SEASONAL VARIATION OF THE GENUS *DINOPHYSIS* WITHIN PUGET SOUND, WASHINGTON: UNDERSTANDING HARMFUL ALGAL BLOOMS THROUGH SPECIES IDENTIFICATION

by

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ABSTRACT

Seasonal Variation of the Genus *Dinophysis* within Puget Sound, Washington: Understanding Harmful Algal Blooms through Species Identification

Jennifer Sun Runyan

Though harmful algal blooms have been present in Puget Sound, Washington for years, it is only since June of 2011 that *Dinophysis* has started causing illnesses. This dinoflagellate exudes dinophysistoxins and okadaic acid which are responsible for diarrhetic shellfish poisoning. The purpose of this study was to examine any seasonal patterns exhibited by *Dinophysis spp*. and to see if the abundance of *Dinophysis spp.* varied by location. Lastly, this study assessed changes in phytoplankton community composition before, during and after the presence of *Dinophysis* blooms. Phytoplankton samples were collected from Sequim Bay, Penn Cove, and Quartermaster Harbor by a citizen science program known as SoundToxins. Results showed *Dinophysis spp.* did vary seasonally and by site. *Dinophysis* was the most abundant during the summer at all sites and had a significantly greater abundance during summer at Sequim Bay and Quartermaster Harbor ($p = 0.04$, $p = 0.037$) during this period relative to other times of the year . Penn Cove had the lowest population of *Dinophysis and* the highest variability in salinity throughout the year, suggesting that *Dinophysis* is likely impaired by too much freshwater. As for community composition *Protoperidinium* was the most positively correlated with *Dinophysis* at all three sites (Sequim Bay $p<0.001$, Quartermaster Harbor $p< 0.001$ Penn Cove $p= 0.024$). Correlations between other species varied by site. Species richness was found to be greater when *Dinophysis* was present than when *Dinophysis* was absent at Quartermaster Harbor and Sequim Bay ($p= 0.001$, $p<0.001$). Currently the literature does not provide any studies in regard to phytoplankton identification down to genus level within Puget Sound. These results suggest that *Dinophysis* abundance varies seasonally, and is affected by variation in salinity, and such knowledge is important Washington's shellfish industry, Native American Tribes, scientists, and recreational clam diggers. It is important to minimize health risks and economic loss through early detection of harmful algal blooms.

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INTRODUCTION

With harmful algal blooms (HABs) increasing in frequency, it is important to gain a better understanding of their function within the marine food web (Lewitus et al. 2012; Smayda & Reynolds 2001). Phytoplankton are not only the base of the marine food web, but also provide a significant amount of oxygen to the atmosphere and the water, and also aid in carbon sequestration (Anderson et al. 2012; Archer 2010). The invisible world in which phytoplankton live in, is a difficult one filled with fierce competition. This includes competition between phytoplankton species for nutrients and competition to avoid predation by zooplankton, small fish, bivalves, or even other phytoplankton (Pitcher et al. 2010; Kozlowky- Suzuki et al. 2006; Smayda & Reynolds 2003; Smayda & Reynolds 2001). Phytoplankton have developed special adaptations to survive these rivalries. One adaptation is through development of toxins. Toxin production not only provides an anti-predatory defense mechanism, but is also a way to combat intraspecific competition (Smayda 1997). Though this toxin production is aimed towards phytoplankton survival, larger marine organisms and humans are caught in the crossfire. Therefore, it is integral to understand what causes harmful algal species to bloom, how our health is affected by them, and to set up proactive measures to combat harmful algal blooms.

Focusing in on the economic impact of HABs on the aquaculture industry alone, in the coastal US from 1987-2000, there was an 82 million dollar loss per year due to harmful algal blooms (Joshens et al. 2010). Puget Sound in Washington is of the largest producers of shellfish, especially for clam and mussel sales. In 2003, there was a 19 million dollar loss (13.5 million pounds) in the local shellfish industry due to a harmful

algal bloom (Trainer et al. 2007). Not only are the economy and the shellfish producers being affected by these blooms but so are other organisms such as marine mammals, marine birds, and humans (Lewitus et al. 2012).

Monitoring and accurately detecting harmful algal blooms is critical to promote human health, the economy, and to ensure the health of the surrounding ecosystems. SoundToxins is a citizen science organization located within Puget Sound, WA, with goals of establishing "a cost-effective monitoring program that will be led by state managers, tribal harvesters, and commercial fish and shellfish farmers. The SoundToxins program aims to provide sufficient warning of harmful algal blooms to enable early or selective harvesting of seafood, thereby minimizing risks to human health and reducing economic losses to Puget Sound fisheries" (www.soundtoxins.org). SoundToxins monitors the presence and abundance of the follow harmful algal genera: *Pseudo-nitzschia*, *Alexandrium*, *Dinophysis*, and *Heterosigma*. These four genera are the main genera that form HABs within Puget Sound.

These harmful algal bloom species have been in Puget Sound for hundreds of years. *Alexandrium*, for instance, was first discovered in Puget Sound in the early 1900s and monitoring for *Alexandrium* has been a common occurrence since 1957 (Moore et al. 2009). *Dinophysis*, another HAB species that has been documented in the global ocean for many years, is just now starting to cause concern in Puget Sound. In July of 2011 at Sequim Bay State Park, the first case of illness caused by *Dinophysis* was reported to Department of Health. Due to this sudden onset of *Dinophysis* producing toxins causing illnesses, scientists have started to pay greater attention to *Dinophysis*. This thesis is one of a very few set of studies that use SoundToxins data to understand the seasonality of

Dinophysis at several locations within Puget Sound. Further, it is one of the few studies that has examined *Dinophysis* distribution in Puget Sound and how it varies seasonally. Studies in Puget Sound have yet to address phytoplankton community composition down to the genus level throughout seasons, which tremendously limits our ability to understand HABs given that community compositional data can provide insight into why HABs occcur. Understanding phytoplankton succession throughout seasons may give us an insight into what other phytoplankton *Dinophysis* commonly occurs with. Therefore, if we see a particular genus that generally co-occurs with *Dinophysis,* we can be on the lookout for *Dinophysis*. Providing baseline phytoplankton resident data in Puget Sound waters will enable scientists to use this information to see how the phytoplankton community might change in the future.

LITERATURE REVIEW

Introduction

Recorded deaths from harmful algal blooms have occurred as early as 1793 when three of Captain George Vancouver's Royal Navy Crew became ill and one died after consuming shellfish from Poison Cave in Canada (Lewitus et al. 2012). Phytoplankton genera such as *Alexandrium, Pseudo-nitschia,* and *Dinophysis* are well- known organisms causing shellfish toxicity to a variety of organisms, including humans. Various monitoring programs have been put in place to understand where, when, and how these organisms produce toxins. This because these organisms do not always exude their toxins.

Long-term monitoring efforts of harmful algal blooms within Puget Sound reveal that several genera of toxic phytoplankton have been present since the early 1900s. The genus *Dinophysis,* which may exude okadaic acid and dinophysistoxins, can cause diarrhetic shellfish poisoning (DSP) in humans, and has existed in the Pacific Northwest for many years (Trainer et al. 2013). The first reported case of DSP in Washington was June *2011* (Eberhart et al. 2013; Taylor et al. 2013; Trainer et al. 2013). Though this DSP case was the first in Washington state, it is unknown to why the emergence of DSP has become so prevalent since *Dinophysis* has been present in Pacific Northwest waters for many years (Trainer et al. 2013). This puzzle of why DSP has only become a recent problem has been addressed by organizations such as SoundToxins (a citizen science harmful algal bloom monitoring network), Washington Department of Health, and various scientists from NOAA and local tribes, but more work is needed to gain a better understanding. Current research within Puget Sound has suggested seasonal patterns of

Dinophysis as well as possible physical conditions in which this genus thrives, but it is still unclear if one particular species of *Dinophysis* causes greater toxin production than another (Reguera et al. 2014). Though we may have a basic understanding of Dinophysis seasonality in Puget Sound, weekly monitoring by SoundToxins enables Department of Health to triage the order in which they test for mussel toxin detection. Not only is there seasonality within *Dinophysis*, but also within the phytoplankton community in general. For example, diatoms dominate in spring, while dinoflagellates dominate in summer. Though this is a coarse outline of phytoplankton seasonality, the resolution may be fine-tuned to observe seasonality at the genus level. Understanding phytoplankton succession at this level may allow greater forecasting of *Dinophysis* if there is a specific genus, or perhaps specific combinations of genera, that proceed *Dinophysis*. This thesis will address seasonal phytoplankton community succession and *Dinophysis* species composition at several locations within Puget Sound. This information is important because the literature has yet to demonstrate patterns of phytoplankton genera that are present right before *Dinophysis.* This thesis will bring us one step closer to understanding if certain genera can predict the presence of *Dinophysis* or if *Dinophysis* alters the community composition significantly after its presence. This literature review will provide essential background knowledge of phytoplankton, phytoplankton ecology, harmful algal bloom dynamics, and how harmful algal blooms have affected the local economy.

Harmful Algal Blooms

Harmful algal blooms (HABs) are defined as algal blooms that negatively affect the health of marine organisms and humans through one of three main mechanisms: physical damage, eutrophication, or toxin production (Joshens et al. 2010). These phytoplankton are generally categorized into two groups, diatoms and dinoflagellates (Table 1). Most HAB species are dinoflagellates. Smayda (1997) suggests that flagellates, including those that are considered as HABS, have a lower nutrient uptake affinity than diatoms, meaning that nutrients are less available to them. Several ways to combat this include using phycotoxins for intraspecific competition and anti-predatory defense mechanisms.

General Characteristics	**Diatoms**	**Dinoflagellates**
Range	Poles to tropics, most abundant in polar to temperate regions	All oceans, most successful in tropics
Habitat	Freshwater, saltwater, and brackish water. Found in benthos, planktos, in sea ice, sediments, and air. Can be free, living, epiphytic, endophytic, epizoic, and endozoic.	Freshwater, saltwater, and brackish water. Found in benthos, planktos, interstially in sand and soil, snow, and sea ice. Can be free living, symbiotic, or parasitic.
Size Class	5-200 µm	2-200 µm , although *Noctiluca* can reach 2mm
Cell Wall	Composed of silica	Composed of cellulose
Identifying Characteristics	Patterned variety of pores, ribs, spines, ridges, and delevations in frustule (shell).	Presence or absence of plates, arrangement and shape of plates, horns, spines, ridges, and reticulations.
Flagella	None	Two
Morphological	Two orders:	Two groups:

Differences	Centrales (centric diatoms) which are radially symmetric, Pennales (pennate diatoms) which are longitudinally symmetric)	Desmokonts- two dis-similar flagella arising from the anterior part of the cell Dinokonts- a transverse and longitudinal flagella
Examples of HAB species in Washington	*Pseudo-nitzschia spp.*	*Alexandrium spp, Dinophysis spp.*

Table 1. Differences between diatoms and dinoflagellates (Horner 2002)

Physical properties of phytoplankton can physically damage marine organisms. An example of this would be the siliceous spines from the genus *Chaeteroceros*. These spines can stick into the gill filaments of a fish causing irritation. Mucous is then created by fish to coat the gills in order to relieve this irritation. In promoting greater mucous production, the gills are no longer efficient enough to extract oxygen from the water, thereby causing the fish to die from suffocation. Though this type of bloom is rare, there was an incident in Dabob Bay, Washington in October of 1991 where cell abundance did reach up to 10^3 cells per liter (Horner et al. 1997).

Eutrophication, is an indirect, non-toxic mechanism of killing organisms. An increase in nutrients brings an increase in all types of phytoplankton. Phytoplankton continue to proliferate until nutrients are depleted. These phytoplankton die and are consumed by bacteria. These bacteria use up the oxygen in the water column thus creating anoxic conditions. With a lack of oxygen, many organisms perish (Valeila 1995). In 2003 at Carr Inlet in Puget Sound, Washington, there was a eutrophic event that was brought upon by a spring bloom and highly stratified waters (Edwards et al. 2007).

Other algal blooms are harmful through toxin production. These toxins are thought to be exuded or are found within the phytoplankton. Zooplankton eat these harmful phytoplankton, and the toxins bioaccumulate and biomagnify up the food chain. The end result can be sick marine mammals, birds, and even humans. Humans may become sick in one of two ways, 1) eating large fish which consumed toxic phytoplankton and/or 2) eat filter-feeding organisms such as clams, oysters, mussels, and crabs which have directly fed upon the toxic phytoplankton (Landsberg 2002).

This next section will go into greater depth about one of the harmful algal bloom species known as *Dinophysis*. Certain species of *Dinophysis* may produce a suite of toxins comprising of okadaic acid, dinophysistoxins, and pectenotoxins (Reguera et al. 2014). This particular genus has only recently become a problem within Puget Sound since 2011. Therefore, understanding the history of this organism in other parts of the world, in a lab setting, and its general ecology will enable further research to occur within Puget Sound.

Dinophysis

There are 120 species of *Dinophysis* in the world, but approximately only twelve species of *Dinophysis* have been found to have okadaic acid and dinophysistoxins in them. Oddly enough, only six species out of the twelve have been identified in causing diarrhetic shellfish poisoning (DSP). Within Puget Sound, there are eight species of *Dinophysis* where groups such as SoundToxins, monitors for on a weekly basis. Of the eight species, only six are considered to have okadaic and dinophysistoxins and are as

follows: *D. fortii*, *D. acuminata*, *D. acuta*, *D. norvegica*, *D. tripos* and *D. rotundata* (Reguera et al. 2014; Lewitus et al. 2012; Trainer et al 2010; Maso & Garces 2006).

Though okadaic acid and dinophysistoxins mainly come from *Dinophysis*, *Prorocentrum* has also been suggested to contribute to DSP as well (Reguera et al. 2014; Trainer et al. 2013). Still, *Dinophysis* is the main culprit for many of the DSP illnesses and not *Prorocentrum* (Reguera et al. 2014). Symptoms of DSP include diarrhea, nausea, vomiting, and abdominal pains. These effects can start as early as 30 minutes to several hours after toxin consumption, with complete recovery taking up to three days. Hospitalization is often rare. Chronic exposure of low levels of okadaic acid has also been identified as a tumor promoter in the digestive system (Trainer et al. 2013; Trainer et al. 2010; Manerio et al 2008; Maso & Garces 2006; Van Dolah 2000).

Most *Dinophysis spp.* are considered as a mixotrophic dinoflagellate, where the organism can photosynthesize as well as consume ciliates (Hattenrath-Lehmann. et al 2013). In a laboratory culture setting, *Dinophysis acuminata* and *Dinophysis norvegica* preyed upon *Myrionecta rubra*, a ciliate, by myzocytosis, also known as cellular vampirism (Imai & Nishitani 2001, Park et al. 2006). *D. acuminata* fed upon this marine ciliate by extracting *M. rubra's* cytoplasm through *D. acuminata*'s peduncle (Park et al. 2006). Dinophysis has yet to be seen to selectively feed upon phytoplankton or ciliates in its natural habitat, but the remains of ciliates have been found in the digestive vacuoles of *D.acuminata*, *D. norvegica*, and *D.fortii* (Pizarro et al. 2008).

Other organisms, such as shellfish and copepods feed upon *Dinophysis*. A study by Kozlowky- Suzuki et al. (2006) showed that copepods readily chose *Dinophysis* as

prey. There was some evidence of copepods decreasing the amount of *Dinophysis* consumed as other phytoplankton availability increased. Their results suggested that copepods ate a significant amount of *Dinophysis*, enough to reduce their populations. Okadaic acid and dinophysistoxins accumulation was minimal in the copepods that ingested *Dinophysis*, therefore suggesting that these toxins became more dilute within the copepod. If the copepod were to be eaten by a fish, the fish would have an even more dilute amount of toxins within it. With this is mind, DSP symptoms can only then be obtained by humans through the direct consumption of shellfish (Manerio et al 2008).

Though many of these early closures and cases were in Europe, the West Coast of the U.S. first reported DSP in 2003. Okadaic acid was first discovered in manila clams grown in British Columbia in low amounts. There have also been cases in California, Mexico, and Washington. The first DSP reported illness in the United States occurred in 2011 from blue mussels (*Mytilus edulis*) collected at a pier at Sequim Bay State Park (Lewitus et al. 2012, Lloyd et al. 2013, Trainer et al. 2013). The first closure in Washington due to okadaic acid and dinophysistoxins was off of the Pacific coast of Washington at Ruby Beach in 2012 (Eberhart et al. 2013). It is difficult to understand why reports of DSP are only recently being reported within the past two years. This could be due to underreporting of illnesses by the public, a lack of understanding of DSP by doctors, or of toxic species of *Dinophysis* only becoming recently present. Unlike many harmful algal blooms, a change in the color of the water is not indicative of the presence of *Dinophysis* (Kozlowsky-Suzuki et al 2006). Cell densities as low as 200 cells/L can cause enough toxin accumulation to cause harm to humans (Trainer et al. 2013).

When toxins reach or exceed their prescribe limits, shellfish harvest areas are either controlled or closed to avoid consumption of toxic shellfish. These measures are used to prevent contaminated shellfish reaching the marketplace and avoidance of gastrointestinal discomfort. Various rapid testing techniques such as the Jellett Rapid Test, ELISA, and the protein phosphatase 2A inhibition assay (PP2A), are currently being used to detect the presence of dinophysistoxins and oakadaic acid from shellfish tissue samples. This allowed shellfish growers to test pre-harvest samples thus preventing any illnesses. A study by Eberhart *et al*. (2013), tested all three techniques to show if one test was more effective than the others. The Jellet Rapid Test is an immuno-chromatographic system similar to pregnancy test strips. The results of the test provided a high number of false negatives. As for the ELISA, the enzyme-linked immunosorbent assay, the test provided a false positive 22% of the time. Lastly, the PP2A, showed the least amount of false negatives and false positives, therefore making it the best choice for rapid testing of dinophysistoxins and oakadaic acid. These three tests were compared to the current regulatory testing methods, liquid chromatography with mass spectroscopy, to ensure accuracy of the rapid tests.

Other Economic and Human Impacts of Harmful Algal Blooms in Washington

Pseudo-nitzschia

Pseudo-nitzschia has been observed off the West Coast of the U.S. since the 1920s (Lewitus et al. 2012). This diatom generally produces the marine biotoxin, domoic acid (DA) and tends to bloom during the late spring and summer (Horner & Postel 1993). Ten out of the twelve species of *Pseudo-nitzschia* that reside off the west coast are known to produce domoic acid. Unfortunately, these species may change in toxin potency depending on the location of *Pseudo-nitzschia*. For example, in Washington, the most toxic *Pseudo-nitzschia* species are *P.pseudodelicatissima*, *P. cuspidata* and *P.australis*, while in California, the most toxic are *P.autralis* and *P.multiseries* (Trainer et al. 2010). Domoic acid poisoning in humans is known as Amnesic Shellfish Poisoning (ASP) and has the following symptoms: headache, gastrointestinal disorders, and short-term memory loss (affecting the hippocampus). These symptoms can occur as early as 24-48 hours from when the toxic shellfish was consumed (Lewitus et al. 2012; Trainer et al. 2010). Though, shellfish and some finfish are the main way to consume DA, other organisms have also been known to be vectors of DA and these include Pacific sardines, northern anchovies (*Engraulis mordax*), krill (*Euphausia pacifica*), market squid (*Loligo opalescens*), and some benthic invertebrates. ASP also affects other higher trophic organisms other than humans, such as California sea lions (*Zalophus californianus*), harbor porpoises (*Phocoena phocoena*), common dolphins (*Delphinus delphis*), grey whales (*Eschrichtius robustus*), western grebes (*Aechmophorus occidentalis*), and other marine birds and mammals (Bargu et al. 2012; Fire et al. 2010; Shumway et al. 2003; Scholin et al. 2000)

Domoic acid within shellfish was first discovered in Canada when three people died and 105 people became ill from eating contaminated blue mussels from Prince Edward Island in 1987 (Lewitus et al. 2012). On the West Coast of the U.S., the first documented case was in the summer of 1991 off Monterey Bay, California (Fritz et al. 1992). Instead of humans being directly affected by domoic acid, this time Brandt's cormorants (*Phalacrocorax penicillatus*) and brown pelicans (*Pelecanus occidentalis*) mortalities occurred from eating anchovies that earlier consumed *Pseudo-nitzschia*. This *Pseudo-nitzschia* event then expanded Northern California, Oregon, and lastly to Washington by fall of 1991 (Wekell et al. 1994). Off of the Washington coastline, 25 human illnesses were reported due to Amnesic Shellfish Poisoning and the crab fishing industry was forced to shut down at a $7 million (Lewitus et al. 2012).

In September 2003 at Kilisut Harbor, Washington, a monospecific bloom of *Pseudo-nitzschia australis* closed shellfish harvesting. The regulatory limit of domoic acid is 20 ppm, but when blue mussels were bioassayed for domoic acid concentrations, levels reached up to 29 ppm. This was the first documented shellfish closure due to domoic acid within Puget Sound (Bill et al. 2006). Not only were blue mussels (*Mytilus edulis*) affected, but so were littleneck clams (*Protothaca staminea*), manilla clams (*Tapes philippinarum*), geoduck clams (*Panopea abrupta*), and Pacific oysters (*Crassostrea gigas*). All of the bivalves listed can expel the toxin over a period of hours to days, but it is unknown how geoduck clams manage domoic acid. Puget Sound is one of the largest producers of shellfish, especially for clam and mussel sales which created at least $19 million (13.5 million pounds) in 2003 (Trainer et al. 2007). *Pseudo-nitzschia* is not the only harmful algal bloom genus that has caused problems in Puget Sound.

Alexandrium, a dinoflagellate, is another key player in harmful algal blooms within the Pacific Northwest.

Alexandrium

Alexandrium produces a saxitoxin derivative compounds causing Paralytic Shellfish Poisoining (PSP) and can be found between May through October all along the U.S. West Coast (Horner et al. 1997). *Alexandrium* is not the only dinoflagellate to produce this toxin but is the main genus associated with PSP in the Pacific Northwest. Other dinoflagellates which can produce saxitoxin compounds include *Gymnodinium* and *Pyrodinium* (Lewitus et al 2012). Nausea, vomiting, light headedness, and incoherent speech are some mild symptoms of PSP. The main symptom of PSP starts with numbness and tingling around the mouth and lips, spreading over the rest of the face, down the neck, and continuing down the body. In severe cases, there is death due to respiratory failure. These symptoms can occur 30 minutes to three hours after tainted seafood consumption (Backer et al. 2006).

The first recorded death of PSP occurred in 1793 when three of Captain George Vancouver's Royal Navy crew became ill and one crew member died after consuming shellfish from Poison Cave (Lewitus et al. 2012). From 1962 to 1989, toxic PSP events occurred in 22 of the 28 years off the coast of California (Horner et al. 1997). These PSP events occur quite often and affect many organisms. Shellfish varieties that are generally affected by PSP include Pacific oysters (*Crassostrea gigas*), manila clams (*Tapes philippinarum*), razor clams (*Siliqua patula*), geoduck clams (*Panopea abrupta*), butter

clams (*Saxidomus giganteus*), littleneck clams (*Protothaca staminea*), varnish clams (*Nuttallia obscurata*), various rock scallops, and various mussel species. Not only are these bivalves affected by PSP, but so are a range of other organisms such as gooseneck barnacles (*Pollicipes polymerus*), moon snails (*Lunatia heros*), spiny lobsters (*Panulirus* spp.), Dungeness crabs (*Metacarcinus magister*), and various whelk and cockle species (Lewitus et al. 2012).

As mentioned previously, the aquaculture industry within Puget Sound is an integral part of the local economy. The Washington coast as well as Puget Sound has been identifying *Alexandrium* in their waters since the early 1900s. A biotoxin monitoring program has been set in place since 1957 by Washington's Department of Health to test mussel tissue for saxitoxin (Moore et al. 2009). The monitoring program became useful right away when the first shellfish closure occurred in 1957 at Sequim Bay and Discovery Bay. There are two possible thoughts on how Alexandrium migrated to south Puget Sound waters. First, is that currents from Whidbey Island basin brought *Alexandrium* further into Puget Sound. Second, *Alexandrium* cells or toxin concentrations were not in high enough to raise any concern until 1957. It was not until 1988 when the first shellfish closure occurred in south Puget Sound at Carr Inlet (Cox et al. 2008).

Phytoplankton Ecology

This section will provide some insight into why certain genera of phytoplankton bloom through the lens of phytoplankton community interactions, temperatures, and salinity.

Phytoplankton Community Interaction

Seasonal changes in diatom and dinoflagellate assemblages are complex. A study by Hutchinson (1961) tried to understand why many species of phytoplankton can coexist while competing for a limited amount of resources. Hutchinson came up with the "paradox of the plankton" suggesting that communities of phytoplankton are organized by processes beyond nutrient competition such as habitat viability, species interaction, and phytoplankton dispersal. A study by Cloern & Dufford (2005) took Hutchinson's concept of the "paradox of the plankton" and applied it to their study in San Francisco Bay where they observed phytoplankton species composition for a decade. Cloern & Dufford came up with eight principles of phytoplankton community assembly and they are as follows 1) Cell size is determined by nutrient supply and selective grazing, 2) Diatoms respond quickly to nutrient pulses, 3) Pelagic habitats select phytoplankton species on the basis of their own form and function, 4) Pelagic communities are shaped by species interactions across trophic levels, 5) Phytoplankton species have mixed nutritional modes, 6) Phytoplankton species have variable life histories, 7) Pelagic ecosystems are open, away from coastal areas , and 8) Communities respond to large-scale climatic periodicity. These principles validated Hutchinson's "paradox of plankton" and strengthened the current knowledge that phytoplankton composition is influenced by more than just community composition and nutrients.

Other studies have focused on phytoplankton community composition at a more coarse scale by trying to understand the seasonal cycle of diatoms and dinoflagellates. These studies suggest diatoms dominate during spring, heterotrophic organisms and dinoflagellates dominate during the summer, and dinoflagellates dominate in the late

summer and early fall (Margalef 1978, Smayda & Reynolds 2003; Pitcher et al. 2010). Margalef's Mandala (1978) supports the seasonality of phytoplankton by suggesting that phytoplankton adaptations are specific to their habitat types, which are defined by turbulence intensity and nutrient concentrations (Cloern & Dufford 2005; Smayda & Reynolds 2001). Though there has been much research on phytoplankton community composition, each study must focus on a variety of factors to understand why a certain species is dominating the water column. Temperature and salinity are two factors that will be discussed in further detail.

Temperature

Since the specific heat capacity of water enables the ocean to mildly fluctuate in temperature, the greatest difference in temperature is found between seasons. Warmer summer waters bring stratification to the water column where the warmer water layer is on top, the cooler and saltier water down below. Since calm waters create stratification which does not allow the diatom frustules to be picked up by the currents, they become too heavy and sink to the bottom (Lehman 2000). Dinoflagellates, on the other hand, do well in these stratified waters since they have two flagella which enable them to move up and down the water column. It is the flagella that enable the dinoflagellates to become the more dominant group of phytoplankton during the summer and early autumn months (Trainer et al. 2010).

Gisselson et al. (2002) suggest that *D.novegica* cells aggregating along the thermocline at 15 to 20 m depth and the presence of digestive vacuoles in up to 22% of the population found there shows that *D.norvegica* can find suitable prey at the

thermocline. There is also some evidence that *Dinophysis spp.* migrate vertically within the water-column at some locations but not at others (Gonzalez-Gil et al. 2010;Pizzaro et al. 2008). Other studies have shown that *Dinophysis spp*. may prefer certain temperature ranges such as *D.acuminata* being significantly correlated with temperatures ranging from 11.1 to 26.6°C (Hattenrath-Lehmann et al. 2013). Another study by Gonzalez-Gil et al. (2010) suggested that *D.acuminata* can be observed at temperatures from 13 to 22°C. This wide temperature range attributes to *D.acuminata*'s long growing season (spring to autumn) (Reguera et. al 2014). Though temperature has been discussed in relation to seasonality or as an independent factor, temperature does not act independently. Salinity may vary depending upon the temperature of the water since warmer water generally holds saltier water.

Nutrients

Salinity may also play a role in phytoplankton ecology where high nutrient concentrations are generally found either at or below the pycnocline (Anderson et al 1995). Many HAB species, such as *Dinophysis*, have been found to take advantage of this nutrient gradiation through a 24 hour vertical migration. Since *Dinophysis*, and all dinoflagellates, have flagella, these flagella enable them to move through the water column. During the night, dinoflagellates will migrate downwards towards the nutrient rich pycnocline region where they can uptake nitrates and other nutrients. During the day, these dinoflagellates migrate back up to the surface waters in order to use the sunlight and the nutrients that they recently acquired for photosynthesis (Anderson 1995).

Dinophysis has been found anywhere between 29 to 34 parts per thousand (Gonzalez-Gil et al. 2010 ; Pizarro et al. 2008; Aubrey et al. 2000; Peperzak 1996). This adaptation also allows them to do well in areas where there's a great amount of freshwater input, where the freshwater creates a layer on top of the salt water (Trainer et al. 2013).

Even with understanding temperature and salinity, nutrients are another important factor in understanding phytoplankton community composition and even toxin production in HABS. Other factors that are also important in understanding harmful algal bloom dynamics and community composition are competition between phytoplankton genera and phytoplankton predation. These three factors were not integrated into this study due to the lack of time and data availability. Therefore, this thesis focused primarily on temperature, salinity, and seasonality data of phytoplankton genera composition collected from the SoundToxins volunteers. This study focuses on phytoplankton community composition in relation to *Dinophysis* species presence in Puget Sound. This study hopes to elucidate on several questions: 1) Does the presence of *Dinophysis* species vary seasonally within Puget Sound 2) does *Dinophysis* species also vary by location with varying salinity levels and 3) are there certain phytoplankton communities that are present either before, during, or after *Dinophysis* presence?

METHODS

Site Description

Three current sampling sites from SoundToxins were chosen out of 15 sites that spread over Puget Sound. The sites are as follows: Penn Cove, Sequim Bay, and Quartermaster Harbor (Figure 1). Each location chosen provided comprehensive data sets on a weekly (March through October) or bi-weekly basis (November- February) throughout 2012—2013. Comprehensive regular phytoplankton sampling, water and air temperature measurements, and salinity measurements were conducted. Furthermore, volunteers for the sites have also been consistent throughout 2012 to 2013.

Quartermaster Harbor

Quartermaster Harbor (47° 22' 20.748" W, -122° 27' 15.6522" N) is a shallow, southward facing bay between Vashon and Maury islands and connects over a shallow sill to the southern end of the Main Bain in south Puget Sound (Figure 1). The shallow inner bay has an average depth of 6 m (Tobin & Horner 2010). Phytoplankton tow from these locations were generally taken at two to three meters in depth from Quartermaster Harbor marina.

Penn Cove

Penn Cove tidelands (48° 14' 1.0782" W, -122° 43' 23.34" N), located in Whidbey Basin, contains the waters east of Whidbey Island and North of the Main Basin. There is no sill across the entrance to the Whidbey Basin, therefore, it is a much deeper basin with depths ranging from 8 m in Skagit Bay to 177 m in Saratoga Passage, between Whidbey

Island and Camano Island. This relative shallowness is accompanied by a high percentage of tidelands (Downing 1983).This location is also intermediate in depth between shallow Quartermaster Harbor and deep Sequim Bay. Penn Cove samples are taken from the Penn Cove Shellfish Farm dock.

Sequim Bay

Sequim Bay (48° 2' 28.1616" W, -123° 1' 32.1378" N) is connected to the ocean by the Strait of Juan de Fuca, with the passage having a maximum depth of 200 m and 160 km in length. A double sill, located in Admiralty Inlet, at the entrance of Puget Sound separates it from the Strait of Juan de Fuca (Moore et al. 2008). Sequim Bay samples are taken from a dock located within Sequim Bay State Park.

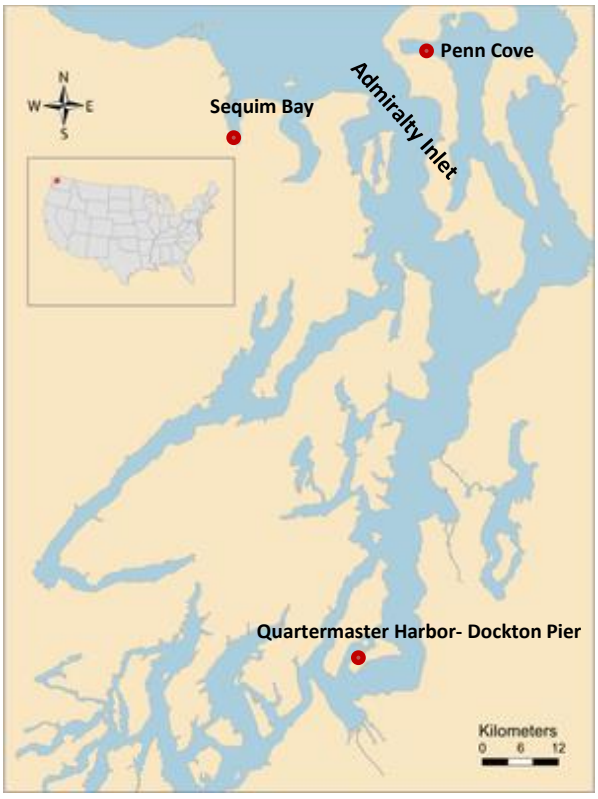


Figure 1. Puget Sound, Washington, showing locations of sampling locations.

SoundToxin Sample and Data Collection

Samples were originally collected by volunteers of the SoundToxins harmful algal bloom monitoring program (Chadsey et al. 2011). SoundToxins protocol had volunteers collect vertical net tow samples using a 20-µm mesh net from a dock several meters from the shore. This tow was conducted by first determining the depth of the water column at the time of the tow. Once the general depth was known, the plankton net was cast down close to the bottom and then towed upwards throughout the water column at approximately 1 meter/second. This was repeated two additional times. Depths of the tow varied, but were generally around one meter to four meters. Net tow samples were poured into 20 mL scintillation vials and preserved by adding 1mL of a 1% buffered formalin solution.

To obtain water temperature and salinity data, a thermometer was put into the bucket holding the surface sea water. The data collector allowed the thermometer to be submerged in the water for one minute and before the reading was taken. In order to determine salinity, one or two drops of the water from the bucket were added to the refractometer. This data was then added to the SoundToxins website.

Laboratory Analysis

Only archived samples collected from April 2012 to April 2013 were analyzed. Samples collected in the field by the volunteers were analyzed and preserved within several hours of sample retrieval. To take a subsample of this preserved net tow sample, the scintillation vial is first mixed at least 20 times to create a homogenous solution.

Relative phytoplankton abundance was determined on a 0.1 ml aliquot of the sample. Though the community composition is recorded by relative abundance, the main focus of SoundToxins is to identify, enumerate, and report the presence of specifically four toxic genera (*Pseudo-ntizschia*, *Alexandrium*, *Heterosigma akashiwo*, and *Dinophysis*).

For this study, all of the phytoplankton present within the sample were identified and enumerated down to genus level, and all *Dinophysis* were identified to species level on a Palmer Maloney counting cell (holding the 0.1 ml aliquot of the sample) on a Zeiss Universal Compound Microscope using phase contrast and light illumination (Hasle 1978). Smaller flagellates and some zooplankton that were difficult to discern due to the formalin preservation were not included in enumeration. Severely dense net tow samples were counted only using half of the chamber (Guillard 1978). Samples were enumerated in triplicates. Net tow enumeration data and cod end volume (the volume of the collection container attached to the phytoplankton net) were used to calculate whole water abundance (Equation 1).

$$\text{Net tow cell concentration } \left(\frac{\text{cells}}{\text{L}}\right) \text{ x Cod end volume(L)/ Total volume filtered (L)=Whole water cell abundance } \left(\frac{\text{cells}}{\text{L}}\right)$$

Equation 1. Calculation for whole water cell abundance from net tow samples.

Whole water cell abundance (henceforth referred to as cell abundance) was used to understand seasonality of phytoplankton and to note any trends of a particular genus that happened to be present, before, during, or after the presence of *Dinophysis*.

In order to calculate variability between subsamples of an aliquot, triplicate whole water abundances were first averaged. Standard deviation, standard error, and coefficient of variation were then calculated for each sample.

Statistical Analysis

All data was checked for normality. That data was not normally distributed even when the data was log and square-root transformed. Therefore, resampling methods were applied. Resampling ANOVAs were used to determine significant difference between sampling sites and seasonality of Dinophysis for each site. Resampling correlations were used to understand if there was any correlation between *Dinophysis* and any other phytoplankton genera. Those genera significantly correlated with *Dinophysis* were used to gain a refined understanding of when those populations bloomed in relation to Dinophysis. Temperature and salinity were two other factors correlated with *Dinophysis.*

MRPP/NMS Ordinations were used to see if there was a pattern between *Dinophysis* and the phytoplankton community at each site. At each site, the seasonal fluctuations of *Dinophysis* abundance provided the breaks in bins by which Dinophysis was grouped. All phytoplankton genera present within each site were also categorized based upon the *Dinophysis* abundance. Pair-wise comparisons were also calculated to highlight any significant differences between each phytoplankton community based on the set criteria. In Quartermaster Harbor, three criteria were set as (1) No *Dinophysis*, (2), *Dinophysis* cell abundance less than or equal to 20 cells/L, (3) *Dinophysis* cell abundance greater than 20 cells/L. T*he change in criterion was set at 20 cells/L because that was the lowest concentration of Dinophysis* present throughout the year. In Sequim Bay, three criteria were set as (1) No *Dinophysis*, (2) *Dinophysis* cell abundance less than or equal to 350 cells/L, and (3) *Dinophysis* cell abundance greater than 350 cells/L. In Penn Cove, three criteria were set (1) No *Dinophysis* and (2) *Dinophysis* population less than 10 cells/L, and (3) *Dinophysis* population greater than 10 cells/L.

Lastly, species evenness, species richness, Simpson's diversity index, and Shannon-Wiener's diversity index were conducted twice, once when *Dinophysis* was present and then again when *Dinophysis* was not present to understand if *Dinophysis* may impact these measurements of phytoplankton community.

RESULTS

Variations in *Dinophysis* Abundance at Different Sampling Locations

All sites exhibited seasonality within *Dinophysis* with higher concentrations during the summer and the lower concentrations during the winter. Specific trends such temperature, salinity, phytoplankton community composition in relation to the presence of *Dinophysis*, and the differences between phytoplankton communities will be described below.

At Quartermaster Harbor, *Dinophysis* abundance ranged from 0 to 184 cells/L over the course of the year, with higher estimates of abundance during the summer. To understand if there was a difference between *Dinophysis* and seasonality at Quartermaster Harbor, a resampling ANOVA was conducted and showed there was a significant difference between seasons (p-value= 0.001, F (3,29)). The fall average was 7± 9 cells/L. The spring combined average was 0± 0 cells/L. In summer, the average was 75± 63cells/L. In winter, the average was 1.5± 2.5 cells/L.

At Sequim Bay, *Dinophysis* abundance ranged from 0 to 1393 cells/L over the course of the year, with the higher estimates of abundance during the early fall. To understand if there was a difference between *Dinophysis* and seasonality, a resampling ANOVA was conducted and showed a significant difference (p=0.05, F (4,34)). In the fall the average was 280 ± 523.5 cells/L. In spring 2012 and 2013 combined average was 200± 309.1 cells/L. In summer, the average was 408± 253.3 cells/L. In the winter, the average was 0± 0 cells/L.

Dinophysis exhibited the lowest abundance measurements over the course of the year at Penn cove with cell counts ranging from 0 to only 36 cells/L. This resampling ANOVA showed no significant viability seasonally (p-value= 0.487, F(3.19). In the fall, the average was 0± 0 cells/L. In spring of 2012 and 2013 combined, the average was 6 ±13.4 cells/L. In summer, the average was 9 ± 15.4 cells/L. In winter, the average was 2 ±2.6 cells/L.

Lastly, a resampling ANOVA was conducted to note the difference of *Dinophysis* populations at each location. *Dinophysis* population data from the entire year was compared from site to site. No significant differences of *Dinophysis* populations were found between locations (p-value= 0.995, F(2,92)). Over the entire data set (April 2012-April 2013), the average at Sequim Bay was 242 ±335.1 cells/L. At Quartermaster Harbor, the average was 23 ±45.7 cells/L. At Penn Cove, the average was 4 ± 9.9 cells/L. Since *Dinophysis* was not identified numerous times, this lended many zeros in the data set which could explain the lack of a significant difference even though there were clear differences in abundance estimates between sites.

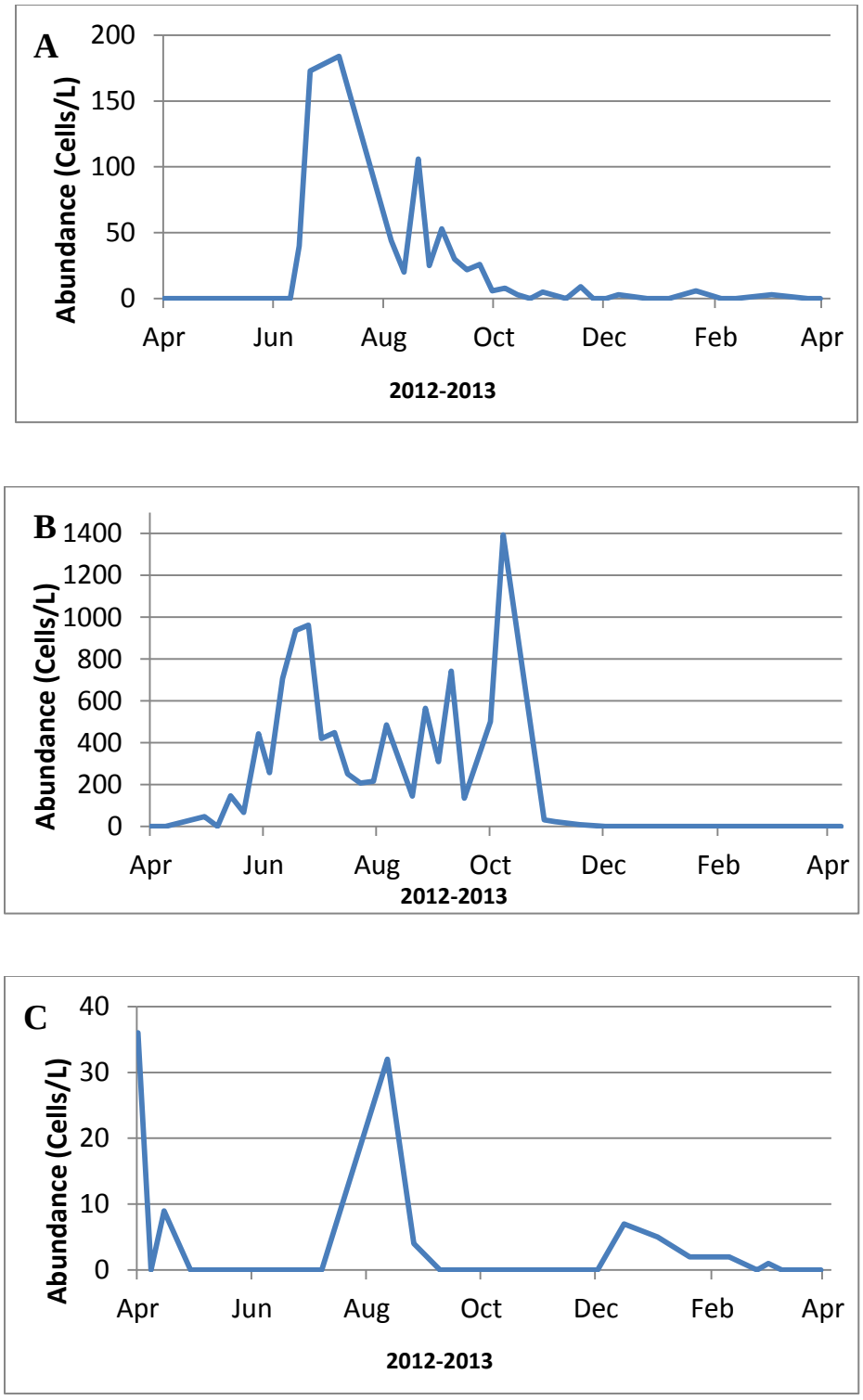


Figure 2. Seasonality of *Dinophysis* during 2012-2013 at the A) Quartermaster Harbor, B) Sequim Bay, and C) Penn Cove.

Dinophysis and Phytoplankton Community Composition Correlations

Quartermaster Harbor

There were several phytoplankton genera that were significantly correlated with *Dinophysis*. *Protoperidinium* and *Scripsiella* were significantly correlated with *Dinophysis* (*Protoperidinium:* $p< 0.001$, $r^2=0.55$, $n =22$; *Scripsiella:* $p = 0.007$, $r^2=0.27$, $n = 20$) Since *Dinophysis*, *Scripsiella*, and *Protoperidinium* are all dinoflagellates, it is common to see them during the summer months (see explanation in discussion). Lastly, *Coscinodiscus*, a diatom, was significantly correlated with *Dinophysis* ($p = 0.02$, $r^2= 0.23$, $n= 21$) (Table 1).

In Quartermaster Harbor, there were several genera of phytoplankton that were significantly correlated with *Dinophysis* but likely arose because there was too little data, or they were a spurious correlation. *Amylax*, a dinoflagellate, was significantly correlated, but to was a spurious correlation since *Amylax* was rarely present in the samples ($r=0.65$, $p =0.02$, $n=3$). *Gonyaulax*, also a dinoflagellate,was also significantly correlated with *Dinophysis*, but this was likely spurious ($r=0.62$, $p = 0.026$, $n=3$).

Sequim Bay

There were several phytoplankton genera that were significantly correlated with *Dinophysis*. *Ceratium*, had a significant correlation ($p = 0.028$, $r^2= 0.13$, $n= 15$). Two other dinoflagellates, as in Quartermaster Harbor, *Protoperidinium* and *Scripsiella*, were also significantly correlated with *Dinophysis* (*Protoperidinium*: $p = 0.001$, $r^2=0.48$, $n= 28$; *Scripsiella*: $p = 0.02$, $r^2= 0.15$, $n= 24$). Several diatoms were also significantly correlated with *Dinophysis*. *Pseudo-nitzschia* large and small cell type both significantly

correlated with *Dinophysis* (*Pseudo-nitzschia* large cell type: r= 0.37, p = 0.04, n= 26; *Pseudo-nitzschia* small cell type: r= 0.31, p = 0.05, n= 12) (Table 1).

In Sequim Bay, there were several spurious correlations. *Kofoidinium*, a dinoflagellate, was significantly correlated with *Dinophysis* (p = 0.013,, r^2= 0.23, n=4). *Oxyphysis*, another dinoflagellate, was also significantly correlated with *Dinophysis* (p= 0.035, r^2=0.15, n=4).

Penn Cove

There were several phytoplankton genera that were significantly correlated with *Dinophysis* including *Ceratium*, *Chaetoceros*, *Protoperidinium*, *Thalassionema*, and *Thalassiosira* (*Ceratium*: p = 0.047, r^2=0.34, n=5; *Chaetoceros*: p = 0.011, r^2 =0.61, n= 21; *Protoperidinium*: p= 0.024, r^2= 0.55, n= 14; *Thalassionema*: p = 0.035, r^2=0.49, n= 16; *Thalassiosira*: p = 0.042, r^2= 0.20, n= 23) (Table 1).

In Penn Cove, *Navicula*, *Licmorpha*, and *Eucampia* were significantly correlated with *Dinophysis (Navicula:* p = 0.031, r^2=0.34, n=4; *Licmorpha* : p = 0.001, r^2= 0.01, n=1; *Eucampia:* p = 0.05, r^2= 0.33, n= 2).

Genera	Penn Cove	Quartermaster Harbor	Sequim Bay
Protoperidinium	0.55	0.48	0.56
Ceratium	0.34	n/a	0.13
Scripsiella	n/a	0.15	0.27
Chaetoceros	0.61	n/a	n/a
Thalassionema	0.5	n/a	n/a
Thalassiosira	0.2	n/a	n/a
Coscinodiscus	n/a	0.23	n/a

Table 2. Presenting R^2 values for significantly correlated genera with *Dinophysis*.

Differences in Phytoplankton Communities

In order to assess differences in phytoplankton community composition in relation to *Dinophysis*, MRPP / NMS Ordinations were conducted for each site.

Quartermaster Harbor

There was a statistically significant difference between the communities (A=0.24, p=0.001) (Figure 2). Pair-wise comparisons showed greatest difference between criterion 2 and 3, therefore suggesting the greatest difference when *Dinophysis* populations were either below 20 cells/L and when *Dinophysis* cells were greater than 20 cells/L (A= 0.04,p= 0.02). Comparisons between criteria 1 and 3 as well as 1 and 2 did not differ significantly (Criteria 1 vs. 3: A=0.06, p< 0.001; Criteria 1 vs. 2: A= 0.019, p= 0.12).

Sequim Bay

There was statistical difference between the communities (A= 0.071, p=0.001) (Figure 2). Pair-wise comparisons showed greatest difference between 1 vs. 2 and 2 vs. 3, therefore suggesting the greatest difference when *Dinophysis* populations are between

Dinophysis being absent and cell abundance less than or equal to 350 cells/L and a difference when *Dinophysis* populations are present at either below or above 350 cells/L (Criteria 1 vs. 2: A= 0.03, p= 0.01; Criteria 2 vs. 3: A= 0.04, p=0.013). There was no difference between criteria 1 and 3 (A =0.11, p<0.001).

Penn Cove

There was not a statistical difference between the communities (A= 0.001, p= 0.67) (Figure 2). This is to be expected since *Dinophysis* abundance was low throughout the year, therefore making it difficult to assess any real pattern.

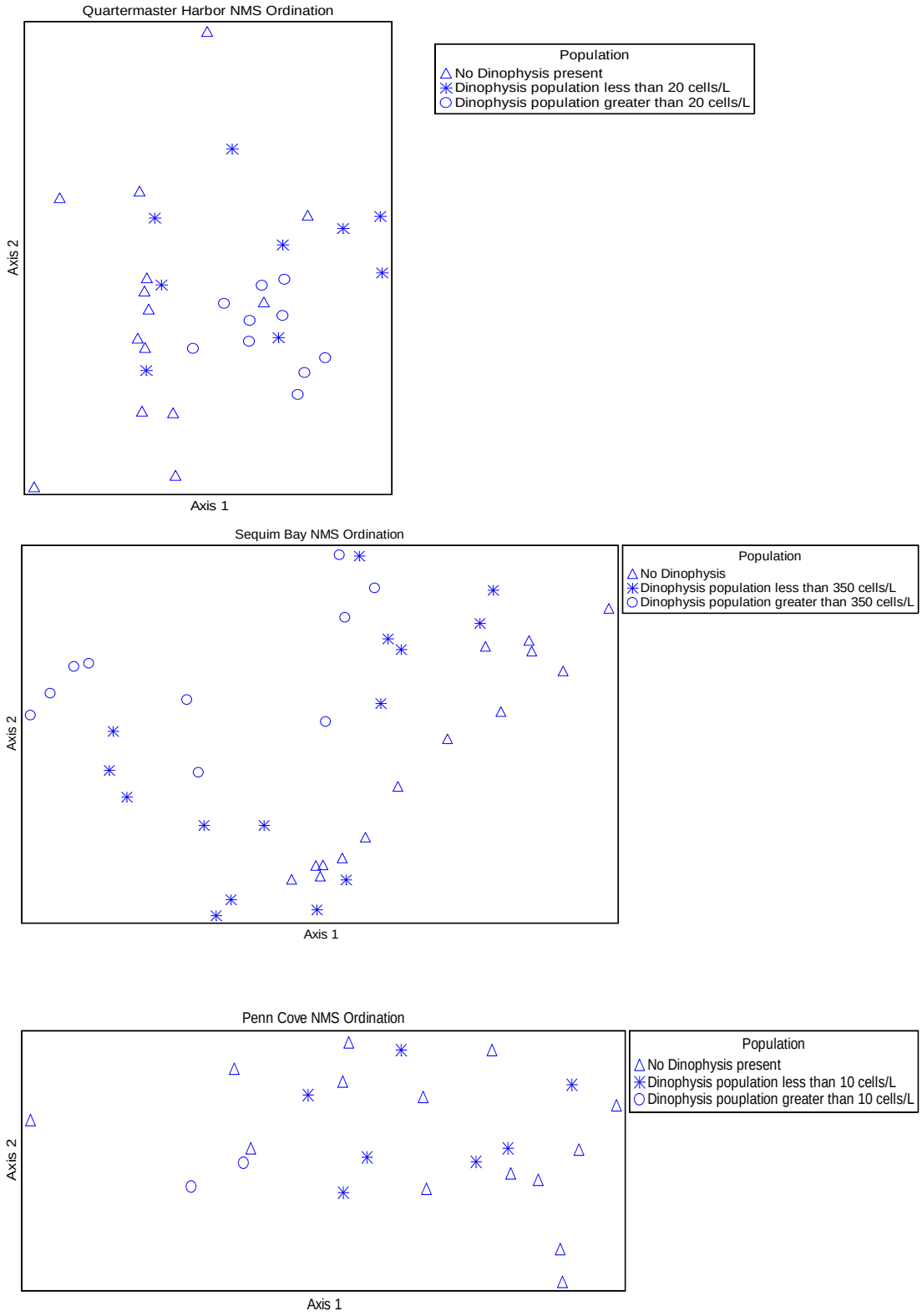


Figure 3. NMS Ordinations at three sites.

Diversity of Phytoplankton Communities

Genera richness, genera evenness, Shannon's diversity index, and Simpson's diversity index were calculated at each site. These calculations were conducted twice, once when *Dinophysis* was present and then again when *Dinophysis* was not present to understand if *Dinophysis* may impact these measurements of phytoplankton community. All tests provided non-significant results, except for richness, which yielded significant differences at Quartermaster Harbor and Sequim Bay (Quartermaster Harbor: $p = 0.001$; Sequim Bay: $p < 0.001$). Richness tended to be greater when *Dinophysis* was present than when *Dinophysis* was absent.

Location	Richness (S)	Evenness (E)	Shannon's Diversity Index (H)	Simpson's Diversity Index (D')
Sequim Bay (P)	17.63 ± 4.08*	0.49 ± 0.24	1.36± 0.68	0.57 ± 0.25
Sequim Bay (A)	11.53 ± 4.13*	0.56 ± 0.31	1.35 ± 0.67	0.59 ± 0.27
Penn Cove (P)	12.33 ± 3.61	0.50 ± 0.17	1.22 ± 0.41	0.53 ± 0.18
Penn Cove (A)	10.36 ± 3.70	0.60 ± 0.19	1.40 ± 0.55	0.61 ±0 .19
Quartermaster Harbor (P)	17.37± 4.54*	0.57 ± 0.26	1.61 ± 0.74	0.62 ± 0.28
Quartermaster Harbor (A)	12.07 ± 5.23*	0.56 ± 0.26	1.22 ± 0.60	0.56 ± 0.26

Table 3. Locations either noted P (*Dinophysis* present) or A (*Dinophysis* absent) with averages and standard deviations for the following tests: (S) Richness, (E) Evenness, (H) Shannon's Diversity Index, and (D') Simpson's Diversity Index. * Showed significant p-values.

Temperature - Salinity Characteristics

In order to gain greater detail into how *Dinophysis* may behave, temperature and salinity data may provide some insight. Temperature within Puget Sound waters varied by locations but all showed a general trend of the water temperature warming during the summer and cooling in the winter. Sequim Bay consistently exhibited the coolest temperatures, which ranged from 6°C to 16°C, thus varying 10°C throughout the year. Quartermaster Harbor temperatures ranged from 8°C to 19°C, with a slightly larger variation of 11°C throughout the year. Lastly, Penn Cove temperature ranged from 7°C to 16°C for 8 months of the year starting on 8/20/2012. Earlier temperature between the dates of 4/9/2012-8/19/2012 was discarded due to the water quality probe malfunctioning (Figure 1).

Surface salinity within Puget Sound also varied by locations. Penn Cove had the most estuarine conditions with salinity measurements varying between 19-30 ppt, while Sequim Bay's salinity was the highest, with measurements of 30-35ppt. Quartermaster Harbor's salinity measurements were in between the other two sites with salinity readings of 23-29 ppt (Figure 1). These salinity measurements are analogous to their locations with Penn Cove's salinity having the greatest variation being located near the Skagit River in a more estuarine environment, while Sequim Bay has much of an oceanic influence (ocean salinity is around 33ppt). Given the large variability in temperature and salinity, these factors could be the cause of variability in abundance of *Dinophysis*.

Temperature and salinity were not significantly correlated with each other (Penn Cove $r=0.46$, $p = 0.996$; Sequim Bay $r= -0.72$, $p = 1$; Quartermaster Harbor $r= -0.27$, $p = 0.929$).

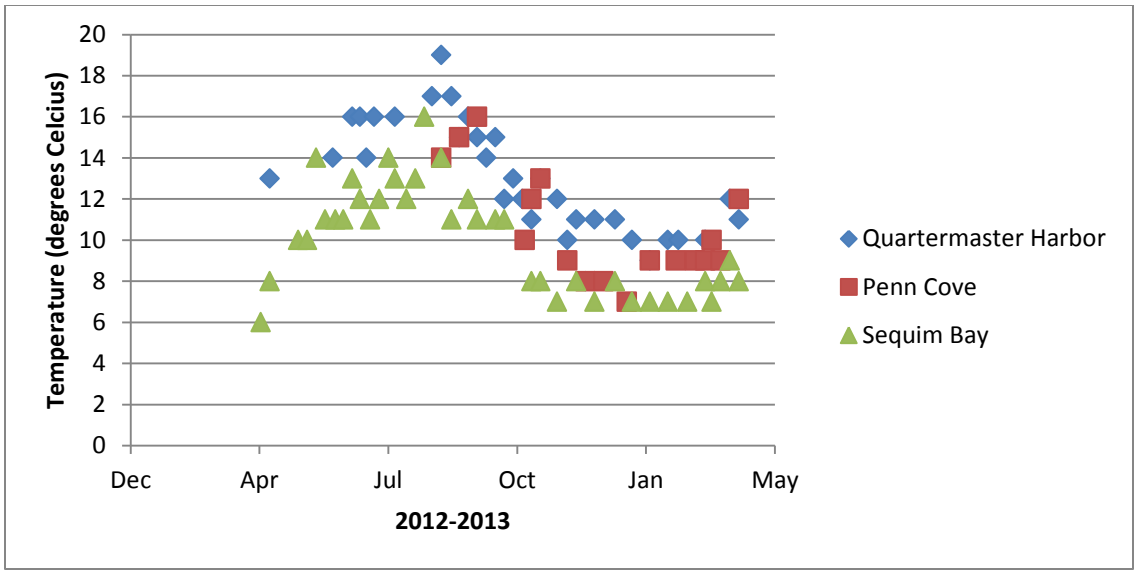


Figure 4. Surface temperature at the three sampling sites.

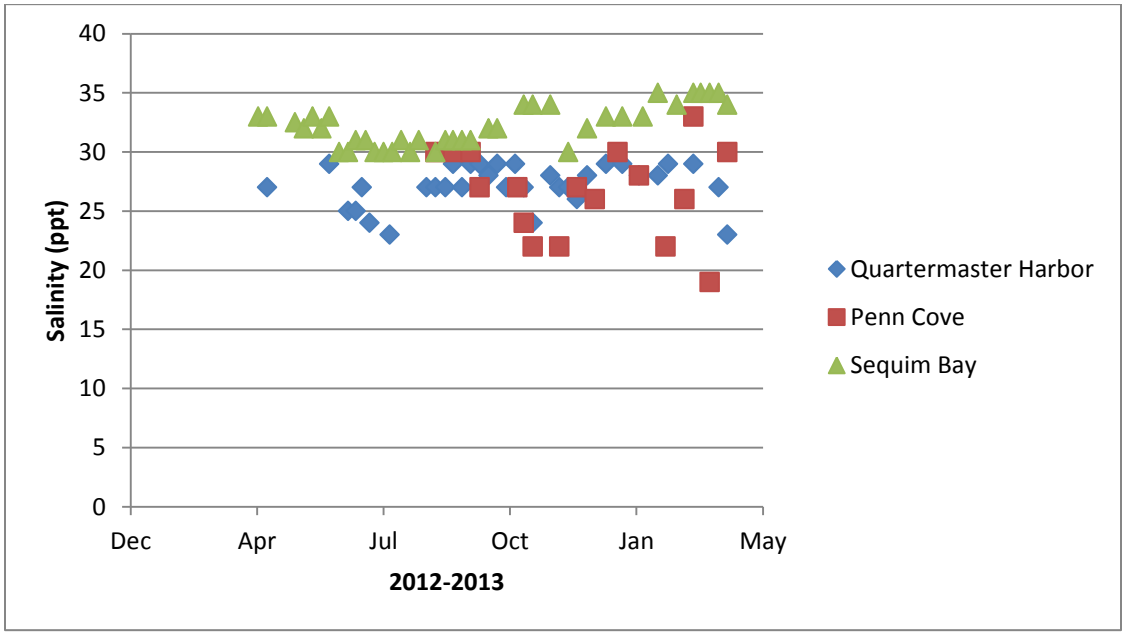


Figure 5. Variability in surface salinity at all three sites.

To assess whether these environmental variables influenced the abundance of Dinophysis, correlation analysis was conducted between *Dinophysis* abundance and these variables. However, *Dinophysis* abundance was not significantly correlated with salinity

at any of the three sites(Penn Cove: r= 0.27, p = 0.137; Sequim Bay: r= -0.49, p =1; Quartermaster Harbor: r= -0.46, p = 0.999).However, *Dinophysis* was significantly positively correlated with temperature for Quartermaster Harbor (p= 0.037, r^2= 0.28). As for Penn Cove, there was not a significant correlation between *Dinophysis* populations and temperature (p = 0.325, r^2= 0.07). Lastly, in Sequim Bay, there was a significant relationship between *Dinophysis* population and temperature (p = 0.004, r^2= 0.31).

Protoperidinium was also significantly correlated with *Dinophysis* at all three sites, therefore, could temperature affect *Protoperidinium*, which then may be influencing the abundance of *Dinophysis*? At Sequim Bay, *Protoperidinium* and temperature were significantly correlated (p =0.001, r^2= 0.53). At Quartermaster Harbor, *Protoperidinium* and temperature were also significantly correlated (p= 0.015, r^2= 0.35). However, *Protoperidinium* was not significantly correlated with salinity at these sites. However, at Penn Cove, *Protoperidinium* and temperature were not significantly correlated (p= 0.067, r^2= 0.60) while *Protoperidinium* and salinity were significantly correlated (p= 0.042, r^2= 24).

DISCUSSION

The focus of this study was to address the following questions: 1) Is there seasonal variation in *Dinophysis* between the three locations within Puget Sound with contrasting influence to the ocean sites, 2) Does the presence of *Dinophysis* species also vary by location, and 3) Are there certain phytoplankton communities present either before, during, or after *Dinophysis* presence. This discussion section will address each question individually in detail.

Seasonality of *Dinophysis*

Dinophysis displayed multiple peaks in abundance (cells/L) at all sites. Quartermaster Harbor and Sequim Bay had the greatest *Dinophysis* abundance for the longest amount of time, spanning almost four months, bracketing the summer dry season, from June to October. As for Penn Cove, *Dinophysis* abundance peaked during three months, April, August, and December. *Dinophysis'* presence in the summer is part of the dinoflagellate seasonality. Dinoflagellates can outcompete diatoms during the summer when waters are more stratified due to non-upwelling conditions that stratify the water and create salinity and temperature gradients. Snowpack melting during the late spring and early summer allows for additional freshwater input while the lack of upwelling creates calm waters enabling surface water temperatures to increase (Lehman 2000; Horner et al. 1997; Moore et al. 2008). Diatoms prefer upwelling conditions (generally during the spring) which allow nutrients, such as silica, to be ready available towards the surface of the water column, where diatoms typically reside (Trigueros & Orive 2001; Cloern & Dufford 2005; Smayda & Reynolds 2001). In contrast, dinoflagellates have flagella that allow them to move up and down the water column, enabling them to

outcompete other phytoplankton when nutrients become stratified within the water column and are depleted from surface waters. This mobility allows them to move towards the top of the water column to photosynthesize and then down a bit further to take up nutrients (Gonzalez-Gil et al. 2010; Anderson 1995). Though a stratified water column generally proves advantageous to *Dinophysis* and other dinoflagellates, an overabundance of freshwater may diminish their populations as shown at Penn Cove.

Dinophysis abundance was 1-2 orders of magnitude less at Penn Cove than the other two sites. The abundance of *Dinophysis* present at Penn Cove also peaked during the summer, but also peaked in April and December. Mackas & Harrison (1997) suggest that approximately one-fourth to one-third of the freshwater input into Puget Sound is due to Skagit river runoff into Skagit Bay. With Penn Cove situated in Skagit Bay, this could be one possible explanation as to why there was such a strong difference in population at this site in comparison to Quartermaster Harbor and Sequim Bay. *Dinophysis* was present throughout the rest of the year, but in small numbers. Maximum abundance reached 36 cells/L in April, then again at 32 cells/L in August, and lastly with the smallest peak with 7 cells/L in December. Penn Cove had the greatest amount of freshwater influence with salinity ranging from 19 to 33 ppt. Though salinity may be a key factor to why *Dinophysis* abundance is so low (see discussion below) , other factors such as temperature may also play a role.

Looking into relationships between *Dinophysis* abundance and temperature / salinity may also provide further support that populations are controlled by seasonal factors since *Dinophysis* are usually associated with warm surface water temperatures, stable salinities, and low nutrients (Trainer et al. 2010). Water temperature can be a

controlling factor for phytoplankton abundance and can reflect seasonal changes (Lehman 2000). As stated earlier, *Dinophysis* prefers warmer, stratified waters, which are found generally during the summer. Sequim Bay is a good example of this for when temperatures reached above 10° C, *Dinophysis* abundance was always greater than 200 cells/L, from June through October. This time period represented the highest cell counts that were found throughout the year. This is consistent with a study by Gonzalez- Gil et al. (2010) which suggested that *Dinophysis acuminata* was observed at temperatures from 13 to 22°C. As such, Sequim Bay's correlation between *Dinophysis* and temperature showed a strong positive relationship. Quartermaster Harbor also showed a strong positive correlation between *Dinophysis* and temperature, where abundance was maximized at temperatures at 16°C during the summer. As for Penn Cove, the temperature probe malfunctioned, therefore providing inaccurate readings. Nonetheless, at Penn Cove during the summer, the second largest peak in *Dinophysis* was in August. Though positive correlations were found between *Dinophysis* and temperature, much of the stratification within the water column in Puget Sound is due to changes in salinity, driven by freshwater inputs instead of temperature driven (Moore et al. 2008).

Salinity gradients also occur depending on the location of the sampling site within Puget Sound. Sequim Bay has the greatest salinity due to its proximity to the ocean, while Penn Cove, has more of an estuarine environment due to the freshwater output from the Skagit River. Studies have suggested that *Dinophysis* prefers salinity levels from approximately 29-34 parts per thousand and/or maybe be found either in or below the pycnocline (Gonzalez-Gil et al. 2010 ; Pizarro et al. 2008; Aubrey et al. 2000;

Peperzak 1996). The phytoplankton community structure may also change depending upon the salinity as well as temperature.

Phytoplankton Community Composition

Phytoplankton communities are shaped by species interactions across trophic levels, their nutritional modes, their form and function, and life histories (Cloern & Dufford 2005). Therefore, understanding which phytoplankton either come before, co-exist, or follow *Dinophysis* may provide additional insight into what we do not currently know about *Dinophysis*. For this study, seasonal phytoplankton succession was noticeable at each site. Diatoms dominated spring, early summer, and fall, while the dinoflagellates dominated during the late summer at all three sites. This pattern is consistent with other reports in temperate regions, where diatoms are also prevalent during spring and fall months (Rynearson et al. 2006). Sequim Bay had the greatest abundance of diatoms with a peak at 289,061 cells/L and dinoflagellates with a peak at 9,068 cells/L . Not only did Sequim Bay have the greatest abundance of diatoms and dinoflagellates, but it also was the most diverse. Sequim bay had 48 genera total, with 31 diatom genera and 17 dinoflagellate genera. Quartermaster Harbor was next with 42 total genera, 26 genera belonging to diatoms and 16 genera belonging to dinoflagellates. Lastly, Penn Cove had 33 total genera with 25 genera belonging to diatoms and 8 genera belonging to dinoflagellates. Though Penn Cove had the most variable range in salinity which were suitable for phytoplankton growth and survival, the temperature range (7-16C) was the coolest out of all three sites. The combination of temperature and select salinity values during the winter and early spring were not as favorable for phytoplankton growth (Cloern & Dufford,2005). Though there was much variability in the amount and

type of phytoplankton present at each site, seasonal community succession was a constant.

Our phytoplankton community succession data followed Margalef's Mandala, which suggests that phytoplankton seasonal variability starts with a general void of phytoplankton in the winter, a diatom bloom during the spring, then a dinoflagellate bloom during the summer and early fall (Pitcher et al. 2010; Smayda & Reynolds 2001; Margalef et al. 1979). This general knowledge of when certain groups of phytoplankton are present throughout the year provides a baseline to when we can supposedly expect certain types of phytoplankton, especially harmful algal blooms. Unfortunately, there is still much uncertainty regarding when harmful algal blooms may occur due to their complex nature. Smayda & Reynolds (2003) suggest that diatom blooms have five major features to their bloom behavior, but that dinoflagellate blooms, in contrast, are unpredictable and ephemeral. Some of the positively correlated phytoplankton with *Dinophysis* in the data set have similar characteristics to *Dinophysis* , such as taxonomy, habitat, temperature, and/or salinity preferences. These may be beneficial in providing a better understanding of this complex dinoflagellate.

In order to gain a finer resolution into the community composition data, correlations between *Dinophysis* and all genera at each site were performed. Certain phytoplankton genera were positively correlated with *Dinophysis,* and they all varied dependent upon the site. *Protoperidinium spp.* was the only genus significantly correlated with *Dinophysis* at all three sites. *Protoperidinium* and *Dinophysis* not only both consume smaller phytoplankton but are also considered neritic species (Gonzalez-Gil et al. 2010; Trigueros & Orive 20010), Therefore, due to their common neritic habitat,

feeding in similar trophic levels, and preference for stratified waters, it would not be uncommon to observe *Dinophysis* around the same time as *Protoperidinium*. Therefore, could *Protoperidinium* be used to forecast the presence of *Dinophysis*? Unfortunately, this was not the case. *Protoperidinium* came before, co-existed, and followed *Dinophysis*.

At Quartermaster Harbor, additional genera that were positively correlated with *Dinophysis* included *Scripsiella trochoidea*, and *Coscinodiscus spp.* (Table 4). *S. trochoidea* is a cosmopolitan species found in coastal temperate waters. *Coscinodiscus spp.* found in Washington are generally considered a cosmopolitan genus found within temperate waters (Horner et al. 2002). *Scripsiella* has been found present before *Dinophysis* due to nutrient control under vertical stratification of the water column (Pitcher et al. 2010; Smayda & Reynolds 2001). Though *Scripsiella* did show a positive relationship with *Dinophysis*, *Scripsiella* was not a consistent precursor to *Dinophysis* at Quartermaster Harbor.

At Sequim Bay, additional genera significantly correlated with *Dinophysis* included *Ceratium fusus*, *Scripsiella trochoidea*, and *Pseudo-nitzschia spp*.(Table 4). *C. fusus* is a cosmopolitan dinoflagellate that can be found in estuarine and oceanic environments (Horner 2002). Therefore, Sequim Bay is a suitable habitat for *C. fusus*. Though some of the literature states that *C. fusus* grows better when temperatures are above 16^0 C and salinity between 12-38 ppt, our data does not show an increase in *C. fusus* abundance above 16^0 C. Other literature has suggested that phytoplankton communities consisting of *Dinophysis acuminata*, *Ceratium spp*, and *Protoperidinium* are all considered larger neritic species that take advantage of a stratified water column

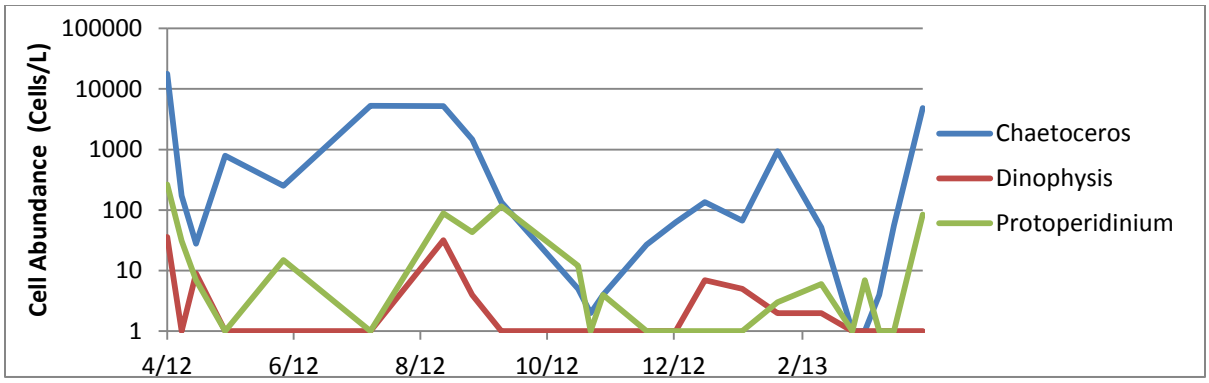
with high seasonal irradiance, which is normally present during the summer (Gonzalez-Gil et a. 2010; Pizarro et al. 2008; Smayda & Reynolds 2001; Trigueros & Orive 2001). Lastly, *Pseudo-nitzschia spp.* is a diatom that can be present throughout summer and that can grow under a wide range of conditions from polar to temperate and equatorial waters to neritic and open ocean environments (Orsini et al. 2004; Horner et al. 2000).

At Penn Cove, additional genera significantly correlated with *Dinophysis* include *Chaetoceros spp., Thalassiosira spp*., and *Thalassionema nitzschioides* (Table 4). C*haetoceros spp.* is a diatom that does well in a variety of conditions from neritic to pelagic ,estuarine or oceanic, and even warm to temperate waters (Horner 2002). *Chaetoceros's* versatility allows it to be a dominant phytoplankton genus for most of the year for all three sites. It is only at Penn Cove, that *Chaetoceros spp.* is significantly correlated with *Dinophysis*. *Thalassiosira* is another versatile diatom abundant at each site, but is only correlated with *Dinophysis* at Penn Cove (Horner 2002). Lastly, *Thalassiosira* is a cosmopolitan diatom found in neritic and coastal waters and been found to bloom in late summer off the coast of British Columbia (Hay 2003; Horner 2002). These correlations provide insight into what is co-occuring alongside of *Dinophysis*, but assessing how these phytoplankton groups distribute themselves in different *Dinophysis* population gradients may provide another avenue to examine how phytoplankton behave.

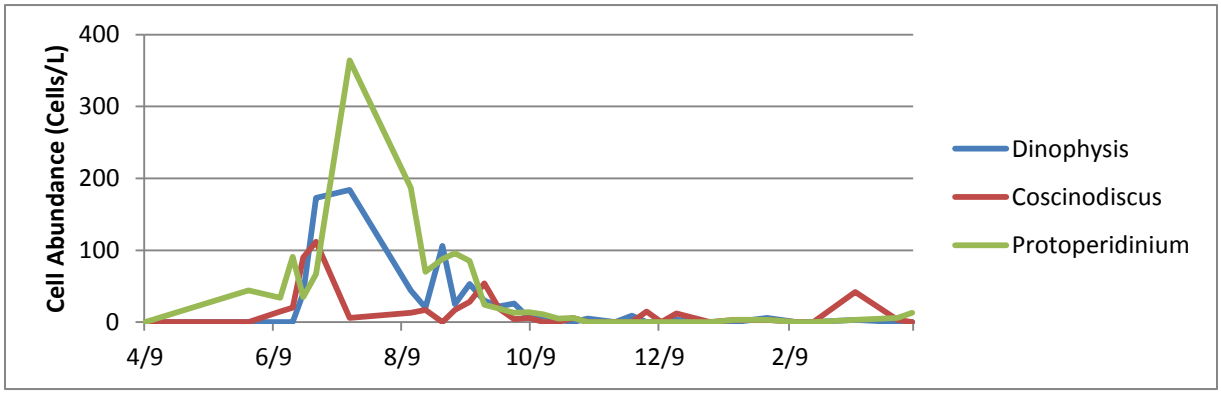
Site	Genus	p-value
Penn Cove	*Protoperidinium*	0.001
Penn Cove	*Chaetoceros*	0.011
Penn Cove	*Thalassionema*	0.035
Quartermaster Harbor	*Protoperidnium*	0.001
Quartermaster Harbor	*Coscinodiscus*	0.02
Quartermaster Harbor	*Scripsiella*	0.007
Sequim Bay	*Protoperidnium*	0.001
Sequim Bay	*Scripsiella*	0.02
Sequim Bay	*Ceratium*	0.028
Sequim Bay	*Pseudo-nitzschia Lg*	0.04
Sequim Bay	*Pseudo-nitzschia Sm*	0.05

Table.4. Phytoplankton genera that are positively correlated with *Dinophysis* at each site. The p-value denotes the significance of the correlation.

A)



B)



C)

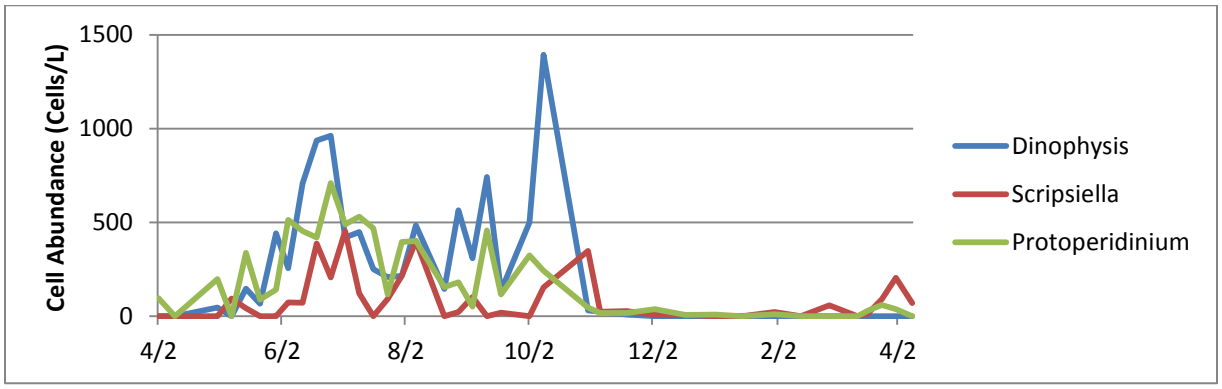


Figure 6. Abundance of *Dinophysis* and the most positively correlated genera at each site. a) Penn Cove, b) Quartermaster Harbor, and c) Sequim Bay.

Referring once more to Cloern & Dufford (2005) they suggest that phytoplankton communities are shaped by species interactions across trophic levels, their nutritional modes, their form and function, and life histories. NMS ordinations were performed to get a better understanding of whether or not the phytoplankton communities were shaped by the presence of *Dinophysis*. These group distributions created by the NMS ordinations allowed us to see if these phytoplankton community groups that might have been similarly influenced by not only *Dinophysis* but other possible factors such as nutritional mode and their form and function.

Each NMS ordination had different *Dinophysis* population criteria due to the different amounts of *Dinophysis* present at each site. At Sequim Bay, the clustering between groups was the most significant. The greatest difference between groupings was between the criteria 1 (No *Dinophysis*) and criteria 2 (*Dinophysis* cells up to 350 cells/L). This could be due to a multiple of reasons from *Dinophysis* using its toxins as a possible

phytoplankton deterent (Smayda 1997), to predation, to physiochemical factors (nutrients, light, currents). Where Sequim Bay had the greatest amount of differences between groups, Penn Cove had the least. There was no significant difference phytoplankton between the presence or absence of *Dinophysis*. Not only is *Dinophysis* population low at this location, the freshwater input from the Skagit River could alter the amount and types of phytoplankton present (Mackas & Harrison 1997). Because of these factors, it is not unlikely for Penn Cove to have a more homogenous pattern between their criteria groups. Lastly, at Quartermaster Harbor, the most significant differences are between criteria 1 (No *Dinophysis*) and criteria 3 (*Dinophysis* cells greater than 20 cells/L). The clustering between these groups also reflect these differences. Since the difference between criteria 1 and criteria 3 is 20 cells/L, this might be enough of a difference, while the difference between criteria 1 and criteria 2 is only 10 cells/L. Much like Sequim Bay, the same reasoning can apply to where these groupings may occur due to the presence of *Dinophysis,* predation, or nutrients. Examining species richness, species evenness, Shannon Weiner's diversity index, and Simpson's index might give us a better idea to why these groupings cluster.

Phytoplankton Diversity

A study by Hutchinson (1961) tried to understand why many species of phytoplankton can coexist while competing for a limited amount of resources. In the "paradox of plankton", when there is a competition for limited nutrients, the superior species should be able to outcompete the others, but in fact, that is generally not the case. Hutchinson came up with a hypothesis for the "paradox of the plankton" suggesting that communities of phytoplankton are organized by processes beyond nutrient competition

such as habitat viability, species interaction, and phytoplankton dispersal. With this concept in mind, species evenness, species richness, Shannon-Wiener's diversity index, and Simpson's index were calculated to gain a better understanding of how the phytoplankton communities were composed when *Dinophysis* was present versus absent.

Species evenness is defined as a measure of relative abundances of species in an assemblage (Gotelli & Ellison 2013). At all sites, there was no significant difference in evenness when *Dinophysis* was present and absent. Though, there was no significant difference between communities when *Dinophysis* was present and absent, their mid-ranging values provides us with information that the phytoplankton community was a bit patchy in their distributions. A possible flaw in separating the groups into presence and absence of *Dinophysis* is that *Dinophysis* will be present when conditions are right for dinoflagellates to grow and when Dinophysis is absent, the conditions will be better suited for diatoms. Consequently, this evenness is measuring more of the seasonal trend, (with dinoflagellates dominating in the summer and early fall and the diatoms dominating in the spring) than the in-between periods of when *Dinophysis* is present for a week and then absent the next.

Species richness will provide another aspect of community composition by measuring the number of species in an assemblage (Gotelli & Ellison 2013). Species richness was significantly higher at Sequim Bay and Quartermaster Harbor when Dinophysis was present, but not Penn Cove. These results seem a bit counterintuitive, since Smayda (1997) suggested to the reason why some dinoflagellates are toxic is to combat intraspecific competition. It may be possible that the presence of *Dinophysis* may deter predators therefore allowing a greater diversity of phytoplankton to survive.

Shannon-Wiener index and the Simpson's index both measure species diversity (Gotelli & Ellison 2013). Both indices at all three sites showed no significant difference between the presence and absence of *Dinophysis*. Once more, it is possible that the diversity measurements could have been compared on a seasonal scale (diatoms vs. dinoflagellates) than on a weekly or bi-weekly scale. Lastly, it could also be due to a small sample size since only one year of data was used. Though species evenness and both diversity indices did not show a significant change in population before and after *Dinophysis*, these analyses do show that there wasn't a monopoly of the phytoplankton by any one particular genus.

Conclusion

Dinophysis abundance varies seasonally and spatially. Seasonal variation was significant at Sequim Bay and Quartermaster Harbor. The highest abundance of *Dinophysis* was at Sequim Bay, the most oceanic site, followed by Quartermaster Harbor, and lastly Penn Cove, the most estuarine site. *Protoperidinium* was the most significantly correlated with *Dinophysis* at each site and correlations between other species varied depending upon the site. Scaling back and looking at the phytoplankton community, the NMS ordinations showed that Sequim Bay showed the greatest evidence of a significant pattern suggesting that the phytoplankton community did change depending upon whether *Dinophysis* was absent or when *Dinophysis* cells reached up to 350 cells/L. Phytoplankton richness was also significantly greater at Sequim Bay when Dinophysis was present, echoing the NMS ordination. Quartermaster Harbor showed slight significance between phytoplankton communities and *Dinophysis* in the NMS ordination and also showed significance in species richness; once again with species richness greater

when *Dinophysis* is present. Penn Cove showed no statistical differences between phytoplankton communities and *Dinophysis* by the NMS ordination and showed no significant difference in species richness. Knowing when *Dinophysis* is present, what species are present, and how the phytoplankton community is being affected will give us insight into how *Dinophysis* behaves within Puget Sound.

Understanding how *Dinophysis* behaves is important to Washington's shellfish industry, Native American Tribes, and the general public. It is important to minimize health risks and economic loss through early detection of harmful algal blooms. One important result from this thesis is that though *Dinophysis* is most abundant during the summer, it is also found at other at other times of the year; therefore, recreational shellfish consumers should ensure that conditions are suitable for shellfish harvesting regardless of season.

Dinophysis studies have been published within Puget Sound, mostly after the DSP event in Sequim Bay State Park from 2011. Since then many scientists from various organizations including NOAA, Washington Department of Health, and the Jamestown S'Klallam Tribe, have been trying to gain a better understanding of why *Dinophysis* has recently been causing DSP events. This thesis is one of a few studies that are able to define *Dinophysis* down to species level at several sites within Puget Sound. Since some species of *Dinophysis* are considered to be more toxic than others, understanding what conditions *Dinophysis spp.* prefers or cataloging trends of where certain *Dinophysis spp.* tend to inhabit, will be quite beneficial. This thesis also provided information on the seasonal genera found within three sites of Puget Sound, which is the first of its kind. Horner & Postel (1993) did provide phytoplankton community composition data along

the Washington coast, while Newton & Horner (2003) provided phytoplankton community composition data within Willapa Bay, but not within Puget Sound. Baseline phytoplankton community composition data will enable future studies to gain a glimpse into what the phytoplankton community was composed of in 2012-2013. This baseline data may have implications in ocean acidification or climate change studies to compare how the diatom, dinoflagellate, or harmful algal bloom community has shifted.

INTERDISCPLINARY STATEMENT AND CONCLUSIONS

Harmful algal blooms have profound consequences stretching from loss of recreational and commercial fishing opportunities, reduction of food supply, and loss of community identity (Bauer et al.2009). Shellfish have provided sustenance, community identity, and an important source of income within the recreation and commercial fishing industries for many coastal communities, but especially local Washington tribes. Therefore, it is imperative that we are able to understand general characteristics and preferable conditions of harmful algal blooms, such as *Dinophysis*, and are able to predict their future patterns. Washington waters are already starting to feel the effects of climate change and ocean acidification (Feely et al. 2010). This chapter will discuss how changing climate conditions will affect harmful algal blooms and how multiple stakeholders, such as Washington tribes, state agencies, federal agencies, community members, citizen science organizations and shellfish farms, work collaboratively to address the complexities of harmful algal blooms. Lastly, I will discuss how this thesis could be improved for future studies.

Ocean Acidification and Climate Change Impacts on Harmful Algal Blooms

Increasing concentrations of greenhouse gases are expected to lower pH, increase surface water temperatures, and cause changes to vertical mixing and upwelling (Moore et al. 2008). The potential consequences of these changes for harmful algal blooms have only recently been explored. Harmful algal blooms have increased around the world and are expected to continue to increase as a result of ocean acidification and climate change. The continuation of an increase of harmful algal blooms will affect the global carbon cycle, tourism, ecosystems, fishing industry, and human health. The aim of this section is

to address how global change (i.e. ocean acidification and climate change) will affect harmful algal blooms.

Ocean Acidification's Impact on Phytoplankton and Harmful Algal Bloom Species

An increase in ocean acidity is likely to influence phytoplankton community composition. This more acidic environment tends to favor certain phytoplankton genera and inhibit others (Schippers et al. 2004; Hallegraeff 2010) . Looking forward in the next hundred years or so, Earth is expected to have similar conditions to the Mesozoic era (Huber et al. 1996). In the Mesozoic era, CO_2 levels in the atmosphere were up towards 800 ppm due to large volcanic eruptions. This era favored dinoflagellate and coccolithophorids because of the low nutrient availability and warm water stratification, in which ocean temperatures slowly rose worldwide ranging from 17°C to 33°C (Huber et al. 1995). Today with the ocean waters becoming more acidic, these calcifying coccolithophorids will find their shells slowly dissolving making it difficult for them to survive. The dinoflagellates, with their cellulose composition will be able to adapt to these new waters.

Since dinoflagellates and diatoms have frustules (shells) made out of either cellulose or silica they are not likely to be susceptible to dissolution under more acidic conditions. A study by Fu et al. (2010) suggests that saxitoxin production by *Alexandrium* was greater under higher pCO_2 conditions and with greater amounts of sunlight due to toxin production being linked to their photosynthetic activity. Other harmful algal bloom species, such as the diatom *Pseudo-nitzschia*, can react negatively to

a change in pH, whether conditions become more acidic or more basic. Sun et al. (2011) suggests that *Pseudo-nitzschia* exude high concentrations of domoic acid in treatments combing high pCO_2 with low pH, which would be expected under conditions of enhanced ocean acidification. Nutrient limitation such as phosphate under these high pCO_2 conditions also increases the amount of domoic acid production by *Pseudo-nitzschia*. Other studies have shown the opposite, that a lower pCO_2 with higher pH can trigger *Pseudo-nitzschia* to create more domoic acid. Lundholm et al. (2004) suggests that under the stress of pH, *Pseudo-nitzschia multiseries* produces similar amounts of domoic acid as it would under silicate or phosphate limitations. It has also been proposed that this greater production of toxin could be encouraged by carbon limitation with increasing pH (Lundholm et al. 2004, Trimborn et al. 2007). Kudela et al. (2002) suggests that environmental stressors, such as silica limitation or even the amount of light, may cause an increase in toxin production by *Pseudo-nitzschia*. Though results of studying both dinoflagellates and diatom species differ, a common theme is that change in the pCO_2 and nutrient limitations can cause an increase in toxin production.

Climate Change Impacts on Harmful Algal Blooms

El Nino/Southern Oscillation and the Pacific Decadal Oscillation (ENSO and PDO) both have warm and cool periods. These periods for ENSO last for six to eighteen months and for PDO, about twenty to thirty years (Mantua & Hare 2002). During these warm periods, the sea surface temperature increases therefore reducing upwelling and increasing stratification (Rasmusson & Carpenter 1982). Since phytoplankton growth is determined by nutrients, vertical mixing, temperature, and sunlight, the reduced

upwelling and increased stratification can influence the phytoplankton community composition (Hallegraeff 2010).

Since warmer waters will increase stratification, this alters the suitable growing conditions for certain phytoplankton genera. Diatoms need a well-mixed water column in order to take advantage of the nutrients and sunlight. When the water column becomes stratified, the nutrients and sunlight are no longer conveniently located. Therefore, diatoms are unable to reach both resources. Dinoflagellates, on the other hand, have two flagella which enable them to migrate through the water column taking advantage of the nutrients near the pycnocline at night and then using those nutrients for photosynthesis during the day (Anderson 1995). Therefore, dinoflagellates are expected to be favored over other phytoplankton under future climate conditions that increase stratification by warming the temperature (Moore et al. 2008).

Dinoflagellates comprise the majority of harmful algal blooms species with many of them residing in tropical waters. *Gambierdiscus toxicus* is an example of a tropical armored dinoflagellate that is associated with ciguatera fish poisoning and is found as an epiphyte on macroalgae. The macroalgae is then eaten by herbivorous/ominivorous fish, which we in turn, consume (Friedman et al. 2008). Ciguatera fish poisoning causes neurological symptoms such as tingling in extremities and heat reversal. Symptoms can last anywhere from days to weeks, but can last for years. Ciguatera has no cure, but the symptoms can be treated (Hokama 1998). Since the toxins within the fish are lipophilic, they bioaccumulate within the fish. This fish can no longer be sold in the market place, therefore causing an economic loss to the fisherman. Tropical unarmored dinoflagellates, such as *Karenia brevis*, can also create a negative influence on the economy and human

health. Since *K.brevis* does not have any armored plates, unlike *Gambierdiscus*, this enables the cells to lyse in wave action, therefore releasing an aerosolized toxin known as brevetoxin. This aerosolized brevetoxin can cause respiratory illnesses, especially those who have asthma (Fleming et al. 2005). Illnesses are not the only drawback to *K. brevis* blooms. Beach closures due to these brevetoxins also affect the local tourism industry. Businesses located on the beach, such as hotels and restaurants, and recreational beach go-ers are unable to take advantage of their prime location (Larkin&Adams 2007). Therefore, as temperatures begin to rise, these tropical HAB species' habitat will continue to increase into higher latitudes and cause even more problems in local communities (Hallegraeff 2010).

Temperate dinoflagellates such as *Alexandrium* are also a cause for concern. *Alexandrium* is responsible for paralytic shellfish poisoning. Water temperatures greater than 13°C have been found to promote *Alexandrium catenella* blooms, thus increasing the possibility for PSP events. In Puget Sound, water temperatures reach their highest during late summer and early fall, therefore providing perfect temperature and habitat (stratified waters) for *A. catenella*, which are actually seen during those seasons (Figure 10). Historically, on average, there's about a 68 day window for prime *Alexandrium* growth at Sequim Bay. As ocean temperatures begin to rise, this will increase the amount of favorable days for *A. catenella* to bloom. It is important to note though, that even though these projections show an increase in *A.catenella's* population, this does not take into considering any other biological or physical factors acting simultaneously in the future (Moore et al. 2008).

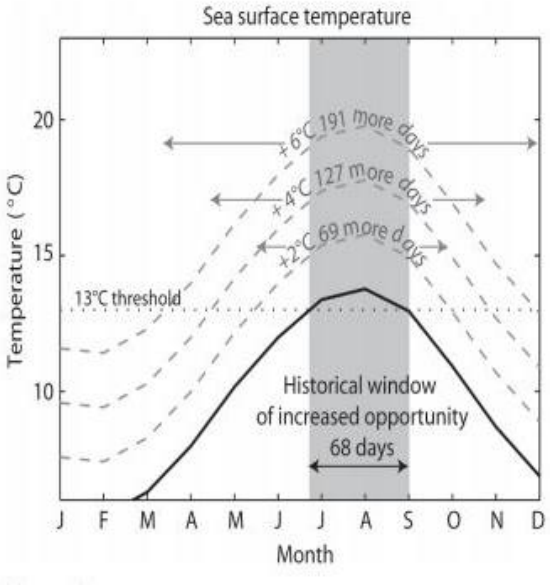


Figure 7. Taken from Moore et al. (2008). Showing This figure shows the potential climate change impacts on Puget Sound shellfish toxicity. Moore et al. (2008) state that "Climatological monthly means of reconstructed sea surface temperature (SST) in Sequim Bay, Puget Sound, using detrended SST records at Race Rocks, British Columbia from 1921 to 2007. The 13°C threshold for accelerated growth of *Alexandrium catenella* is shown, and the mean annual window of favorable SST conditions is shaded for present day conditions. Scenarios for warmer SSST conditions by 2, 4, and 6C are shown in gray with the associated widening of the window of increased opportunity for A.catenella growth."

Impacts of Phytoplankton on the Global Carbon Cycle

As the sea surface temperatures rise and the pH lowers, not only does the phytoplankton community composition likely change to favor dinoflagellates and an increase in toxin production, but conditions can also favor other organisms such as blue-green algae (Schippers et al. 2004). When conditions become favorable, there can be large blooms. These blooms may last several days to several weeks, but when all of the nutrients are consumed, these organisms are either eaten by larger organisms (such as phytoplankton, clams, or small fish), where they may ultimately be respired to carbon

dioxide, or they may sink to the bottom of the ocean repackaged as fecal pellets. They may be degraded along the way, but a fraction of the carbon may eventually reach the ocean floor. The death of these phytoplankton and blue-green algae may contribute a significant amount to the carbon buried in the ocean sediment. A study by Menden-Deuer and Lessard (2000) suggest that dinoflagellates are significantly denser in carbon than diatoms. Therefore, as we consider the future of the possible phytoplankton communities, there may be greater oceanic carbon sequestration by dinoflagellates through larger blooms and their structural ability to hold greater amounts of carbon.

On the other hand, it is possible that warmer waters, could mean a diminishing winter convection. This loss of convection, translates into weaker upwelling and less nutrients reaching the surface of the ocean. Since phytoplankton, especially diatoms, rely upon the nutrients being swept up from the depths and brought to the surface this can severely reduce the amount of primary production leading to a weaker biological pump (Woods & Barkmann 1993; Anderson et al. 2012). This positive feedback loop may reduce the amount of carbon sequestration by phytoplankton.

Anthropogenic Inputs

In order to predict how future harmful algal blooms will change in the future we must understand human behavior. Run-off, pollution, and even the distribution of plastic debris in the ocean can promote harmful algal growth. A study by Maso et al. (2003) showed that *Alexandrium* and other dinoflagellates were found to bind to plastics floating in the ocean. The cysts (resting spores) of *Alexandrium* were found in clumps on plastic

debris and other dinoflagellates embedded themselves on macroalgal growth found on plastic debris. These pieces of debris can then travel into new waters and establish new harmful algal bloom colonies. For a long time, ballast water has been the main contributor for transportation of harmful algal bloom species to a new location. Now, we must consider other anthropogenic inputs such as plastic pollution.

Due to the amount of variability in biotic and abiotic factors in understanding the current and future phytoplankton ecological processes, not one specific study or model holds all the answers. It is imperative to continue to model and collect evidence as to how phytoplankton behave in various conditions. It is also important to be able to have baseline phytoplankton community composition data and harmful algal bloom monitoring programs in place. Knowing what the current phytoplankton community structure is composed of and tracking changes throughout the year might provide insight to the direction of how the phytoplankton communities change. Harmful algal bloom monitoring programs, such as Washington's SoundToxins, enable communities to be aware of what harmful algal blooms are currently present though weekly phytoplankton observation. These monitoring programs provide a better understanding of how phytoplankton communities change through time. Harmful algal blooms are a multi-faceted problem that will definitely need interdisciplinary tools to predict their future behavior.

Summary

Though many studies have shown a variety of results in regards to phytoplankton community composition in greater acidified waters and under certain climate change conditions, it is uncertain to how quickly these changes will occur. In order to address how global change (i.e. ocean acidification and climate change) will affect harmful algal blooms, it was difficult to focus only on factors of ocean acidification. Climate change is another problem that will also affect phytoplankton in the future, therefore, it is important to address both issues in this complex environmental puzzle. Currently, studies have suggested the following answers: (1) with greater stratified waters due to warming of the sea surface and possibly less upwelling that these conditions will favor dinoflagellates, (2) with sea water pH becoming more acidic, calcifying organisms such as coccolithophorids will have to combat their shells slowly dissolving, (3) greater amounts of CO_2 and acidic waters can increase the amount of toxin production from harmful algal genera, (4) a change in the amount and types of nutrients available in the future will favor certain phytoplankton genera, (5) warmer waters will enable tropical harmful algal bloom dinoflagellates to increase from their current habitat range, and (5) an increase in temperature will increase the amount of favorable days for *A.catenella* to bloom. These results are only taking into consideration of the abiotic factors affecting phytoplankton, but there are biotic factors that can influence phytoplankton populations and community structure.

Washington Tribes

> "Shellfish figure prominently in the Northwest Native American myths and legends. In one creation story, humankind is said to have colonized the planet after escaping from a tightly sealed clam's shell. In another, more light-hearted tale, shellfish are banished to a life in beach sand, after being sentenced by other animals for malicious gossiping. This, the story explains, is why beach walkers frequently see small spurts of water shooting up from the sand. The clams are trying to clear the silt and seawater they've swallowed while attempting to tell their spiteful tales." – *Heaven on the Half Shell: The Story of the Northwest's Love Affair with the Oyster*

For centuries, Washington tribes have depended on the bivalves residing along the coastline for subsistence, trade, woodcarvings, and for ceremonial apparel and rites. Many of the mussels, clams, abalone, and oyster shells brought in substantial revenue to the various coastal tribes from trade and shellfish harvest. Unfortunately, harmful algal blooms have affected much of their shellfish harvest. Since many of the harmful algal blooms tend to be seasonal, tribal elders were believed to know when it was safe to harvest. This information was then passed down generation to generation. Though the seasonal bloom information was consistently and accurately passed down, the frequency of when blooms occurred did not. With populations growing and expanding over decades, anthropogenic inputs into the local waterways have increased the amount and frequency of blooms (Bauer et al. 2009). Decadal patterns of shellfish toxicity have indicated that the frequency, magnitude, and geographical scope of saxitoxin, exuded by *Alexandrium catenella* in Puget Sound, has been increasing since the 1950s (Moore et al. 2009).

Fortunately, programs such as the Olympic Region Harmful Algal Blooms (ORHAB) partnership, collaborate with Indian Tribes, state resource managers, coastal communities, researchers, and shellfish-dependent groups, to help provide harmful algal bloom monitoring coordination and dissemination of information (Chadsey et al. 2011).

The Quileute tribe has a heavy dependence on clams. They not only have a phrase for "clam hungry", *ta'a Wshi xa' iits 'os,* but 20% of their total annual harvest goes to subsistence while the other 80% is a source of earnings from clam sales for tribal members (Bauer et al. 2009). The Quileute Indian Tribe has explored methods for rapid detection of certain biotoxins, such as domoic acid and saxitoxin, using stick-like instant read indicators. These stick assays, taking about one hour to produce a result, have provided adequate forewarning of possible shellfish biotoxin contamination. These stick indicators are of extra importance for tribes such as the Quileute, Quinalt, and the Makah due to the remoteness of their locations (Northwest Fisheries Science Center & Washington Sea Grant 2002). Though the stick indicator is a step in the right direction, it is not always 100% accurate. Other monitoring techniques, though they take more time, provide greater accuracy. Understanding when the toxic phytoplankton are present and if the toxins exuded are above the toxin threshold is of vital importance. If harmful algal blooms can be detected earlier enough or their mechanisms under which they bloom can be better understood, it may allow a more economically robust industry and less negative impacts to the regional Native American tribes.

In 1998, the Quinault Indian Nation suffered great economic loss from the ASP closures. This long episode of domoic acid presence within the clams created a prolonged hiatus in harvesting. This hiatus created a disinterest within the commercial markets therefore leaving the Quinault for other clam distributors temporarily (Northwest Fisheries Science Center and Washington Sea Grant 2002).

The tribal clam industry was not the only industry hit by the increase of harmful algal blooms. In that same year, the Quileute Indian Nation's Dungeness crab fishery had

similar setbacks. The domoic acid levels from *Pseudo-nitzschia* blooms were above the regulatory limit within the viscera of the crab. Because this domoic acid was concentrated within the viscera, the crab processors removed the gut to save what was left of the crab harvest. Because much of the weight was removed from the crab, the per pound value return of the crab was half the amount they expected to receive (Northwest Fisheries Science Center and Washington Sea Grant 2002). Domoic acid is not the only toxin creating problems for the shellfish industry.

Commercial geoduck fisheries ran by Jamestown S'Klallam, Puyallup, and Suquamish tribes have been greatly impacted by PSP closures. A recall of the tainted geoducks caused a loss of $30,0000. Butter clams, littleneck clams, horse clams and manila clams used for ceremonial events and as a part of the Jamestown S'Klallam tribe's traditional diet. Clams are also an integral part of the Puyallup tribe's culture, being used in weddings, funerals, and ceremonial dinners (Lewitus et al. 2012). With PSP limiting the amount of bivalves being consumed, saxitoxin monitoring is now common practice for Washington Department of Health. In order to be able to monitor these various HAB species, it is important to understand what conditions are optimal for phytoplankton growth and to see if seasonality may play a role in this ever changing marine environment. Citizen science programs, such as SoundToxins, can provide an inexpensive monitoring network to look for seasonal changes in phytoplankton growth and report back to Washington's Department of Health.

Citizen Science

Citizen science, research conducted by amateur or non-professional scientists, allows organizations to broaden their sample sizes and create community awareness of a particular environmental issue or study. Harmful algal blooms are an environmental issue that affects the coast of Washington and Puget Sound. These blooms blanket the coastline seasonally and pose as a threat to the shellfish industry and human health. Awareness of various scientific topics, such as harmful algal blooms, can create greater ecosystem stewardship through community involvement in research (Conrad&Hilchey 2011). Using citizen scientists could allow community members insight into how harmful algal blooms affect the local community as well as how to mitigate their occurrence. The benefits and challenges of integrating volunteers must be assessed in order to see if the data collected is legitimate. Proper training on data collection and subject matter is imperative for a successful citizen science program.

Citizen science volunteer programs have grown in number in recent years; they incorporate one or more of the following: government agencies, industry, academia, community groups, and local institutions to collaborate by monitoring, tracking, and responding to local environmental issues (Whitelaw et al. 2003). Creating citizen awareness of environmental issues often pressures policy makers to support environmental measures and develops more informed citizens when voting for environmental initiatives. It also increases environmental democracy, scientific literacy, social capital, citizen inclusion in local issues, benefits to government, and benefits to ecosystems being monitored (Conrad&Hilchey 2011) For example, on Martha's Vinyard in Massachusetts, a neighborhood pond association formed a citizen science organization

out of concern for declining water quality. Nonprofit organizations, local environmental managers, and the pond association worked on a number of pollution initiatives to improve the water quality. Newsletters and annual reports now feature "Pond Reminders" which help the community remember how to provide proper boat maintenance and general green gardening techniques to minimize polluted run-off (Karney 2000, Conrad&Hilchey 2011). With the knowledge gained through observation, sampling, and data collecting, these citizens can take this experience and apply it to other environmental issues they observe or are able to understand an ecosystem better due to the organisms in which they studied and surveyed.

SoundToxins implements harmful algal bloom monitoring within the Puget Sound that includes professional scientists, from organizations such as NOAA, Washington Department of Health, and Washington Sea Grant, as well as individuals from informal education facilities, formal educational facilities, local Native American tribes, the aquaculture industry, and concerned citizens. Their annual two-day training consists of an introduction to phytoplankton the methods used by SoundToxins to monitor phytoplankton, including microscope training and phytoplankton ID training. The second day is used to report back to the volunteers on how the SoundToxins data is used. Due to the rigorous standards set by the SoundToxins sampling protocol, the constant feedback volunteers receive from sending in their data, and professional support for volunteer concerns, the data coming from SoundToxins is able to be used to aid in early warning of harmful algal blooms. Understanding how and when these phytoplankton blooms occur using SoundToxins data can help decrease the amount of toxic shellfish related illnesses and possibly even the frequency of which these blooms occur.

Conclusion

This thesis tries to understand if there is a way to forecast the presence of *Dinophysis* and where certain species tend to reside. Though no particular genus was identified a constant precursor to the presence of *Dinophysis* it is important to note that *Dinophysis* generally conforms to the traditional diatom-dinoflagellate seasonal patterns. The year round presence of *Dinophysis* and the fact that this species can exude its biotoxins within a small population presents a unique challenge in understanding its behavior.

In order to gain a better understanding where certain species of *Dinophysis* resides and if there are any phytoplankton genera that can provide us with a forewarning of *Dinophysis*, this study would have to look at a larger time scale of at least five years and at several other locations, preferably another station in the middle of Puget Sound and several in south Puget Sound. Setting a wider cast of sampling stations will allow us to gain a finer resolution of the phytoplankton community present throughout all seasons. Also, incorporating nutrient, chlorophyll, and weather data into the study will provide greater strength and open up other possible explanations for the patterns exhibited by phytoplankton.

Overall, a holistic approach needs to be taken to further understand harmful algal bloom ecology, how anthropogenic sources and climate change affect harmful algal blooms, harmful algal bloom toxin mechanisms, understand what can evoke a sense of responsibility and change of behaviors to ensure less pollution and nutrients enter the ocean. This thesis is only a small piece to this very complex puzzle. Hopefully through

the continuation of citizen science programs, such as SoundToxins, communities can find ways to gain a better understanding of this ever evolving phenomenon.

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APPENDICES

APPENDIX 1. SEQUIM BAY PHYTOPLANKTON COMPOSITION

Date	Genus	Average (Cells/L)	SE
6/5/2012	*Chaetoceros*	48563.3	6036.6
	Cyindrotheca	293.9	73.5
	Detonula	9110.2	902.8
	Dinophysis	257.1	132.4
	Ditylum	257.1	97.2
	Eucampia	330.6	168.3
	Leptocylindrus	5804.1	1182.4
	Noctiluca	220.4	73.5
	Odontella	293.9	194.4
	Proboscia	36.7	36.7
	Protoperidinium	514.3	146.9
	Pseudo-nitzschia Lg. Cell type	14914.3	320.2
	Pseudo-nitzschia Sm. Cell type	4298.0	63.6
	Rhizosolenia	12269.4	847.3
	Scripsiella	73.5	73.5
	Skeletonema	1836.7	573.8
	Thalassionema	1028.6	194.4
	Thalassiosira	2130.6	446.9
Date	**Genus**	**Average (Cells/L)**	**SE**
6/12/2012	*Akashiwo sanguinea*	18.1	18.1
	Chaetoceros	979.6	83.1
	Cylindrotheca	1741.5	268.5
	Dinophysis	707.5	158.1
	Ditylum	108.8	31.4
	Eucampia	18.1	18.1
	Kofoidinium	18.1	18.1
	Leptocylindrus	2258.5	583.0
	Minescula	18.1	18.1
	Navicula	36.3	18.1
	Noctiluca	54.4	31.4
	Odontella	254.0	18.1
	Pleurosigma	108.8	0.0
	Protoperidinium	453.5	48.0
	Pseudo-nitzschia Lg. Cell type	23673.5	349.9

	Pseudo-nitzschia Sm. Cell type	9378.7	1702.0
	Rhizosolenia	9759.6	414.9
	Scripsiella	72.6	18.1
	Skeletonema	72.6	36.3
	Thalassionema	90.7	18.1
	Thalassiosira	199.5	18.1
Date	**Genus**	**Average (Cells/L)**	**SE**
6/19/2012	*Alexandrium*	32.3	32.3
	Chaetoceros	32.3	32.3
	Dinophysis	937.1	85.5
	Leptocylindrus	1744.9	335.8
	Noctiluca	48.5	32.3
	Odontella	64.6	32.3
	Pleurosigma	32.3	32.3
	Protoperidinium	420.1	116.5
	Pseudo-nitzschia Lg. Cell type	83238.1	5490.9
	Pseudo-nitzschia Sm. Cell type	3134.4	520.0
	Scripsiella	387.8	223.9
	Rhizosolenia	14637.8	824.5
	Thalassionema	96.9	96.9
Date	**Genus**	**Average (Cells/L)**	**SE**
6/26/2012	*Actinoptychus*	148.0	78.3
	Ceratium	44.4	0.0
	Chaetoceros	66.6	14.8
	Cylindrotheca	44.4	25.6
	Dinophysis	961.7	53.3
	Kofoidinium	29.6	14.8
	Leptocylindrus	177.6	51.3
	Licmorpha	14.8	14.8
	Minescula	14.8	14.8
	Odontella	88.8	0.0
	Protoperidinium	710.2	51.3
	Pseudo-nitzschia Lg. Cell type	72381.6	313.2
	Pseudo-nitzschia Sm. Cell type	1035.7	411.1
	Rhizosolenia	24265.3	363.6
	Scripsiella	207.1	29.6
	Thalassionema	44.4	25.6

	Thalassiosira	14.8	14.8
Date	**Genus**	**Average (Cells/L)**	**SE**
7/3/2012	*Cylindrotheca*	350.3	70.1
	Dinophysis	420.4	60.7
	Pleurosigma	175.2	35.0
	Protoperidinium	490.5	70.1
	Pseudo-nitzschia Lg Cell type	70873.8	4245.2
	Scripsiella	455.4	126.3
	Rhizosolenia	68316.3	7355.1
	Thalassionema	105.1	60.7
Date	**Genus**	**Average (Cells/L)**	**SE**
7/10/2012	*Akashiwo sanguinea*	61.2	35.3
	Ceratium	81.6	40.8
	Chaetoceros	20.4	20.4
	Coscinodiscus	61.2	35.3
	Cylindrotheca	40.8	40.8
	Dinophysis	449.0	20.4
	Heterocapsa	40.8	20.4
	Leptocylindrus	653.1	81.6
	Noctiluca	40.8	40.8
	Odontella	40.8	20.4
	Polykrikos	102.0	54.0
	Protoperidinium	530.6	108.0
	Pseudo-nitzschia Lg. Cell type	201346.9	3396.6
	Rhizosolenia	86244.9	825.5
	Scripsiella	122.4	70.7
	Thalassionema	81.6	81.6
	Thalassiosira	571.4	40.8
Date	**Genus**	**Average (Cells/L)**	**SE**
7/17/2012	*Actinoptychus*	144.2	72.1
	Asterionellopsis	108.2	62.4
	Chaetoceros	10203.4	593.5
	Coscinodiscus	360.5	144.2
	Cylindrotheca	180.3	72.1
	Detonula	72.1	36.1
	Dinophysis	252.4	44.2
	Ditylum	288.4	95.4
	Eucampia	937.4	157.2
	Gonyaulax	36.1	36.1

	Heterocapsa	108.2	62.4
	Leptocylindrus	1045.6	157.2
	Protoperidinium	468.7	95.4
	Pseudo-nitzschia Lg. Cell type	4651.0	390.0
	Rhizosolenia	172592.5	1724.2
	Skeletonema	504.8	236.4
	Tropedoneis	108.2	62.4
	Stephanopyxis	72.1	36.1
	Thalassionema	108.2	0.0
	Thalassiosira	685.0	200.7
Date	**Genus**	**Average (Cells/L)**	**SE**
7/24/2012	*Alexandrium*	115.6	61.2
	Asteromphalus	115.6	61.2
	Chaetoceros	693.9	40.1
	Cylindrotheca	185.0	61.2
	Detonula	46.3	23.1
	Dinophysis	208.2	40.1
	Dissodinium	23.1	23.1
	Ditylum	92.5	23.1
	Heterocapsa	23.1	23.1
	Leptocylindrus	346.9	0.0
	Pleurosigma	46.3	23.1
	Prorocentrum	1665.3	80.1
	Protoperidinium	115.6	46.3
	Pseudo-nitzschia Lg. Cell type	23.1	23.1
	Rhizosolenia	108453.1	884.1
	Scripsiella	92.5	61.2
	Stephanopyxis	69.4	40.1
	Thalassionema	23.1	23.1
	Thalassiosira	809.5	61.2
	Tropedoneis	23.1	23.1
Date	**Genus**	**Average (Cells/L)**	**SE**
7/31/2012	*Actinoptychus*	360.5	190.8
	Alexandrium	252.4	36.1
	Chaetoceros	2271.4	62.4
	Cylindrotheca	216.3	62.4
	Dinophysis	216.3	0.0
	Ditylum	288.4	95.4
	Gonyaulax	216.3	62.4

	Guinardia	432.7	62.4
	Heterocapsa	396.6	36.1
	Leptocylindrus	649.0	124.9
	Pleurosigma	288.4	36.1
	Prorocentrum	5119.7	406.3
	Protoperidinium	396.6	36.1
	Pseudo-nitzschia Lg. Cell type	72.1	36.1
	Rhizosolenia	180921.1	11060.4
	Scripsiella	216.3	62.4
	Skeletonema	144.2	36.1
	Stephanopyxis	180.3	95.4
	Thalassionema	432.7	165.2
	Thalassiosira	2235.4	72.1
Date	**Genus**	**Average (Cells/L)**	**SE**
8/7/2012	*Actinoptychus*	1138.8	193.3
	Asterionellopsis	63.3	63.3
	Ceratium	21.1	21.1
	Chaetoceros	8456.5	750.6
	Coscinodiscus	780.3	55.8
	Cylindrotheca	738.1	117.4
	Dactyliosen	21.1	17.2
	Detonula	147.6	21.1
	Dinophysis	485.0	55.8
	Ditylum	147.6	21.1
	Heterocapsa	42.2	42.2
	Leptocylindrus	2530.6	73.1
	Pleurosigma	168.7	42.2
	Prorocentrum	7718.4	562.3
	Protoperidinium	400.7	76.0
	Pseudo-nitzchia Lg. Cell type	126.5	36.5
	Pseudo-nitzschia Sm. Cell type	379.6	239.5
	Rhizosolenia	49199.3	1921.1
	Scripsiella	400.7	76.0
	Skeletonema	210.9	55.8
	Thalassionema	316.3	63.3
	Thalassiosira	2087.8	131.7
Date	**Genus**	**Average (Cells/L)**	**SE**
8/21/2012	*Alexandrium*	51.9	8.5

	Asteromphalus	103.7	10.4
	Bacteriastrum	31.1	0.0
	Cerataulina	10.4	10.4
	Ceratium	321.6	51.9
	Chaetoceros	228.2	57.8
	Coscinodiscus	31.1	18.0
	Cylindrotheca	20.7	10.4
	Dactyliosen	145.2	74.8
	Dinophysis	145.2	10.4
	Ditylum	10.4	10.4
	Kofoidinium	10.4	10.4
	Leptocylindrus	93.4	18.0
	Noctiluca	10.4	10.4
	Pleurosigma	10.4	10.4
	Prorocentrum	1089.3	95.1
	Protoperidinium	155.6	53.9
	Pseudo-nitzschia Lg. Cell type	83.0	10.4
	Rhizosolenia	10.4	10.4
	Skeletonema	166.0	10.4
	Thalassiosira	197.1	20.7
	Tropidoneis	10.4	10.4
Date	**Genus**	**Average (Cells/L)**	**SE**
8/28/2012	*Alexandrium*	339.3	103.7
	Asteromphalus	248.8	59.8
	Ceratium	791.7	254.9
	Chaetoceros	22.6	22.6
	Cylindrotheca	67.9	0.0
	Dinophysis	565.5	59.8
	Gonyaulax	45.2	22.6
	Leptocylindrus	22.6	22.6
	Noctiluca	22.6	27.7
	Pleurosigma	226.2	22.6
	Prorocentrum	248.8	59.8
	Protoperidinium	181.0	22.6
	Pseudo-ntizschia Lg. Cell type	22.6	22.6
	Scripsiella	22.6	22.6
	Skeletonema	22.6	22.6
	Thalassiosira	90.5	90.5
Date	**Genus**	**Average (Cells/L)**	**SE**

9/4/2012	*Alexandrium*	3748	230
	Amylax	26	26
	Ceratium	698	157
	Chaetoceros	52	26
	Coscinodiscus	52	52
	Cylindrotheca	52	26
	Dinophysis	310	78
	Leptocylindrus	78	45
	Oxyphysis	52	26
	Pleurosigma	78	45
	Prorocentrum	155	45
	Protoperidinium	52	26
	Pseudo-nitzschia Lg. Cell type	26	26
	Scripsiella	103	26
Date	**Genus**	**Average (Cells/L)**	**SE**
9/11/2012	*Akashiwo sanguinea*	429	70
	Alexandrium	3686	429
	Asterionellopsis	86	49
	Asteromphalus	29	29
	Ceratium	1857	273
	Chaetoceros	2714	206
	Coscinodiscus	86	49
	Cylindrotheca	314	76
	Detonula	57	29
	Dinophysis	743	114
	Ditylum	257	49
	Eucampia	86	49
	Kofoidinium	29	29
	Leptocylindrus	229	57
	Oxyphysis	57	57
	Pleurosigma	143	143
	Prorocentrum	29	29
	Protoperidinium	457	29
	Pseudo-nitzschia Lg. Cell type	171	49
	Pseudo-nitzschia Sm. Cell type	29	29
	Rhizosolenia	114	29
	Skeletonema	229	29
	Thalassiosira	1257	234

Date	Genus	Average (Cells/L)	SE
9/18/2012	*Akashiwo sanguinea*	96	19
	Alexandrium	39	19
	Asteromphalus	19	19
	Ceratium	328	126
	Chaetoceros	675	84
	Coscinodiscus	347	0
	Cylindrotheca	19	19
	Detonula	19	19
	Dinophysis	135	19
	Ditylum	598	168
	Eucampia	77	19
	Lauderia	58	33
	Leptocylindrus	19	19
	Pleurosigma	116	33
	Protoperidinium	116	33
	Pseudo-nitzschia Lg. Cell type	29	24
	Pseudo-nitzschia Sm. Cell type	19	24
	Rhizosolenia	96	96
	Scripsiella	19	19
	Stephanopyxis	19	19
	Thalassionema	39	19
	Thalassiosira	906	184

Date	Genus	Average (Cells/L)	SE
10/2/2012	*Akashiwo sanguinea*	413	53
	Alexandrium	29	29
	Asteromphalus	22	15
	Ceratium	973	68
	Chaetoceros	15	15
	Coscinodiscus	133	44
	Cylindrotheca	1739	103
	Dinophysis	501	78
	Leptocylindrus	59	59
	Noctiluca	44	26
	Oxyphysis	29	15
	Pleurosigma	15	15
	Protoperidinium	324	15
	Pseudo-nitzcshia Lg. Cell type	29	29

	Stephanopyxis	15	15
	Scripsiella	206	64
	Thalassionema	15	15
Date	**Genus**	**Average (Cells/L)**	**SE**
10/9/2012	*Akashiwo sanguinea*	22	22
	Alexandrium	88	88
	Ceratium	862	108
	Cylindrotheca	133	38
	Dinophysis	1393	138
	Licmorpha	22	22
	Oxyphysis	22	22
	Protoceratium	44	44
	Protoperidinium	243	22
	Scripsiella	155	58
Date	**Genus**	**Average (Cells/L)**	**SE**
10/31/2012	*Amylax*	16	16
	Ceratium	1629	202
	Chaetoceros	403	57
	Coscinodiscus	16	16
	Cylindrotheca	127	32
	Dinophysis	32	32
	Eucampia	16	16
	Heterocapsa	16	16
	Leptocylindrus	47	27
	Minuscula	16	16
	Protoperidinium	47	27
	Pseudo-nitzschia Lg Cell type	79	57
	Scripsiella	348	84
	Thalassiosira	158	42
Date	**Genus**	**Average (Cells/L)**	**SE**
11/6/2012	*Cerataulina*	12	12
	Ceratium	179	36
	Chaetoceros	71	21
	Coscinodiscus	12	12
	Cylindrotheca	36	24
	Detonula	12	12
	Dinophysis	24	24
	Lauderia	24	24
	Leptocylindrus	12	12
	Pleurosigma	12	12

	Protoperidinium	18	12
	Scripsiella	24	24
	Skeletonema	24	29
	Thalassiosira	24	12
Date	**Genus**	**Average (Cells/L)**	**SE**
11/19/2012	*Ceratium*	129	60
	Cerataulina	9	9
	Chaetoceros	156	34
	Cylindrotheca	18	9
	Detonula	9	9
	Dinophysis	9	9
	Ditylum	28	22
	Leptocylindrus	46	24
	Navicula	46	9
	Pleurosigma	18	9
	Protoperidinium	18	9
	Pseudo-nitzschia Lg. Cell type	9	9
	Scripsiella	28	28
	Skeletonema	55	16
	Thalassionema	37	24
	Thalassiosira	9	9
Date	**Genus**	**Average (Cells/L)**	**SE**
12/3/2012	*Alexandrium*	235	59
	Ceratium	39	8
	Chaetoceros	180	34
	Coscinodsicus	23	0
	Cylindrotheca	47	14
	Leptocylindrus	8	8
	Paralia	8	8
	Protoperidinium	39	21
	Pseudo-nitzschia Lg. Cell type	16	16
	Scripsiella	8	10
	Skeletonema	23	14
	Thalassionema	39	8
	Thalassiosira	47	14
Date	**Genus**	**Average (Cells/L)**	**SE**
12/18/2012	*Chaetoceros*	76	30
	Cylindrotheca	69	13
	Gonyaulax	38	20

	Leptocylindrus	23	0
	Navicula	15	8
	Paralia	30	8
	Pleurosigma	8	8
	Protoperidinium	8	8
	Pseudo-nitzschia Lg. Cell type	38	8
	Scripsiella	8	8
	Thalassionema	61	15
Date	**Genus**	**Average (Cells/L)**	**SE**
1/2/2013	*Akashiwo sanguinea*	14	9
	Chaetoceros	9	9
	Cylindrotheca	18	18
	Paralia	28	16
	Protoperidinium	9	9
	Pseudo-nitzshcia Lg. Cell type	18	9
	Thalassionema	9	9
	Thalassiosira	18	9
Date	**Genus**	**Average (Cells/L)**	**SE**
1/15/2013	*Chaetoceros*	13	13
	Cylindrotheca	102	34
	Paralia	63	25
	Skeletonema	63	25
	Thalassionema	76	44
	Thalassiosira	25	13
Date	**Genus**	**Average (Cells/L)**	**SE**
1/31/2013	*Chaetoceros*	34	0
	Coscinodiscus	11	14
	Cylindrotheca	79	41
	Leptocylindrus	57	23
	Navicula	11	11
	Paralia	45	11
	Protoperidinium	11	11
	Scripsiella	23	23
	Skeletonema	45	11
	Thalassionema	45	11
	Thalassiosira	11	11
Date	**Genus**	**Average (Cells/L)**	**SE**
2/13/2013	*Alexandrium*	14	14
	Asterionellopsis	204	62

	Chaetoceros	177	36
	Corethron	14	14
	Coscinodiscus	14	14
	Cylindrotheca	27	27
	Eucampia	14	14
	Leptocylindrus	136	59
	Paralia	27	27
	Skeletonema	82	0
	Thalassionema	27	14
	Thalassiosira	109	27
Date	**Genus**	**Average (Cells/L)**	**SE**
2/27/2013	*Asterionellopsis*	522	115
	Chaetoceros	784	242
	Cylindrotheca	174	0
	Paralia	73	29
	Pseudo-nitzschia Sm. Cell type	29	15
	Scripsiella	58	38
	Skeletonema	44	25
	Thalassionema	102	38
	Thalassiosira	319	52
Date	**Genus**	**Average (Cells/L)**	**SE**
3/13/2013	*Asterionellopsis*	576	121
	Chaetoceros	16359	695
	Corethron	37	21
	Cylindrotheca	208	68
	Ditylum	37	21
	Eucampia	12	12
	Leptocylindrus	61	24
	Odontella	24	15
	Paralia	12	12
	Pleurosigma	24	12
	Pseudo-nitzschia Sm. Cell type	98	12
	Stephanopyxis	49	12
	Thalassionema	416	53
	Thalassiosira	6208	127
Date	**Genus**	**Average (Cells/L)**	**SE**
3/18/2013	*Asterionellopsis*	1541	83
	Bascillaria	15	15
	Chaetoceros	25667	342

	Coscinodiscus	45	26
	Cylindrotheca	688	60
	Leptocylindrus	75	15
	Navicula	45	26
	Paralia	15	15
	Protoperidinium	30	15
	Pseudo-nitzschia Lg. Cell type	15	15
	Pseudo-nitzschia Sm. Cell type	195	40
	Scripsiella	15	15
	Skeletonema	30	15
	Stephanopyxis	150	15
	Thalassionema	703	40
	Thalassiosira	13050	184
Date	**Genus**	**Average (Cells/L)**	**SE**
3/25/2013	*Asterionellopsis*	471	66
	Chaetoceros	25357	785
	Cylindrotheca	471	15
	Ditylum	46	0
	Leptocylindrus	15	15
	Licmorpha	15	15
	Paralia	61	40
	Protoperidinium	61	15
	Pseudo-nitzschia Lg. Cell type	15	19
	Pseudo-nitzschia Sm. Cell type	106	40
	Scripsiella	91	26
	Stephanopyxis	61	40
	Thalassionema	760	106
	Thalassiosira	6214	132
Date	**Genus**	**Average (Cells/L)**	**SE**
4/9/2013	*Asterionellopsis*	71	0
	Cerataulina	24	24
	Chaetoceros	47286	1256
	Cylindrotheca	595	63
	Detonula	48	48
	Leptocylindrus	107	24
	Licmorpha	24	24
	Odontella	24	19
	Navicula	71	71

Paralia	48	24
Pleurosigma	405	48
Pseudo-nitzschia Lg. Cell type	214	41
Pseudo-nitzschia Sm. Cell type	143	41
Scripsiella	71	41
Thalassionema	167	48
Thalassiosira	952	104

APPENDIX 2. QUARTERMASTER HARBOR PHYTOPLANKTON COMPOSITION

Date	Genus	Average (Cells/L)	SE
4/9/2012	*Actinoptychus*	30	17
	Chaetoceros	554	54
	Cylindrotheca	40	10
	Detonula	29413	268
	Eucampia	60	17
	Leptocylindrus	20	10
	Odontella	239	35
	Pleurosigma	40	10
	Pseudo-nitzschia Lg. cell type	459	53
	Pseudo-nitzschia Sm. Cell type	70	26
	Skeletonema	90	17
	Thalassionema	190	26
	Thalassiosira	1098	70
	Tropodeneis	10	10
Date	**Genus**	**Average (Cells/L)**	**SE**
5/28/2012	*Actinoptychus*	13	13
	Chaetoceros	8546	350
	Cylindrotheca	70	6
	Ditylum	13	3
	Leptocyclindrus	19	5
	Licmorpha	16	3
	Noctiluca	25	3
	Odontella	10	5
	Pleurosigma	3	3
	Protoceratium	3	3
	Protoperidinium	44	8
	Pseudo-nitzschia Lg. cell type	67	5
	Rhizosolenia	22	22
	Scripsiella	25	11
	Skeletonema	10	5
	Thalassionema	6	3
	Thalassiosira	10	0
Date	**Genus**	**Average (Cells/L)**	**SE**
6/12/2012	*Actinoptychus*	7	7

	Ceratium	2	2
	Chaetoceros	128	11
	Coscinodiscus	15	4
	Cylindrotheca	82	6
	Leptocylindrus	7	4
	Noctiluca	7	0
	Pleurosigma	5	2
	Protoperidinium	34	2
	Pseudo-nitzschia Lg. cell type	5	2
	Rhizosolenia	15	0
	Scripsiella	7	7
	Skeletonema	5	2
	Thalassiosira	44	7
Date	**Genus**	**Average (Cells/L)**	**SE**
6/18/2012	*Akashiwo sanguinea*	8	4
	Astrionellopsis	4	4
	Ceratium	333	54
	Chaetoceros	36	0
	Coscinodiscus	20	14
	Ditylum	4	4
	Eucampia	8	4
	Oxyphysis	8	4
	Polykrikos	4	4
	Pleurosigma	4	4
	Prorocentrum	325	60
	Protoperidinium	91	28
	Scripsiella	56	21
	Skeletonema	24	12
	Stephanopyxis	4	4
	Thalassionema	4	4
	Thalassiosira	36	12
Date	**Genus**	**Average (Cells/L)**	**SE**
6/23/2012	*Actinoptychus*	96	12
	Alexandrium	45	10
	Amylax	3	3
	Ceratium	5	3
	Chaetoceros	66	3
	Coscinodiscus	90	15
	Cylindrotheca	578	74
	Dinophysis	40	5

	Heterocapsa	5	5
	Kofoidinium	3	3
	Noctiluca	13	3
	Pleurosigma	19	7
	Protoperidinium	34	3
	Pseudo-nitzschia Lg. cell type	32	9
	Rhizosolenia	122	11
	Scripsiella	133	10
	Skeletonema	3	3
	Stephanopyxis	3	3
	Thalassiosira	157	12
	Tropidoneis	3	3
Date	**Genus**	**Average (Cells/L)**	**SE**
6/29/2012	*Alexandrium*	780	27
	Amylax	20	7
	Asteromphalus	3	3
	Ceratium	8	0
	Chaetoceros	17	8
	Coscinodiscus	112	7
	Cylindrotheca	20	7
	Dinophysis	173	20
	Ditylum	6	6
	Kofoidinium	3	3
	Licmorpha	8	5
	Noctiluca	14	7
	Oxyphysis	6	3
	Pleurosigma	14	6
	Polykrikos	3	3
	Prorocentrum	59	13
	Protoperidinium	67	5
	Rhizosolenia	6	6
	Scripsiella	235	30
	Skeletonema	3	3
	Thalassiosira	22	15
	Tropedoneis	*3*	*3*
Date	**Genus**	**Average (Cells/L)**	**SE**
7/15/2012	*Alexandrium*	138	11
	Ceratium	178	27
	Coscinodiscus	6	3
	Dinophysis	184	11

	Gonyaulax	21	3
	Noctiluca	89	13
	Pleurosigma	28	5
	Prorocentrum	95	11
	Protoperidinium	364	22
	Pseudo-nitzschia Lg. Cell type	77	6
	Thalassiosira	31	8
Date	**Genus**	**Average (Cells/L)**	**SE**
8/6/2012	*Akashiwo sanguinea*	9	5
	Asterionellopsis	5	5
	Ceratium	381	61
	Chaetoceros	41	0
	Cosdinodiscus	23	16
	Cylindrotheca	14	8
	Dinophysis	5	5
	Ditylum	5	5
	Eucampia	9	5
	Oxyphysis	9	5
	Pleurosigma	5	5
	Polykrikos	5	5
	Prorocentrum	390	67
	Protoperidinium	109	28
	Scripsiella	63	24
	Skeletonema	50	32
	Stephanopyxis	5	5
	Thalassionema	5	5
	Thalassiosira	41	14
Date	**Genus**	**Average (Cells/L)**	**SE**
8/13/2012	*Akashiwo sanguinea*	10	10
	Alexandrium	63	36
	Ceratium	324	31
	Chaetoceros	48	10
	Cosdinodiscus	13	8
	Cylindrotheca	32	13
	Dinophysis	44	6
	Pleurosigma	51	18
	Prorocentrum	124	5
	Protoperidinium	187	49
	Pseudo-nitzschia Sm. Cell type	3	3

	Rhizosolenia	48	10
	Scripsiella	156	11
	Skeletonema	95	29
	Thalassionema	13	8
	Thalassiosira	25	3
Date	**Genus**	**Average (Cells/L)**	**SE**
8/20/2012	*Alexandrium*	95	15
	Asteromphalus	3	3
	Ceratium	441	15
	Chaetoceros	70	16
	Cosdinodiscus	17	8
	Dinophysis	20	7
	Leptocylindrus	173	20
	Lingulodinium	11	3
	Oxyphysis	28	16
	Pleurosigma	6	6
	Prorocentrum	148	16
	Protoperidinium	70	16
	Rhizosolenia	120	12
	Scripsiella	134	13
	Skeletonema	14	6
	Stephanopyxis	3	3
	Thalassiosira	6	3
Date	**Genus**	**Average (Cells/L)**	**SE**
8/28/2012	*Akashiwo sanguinea*	91	45
	Alexandrium	121	20
	Amylax	23	4
	Asteromphalus	13	5
	Ceratium	451	51
	Chaetoceros	53	8
	Cylindrotheca	3	3
	Dinophysis	106	12
	Leptocylindrus	161	48
	Oxyphysis	60	8
	Pleurosigma	10	5
	Prorocentrum	93	13
	Protoperidinium	88	5
	Pseudo-nitzschia Sm. Cell type	10	3
	Rhizosolenia	171	44
	Scripsiella	53	4

	Skeletonema	23	13
	Thalassionema	3	3
	Thalassiosira	18	9
Date	**Genus**	**Average (Cells/L)**	**SE**
9/3/2012	*Akashiwo sanguinea*	3	3
	Alexandrium	3001	71
	Asteromphalus	96	9
	Ceratium	232	13
	Chaetoceros	83	17
	Coscinodiscus	17	7
	Dinophysis	25	6
	Ditylum	13	7
	Eucampia	3	3
	Gonyaulax	3	3
	Oxyphysis	60	34
	Pleurosigma	25	6
	Polykrikos	7	7
	Prorocentrum	96	23
	Protoperidinium	129	11
	Pseudo-nitzschia Lg. Cell type	20	6
	Rhizosolenia	50	21
	Scripsiella	10	6
	Skeletonema	17	9
	Thalassionema	30	6
	Thalassiosira	20	6
	Silicoflagellate	3	3
Date	**Genus**	**Average (Cells/L)**	**SE**
9/10/2012	*Akashiwo sanguinea*	558	15
	Alexandrium	188	20
	Asteromphalus	11	11
	Ceratium	817	28
	Chaetoceros	618	27
	Coscinodiscus	28	4
	Cylindrotheca	4	4
	Dinophysis	53	6
	Ditylum	75	11
	Kofoidinium	4	4
	Leptocylindrus	36	7
	Oxyphysis	128	9
	Pleurosigma	85	12

	Prorocentrum	25	4
	Protoperidinium	85	18
	Pseudo-nitzschia Lg. cell type	476	22
	Pseudonitzschia Sm. Cell type	39	9
	Rhizosolenia	21	6
	Scripsiella	36	9
	Skeletonema	96	16
	Thalassionema	135	19
	Thalassiosira	274	13
Date	**Genus**	**Average (Cells/L)**	**SE**
9/17/2012	*Akashiwo sanguinea*	109	26
	Alexandrium	21	6
	Ceratium	224	13
	Chaetoceros	194	13
	Coscinodiscus	54	5
	Cylindrotheca	6	6
	Dinophysis	30	6
	Ditylum	6	3
	Eucampia	6	6
	Kofoidinium	3	3
	Leptocylindrus	18	0
	Licmorpha	3	3
	Odontella	3	3
	Oxyphysis	51	18
	Pleurosigma	24	3
	Prorocentrum	15	11
	Protoperidinium	24	6
	Pseudo-nitzschia Lg. Cell type	160	26
	Pseudo-nitzschia Sm. Cell type	9	5
	Rhizosolenia	15	6
	Scripsiella	9	0
	Skeletonema	21	11
	Thalassionema	6	3
	Thalassiosira	54	18
Date	**Genus**	**Average (Cells/L)**	**SE**
9/24/2012	*Akashiwo sanguinea*	563	81
	Alexandrium	11	7
	Asteromphalus	7	7

	Ceratium	441	32
	Chaetoceros	185	32
	Coscinodiscus	19	7
	Cylindrotheca	4	4
	Dinophysis	22	6
	Eucampia	7	4
	Guinardia	7	4
	Kofoidinium	4	4
	Noctiluca	4	4
	Oxyphysis	44	22
	Pleurosigma	15	10
	Prorocentrum	33	6
	Protoperidinium	19	4
	Pseudo-nitzschia Lg. Cell type	67	19
	Scripsiella	19	13
	Skeletonema	22	11
	Thalassionema	7	7
	Thalassiosira	7	7
Date	**Genus**	**Average (Cells/L)**	**SE**
10/1/2012	*Akashiwo sanguinea*	1325	133
	Ceratium	385	69
	Chaetoceros	179	36
	Coscinodiscus	4	4
	Dinophysis	26	8
	Ditylum	4	4
	Eucampia	4	4
	Guinardia	4	4
	Kofoidinium	9	4
	Leptocylindrus	22	4
	Oxyphysis	4	4
	Pleurosigma	22	9
	Prorocentrum	13	8
	Protoperidinium	13	8
	Pseudo-nitzschia Lg. Cell type	22	9
	Rhizosolenia	20	4
	Scripsiella	22	16
	Skeletonema	13	13
	Thalassionema	4	4
	Thalassiosira	26	15

Date	Genus	Average (Cells/L)	SE
10/8/2012	*Akashiwo sanguinea*	872	27
	Asteromphalus	6	3
	Ceratium	83	11
	Chaetoceros	46	5
	Coscinodiscus	6	3
	Cylindrotheca	3	3
	Detonula	3	3
	Dinophysis	6	3
	Ditylum	6	3
	Eucampia	9	0
	Kofoidinium	3	3
	Leptocylindrus	6	3
	Oxyphysis	6	6
	Pleurosigma	3	3
	Prorocentrum gracile	15	8
	Prorocentrum reticulatum	3	3
	Protoperidinium	14	8
	Rhizosolenia	3	3
	Scripsiella	9	5
	Skeletonema	3	3
	Stephanopyxis	3	3
	Thalassiosira	34	13
Date	**Genus**	**Average (Cells/L)**	**SE**
10/15/2012	*Akashiwo sanguinea*	6946	638
	Ceratium	60	4
	Chaetoceros	72	15
	Detonula	4	4
	Dinophysis	8	4
	Ditylum	4	4
	Eucampia	8	8
	Kofoidinium	4	4
	Oxyphysis	11	7
	Pleurosigma	4	4
	Prorocentrum	45	17
	Protoperidinium	11	11
	Scripsiella	8	8
	Skeletonema	53	15
	Thalassionema	6	4
	Thalassiosira	57	17
	Silicoflagellate	4	4

Date	Genus	Average (Cells/L)	SE
10/22/2012	*Akashiwo sanguinea*	2119	173
	Ceratium	25	7
	Chaetoceros	28	9
	Detonula	8	4
	Dinophysis	3	3
	Ditylum	5	5
	Eucampia	3	3
	Kofoidinium	5	3
	Leptocylindrus	3	3
	Oxyphysis	3	3
	Prorocentrum	3	3
	Protoperidinium	5	3
	Pseudo-nitzschia Lg. Cell type	3	3
	Scripsiella	5	3
	Skeletonema	5	3
	Thalassiosira	18	3
Date	**Genus**	**Average (Cells/L)**	**SE**
10/29/2012	*Akashiwo sanguinea*	1283	68
	Ceratium	14	3
	Chaetoceros	19	11
	Coscinodiscus	6	3
	Cylindrotheca	3	3
	Detonula	3	3
	Kofoidinium	3	3
	Lauderia	3	3
	Leptocylindrus	8	5
	Protoperidinium	6	3
	Thalassiosira	33	8
Date	**Genus**	**Average (Cells/L)**	**SE**
11/5/2012	*Akashiwo sanguinea*	2158	195
	Ceratium	11	3
	Chaetoceros	5	3
	Dinophysis	5	3
	Gonyaulax	3	3
	Kofoidinium	3	3
	Leptocylindrus	3	3
	Pleurosigma	3	3
	Prorocentrum	16	12
	Scripsiella	8	5

	Thalassiosira	16	5
	Silicoflagellate	3	3
Date	**Genus**	**Average (Cells/L)**	**SE**
11/18/2012	*Akashiwo sanguinea*	8	0
	Pleurosigma	3	16
Date	**Genus**	**Average (Cells/L)**	**SE**
11/26/2012	*Akashiwo sanguinea*	77	23
	Ceratium	6	3
	Chaetoceros	6	3
	Dinophysis	9	5
	Pseudo-nitzschia Lg. Cell type	3	3
	Stephanopyxis	3	3
	Thalassiosira	6	6
Date	**Genus**	**Average (Cells/L)**	**SE**
12/3/2012	*Akashiwo sanguinea*	6	3
	Chaetoceros	9	9
	Coscinodiscus	15	3
	Cylindrotheca	6	3
	Leptocylindrus	9	5
	Pseudo-nitzschia Sm. Cell type	6	6
	Rhizosolenia	18	10
	Skeletonema	3	3
	Thalassiosira	9	5
Date	**Genus**	**Average (Cells/L)**	**SE**
12/10/2012	*Ceratium*	3	3
	Chaetoceros	18	5
	Cylindrotheca	9	5
	Silicoflagellate	3	3
Date	**Genus**	**Average (Cells/L)**	**SE**
12/17/2012	*Asteromphalus*	6	3
	Chaetoceros	34	11
	Coscinodiscus	12	3
	Dinophysis	3	3
	Ditylum	3	3
	Melosira	6	3
	Paralia	6	3
	Pleurosigma	3	3
	Pseudo-nitzschia Lg. Cell type	3	3

	Rhizosolenia	6	6
	Scripsiella	6	3
	Skeletonema	6	6
	Thalassiosira	18	5
	Silicoflagellate	6	3
Date	**Genus**	**Average (Cells/L)**	**SE**
1/2/2013	*Asteromphalus*	3	3
	Ceratium	3	3
	Chaetoceros	99	21
	Cylindrotheca	12	3
	Leptocylindrus	3	3
	Paralia	12	8
	Pleurosigma	3	3
	Pseudo-nitzschia Sm. Cell type	6	3
	Skeletonema	174	11
	Thalassionema	9	5
	Thalassiosira	93	17
	Silicoflagellate	3	3
Date	**Genus**	**Average (Cells/L)**	**SE**
1/14/2013	*Chaetoceros*	350	43
	Coscinodiscus	3	3
	Cylindrotheca	28	5
	Detonula	9	5
	Ditylum	3	3
	Leptocylindrus	3	3
	Protoperidinium	3	3
	Pseudo-nitzschia Lg. cell type	15	6
	Skeletonema	93	19
	Thalassiosira	456	28
Date	**Genus**	**Average (Cells/L)**	**SE**
1/29/2013	*Asteromphalus*	3	3
	Chaetoceros	161	26
	Coscinodiscus	3	3
	Cylindrotheca	26	6
	Detonula	10	0
	Dinophysis	6	3
	Leptocylindrus	10	6
	Navicula	3	3
	Paralia	3	3

	Pleurosigma	3	3
	Protoperidinium	3	3
	Skeletonema	26	3
	Thalassionema	29	3
	Thalassiosira	74	8
	Silicoflagellate	3	3
Date	**Genus**	**Average (Cells/L)**	**SE**
2/12/2013	*Asteromphalus*	3	3
	Chaetoceros	190	12
	Cylindrotheca	194	53
	Detonula	3	3
	Leptocylindrus	7	7
	Paralia	7	7
	Pleurosigma	7	7
	Pseudo-nitzschia Sm. cell type	3	3
	Skeletonema	61	10
	Thalassionema	20	6
	Thalassiosira	48	15
Date	**Genus**	**Average (Cells/L)**	**SE**
2/20/2013	*Asteromphalus*	7	7
	Chaetoceros	598	96
	Cylindrotheca	504	90
	Detonula	26	9
	Eucampia	7	7
	Leptocylindrus	13	9
	Paralia	3	3
	Pleurosigma	10	6
	Pseudo-nitzschia Lg. cell type	7	7
	Skeletonema	42	23
	Thalassionema	13	7
	Thalassiosira	55	14
	Silicoflagellate	3	3
Date	**Genus**	**Average (Cells/L)**	**SE**
3/12/2013	*Asteromphalus*	6	6
	Chaetoceros	184	10
	Coscinodiscus	42	28
	Cylindrotheca	1028	50
	Dinophysis	3	3
	Eucampia	78	19

	Leptocylindrus	6	3
	Odontella	13	6
	Pleurosigma	32	9
	Protoperidinium	3	3
	Pseudo-nitzschia Lg. cell type	32	6
	Pseudo-nitzschia Sm. cell type	6	3
	Rhizosolenia	26	12
	Scripsiella	13	13
	Skeletonema	13	13
	Stephanopyxis	42	12
	Thalassionema	48	15
	Thalassiosira	837	157
	Tropedoneis	3	3
Date	**Genus**	**Average (Cells/L)**	**SE**
4/1/2013	*Chaetoceros*	1874	55
	Coscinodiscus	3	3
	Cylindrotheca	174	44
	Ditylum	21	3
	Eucampia	284	6
	Leptocylindrus	9	0
	Noctiluca	3	3
	Odontella	75	6
	Pleurosigma	6	3
	Protoperidinium	6	3
	Pseudo-nitzschia Lg. cell type	317	61
	Pseudo-nitzschia Sm. cell type	15	6
	Rhizosolenia	9	0
	Scripsiella	3	3
	Skeletonema	21	8
	Stephanopyxis	150	11
	Thalassionema	48	11
	Thalassiosira	1344	67
Date	**Genus**	**Average (Cells/L)**	**SE**
4/8/2013	*Chaetoceros*	2637	107
	Cylindrotheca	120	17
	Ditylum	13	3
	Eucampia	123	28
	Lauderia	10	0

Leptocylindrus	93	12
Lingulodinium	3	3
Noctiluca	3	3
Odontella	50	6
Pleurosigma	17	3
Prorocentrum	3	3
Protperidinium	13	3
Pseudo-nitzschia Lg. cell type	47	9
Pseudo-nitzschia Sm. cell type	7	3
Rhizosolenia	27	9
Scripsiella	53	18
Skeletonema	502	103
Stephanopyxis	103	24
Thalassionema	13	3
Thalassiosira	279	15

APPENDIX 3. PENN COVE PHYTOPLANKTON COMPOSITION

Date	Genus	Average (Cells/L)	SE
6/4/2012	*Chaetoceros*	251	56
	Detonula	236	22
	Leptocylindrus	82	6
	Noctiluca	257	16
	Protoperidinium	15	3
	Pseudo-nitzschia Lg. Cell type	82	38
	Pseudo-nitzschia Sm. Cell type	20	3
	Rhizosolenia	1131	99
	Skeletonema	76	6
	Thalassionema	3	3
	Thalassiosira	262	4
	Silicoflagellate	6	3
Date	**Genus**	**Average (Cells/L)**	**SE**
7/16/2012	*Chaetoceros*	5247	116
	Cylindrotheca	110	12
	Detonula	873	29
	Ditylum	520	53
	Leptocylindrus	207	18
	Pseudo-nitzschia Lg. Cell type	224587	1883
	Rhizosolenia	367	7
	Skeletonema	10820	164
	Thalassiosira	780	35
Date	**Genus**	**Average (Cells/L)**	**SE**
8/20/2012	*Alexandrium*	53	10
	Astrerionellopsis	53	0
	Ceratium	24	6
	Chaetoceros	5194	68
	Detonula	41	21
	Dinophysis	32	15
	Ditylum	330	16
	Eucampia	77	12
	Leptocylindrus	47	12
	Navicula	12	12
	Noctiluca	118	16
	Pleurosigma	6	6
	Protoperidinium	88	10

	Pseudo-nitzschia Lg. Cell type	707	10
	Rhizosolenia	200	16
	Skeletonema	2007	136
	Thalassionema	35	10
	Thalassiosira	1733	84
Date	**Genus**	**Average (Cells/L)**	**SE**
9/3/2012	*Actinoptychus*	54	31
	Asterionellopsis	43	13
	Chaetoceros	1473	23
	Coscinodiscus	6	4
	Cylindrotheca	34	17
	Dinophysis	4	2
	Ditylum	11	6
	Leptocylindrus	40	2
	Noctiluca	117	33
	Pleurosigma	23	6
	Proboscia	32	6
	Protoperidinium	43	2
	Pseudo-nitzshcia Sm. Cell type	4	4
	Rhizosolenia	6	4
	Skeletonema	16	2
	Stephanopyxis	6	4
	Thalassiosira	104	21
Date	**Genus**	**Average (Cells/L)**	**SE**
9/17/2012	*Actinoptychus*	7	7
	Asterionellopsis	102	5
	Asteromphalus	2	2
	Chaetoceros	136	14
	Cyindrotheca	13	2
	Detonula	7	5
	Ditylum	65	3
	Eucampia	24	4
	Gymnodinium	56	5
	Heterocapsa	2	2
	Kofoidinium	2	2
	Leptocylindrus	13	5
	Pleurosigma	7	4
	Protoperidinium	116	7
	Pseudo-nitzshcia Lg. Cell	16	0

	type		
	Rhizosolenia	80	7
	Scripsiella	9	2
	Skeletonema	34	2
	Stephanopyxis	2	2
	Thalassionema	13	2
	Thalassiosira	38	3
Date	**Genus**	**Average (Cells/L)**	**SE**
10/24/2012	*Asteromphalus*	5	3
	Ceratium	2	2
	Chaetoceros	5	3
	Cylindrotheca	2	2
	Diylum	8	3
	Paralia	2	2
	Pleurosigma	2	2
	Protoperidinium	12	2
	Scripsiella	77	10
	Skeletonema	12	2
	Thalassiosira	6	2
	Silicoflagellate	11	4
Date	**Genus**	**Average (Cells/L)**	**SE**
10/30/2012	*Ceratium*	5	5
	Chaetoceros	2	2
	Cylindrotheca	13	7
	Leptocylindrus	5	3
	Paralia	2	2
	Pseudo-nitzschia Lg. Cell type	5	3
	Scripsiella	7	2
	Skeletonema	8	3
	Thalassiosira	12	2
	Silicoflagellate	65	13
Date	**Genus**	**Average (Cells/L)**	**SE**
11/5/2012	*Actinoptychus*	2	2
	Alexandrium	40	7
	Asterionellopsis	1	1
	Chaetoceros	4	0
	Cylindrotheca	4	2
	Ditylum	2	1
	Protoperidinium	4	4
	Scripsiella	135	16

	Skeletonema	6	1
	Thalassiosira	6	1
	Silicoflagellate	37	4
Date	**Genus**	**Average (Cells/L)**	**SE**
11/26/2012	*Alexandrium*	1	1
	Asterionellopsis	1	1
	Cerataulina	1	1
	Ceratium	1	1
	Chaetoceros	27	5
	Cylindrotheca	3	2
	Dinophysis	2	2
	Ditylum	7	1
	Heterocapsa	1	1
	Leptocylindrus	13	3
	Scripsiella	3	2
	Skeletonema	11	4
	Thalassionema	1	1
	Thalassiosira	7	2
	Silicoflagellate	16	7
Date	**Genus**	**Average (Cells/L)**	**SE**
12/10/2012	*Cerataulina*	4	2
	Chaetoceros	63	2
	Coscinodiscus	1	1
	Cylindrotheca	4	2
	Ditylum	2	1
	Leptocylindrus	12	2
	Meringosphaera	1	1
	Pleurosigma	1	1
	Skeletonema	6	3
	Thalassionema	1	1
	Thalassiosira	5	3
	Silicoflagellate	15	2
Date	**Genus**	**Average (Cells/L)**	**SE**
12/24/2012	*Actinoptychus*	7	5
	Cerataulina	8	0
	Chaetoceros	135	6
	Cylindrotheca	4	2
	Dinophysis	7	1
	Ditylum	7	1
	Leptocylindrus	14	1
	Pleurosigma	7	3

	Protoperidinium	1	1
	Scripsiella	4	0
	Skeletonema	5	3
	Thalassionema	8	4
	Thalassiosira	15	6
	Silicoflagellate	65	19
Date	**Genus**	**Average (Cells/L)**	**SE**
1/11/2013	*Cerataulina*	18	6
	Chaetoceros	67	6
	Cylindrotheca	2	1
	Dinophysis	5	2
	Leptocylindrus	4	2
	Navicula	1	1
	Odontella	1	1
	Paralia	1	1
	Pleurosigma	1	1
	Skeletonema	7	6
	Thalassionema	1	1
	Thalassiosira	15	2
	Silicoflagellate	18	4
Date	**Genus**	**Average (Cells/L)**	**SE**
1/28/2013	*Cerataulina*	10	5
	Chaetoceros	942	37
	Cylindrotheca	20	8
	Dinophysis	2	2
	Ditylum	48	12
	Leptocylindrus	20	5
	Pleurosigma	2	2
	Protoperidinium	3	3
	Pseudo-nitzschia Lg. Cell type	2	2
	Rhizosolenia	5	3
	Skeletonema	34	6
	Thalassionema	27	15
	Thalassiosira	199	19
	Silicoflagellate	24	2
Date	**Genus**	**Average (Cells/L)**	**SE**
2/18/2013	*Cerataulina*	17	2
	Chaetoceros	52	8
	Cylindrotheca	37	9
	Detonula	3	3

	Dinophysis	2	2
	Ditylum	10	3
	Leptocylindrus	3	3
	Pleurosigma	5	0
	Protoperidinium	6	2
	Skeletonema	6	2
	Thalassionema	79	6
	Thalassiosira	2375	89
	Silicoflagellate	43	14
Date	**Genus**	**Average (Cells/L)**	**SE**
3/5/2013	*Coscinodiscus*	1	1
	Cylindrotheca	6	1
	Scripsiella	1	1
	Thalassiosira	13	4
	Silicoflagellate	2	2
Date	**Genus**	**Average (Cells/L)**	**SE**
3/11/2013	*Cerataulina*	4	4
	Cylindrotheca	11	5
	Dinophysis	1	1
	Odontella	1	1
	Protoperidinium	7	1
	Scripsiella	10	4
	Thalassiosira	27	4
	Silicoflagellate	22	6
Date	**Genus**	**Average (Cells/L)**	**SE**
3/18/2013	*Chaetoceros*	4	2
	Cylindrotheca	140	10
	Ditylum	1	1
	Navicula	2	1
	Pleurosigma	3	0
	Skeletonema	6	2
	Thalassionema	18	1
	Thalassiosira	77	5
	Silicoflagellate	2	1
Date	**Genus**	**Average (Cells/L)**	**SE**
3/25/2013	*Cerataulina*	12	2
	Chaetoceros	56	8
	Cylindrotheca	9	3
	Heterocapsa	5	5
	Leptocylindrus	40	5
	Licmorpha	2	2

	Navicula	2	2
	Skeletonema	2	2
	Thalassionema	7	5
	Thalassiosira	360	36
	Silicoflagellate	10	0
Date	**Genus**	**Average (Cells/L)**	**SE**
4/8/2013	*Chaetoceros*	4818	88
	Cylindrotheca	23	7
	Heterocapsa	4	5
	Leptocylindrus	27	4
	Protoperidinium	84	8
	Skeletonema	145	21
	Stephanopyxis	11	7
	Thalassionema	214	17
	Thalassiosira	2801	43