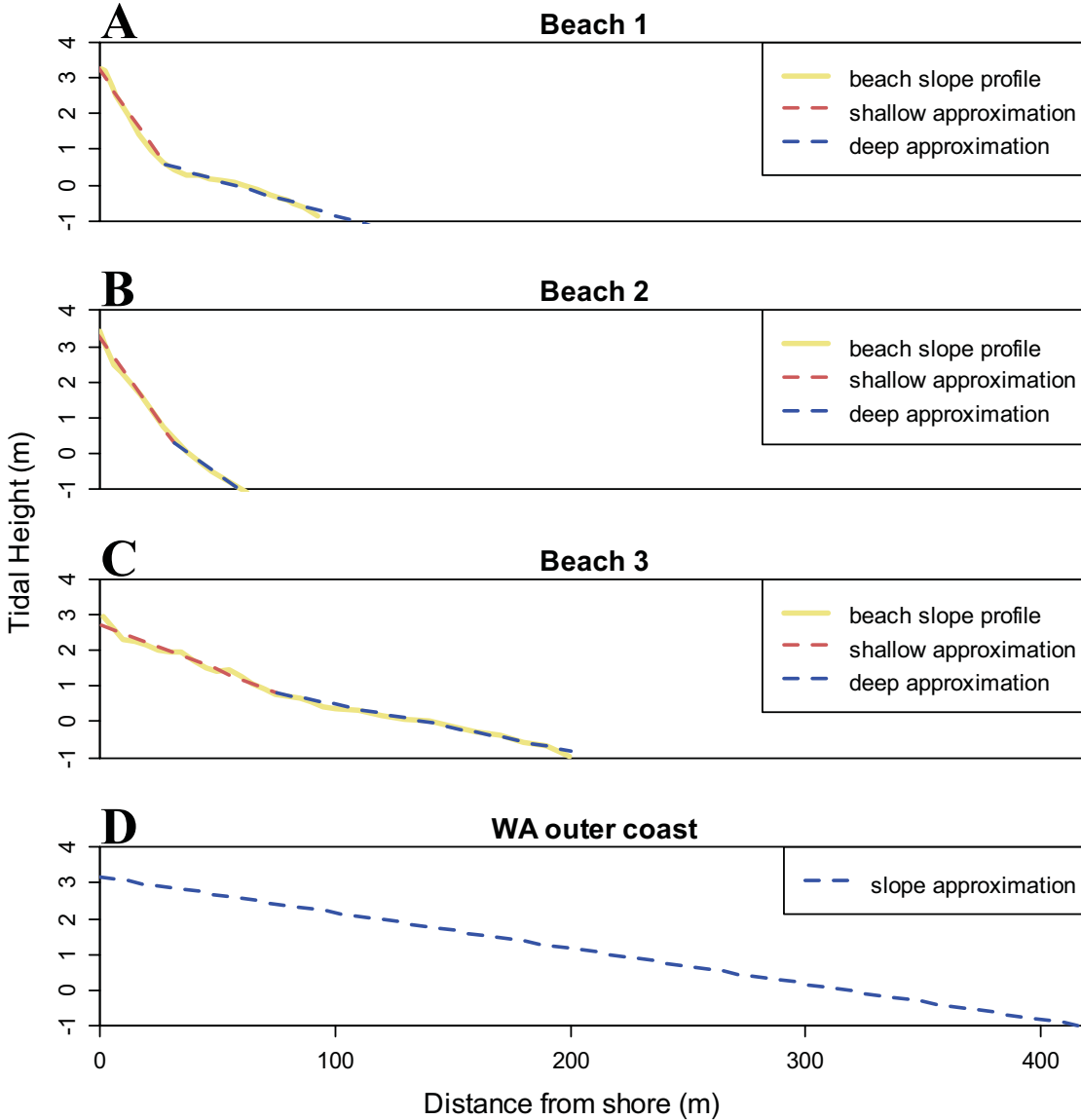


Figure 6. Beach slope profile data for Lofall beaches (panels A through C, shown in tan) with the fit of shallow (maroon) and deep (blue) linear approximations superimposed on the data. Panel D shows a Washington outer coast beach slope profile approximation.

A beach slope profile for the Washington outer coast was approximated using descriptive data obtained from WDFW (WDFW 2011). WDFW completes annual razor clam surveys along Washington outer coast beaches for the purpose of setting harvest quotas. Survey descriptions

indicate that the distance (in the x-direction) from the top of the clam beds to the edge of the surf during the lowest tides is approximately 600 feet (182.9m). During the most dramatic spring tides, the lowest exposed depth in the intertidal on Washington outer coast beaches is -0.88m above MLLW. The upper elevations inhabitable by razor clams in the Pacific Northwest were estimated as 3.1 feet (0.94m) above MLLW at Long Beach, WA (Nickerson 1975, Lassuy & Simons 1989). Hence, I assumed that the vertical range inhabited by razor clams is -0.88m to +0.94m above MLLW. Since razor clam beaches along the outer Washington coast are very flat, I approximated the beach slope profile for the outer coast with a single linear equation (Fig. 6D):

Washington outer coast:
$$\eta = -0.00996 \cdot x + 3.15 \quad \text{(Eqn 6)}$$

Clam Species Composition and Abundance Data. Clam assemblages, in terms of species and size composition, as well as standing stock, determine how much potential exists for competition. Data for Lofall beaches are presented in Chapter I: Intertidal Zonation of Suspension-Feeding Epi- and Infauna at Lofall, Hood Canal. For three or four transects parallel to shore at each site (in the y-direction, Fig. 5) I estimated mean organism density (no. · m⁻²) categorized by species and size.

Because the model calculations are all based on tidal height, mean organism densities were converted into mean number of animals per centimeter of tidal height by meter of beach width in the y-direction (no. · cm⁻¹ · m⁻¹). This will be referred to as “mean density · cm⁻¹ of tidal height” throughout this chapter. Calculations of mean density · cm⁻¹ of tidal height were achieved for each beach and transect by dividing the mean areal organism density by the shore distance (in

the x-direction) at that beach and tidal height (Fig. 7). For Beach 1, meters of shore distance per centimeter of tidal height ranged from 0.10 to 0.51, for Beach 2 from 0.11 to 0.22, and for Beach 3 from 0.39 to 0.76.

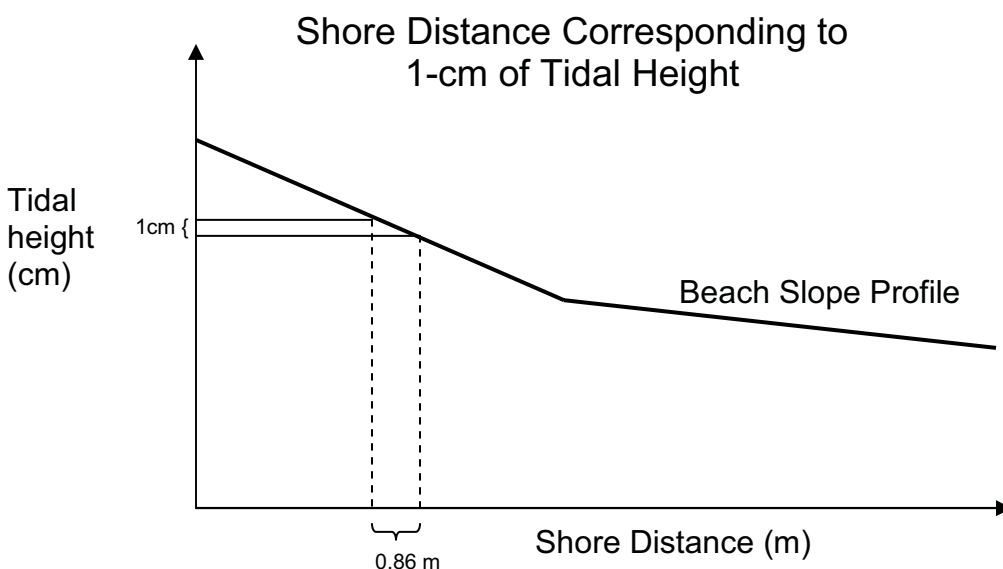


Figure 7. Relationship between one centimeter of tidal height and the corresponding shore distance.

Data on average abundance and size distribution for razor clams at Twin Harbors, an outer Washington coast beach, were obtained from WDFW (WDFW 2011)(Fig. 8). According to the protocol described above, mean areal razor clam density was converted to mean density $\cdot \text{cm}^{-1}$ tidal height. Approximately 1.00 meters of shore distance corresponds to one centimeter of tidal height along the outer Washington coast. Thus, mean razor clam density $\cdot \text{cm}^{-1}$ tidal height at Twin Harbors was approximately $3.2 \text{ clams} \cdot \text{cm}^{-1} \cdot \text{m}^{-1}$.

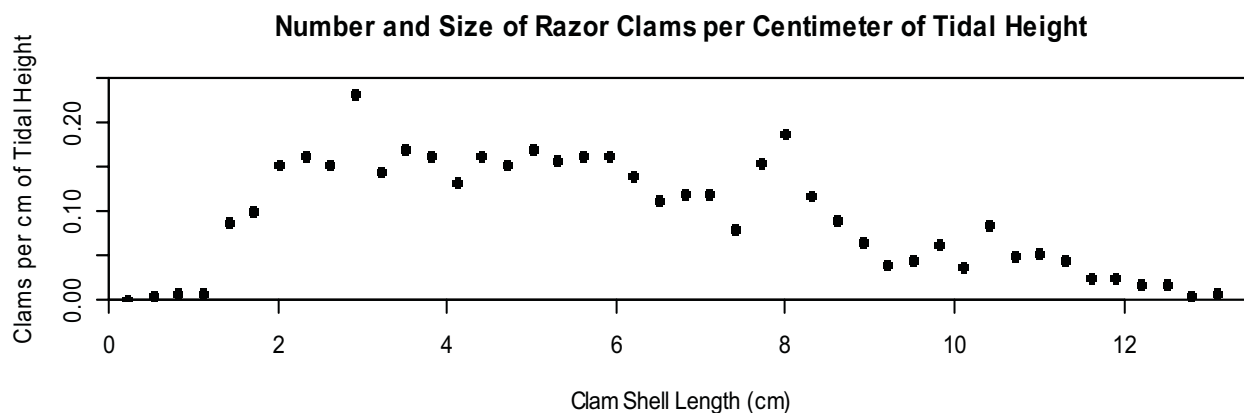


Figure 8. Razor clam size distribution and density $\cdot \text{cm}^{-1}$ of tidal height for Twin Harbors on the Washington outer coast.

At Lofall and along the Washington outer coast, the tidal range over which I found each species in the intertidal zone is listed in Table 2. The tidal heights provided in the table for all species except razor clam and purple varnish clam are actual tidal heights where these species were observed during Lofall clam abundance and species composition surveys. For razor clam, minimum tidal height within the intertidal zone was estimated as the lowest spring tide, and maximum tidal height was estimated as +0.94m at Long Beach, Washington (Nickerson 1975, Lassuy & Simons 1989). For purple varnish clam, tidal range was obtained from surveys conducted at Departure Bay, British Columbia by Gillespie et al. (1999), and translated to the corresponding tidal heights in Lofall, Washington.

Table 2. Highest and lowest tidal heights (relative to MLLW) at which each clam species was found during intertidal species composition surveys. Data for all species except purple varnish clam and razor clam are from Lofall, Hood Canal.

Species	Latin name	Highest Tidal Height (m)	Lowest Tidal Height (m)	Source
purple varnish clam	<i>Nuttallia obscurata</i>	1.86	-0.04	Gillespie <i>et al.</i> 1999
Pacific oyster	<i>Crassostrea gigas</i>	1.47	0.51	Lofall survey
bay mussel	<i>Mytilus trossulus</i>	1.47	0.51	Lofall survey
soft-shell clam	<i>Mya arenaria</i>	1.05	0.38	Lofall survey
Manila clam	<i>Ruditapes philippinarum</i>	1.05	0.07	Lofall survey
Nuttall's cockle	<i>Clinocardium nuttallii</i>	1.05	-0.91	Lofall survey
littleneck clam	<i>Leukoma staminea</i>	1.05	-0.91	Lofall survey
butter clam	<i>Saxidomus giganteus</i>	1.05	-0.91	Lofall survey
horse clam	<i>Tresus capax</i>	1.05	-0.91	Lofall survey
razor clam	<i>Siliqua patula</i>	0.94	-0.88	WDFW

Clearance Rate Parameters. Clearance rate, defined as liters of water cleared of phytoplankton per hour, was used in the model to calculate clam feeding on *Pseudo-nitzschia*. Species-specific clearance rates as a function of tissue dry weight, based on laboratory experiments described in Chapter III: Clearance Rate Measurements on Thirteen Intertidal Clams a **(Eqn 7)** Barnacles Fed on the Toxin-Producing Diatom *Pseudo-nitzschia* (Table 3), were estimated using allometric equations of the form:

$$CR = a \cdot W^b$$

where:

CR = clearance rate,

W = tissue dry weight (g), and,

a & b = species-specific parameters to describe the allometric relationship.

Although clearance rates have been shown to vary with respect to a number of parameters, including phytoplankton concentration (e.g., Kiorboe & Mohlenberg 1981, Riisgard et al. 2003), seston composition (e.g., Arifin & Bendell-Young 1997), temperature (e.g., Bougrier et al. 1995, Han et al. 2008), and flow rate (e.g., Jones et al. 2011), the effects of these parameters on clearance rate are not clearly defined for most species. For the purposes of this model, I assumed that clearance rate remains constant while clams are submerged, regardless of environmental conditions.

Table 3. Species-specific allometric parameters for clearance rate, with standard errors (in parenthesis).

Species	size range tested (tissue dry wt, g)	a (\pm sd)	b (\pm sd)
bay mussel	0.03 - 2.79	5.98 (\pm 0.38)	0.60 (\pm 0.04)
Pacific oyster	0.95 - 7.18	6.03 (\pm 0.71)	0.72 (\pm 0.08)
Nuttall's cockle	0.69 - 14.11	2.25 (\pm 0.28)	0.42 (\pm 0.07)
Manila clam	0.61 - 4.03	1.97 (\pm 0.29)	0.21 (\pm 0.21)
butter clam	1.63 - 23.45	1.58 (\pm 0.24)	0.45 (\pm 0.08)
purple varnish clam	0.33 - 3.24	1.53 (\pm 0.19)	0.41 (\pm 0.14)
littleneck clam	0.41 - 5.75	1.64 (\pm 0.15)	0.15 (\pm 0.05)
horse clam	13.07 - 37.22	0.29 (\pm 0.30)	0.89 (\pm 0.31)
soft-shell clam	0.19 - 3.00	0.80 (\pm 0.13)	0.44 (\pm 0.13)
razor clam	0.59 - 13.31	0.50 (\pm 0.12)	0.58 (\pm 0.12)

Suspension-Feeding Capacity. The model required a function relating beach clearance rate to tidal height, to calculate phytoplankton depletion over specific points in the intertidal. For sampled transects along Lofall beaches, clam densities \cdot cm⁻¹ tidal height for each species and size were multiplied by the appropriate clearance rates and summed, to yield total suspension-feeding capacity at each tidal height (clearance rate \cdot cm⁻¹ tidal height \cdot m⁻¹). Because data were only available for three or four discrete tidal heights along each beach, suspension-feeding

capacity for the tidal heights between or above/ below sampled transects were calculated using linear interpolation between adjacent tidal heights. At Lofall beaches, the observed upper limit of the clam tidal range was +2.28m above MLLW, so suspension-feeding capacity at this tidal height was set to zero. For tidal heights lower than the last sampled transect, I assumed that suspension-feeding capacity was equal to that of the lowest sampled transect.

For Twin Harbors beach on the outer Washington coast, total suspension-feeding capacity was obtained as above. Suspension-feeding capacity was assumed to be constant throughout the whole range of razor clam inhabitance, including at tidal heights below the sampled region, because data on razor clam density at different tidal heights were not available. At tidal heights above the top of the razor clam beds (+0.94m), suspension-feeding capacity was assumed to be zero.

DA Assimilation Efficiencies. Domoic acid assimilation efficiency (AE) is the percentage of consumed DA, contained within *Pseudo-nitzschia* cells, that is assimilated into shellfish tissue. As presented in Chapter IV: Domoic Acid Uptake and Depuration by Nine Intertidal Clams Experimentally Fed on Toxin-Producing *Pseudo-nitzschia multiseriata*, I estimated DA AE for four species using maximum likelihood estimation (Table 4). For bay mussel, I used AE = 39%, which had been experimentally measured for blue mussels (Mafra et al. 2010a). For other species where data were not available, I made the conservative assumption that 100% of consumed DA is assimilated into clam tissue.

Table 4. DA assimilation efficiency for five species; estimates from the “2009 expt” are described in Chap. IV.

Species	Assimilation Efficiency (%)	Source
soft-shell clam	59%	2009 expt
bay mussel	39%	Mafra <i>et al.</i> 2010
purple varnish clam	100%	2009 expt
Manila clam	100%	2009 expt
razor clam	11%	2009 expt

Depuration Rate Parameters. As described in Chapter IV: Domoic Acid Uptake and Depuration by Nine Intertidal Clams Experimentally Fed on Toxin-Producing *Pseudo-nitzschia multiseriis*, based on laboratory experiments, I estimated depuration of DA from shellfish tissue using a one-compartment exponential decay model,

$$DA_t = DA_0 e^{-rt} \quad \text{(Eqn. 8)}$$

where DA_t is domoic acid concentration after t days, DA_0 is DA concentration at the beginning of the depuration time, r is the exponential depuration rate, and t is days elapsed. DA_0 and r were then estimated using linear regression after log-transformation. Data on DA depuration were only collected for one size class per species (Table 5). Depuration rates for bay mussel were not available, so depuration measurements for blue mussel were used as a proxy (Mafra et al. 2010a). Mafra et al. present depuration rates by mussel size, with estimates of 1.4 to $1.6 \cdot \text{day}^{-1}$ (± 0.09 to 18). Thus, for bay mussels, I used a depuration rate of $1.5 \cdot \text{day}^{-1}$ (± 0.18).

Table 5. Depuration rates by clam species, with standard error; the “2008 expt” and “2009 expt” are described in Chap. IV.

Species	Shell Length (cm)	Exponential Rate (day ⁻¹)	Depuration SE	Source
Pacific oyster	6.9	0.86	0.08	2008 expt
Nuttall's cockle	6.9	0.49	0.04	2008 expt
soft-shell clam	7.7	0.35	0.03	2009 expt
bay mussel	5.0	1.50	0.18	Mafra <i>et al.</i> 2010
purple varnish clam	4.5	0.11	0.05	2009 expt
littleneck clam	5.1	0.40	0.03	2008 expt
butter clam	7.1	0.84	0.06	2008 expt
horse clam	11.6	0.78	0.03	2008 expt
Manila clam	5.0	0.44	0.03	2009 expt
razor clam	10.0	0.03	0.09	2009 expt

Historical 2005 Sequim Bay *Pseudo-nitzschia* Bloom Data. I used published data from the 2005 Sequim Bay bloom (Trainer et al. 2007) to obtain realistic estimates of *Pseudo-nitzschia* concentration and particulate DA (pDA; DA contained within *Pseudo-nitzschia* cells) during a Puget Sound bloom. In that study, a time series of *Pseudo-nitzschia* cell counts and pDA measurements were based on weekly water sampling at Sequim Bay. I selected a *Pseudo-nitzschia* bloom concentration of 6,500 cells · ml⁻¹, as well as the associated pDA of 0.76 pg · cell⁻¹ (Fig. 9) for the model. This bloom concentration represented a medium *Pseudo-nitzschia* density, rather than the peak density during the Sequim Bay bloom. However, *Pseudo-nitzschia* concentration and pDA are simple multipliers within the model, so the effects of different phytoplankton concentrations and pDA levels can easily be explored.

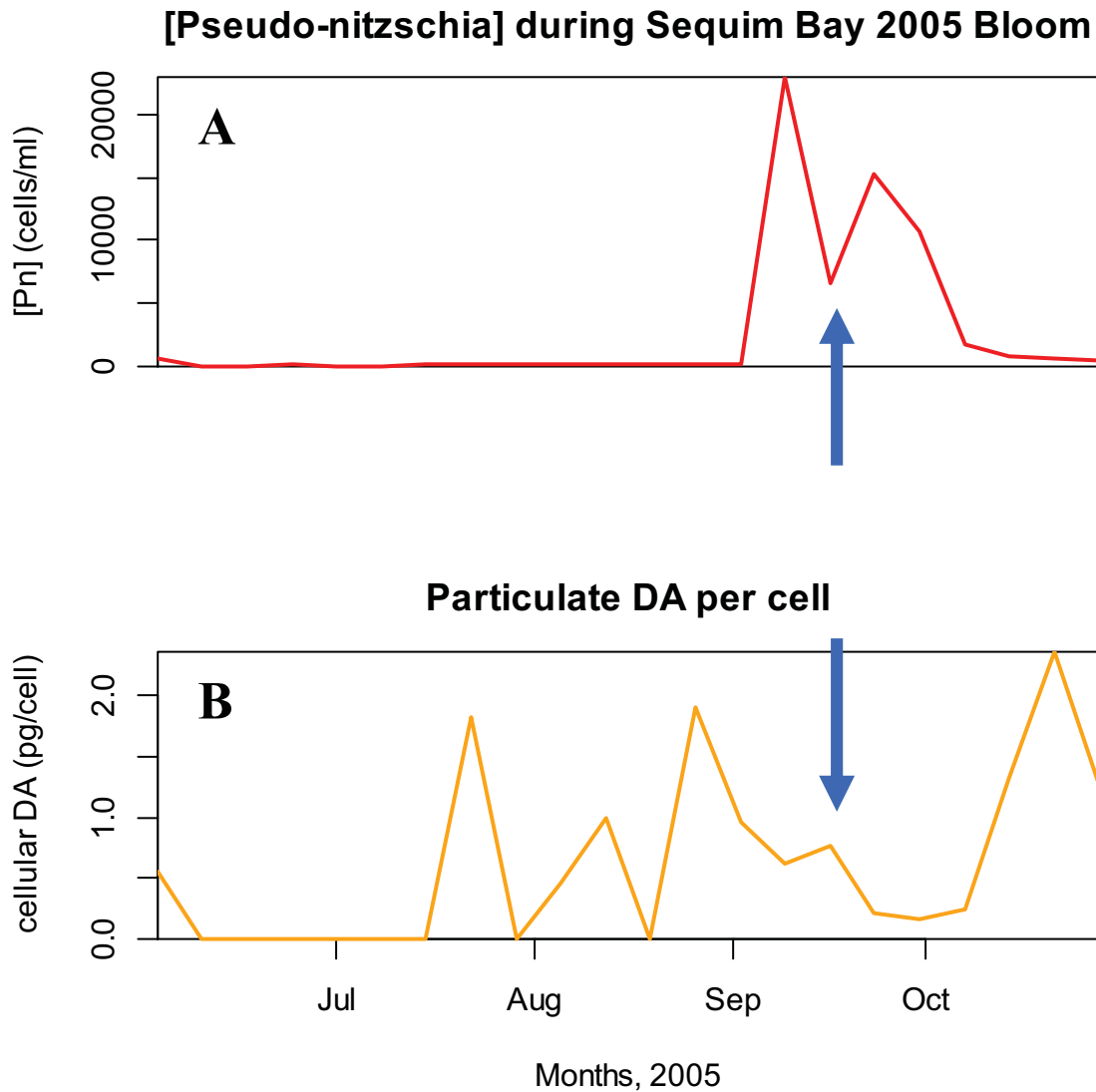


Figure 9. Time series of *Pseudo-nitzschia* concentration (brown) and particulate DA (orange) during the Sequim Bay bloom, September 2005, based on (Trainer et al. 2007). The blue arrow points to the *Pseudo-nitzschia* concentration and pDA used in the model.

Initial Phytoplankton Concentration Profile. In Chapter II: Phytoplankton Advection into the Intertidal Zone of a Temperate Fjord, I observed gradual tapering of chlorophyll concentrations around the margins of a phytoplankton bloom. While decreased phytoplankton concentrations around bloom margins are likely a result of several physical processes, I used only the process of suspension-feeding to create a tapered *Pseudo-nitzschia* profile around the bloom. I ran the

model over Beach 2 for one day using a uniform *Pseudo-nitzschia* concentration profile in each column, and then determined the level of decline in *Pseudo-nitzschia* concentration in the first 29 columns (Fig. 10). All columns seaward from the first 29 had only small amounts of *Pseudo-nitzschia* decline, so they were assigned an initial *Pseudo-nitzschia* concentration of 6500 cells · ml⁻¹ in the model. The total number of columns was different at each beach, as a result of different beach slope profiles: Beach 2, the steepest beach, had 40 columns of water; Beach 1 had 81 columns; Beach 3 had 153 columns; and the Washington outer coast, which was the shallowest beach, had 174 columns.

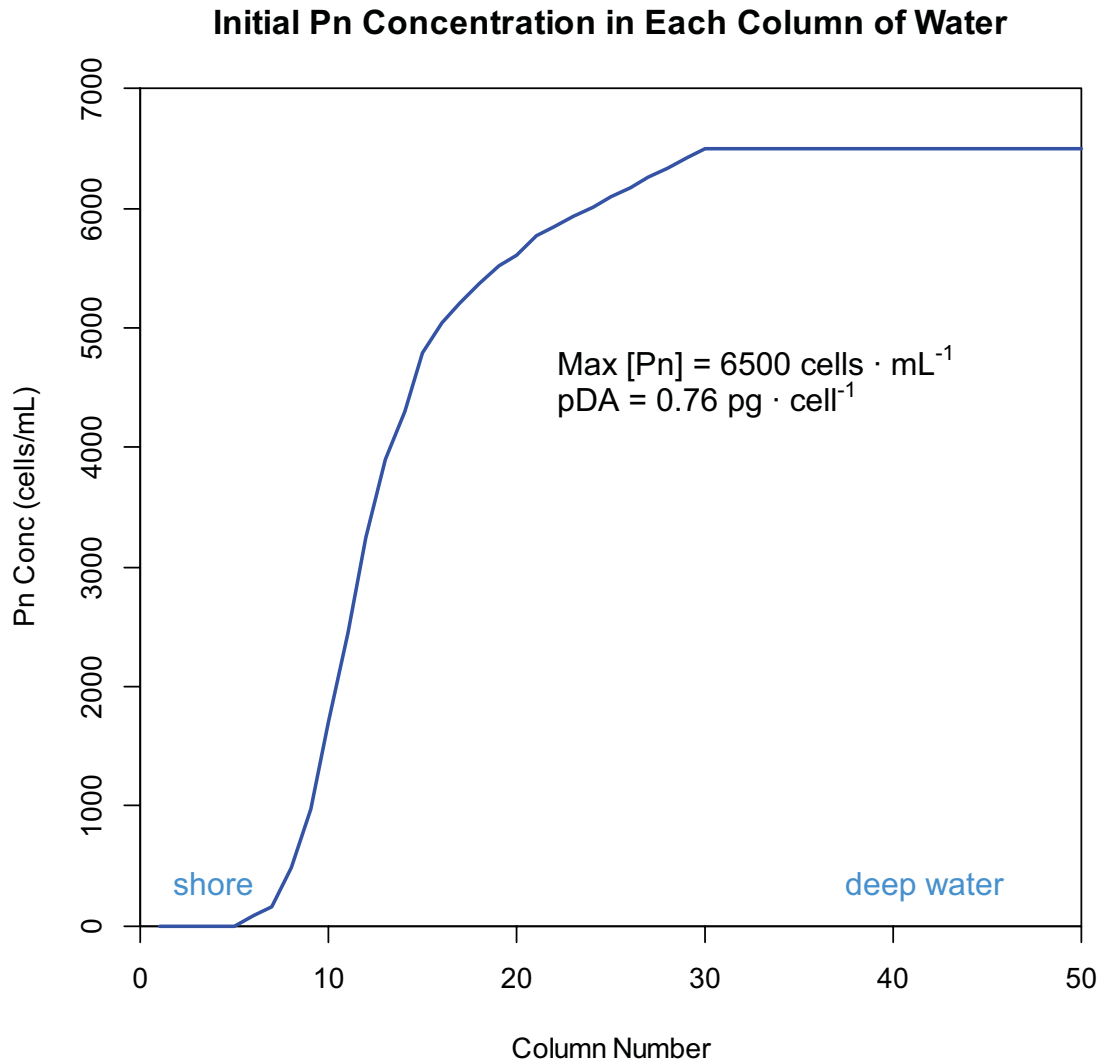


Figure 10. Initial *Pseudo-nitzschia* concentration in each column of water in the Lagrangian *Pseudo-nitzschia* bloom model. All columns seaward from the first 29 were assigned the same initial *Pseudo-nitzschia* concentration.

Model Assumptions & Justification

As described in Chapter II: Phytoplankton Advection into the Intertidal Zone of a Temperate Fjord, advection of phytoplankton into the intertidal in Lofall, Hood Canal has the following characteristics:

1. the depth of maximum chlorophyll (highest phytoplankton density) is ~2m;

2. tidal currents flow almost perfectly parallel to shore (in the y-direction) as the tide rises and falls, with only a small fraction of their flow (~10%) directed towards or away from shore (in the x-direction); thus, a tidally advected bloom passes over many clams at similar tidal heights along its path during tidal flood, and approximately retraces the path during tidal ebb; and,
3. the water column over the shallow intertidal is well mixed.

As a result of these findings, the Lagrangian *Pseudo-nitzschia* bloom model is structured to make the following assumptions:

1. the water column is perfectly mixed within each fixed-volume column of water;
2. the only consumers of *Pseudo-nitzschia* are benthic clams;
3. when submerged, clams feed at a constant rate, regardless of external factors;
4. all ingested *Pseudo-nitzschia* is consumed; none is rejected as pseudofeces;
5. phytoplankton production is zero; and,
6. *Pseudo-nitzschia* toxicity is constant over time.

Lagrangian Model for *Pseudo-nitzschia* Concentrations

A strip of water one-meter wide in the y-direction (along shore), going from shore toward deeper water (in the x-direction), was divided into equal-volume adjacent 3-dimensional columns of water (Fig. 3). As the tide rises and falls, the same water is contained within each column, even though column dimensions might change to accommodate the different slopes of a beach slope profile. Along the Washington outer coast, columns of water do not change in dimension, as the beach has a constant slope.

To advance the model to the next time-step, the following occurs:

1. tidal height is calculated for the new time-step, t ;
2. the location of each column's edges are calculated, along with column width (in the x -direction);
3. suspension-feeding by all clams is calculated for each column based on column location and width;
4. based on suspension-feeding rate by clams under each column, the appropriate amount of *Pseudo-nitzschia* is removed from each column of water; the new *Pseudo-nitzschia* concentration is then adjusted accordingly:

$$[Pn]_{x,t} = \frac{[Pn]_{x,t-1} \cdot (V - SFC_{x,t} \cdot \Delta t)}{V} \quad (\text{Eqn. 9})$$

where $[Pn]_{x,t}$ is *Pseudo-nitzschia* concentration in column x at time step t , $[Pn]_{x,t-1}$ is *Pseudo-nitzschia* concentration in column x at the previous time step, V is volume of water in each column, $SFC_{x,t}$ is suspension-feeding capacity in column x at time step t , and Δt is the length of one time step; and,

5. for specific tidal heights (+1.5m, +1.0m, +0.5m, +0m, -0.5m, -1.0m above MLLW), the *Pseudo-nitzschia* concentration over these tidal heights is recorded for the current time step. The *Pseudo-nitzschia* concentrations at specific tidal heights over time become the output of the Lagrangian *Pseudo-nitzschia* bloom model.

Eulerian Model for DA Concentrations in Bivalves

The Eulerian model for DA concentration in bivalves takes the matrix of *Pseudo-nitzschia* concentration over time at specific tidal heights (+1.5m, +1.0m, +0.5m, +0m, -0.5m, -1.0m above MLLW), to:

1. determine which tidal heights are submerged at that point in time;
2. for submerged tidal heights, calculate accumulation of DA for one clam of each species, using species-specific parameters for clearance rate and DA AE:

$$DA_t = DA_{t-1} + AE \cdot CR \cdot \Delta t \cdot [Pn]_t \cdot pDA \quad (\text{Eqn. 10})$$

where DA_t and DA_{t-1} are DA (mg) accumulated in the clam at times t and $t-1$, AE is assimilation efficiency, CR is clearance rate, Δt is the length of the time step, $[Pn]_t$ is *Pseudo-nitzschia* concentration over the clam at time t , and pDA is particulate DA contained within the *Pseudo-nitzschia* cells;

3. for submerged tidal heights, calculate depuration of DA for each clam according to Eqn. 8; and,
4. record DA concentration (mg DA · kg⁻¹ clam tissue) within each clam. This forms the output of the Eulerian model.

Integrating Variability in Clearance Rate and Depuration Rate into the Model

In order to include variability around species-specific clearance and depuration rates into the model, I:

1. repeated each of the following steps 10,000 times:

- a. for each of the nine Lofall clam species, sampled from the error distributions around the two parameters that make up clearance rate, obtaining a total of 18 parameters (Eqn. 7);
 - b. using each set of 18 sampled parameters, calculated the total suspension-feeding capacity for each Lofall transect as described above;
 - c. summed suspension-feeding capacities for all transects over all beaches, to obtain one grand sum for each sampled set of parameters;
2. sorted the 10,000 grand sums and created a probability density function;
 3. selected 100 percentiles from the probability density function (a sequence from 0.5 to 99.5, incrementing by 1%) and obtained the 100 corresponding sets of clearance rate parameters; these sets of clearance rate parameters were used to calculate suspension-feeding capacity in the Lagrangian model, and DA uptake in the Eulerian model; and,
 4. incorporated variability around depuration rate for each of the 100 runs of the Eulerian model, by sampling once per species from the distribution around depuration rate.

Thus each model run had one set of estimated clearance rate parameters, and one set of estimated depuration rate parameters.

Model Scenarios

I selected 16 scenarios to examine six factors influencing *Pseudo-nitzschia* cycling and DA uptake by intertidal clams (Table 6). First, I examined each of the three Lofall beaches and the Washington outer coast in terms of *Pseudo-nitzschia* concentration over time and clam DA concentrations for each species. I then parsed the effects of beach slope profile and clam assemblage composition on *Pseudo-nitzschia* concentration and clam DA content. This involved

setting one of the factors (either beach slope profile or clam assemblage) to a “baseline” value, while changing the other factor to test different conditions. The clam assemblage composition for Beach 2 was selected as the baseline, because this beach and Beach 3 showed very similar suspension-feeding profiles, but Beach 2 had slightly higher clam density. The beach slope profile at Beach 1 was selected as the baseline, because it is steep at the top of the intertidal zone, with a low tide terrace at the bottom of the intertidal zone, which is quite common in Puget Sound.

In all of the previous scenarios, I permitted *Pseudo-nitzschia* concentration in each column of the bloom model to decline as a result of clam feeding. I then investigated the effect of providing a more constant source of *Pseudo-nitzschia* to clams, to examine patterns of DA concentration in clam tissue. Thus, I ran the model for each Lofall beach site with daily revival of initial *Pseudo-nitzschia* concentrations in each column of the bloom model.

Despite their importance to clam DA concentrations, the effects of different *Pseudo-nitzschia* and pDA concentrations were not modeled because these values are directly proportional to clam DA concentration. Similarly, the effects of different DA AEs are important to clam DA concentrations, but AE is also directly proportional to clam DA concentrations.

Table 6. Model scenarios used to examine factors influencing *Pseudo-nitzschia* cycling and DA concentrations in intertidal clams.

Effect Under Examination	Contrasting Options
1.) tidal height	Each species was tested under the range of tidal heights where it is found in the intertidal
2.) site/ species composition	Lofall, Beach 1
	Lofall, Beach 2
	Lofall, Beach 3
	Washington outer coast
3.) beach slope profile	Beach slope profile for Beach 1, with Beach 2 clams
	Beach slope profile for Beach 2, with Beach 2 clams
	Beach slope profile for Beach 3, with Beach 2 clams
4.) clam assemblage composition	Beach slope profile for Beach 1 with Beach 1 clams
	Beach slope profile for Beach 1 with Beach 2 clams
	Beach slope profile for Beach 1 with Beach 3 clams
5.) clam abundance	Beach 2 mean clam abundance
	Beach 2 mean clam abundance + 1 standard deviation
6.) bloom renewal	Beach 1 with daily bloom renewal
	Beach 2 with daily bloom renewal
	Beach 3 with daily bloom renewal

Comparing Species by Their Potential to Accumulate DA

Lastly, I explored clam interspecies differences in ability to accumulate and retain DA, using a model simulation where all clams were fed a constant diet of the same concentrations of *Pseudo-nitzschia* at constant toxicity. If clam DA concentration is viewed as a combination of DA uptake and DA loss, when these two processes are equal, no change occurs in DA concentration within the clam, and an asymptotic concentration of DA is reached. For each species, the DA

concentration asymptote can be obtained analytically by combining the differential equation for DA uptake with the differential equation for DA depuration and setting them to zero:

$$\frac{dC}{dt} = 0 = CR \cdot 24 \cdot AE \cdot [Pn] \cdot pDA - rC_{Asymptote} \quad (\text{Eqn. 11})$$

where:

dC/dt = change in DA content (in mg) in a clam over time

CR = clearance rate (per hour)

AE = assimilation efficiency of ingested DA

$[Pn]$ = *Pseudo-nitzschia* concentration

pDA = particulate DA contained in each *Pseudo-nitzschia* cell

$-r$ = DA depuration rate (per day)

$C_{Asymptote}$ = asymptotic DA content (mg) in a clam

Solving for $C_{Asymptote}$ and then dividing by the wet weight of the clam (in order to obtain mg DA per kg of clam tissue), we obtain:

$$\frac{1}{wetWt} \cdot \frac{CR \cdot 24}{r} \cdot AE \cdot [Pn] \cdot pDA = [DA]_{Asymptote} \quad (\text{Eqn. 12})$$

where:

$wetWt$ = tissue wet weight of the clam

$[DA]_{Asymptote}$ = asymptotic DA concentration in the clam

Thus, if clam size, clearance rate, AE, DA depuration rate, and wet weight are known, it is possible to calculate maximum DA concentration under a constant and known diet of *Pseudo-nitzschia* concentration and toxicity. Since *Pseudo-nitzschia* concentration and particulate DA are direct proportionality constants in the equation, the magnitude of difference between asymptotes of different species is constant. This magnitude of difference between clam species varies based on clam size, as well.

RESULTS

Suspension-Feeding Capacity at Various Tidal Heights

Suspension-feeding capacity at Beaches 2 and 3 was highest at approximately +1.5m above MLLW, where oyster and mussel beds occur (mean = 141 & 94 $\text{l}\cdot\text{hr}^{-1}\cdot\text{cm}^{-1}\cdot\text{m}^{-1}$, respectively; Fig. 11). Clearance rates for mussels and oysters are also high compared to infaunal clams (Table 3). Suspension-feeding capacity by clams in sandy-gravel substrates between +1.0 to 0m MLLW was slightly higher at Beach 1 (mean=30 to 55 $\text{l}\cdot\text{hr}^{-1}\cdot\text{cm}^{-1}\cdot\text{m}^{-1}$ tidal height $\cdot\text{m}^{-1}$) than at Beaches 2 or 3 (mean=36 & 19 to 52 $\text{l}\cdot\text{hr}^{-1}\cdot\text{cm}^{-1}\cdot\text{m}^{-1}$, respectively). Transects over eelgrass beds, which occurred at tidal heights of approximately -0.5m, were relatively low in suspension-feeding capacity at Beaches 1 and 2 (mean=0 & 9 $\text{l}\cdot\text{hr}^{-1}\cdot\text{cm}^{-1}\cdot\text{m}^{-1}$, respectively). In contrast, Beach 3 had a higher suspension-feeding capacity in the eelgrass beds \sim -1m MLLW, due to the presence of large infaunal clams (mean=36 $\text{l}\cdot\text{hr}^{-1}\cdot\text{cm}^{-1}\cdot\text{m}^{-1}$). At all three Lofall beaches, variability in clam abundance was high, leading to high variability in suspension-feeding capacity, such that

standard deviations often equaled or exceeded mean suspension-feeding capacity per centimeter of tidal height (Fig. 11).

Mean suspension-feeding capacity of razor clam beds at Twin Harbors along the Washington outer coast was $2.84 \text{ l}\cdot\text{hr}^{-1}\cdot\text{cm}^{-1}\cdot\text{m}^{-1}$. I assumed that the suspension-feeding capacity was zero at tidal heights above razor clam beds ($> +0.94\text{m}$ above MLLW). I also assumed that razor clam density in the shallow subtidal, which was covered during low tide by the Lagrangian bloom model, was equal to razor clam density in intertidal clam beds.

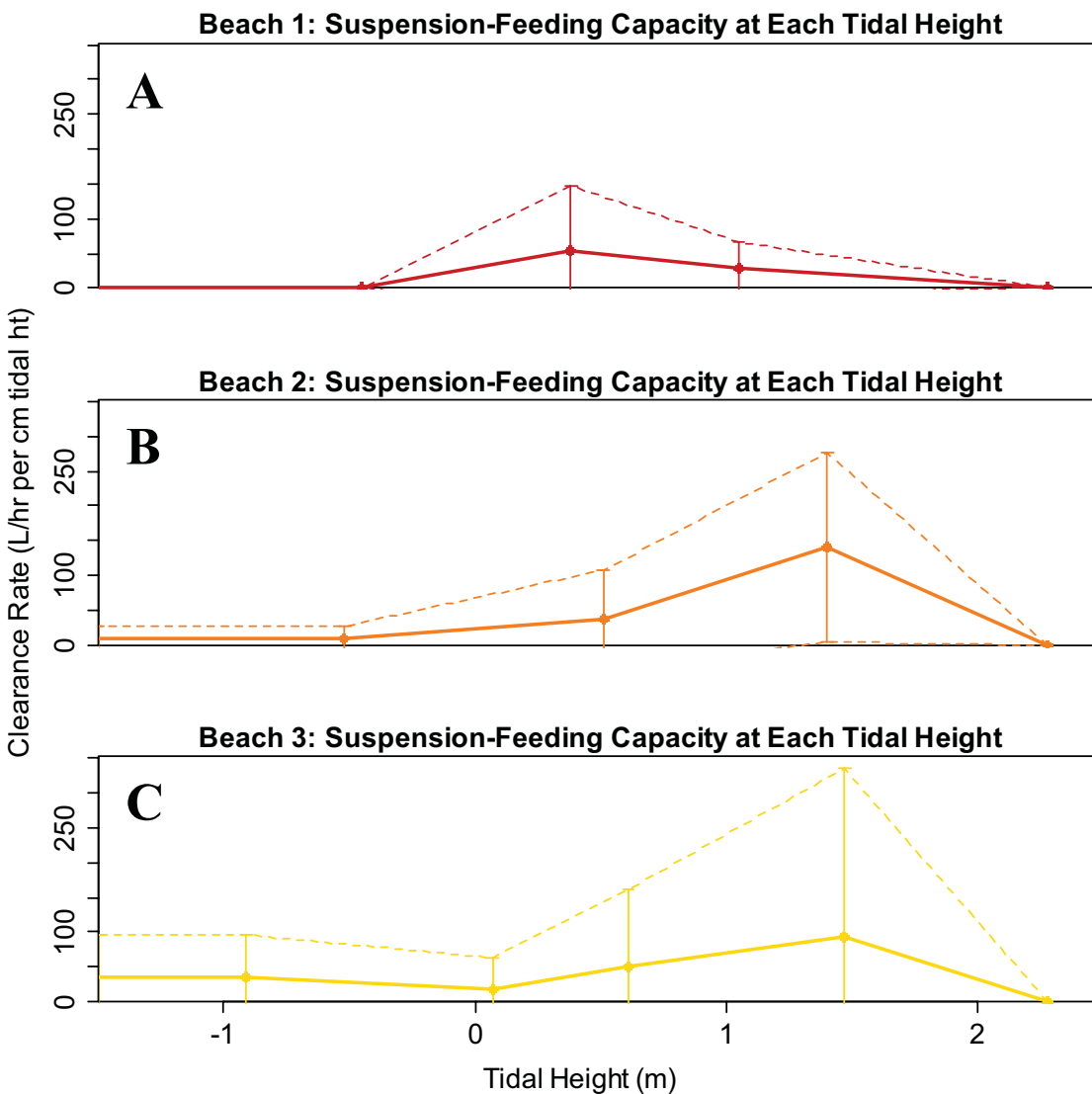


Figure 11. Suspension-feeding capacity (clearance rate \cdot cm⁻¹ tidal height \cdot m⁻¹) at Beach 1 (A), 2 (B) and 3 (C). Error bars and dashed lines represent one standard deviation, where the variability is solely from variability in clam abundance.

Variability in Clearance Rate and its Effect on Suspension-Feeding Capacity

The effect of stochasticity in clearance rate parameters on suspension-feeding capacity is shown for each Lofall beach in Figure 12. Based on the resulting spectrum of suspension-feeding capacities at each site, the sampling scheme that I used to draw 100 sets of clearance rate parameters appears to be adequate. Compared to the amount of variability that was observed in

clam abundance (Fig. 11), stochasticity in clearance rate parameters resulted in substantially lower variability around suspension-feeding capacity.

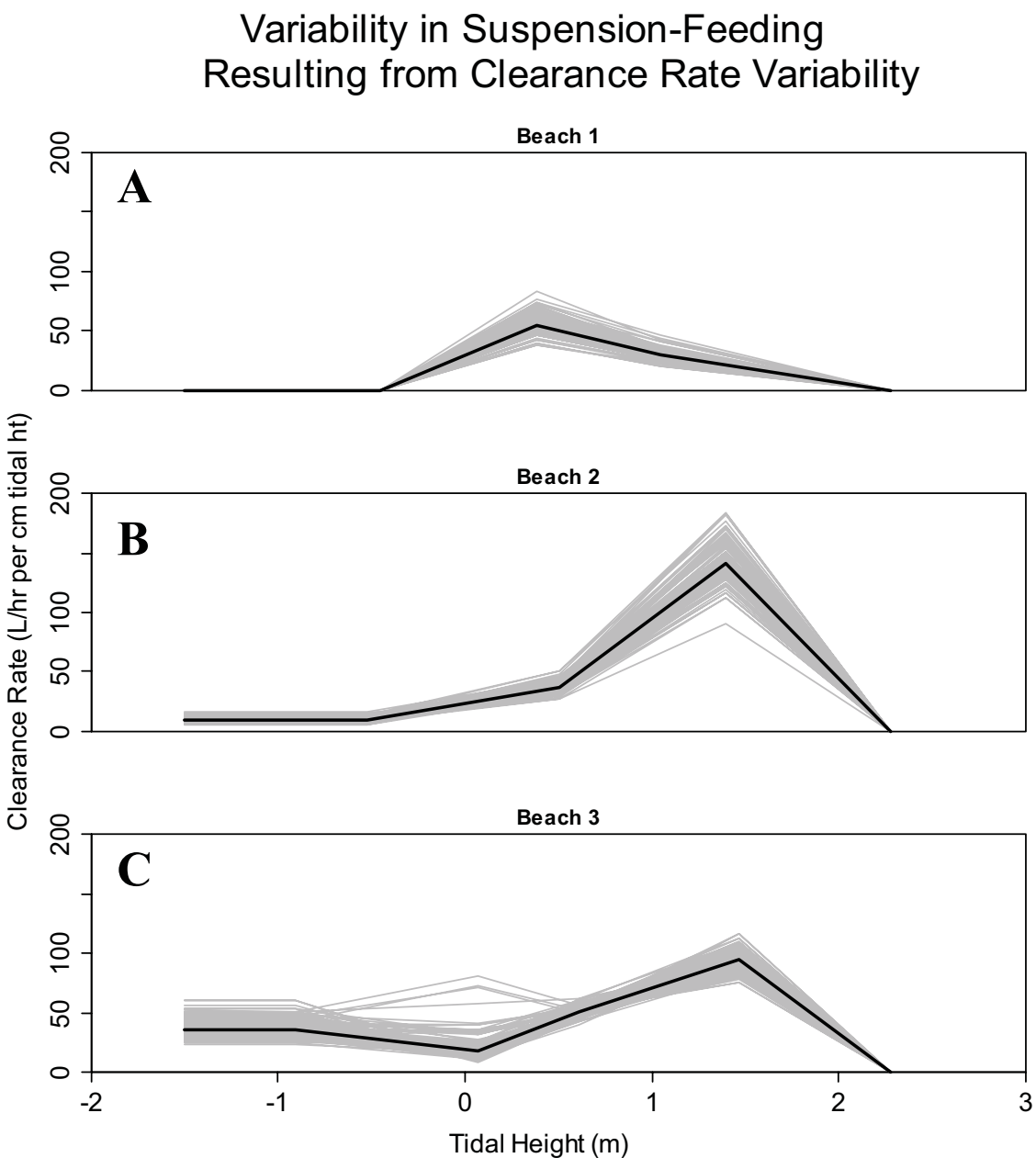


Figure 12. Suspension-feeding capacity at Beaches 1 (A), 2 (B) and 3 (C), calculated from 100 sets of stochastically generated clearance rate parameters. Black lines show deterministic suspension-feeding capacity.

Columns of the Lagrangian Model for *Pseudo-nitzschia* Concentrations

Different volumes of water were necessary to fill the intertidal in the four study locations because each site had a different beach slope profile. In ascending order, these water volumes were: Beach 2: 195×10^3 l; Beach 1: 400×10^3 l; Beach 3: 760×10^3 l; and Washington outer coast: 865×10^3 l. Each column of the Lagrangian model contained 5×10^3 l of water, thus the number of columns modeled at each site was: Beach 2: 40 columns, Beach 1: 81 columns, Beach 3: 153 columns, and Washington outer coast: 174 columns.

In the model, the *Pseudo-nitzschia* bloom is assumed to extend past the last column of water that enters the intertidal zone. At sites that require lower volumes of water to fill the intertidal (Beaches 1 and 2), the integrated quantity of *Pseudo-nitzschia* is low compared to sites that require larger volumes of water to fill the intertidal zone. In contrast, shallow beaches such as Beach 3 and the Washington outer coast have more columns of water passing over each point in the intertidal, resulting in greater access to *Pseudo-nitzschia*. For example, at a tidal height of +1.0m, clams at Beaches 1 and 2 had access to *Pseudo-nitzschia* cells within the first 10 columns of the Lagrangian bloom model, while at Beach 3 and the Washington outer coast, clams at +1.0m had access to *Pseudo-nitzschia* cells within the first 36 and 47 columns, respectively (Fig. 13).

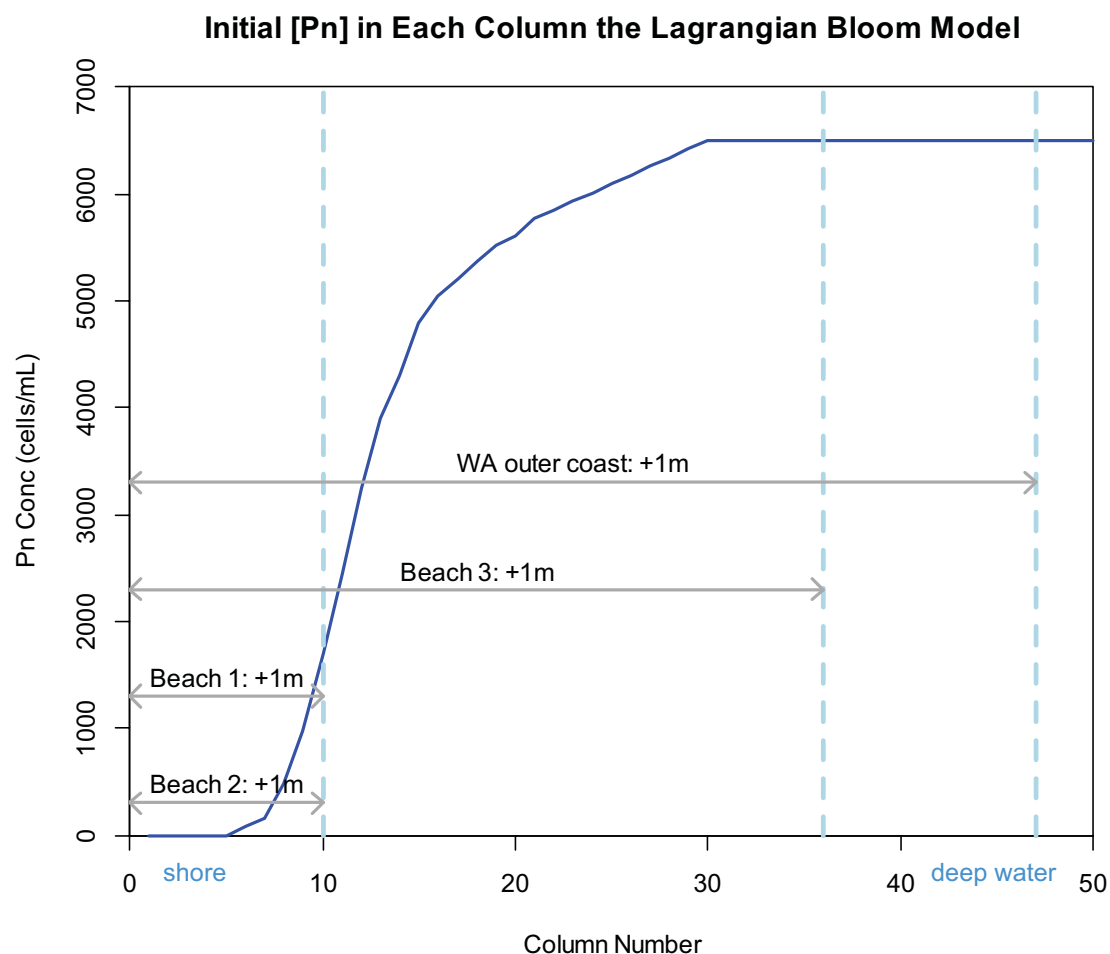


Figure 13. Initial *Pseudo-nitzschia* concentration in each column of the Lagrangian bloom model. Blue dashed lines, grey arrows and text indicate the range of columns that pass over suspension-feeders at +1m above MLLW throughout a tidal cycle.

Effect of Tidal Height

DA concentration over each clam species' tidal range was inversely related to tidal height. The model output indicated *Pseudo-nitzschia* concentrations decline over time at a faster rate at higher tidal elevations than at lower tidal elevations (Fig.14A). The effect on DA concentrations in each species was the same: DA concentrations were highest at the lowest tidal elevation for the species (illustrated for littleneck clam, Fig. 14B). As a result, all other plots show DA at the lowest observed tidal height for each species.

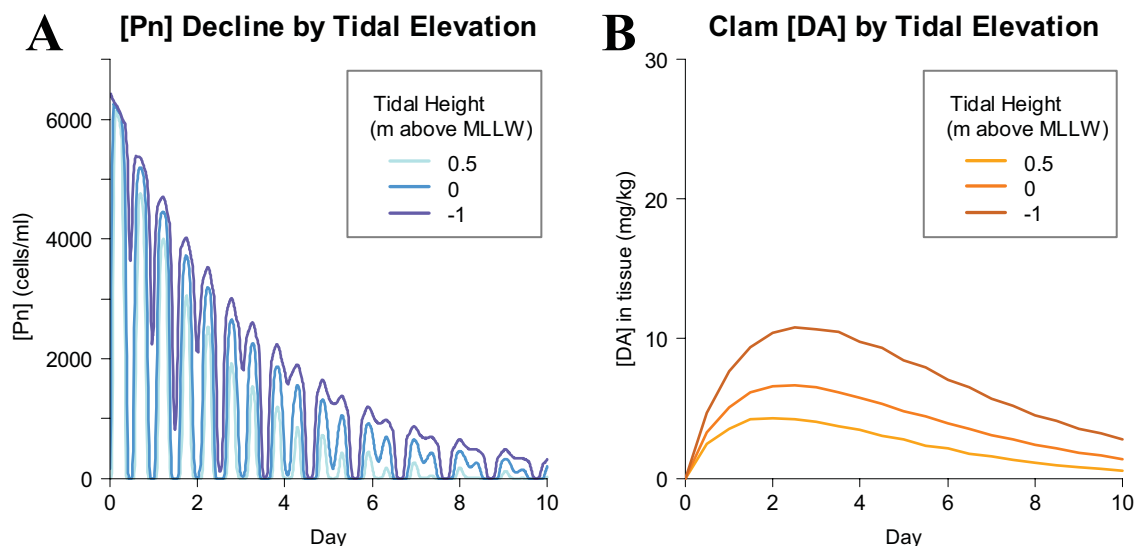


Figure 14. Deterministic model results for *Pseudo-nitzschia* concentration over time at various tidal heights (A) and resulting DA concentrations in littleneck clams at the same tidal heights (B) shown for Beach 3 at Lofall.

Effect of Beach Slope Profile

The strength of the beach slope effect on *Pseudo-nitzschia* concentrations and clam DA over time was explored by modeling the clam assemblage from Beach 2, with beach slope profiles from all three Lofall sites (Fig. 15). The Beach 2 clam assemblage was chosen because clams were present at all tidal elevations and in relatively dense communities.

The shallow beach slope profile at Beach 3 (Fig 15C,F) resulted in slower decline of *Pseudo-nitzschia* concentrations, and higher overall DA concentrations in clams. This effect was disproportionately stronger at higher tidal elevations; indeed, even bay mussel and Pacific oyster, at +0.5m above MLLW, contained relatively high levels of DA during the early part of the modeled time series (Fig. 15F). In contrast to the slow decline of *Pseudo-nitzschia*

concentrations at Beach 3, the more steeply inclined Beach 2 had a quick decline of *Pseudo-nitzschia* and low overall levels of DA in the clams (Fig. 15A,D).

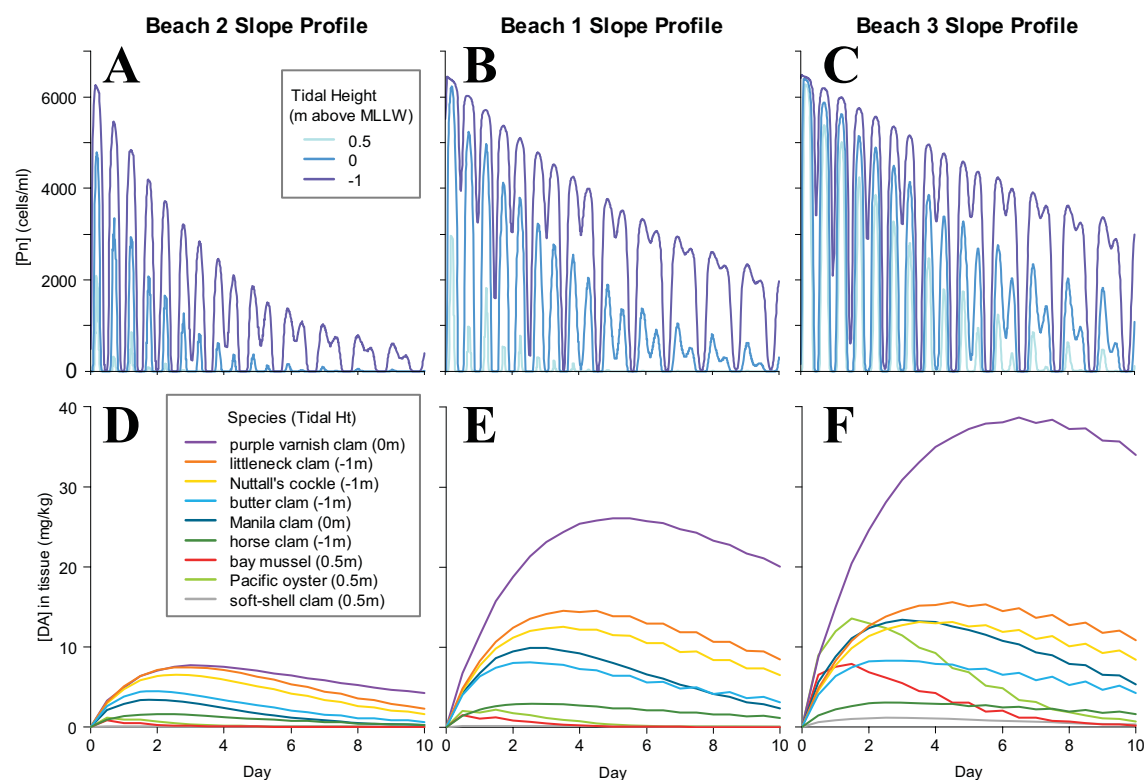


Figure 15. Deterministic model results examining the effect of beach slope profile on *Pseudo-nitzschia* concentrations over time (A,B,C), and on DA concentrations in nine species of bivalves at the lowest part of their elevation range in the intertidal (D,E,F). Model results combine the bivalve assemblage from Beach 2, with beach slope profiles from Beaches 1 through 3. Beaches are arranged in order of decreasing steepness.

Effect of Clam Assemblage Composition

Beach 1 had the lowest suspension-feeding capacity of the three Lofall sites, resulting in the highest *Pseudo-nitzschia* concentrations in the water and DA concentrations in clams (Fig. 16A,D). Although Beaches 2 and 3 both had a high total suspension-feeding capacity, the vertical distribution of clams at the two sites differed (Fig 11). At Beach 2, the majority of the

suspension-feeding capacity was concentrated in the oyster distribution, around +1.5m above MLLW, with a steady decline in suspension-feeding capacity with tidal elevation. Similarly, Beach 3 had much of its suspension-feeding capacity in an upper-mid intertidal concentration of mussel, but the lower intertidal at Beach 3 also had relatively high suspension-feeding capacity. Clams in the lower intertidal at Beach 3 caused a more dramatic decline of *Pseudo-nitzschia* at all tidal heights and reduced DA concentrations (Fig. 16C,F). Thus, the tidal height of suspension-feeders is important in dictating how much *Pseudo-nitzschia* will be available to suspension-feeders at all tidal heights, and thus how much DA they can accumulate.

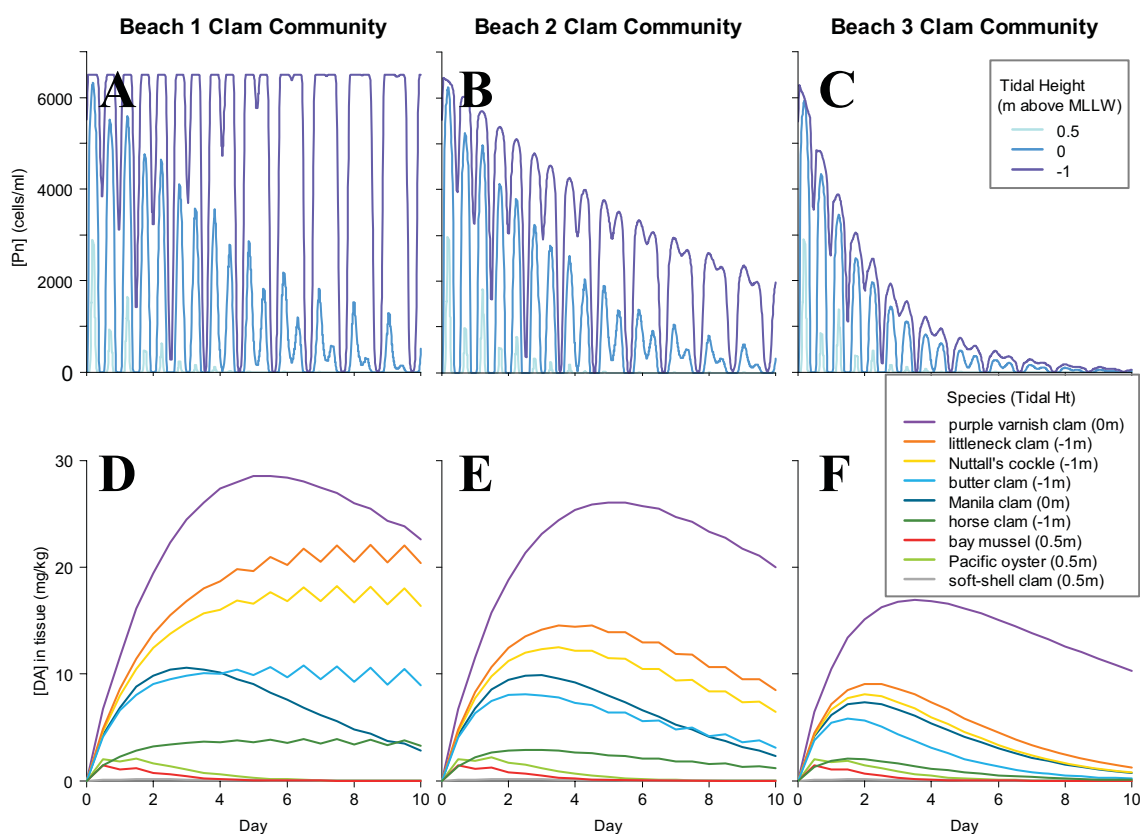


Figure 16. Deterministic model results of the effect of clam assemblage composition on *Pseudo-nitzschia* concentrations over time (A,B,C), and on DA concentrations in clams at the lowest part of their tidal range (D,E,F). Model results combine the beach slope profile from Beach 1, with clam assemblage compositions from Beaches 1 through 3. Beaches are arranged in order of increasing suspension-feeding capacity in the lower intertidal.

Effect of Clam Abundance

Increasing the clam density $\cdot \text{cm}^{-1}$ tidal height by one standard deviation at all tidal heights resulted in faster decline of *Pseudo-nitzschia* concentrations, and lower DA in clams (Fig. 17C,F). In purple varnish clams at 0m above MLLW, maximum DA concentrations declined by 55% (from 26.5 to 11.9) in response to the beach-wide increase of clam density $\cdot \text{cm}^{-1}$ tidal height by one standard deviation. Because of the disproportionately strong impact of suspension feeding at low intertidal heights, the impact of increasing only low intertidal clam density $\cdot \text{cm}^{-1}$ tidal height (at -0.91m above MLLW) by one standard deviation was also tested. Model results for this scenario indicate a decline in maximum DA concentrations by 37% relative to the scenario with mean density $\cdot \text{cm}^{-1}$ tidal height at all tidal heights (from 26.5 to 16.7 $\text{mg} \cdot \text{kg}^{-1}$) (Fig. 17B,E). This indicates that low intertidal clam abundance has a disproportionately stronger effect on *Pseudo-nitzschia* availability to the whole intertidal.

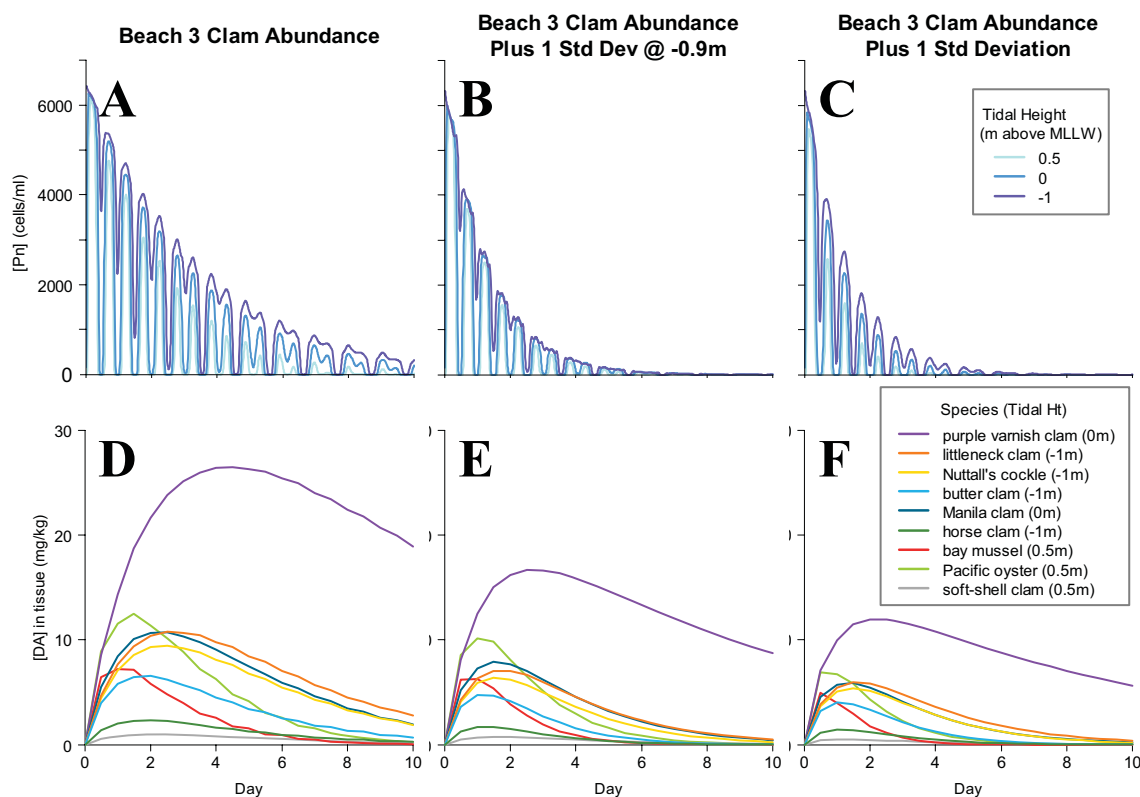


Figure 17. Deterministic model results examining the effect of clam density on *Pseudo-nitzschia* concentrations over time (A,B,C), and on DA concentrations in clams at the lowest part of their tidal range (D,E,F). Panels are arranged in order of increasing clam density at Beach 3, showing: mean clam density \cdot cm^{-1} tidal height (A,D); mean clam density \cdot cm^{-1} tidal height, plus one standard deviation at the lowest intertidal transect (-0.91m above MLLW) (B,E); and mean clam density \cdot cm^{-1} tidal height, plus one standard deviation at all tidal heights (C,F).

Comparison of Suspension-Feeding Capacity of Lofall Beaches and the Washington Outer Coast

Comparison of model results for Lofall beaches and the Washington outer coast indicated that *Pseudo-nitzschia* concentrations declined over time at all sites, but the rate of decline differed by site and by tidal elevation. At all Lofall beaches, *Pseudo-nitzschia* concentration declined rapidly at all tidal heights (Fig. 18A-C), with the exception of the lowest tidal height at Beach 1, where the suspension-feeding capacity was zero (Fig. 11A). On the Washington outer coast, *Pseudo-nitzschia* concentration declined gradually at all tidal heights (Fig. 18D). The

Washington outer coast has a higher volume intertidal area than the Lofall beaches, and also low suspension-feeding capacity at each tidal height. These two factors account for the more gradual pattern of *Pseudo-nitzschia* decline observed for the Washington outer coast.

Within Lofall, Beaches 2 and 3 were similar in their pattern of suspension-feeding capacity, but differed greatly in terms of beach slope profile. This led to differences in *Pseudo-nitzschia* availability at 0m and +0.5m above MLLW, whereby *Pseudo-nitzschia* declined more rapidly at Beach 2 than at Beach 3 (Fig. 18B,C). Beach 3 is a shallow delta that requires approximately four times as much water to fill the intertidal as Beach 2, resulting in a fourfold faster rate at which water passes over each point in the intertidal zone. This reduces the amount of time suspension-feeders have to draw down *Pseudo-nitzschia* in any one column of the Lagrangian model, and results in a greater availability of *Pseudo-nitzschia* at each tidal height over time. Despite the vastly different beach slope profiles at Beaches 2 and 3, *Pseudo-nitzschia* concentration decline at -1m above MLLW was almost identical at the two beaches. This is because Beach 2, in spite of its lesser intertidal volume, has approximately one fourth the low intertidal suspension-feeding capacity of Beach 3 (Fig. 11B,C), which compensates for the lesser water volume over the intertidal zone at Beach 2.

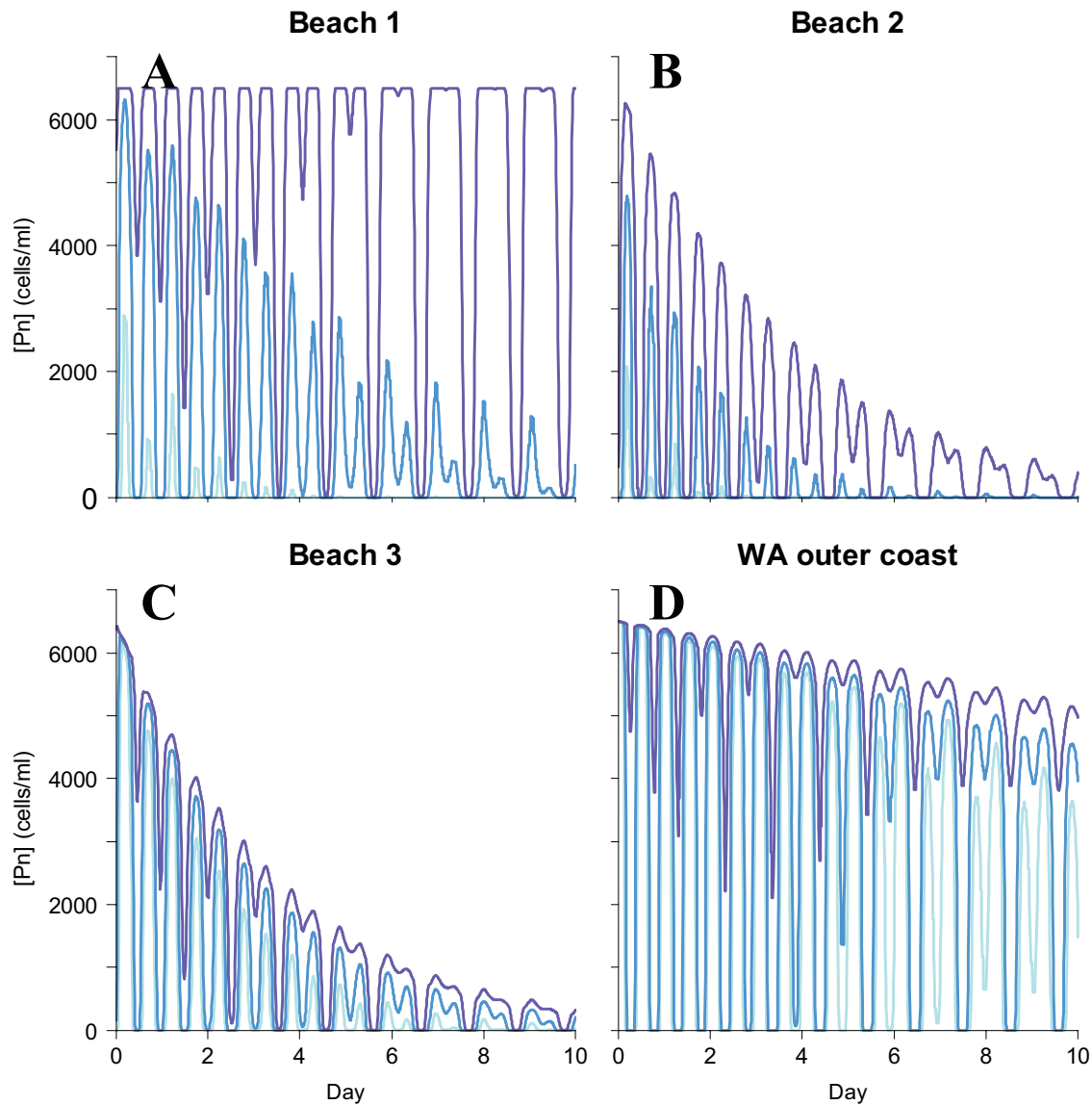


Figure 18. Deterministic model results for *Pseudo-nitzschia* concentrations over time at three Lofall beaches (A-C) and the Washington outer coast (D).

Domoic acid concentrations in clams corresponded predictably to *Pseudo-nitzschia* concentrations by site and tidal height for the three Lofall beaches. Beaches and tidal heights with higher available concentrations of *Pseudo-nitzschia* had higher DA concentrations in clam tissues (Fig. 19A-C). Despite high availability of *Pseudo-nitzschia* on the Washington outer

coast, DA concentrations in razor clams were low over the ten day course of the model (Fig. 19D). This was a result of slow feeding rates in razor clams, combined with low DA AE (11% in razor clams).

At all Lofall beaches, purple varnish clam had the highest DA concentrations at most time points, followed by littleneck clam, Nuttall's cockle, butter clam, and Manila clam. At Beach 3, Pacific oyster and bay mussel had a spike in DA concentrations early in the modeled time series, but their tissue DA concentrations declined quickly due to their fast depuration rates (Fig. 19C).

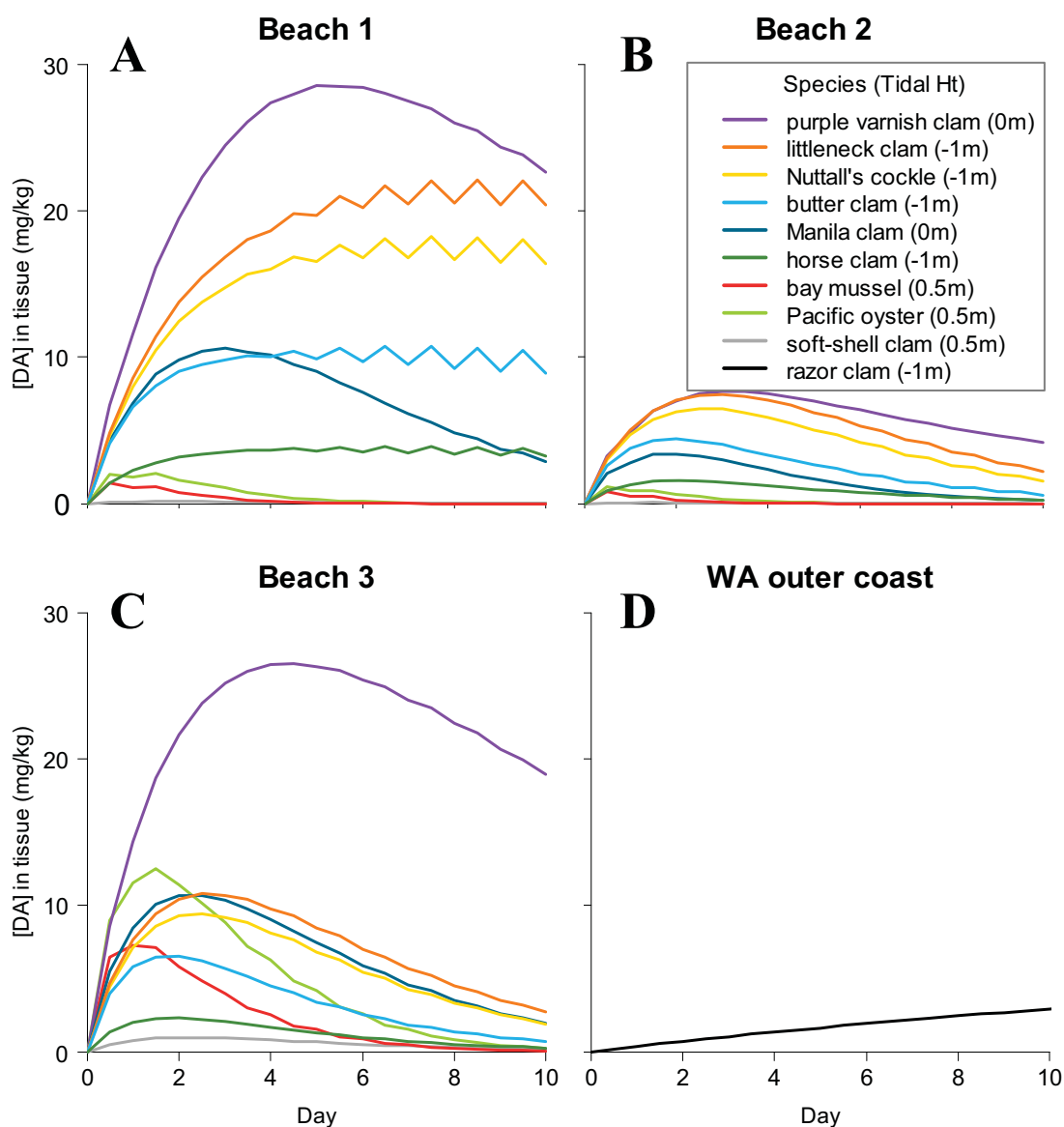


Figure 19. Deterministic model results for DA concentrations in clams at the lowest part of their tidal range. Results are shown for three Lofall beaches (A-C) and the Washington outer coast (D).

Effect of Stochasticity in Clearance and Depuration Rates on Clam DA

Variability around clam DA concentrations increased over time in the stochastic model at all Lofall beaches and in all species except horse clams (Fig. 20). The coefficient of variation (CV) in horse clam DA concentration was highest of all tested species, and had a general trend of

declining over time. This was caused by especially high variability in clearance rate parameters for horse clam (Table 3), which became less important over time at Beaches 2 and 3 as *Pseudo-nitzschia* concentrations declined.

CVs remained nearly constant over time in four species (littleneck clam, Nuttall's cockle, butter clam, horse clam) at Beach 1, which attained a maximum DA concentration around day five and then maintained that concentration over the duration of the model, albeit with oscillations because of tidal flow (Fig. 19A). CVs changed more dramatically in species and sites where a maximum DA concentration was reached, and then DA subsequently declined (e.g., Beach 1, bay mussel, purple varnish clam; Beaches 2 and 3, all species). Bay mussel variability in DA concentration increased particularly rapidly at Beaches 1 and 2, as DA concentrations approached zero. The effective level of variability in milligrams of DA per kilogram of shellfish tissue was very low at that stage, however.

Although the CVs for most species and time points were below 0.5, this resulted in a relatively wide range of possible DA concentrations when the DA was already high (Fig. 21). In purple varnish clam, which had the highest DA concentrations, the estimated final DA concentration range was between 10 and 45 mg · kg⁻¹.

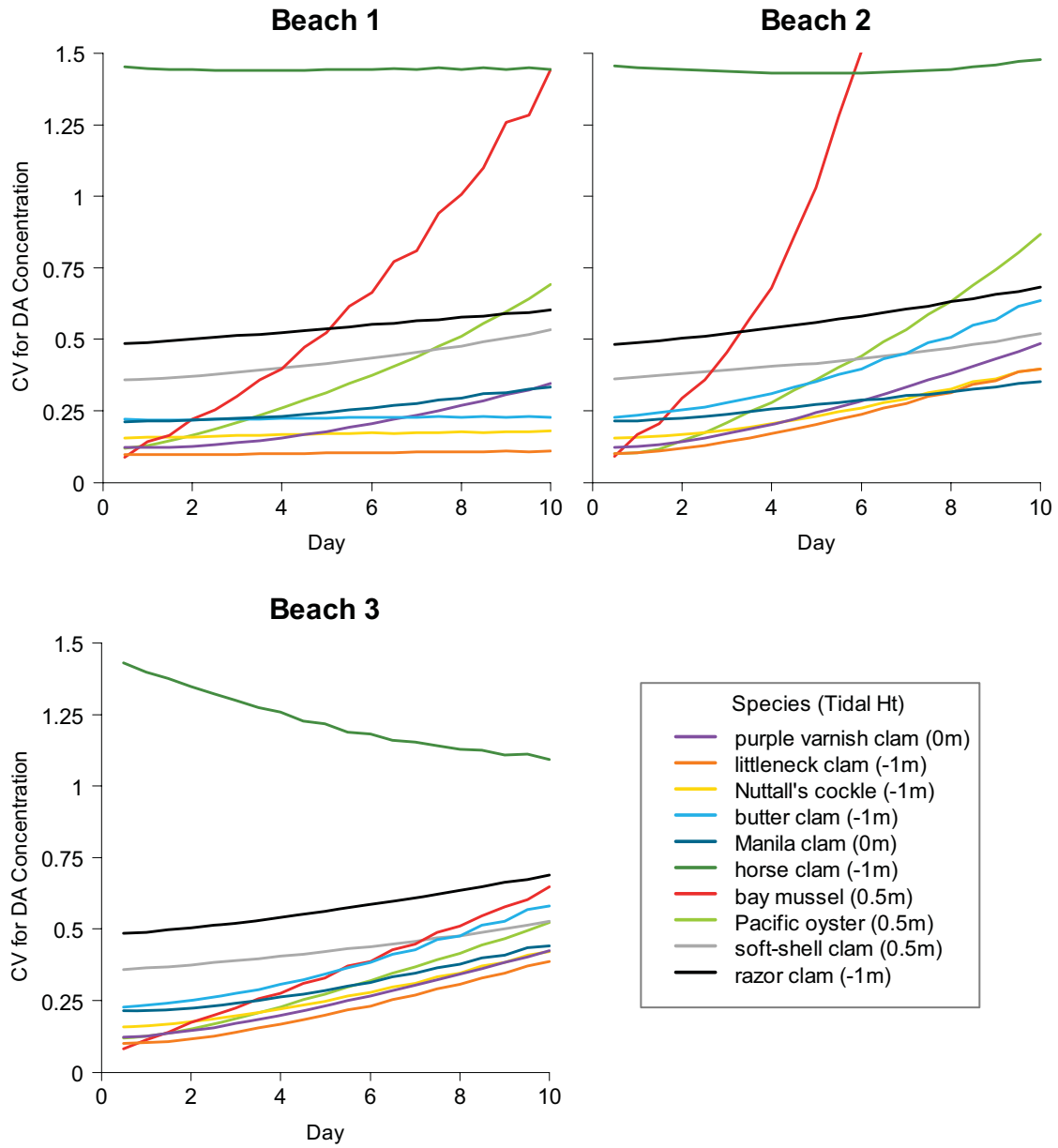


Figure 20. Coefficients of variation in clam DA concentrations over time from stochastic model output for Lofall beaches. Washington outer coast razor clams are modeled for each beach, for comparison with Puget Sound species.

Stochastic Clam DA Concentrations: Beach 1

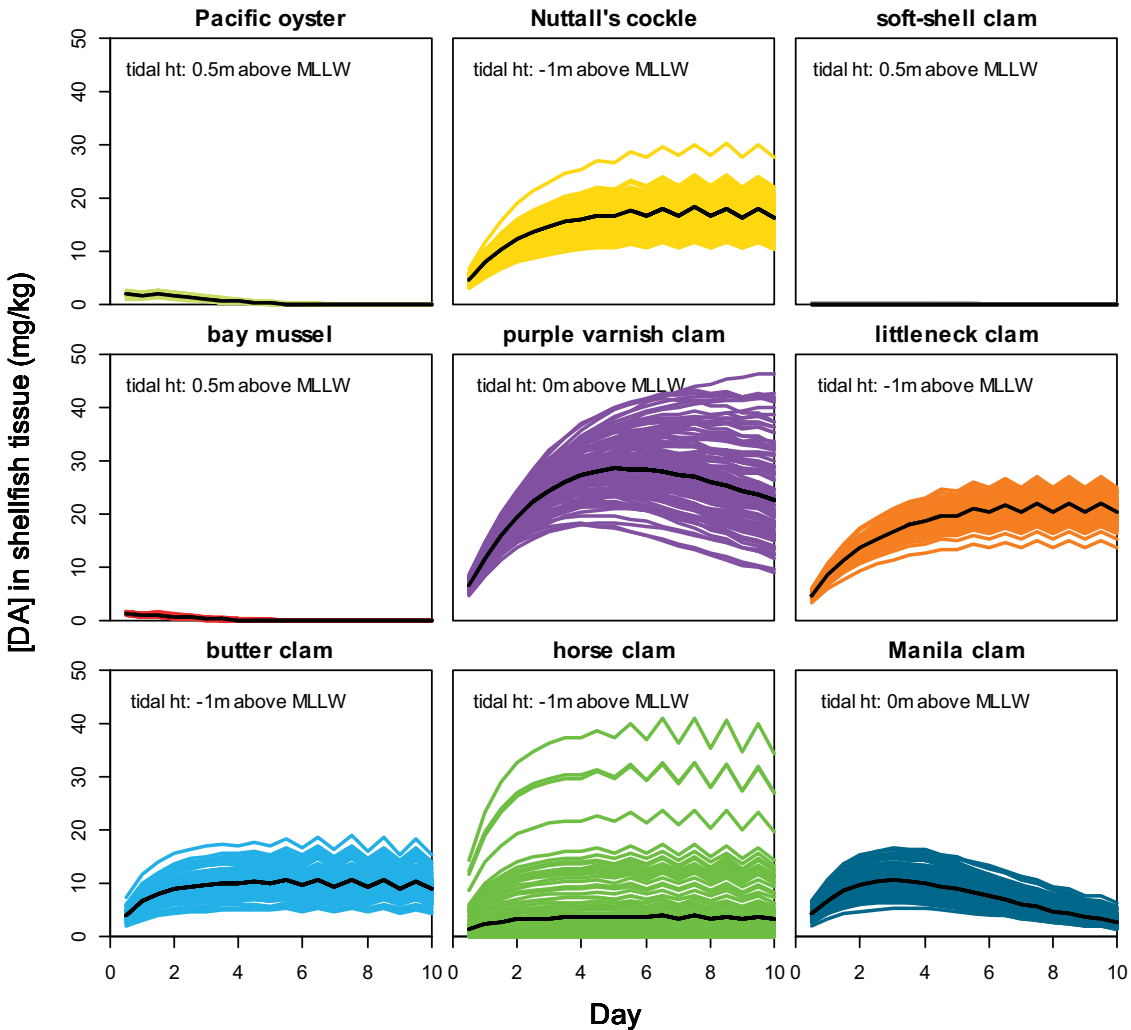


Figure 21. Stochastic model results for DA concentration in clam tissue at Beach 1, showing 100 lines produced by sampling from the variability around clearance rate and DA depuration rate.

Comparing Species by Their Potential to Accumulate DA

Under a modeled scenario where all species received a constant diet of *Pseudo-nitzschia* of $6,500 \text{ cells} \cdot \text{ml}^{-1}$, and pDA of $0.76 \text{ pg} \cdot \text{cell}^{-1}$, the highest maximum DA concentrations occurred in purple varnish clam (Table 7). This species also had the lowest depuration rate among the Puget

Sound clams. Species with the lowest maximum DA concentrations were horse clam, soft-shell clam and butter clam.

Puget Sound species achieved their maximum, steady-state DA concentration within the first five to 50 days of the model, whereas razor clam took nearly 150 days to reach their maximum, steady-state DA concentration (Fig. 22). The slow depuration rate in this species meant that a relatively high DA concentration had to accumulate in this clam's tissue before DA uptake equaled DA depuration and a steady state could be achieved. Feeding rate and DA assimilation are also low in razor clams, resulting in a long time interval before the maximum DA concentration was attained.

Table 7. Modeled clearance rate, assimilation efficiency (AE), depuration rate, and maximum DA concentration in clams at various shell lengths fed on a constant diet of *Pseudo-nitzschia* at $6500 \text{ cells} \cdot \text{ml}^{-1}$, with a pDA of $0.76 \text{ pg} \cdot \text{cell}^{-1}$.

	Shell Length (cm)	Clearance Rate (L/hr)	AE	Depuration Rate (per day)	Max [DA] (mg/kg)
horse clam	11.6	3.8	100%	0.78	5
soft-shell clam	7.7	1.7	59%	0.35	7
butter clam	7.1	3.7	100%	0.84	13
Nuttall's cockle	6.9	4.4	100%	0.49	24
littleneck clam	5.1	1.8	100%	0.40	30
Manila clam	5.0	2.3	100%	0.44	46
Pacific oyster	6.9	8.4	100%	0.86	58
bay mussel	5.0	4.6	39%	1.50	30
razor clam	10.0	2.2	11%	0.03	15
purple varnish clam	4.5	1.6	100%	0.11	255

Maximum DA Concentration by Clam Species

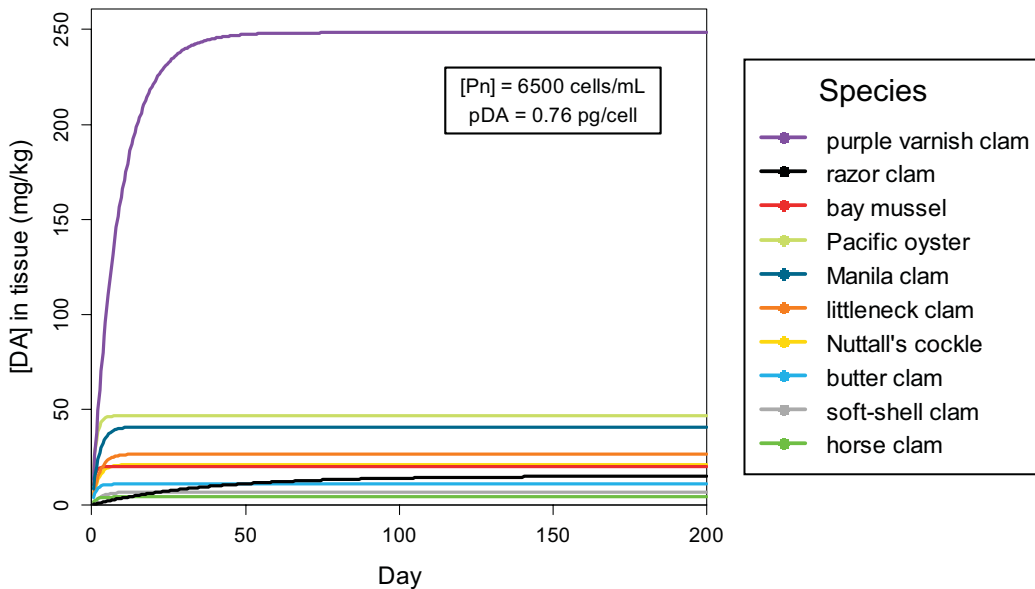


Figure 22. DA concentrations over 200 modeled days for various clam species fed on a constant supply of *Pseudo-nitzschia* at $6500 \text{ cells} \cdot \text{mL}^{-1}$, with a pDA of $0.76 \text{ pg} \cdot \text{cell}^{-1}$. Shell length, clearance rate, AE, and depuration rate for each species are provided in Table 7.

DISCUSSION

The purpose of this modeling study was to determine which aspects of bivalve assemblage and beach physical characteristics impact domoic acid concentrations, and to compare Puget Sound to the Washington outer coast in terms of potential for domoic acid toxicity in harvested shellfish. My models showed that beach slope, clam abundance in the low intertidal zone, and tidal elevation all affect the amount of *Pseudo-nitzschia* available to suspension-feeding clams, and thus the level of competition for *Pseudo-nitzschia* and subsequent domoic acid concentrations in clam tissues. In addition to factors that affect the level of competition for *Pseudo-nitzschia*, there are physiological factors, including DA AE and depuration rate, that make certain clam species more or less toxic following a *Pseudo-nitzschia* bloom.

Factors Affecting *Pseudo-nitzschia* Availability to Clams

Model results indicated that clam DA concentration should be inversely related to tidal height: suspension-feeders at the bottom of their tidal ranges are predicted to be more toxic than the same species higher in their tidal ranges. Thus, if harvested clams must be tested for DA concentrations, my model suggests that they should be collected from the deepest elevations of their tidal range in order to maximize DA concentrations in the clams. Conversely, clams for consumption should be collected at the highest elevations of their intertidal range, to minimize cumulative effects of DA.

Shallow beaches should have higher DA concentrations in clams than steep beaches. On a shallow beach, more water passes over each point in the intertidal zone during a tidal cycle than at a steep beach. Thus, more *Pseudo-nitzschia* passes over shallow beaches, which allows all suspension-feeders to accumulate higher levels of DA in their tissues. Clams for DA testing should be collected from shallow beaches, while clams for consumption should be collected from steep beaches to minimize cumulative effects of DA.

Clam densities in the low and mid-intertidal zone are important in determining DA concentrations on a broader regional scale. Regions with high suspension-feeding in low- to mid-intertidal waters result in a gauntlet for *Pseudo-nitzschia*, limiting its availability to all clams in the intertidal zone. Clams that are situated at higher tidal elevations feed from waters that already receive reduced *Pseudo-nitzschia* concentrations, thus these animals do not contribute as

much to the draw-down of a bloom, at least not under conditions of little or no horizontal mixing.

In addition to the factors discussed above, I also tested the effects of season and of bloom footprint on *Pseudo-nitzschia* availability to suspension feeders. Comparing strong tidal signals during summer/winter spring tides to weak tidal signals during spring/fall neap tides resulted in slightly higher phytoplankton concentrations (because intertidal areas were exposed for longer periods during extreme low tides) and slightly higher clam DA concentration (~10%), with almost identical shapes of clam DA concentration curves. Comparison of smaller bloom footprints to larger bloom footprints yielded a predictable outcome: smaller blooms result in lower clam DA concentrations.

Differential DA Accumulation by Species

Purple varnish clams had a very high maximum DA concentration relative to other species, when all clams were modeled with continuous submersion and constant *Pseudo-nitzschia* concentration and toxicity. This ability to take up and retain DA longer than other species was also apparent in all of the model scenarios where, despite their modeled depth at 0m above MLLW, purple varnish clam had a higher DA than all other species. The slow depuration rate and moderate clearance rate in purple varnish clam results in toxicity being reached faster in this species than in other species.

The model estimated that razor clams reached a maximum DA concentration of $15 \text{ mg} \cdot \text{kg}^{-1}$ very slowly, over a period of about 150 days. This does not seem to fit with data from a Washington

outer coast *Pseudo-nitzschia* bloom that occurred in fall 2002, where razor clams reached DA concentrations of $99 \text{ mg} \cdot \text{kg}^{-1}$ over a course of 25 days (Table 8) (Trainer & Suddleson 2005). In both the modeled scenario as well as the 2002 bloom event, as a product of *Pseudo-nitzschia* concentration and particulate DA per cell, the total accessible DA was the same order of magnitude. This indicates a need to re-examine aspects of DA uptake in razor clams. During the DA uptake and depuration experiments, I obtained a model result for assimilation efficiency of 11% in razor clams. If the actual assimilation efficiency is closer to 100% in razor clams, the maximum DA should be around $150 \text{ mg} \cdot \text{kg}^{-1}$ and will be reached sooner. The *in situ* feeding rate could also be higher than measured in laboratory experiments, causing a higher maximum DA concentration as well.

Table 8. Domoic acid levels and *Pseudo-nitzschia* cell numbers preceding and during the late summer 2002 bloom event at Kalaloch Beach, Washington (Trainer & Suddleson 2005).

Date	<i>Pseudo-nitzschia</i> (cells/L · 10 ⁶)	Domoic Acid	
		pDA (pg/cell)	Razor clam (mg/kg)
30-Aug-02	0.16		
3-Sep-02	0.23		
5-Sep-02	0.19	0.16	2
9-Sep-02	0.01	3.11	2
12-Sep-02	0.02	1.56	
16-Sep-02	0.04	5.45	
18-Sep-02			2
20-Sep-02	0.25	2.49	
23-Sep-02	0.53	4.29	8
25-Sep-02	0.64	5.11	17
30-Sep-02	0.72	0.43	26
3-Oct-02	1.56	1.96	
6-Oct-02	4.27	0.79	52
10-Oct-02	3.05	1.33	
13-Oct-02			99

Comparing Washington outer coast to Puget Sound

In terms of clam assemblage, beach habitat, and clam physiology, there are three primary differences between the Washington outer coast and Puget Sound, which could lead to differences in DA harvest closure frequency: 1) beach slopes on the outer coast are shallower than most Puget Sound beaches, resulting in more water that inundates the intertidal during every tidal cycle; this results in a larger quantity of *Pseudo-nitzschia* that passes over each clam for potential consumption; 2) suspension-feeding capacity is greater in Puget Sound than on the Washington outer coast, both in terms of clam density as well as clearance rate of clams; the lower suspension-feeding capacity on the outer coast means that more *Pseudo-nitzschia* will be available to clams on the beach; and, 3) DA depuration rate of razor clams on the outer coast is much slower than that of clams in Puget Sound; once toxic, clams on the outer Washington coast will hold onto toxin much longer than clams in Puget Sound. These three factors all increase the chances of DA harvest closures on the Washington outer coast when compared to Puget Sound.

Ability of bivalves to limit exposure of other bivalves to *Pseudo-nitzschia*—a case for competition

Within whole assemblages of intertidal clams, co-occurring species can be important competitors for available food resources (Peterson & Andre 1980). In Willapa Bay, an outer Washington coast estuary that produces much of the oyster harvest from Washington state, oysters near the mouth of the bay consume nearly all of the oceanic phytoplankton that is brought into the bay by tidal currents, limiting the availability of phytoplankton to oysters further in the bay (Banas et al. 2007). While Puget Sound is much deeper than Willapa Bay and has different water circulation dynamics, there may still be phytoplankton draw-down in intertidal waters to which clams have

access. This would result in generally lower toxicity in all clams. Peterson and Black (1987) observed that suspension feeders in the low intertidal zone are capable of depleting the food supply during incoming tides, limiting food to suspension-feeders in the high intertidal zone. In Puget Sound, this type of resource competition during a *Pseudo-nitzschia* bloom could be responsible for limiting DA concentrations in clams, as predicted by my models.

Future work

The model presented in this chapter was a scenario case study for *Pseudo-nitzschia* availability to clams in Puget Sound and on the Washington outer coast, and subsequent DA accumulation and cycling in clam tissues. All models require simplifications, some of which may decrease the accuracy of the model predictions. The three model assumptions most likely to cause inaccuracy are: 1) assuming no *Pseudo-nitzschia* production during the bloom; 2) values used for DA assimilation efficiency; and, 3) assuming perfect vertical mixing of each water column in the Lagrangian bloom model.

***Pseudo-nitzschia* production.** In a laboratory setting, DA production by *Pseudo-nitzschia* cells occurs under nutrient limitation (Pan et al. 1996a, Pan et al. 1996b). Thus, I modeled a situation where the *Pseudo-nitzschia* bloom had reached stationary phase (little to no growth was occurring) and was producing DA. I assumed that any production by the bloom would be balanced by zooplankton feeding on the bloom. This assumption may not be accurate under natural conditions, where water circulation and mixing bring nutrient influxes, spurring renewed bloom growth. Nutrient influxes may have reinvigorated the Sequim Bay bloom in 2005, which showed one rapid increase and subsequent decline, followed by a second rapid increase several

days later, and a subsequent decline (Fig. 9A). Interestingly, the rapid declines in *Pseudo-nitzschia* concentration recorded during the Sequim Bay bloom are within the same order of magnitude as some of the *Pseudo-nitzschia* declines that I modeled at the three Lofall beaches. The first Sequim Bay rapid decline, from 23×10^6 cells \cdot L⁻¹ to 6.5×10^6 cells \cdot L⁻¹, or a 354% decline, occurred over a seven-day period, and the second decline, from 15.3×10^6 cells \cdot L⁻¹ to 1.7×10^6 cells \cdot L⁻¹, or a 900% decline, occurred over a 14-day period. Over the first seven-day period in my models, the peak *Pseudo-nitzschia* concentrations declined at -1m above MLLW by 623% at Beach 2 and 723% at Beach 3 (Beach 1 had no *Pseudo-nitzschia* decline at -1m). The peak *Pseudo-nitzschia* concentration declines at 0m above MLLW were: 382% at Beach 1; 8377% at Beach 2; and 987% at Beach 3. The modeled *Pseudo-nitzschia* concentration declines are greater than those documented at Sequim Bay. Part of the difference may be caused by sampling methodology: the modeled *Pseudo-nitzschia* declines represent the *Pseudo-nitzschia* concentration peaks, while the Sequim Bay samples may not be characteristic of actual peak concentrations within the bloom, since they were collected by bucket from the end of a pier at Sequim Bay (Trainer et al. 2007).

Assimilation Efficiency Estimates. Assimilation efficiency has a strong effect on DA concentrations in clam tissues because the two are directly proportional to each other. No data on AE were available for five of the ten species in the models, so I assumed AE=100%. I obtained AE estimates for four species by fitting a model to the DA uptake and depuration data, and using maximum likelihood to determine how much the uptake phase of the model should be shifted up or down to fit the data. This shift corresponded to the estimates of AE. A better way to obtain AE estimates would be through direct experiments on individual clams: feeding

individual clams a constant diet of *Pseudo-nitzschia*, constantly tracking their feeding rate through phytoplankton decline, and then testing the DA concentration in each clam's tissue. While this methodology would still employ maximum likelihood estimation to obtain AE, constantly measuring phytoplankton concentrations in the tank and performing the experiment on numerous individuals would provide more accurate estimates.

Vertical Mixing in the Lagrangian Bloom Model. I assumed that the Lagrangian bloom model was perfectly mixed within each column, and that intertidal clams had access to all phytoplankton in the water directly above them. This may be accurate for the shallow intertidal zone, which is more likely to be vertical well mixed by wind and waves, but a ten-meter deep water column on a calm day is unlikely to have perfect vertical mixing. One way to account for different *Pseudo-nitzschia* concentrations by depth is to include depth as a second dimension in the model, and divide each column into an upper and a lower box with a mixing component between them. The mixing component would probably require measurement in the field to be realistic over a variety of weather conditions.

Additional Improvements to the Model. Additional topics that could be explored, but are likely to be less important than the other three are: 1) suspension-feeding impact of non-bivalve taxa, including barnacles and polychaetes; 2) effect of *Pseudo-nitzschia* consumption by zooplankton; 3) effects of different initial conditions for *Pseudo-nitzschia* concentrations at the edge of the bloom; 4) effects of high and low phytoplankton concentrations on clam feeding rates and assimilation efficiencies (e.g., Riisgard et al. 2003); 5) evaluation of DA depuration rate by clam

size (e.g., Mafra et al. 2010a); and, 6) including horizontal mixing between adjacent columns of the Lagrangian bloom model.

Synthesis

INTRODUCTION

The motivation for this study was to explore why *Pseudo-nitzschia* and domoic acid (DA) have caused numerous and extensive shellfish harvest closures on the Washington outer coast since 1991, but have only caused three harvest closures in Puget Sound. To extend this question further, I wanted to also assess the relative risk of harvest closures in Puget Sound by determining whether there are inherent aspects of Puget Sound's beach suspension-feeding benthic macroinvertebrate (SFBM) assemblages, which make it a less likely candidate for DA harvest closures than the outer Washington coast.

In this dissertation, I explored the effect of SFBM assemblages, clam physiology, and beach gradients on DA concentrations in bivalve tissue, in Puget Sound and on the outer Washington coast. My hypotheses were that: 1) competition between benthic intertidal SFBMs in Puget Sound reduces availability of *Pseudo-nitzschia* to harvestable clams, including oysters and mussels, thus reducing DA concentrations in clam tissue; and, 2) the physiology of Puget Sound clams reduces DA uptake or retention compared to razor clams on the Washington outer coast. These hypotheses were addressed in a mathematical model to predict DA concentrations in Puget Sound intertidal clams after feeding on a *Pseudo-nitzschia* bloom, for comparison to modeled DA concentrations in outer Washington coast clams.

I used a combination of field, laboratory, and modeling approaches to address these hypotheses. The approaches included: an ecological field study to obtain data on clam community assemblages and beach slope profiles at Lofall, Hood Canal; an oceanographic field study to determine phytoplankton flux into the intertidal; laboratory experiments to measure clearance rate, and domoic acid uptake and depuration rates; and, numerical modeling to link all of these data together for Puget Sound and the outer Washington coast.

I considered three factors that affect DA concentrations in bivalve tissues: 1) exposure of bivalves to *Pseudo-nitzschia*; 2) uptake of domoic acid into bivalve tissue; and, 3) retention of DA in bivalve tissues (Fig. 1). Each of these categories produced one or more parameters, leading to the outcome of *Pseudo-nitzschia* concentration decline across a beach as a result of clam feeding, and domoic acid concentrations over time in a variety of clam species. The purpose of the synthesis section is to discuss lessons learned from this study, and to explore aspects of Puget Sound and Washington outer coast bivalve physiology, intertidal suspension-feeding communities, and beach gradients that affect the likelihood of domoic acid buildup in bivalves.

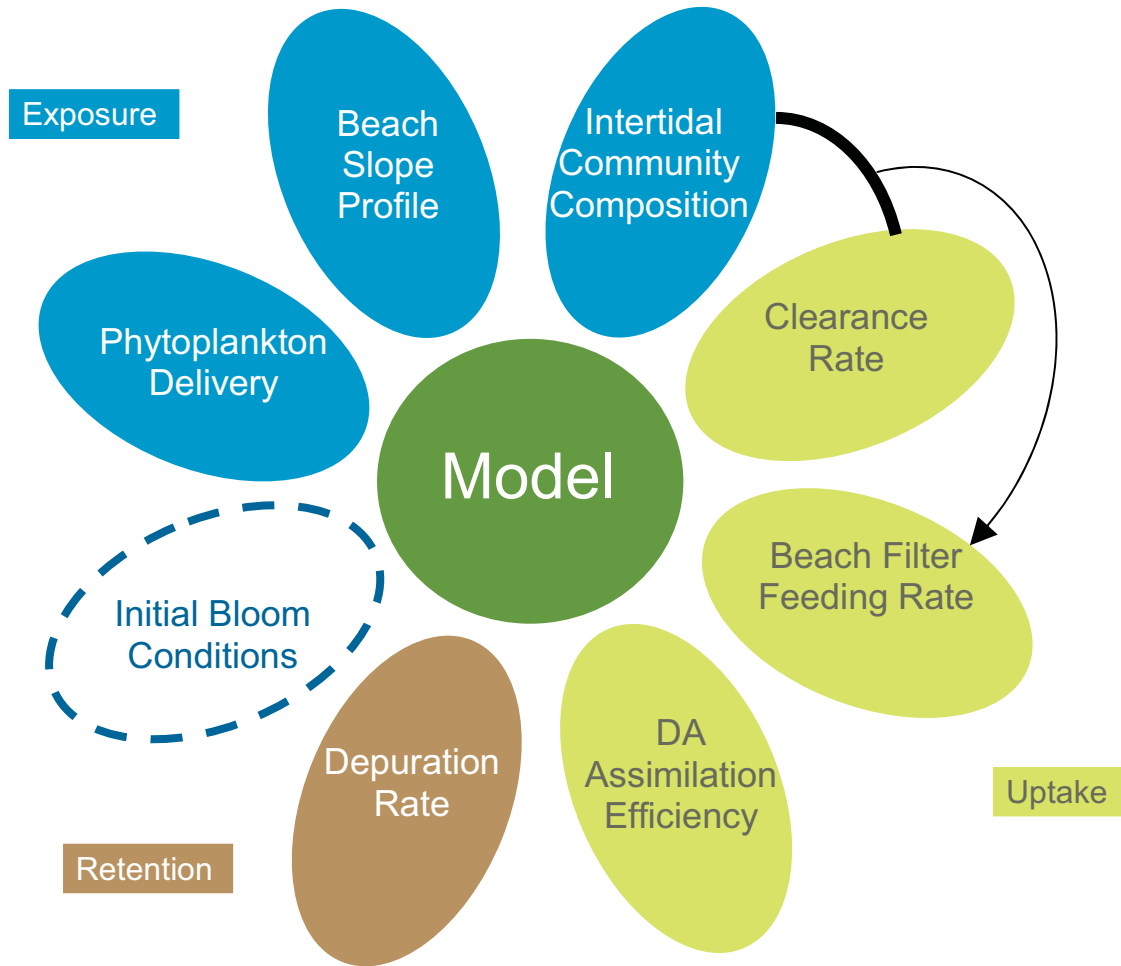


Figure 1. Conceptual model of data inputs that were used as parameters in the model. Filled blue ovals represent data inputs that relate to exposure of clams to *Pseudo-nitzschia*, light green filled ovals represent data inputs that relate to domoic acid uptake by bivalves, and the brown filled oval represents the data input that relates to retention of domoic acid in bivalve tissues. The oval with the dashed outline represents a parameter that was obtained from literature values. The beach suspension-feeding rate was calculated as a combination of intertidal community composition and clearance rate.

PRIMARY RESULTS FROM THE DISSERTATION CHAPTERS

Clearance rate and domoic acid depuration experiments revealed several important differences in the physiology of bivalves found in Puget Sound, compared to those on the Washington outer coast. Firstly, clearance rate by organism size (tissue dry weight) for razor clams is slower than for all other Puget Sound bivalves (Fig. 2). From the perspective of an individual clam, this may

reduce *Pseudo-nitzschia* consumption; however, from a beach perspective, this also reduces the potential for competition between bivalves for *Pseudo-nitzschia*. This competition may be important to reducing domoic acid concentrations in all bivalves at a beach. Secondly, depuration rates for razor clams are slower than for all other Puget Sound clams (Fig. 3). This had been discussed in the literature, although an exact depuration rate had not been presented for razor clams (Wekell et al. 1994, Adams et al. 2000, Trainer & Bill 2004). Thus, my domoic acid depuration data confirmed this finding. Slower depuration rates in razor clams mean that, once they accumulate toxin in their tissues, they will remain toxic for longer periods of time.

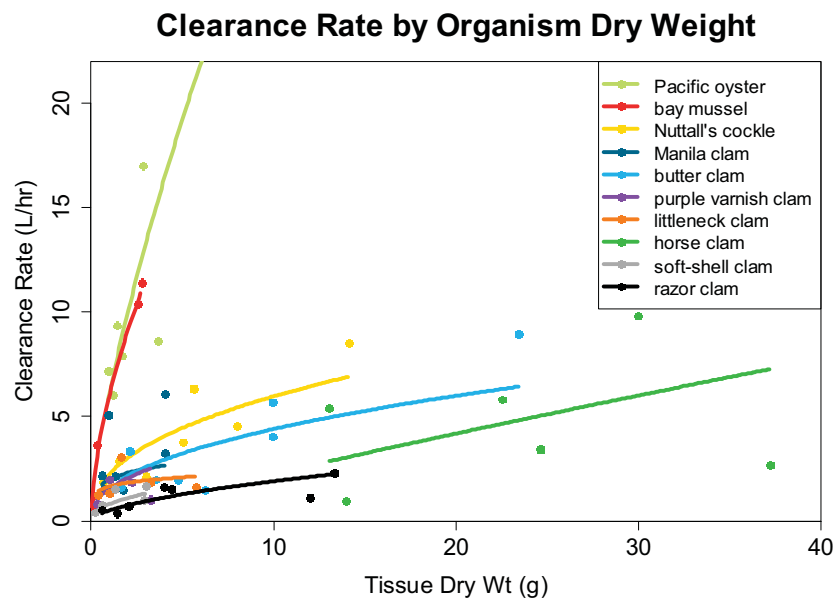


Figure 2. Clearance rate on *Pseudo-nitzschia* by organism size (tissue dry weight) for Puget Sound bivalves (shown in colors), and Washington outer coast razor clams (shown in black). Points represent individual clearance rate experiments, while lines represent allometric model fit to the data.

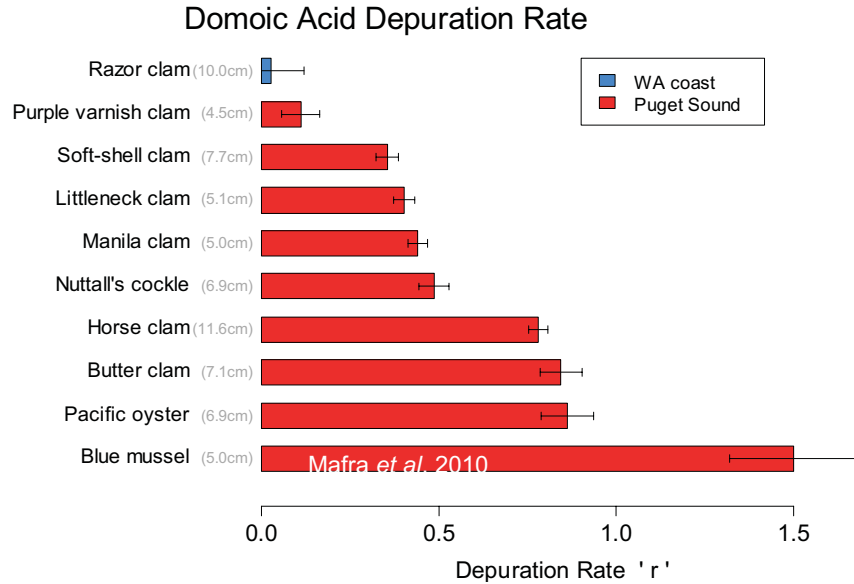


Figure 3. Domoic acid depuration rates for Puget Sound bivalves (shown in red) and Washington outer coast razor clams (shown in blue). Depuration rate for blue mussels (calculated by Mafra et al. 2010a) is shown as an approximation for the closely related bay mussel found in Puget Sound.

Modeling allowed me to test the effect of four primary factors that affect competition for *Pseudo-nitzschia* between bivalves on a beach, and thus influence domoic acid concentrations in bivalve tissue:

1. tidal elevation: within each bivalve species, individuals that are lower in the intertidal zone will have better access to *Pseudo-nitzschia*, and will thus accumulate more domoic acid in their tissues than individuals of the same species that live higher in the intertidal;
2. beach slope profile: beaches with a shallow gradient have a higher volume of water that covers them during high tide; thus, bivalves living on a shallow gradient beach will have access to more *Pseudo-nitzschia* than bivalves living on a steep gradient

beach. This results in higher domoic acid concentrations in bivalves on a shallow gradient beach when a *Pseudo-nitzschia* bloom intersects the beach;

3. the region-wide presence of suspension-feeders at low intertidal or shallow subtidal elevations (such as Hood Canal's farmed geoduck beds; geoducks were not included in this analysis because geoducks could not be obtained for clearance rate and depuration rate experiments): as the tide rises, bivalves situated at low intertidal elevations have access to *Pseudo-nitzschia* first, and can act as a gauntlet for *Pseudo-nitzschia* getting to the upper intertidal zone. This clearly results in lower availability of *Pseudo-nitzschia* for bivalves at higher tidal elevations, but also decreases *Pseudo-nitzschia* availability over time at all tidal elevations, thereby reducing domoic acid accumulation in all bivalves. This factor works on a region-wide basis (as opposed to a beach-wide basis) because phytoplankton travel predominantly in a long-shore direction, with only a small component of its travel directed towards shore as the tide rises (see Chapter II: Phytoplankton Advection into the Intertidal Zone of a Temperate Fjord). Thus, the competitive effect of suspension-feeding bivalves at low tidal elevations on one beach impacts suspension-feeding bivalves at higher tidal elevations on a different beach (located down-current of the first beach, where currents are measured during tidal flood);
4. bivalve density on a region-wide basis: high bivalve densities within a region result in lower availability of *Pseudo-nitzschia* for all suspension-feeders, and lower accumulation of domoic acid in bivalve tissues.

The importance of competition for phytoplankton among bivalves has been shown in other areas as well. In Willapa Bay on the outer Washington coast, oysters near the mouth of the bay consume nearly all of the oceanic phytoplankton that is brought into the Bay by tidal currents, limiting the availability of phytoplankton to oysters further in the bay (Banas et al. 2007). In Shark Bay, Australia, Peterson and Black (1987) observed that suspension feeders situated at low intertidal elevations are capable of depleting the food supply during incoming tides, limiting food to suspension feeders situated at high intertidal elevations. This type of resource competition during a *Pseudo-nitzschia* bloom could be responsible for limiting domoic acid concentrations in Puget Sound bivalves, as predicted by my models.

IMPLICATIONS OF MY FINDINGS FOR PUGET SOUND

From the perspectives of beach gradient, intertidal suspension-feeding community, and bivalve physiology, there are several factors that reduce Puget Sound's relative likelihood for domoic acid harvest closures compared to the Washington outer coast. Most Puget Sound sand/ cobble beaches have a steeper gradient than sandy beaches on Washington's outer coast (Fig. 4). Less water passes over each tidal elevation on a steep gradient Puget Sound beach than on a shallow gradient Washington outer coast beach. As a result, we should expect lower *Pseudo-nitzschia* availability to suspension-feeding bivalves on most Puget Sound beaches, and thus lower domoic acid accumulation in Puget Sound bivalves. Within Puget Sound, there are some river deltas that have shallow gradients, and will be more similar to the Washington outer coast in terms of water advection rates over the intertidal. At these river delta beaches, we can expect higher *Pseudo-nitzschia* availability to bivalves, thus higher domoic acid toxicity in bivalve tissues.

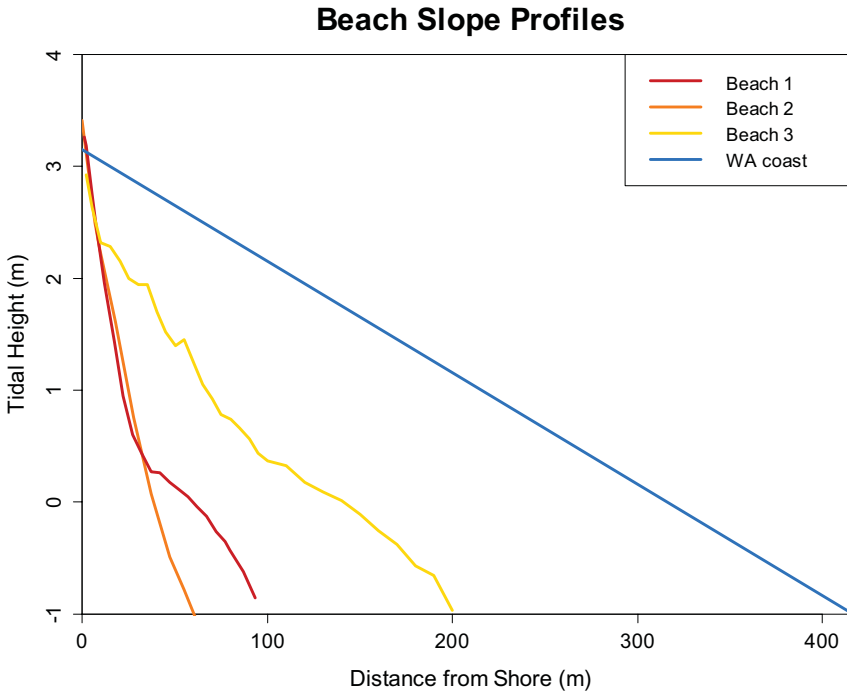


Figure 4. Beach slope profiles for three Lofall, Hood Canal beaches (red, orange, yellow) and a beach slope approximation for the Washington outer coast (blue) based on data from WDFW. The yellow line represents a small delta in Lofall, showing a more similar beach gradient to the Washington outer coast.

Differences in suspension-feeding bivalve abundance between Puget Sound and the Washington outer coast may also affect the relative risks of domoic acid harvest closures in the two locations. The Puget Sound beaches that I examined had substantially higher density of bivalves than the Washington outer coast (Fig. 5). The higher bivalve density, combined with faster clearance rates in Puget Sound bivalves, results in stronger competition by Puget Sound suspension-feeding communities for available *Pseudo-nitzschia*, thus lower accumulation of domoic acid in all suspension-feeding bivalves.

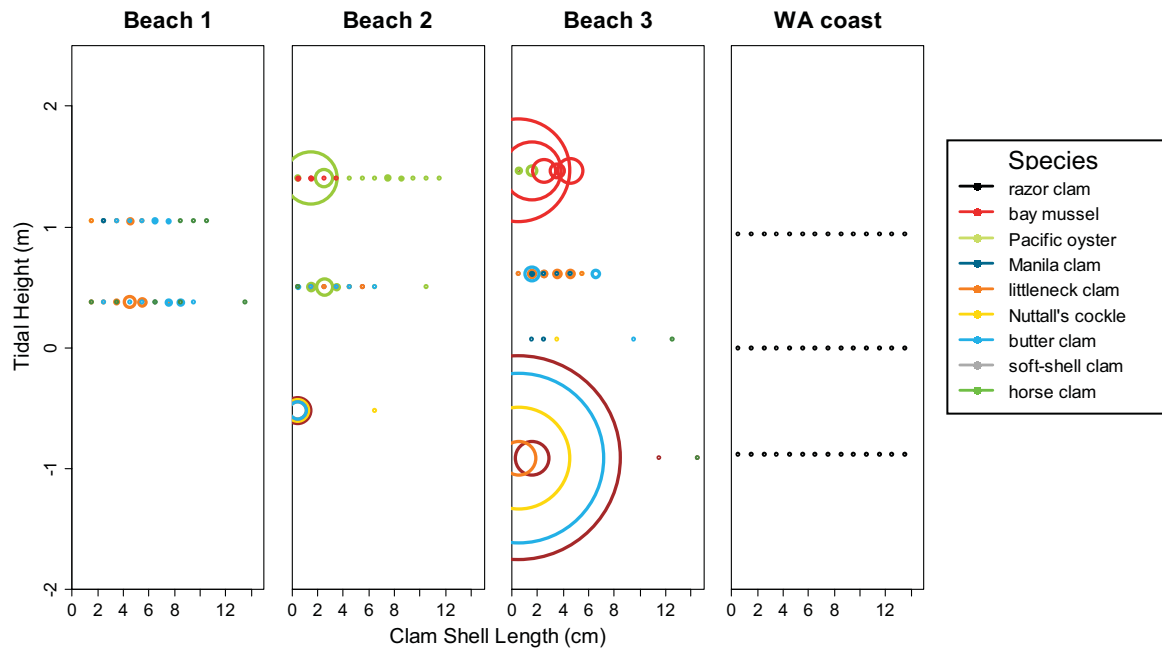


Figure 5. Relative clam density $\cdot \text{cm}^{-2}$ of tidal height and size composition for three or four tidal heights at Lofall beaches (Beach 1,2,3) and the Washington outer coast. Circle diameter represents clam density $\cdot \text{cm}^{-2}$ of tidal height, with bigger circles representing higher density. Different colors represent different clam species.

In cases where Puget Sound bivalves accumulate enough domoic acid to cause a bivalve harvest closure, the closures should be shorter in duration than those on the Washington outer coast. I measured depuration rates in eight species of Puget Sound bivalves, all of which depurated domoic acid faster than Washington outer coast razor clams (Fig. 3). My experimental results are supported by *in situ* evidence from Puget Sound and the Washington outer coast. During the three harvest closures that have occurred in Puget Sound, bivalves have only remained toxic for a few days to several weeks (Bill et al. 2006, Trainer et al. 2007). In contrast, some Washington outer coast razor clam harvest closures have lasted many months (WDFW 2011). Once outer coast razor clams become toxic, they tend to stay toxic for a long time.

IMPLICATIONS FOR MONITORING AND MANAGEMENT

Within Puget Sound, sampling for domoic acid toxicity in bivalves occurs at 30 sites across Puget Sound on a biweekly basis (F Cox, pers comm. June 12, 2012). At each site, bay mussels are used as the sentinel organism for testing. While bay mussels are quick to accumulate domoic acid in their tissues, their fast depuration rate and their location at upper tidal elevations may result in lower domoic acid concentrations in bay mussels than in other species. I tested the relative domoic acid concentrations in bivalves under a simulated common garden scenario, where all Puget Sound and outer coast bivalve species were modeled under conditions of constant submersion and constant diet of *Pseudo-nitzschia*, and found that even with all environmental factors being equal, bay mussels still accumulated less domoic acid than purple varnish clams and Pacific oysters (Fig. 6). My modeling results indicate that the Washington Department of Health sampling protocol has the potential to underestimate maximum DA concentrations in clams at a beach.

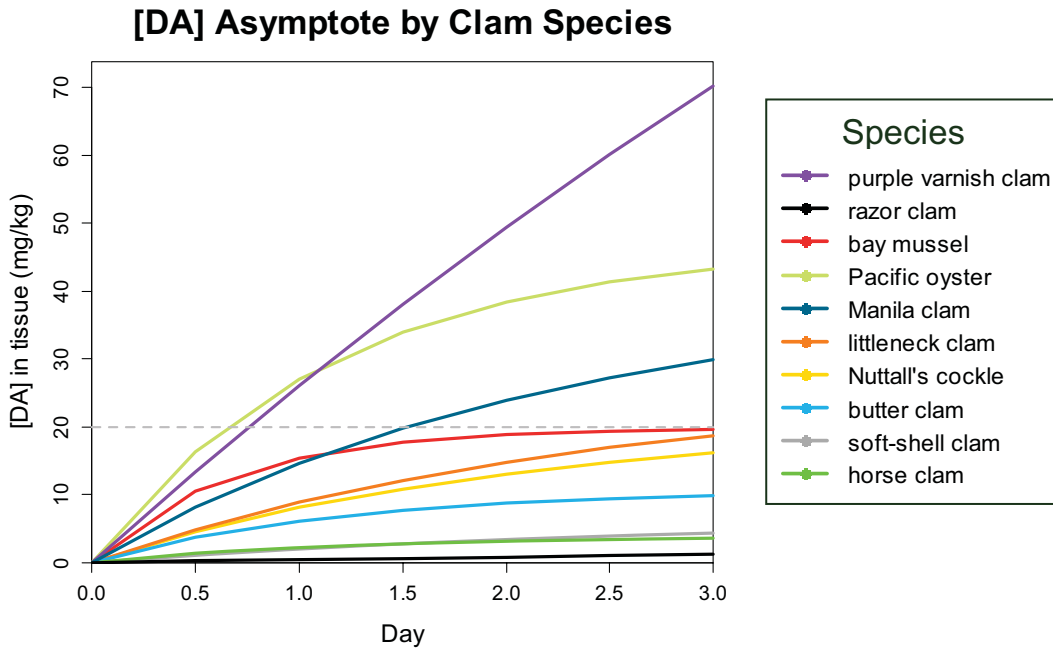


Figure 6. Domoic acid concentrations over three modeled days for Puget Sound and Washington outer coast clam species fed on a constant supply of *Pseudo-nitzschia* at $6500 \text{ cells} \cdot \text{ml}^{-1}$, with a particulate domoic acid concentration (pDA) of $0.76 \text{ pg} \cdot \text{cell}^{-1}$.

Other bivalves would likely be better diagnostic test organisms for a more accurate measure of potential DA exposure. Purple varnish clam may be an especially good sentinel species for domoic acid testing. Compared to other Puget Sound species, purple varnish clam feeds at a medium rate for its body mass (Fig. 2), but depurates domoic acid slowly; this results in high domoic acid concentrations in purple varnish clam tissue. In fact, purple varnish clam may be the closest Puget Sound equivalent to Washington outer coast razor clam in terms of depuration rate (Fig. 3). While Pacific oyster also had a higher domoic acid concentration than bay mussel in the common garden model analysis, this species is more similar to bay mussels. Pacific oyster is found at high tidal elevations, feeds at a fast rate, and depurates domoic acid quickly. Domoic

acid assimilation efficiency is unknown for Pacific oyster (I assumed 100% assimilation efficiency in my modeling analyses); if domoic acid assimilation efficiency in Pacific oyster is similarly low as for eastern oyster (1.3 to 43%, depending on oyster size and duration of exposure to *Pseudo-nitzschia*) (Mafra et al. 2010a), the domoic acid concentration profile over time in Pacific oyster may be very similar to that of bay mussel.

Regardless of which species is tested, specimens should be collected as low as possible in the intertidal, to obtain conservative estimates of domoic acid in shellfish. If possible, bivalves should also be collected from sites with shallow gradients, such as river deltas, rather than sites with steep beach slopes.

Since domoic acid toxicity in shellfish is inversely related to tidal elevation, it may be possible in the future to create a harvest closure protocol whereby only certain tidal heights of a beach would be closed for collection. My model results for littleneck clam at a shallow gradient beach (Beach 3) indicate that clams living at +0.5m above MLLW may have less than half of the domoic acid concentrations of littleneck clams at -1m above MLLW (Fig. 7). These results will need to be verified by field studies to determine whether there are other important forces to model, before creating partial bivalve harvest closures by tidal height.

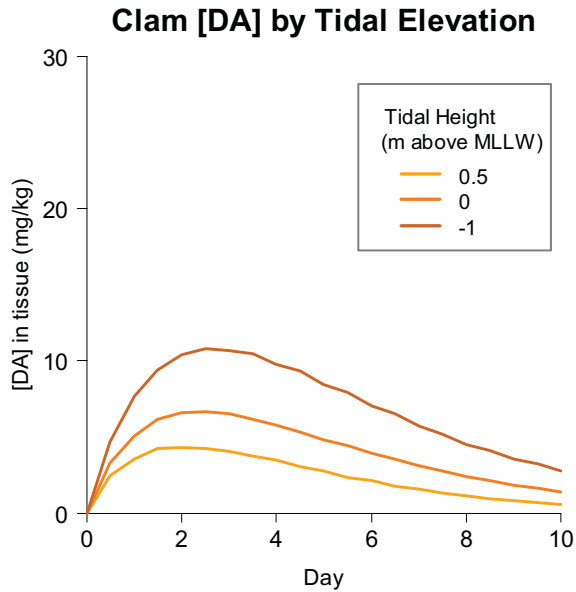


Figure 7. Model results for domoic acid concentrations over time in littleneck clams at three tidal heights: +0.5m above MLLW, 0m (at MLLW) and -1m above MLLW. Model results are shown for Beach 3.

DISCUSSION OF THE STUDY APPROACH AND FUTURE DIRECTIONS

This is the first model of its kind to predict domoic acid concentrations in intertidal shellfish at different tidal heights following exposure to toxin-producing *Pseudo-nitzschia* over many tidal cycles. It is also the first model that compares Puget Sound to the Washington outer coast in terms of their susceptibility to high domoic acid concentrations in bivalves. Through a modeling framework, I was able to identify several factors that are important to domoic acid concentrations in bivalves, and aspects of Puget Sound suspension-feeding communities and beaches that may decrease risk of domoic acid harvest closures compared to the Washington outer coast.

The phytoplankton delivery dynamics were the least understood part of the model. Without data on the vertical and horizontal mixing within the water column, I assumed perfect vertical mixing

in each column of the (Lagrangian) phytoplankton bloom model, and no horizontal mixing between adjacent columns. This is certainly an oversimplification of the complex dynamics that govern water mixing over intertidal and shallow subtidal areas. Model accuracy and predictive capability would be improved by adding realistic parameters for vertical and horizontal mixing. As a first step, each column of the bloom model could be divided into two boxes: 1) a lower box that extends from the seafloor up to the height that clams can access with extended siphons; and, 2) an upper box that has a certain mixing rate with the lower box. Adjacent boxes in the model could also exchange water per a realistic horizontal mixing parameter. Adding a vertical mixing parameter will have a net effect of lowering predicted domoic acid concentrations in bivalves (since bivalves will no longer have access to all phytoplankton within the water column), while adding a horizontal mixing parameter may increase predicted domoic acid concentrations in bivalves.

In the model framework, I assumed no phytoplankton growth and no pelagic consumption of *Pseudo-nitzschia*. The assumption of zero growth was based on the laboratory observation that *Pseudo-nitzschia* tend to produce domoic acid in response to nutrient limitation, which coincides with the diatom culture reaching stationary phase (zero population growth)(e.g., Pan et al. 1996b). However, *in situ* toxin production and bloom growth dynamics are not so straightforward. For example, the Sequim Bay bloom showed a peak in *Pseudo-nitzschia* concentration in early September 2005, followed by a rapid decline in mid-September, then a resurgence of *Pseudo-nitzschia* growth in late-September (Trainer et al. 2007)(Fig. 8). These dynamics of renewed growth may be much more common in natural conditions, where nutrient concentrations are linked to water mixing caused by ocean currents and weather.

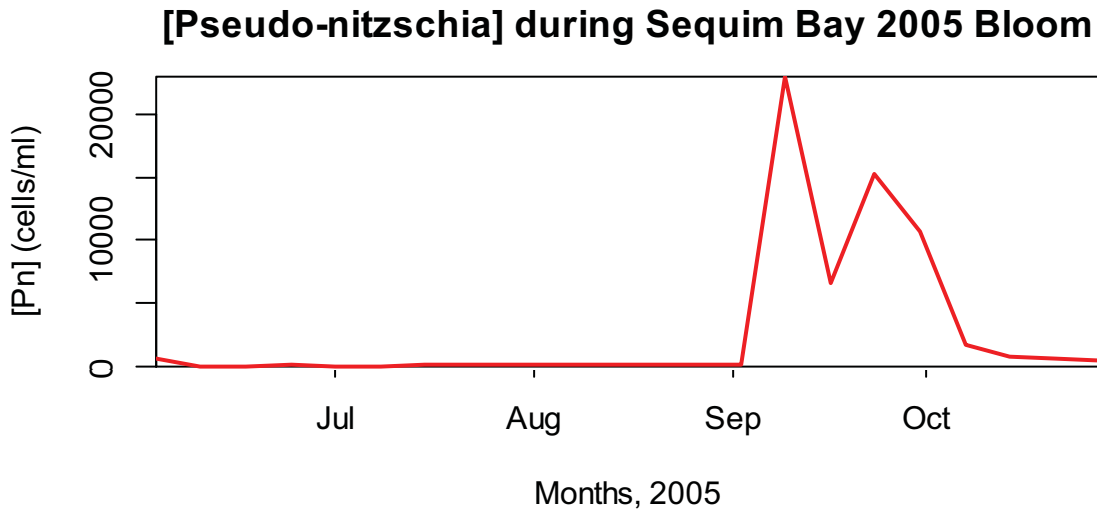


Figure 8. *Pseudo-nitzschia* concentration dynamics during the Sequim Bay bloom in September, 2005. Data from Trainer et al. (2007).

Just off-shore of the Washington outer coast, measurements of *in situ Pseudo-nitzschia* growth rates indicate a mean growth rate of 1.20 d^{-1} (one cell division every 14 hours), with a maximum rate of 2.0 d^{-1} (one cell division every 8 hours)(Olson 2006). *In situ* measurements of microzooplankton (e.g., ciliates, heterotrophic dinoflagellates and heterotrophic flagellates) feeding rates on *Pseudo-nitzschia* ranged between 0 and 1.7 d^{-1} , with a mean of 0.34 d^{-1} (Olson 2006). Copepod feeding on *Pseudo-nitzschia* was also explored as part of the same study; copepods showed neutral preference or discrimination against *Pseudo-nitzschia*, but a high preference for microzooplankton, which could result in interesting trophic cascades. Based on these results, Olson inferred that only under highly specific conditions will the population ecology of *Pseudo-nitzschia* be significantly affected by zooplankton grazing. Thus, for the purposes of the model, a stochastic variable for net *Pseudo-nitzschia* growth rate (growth minus

zooplankton feeding) could be added, to allow exploration of the effect of *Pseudo-nitzschia* growth on domoic acid concentrations in bivalves.

In order to model clam domoic acid concentrations, I needed to use values for assimilation efficiency of domoic acid into bivalve tissue. Assimilation efficiency is the percentage of ingested domoic acid, contained within *Pseudo-nitzschia* cells, that is incorporated into bivalve tissues. In four species (razor clam, soft-shell clam, purple varnish clam, Manila clam), I obtained assimilation efficiency estimates through model fitting to domoic acid uptake and depuration data; and for bay mussel, I used assimilation efficiency reported by Mafra et al (2010) for blue mussels. For all other species, I assumed 100% assimilation efficiency. This assumption was based on domoic acid uptake and depuration experiments where I lacked sufficient data to fit a model to the data and obtain assimilation efficiency, but the data suggested that all ingested domoic acid was assimilated into tissues. Addressing this parameter with experiments designed to specifically obtain the assimilation efficiency would be preferable, as this parameter is extremely important in determining domoic acid concentrations in bivalve tissue, and has significant impacts on inter-species comparisons of bivalve domoic acid concentrations.

In this dissertation, I explored the effects of bivalve abundance, bivalve physiology, and beach gradient on *Pseudo-nitzschia* availability at different tidal elevations, as well as the resulting domoic acid concentrations in bivalves at those tidal elevations. There are other equally relevant aspects of the *Pseudo-nitzschia* bloom and domoic acid toxicity puzzle that may be responsible for domoic acid harvest closure differences between Puget Sound and the Washington outer coast. *Pseudo-nitzschia* assemblages and domoic acid generating capabilities for the two regions

differ (Hubbard 2010)(Olson 2006). On a finer scale, research by Hubbard (2010) also shows that *Pseudo-nitzschia* assemblages vary in the different reaches of Puget Sound, with possible greater likelihood of toxin production in the northern reaches. Within *Pseudo-nitzschia* assemblages, environmental conditions, temporal variability, and chance determine which particular species will dominate the assemblage at any given time (Olson 2006). Factors such as these will also be important in determining the risks of domoic acid harvest closures in Puget Sound.

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VITA

Eva Dusek Jennings was born in Brno, Czech Republic, and immigrated to the United States with her parents and sister in 1981. She attended Stanford University, where she earned a Bachelor of Sciences degree in Biology in 2000. In 2004 she entered the University of Washington School of Aquatic and Fishery Sciences, earning her Doctor of Philosophy in 2012. She currently lives in Seattle with her husband and daughter.