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# NOAA Phytoplankton Monitoring Program: Understanding Ecosystem Conditions for Phytoplankton in Kachemak Bay

by

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## DEDICATION

I want to dedicate my thesis work to my family and friends who have stuck by and supported me no matter what struggles I have faced. I am especially thankful to my wonderful, loving parents, Karen and Peter Rosenberg, who always encourage me to do my best and counsel me when I am unsure of myself. They are my guiding light and for them I strive to do and be better every day because their pride and belief in me at the end of the day makes it all worth it.

I also want to dedicate my thesis to my friends, especially Jess Schiller, Patricia Walchessen, and Ally Madden, my best friends, who have put up with me no matter how stressed I am, and always lend amazing helping hands no matter the task. Without their help and imagination, I would not be so successful nor so proud of this piece.

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**ABSTRACT**

My research focuses on the two most common and harmful phytoplankton genera in Kachemak Bay, Alaska, *Chaetoceros spp.* and *Pseudo-nitzschia spp*. *Chaetoceros spp.* can be deadly to fish in high concentrations because they clog and destroy fish's gills. *Pseudo-nitzschia spp.* are more dangerous because they produce a deadly toxin, domoic acid, which causes amnesic shellfish poisoning to mammals. I compared these genera to environmental variables to see which parameters affected them the most. The environmental parameters that I focused on were photosynthetically active radiation (PAR), water temperature, salinity, and nutrients because they have been found to influence the presence of harmful phytoplankton species. I found that PAR, orthophosphate, and nitrite+nitrate were significantly correlated with the presence of *Chaetoceros*, which was also correlated with water temperature in certain instances. *Pseudo-nitzschia spp.* are significantly correlated to water temperature, PAR, and nitrite+nitrate. Over time, we have seen more and more *Pseudo-nitzschia* in the bay, possibly caused by temperature change regimes. Increasing temperatures from climate change will lead to changes in species dynamics and competition, which we found, and is expected to lead to more harmful algal blooms, which we saw.

# INTRODUCTION

## *1.1 Phytoplankton Dangers*

My thesis focuses on the distribution of two harmful phytoplankton genera and the environmental variables that might influence their prevalence. Phytoplankton of all species are critical primary producers at the base of the marine food chain and are the source of half of the carbon dioxide fixed and oxygen produced on the planet each year (Backer *et al.,* 2015; Moroney and Ynalvez, 2009). For instance, one of the genera I focused on, *Chaetoceros* belongs to the most species rich phytoplankton genera and contributes to 20-25% of the primary production in the oceans (Suto, 2005). As primary producers, phytoplankton growth relies on photosynthetically active radiation (PAR), temperature, salinity, and nutrients, which were the main parameters I focused on (Macedo and Duarte, 2006; Zonnevald, 1998). Scientists need to understand these relationships in order to understand how the changing climate can affect the marine food web. The focus of my work was on species that can have adverse effects, as some species of phytoplankton can be some of the deadliest killers in the ocean.

Due to their position at the base of the food chain, phytoplankton fuel almost the entire food web of the ocean, but they are also the source of dangerous compounds that can accumulate up through the levels of the food web (Anderson *et al.,* 2012). Phytoplankton blooms-- increased algal populations due to rapid increases in growth and cell accumulation under optimal nutrient and sunlight

conditions-- provide an all you can eat buffet for zooplankton that are in turn food for benthic filter-feeders such as oysters, mussels, scallops, and clams, which are all ingested by fish and/or mammals (McLean and Sinclair, 2012). However, some blooms are designated as harmful algal blooms (HAB), because some phytoplankton species can produce some of the most potent natural toxins that have ever been found (Anderson *et al.,* 2012; Backer *et al.,* 2015). During most of these blooms, rapid increases in phytoplankton concentrations lead to the release of toxic/noxious chemicals, but HABs can also occur when there are low numbers of cells in particularly toxic species (Anderson *et al.,* 2012). In these cases, it can be difficult to track what is causing toxin-associated deaths (Anderson *et al.,* 2012). For instance, as little as 500 micrograms of saxitoxin, created by *Alexandrium spp.*, can be fatal to humans and with such small concentrations it is hard to observe and monitor for *Alexandrium* (Hallegraeff, 1993).

### *1.1.1 Example of Harmful Phytoplankton Species*

There are a number of harmful effects that phytoplankton can cause to fish and mammals. The first example being mechanical damage such as that done by *Chaetoceros spp.* to some fish species, first reported in 1961 (Bell, 1961). *Chaetoceros spp.,* a genus that I focused on in my thesis, are sporulating diatoms that are not toxic to mammals, but can be very harmful to fish and some invertebrates (Montresor *et al.,* 2013). *Chaetoceros spp.* were found mostly in near-shore upwelling and coastal areas around the world, in locations ranging from the North Atlantic to the North Pacific as well as the Mediterranean and

Antarctic (Daniels *et al.,* 2015; Ferrario, Sar, and Vernet, 1998; Hondolero *et al.*, 2014; Suto, 2015). When *Chaetoceros* is highly concentrated, they forms spiny chains that allow them to avoid predation as well as increase their surface area, and these chains catch on fish gills when the fish attempt to eat them or swim through areas where they are highly concentrated (Bell, 1961). This causes various problems to fish, including capillary hemorrhage, lack of proper gas exchange, suffocation from too much mucus produced in response, and secondary infections (Hallegraeff, 1993). Fish that are part of aquaculture or are in cages are especially vulnerable because they are in limited spaces (Hallegraeff, 1993). For instance, *Chaetoceros spp.* have been implicated in the deaths of Lingcod, Sockeye, Coho, Chinook, and pink salmon in culture (Hallegraeff, 1993).

Other species of phytoplankton produce physical toxins that can affect a wide range of animals such as humans, whales, porpoises, manatees, seabirds, and fish, which can become ill or die due to either direct ingestion of toxins or, more commonly, via the toxin accumulating through the food web by the ingestion of zooplankton or fish (Anderson *et al.,* 2012). Examples of this are several species of *Dinophysis* that cause Diarrhetic Shellfish Poisoning (DSP), which is attributed to okadaic acid and/or dinophysis toxin (Hallegraeff, 1993). DSP is found in the oceans surrounding Japan, Europe, Chile, Thailand, Nova Scotia, Australia, and New Zealand (Hallegraeff, 1993). DSP can affect humans who ingest shellfish that have accumulated the toxin through filter feeding (Hallegraeff, 1993). DSP causes symptoms of diarrhea, nausea, vomiting, abdominal pain, and tumor

formation (Hallegraeff, 1993). It creates these symptoms by inhibiting protein phosphatases in the digestive system, which can lead to chronic problems such as stomach tumors, but it has never been linked to human fatalities (Yasumoto and Murata, 1993).

Another example is Ciguatera Poisoning, caused by the species *Gambierdiscus toxicus*, which produces ciguatoxin and other toxins such as maitotoxins (Lewis, 2001). Ciguatera Poisoning is common in lower latitude areas of the Pacific Ocean, Western Indian Ocean, and Caribbean Sea, and is caused particularly by ingesting tropical and subtropical fish that have accumulated the toxins through the food chain (Lewis, 2001). The toxins are sodium channel activators that cause a loss of polarization in neurons, which leads to a general malfunctioning of the cells (Lewis, 2001). The initial symptoms of poisoning are diarrhea, abdominal pain, nausea, and vomiting (Hallegraeff, 1993). As the condition progresses, more severe symptoms can include numbness and tingling of hands and feet, difficulty balancing, low heart rate and blood pressure, and/or death through respiratory failure (Hallegraeff, 1993).

*Alexandrium spp.* release a neurotoxin that can have a significant impact at lower densities (Anderson *et al.,* 2012). These species along with some *Gymnodium spp.* and *Pyrodinium spp.* cause Paralytic Shellfish Poisoning (PSP) (Hallegraeff, 1993). PSP can be found around the world and is present in every ocean (Figure 1). There are several toxins that can cause PSP one such toxin, saxitoxin, created by *Alexandrium spp.* blocks depolarization of neurons causing

complete muscle relaxation that leads to paralysis, which can cause death if it affects the muscles that control breathing (McLean and Sinclair, 2012). PSP is characterized by the sensation of numbness along the body that leads to muscular paralysis, choking, and breathing difficulty that can result in death (McLean and Sinclair, 2012).

Lastly, the second genus that I will be focusing on in my thesis is, *Pseudo-nitzschia,* which produces domoic acid, a toxin that bioaccumulates and causes Amnesic Shellfish Poisoning (ASP) (Yoshida *et al.,* 2002). *Pseudo-nitzschia spp.* are found along the west coast of the United States and Canada as well as throughout the Atlantic and Gulf of Mexico (Anderson *et al.,* 2012; McLean and Sinclair, 2012). In some studies, domoic acid was the most commonly reported marine toxin found in all 11 states in the US that had been a part of this study (Backer *et al.,* 2015). Domoic acid is believed to be an iron or copper chelator, allowing it to keep iron soluble and available for metabolism, and act as a copper detoxifier (Rue and Bruland, 2001; Trainer *et al.,* 2012). Therefore, *Pseudo-nitzschia* only releases DA when in low iron/nutrient conditions (Trainer *et al.,* 2012). DA affects the nervous system of marine mammals, humans, and in some cases fishes, by binding to kainate receptors in the central nervous system interfering with neurochemical transmission in the vertebrate brain (Yoshida *et al*., 2002). This leads to a continuous depolarization of neurons that causes them to degenerate (Anderson, 1994). The symptoms of ASP are a result from lesions in the hippocampus and include amnesia, disorientation, and complete memory

loss (Hallegraeff, 1993). As little as 20 micrograms of domoic acid per gram of shellfish meat is considered dangerous for human consumption (Hallegraeff, 1993). The first recognized case of ASP was in 1987, in Prince Edward island, Canada where there were 105 cases of acute human poisonings that led to three deaths (Hallegraeff, 1993). In addition to these human deaths, there were 14 unexpected deaths of humpback whales that were equivalent to 50 years of natural mortality and were tentatively attributed to ASP (Geraci *et al.,* 1989).

### *1.1.2 HAB Prevalence*

Only 2% of phytoplankton species have the capacity to form harmful algal blooms, yet they have done so for thousands of years causing widespread effects (Anderson *et al.,* 2012). This is not only a modern problem as HABs are thought to be first reported in the Bible (Hallegraeff, 1993). Although the species responsible is unknown, scientists believe a HAB caused the Nile River to turn the color of blood, the death of all of the fish, and people to not be able to drink from the water (Hallegraeff, 1993). In current times, 77% of the harmful algal bloom reports from the Harmful Algal Bloom-Related Illness Surveillance System (HABISS) monitoring program are from freshwater systems like the Nile; whereas, 21% of the reports are from brackish systems, with mixed salt and freshwater (Backer *et al.*, 2015). Modern monitoring programs have found that upwards of 10% of all reported blooms end up leading to human associated illnesses, and 3% of the time these cases also lead to animal morbidity and mortality events (Backer *et al.,* 2015; McLean and Sinclair, 2012). Most human

cases of illness result particularly from seafood, such as finfish (89%) and shellfish (11%), producing gastrointestinal symptoms for 47% of cases, and neurological symptoms in 28% (Backer *et al.,* 2015). In the detailed domestic animal reports, 43% of patients had gastrointestinal symptoms, and 18% of victims had neurological symptoms that in 57% of the cases were fatal (Backer *et al.,* 2015).

While HABs are natural and have occurred throughout recorded history, they are increasing in frequency, intensity, and global distribution (Anderson *et al.,* 2012; McLean and Sinclair, 2012). For instance, up to 1970, PSP-producing dinoflagellates were only found in the Northern temperate waters of Europe, North America, and Japan (Hallegraeff, 1993). However, 20 years later, they have spread throughout all of the Southern Hemisphere and are now found in South Africa, Australia, India, Thailand, Brunei, Sabah, Philippines, and Papua New Guinea (Hallegraeff, 1993) (Figure 1). Furthermore, in areas where PSP was previously found, such as in Japan, they have spread from two common areas with a few blooms a year to ten areas with multiple large blooms a year (Hallegraeff, 1993). There are a variety of reasons that these blooms are increasing, and, unfortunately, they are only expected to continue to increase (Hallegraeff, 1993).

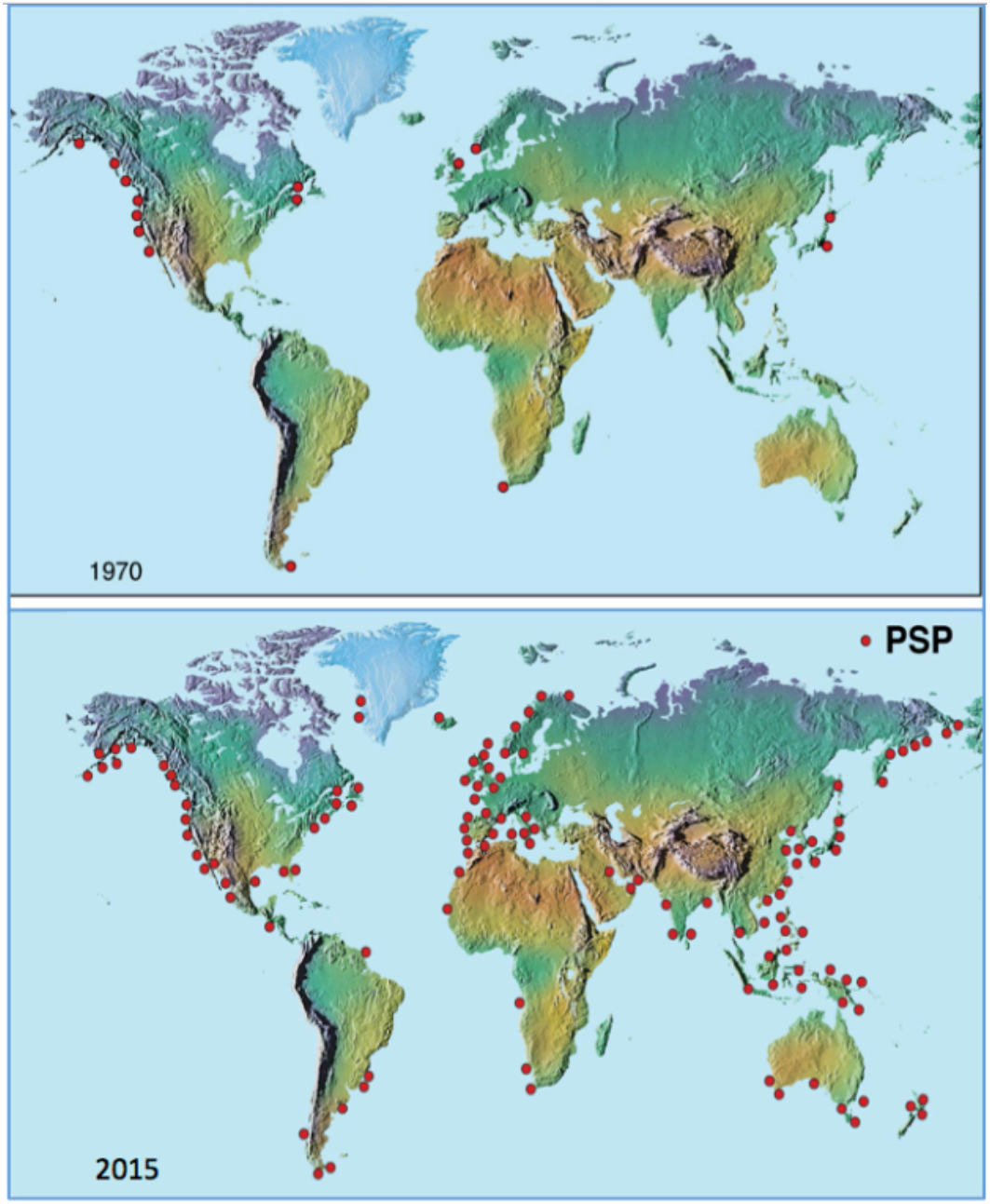


**Figure 1:** This map illustrates the distribution of Paralytic Shellfish Poisoning toxins, caused by *Alexandrium spp.*, via their detection in shellfish and/or fish. From (Woods Hole Oceanographic Institute, 2016)

*1.2 HAB Bloom Reasons for Increase*

In my thesis, I looked at *Chaetoceros,* the genus which causes mechanical damage to fish, and *Pseudo-nitzschia,* the genus that releases domoic acid causing amnesic shellfish poisoning (ASP), and like other HABs both are notably increasing in prevalence, worldwide. For all HAB species, there are many reasons for this increase in prevalence, including more scientific awareness of HAB species, increased aquaculture and nutrient stimulation of blooms, transport of dinoflagellate cysts from ships or other forms of transportation, and climate change (McLean and Sinclair, 2012; Tester and Litaker, 2014). However, in my study area there is a small sustainable human population, and in this area, *Chaetoceros* and *Pseudo-nitzschia* are believed to be more profoundly affected by environmental drivers than anthropogenic sources (Holderied, Brainard, and Ko, 2014). This indicates that the reasons for the apparent bloom increase in Alaska are mostly due to increased scientific/medical awareness, better monitoring of these species, and climate change.

For all HABs, scientific/medical awareness of illnesses caused by these blooms has greatly increased in the past few decades allowing for the correct identification of illnesses, which led to more effective monitoring of these species and blooms (Backer *et al.,* 2015). For instance, in 1987, there were around a hundred cases of human illness and death after consumption of mussels from the Atlantic Ocean (Anderson *et al.,* 2012). Domoic acid was identified as the cause of this event, and afterwards, new methods were discovered to test for this

substance in water prior to fishing (Anderson *et al.,* 2012). This has allowed scientists to better define where *Pseudo-nitzschia* species are found and have their greatest ill effects (Anderson *et al.,* 2010; Anderson *et al.,* 2012). This has also led to the identification of domoic acid in California, which has been found to be a major cause of death for sea-birds (Anderson *et al.,* 2012). It is now recognized that this toxin was present in these waters for a very long time before it was properly identified, which contributes to it appearing more prevalent now (Anderson *et al.,* 2012).

Monitoring programs allow scientists and local community members to understand what is present in the water and if it is safe to fish, clam, or swim etc. Phytoplankton monitoring in Kachemak Bay is conducted at weekly, monthly, and quarterly frequencies in different parts of the bay with water samples collected to estimate species concentrations (Bursch, 2014). Fisherman and clam diggers also have domoic acid field tests kits that allow them to estimate the DA levels in shellfish tissues (Scanlan, 2014). In addition, laboratory tests are required to confirm safe levels for any shellfish that is sold commercially (Litaker *et al.,* 2008). Worldwide, more and more researchers are now testing and monitoring their local waters for HAB species and distributing this news to the media, indicating whether or not the blooms are toxic (Hallegraeff, 1993).

Although not a big driver in the Kachemak Bay system, in other systems, aquaculture has been implicated in increases in HABs in freshwater and estuary environments because it increases nutrient inputs (Anderson *et al.,* 2012; McLean

and Sinclair, 2012). Due to overfishing, many countries are turning towards aquaculture as a way to acquire enough fish to meet public demand (Hallegraeff, 1993). Aquaculture often leads to overfeeding of their fish, supplying excess nutrients to phytoplankton that can form harmful blooms (Anderson *et al.,* 2012). These fish are confined in tighter spaces, unable to move freely, and exposed to concentrated amounts of phytoplankton allowing toxins to accumulate in their bodies' faster (Maldonado *et al.*, 2002). This phenomenon resulted in the discovery of algal groups, such as *Heterosigma spp*, *Chatonella sp*., *Prumnesium parvum*, and *Gymnodium sp*., that form red blood cell destroying molecules in fish species that under natural concentrations would not be hazardous (Hallegraeff, 1993).

Similarly, eutrophication from domestic, industrial, and agricultural wastes stimulates HAB growth by increasing nutrient inputs (Trainer *et al.,* 2012; Sathicq, Bauer, and Gomez, 2015). Nutrient loading changes nutrient ratios allowing harmful species to potentially increase by outcompeting native species (Sathicq, Bauer, and Gomez, 2015). For instance, in Hong Kong Harbour there was an eightfold increase in the number of HABs from 1976-1986, which was strongly correlated to the increase in human population and nutrient loading that occurred in the area (Hallegraeff, 1993). Since then, controls have been placed on the waste disposal and HABs have declined (Hallegraeff, 1993). Some studies found that changing nutrient ratios can force species already in the area to change

from their non-toxic forms to their toxic forms because of the sudden and sometimes unfavorable environmental changes (Hallegraeff, 1993).

Furthermore, HABs can be introduced to new areas by human transportation channels. Transport of dinoflagellate cysts to novel locations has led to the increased geographical distribution of many HAB species (Anderson *et al.,* 2012). With increased shipping across the oceans, cargo vessel ballast water can hold non-indigenous marine plankton and transfer these spores to new waters (McLean and Sinclair, 2012). For instance, in the mid-1970s a ship transported the toxic dinoflagellate *Gymnodinium catenatum* into Tasmania where it had never been seen before, and from there it spread to Australia (Anderson *et al.,* 2012). In another case, *Odontella sinensis*, which is usually found on the tropical and subtropical coasts of the Indo-Pacific, was confirmed to have been transported to Europe by a specific boat (Hallegraeff, 1993).

The problem of human transportation is exacerbated by other anthropogenic changes, the biggest of which is climate change (Committee on Ecological Impacts of Climate Change, 2009). Climate change is caused by humans continuing to add carbon dioxide to the atmosphere, which has led to significant increases in air and sea temperatures worldwide. (Alexander *et al.,* 2006). Climate change is leading to rising average temperatures, as well as new precipitation and ocean circulation patterns (Alexander *et al.,* 2006; Broecker 1997). These extraordinary physical changes are leading to changes in the species

composition of phytoplankton (Sathicq, Bauer, and Gomez, 2015), with some species expanding their ranges while others are going extinct (Dybas, 2006).

Climate change is leading to changes in species composition and trophic dynamics because of increased sea surface temperatures. With increases in sea surface temperatures phytoplankton species are able to expand their ranges. For example, *Pyrodinium spp.* blooms were once confined to the tropical, mangrove-fringed coasts of the Atlantic and Indo-West Pacific; however, in more recent times this genus has traveled to the Pacific East Coast probably due to ship transport (Hallegraeff, 1993). Subsequent to that, each year these species are sweeping northward on this coast causing illness and death as water temperature permits (Hallegraeff, 1993). These northward range expansions are not the only events attributed to climate change, there are also incidents in which phytoplankton have crossed oceans due to climate change related storm events.

Increased sea surface temperatures have led to an acceleration of the hydrological cycle increasing El Niño and hurricanes in frequency and intensity, which can spread phytoplankton or change ecosystem conditions in favor of already present HAB species (Cai *et al.*, 2013; Sathicq, Bauer, and Gomez, 2015). El Niño is caused by atmospheric forcing from warmer temperatures, which leads to the collapse of the trade winds over the Pacific Ocean (Wyrtki, 1985). When the trade winds collapse, large volumes of warm water spread from West to East towards higher latitudes potentially carrying HAB species (Wyrtki, 1985). During El Niño, native phytoplankton populations experience dramatic population

declines because of water column stratification, which prevents nutrients from entering the upper surface waters giving the nonnative HAB species an advantage over native as they can outcompete them under nutrient limited conditions (Chavez *et al.,* 1999).

Typhoons (referred to as hurricanes in the Atlantic Ocean) can also affect phytoplankton, both by physically moving them and by changing their ecosystem conditions. In 1972, an uncommonly large tropical storm resulted in the dispersal of an *Alexandrium* bloom across the Pacific basin to an area where it had not been previously (Anderson *et al.,* 2012) (Figure 1). *Alexandrium spp.* were then able to become established in the area producing cysts that were present in the sediment allowing them to bloom in subsequent years (Anderson *et al.,* 2012). Typhoons can also induce cold water upwelling, which allows nutrient-rich water from deep offshore regions to replace displaced surface waters close to the coast, thereby nourishing phytoplankton and increasing biomass (Danling and Sui, 2010; Hu *et al.,* 2006; McLean and Sinclair, 2012). Finally, typhoons can provide nourishment for marine phytoplankton via rain, which brings nitrogen and carbon from terrestrial and atmospheric sources (Danling and Sui, 2010; Hu *et al.,* 2006).

### *1.3 HABS in Kachemak Bay Alaska*

#### *1.3.1 Chaetoceros and Pseudo-nitzschia phytoplankton species*

Two of the potentially dangerous phytoplankton genera mentioned above, *Chaetoceros* and *Pseudo-nitzschia,* are present in Kachemak Bay, South Central Alaska, where I did my thesis work. These two phytoplankton genera are

commonly ingested by a variety of shellfish found and harvested in Alaskan ocean waters including tunicates, clams, and mussels (Mariculture, Alaska Department of Fish and Game).

These genera are monitored due to their potential for detrimental effects, especially considering that the nearby Kodiak Island, Alaska have had devastating *Pseudo-nitzschia* blooms leading to the poisoning of animals and humans (Morton and Bursch, 2015; Trainer, 2002). So far, Kachemak Bay has continually had non-dangerous blooms of *Pseudo-nitzschia*, and research indicates that toxicity has been shown to be associated with iron limitation suggesting the area is not iron limited (Trainer, 2002). In comparison, nutrient-rich conditions favor and may have led to harmful blooms of the spiny-structured *Chaetoceros*, a species found in a variety of estuary environments including both temperate and subarctic environments, especially in Alaskan bays where harvesters have attempted to rear salmon (Morton and Bursch 2015; Trigueros and Orive, 2001; and Waite, Bienfang, and Harrison, 1992). However, *Pseudo-nitzschia* is not expected to be as abundant in this area given that their optimum temperature is $10^{o}$C (Anderson *et al.*, 2010), as compared to the average high of $7.4^{o}$C that is typically recorded here (U.S. Climate DATA, 2002). Nonetheless, in recent years, particularly 2015, increased *Pseudo-nitzschia* concentrations have been observed with increasing water temperatures. We expect that this phenomenon will occur more frequently with increasing temperatures, and earlier spring warming.

Due to *Pseudo-nitzschia'*s lifecycle, earlier warmer temperatures means that this species may outcompete other species and possibly limit the nutrients in the environment leading to domoic acid release and the decline in other species. *Pseudo-nitzschia* is a pennate diatom that has two characteristic life stages, an asexual phase and a sexual phase without a known resting benthic stage, unlike other diatoms (Amato *et al.,* 2005; Montressor *et al.,* 2013; Quijano-Scheggia *et al.,* 2008). *Pseudo-nitzschia*, like many other diatoms, have a period of rapid division that leads to progressively smaller and smaller cells that began from an original large cell (Amato *et al.,* 2005; Quijano-Schegiia *et al.,* 2008). Large cell size is recovered during their sexual reproduction period, which occurs during different times of the year depending on the particular species, and is observed as multiple *Pseudo-nitzschia* blooms throughout the year (Garces *et al.,* 2001; Quijano-Schegiia *et al.,* 2008). In the beginning of spring, when other phytoplankton, predominately *Chaetoceros*, are blooming, *Pseudo-nitzschia* concentrations are especially low until conditions, especially temperature, favor *Pseudo-nitzschia* (Montressor *et al.,* 2013). Once the conditions are favorable, they are able to establish themselves and outcompete smaller phytoplankton such as *Chaetoceros* because when *Pseudo-nitzschia* are most abundant their cells tend to be at their largest size (Montressor *et al.,* 2013; Quijano-Schegiia *et al.*, 2008). While *Pseudo-nitzschia* are not known to have a resting benthic stage, their large-sized-cells found in spring in similar composition and concentration patterns that were found in previous years suggests that they rest somewhere, but where they

rest has yet to be found (Montressor *et al.,* 2013). Therefore, with warmer water temperatures, if *Pseudo-nitzschia* blooms occur earlier in the spring, they may outcompete other species earlier leaving fewer nutrients available for their own smaller sized phytoplankton later in the season. Under such low nutrient/iron conditions, *Pseudo-nitzschia* releases deleterious domoic acid to concentrate iron for itself allowing it to further outcompete other phytoplankton (Trainer *et al.,* 2012). Studies have shown that when domoic acid is released other plankton species decline in abundance; it is not known if this is because they are directly affected by domoic acid or if they are indirectly inhibited by a secondary metabolite (Prince *et al.,* 2013).

Unlike *Pseudo-nitzschia*, which is able to thrive in warm nutrient poor conditions, *Chaetoceros'* life cycle allows it to thrive in colder more nutrient rich conditions; furthermore, the genus has many diverse species that allow it to survive in a wider range of growing conditions compared to *Pseudo-nitzschia* (Daniels *et al.,* 2015). This means that *Chaetoceros* responds quickly to the improved growing conditions of spring, and *Pseudo-nitzschia* is only able to dominate once it becomes fully established (Daniels *et al.,* 2015). In the spring, *Chaetoceros* quickly responds to warming temperatures because of its abundant spores that are created in autumn when nutrients, particularly nitrogen, are low (Montresor *et al.,* 2013). These spores remain in the sediments in areas of upwelling where they do not exhibit much if any vegetative growth until they sense the correct combination of temperature, light, and nutrients (Pitcher, 1990).

This is part of the reason *Chaetoceros* dominates in newly upwelled waters, such as those found in Kachemak Bay, because they have this ability to capitalize quickly on favorable conditions due to their seed population (Pitcher, 1990). In addition, the small size of *Chaetoceros* cells help it to be successful earlier in the warm season because it can better compete for light and nutrients with a higher surface area to volume ratio, which allows it to absorb more light at max PAR (Trigueros and Orive, 2001).

### *1.3.2 Environmental Factors*

In order to better understand the patterns of these phytoplankton blooms, I studied their species composition and concentration throughout the Kachemak Bay region, in relation to temperature, photosynthetically active radiation (PAR), salinity, and nutrients. This is an important area of study because it is a recreational shellfish harvest area, and blooms of HAB species are not only a problem currently, but are expected to increase in frequency as the plankton community changes due to climate change (Hays *et al.*, 2005).

The study area consists of most of the Lower Cook Inlet, with intensive studies done particularly in Kachemak Bay. Kachemak Bay and the larger Cook Inlet are sub-arctic environments located in South Central Alaska (Walker and Field, 2003) (Figure 2). Humans use this area for recreational harvest of fish and shellfish, shellfish farming, and for hunting caribou and moose (Walker and Field, 2003). The area has heavy harvesting of a wide variety of marine life including mussel and oyster farming as well as an avid fishing center for various species of

salmon and halibut (Walker and Field, 2002). Kachemak Bay is intensely studied because it is one of the most biologically productive areas due to the combination of nutrient upwelling from incursions of the Alaska Coastal Current, significant freshwater input, and mixing from strong tidal currents (5.5-8.5 meter tidal range and storms). Figure 3 shows the general pattern of subtidal surface ocean circulation in the area (Walker and Field, 2003). This current flows along the Gulf of Alaska coastline from east to west and causes vertical mixing and upwelling, especially as the current moves up over the shelf (Walker and Field, 2003). Given the direction and location of the current, predominantly on the eastern coast, much of this nutrient rich water enters Kachemak bay where it mixes with freshwater input from rain, snowpack melt, and glacial meltwater (Walker and Field, 2003).


**Figure 2:** Map of Alaska and Cook Inlet with blue arrows indicating one path of occasional movement of the Alaska Coastal Current into lower Cook Inlet that provides nutrients, especially nitrate, from ocean upwelling (*pers. comm.* Holderied, 2015).

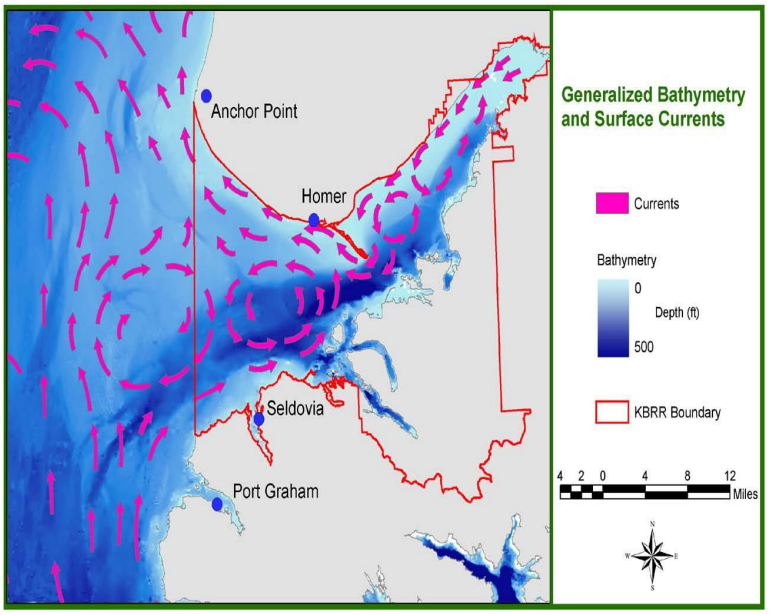


**Figure 3:** Conceptual diagram of surface currents in southeastern Cook Inlet and Kachemak Bay. Note in particular that the offshore surface water in Kachemak moves eastward (into the bay) along the south coast in contrast to the westward (seaward) movement along the northern coast (Walker and Field, 2003)

Kachemak Bay is particularly important to study due to the economic importance of marine resources for both harvest and ecotourism, and since it is the only fjord estuary that is protected under the National Estuarine Research Reserve System (Walker and Field, 2003). This fjord was created by glaciers, with an ice field and several glaciers still remaining on the southern part of the bay (Walker and Field, 2003). Snowpack and glacial melt inputs of freshwater and sediment occur during the spring and summer as temperatures increase, which stratifies the water column with less saline water on the surface (Walker and Field, 2003).

At Kachemak Bay, 2015 was particularly noteworthy because *Chaetoceros,* which typically dominates throughout the year, was supplanted by *Pseudo-*

*nitzschia* in some locations, and it is important for us to understand why. I hypothesize that PAR, water temperature, salinity, and nutrients are significant factors affecting phytoplankton species abundance and composition (Macedo and Duarte, 2006; Zonnevald, 1998). Therefore, I conducted a comparative study of two locations in Kachemak Bay including Homer harbor and Kasitsna Bay. I expected Homer to be less saline and slightly warmer than Kasitsna, because Homer is located closer to rivers and glaciers that deposit freshwater, which is then warmed in the surface layer as it flows out of the bay. I believed Kasitsna Bay would have a much higher salinity on average because of its proximity to the ocean and the fact that it has fewer surrounding glaciers feeding into it. Therefore, I expected there to be less *Chaetoceros* at Homer than Kasitsna because *Chaetoceros* does much better in more saline waters and it outcompetes other species in colder water (Trigueros and Orive, 2001). I hypothesized that *Pseudo-nitzschia* would be found more predominantly at Homer because I expected Homer to have warmer temperatures, and *Pseudo-nitzschia* grows better at warmer temperatures (Anderson *et al.*, 2010).

# METHODS

## *2.1 Data Collection Area*

I was able to investigate the patterns of the two phytoplankton species by analyzing data from phytoplankton monitoring efforts at the National Oceanic and Atmospheric Administration (NOAA), Kasitsna Bay Laboratory, and Kachemak bay Research Reserve, which are also supported by the NOAA Phytoplankton Monitoring Program (*pers. comm.* Kris Holderied). These monitoring efforts aim to document important phytoplankton species that can drive changes in marine food webs, and also detrimentally affect the health of humans and marine life as the environment changes (*pers. comm.* Kris Holderied). Particular attention was focused on phytoplankton that could be detrimental to humans and other large marine mammals that ingest shellfish and fish, which have accumulated more of the toxins.

## *2.2 Information Collection*

### *2.2.1 System Wide Monitoring Program (SWMP) Data*

Light, temperature, salinity, and nutrients have been known to influence the abundance and species composition of phytoplankton. The Kachemak Bay National Estuarine Research Reserve has a System-Wide Monitoring Program (SWMP) consisting of one meteorological station, four-water quality stations, and four nutrient stations.

The meteorological station is located at the end of the Homer Spit and this collection began in 2003. It collects air temperature, relative humidity, barometric

pressure, wind speed/max wind speed, wind direction, precipitation, photosynthetic active radiation (PAR), and total solar radiation every 15 minutes. The standard deviation for wind direction and cumulative precipitation were also derived.

There are two water quality and nutrient stations each in Homer and Seldovia, which are located 1 meter below the surface and 1 meter from the bottom (Figure 3). These stations are located thusly because the Homer Spit separates Kachemak Bay into two regions with the inner bay being more influenced by freshwater, and the outer bay more influenced by oceanic conditions. The water quality stations use a sonde, or multi-sensor water quality-monitoring instrument package, at each depth, and they have been in place since 2001. The water quality stations measure water temperature, conductivity, salinity, dissolved oxygen, depth, pH, turbidity, and chlorophyll fluorescence every 15 minutes. I only used the data from the deep sonde at each station since both shallow and deep stations lie in the photic zone and other studies using statistical measurements have shown that measurements at the surface did not significantly differ from those at the bottom (Quijano-scheggia *et al.,* 2008). In addition, the surface stations tended to have more human interaction with boats and ferries.

The nutrient measurements are taken monthly using a 5-liter Niskin bottle, an instrument used to collect samples, and this part of the program collects samples monthly and has been active since 2002. Water samples collected for

nutrients are analyzed for nitrite, nitrate, nitrite+nitrate, ammonium, orthophosphate, and chlorophyll-a concentrations. During each sampling period three samples were collected at the shallow and deep-water stations with a Niskin bottle during high tide. Special attention was given to the parameters nitrite+nitrate ($NO_2^3$) and orthophosphate ($PO_4$), from which I calculated the nitrogen to phosphorous (N:P) ratio. I calculated the N:P ratio because other studies suggested that this ratio was an important determinant in nutrient limitation among phytoplankton (Tilman *et al.*, 1982).


**Figure 4:** Satellite Image of Kachemak Bay Laboratory, Alaska illustrating the SWMP stations at Homer and Seldovia as well as the nearby locations for phytoplankton collection at Kasitsna Bay Lab and Station 9-10.

Initial compilations of the data were taken from an internal ocean workspace site as part of the Gulf Watch Alaska long-term ecosystem monitoring program http://www.gulfwatchalaska.org. The most recent meteorological, water quality, and nutrient data at the Kachemak Bay National Estuarine Research Reserve (NERR) were obtained directly from the NERR Centralized Data Management Office (CDMO) (http://cdmo.baruch.sc.edu/ get/export.cfm). All of the SWMP data can be found on the CDMO website, and these data have been through tertiary review by an automatic and two manual Quality Assurance Quality Control (QAQC) programs. In the analysis, I excluded around 5-10% of the data that had been deemed problematic by QAQC, for example measurements with flags that indicated the data were outside of the possible measurement range.

*2.2.2 Phytoplankton Sampling and Data*

Phytoplankton sampling is conducted in Kachemak Bay at both shore stations and from boats very close to where the SWMP data was collected, but not the exactly same. However, the data can be compared since the changes over time of the two locations have been observed to be relatively coherent across the bay (*pers. comm.* Holderied, 2016). My thesis focused on phytoplankton data collected during routine shore station sampling conducted by Kasitsna Bay Laboratory researchers, as well as selected data from shipboard surveys conducted monthly in Kachemak Bay and seasonally in lower Cook Inlet, as part of the Gulf Watch Alaska program beginning in May 2012 (Figure 4). During the summer, the Homer phytoplankton station (Transect 9 Station 10) was sampled

biweekly/monthly. Phytoplankton samples were also collected at Kasitsna Bay Laboratory on a weekly/biweekly basis throughout the year.

Phytoplankton were collected via 40L surface water grabs, which were passed through 20 cm diameter, 20 μm mesh net and concentrated into a 250mL bottle. These samples were preserved with 10% lugol solution and were then stored at $4^{o}$C until visual analysis occurred within a year. A Palmer-Maloney counting cell (Ward's, Rochester, New York) with a 0.1mL volume was used for counting and calculating phytoplankton concentrations. Each sample was counted three times and an average cell concentration for each phytoplankton genus was calculated.

*2.3 Data Analysis*

In order to determine the significance of the various environmental parameters, I used STATA to create a variety of models to quantify whether or not environmental conditions can be used to predict the concentrations of the two species. I used biweekly data in order to have enough data points to create the model, which were derived from the biweekly averages of meteorological, water quality, and phytoplankton at Kasitsna. For the nutrient data at both places, and phytoplankton data at Homer and Kasitsna in a couple instances, I compared the averages from the measurement from one month to the next using the intermediate of the two for the biweekly period in between. I quantified significant values as p values that were less than 0.05, but I also did note if p values were close to this value if they were less than 0.075. I created several

models, and in all of the models I used tobit regression with a lower limit of 0 in order to see the significance of the parameters only when the phytoplankton were present in the water. In order for the models to work, I had to remove outlying data to find any significance present during normal cycling leading me to have to delete a large *Chaetoceros* bloom in 2012. The first model I created was an individual model where I looked at each environmental parameter as independent variables with each species as dependent variables at each location, in order to see if each parameter influenced the presence of phytoplankton. For the final model, I made a combined model using PAR, water temperature, salinity, location, and nitrite+nitrate as independent variables with phytoplankton as the dependent variable to get a more accurate understanding of these parameters overall influence on these species. I also did a nutrient model looking at orthophosphate and nitrite+nitrate individually using a xtreg application, which looks at the random individual differences over a time series to see how the two-phytoplankton species influenced these nutrient concentrations. I used lag variables for the combined and nutrient model to see if there was a certain time that environmental conditions and/or phytoplankton would more likely influence the other parameters. Lastly, to see if there was a significant difference in the environmental parameters at the locations I used a t-test in STATA.

*2.4 Graphical Representations*

To determine if there were temporal patterns in the two species' blooms, I graphed their concentrations relative to the total phytoplankton concentration in

the water based on the sample estimate percentages as well as their actual concentrations for Homer and Kasitsna. Then, to understand the conditions in which *Chaetoceros* and *Pseudo-nitzschia* species are present, I created graphs that looked at the biweekly/monthly abundance of these two species relative to daily PAR, daily water temperature, daily salinity, and monthly nutrients between January 2012 and June/December 2015. In addition, in order to see how each species interacted with each parameter individually, I created scatterplots of the monthly phytoplankton and environmental data comparing them to look at what thresholds phytoplankton appeared.

To understand how water temperature influences phytoplankton and will influence it in the future, it is necessary to look at how water temperature changes over long periods of time. From the fifteen years of data, I averaged all the data for one month for each month and each month had data points ranging from 7 (there was only 7 months total between both locations that had less than 15 days of data) to 31 days of data. I compared the monthly data over the 15 years to the data for the average values that a particular month had over the years creating an anomaly plot. To see how water temperature influences salinity over time, I graphed them together from 2001 to 2015 at Homer and Seldovia.

Finally, in order to see how nutrients have changed during the time that we measured phytoplankton, I looked at the yearly nitrite+nitrate, orthophosphate, and N:P data at Homer and Kasitsna. In order to see how the different nutrient parameters changed over time I used an ANOVA, analysis of variance to look at

the difference between the levels of nutrients for the different years, from monthly data in SPSS.

## Results

### *3.1 Significance of Parameters According to Model*

Based on the individual model of temporal and environmental measurements at both locations, *Pseudo-nitzschia spp.* were significantly and positively correlated with year at Kasitsna Bay (Appendix Table 1: Model 2). Positive correlations such as this suggest that *Pseudo-nitzschia* increases over time at Kasitsna. Air temperature was positively and significantly correlated with both species at both Homer and Kasitsna Bay (Table 1a: Models 1 & 2). Barometric pressure was positively and significantly correlated with *Pseudo-nitzschia* at both locations and *Chaetoceros* only at Kasitsna (Appendix Table 1: Models 1 & 2). Interestingly enough, wind speed and maximum wind speed were negatively correlated with phytoplankton at both locations (Appendix Table 1: Models 1 & 2). Negative correlations like this suggest that higher wind speeds are associated with less phytoplankton. Wind direction and the standard deviation of wind direction were positively and significantly correlated with both species at both locations (Appendix Table 1: Models 1 & 2). In addition, both species at both locations were positively and significantly correlated with total PAR and total solar radiation (Appendix Tables 1 and 1a: Models 1 & 2).

In regards to the water quality data, water temperature was found to be positively and significantly correlated with *Pseudo-nitzschia* at both locations, and *Chaetoceros* only at Kasitsna (Table 1a: Models 1 & 2). The percent of dissolved oxygen was found to be positively and significantly correlated with

both species at both locations (Appendix Table 1: Models 1 & 2). *Chaetoceros spp.* were significantly correlated with high dissolved oxygen concentration percentages at both locations and *Pseudo-nitzschi*a only slightly significant at Kasitsna. Corrected depth was found to be negatively and significantly correlated with *Chaetoceros* at Kasitsna Bay. PH was positively and negatively significantly correlated with *Chaetoceros* at Homer and Kasitsna, respectively. Turbidity was found to be negatively and significantly correlated to *Chaetoceros* only at Homer (Appendix Table 1: Models 1 & 2).

In regards to nutrients, orthophosphate was negatively and significantly correlated with *Chaetoceros* at both locations and with *Pseudo-nitzschia* at Homer, but orthophosphate was only slightly significant with *Pseudo-nitzschia* at Kasitsna (Table 1a: Models 1 & 2- Nutrients). Ammonia was positively correlated with *Chaetoceros* and negatively significant with *Pseudo-nitzschia*, at Homer. Nitrite+Nitrate was negatively and significantly correlated with both species at both locations, and N:P ratios were negatively and significantly correlated with *Pseudo-nitzschia* and *Chaetoceros* only at Kasitsna (Table 1a: Models 1 & 2- Nutrients). At Homer and Kasitsna, we found that when phytoplankton were lagged 2 weeks and compared to the current orthophosphate and nitrite+nitrate levels there were significant negative correlations for both species (Table 1a: Models 1 & 2- Reverse Nutrients). This means that increasing phytoplankton from two weeks ago were associated with current decreasing nutrients.

Looking at the combined model, we see a slightly different outcome. I found that *Chaetoceros spp.* were positively and significantly correlated with PAR when lagged 2 weeks (Table 1a: Model 4). This suggests that *Chaetoceros* concentrations were more correlated with earlier PAR than current PAR and *Pseudo-nitzschia* were positively and significantly correlated with current PAR. Water temperature was positively and significantly correlated with *Pseudo-nitzschia* and negatively correlated with *Chaetoceros spp.* when they were described quadratically (Table 1a: Model 4). While phytoplankton seem to be influencing nutrient concentrations according to the previous models, it appears that nutrient concentrations, particularly nitrite+nitrate, are only positively and significantly associated with *Chaetoceros* when lagged a month (Table 1a: Model 4). This means that increasing nutrient concentrations from a month ago are associated with current increases in *Chaetoceros*. *Chaetoceros* and *Pseudo-nitzschia* were both significantly correlated with location; *Chaetoceros spp.* were significantly higher at Kasitsna and *Pseudo-nitzschia spp.* were higher at Homer (Table 1a: Model 4). Finally, I found that there was a significant difference between the two locations in regards to water temperature (somewhat significant $p=0.072$), salinity, and *Chaetoceros* which were all higher at Homer, as well as the N:P ratios, which were higher at Kasitsna (Table 1a: T-test).

**Table 1a:** The impact of environmental parameters on phytoplankton concentrations (cells/L) of *Chaetoceros* and *Pseudo-nitzschia*. The results of model analysis with an individual species model for each location and the combined model for both locations, as well as t-test analysis comparing conditions between the locations. Three asterisks indicates highly significant results with p values less than 0.025, two asterisks indicate significant values with p values less than 0.05, and one asterisk indicates p values greater than 0.05 but less than 0.075.

| **Model:** | **Model 1 (Individual) Homer** | | **Model 2 (Individual) Kasitsna** | | **Model 4 (Both Locations)** | | **T-test** |
|---|---|---|---|---|---|---|---|
| Parameter | *Chaetoceros* | *Pseudo-nitzschia* | *Chaetoceros* | *Pseudo-nitzschia* | *Chaetoceros* | *Pseudo-nitzschia* | *Location* |
| **Meteorological** | | | | | | | |
| Air Temperature | Coeff.=28100.3 p=0.014*** | Coeff.=2136.8 p=0.001*** | Coeff.=16943.4 p=0.000*** | Coeff.=1108.8 p=0.004*** | N/A | N/A | N/A |
| Total PAR | Coeff.=1267.8 p=0.001*** | Coeff.=70.4 p=0.000*** | Coeff.=764.5 p=0.000*** | Coeff.=53.3 p=0.000*** | Lag 1 Coeff=690.1 p=0.00*** | Coeff=51.68 p=0.00*** | N/A |
| **Water Quality** | | | | | | | |
| Water Temperature | Coeff.=26153.7 p=0.215 | Coeff.=3450.9 p=0.003*** | Coeff.=17296.42 p=0.042** | Coeff.=1167.5 p=0.044** | Squared Coeff= -3996.3 p=0.009*** | Coeff.=1944.7 p=0.006*** | dF=171 t=1.8126 p=0.0716* |
| Salinity | Coeff.=129370.4 p=0.165 | Coeff.=1609.8 p=0.723 | Coeff.=18755.4 p=0.560 | Coeff.=599.4 p=0.801 | Coeff= -22200.8 p=0.243 | Not included | dF=169 t=3.1009 p=0.0023*** |
| **Nutrients** | | | | | | | |
| Phosphate | Coeff.= -1.27x10$^{7}$ p=0.005*** | Coeff.= -590313 p=0.001*** | Coeff.= -8193964 p=0.000*** | Coeff.= -288639.7 p=0.063* | Not included due to similarity to $NO_2^3$ | Not included due to similarity to $NO_2^3$ | dF=147 t=0.7792 p=0.437 |
| Nitrate+Nitrite | Coeff.= -1661583 p=0.010*** | Coeff.= -95428 p=0.000*** | Coeff.= -1253718 p=0.000*** | Coeff.= -57528.5 p=0.008*** | Lag 2 Coeff.=598113.2 p=0.023*** | Coeff.=52119.9 p=0.101 | dF=147 t=1.45 p=0.159 |
| N:P | Coeff.=1.924 p=0.821 | Coeff.= -0.1835 p=0.549 | Coeff.= -32812.3 p=0.005*** | Coeff.= -2034.3 p=0.011*** | Not included due to similarity to $NO_2^3$ | Not included due to similarity to $NO_2^3$ | dF=145 t= -2.166 p=0.0456** |
| **Phytoplankton** | | | | | | | |
| *Chaetoceros* | NA | Coeff.=0.00123 p=0.923 | NA | Coeff.=0.047 p=0.002*** | N/A | N/A | dF=148 t=2.3226 p=0.0216*** |
| *Pseudo-nitzschia* | Coeff.=0.50 p=0.943 | NA | Coeff.=2.18 p=0.066* | NA | N/A | N/A | dF=149 t= -1.035 p=0.3024 |
| **Reverse Nutrient** | | | | | | | |
| Phosphate | Phyto. Lag 1 Coeff.= -3.37x10$^{-8}$ p=0.00*** | Phyto. Lag 1 Coeff.= -5.14x10$^{-7}$ p=0.000*** | Phyto. Lag 1 Coeff.= -3.37x10$^{-8}$ p=0.00*** | Phyto. Lag 1 Coeff.= -5.14x10$^{-7}$ p=0.000*** | N/A | N/A | N/A |
| Nitrate+Nitrite | Phyto. Lag 1 Coeff.= -2.48x10$^{-7}$ p=0.00*** | Phyto. Lag 1 Coeff.= -4.22x10$^{-6}$ p=0.000*** | Phyto. Lag 1 Coeff.= -2.48x10$^{-7}$ p=0.00*** | Phyto. Lag 1 Coeff.= -4.22x10$^{-6}$ p=0.000*** | N/A | N/A | N/A |
| Location | N/A | N/A | N/A | N/A | Coeff.= -53384.2 p=0.017*** | Coeff=5375 p=0.013*** | N/A |

*3.2 Temporal Phytoplankton Concentrations and Compositions*

I looked at *Chaetoceros* and *Pseudo-nitzschia*, more specifically, their abundance in Kachemak Bay and the seasonal timing of their blooms, because of the dangers they pose to the area (Figures 5a & 5b). These graphs show the changes in the timing of blooms and the spring emergence of *Chaetoceros* and

*Pseudo-nitzschia* illustrating that in more recent years *Pseudo-nitzschia* bloomed earlier at both comparison sites (Figure 5a & 5b).

In Homer, there was a consistent pattern of *Chaetoceros* blooms that were more dominating of the environment, albeit less frequent than *Pseudo-nitzschia*; in contrast, *Pseudo-nitzschia spp.* dominance fluctuated but were always less than *Chaetoceros spp.* (Figure 5a). While the pattern was more variable in Kasitsna, at both locations *Chaetoceros* and *Pseudo-nitzschia* concentrations began rising earlier while *Pseudo-nitzschia* became the last to bloom (Figure 5a & 5b). For instance, *Pseudo-nitzschia* blooms have come earlier as they were only present between June and September in 2012, and in more recent years, they have shifted to April and even February (Figure 5a & 5b). In previous years, *Pseudo-nitzschia* was most abundant in September; while in later years, they became highly concentrated as early as July and August (Figure 5a & 5b). Another notable trend in Homer was that in June 2012, *Chaetoceros* completely dominated the waters with estimated relative concentrations of around 100%, but by April 2015 the usual large spring bloom only comprised 50% of the estimated phytoplankton population (Figure 5a).

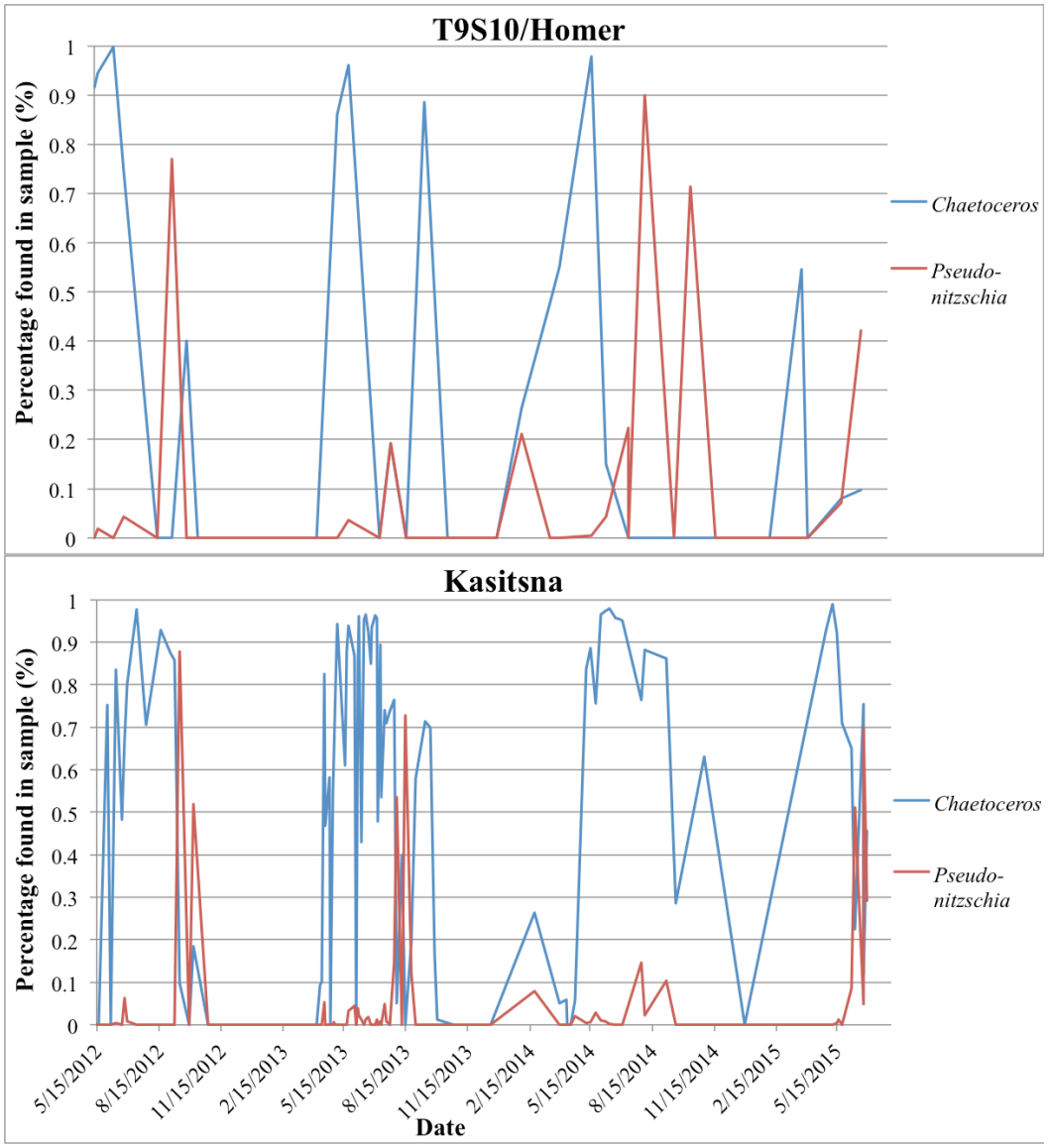


**Figure 5a & 5b:** *Chaetoceros* vs. *Pseudo-nitzschia* concentrations in a) Transect 9 Station 10/Homer with monthly samples and b) Kasitsna Bay with biweekly samples throughout 2012-2015.

In order to compare the abundance of the two genera, we graphed *Chaetoceros* and *Pseudo-nitzschia* cell concentrations (Figure 6a & 6b). We found that phytoplankton concentrations usually began to rise in early April or June and were mostly gone by early October (Figure 6a). During the summer,

*Chaetoceros spp.* were typically the dominant genus present especially in the earlier periods of the summer, March through June (Figure 6a & 6b). Towards the later part of the summer, in August, when *Chaetoceros* numbers were fairly low, *Pseudo-nitzschia* often had population spikes (Figure 6a & 6b) as well as other species (Appendix Figure 3&4).

While this program was active, *Chaetoceros* had its largest recorded bloom in June of 2012 at Homer, which was four times larger than what was observed in subsequent years (Figure 6a). However, in 2012, the data show a much smaller bloom at Kasitsna Bay, a quarter of the size (Figure 6b). The bloom at Homer was particularly noteworthy because there were very few to no other phytoplankton species found during this bloom (Appendix Figure 1). It was also interesting that while no other kind of phytoplankton were viewed at Homer there were blooms of other species during the *Chaetoceros* bloom at Kasitsna (Appendix Figure 2).

At Kasitsna Bay, most of the *Chaetoceros* blooms were all of relatively equal size and were also usually larger than what was seen at Homer, with the exception of the June 2012 bloom in Homer (Figure 6a & 6b). In addition, short-term variability in cell concentrations and blooms are better captured by the higher frequency sampling at Kasitsna Bay than Homer (Figure 6a & 6b).

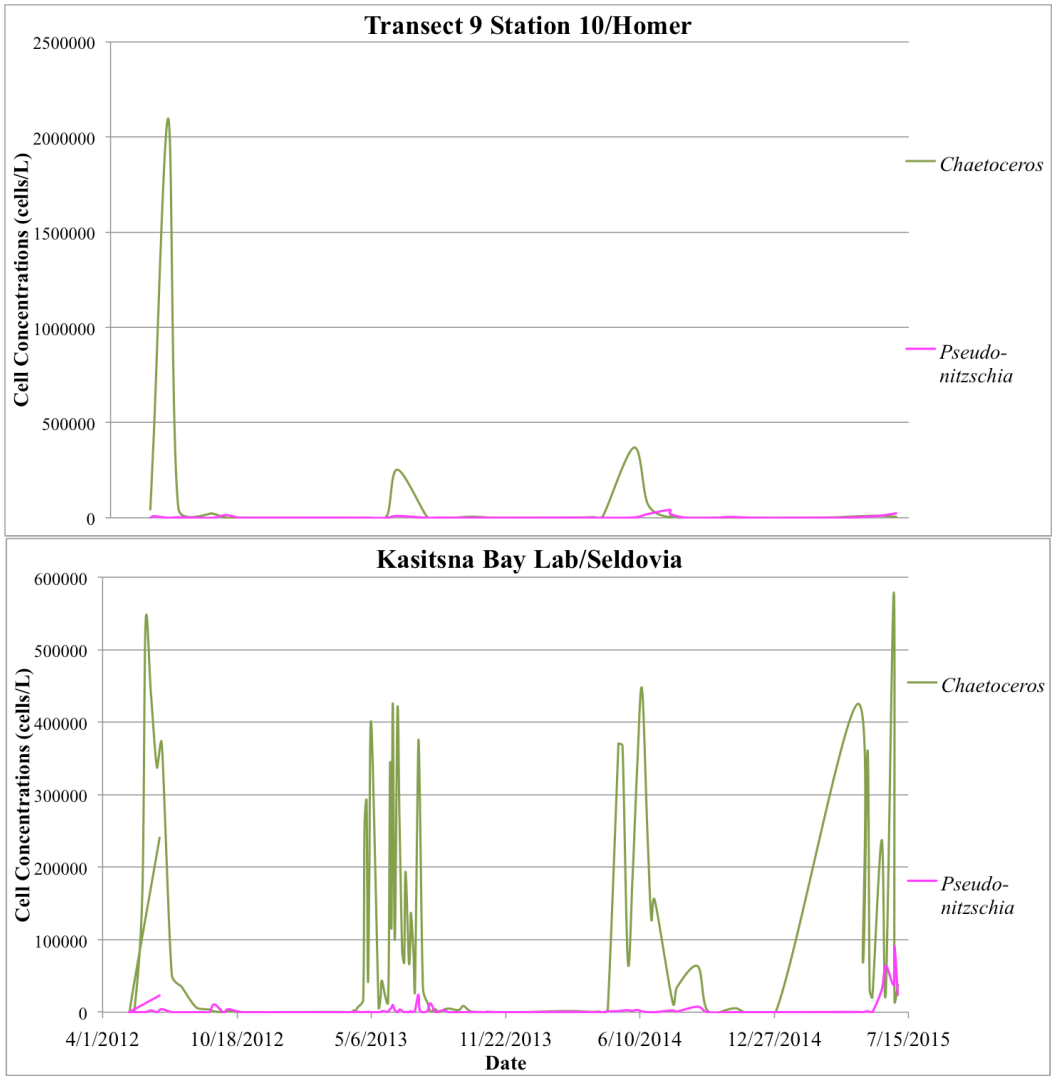


**Figure 6a & 6b:** Phytoplankton Cell Concentrations at: a) Transect 9 Station 10, near Homer harbor, and at b) Kasitsna Bay Laboratory, on the south side of Kachemak Bay about nine miles from Seldovia between the years 2012-2015**.**

*3.2.1 Phytoplankton vs. Environmental Variables at Homer*

Since *Pseudo-nitzschia* has larger cells than *Chaetoceros,* there is more biomass and/or chlorophyll with fewer cells; for that reason, in order to compare the timing of the blooms of both species to the main environmental parameters, I created graphs scaled to *Pseudo-nitzschia's* concentrations (Figure 7 & 8). In the

superimposed graphs, the top graph shows phytoplankton composition and cell concentration at Homer's Transect 9-Station 10 (Figures 7a & 8a). Figures 7b and 8b show air temperature and PAR, 7c and 8c show water temperature and salinity, and 7d and 8d show orthophosphate and nitrite+nitrate (Figure 7 & 8).

Figure 7a shows the phytoplankton with similar patterns as seen above (Figure 5 & 6), with lower cell concentrations in winter and blooms mostly during the summer months, April to October. Figure 7b shows PAR quickly increasing after the solstice in December from 14 mmol/$m^2$ till its peak in June of around 600 mmol/$m^2$ (Figure 7b). Air and water temperature (7b & 7c) typically began to rise in February lagging behind PAR. Comparing this to the phytoplankton graph, there was a difference in the timings of when certain phytoplankton appeared (Figure 7a). For instance, *Chaetoceros* normally bloomed in May at peak PAR and died off around the time when the air and water temperature were highest, in late July and early August (Figure 7a, 7b & 7c). In these higher temperatures *Pseudo-nitzschia* dominated (Figure 7a). For the most part, *Pseudo-nitzschia spp.* bloomed at the minimum temperatures of around 7$^{o}$C, but were mostly found at temperatures of 9.5-9.7$^{o}$C, when nutrients were declining after the summer high (Figure 7a, 7b, & 7c). Over the course of the study, 2012-2015, the water and air temperatures had highs that increased and occurred earlier in the year during each consecutive year (Figure 7b & 7c). In the year of the record *Chaetoceros* bloom, 2012, temperatures increased slowly after the winter compared to subsequent years and peak temperature was the lowest of the period (Figure 7b). At the time

of the 2012 *Chaetoceros* bloom, the water temperature was around 6-7$^{o}$C, which was the temperature at which *Chaetoceros spp.* were usually found (Figure 7c). When *Chaetoceros* cell concentrations were highest, the nitrite+nitrate and phosphate were low or declining (Figure 7a & 7d). *Chaetoceros spp.* were also usually found only when salinity was over 29 psu (Figure 7a & 7c). Since 2012, the high summertime water temperatures increased with each year from 10-11$^{o}$C to 12$^{o}$C (Figure 7c). The 2012 winter also had its lowest air temperature recorded in this time cycle, and each winter afterwards was warmer than the last (Figure 7b). In general, the environmental changes during the 2012-2015 time period began to favor *Pseudo-nitzschia*, as the temperature increased so did *Pseudo-nitzschia*.

In comparison, salinity dropped when air temperatures were highest and it reached as low as 29 psu, which was the lowest salinity in which *Chaetoceros* had been found (Figure 7c). Unlike salinity, nutrients increased throughout the winter months and reached their peak in March (Figure 7d). The nitrite+nitrate best illustrates the winter and summer changes in nutrients and so this was used for the rest of the nutrient comparisons (Figure 7 & 8). Orthophosphates follow the nitrite+nitrate's general patterns of being high in the winter and low in the summer, but the range of variability was much smaller than nitrite+nitrate's (Figure 7d). After March, the nutrients dropped fast until about July when the nitrite+nitrate reached their minimum of around 0.005-0.007 mg/L. Nitrite+nitrate then increased in July and August reaching another, much smaller peak in

October, before they dropped to another minimum in November of around 0.02-0.04 mg/L. They then increased until the next February/March where it was around 0.21-0.23 mg/L, on average.

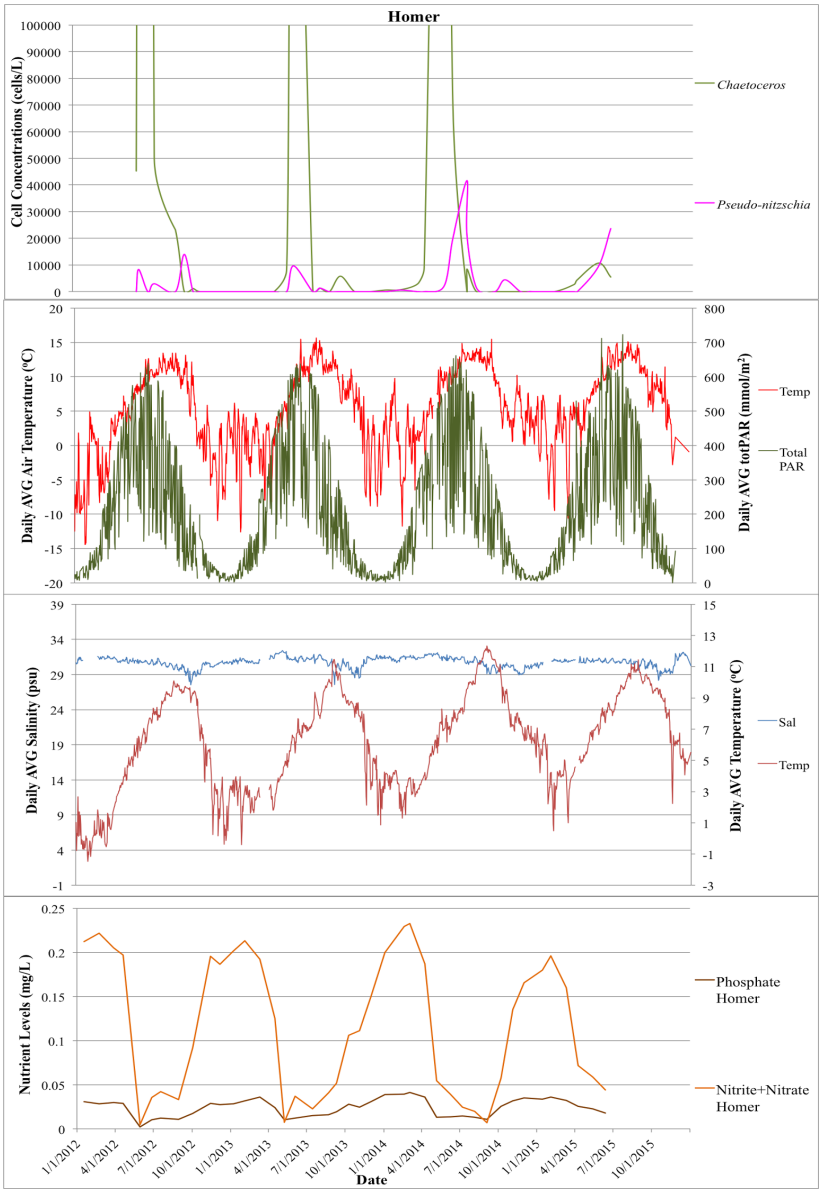


**Figure 7** a) Phytoplankton composition and species that has been zoomed in for a better view at T9S10 b) Air temperature and PAR from Homer Meteorological Station 2012-2015. c) Water Temperature and salinity from Homer deep-water quality station 2012-2015. d) Orthophosphate and nitrite+nitrate from combined Homer Shallow and Deep nutrient stations 2012-2015.

*3.2.2 Phytoplankton vs. Environmental Variables at Kasitsna*

Like Homer, Kasitsna Bay, where phytoplankton were measured, and Seldovia, where environmental parameters were measured, had lower phytoplankton concentrations in the wintertime as well as early spring blooming of *Chaetoceros* and later blooming of *Pseudo-nitzschia* (Figure 8a). In addition, the environmental conditions of air and water temperature, PAR, salinity, precipitation, and nutrients illustrated similar general patterns (Figure 8a, 8b, 8c, and 8d). Interestingly enough, at Seldovia the highest summertime nutrients in 2012 corresponded to the lowest nutrients in the summertime at Homer (Figure 8d). Like Homer, major blooms of *Chaetoceros* and *Pseudo-nitzschia* at Kasitsna Bay were associated with a reduction in nutrient concentrations (Figure 8a and 8d). For temperature, in comparison to Homer, where *Chaetoceros spp.* were mostly found at temperatures between 6-7$^{o}$C, at Kasitsna they were found at broader ranges of temperatures from 4-8.5$^{o}$C. At Homer, *Pseudo-nitzschia spp.* were found at temperatures as low as 7$^{o}$C, but were most commonly found at temperatures of 9.5-9.7$^{o}$C. In comparison, at Kasitsna, *Pseudo-nitzschia spp.* were found at higher temperatures around 10-12$^{o}$C.

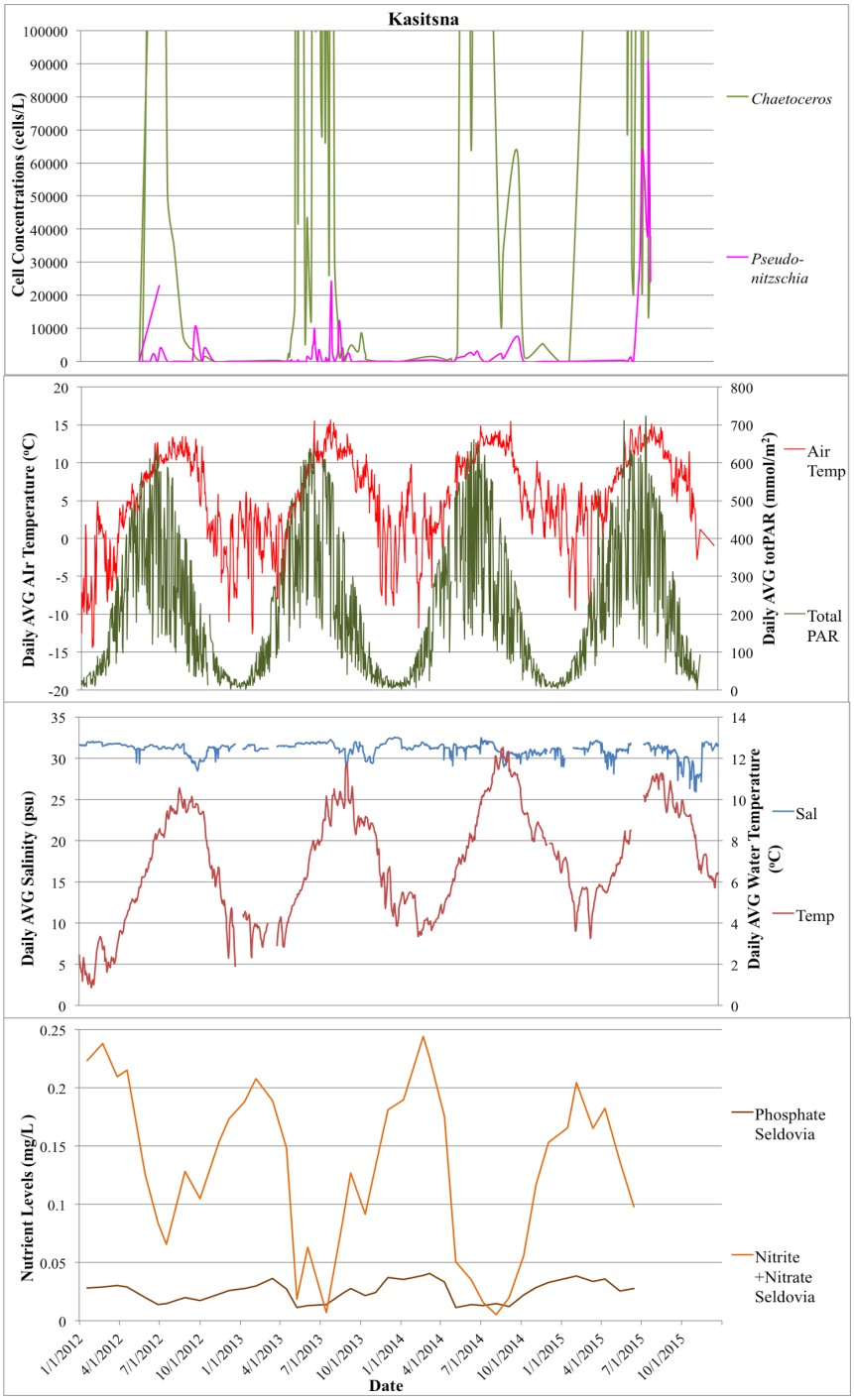


**Figure 8:** a) Phytoplankton composition and species that has been zoomed in for a better view at Kasitsna b) Air temperature and PAR from Homer Meteorological Station 2012-2015. c) Water Temperature and salinity from Seldovia deep-water quality station 2012-2015. d) Orthophosphate and nitrite+nitrate from combined Seldovia Shallow and Deep nutrient stations 2012-2015.

*3.3 Abiotic and Environmental Factors that influence phytoplankton*

*3.3.1 Meteorological Factors: PAR*

Both species showed blooms between 350 and 450 mmol/m$^2$ at both sites. At Kasitsna there was another major peak of *Pseudo-nitzschia* around 150 mmol/m$^2$ (Figure 10b), and *Pseudo-nitzschia* had its largest bloom at both locations at around 450 mmol/m$^2$ (Figure 9b and 10b). *Chaetoceros* appeared to more closely follow PAR parameters as it appeared only above the threshold of around 350 mmol/m$^2$, with a peak at 475 mmol/m$^2$ (Figure 9a and 10a).

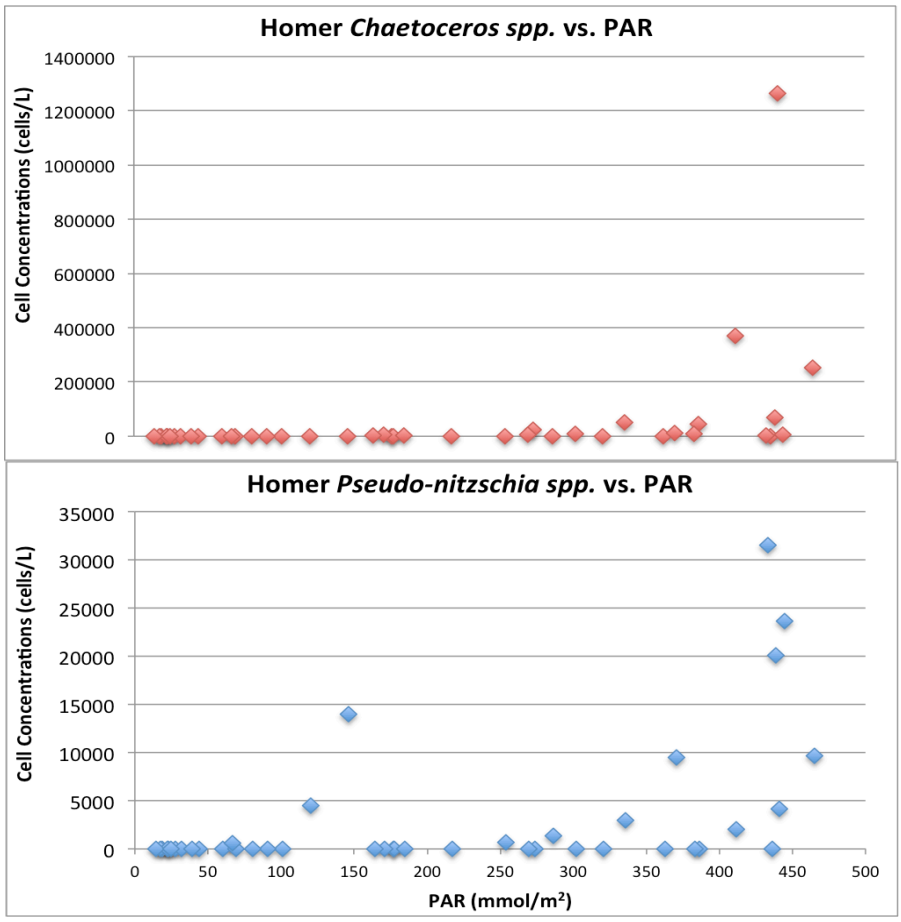


**Figure 9a & 9b:** Transect 9 Station 10 monthly average a) *Chaetoceros* and b) *Pseudo-nitzschia* concentrations vs. Homer meteorological PAR 2012-2015.

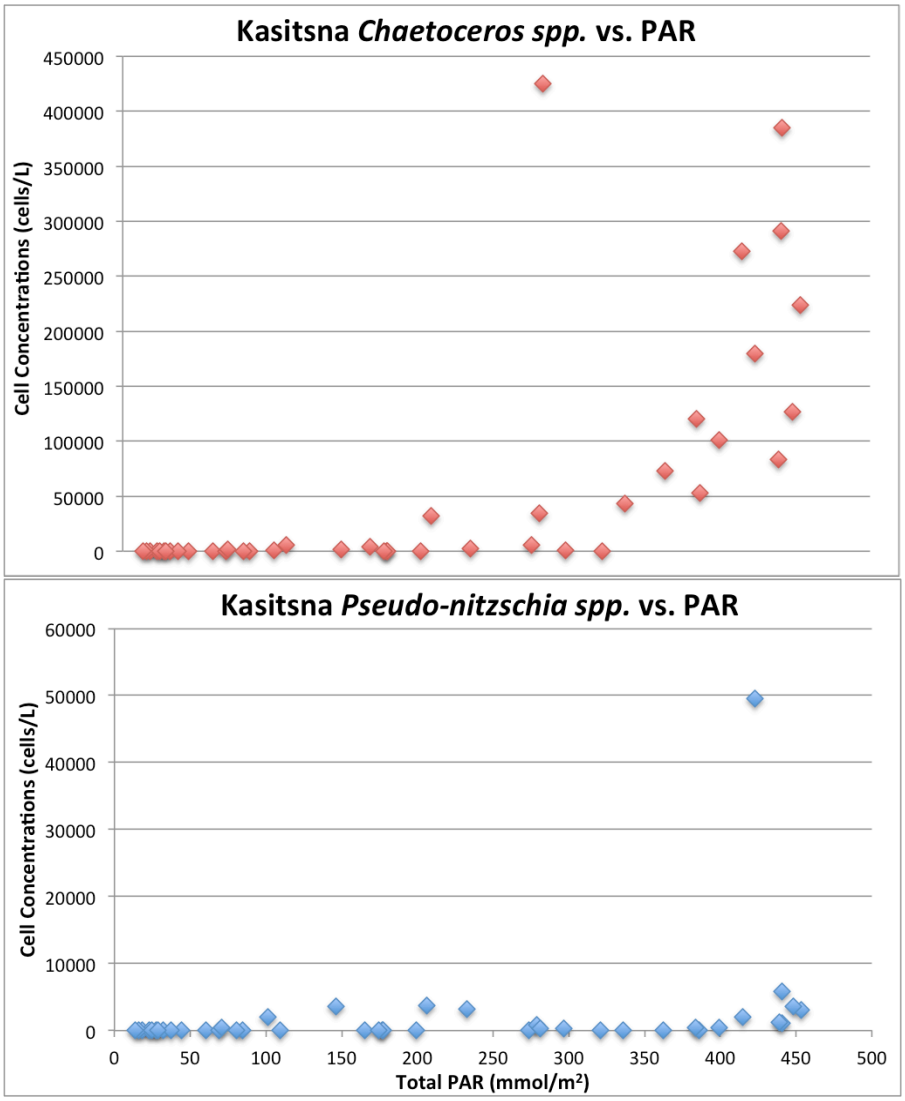


**Figure 10a & 10b:** Kasitsna Bay monthly average a) *Chaetoceros* and b) *Pseudo-nitzschia* concentrations vs. Homer meteorological PAR 2012-2015.

### *3.3.2 Water Quality Factors*

#### *3.3.2.1 Water Temperature*

At Homer, there appeared to be an inter-annual temperature cycle with warmer than average temperatures from 2003-2006 and cooler temperatures from 2007 to the autumn of 2013 with the lowest temperature anomalies found in

January 2009 (Figure 11). There was a transition to warmer than average conditions and there have been consistently warmer summers and winters since October 2013, which has persisted since then with the warmest anomalies seen in the winter months (Figure 11a). Seldovia water temperatures showed a similar pattern compared to Homer, but with some differences in timing and magnitude of the temperature anomalies (Figure 11 & 12). For instance, in Seldovia the coldest temperature anomalies were observed in the winters of 2007 and 2012, versus 2009 in Homer. The transition to warmer than average water temperatures in fall 2013 was observed at both Homer and Seldovia and was part of anomalously warm conditions observed throughout the northeast Pacific Ocean that have continued into early 2016 (Figure 11 & 12).

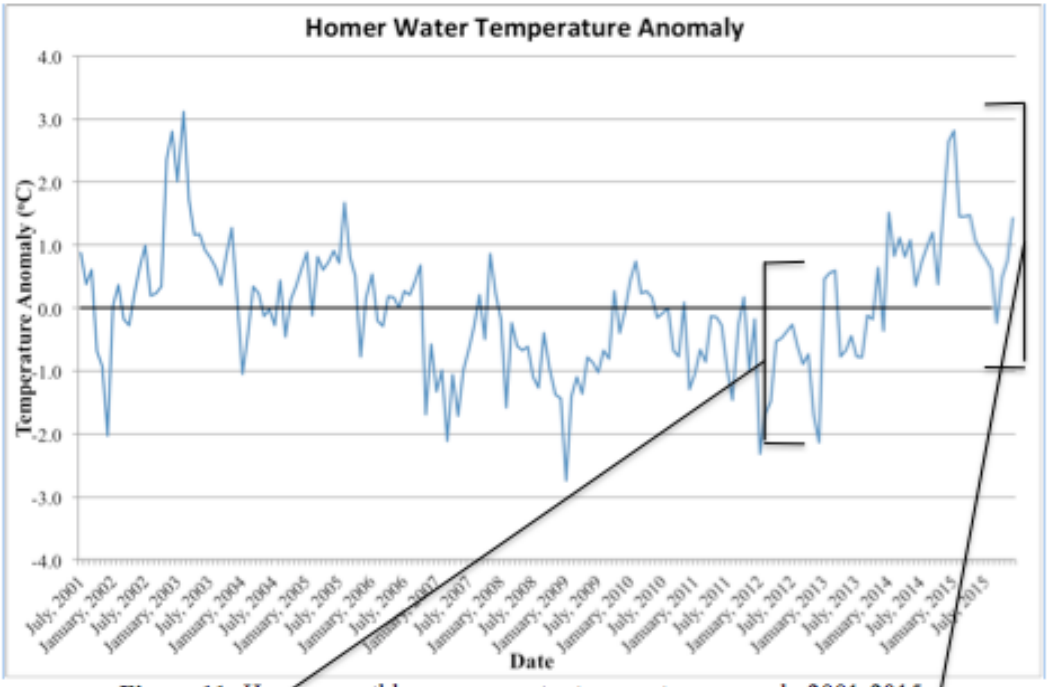


**Figure 11:** Homer monthly average water temperature anomaly 2001-2015.

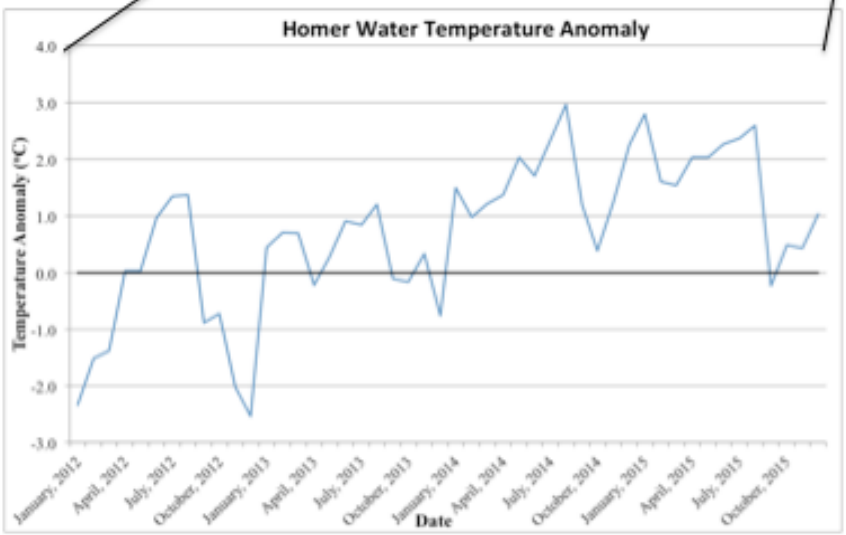


**Figure 11a:** Zoomed in version of Homer monthly average water temperature anomaly 2012-2015.

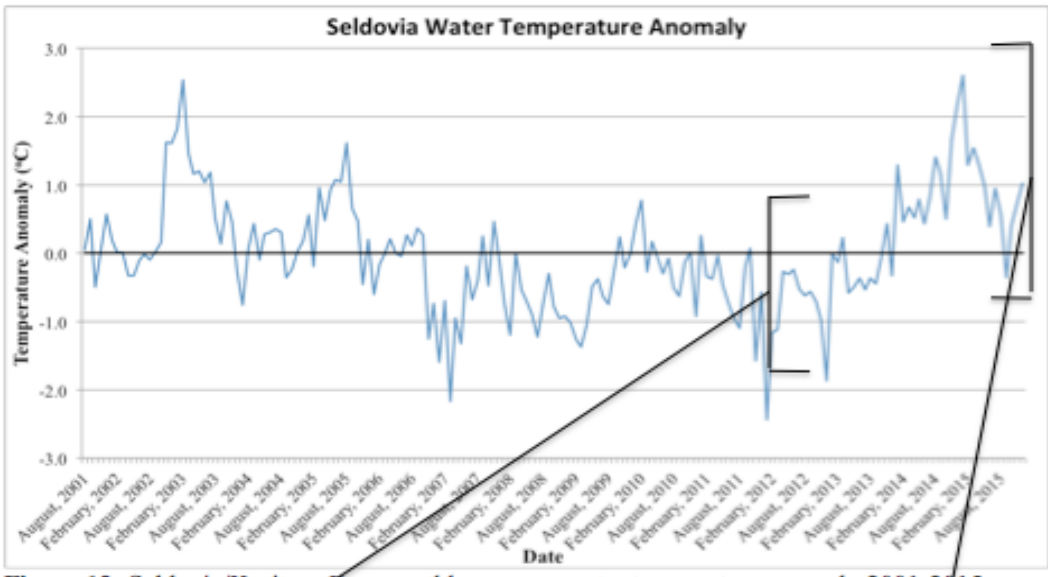


**Figure 12:** Seldovia/Kasitsna Bay monthly average water temperature anomaly 2001-2015.

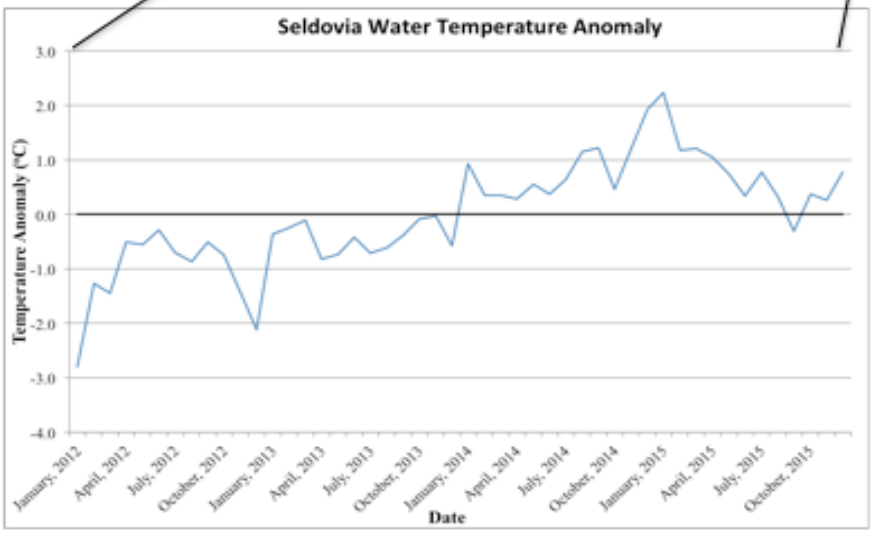


**Figure 12a:** Zoomed in version of Seldovia/Kasitsna Bay monthly average water temperature anomaly 2012-2015.

*Chaetoceros* and *Pseudo-nitzschia* both appear to closely follow temperature albeit at different ranges (Figure 13 and 14). At Homer, *Chaetoceros spp.* were only seen around their peak at 7°C; unlike at Kasitsna, where they were present around a variety of temperatures, 4-12°C peaking between 7-8°C (Figure

13a and 14a). At Homer, *Pseudo-nitzschia spp*. were found between 7-9.5°C and peaked at 9.5°C, but in Kasitsna they were found at a peak of 8°C (Figure 13b and 14b).

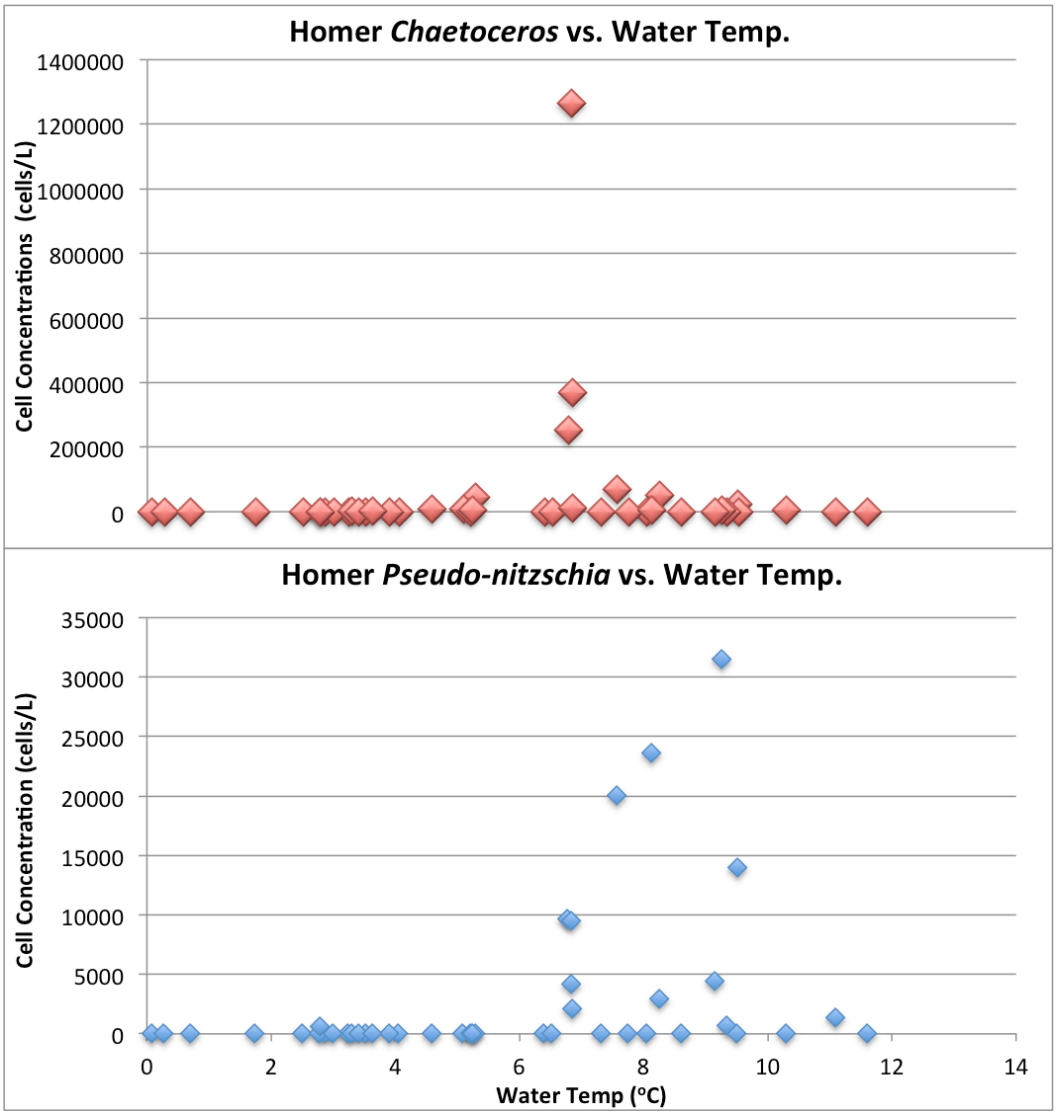


**Figure 13a & 13b:** Transect 9 Station 10 monthly average a) *Chaetoceros* concentrations and b) *Pseudo-nitzschia* concentrations vs. Homer Harbor SWMP water temperature 2012-2015.

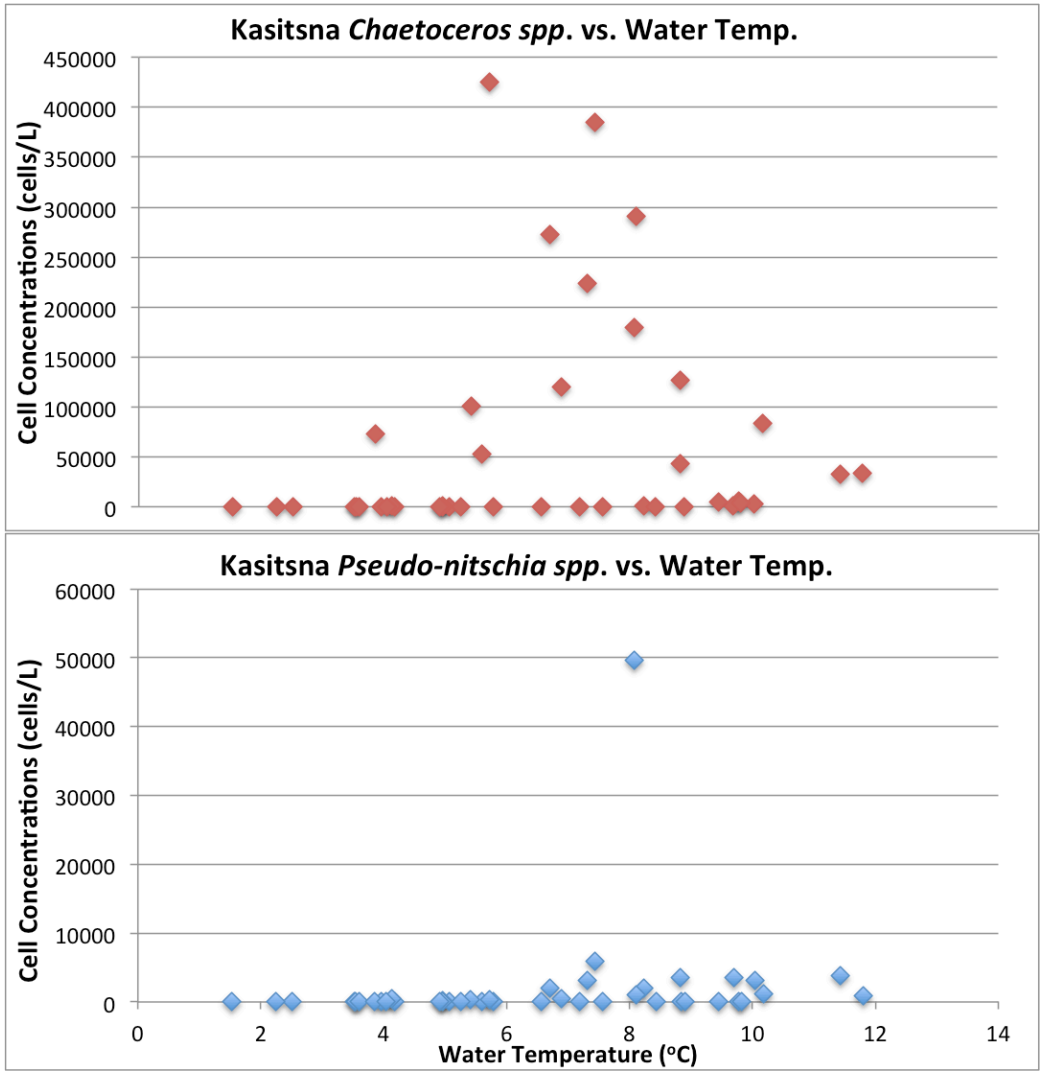


**Figure 14a & 14b:** Kasitsna Bay Lab monthly average a) *Chaetoceros* and b) *Pseudo-nitzschia* concentrations vs. Seldovia SWMP water temperature 2012-2015.

*3.3.2.2 Salinity*

At both sites, salinity decreased in the spring and summer time and increased during the fall and winter-time (Figure 15a and 15b). Salinity decreased in the spring and summer most likely due to the spring melting (Figure 15a and 15b). During the warmer years of the inter-annual cycle, salinity was lower than

in the colder years, especially in 2015 there was a pronounced decrease (Figure 15a and 15b).

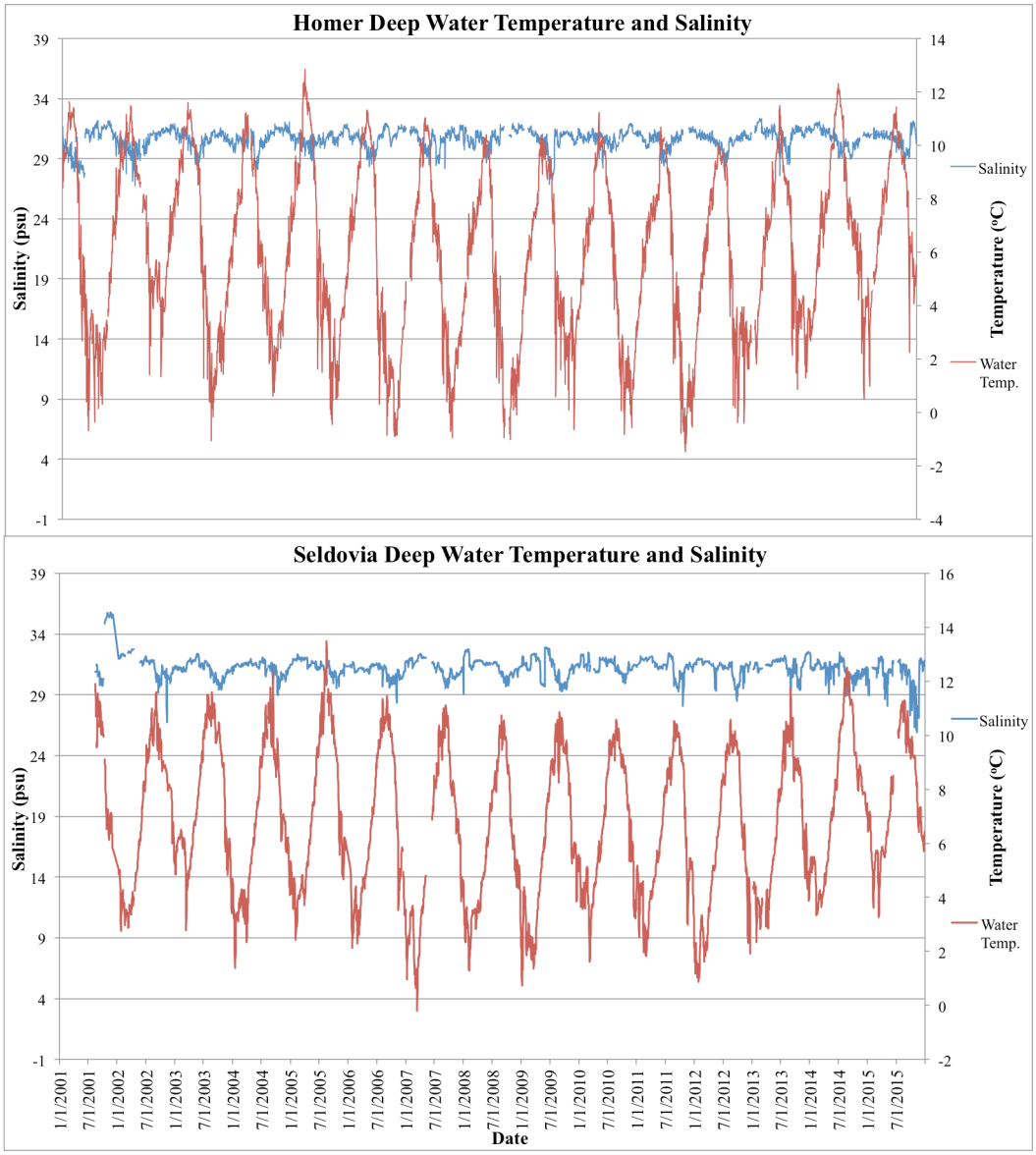


**Figure 15a & b:** Graph of a) Homer Deep and b) Seldovia Deep Water Quality Station's daily average temperature and salinity throughout 2001-2015.

At Homer, *Chaetoceros spp.* first appeared and were most abundant at 31.5 psu; although, they were present up until 32 psu (Figure 16a). At Kasitsna,

*Chaetoceros spp.* were present anywhere between 30-32 psu peaking at around 31-31.5 psu (Figure 17a). At Homer, *Pseudo-nitzschia spp.* were present anywhere between 29.5-32 psu, and were most abundant between 31-32 psu (Figure 16b). Whereas at Kasitsna, *Pseudo-nitzschia* were present between 31-31.5 psu and peaked at 31.25 psu (Figure 17b).

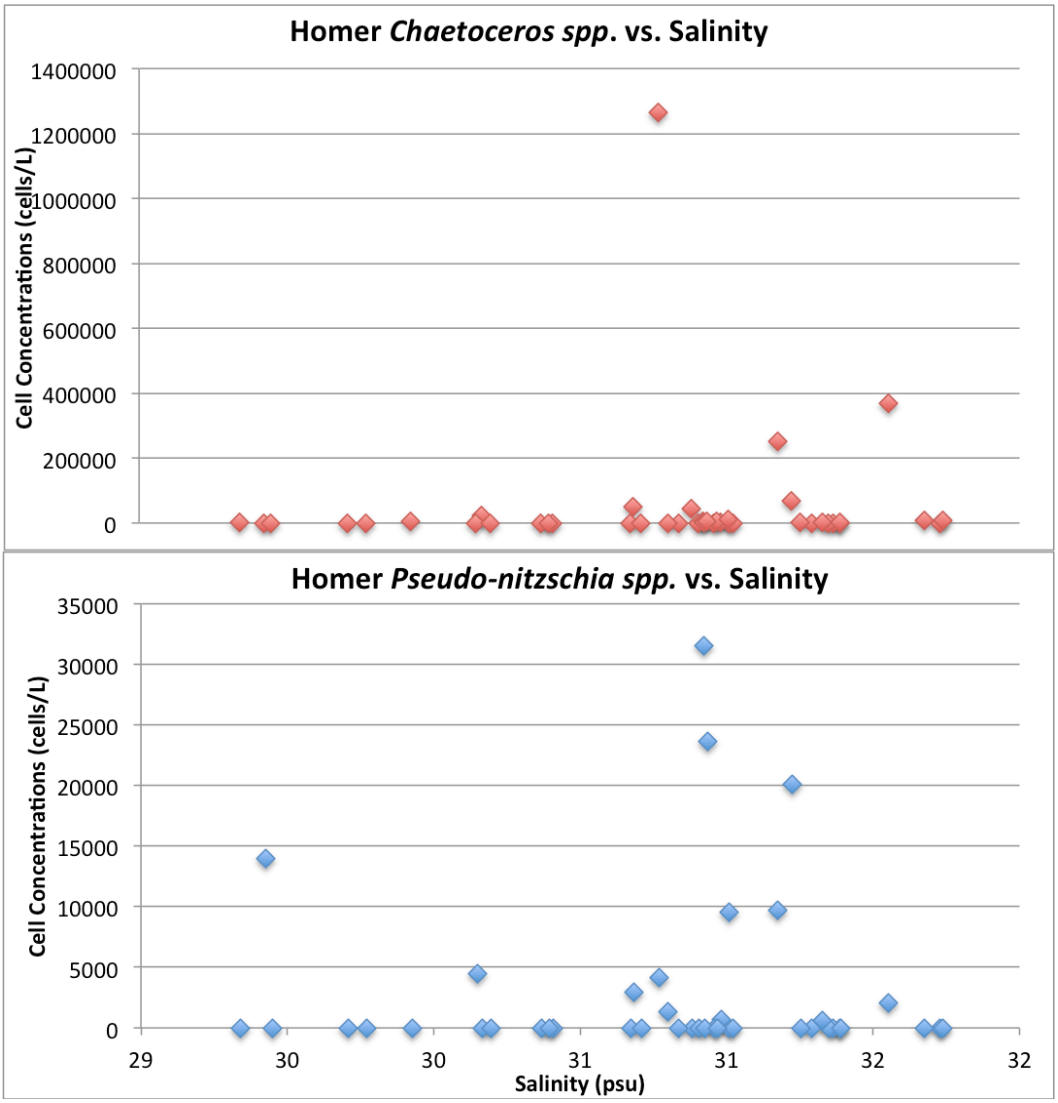


**Figure 16a & b:** Transect 9 Station 10 monthly average a) *Chaetoceros* concentrations and b) *Pseudo- nitzschia* concentrations vs. Homer Harbor SWMP salinity 2012-2015.

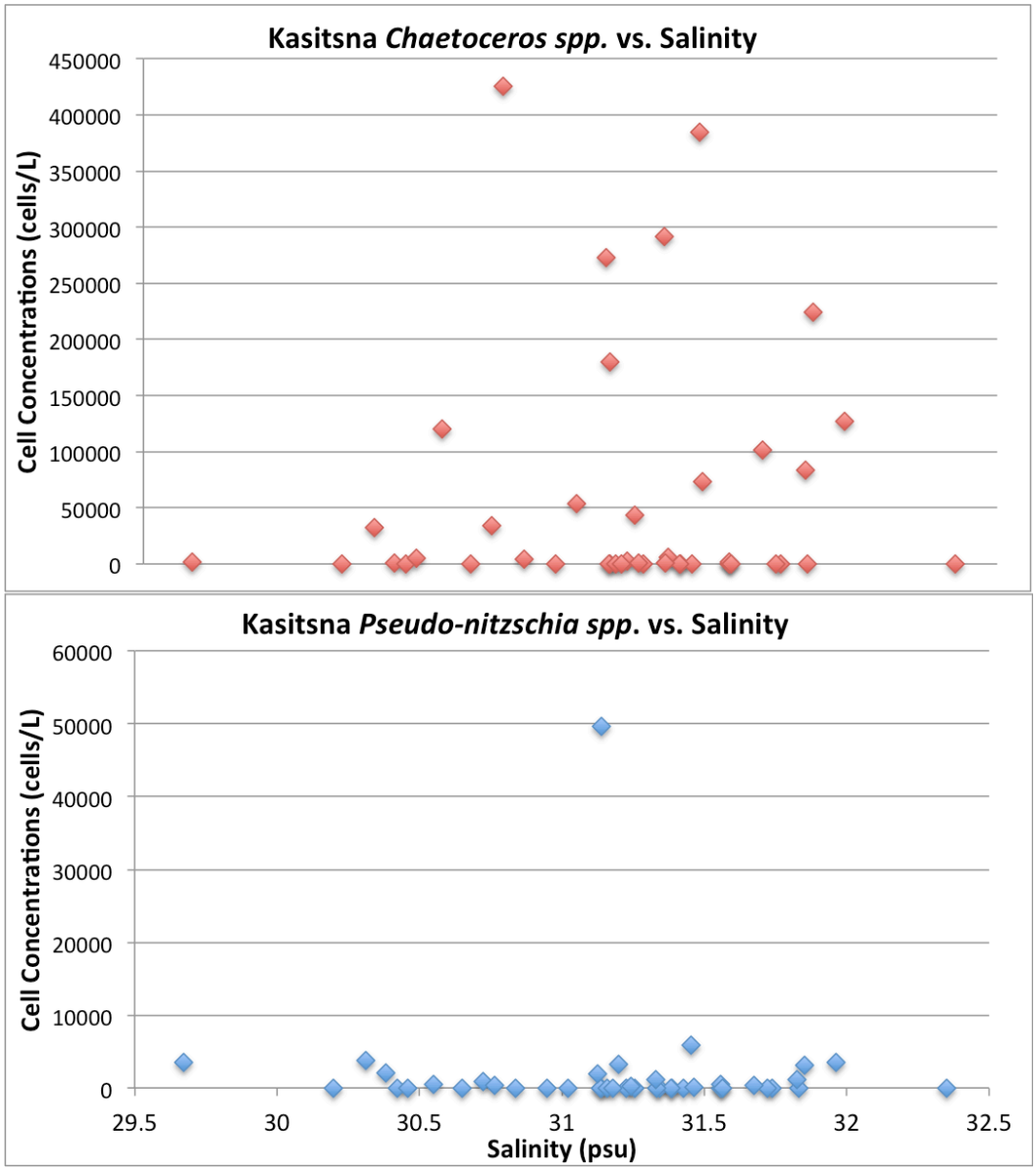


**Figure 17a & b:** Kasitsna Bay monthly average a) *Chaetoceros* concentrations and b) *Pseudo- nitzschia* concentrations vs. Seldovia SWMP salinity 2012-2015.

*3.3.3 Nutrient Influences*

At Homer, nitrite+nitrate concentrations decreased each year while orthophosphate concentrations increased from 2012-2014, but the standard error for both was very large (Figure 18a & b). When I conducted an ANOVA to see if

there was a significant difference between the years at Homer, I found that there was no difference in the average orthophosphate concentrations (F=.954, and p=0.425), nor was there a difference in the average nitrite+nitrate concentrations (F=1.85 and p=0.906). In comparison, N:P ratio decreased each year from 2012 to 2014, but this was not found to be significant (F=1.23 and p=0.313) (Figure 18c). When I conducted an ANOVA to see if there was a difference at Kasitsna, I found that there was no significant difference in the average orthophosphate concentrations (F=2.018, and p=0.128), nor was there a difference in the average nitrite+nitrate concentrations (F=1.27 and p=0.299). The N:P ratio decreased over time, and they were significantly different between the years (F=7.99 P=.001) (Figure 19a and 19c).

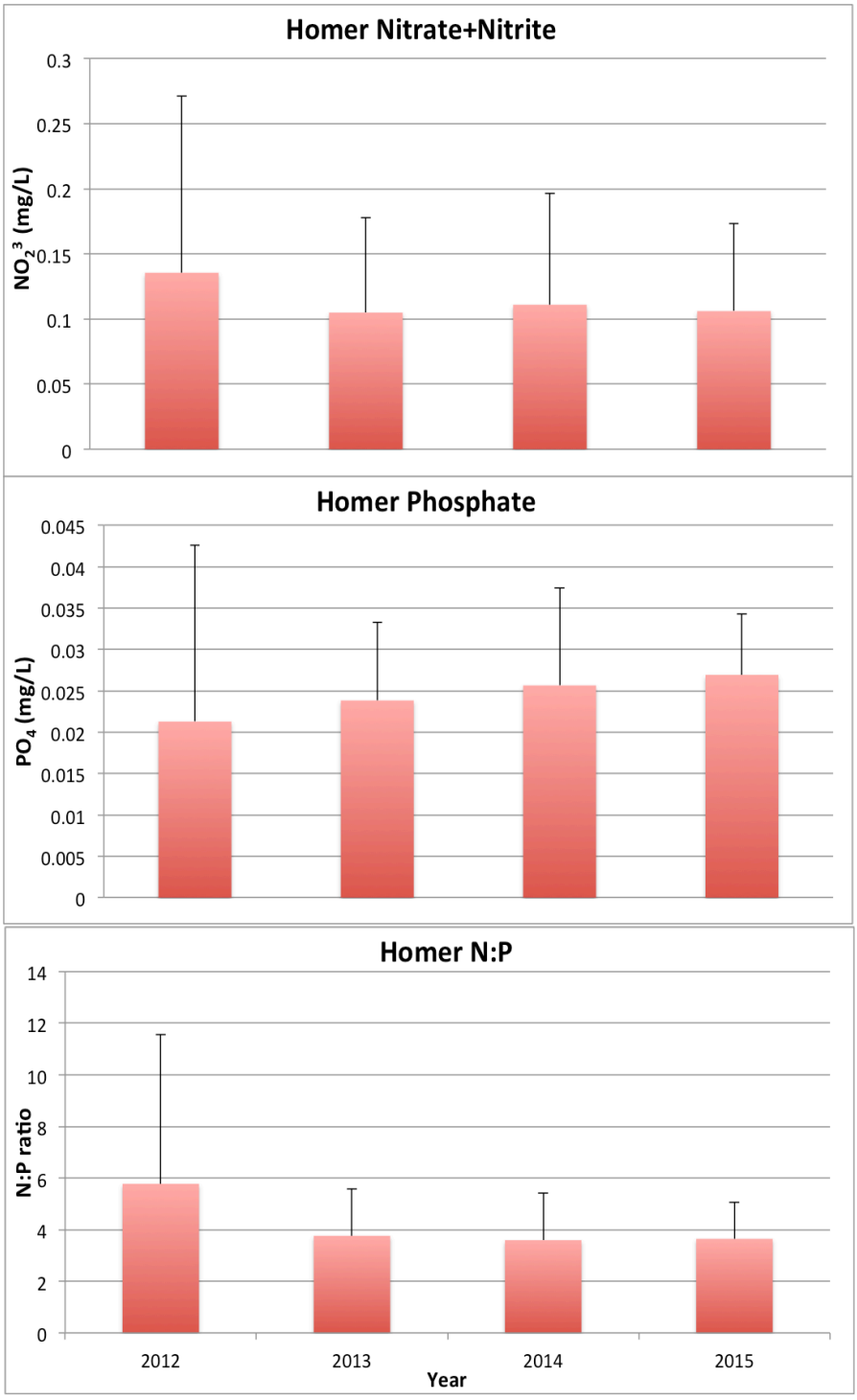


**Figure 18a, 18b, & 18c:** Homer yearly average a) nitrite+nitrate concentrations b) orthophosphate concentrations and c) N:P ratio comparisons with error bars illustrating the standard error.

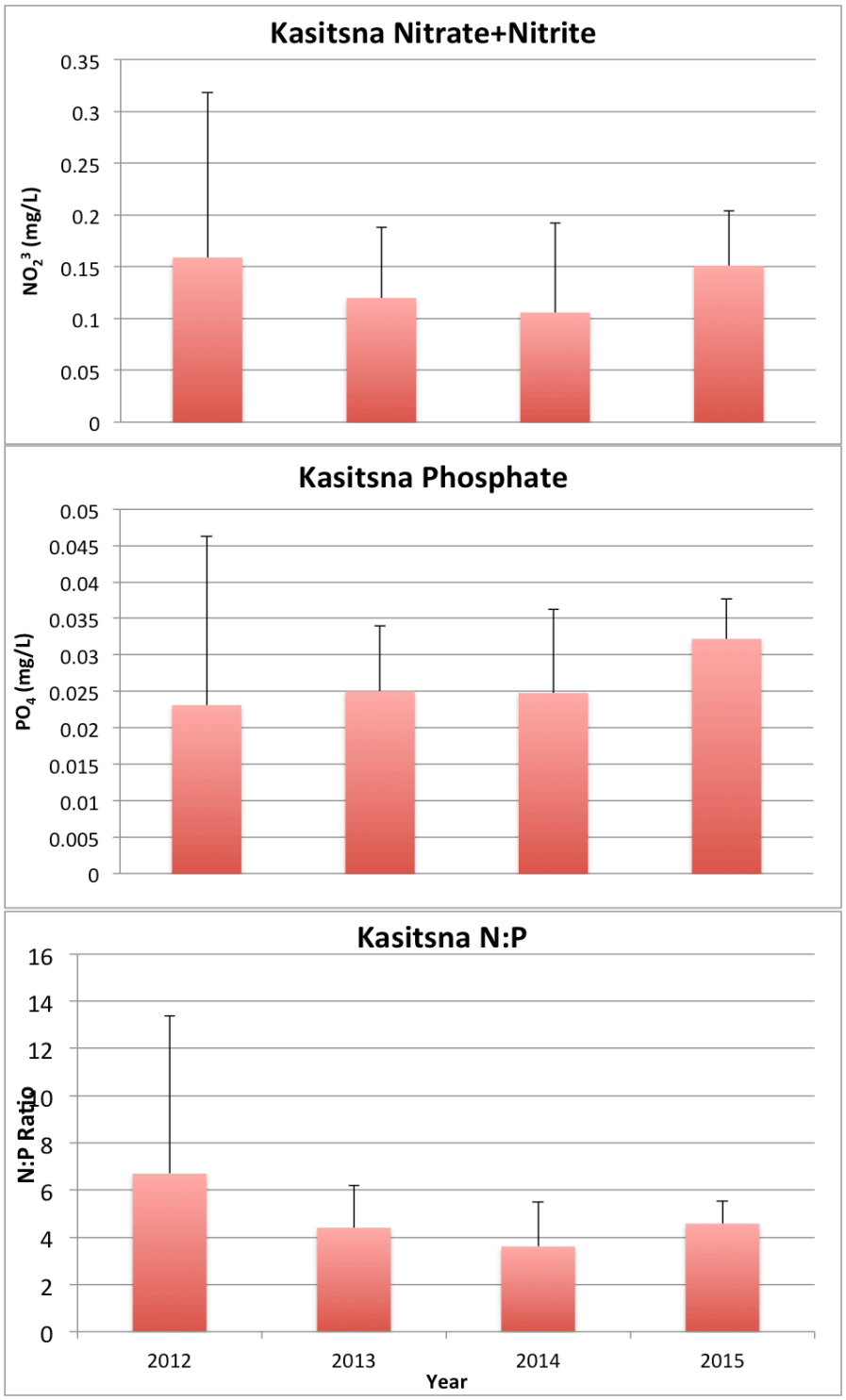


**Figure 19a, 19b, and 19c:** Kasitsna yearly average a) nitrite+nitrate concentrations b) orthophosphate concentrations and c) N:P ratio comparisons with error bars illustrating the standard error.

Neither *Chaetoceros* nor *Pseudo-nitzschia* cell concentration patterns appeared to particularly follow the N:P ratio closely with wide variations in the ratios (Figure 20 and 21). At Homer, *Chaetoceros* was present particularly in areas with an N:P ratio between 1-3, with a peak around 2.5 (Figure 20a). At Kasitsna, *Chaetoceros spp.* were prevalent anywhere between 0.5-6, with no apparent peak (Figure 21a). At Homer, *Pseudo-nitzschia spp.* were most prevalent between N:P ratios of 1-3, but were also present at a ratio of 5.5 (Figure 20b). At Kasitsna, *Pseudo-nitzschia spp.* were found anywhere between .5-7 but were at peak at around 6-7 (Figure 21b).

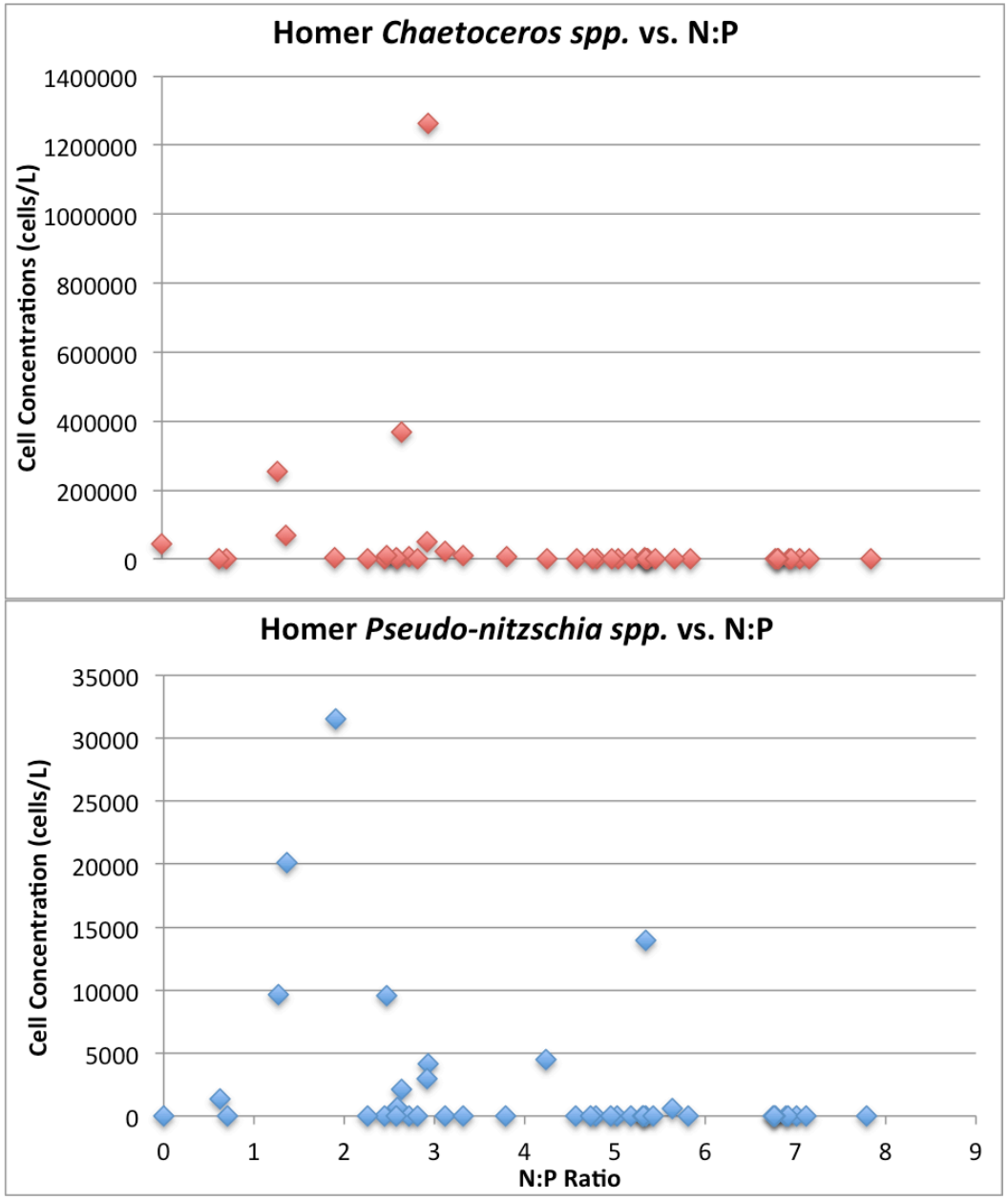


**Figure 20a & b:** Homer monthly average a) *Chaetoceros* concentrations and b) *Pseudo- nitzschia* concentrations vs. Homer Harbor SWMP N:P ratios 2012-2015

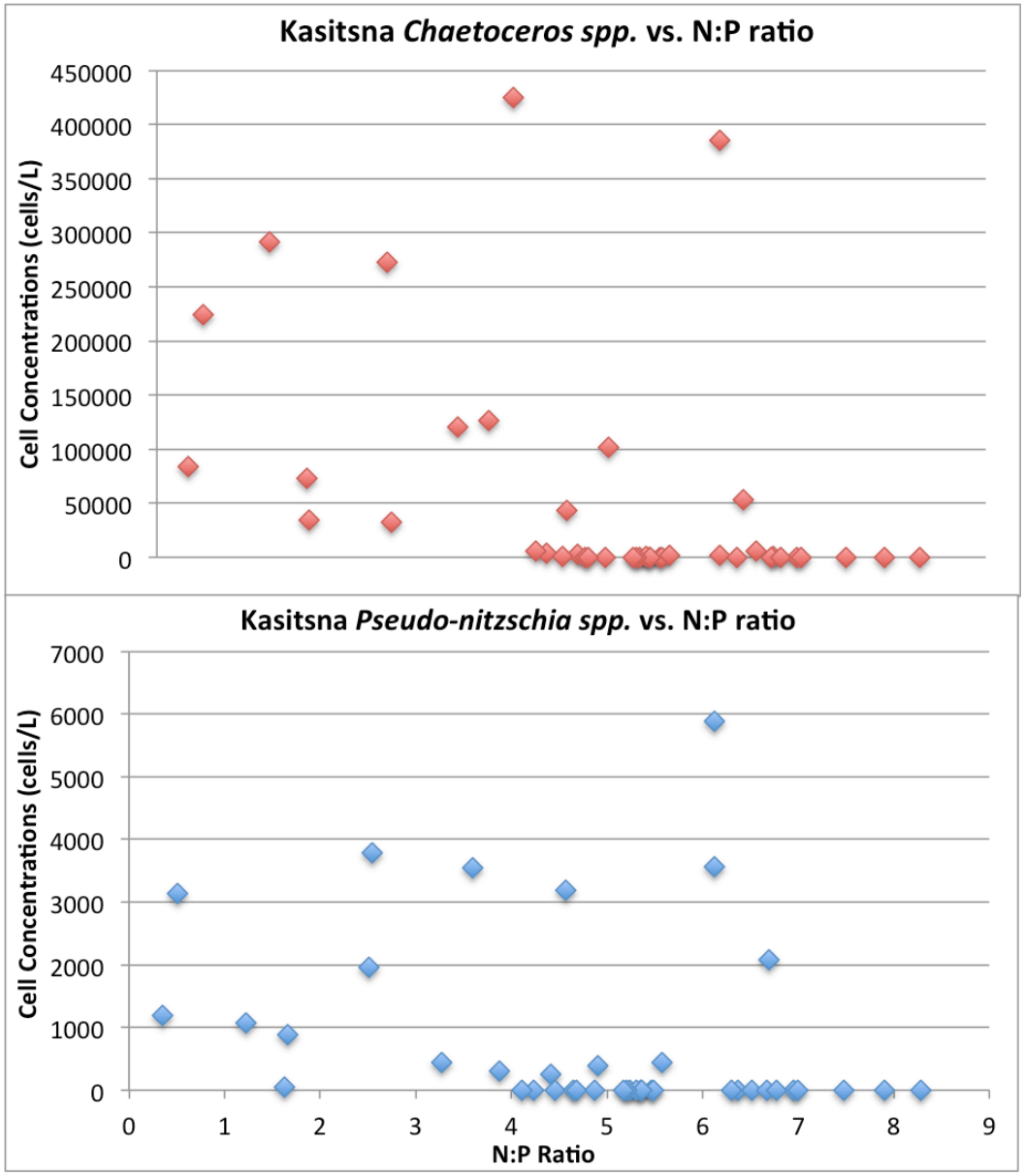


**Figure 21a & 21b:** Kasitsna monthly average a) *Chaetoceros* concentrations and b) *Pseudo- nitzschia* concentrations vs. Seldovia SWMP N:P ratios 2012-2015.

# DISCUSSION

Harmful algal blooms (HABs) are increasing in frequency, intensity, and global distribution, and there are a variety of factors that are believed to lead to this increase such as climate change and eutrophication (Anderson *et al*., 2012; McLean and Sinclair, 2012; Hallegraeff, 1993). Kachemak Bay is an important location to study because many of the common causes of HAB increases, such as eutrophication and major shipping, are not a significant problem for Alaska, making it the perfect place to study the effects of climate variables on phytoplankton in isolation. The location has huge commercial and recreational fishing and hunting enterprises; however, while commercial harvests are tested for harmful substance, recreational harvests are not, which means that people who dig for clams are at risk for amnesic shellfish poisoning and other phytoplankton toxin poisoning (Walker and Field, 2003). That is why it is crucial to understand the environmental conditions that lead to HABs throughout the area, in order to anticipate when they are more likely to occur, which will help researchers know when to notify recreational clammers. The environmental conditions that have been shown to have a strong influence on phytoplankton concentrations and species composition are photosynthetically active radiation (PAR), temperature, salinity, and nutrients (Macedo and Duarte, 2006; and Zonnevald, 1998). It is important to focus on Homer because it is a common location for fishing, and Kasitsna because it is a common location for shellfish farming and recreational shellfish harvests. I expected the phytoplankton concentrations and compositions

at the two locations to be different because the Kasitsna Bay experiences more oceanic conditions and Homer is influenced more by freshwater input, so the two locations may differ in salinity, water temperature, and nutrients.

*4.1 PAR and Phytoplankton*

PAR, measured at Homer, rose after the winter solstice and peaked in June, around the summer solstice (Figure 7b & 8b). Other studies found that *Chaetoceros* begins to increase at a PAR threshold of approximately 380 millimoles/$m^2$ (Hondolero *et al.*, 2014), and the species bloomed at the max PAR of the season, which is usually in May or June. These results were very similar to what I found as *Chaetoceros* only appeared between 350-475 mmol/$m^2$ (Figures 9a & 10a). I also found that *Chaetoceros* significantly increased when PAR increased at both locations (Table 1a: Models 1 & 2). In the compiled model, *Chaetoceros spp.* were most correlated with PAR from two weeks previous, suggesting that earlier PAR is much more important to *Chaetoceros* appearance and growth (Table 1a: Model 4). In comparison, *Pseudo-nitzschia* can be found in a wider range of PAR conditions ranging anywhere between 150-450 mmol/$m^2$ (Figures 9b & 10b). This lag gives an indication of how fast the phytoplankton respond to increasing light, as *Pseudo-nitzschia spp.* grew at lower light levels and responded slower than *Chaetoceros* (*pers. comm.* Holderied, 2016). This coincided with studies that found PAR to have a strong positive correlation with both species and that *Chaetoceros* more quickly responded to environmental conditions (Daniels *et al.*, 2015).

The individual and compiled model both have their strengths and weaknesses. For instance, with the individual model you are able to see the individual parameters and how they impact phytoplankton, but when you do this there is the possibility of illusory correlation because of omitted variable bias. Illusory correlation occurs when you are not taking into account other factors that may have a more significant impact on phytoplankton concentrations, leading you to think that there is a relationship even when one does not exist. I attempted to overcome this problem by using a compiled model, but the compiled model creates a new problem associated with multi-colinearity bias. This means that if you use two related environmental variables in a model there is the possibility that the independent variables are more closely related to each other than to the dependent variable, which will change the significance of your results. In order to overcome this, I did not include as many variables in the compiled model, such as several nutrient factors, in order for it to work properly. In addition, in order for this model to work I had to pull out the "outlying" 2012 *Chaetoceros* bloom, because statistically it quantified as an outlier; although, biologically it can be explained. Another problem with the compiled model is that something that might be impacting phytoplankton is considered insignificant in the model, because other included factors might be more strongly associated with the phytoplankton. Considering the differences in the two types of models, there were some discrepancies in the models that led to opposing results, such as the negative correlation between water temperature and *Chaetoceros* in the compiled model

compared to the expected positive correlation in the individual models (Table 1a: Models 1, 2, & 4). This may be because the rapid response of phytoplankton to multiple environmental factors are complex, and the sampling frequency is not always sufficient to capture rapid changes in environmental conditions and cell concentrations (*pers. comm.* Holderied, 2016).

*4.2 Temperature and phytoplankton*

Air and water temperatures began to rise quickly in April, and peaked around July, and in the graph they appeared to lag behind PAR (Figures 7b & 8b). The compiled model also showed a lag, and in the model the lag was found to be most significant at 2 weeks (Table 1a: Model 4). This exact timing might not be as precise considering the graph and the model are using two different time averages, as the graphs were daily and the model was biweekly. With more data, we could more accurately tell how long it was lagged. However, the lag of two weeks suggest that the atmosphere and ocean respond very quickly to environmental changes leading to fast changes that may make it difficult or beneficial for certain phytoplankton to respond to. In addition to this yearly pattern, there is an overlaying inter-annual variability in temperature that persists over multiple years impacting air and water temperatures (*pers. comm.* Holderied 2015).

This inter-annual cycle was relatively similar between the two locations with some differences occurring in regards to when exactly temperatures were anomalously cold in the cycle, which occurred for unknown reasons. However,

beginning in the fall of 2013, the Pacific Ocean "Blob," a natural ocean circulation pattern associated with an influx of warm air and water, was attributed to the transition to warmer temperature in our data (Bond *et al.,* 2015; *pers. comm.* Holderied). In addition, our data also illustrated historic highs in 2015 that were more than 2$^{o}$C above normal, (*pers. comm.* Holderied, 2015; Sinclaire, 2015), which is believed to be a result of the compounding of the "Blob" as well as El Niño (Sinclaire, 2015). This illustrates that the estuary waters of Kachemak Bay are tightly connected to changes in the adjacent ocean waters (*pers. comm.* Holderied, 2015).

I found that temperature influences phytoplankton, as phytoplankton appear in early April and/or June when temperatures are increasing, and they disappear by early October, when temperatures are decreasing. From four years of monitoring data, I found that *Chaetoceros* dominated the phytoplankton community in Kachemak Bay. In such subarctic environments, *Chaetoceros* has been found to dominate in the early spring and summer (Waite, Bienfang, and Harrison, 1992), which was similar to what I found. Other studies also found that *Chaetoceros* had a temperature threshold of 6-7$^{o}$C (Nedwell, 1999), which was also similar to what I found, especially at Homer (Figure 13b). At Kasitsna, *Chaetoceros spp.* were present at a wider variety of temperatures. This may be because this location has other favorable factors, such as nutrients, that make it favorable for the low temperature-tolerant species of this genus to bloom (Figure 13b). This was corroborated by *Chaetoceros* having a significant correlation with

water temperature only at Kasitsna suggesting that of the two places, *Chaetoceros spp.* may be more limited by temperature at Kasitsna than Homer, but they were more numerous at Kasitsna, as expected (Table 1a: Model 2). Higher temperatures have been found to disfavor *Chaetoceros* (Trigueros and Orive, 2001), most likely because at higher temperatures other species can outcompete *Chaetoceros* (Daniels *et al.,* 2015).

In the combined model, this pattern is quite different. In this model, I found that *Chaetoceros* had a negative correlation with temperature, but only when it was in a quadratic form (Table 1: Model 4). The negative correlation in the combined model is unusual for my results, which usually had positive correlations indicating that increasing temperatures were associated with increasing phytoplankton. This negative quadratic result may be because I combined both locations in this model, or because this is such a complex system. Phytoplankton are only found above certain thresholds, which may make it difficult for the model to correlate the cycling of temperature in periods like the winter, which was captured in the quadratic formula, to coincide with phytoplankton.

*Pseudo-nitzschia spp.* were mostly found in the later periods of the summer, August and September (Figures 7 & 8). Other studies have found that *Pseudo-nitzschia* has been found in temperatures between $5^{o}$C-$27^{o}$C, with an optimum temperature of around $10^{o}$C (Anderson *et al.*, 2010). This optimum coincided to the temperatures that I found, 7-9.5$^{o}$C at Homer, and 10-12$^{o}$C at

Kasitsna. The temperature range for *Pseudo-nitzschia* was not as wide as what others have found, possibly because of the particular *Pseudo-nitzschia spp.* in the area. Air and water temperatures at both locations in the individual model and compiled model were significantly correlated with *Pseudo-nitzschia,* more so than *Chaetoceros,* most likely because *Pseudo-nitzschia* is more sensitive to temperature (Table 1a: Models 1, 2, &3), as expected (Anderson *et al.*, 2010).

*4.3 Salinity and Phytoplankton*

In correspondence with the seasonal temperature changes, there are also seasonal changes in salinity. For instance, salinity drops in the summer time and is usually lowest in August/September (Figures 7 & 8), because of the melting of freshwater glaciers and snow from the nearby mountains into the ocean (Scott *et. al.,* 2002), which makes it less salty, especially at Homer. In the model, I found that there was a significant difference in the salinity at the two locations, which I had expected to be attributed to a stronger influence of freshwater input from rivers, glaciers, and snow melt at Homer versus a stronger influence from ocean upwelling at Seldovia (Table 1a: T-test). However, the model showed that salinity had a positive relationship from Seldovia to Homer suggesting that it was saltier at Homer, for reasons that are unknown (Table 1a: T-test). Despite their salinity differences, at both locations, they illustrate the same pattern where in the fall and wintertime it gets saltier because of a reduction in freshwater inputs from snowpack and glacier melt (Scott *et al.,* 2002).

Other studies found that lower salinities disfavor *Chaetoceros* (Trigueros and Orive, 2001), which might explain why earlier in the spring when the salinity was higher, *Chaetoceros* tended to dominate. This reasoning contradicts what we found in the combined model, as *Chaetoceros spp.* were significantly different between the two locations (Table 1a t-test), and their concentrations were actually higher at Kasitsna. When I looked at the two variables side by side I found a distinct salinity threshold for *Chaetoceros* at 31 psu (Figures 16a & 16b). Other studies have shown a positive correlation between *Chaetoceros* and salinity (Trigueros and Orive, 2001), even though they were not significantly correlated in the model (Table 1a: Models 1, 2, & 3). Given the typical high range of salinity in my study location, salinity may not significantly limit *Chaetoceros* or *Pseudo-nitzschia* growth (*pers. comm.* Holderied, 2016). This means that salinity is not a limiting factor in Kachemak Bay, which may also make it a more favorable environment for *Chaetoceros*, since they have been shown to prefer higher salinities. I also looked at salinity and *Pseudo-nitzschia* and found that there was no significant correlation between them, and from my observations, I found that *Pseudo-nitzschia spp.* were present at a wide range of salinities, which other studies have found as well (Thessen *et al.,* 2005).

*4.4 Nutrients and phytoplankton*

Nutrient patterns were similar to salinity as they increased and reached their peak in the winter and decreased in the summer (Figures 7 & 8). However, unlike salinity and the other independent environmental parameters, nutrient

patterns are strongly influenced by phytoplankton (Tilman *et al.,* 1982). Due to frequent upwelling in the area nutrients build up over the winter when it is too cold for the phytoplankton to survive, then when phytoplankton appear in the spring, they take up most of the nutrients (Tilman *et al.,* 1982; Trigueros and Orive, 2001). Other studies found that spikes in phytoplankton preceded low points in nutrients (Tilman *et al*., 1982). In this study, I captured this strong association finding that increased phytoplankton from two weeks previous correlated to current decreases in nutrients suggesting that both species significantly impact nutrient concentrations (Table 1a: Models 1 & 2- Reverse Nutrients)

Not only do nutrients rely on phytoplankton, but also phytoplankton rely on nutrients. For instance, starting in early spring when *Chaetoceros* began to bloom, the nutrients rapidly decrease, most likely because of the high concentrations of *Chaetoceros* utilizing them (Hondolero *et al.*, 2014). In July, the nutrients were lowest and there is low phytoplankton abundance (Hondolero *et al.*, 2014). Then, the nutrients rose a little during August, which coincides with a second albeit smaller bloom of another species, usually *Pseudo-nitzschia* (Hondolero *et al.*, 2014). In the individual model, I found that both species were negatively associated with nitrite+nitrate at both locations. This means that both species are found to increase while nitrite+nitrate decreases, which is most likely due to phytoplankton taking in the nutrients (Table 1a: Models 1 & 2). When I looked at this in greater detail in the compiled model, I found that *Chaetoceros*

*spp.* were significantly correlated with nitrite+nitrate from the month before at both locations, but *Pseudo-nitzschia spp.* were not correlated to current or previous nitrite+nitrate concentrations (Table 1a: Model 4). In comparison to the rest of the environmental factors, *Chaetoceros* very strongly depends on the nutrient concentrations while *Pseudo-nitzschia* does not appear to (Table 1a: Model 4) This most likely means that nutrient concentrations may not get too low for *Pseudo-nitzschia* but may get too low for *Chaetoceros* suggesting that these two species are optimized for different temperature ranges (*pers. comm.* Holderied, 2015). In the individual models, orthophosphate was negatively associated at both locations with *Chaetoceros* and was significantly and somewhat significantly correlated with *Pseudo-nitzschia* at Homer and Kasitsna, respectively (Table 1: Models 1 & 2). While I was unable to include orthophosphate in the compiled model because of multi-collinearity, you can see that *Pseudo-nitzschia* does not seem to rely on this nutrient as much suggesting that it may not be as limiting as nitrite+nitrate (Tilman *et al.*, 1982).

*Pseudo-nitzschia* may not need high nutrient conditions because it has evolved to better uptake nutrients in nutrient poor conditions by blooming later on and using domoic acid. That is why once *Chaetoceros* originally dies off, *Pseudo-nitzschia* is able to establish and outcompete *Chaetoceros* filling the niche it left and competing against *Chaetoceros spp.* (Daniels *et al.,* 2015). In some cases, it was found that domoic acid, the toxin released by *Pseudo-nitzschia* that causes ASP, actually helped *Pseudo-nitzschia* compete with other diatoms by reducing

the other species' growth and boosting *Pseudo-nitzschia'*s growth as they may be better able to acquiesce iron (Prince *et al.*, 2013). In Kachemak Bay, given the sources of freshwater input in the estuary, we do not expect iron to be limiting to phytoplankton growth (*pers. comm.* Holderied, 2016).

Interestingly enough, according to the compiled model *Pseudo-nitzschia spp.* were the only genus found to significantly increase over time and it did so at Kasitsna (Table 1a: Model 4). This may be because of increasing temperatures allowing *Pseudo-nitzschia* to better survive there. This kind of change in species composition may affect the nutrient availability of the location as *Pseudo-nitzschia* strongly relies on nitrogen forms and readily depletes them in environments where they are present (Quijano-Scheggia *et al.,* 2008). I was actually able to see this difference between the species when looking at *Pseudo-nitzschia*'s higher N:P ratio threshold (Figure 20a and 20b). When looking at nutrient comparisons, while there was no significant difference seen in nitrite+nitrate over time, there was a significant decrease in N:P over time at Kasitsna (Figure 19). In addition, the N:P ratio was only significantly correlated with phytoplankton at Kasitsna and it was correlated with both species suggesting that the larger *Pseudo-nitzschia* cells may currently be causing a shift in nutrient regimes at Kasitsna, as they uptake more nutrients (Table 1a: Model 2). When looking at the two locations, there was a significant difference in the N:P ratio between the two locations (Table 1a: T-test). At Homer, where *Pseudo-nitzschia spp.* were more abundant (Table 1a: Model 4), there was a significantly lower N:P

ratio, perhaps because of *Pseudo-nitzschia* more readily using resources at this location (Table 1a: T-test). The changing nutrient dynamics might explain why there was a wider distribution of the two species in regards to N:P, at Kasitsna (Figure 21a and 21b).

*4.5 Environmental Parameters Explain the 2012 Chaetoceros Bloom*

The noteworthy 2012 *Chaetoceros* bloom in Homer can be explained by looking at a mix of the environmental factors during that time. In the winter of 2012, which was during the cold period of this study, there was the highest accumulation of nutrients and the highest significant N:P ratio found (Figure 18c). Despite the fact that we found that the N:P ratio was not significant, therefore not as limiting to *Chaetoceros*, other studies have found that larger N:P ratios have been found to correlate with larger phytoplankton biomasses since nitrogen is commonly a limiting factor (Tilman *et al.,* 1982). In this case, given the cold temperature limiting other competition and the ample nutrients, I believe it led to the high *Chaetoceros* bloom of over 2 million cells/L in June of that year. This is compounded by the fact that when looking at the nutrient levels during the late summer that year, unlike most years, they were very close to zero. *Chaetoceros spp.* were most likely so successful during this year because of their sporulation life cycle allowing them to leave the resting state and respond quickly to favorable conditions, which for these species are a wider range of conditions than for many other species (Montresor *et al.,* 2013; Daniels *et al.,* 2015; Trigueros and Orive, 2001).

*4.6 Recent Species Compositional Changes*

In more recent years, the blooms of both *Chaetoceros* and *Pseudo-nitzschia* are coming earlier and earlier in the year, with *Chaetoceros* even found in February and the normal pattern of *Pseudo-nitzschia* changing from blooming in August to June. 2015 illustrated the greatest shift in this pattern as the water temperature was the highest it has ever been with water temperatures 1-2$^{o}$C warmer than normal around the world (NOAA, 2015). Also in 2015, there was a large *Pseudo-nitzschia* bloom in June and August, with little to no *Chaetoceros* blooms found (Morton and Bursch, 2015). This coincided with an unusual mortality event in Alaska, off the coast of Kodiak island, which encompassed the death of 30 large whales, three times higher than the historical average for this kind of event (NOAA Fisheries, 2015). These events could not be directly correlated to the toxic algal bloom that occurred, but this was because of the difficulty in retrieving the stranded animals and testing the carcasses (*pers. comm.* Kris Holderied, 2015; Yuhas, 2015). There appears to be a species composition pattern shift, from the old pattern where *Chaetoceros* blooms first, strongly overshadowing *Pseudo-nitzschia*, to the more recent pattern where *Pseudo-nitzschia* is significantly increasing, especially at Kasitsna, having earlier and longer blooms that may someday result in them becoming the most dominate species in the bay. This may become a problem in the future, as it may create the likelihood for more harmful algal blooms (NOAA Fisheries, 2015).

*4.5 Future Work*

For future work, researchers could measure the levels of silicate and iron in the water and compare them to the phytoplankton species. Some studies have illustrated that these two molecules are very important for small diatom blooms during the in-between peak nutrient periods in the summer (Trigueros and Orive, 2001). In addition, silicate levels have been shown to strongly indicate when, where, and how big the *Pseudo-nitzschia* blooms will be (Anderson *et al.,* 2010).

Future studies could also focus in more detail on many of the other environmental conditions, and phytoplankton species that were collected, which may help researchers to understand what other parameters are correlated with phytoplankton. In addition, others could expand on my compiled model to quantify the correlations of other parameters. This would help get a better sense of the whole picture of the ecosystem and all environmental variables that play a part and how strongly they do so.

Lastly, other researchers could study the zooplankton samples we had collected to get a better sense of the bigger picture trophic dynamics. Looking at zooplankton will allow scientists to see how grazing pressure affects phytoplankton biomass and how phytoplankton species composition and concentration changes affect higher trophic species, which might allow scientists to understand how climate change affects each level of the food web.

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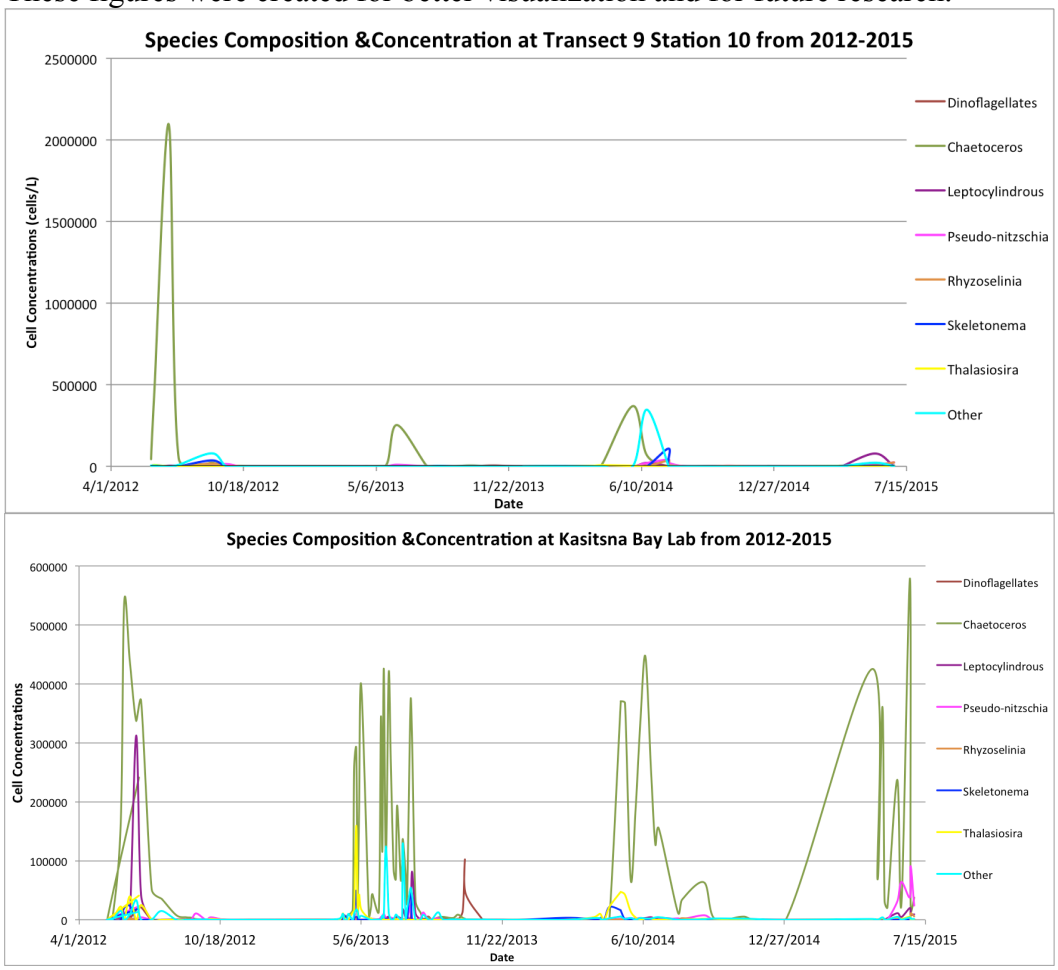
# APPENDIX

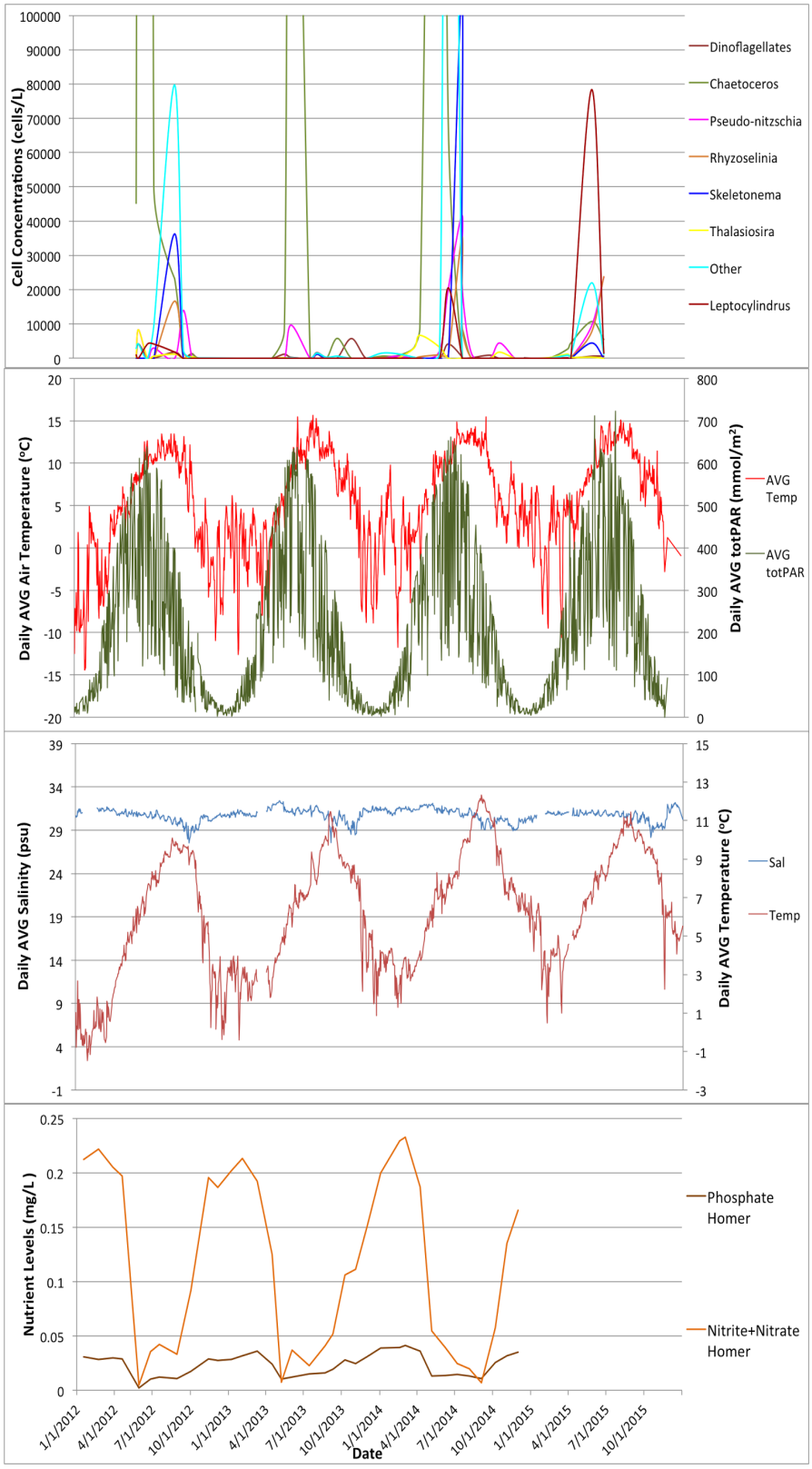
**Table 1:** Expanded table with all results from model analysis.

| Model | Model 1 (Individual) Homer | | Model 2 (Individual) Kasitsna | | T-test | Model 4 (Sal., Temp., PAR, Nut.) | |
|---|---|---|---|---|---|---|---|
| Parameter | *Chaetoceros* | *Pseudo-nitzschia* | *Chaetoceros* | *Pseudo-nitzschia* | *Location* | *Chaetoceros* | *Pseudo-nitzschia* |
| **Time** | | | | | | | |
| Biweek # | Coeff.=-15779.8 p=0.090 | Coeff.= -21.66 p=0.963 | Coeff.= -4198.8 p=0.169 | Coeff.= -287.2 p=0.36 | N/A | Coeff.=502.2 p=0.25 | Coeff=130.976 p=0.004*** |
| Year | Coeff.= -41071 p=0.441 | Coeff.=4073.9 t=1.35 p=0.180 | Coeff.=-23214.8 p=0.236 | Coeff.=4672.8 p=0.014*** | N/A | N/A | N/A |
| **Meteorological** | | | | | | | |
| Air Temperature | Coeff.=28100.27 p=0.014*** | Coeff.=2136.8 p=0.001*** | Coeff.=16943.4 p=0.000*** | Coeff.=1108.8 p=0.004*** | N/A | N/A | N/A |
| Relative Humidity | Coeff.=2697.5 p=0.767 | Coeff.=1011.7 p=0.064* | Coeff.=445.7 p=0.886 | Coeff.=178.3 p=0.551 | N/A | N/A | N/A |
| Barametric Pressure | Coeff.=8950.3 p=0.273 | Coeff.=1005.0 p=0.037** | Coeff.=6468.7 p=0.027* | Coeff.=734.7 p=0.015*** | N/A | N/A | N/A |
| Wind Speed | Coeff.=-161393.9 p=0.002*** | Coeff.=-9081.03 p=0.002*** | Coeff.= -78950.8 p=0.000*** | Coeff.=-5766.7 p=0.002*** | N/A | N/A | N/A |
| Max Wind Speed | Coeff.=-121067.7 p=0.004*** | Coeff.=-7383.6 p=0.002*** | Coeff.=-60724.6 p=0.000*** | Coeff.=-4470.5 p=0.002*** | N/A | N/A | N/A |
| Max Wind Speed Time | Coeff.=992140.9 p=0.562 | Coeff.=336575.7 p=0.232 | Coeff.=503644.9 p=0.400 | Coeff.=201602.2 p=0.044* | N/A | N/A | N/A |
| Wind Direction | Coeff.= 4586.8 p=0.003*** | Coeff.=257.2 p=0.001*** | Coeff.=2649.9 p=0.000*** | Coeff.=193.04 p=0.000*** | N/A | N/A | N/A |
| Standard Deviation Wind | Coeff.=813538.0 p=0.005*** | Coeff.=3012.9 p=0.041** | Coeff.=41179.9 p=0.000*** | Coeff.=2404.4 p=0.013*** | N/A | N/A | N/A |
| Total PAR | Coeff.= 1267.8 p=0.001*** | Coeff.=70.4 p=0.000*** | Coeff.=764.5 p=0.000*** | Coeff.=53.3 p=0.000*** | N/A | Lag 1 Coeff=690.08 p=0.00*** | Coeff=51.68 p=0.00*** |
| Total Solar Radiation | Coeff.=2334.5 p=0.001*** | Coeff.=130.0 p=0.000*** | Coeff.=1425.6 p=0.000*** | Coeff.=98.7 p=0.000*** | N/A | N/A | N/A |
| Total Precipitation | Coeff.=-3519859 p=0.311 | Coeff.=-95902.8 p=0.555 | Coeff.=-1166241 p=0.240 | Coeff.=-127723.9 p=0.296 | N/A | N/A | N/A |
| **Water Quality** | | | | | | | |
| Water Temperature | Coeff.=26153.7 p=0.215 | Coeff.=3450.9 p=0.003*** | Coeff.=17296.42 p=0.042** | Coeff.=1167.5 p=0.044** | dF= 171 t=1.8126 p=0.0716* | Squared Coeff=-3996.3 p=0.009*** | Coeff.=1944.7 p=0.006*** |
| Specific Conductivity | Coeff.=1994.3 p=0.944 | Coeff.=494.8 p=0.781 | Coeff.= -6315.8 p=0.743 | Coeff.=-601.7 t=-0.46 p=0.648 | dF=169 t=2.6619 p=0.0085*** | N/A | N/A |
| Salinity | Coeff.=129370.4 p=0.165 | Coeff.=1609.8 p=0.723 | Coeff.= 18755.4 t=0.59 p=0.560 | Coeff.=599.4 p=0.801 | dF=169 t=3.1009 p=0.0023*** | Coeff=-22200.8 p=0.243 | Not included |
| Dissolved Oxygen % | Coeff.=27260.9 p=0.000*** | Coeff.=845.0 p=0.029** | Coeff.=11646.98 p=0.000*** | Coeff.=347.0 p=0.051* | dF=171 t=2.5535 p=0.0115*** | N/A | N/A |
| Dissolved Oxygen | Coeff.=120834 p=0.035** | Coeff.=753.2 p=0.794 | Coeff.=60807 p=0.001*** | Coeff.=864.5 p=0.541* | dF=171 t=1.4661 p=0.1445 | N/A | N/A |
| Depth | Coeff.= -593983.6 p=0.248 | Coeff.=-22232.6 p=0.411 | Coeff.= -279816.3 p=0.187 | Coeff.=18393.5 p=0.242 | dF=171 t=-0.022 p=0.000*** | N/A | N/A |
| Corrected Depth | Coeff.=-551850.8 p=0.150 | Coeff.=-35274.0 p=0.091 | Coeff.=-352988.1 p=0.031** | Coeff.=-4403.6 p=0.714 | dF=168 t=-22.02 p=0.000*** | N/A | N/A |
| pH | Coeff.=1199897 p=0.049** | Coeff.=41850.1 p=0.219 | Coeff.=-459160.8 p=0.035** | Coeff.=86.4 p=0.995 | dF=170 t=-0.9619 t=0.3375 | N/A | N/A |
| Turbidity | Coeff.=-62967.1 p=0.023*** | Coeff.=-2545.8 p=0.059 | Coeff.=2036.74 p=0.789 | Coeff.=289.7 p=0.612 | dF=171 t=-3.25 p=0.0014*** | N/A | N/A |
| **Nutrients** | | | | | | | |
| Phosphate | Coeff.=-1.27x10$^{7}$ p=0.005*** | Coeff.= -590313 p=0.001*** | Coeff.=-8193964 p=0.000*** | Coeff.= -288639.7 p=0.063* | dF=147 t=0.7792 p=0.437 | Not included due to similarity to NO23 | Not included due to similarity to NO23 |
| Ammonia | Coeff.=1.06x10$^{7}$ p=0.015*** | Coeff.=-519948.0 p=0.006*** | Coeff.=-1439773 p=0.357 | Coeff.=-58253.1 p=0.586 | dF=147 t= -0.35 p=0.7255 | N/A | N/A |
| Nitrate+Nitrite | Coeff.=-1661583 p=0.010*** | Coeff.= -95428 p=0.000*** | Coeff.=-1253718 p=0.000*** | Coeff.= -57528.5 p=0.008*** | dF=147 t=1.45 p=0.159 | Lag 2 Coeff.=598113.2 p=0.023*** | Coeff.=52119.9 p=0.101 |
| N:P | Coeff.=1.924 p=0.821 | Coeff.=-0.1835 p=0.549 | Coeff.=-32812.3 p=0.005*** | Coeff.=-2034.3 p=0.011*** | dF=145 t=-2.166 p=0.0456** | Not included due to similarity to NO23 | Not included due to similarity to NO23 |
| **Phytoplankton** | | | | | | | |
| *Chaetoceros* | NA | Coeff.=0.00123 p=0.923 | NA | Coeff.=0.047 p=0.002*** | dF=148 t=2.3226 p=0.0216*** | N/A | N/A |
| *Pseudo-nitzschia* | Coeff.=0.50 p=0.943 | NA | Coeff.=2.18 p=0.066* | NA | dF=149 t=-1.035 p=0.3024 | N/A | N/A |
| **Reverse Nutrient** | | | | | | | |
| Phosphate | Phyto. Lag 1 Coeff.=-3.37x10$^{-8}$ p=0.00*** | Phyto. Lag 1 Coeff.= -5.14x10$^{-7}$ p=0.000*** | Phyto. Lag 1 Coeff.=-3.37x10$^{-8}$ p=0.00*** | Phyto. Lag 1 Coeff.= -5.14x10$^{-7}$ p=0.000*** | N/A | N/A | N/A |
| Nitrate+Nitrite | Phyto. Lag 1 Coeff.= -2.48x10$^{-7}$ p=0.00*** | Phyto. Lag 1 Coeff.=-4.22x10$^{-6}$ p=0.000*** | Phyto. Lag 1 Coeff.= -2.48x10$^{-7}$ p=0.00*** | Phyto. Lag 1 Coeff.=-4.22x10$^{-6}$ p=0.000*** | N/A | N/A | N/A |
| **Location** | N/A | N/A | N/A | N/A | N/A | Coeff.=-53384.2 p=0.017*** | Coeff=5375. p=0.013*** |

These figures were created for better visualization and for future research.




**Appendix Figure 1 & 2:** Expanded view of Figure 6a and 6b including the other 5 main genera that were found at these locations, Homer and Kasitsna.


Cell Concentrations (cells/L)
100000
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
Dinoflagellates
Chaetoceros
Pseudo-nitzschia
Rhyzoselinia
Skeletonema
Thalasiosira
Other
Leptocylindrus
Daily AVG Air Temperature (°C)
20
15
10
5
0
-5
-10
-15
-20
Daily AVG totPAR (mmol/m²)
800
700
600
500
400
300
200
100
0
AVG Temp
AVG totPAR
Daily AVG Salinity (psu)
39
34
29
24
19
14
9
4
-1
Daily AVG Temperature (°C)
15
13
11
9
7
5
3
1
-1
-3
Sal
Temp
Nutrient Levels (mg/L)
0.25
0.2
0.15
0.1
0.05
0
Phosphate Homer
Nitrite+Nitrate Homer
1/1/2012
4/1/2012
7/1/2012
10/1/2012
1/1/2013
4/1/2013
7/1/2013
10/1/2013
1/1/2014
4/1/2014
7/1/2014
10/1/2014
1/1/2015
4/1/2015
7/1/2015
10/1/2015
Date

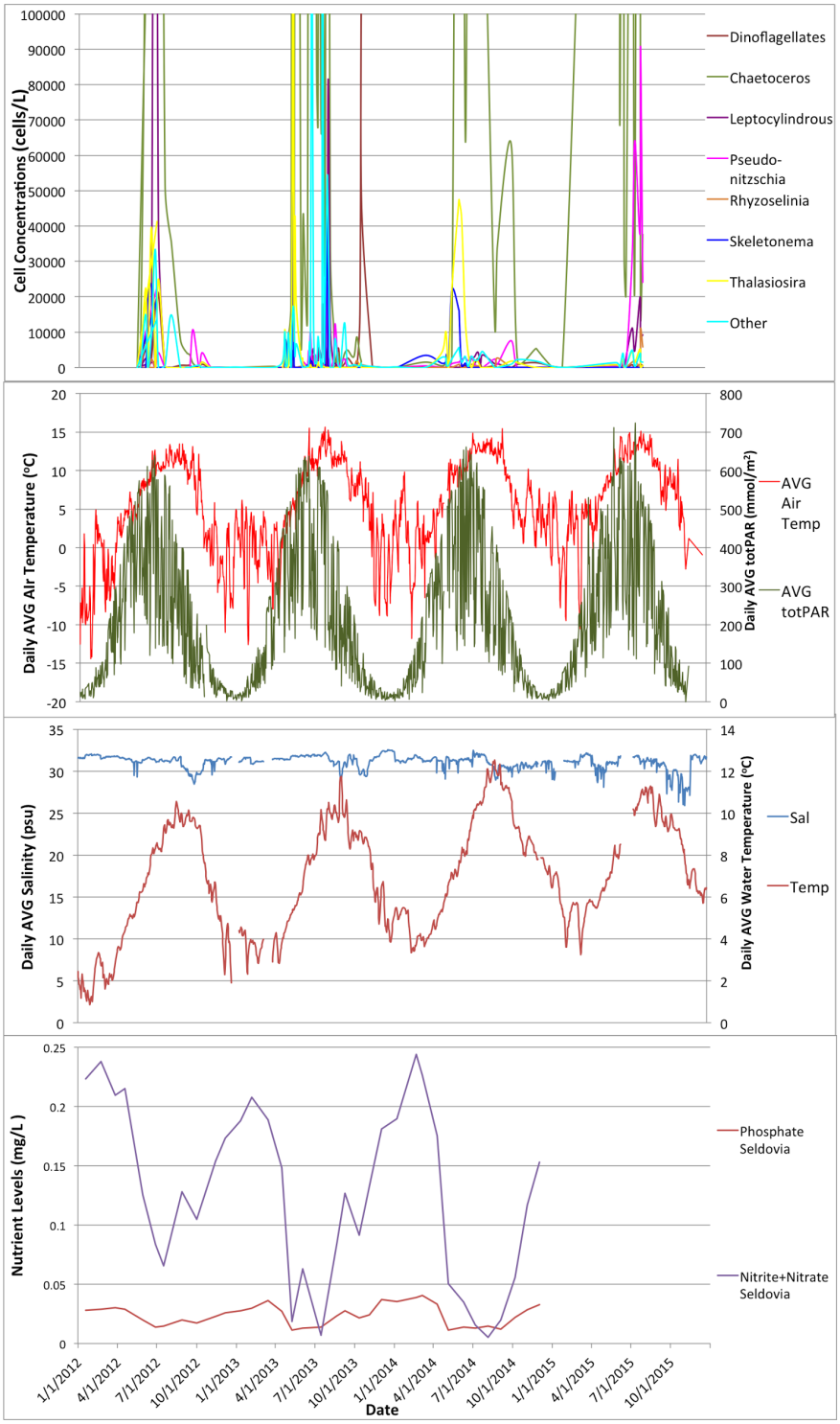



**Figure 3 & 4:** These two graphs illustrate the other 5 genera present in the water at Homer and Kasitsna from 2012-2015. These nutrients have not been updated up till June, like the rest, and only show from 2012-2014.