THE RELATIONSHIP BETWEEN El NIÑO SOUTHERN OSCILLATION AND LEVELS OF PARALYTIC SHELLFISH POISONING PRESENT IN WASHINGTONS MARINE WATERS

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Can large scale weather phenomenon such as El Nino Southern Oscillation be used to predict when levels of paralytic shellfish poisoning will be higher, thus mitigating their harmful impacts? This thesis examines the relationship between levels of Paralytic Shellfish Poisoning (P.S.P.) present in Washington State's marine waters and large scale weather phenomena or Oceanic circulation patterns such as the El Niño Southern Oscillation (ENSO). It questions whether large scale climatic conditions such as those produced by ENSO affect blooms of algae, such as *Alexandrium catenella*. Once we have a better working knowledge of the ecology of *A. catenella* we can better predict and monitor outbreaks of P.S.P., thus reducing the amount of human illnesses and economic hardships associated with P.S.P. The P.S.P. data is compared to sea surface temperatures departures from the normal in Niño region 3.4, and localized weather parameters to see if any significant relationship exists. As indicated by the results of this study P.S.P. levels in Washington State are not influenced by the phases of ENSO.

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Chapter 1

Introduction


Figure 1: Alexandrium catenella Photo: by Jan Rimes**.**

Millions of microscopic plant cells, commonly known as algae, thrive in nearly every drop of seawater. Algae (phytoplankton) are the primary energy producers in the ocean, forming the base of the marine food chain. These single-celled plants photosynthesize and multiply, creating a bloom that feeds everything from fellow microbes to larger fish species. Primary production is the synthesis and storage of organic molecules during the growth and reproduction of photosynthetic organisms such as algae. Seasonal phytoplankton dynamics are most often characterized by low biomass and primary productivity during winter-spring seasons of high river flow, followed by a slow (2-3 months) accumulation of biomass during summer (Alpine, 1992). Typically during late spring and early summer the dominate phytoplankton is diatoms, a species that thrives in un-stratified environments and is unable to migrate below the thermocline when nutrients become depleted in the surface layer. Once nutrients become depleted in the surface layers, more motile species such as dinoflagellates become dominate, due to their ability to migrate below the thermocline and carry on photosynthesis when other algae are dying off. Among the thousands of species of algae in the sea, a few dozen produce toxins and pose a formidable natural hazard. In Washington's Puget Sound basin the dinoflagellate *Alexandrium catenella* (Figure 1) is one such species.

This thesis looks at the relationship between the dinoflagellate *A. catenella* and the climate phenomenon know as El-Nino Southern Oscillation (ENSO). ENSO, a global-scale weather phenomenon influences local climate, which in turn influences the physical properties of the environment where *A. catenella,* is found. This thesis asks the question, is there a relationship between the phases of ENSO, and levels of paralytic shellfish poisoning present in Washington States marine waters? If a relationship exists between the phases of ENSO or a parameter of *A. catenellas* environment and the paralytic shellfish poisoning levels produced by *A. catenella*, then ENSO can be used to predict when blooms of Paralytic shellfish poisoning are likely to occur. If this is the case then regulators of the shellfish industry may be able to use this information to mitigate the economic and health impacts from blooms *A. catenella* and the paralytic shellfish poisoning that it produces.

1.1 Background and Problem Description

Early records, partially based on local native lore passed down by word of mouth, suggest that harmful algal blooms have been present along the coast of the United States for hundreds of years (Trainer, 2003), but actual historic scientific data are non-existent.

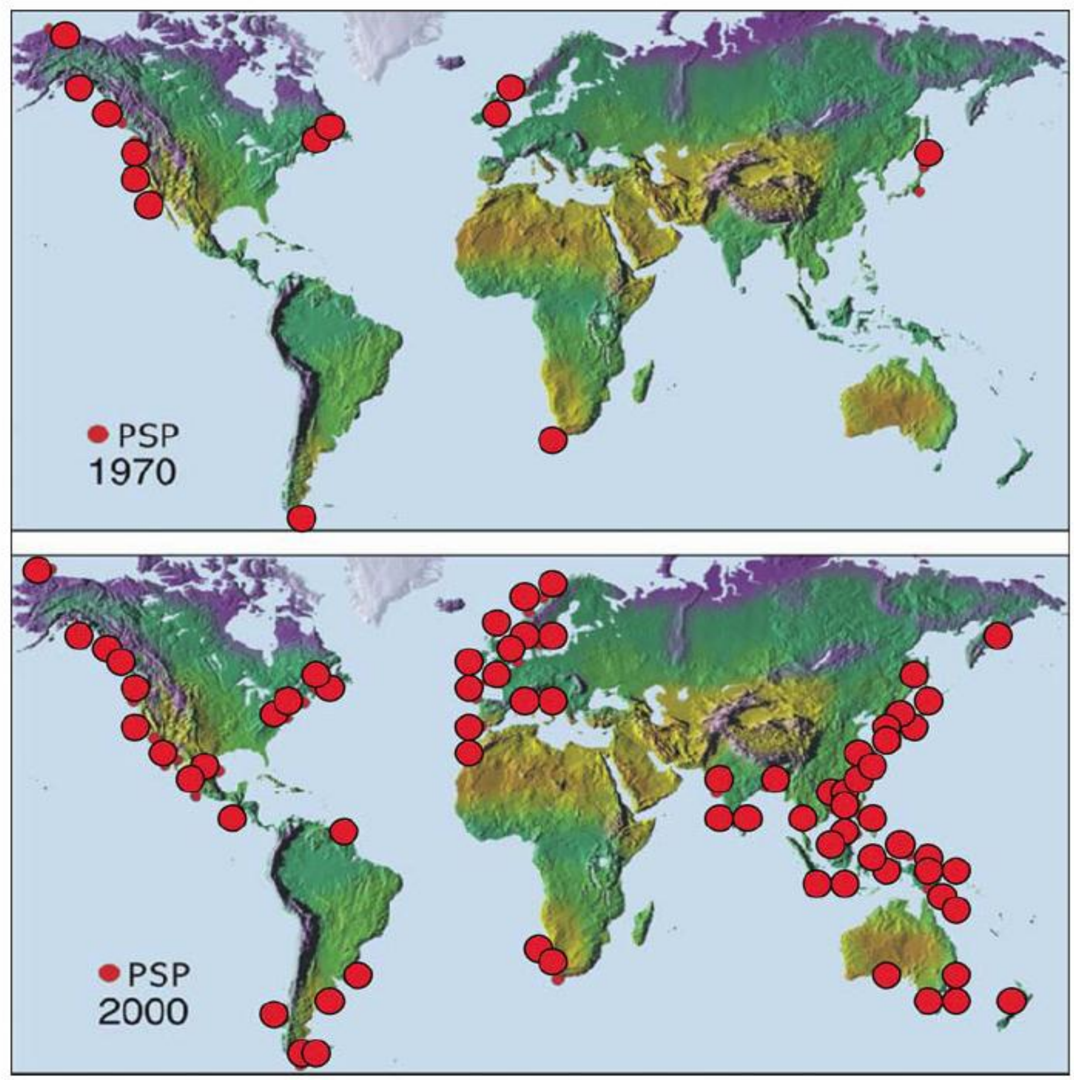


Figure 2: The world wide distribution of P.S.P. during 1970 and 2000, Glibert 2005.

> "Historically, P.S.P. has been known in the Pacific Northwest and Alaska for centuries. Records of P.S.P. events date back as early as June 15, 1793 (Vancouver 1798), when a member of Captain George Vancouver's exploration team died after eating contaminated mussels

> harvested in the uncharted coastline of what is now known as British Columbia (Trainer, 2003)".

Washington State's only three fatalities due to paralytic shellfish poisoning were recorded in 1942 near the entrance to the Strait of Juan de Fuca, since then there has been no illnesses resulting in death.

Harmful algal blooms have been known throughout the world and recorded history, but the nature of the problem has changed considerably over the past several decades, both in extent (Figure 2) and in public perception. Over the past three decades, the occurrence of harmful or toxic algal incidents has increased in many parts of the world, both in frequency and in geographic distribution (Van Dolah, 2000). This increase in occurrence and awarness has public officials and citizens both calling for a greater scientific understanding of the biological and physical properties associated with harmful algal blooms.

There are many theories to what sets the stage for large scale blooms of *A. catenella* and other harmful algal blooms around the world.

> "One cause for the increasing frequency of harmful algal blooms seems to be nutrient enrichment-that is, too much "food" for the algae. Just as the application of fertilizer to lawns can enhance grass growth, marine algae grow in response to nutrient inputs from domestic, agricultural, and industrial runoff (Anderson, 2004)".

Though nutrient enrichment increases the amount of available food for *A. catenella* and other harmful algal blooms, it does not necessarily allow the blooms to persist for longer durations or increase the frequency of blooms inter-annually. Recent studies suggests that not only do nutrients play a role, but that physical process may be important in creating a favorable environment for large scale blooms, and may be what is enabling blooms to persist in the environment longer than historically recorded. Currently, not much is known about how physical processes such as wind, precipitation, salinity, and water temperature, limit or enhance the duration and frequency of harmful algal blooms. However, a growing body of laboratory, field, and theoretical work suggests that the dynamics of harmful algal blooms and their impacts on other organisms are frequently controlled not only by physiological responses to local environmental conditions as modified by trophic interactions, but also by a series of interactions between biological and physical processes occurring over an extremely broad range of temporal an spatial scales (Donaghay, 1997). Some of these physical processes are not yet defined at the appropriate scale, yet may be crucial in the formation of harmful blooms (Gentein, 2005). Physical factors affecting *A. catenella* will be even harder to quantify in the future as we live in a world of changing climate.

1.2 Thesis Statement and Layout

Do the phases of ENSO have an influence on paralytic shellfish poisoning levels in Washington State? It is undetermined at this time if this is the case, however, by performing statistical analysis of paralytic shellfish poisoning levels in Washington States marine waters versus ENSO it may be possible to determine if such a relationship exists.

The layout of this thesis was designed in the following order to provide a logical path to answering the question above. The first two chapters break down the life cycle of *A. catenella*, and how the physical properties of its environment influence this life cycle. Chapter 3 looks at how the different phases of ENSO affect local weather parameters and patterns, and thus the physical properties of *A. catenella* environment. Chapter 4 discusses how climate change influences ENSO and local weather. Chapter 5 describes the data sources used for this study. Chapter 6 includes a statistical analysis of ENSO and associated weather patterns versus paralytic shellfish poisoning levels in Washington State and the results obtained from this analysis. The following chapter, Chapter 7, is a discussion of what the implications of this study may have for individuals and industries impacted by paralytic shellfish poisoning and those who regulate them. Finally, Chapter 8 states the conclusions and discusses the implications that a relationship between ENSO and paralytic shellfish poisoning levels in Washington State may have and how this study may be strengthened in the future.

Chapter 2

Paralytic Shellfish Poisoning and Alexandrium Catenella Life Cycle

A. catenella is an armored, marine, planktonic dinoflagellate associated with toxic Paralytic Shellfish Poisoning (P.S.P.). *A. catenella* occurs either as single cells or as a chain of cells (Figure 1). *A. catenella* produces an array of chemically similar neurotoxins that are responsible for P.S.P., which are transmitted via tainted shellfish. Neurotoxins are so named because they disrupt nerve impulses. P.S.P. toxin accumulates in marine animals that feed either directly on toxic phytoplankton or on consumers of toxic phytoplankton. Bivalve shellfish concentrate biotoxin's when they filter toxic phytoplankton (*A. catenella)* out of the water while feeding.

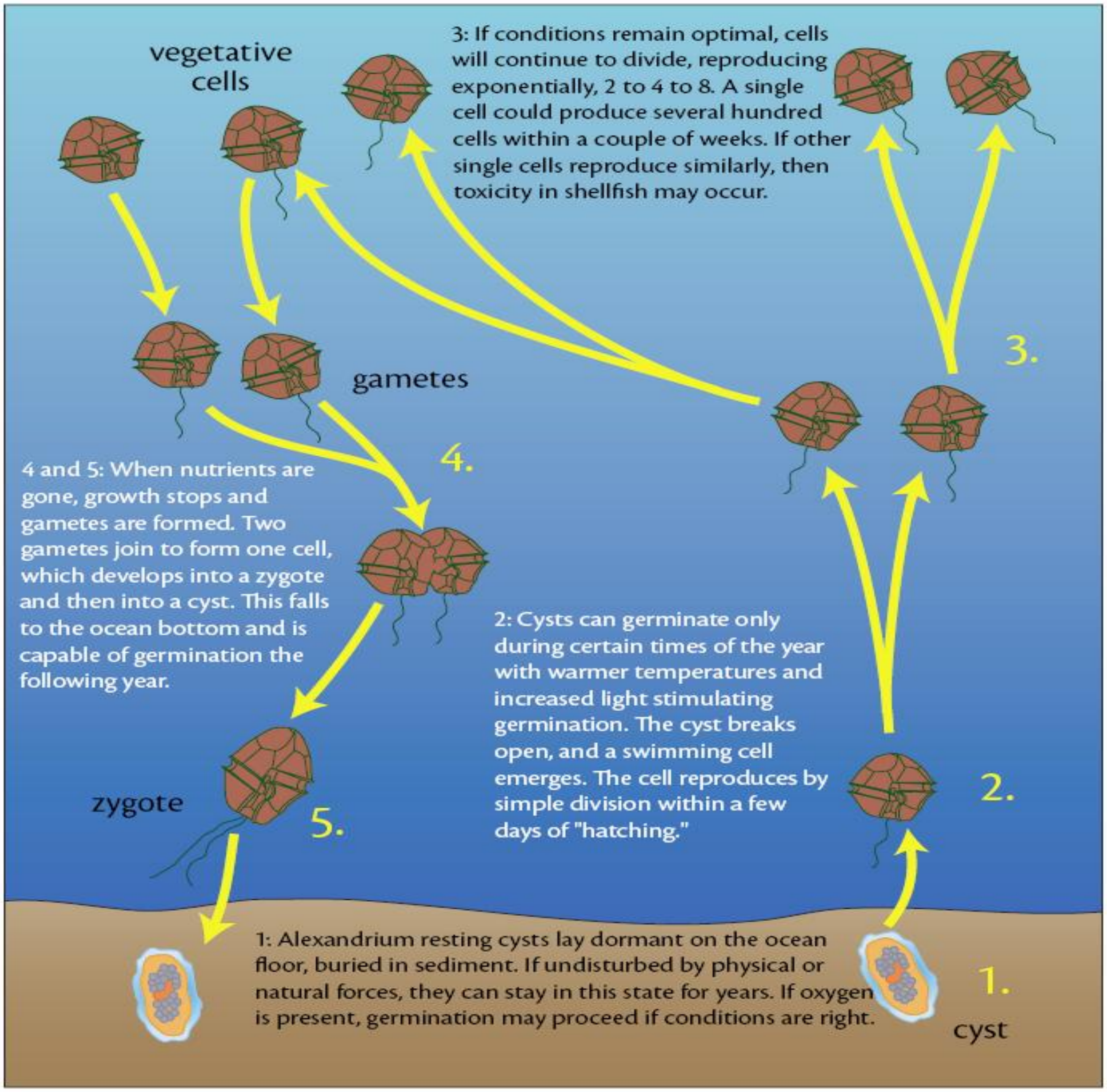
> "These consumers include zooplankton, bivalve shellfish, predatory marine snails, crabs, fish, birds, and marine mammals. Mass mortalities among other shellfish-eating animals including birds, fur seals, foxes, sea otters, and humpback whales have been traced to P.S.P. (Determan, 2003)".

These toxins can make their way up the food chain and end up affecting humans, other mammals, fish and birds. On a global basis, almost 2,000 cases of human poisonings are reported per year, with a 15% mortality rate (Van Dolah, 2000). "Human illness and death are the primary impact of Harmful algal blooms, but effects on other wildlife are also important. Some fish kills due to

Harmful algal blooms can be spectacular is size, with millions of fish and millions of dollars lost to local economies (Glibert, 2005)". In the Puget Sound the seasonal occurrence of *A. catenella* follows a scenario like this and the one in Figure 3. Wintertime rain storms, rivers, and storm water runoff carry nutrients into Puget Sound from uplands and watersheds. Strong winds mix the freshwater with nutrient rich water from the open sea. Typically sunlight is the most dominate factor limiting growth in the winter, however strong winds and colder water temperatures may have an affect (Determan, 2003). In early spring, winds become lighter reducing mixing, and the sun warms the water raising water temperatures.

This causes the water column to stabilize and vertical mixing to slow down or stop. A second influx of nutrients is then introduced in the form of snowmelt, which increases the flow of most Puget Sound Rivers. The addition of these abundant dissolved nutrients in a stable water column lead to blooms of many phytoplankton species in the surface waters (Determan, 2003).

These blooms can be so dense that they can color the water red, brown, green etc; this condition is called a "red tide", and is often thought to be associated with P.S.P., which is a common misconception. *A. catenella* may be present in shellfish at dangerous levels, long before the water becomes discolored (Determan, 2003). By mid to late summer, surface water is frequently depleted of nutrients and many species of phytoplankton die back.



3: If conditions remain optimal, cells will continue to divide, reproducing exponentially, 2 to 4 to 8. A single cell could produce several hundred cells within a couple of weeks. If other single cells reproduce similarly, then toxicity in shellfish may occur.

4 and 5: When nutrients are gone, growth stops and gametes are formed. Two gametes join to form one cell, which develops into a zygote and then into a cyst. This falls to the ocean bottom and is capable of germination the following year.

2: Cysts can germinate only during certain times of the year with warmer temperatures and increased light stimulating germination. The cyst breaks open, and a swimming cell emerges. The cell reproduces by simple division within a few days of "hatching."

1: Alexandrium resting cysts lay dormant on the ocean floor, buried in sediment. If undisturbed by physical or natural forces, they can stay in this state for years. If oxygen is present, germination may proceed if conditions are right.

Figure 3: Meroplanktonic life cycle of the dinoflagellate species Alexandrium (Anderson, 2005).

However, two flagella enable *A. catenella* to vertically migrate to the surface during the day and to depth at night. Because of this, *A. catenella* is able to journey to deeper water via its flagella, where nutrients remain in abundance **(**Trainer, 2003). Thus when nutrients become depleted at the surface during summer *A. catenella* can simply move down to where nutrients are still abundant, giving it an advantage over non-flagellated phytoplankton such as diatoms. *A. catenella then* returns to the surface layer to carry on photosynthesis. As a result of this *A. catenella* is able to bloom longer typically until late autumn. At this time water temperatures begin to cool off and winds speeds increase, inducing vertical mixing that leads to the breakdown of the stratification of the water column, while sunlight starts to become too dim thus limiting or shutting down the blooms production. At which time *A. catenella* may form resting cysts that settle to the bottom awaiting the return of more favorable growing conditions (Figure 3).

2.1 The Life Cycle of Alexandrium Catenella and the Physical Processes of its Environment

The physical properties of aquatic environments play a fundamental role in driving the dynamics of planktonic communities. The physical properties of an aquatic environment not only shape the structure of the pelagic zone but also affect the biological processes of phytoplankton, such as *A. catenella* (figure 1), in many direct and indirect ways. In order to understand how the physical properties of *A. catenella* environment affect its biological processes, it is important to understand how physical processes such as turbulence, precipitation/runoff, salinity and water temperature influence the aquatic environment in which *A. catenella* thrives.

The environment most favorable to blooms of *A. catenella* is a nutrient rich heavily stratified body of water. Generally, dinoflagellates thrive in stratified waters because of their motility which enables it to move to nutrient-rich areas within the water column (Trainer, 2003). Stratification of the water column can be found in upwelling systems, coastal embayments, estuaries, and in retentive zones such as gyres in the open oceans. Typically a stratified environment is composed of layers of water, which represent natural boundaries to phytoplankton. The top layer is known as the mixed layer and consists of a thin band of water that extends from the ocean's surface to the thermocline and can be up to several tens of meters thick.

> "The mixed layer is the boundary layer between the atmosphere and the Deep Ocean, and results from solar radiation and surface cooling. Winds help mix the heat through the layer by generating waves, inducing Langmuir circulation, producing convection, and generating turbulence" (Gentien, 2005).

The thermocline represents the boundary between the surfaced mixed layer and more dense subsurface cold waters. It is produced by sudden changes in water temperature with depth and is the area of greatest and most rapid temperature change with depth in the oceans. The rapid changes in temperatures that produce the thermocline, also creates density changes in the seawater over the same depth range; such a zone of density change is known as the pycnocline.

> "The seasonal thermocline is an important physical barrier in the ocean, separating the surface mixed layer for the deeper water. As the transition region between the nutrient-poor, well-lit surface layer (mixed layer), and the darker, nutrient-rich deeper water, the thermocline plays a role in determining the biological properties of the water column" (Sharples, 2001).

In order for a body of water to become stratified, to a degree that would support blooms of *A. catenella,* certain physical processes in the environment must be maintained for extended periods of time. In Washington State's Puget Sound Basin, stratification occurs on a seasonal time scale. In early spring winds become lighter; this reduction in wind is associated with reduced turbulence, which in turn reduces the mixing occurring in the water column. At the same time increased solar radiation raises the water temperatures and in turn increases the mixed layer's thickness. Reduced amounts of precipitation influences the water column's surface layer by allowing the water temperatures at the surface to warm increasing the depth of the mixed layer, as well as reducing the amount of nutrients available in the surface layer. These processes together cause the water column to stabilize and vertical mixing to slow down or stop. A second influx of nutrients is then introduced in the form of snowmelt, which increases the flow of most Puget Sound Rivers, this colder more nutrient rich waters will settle to the bottom layer below the thermocline due to its relative density. Understanding how these environmental drivers shape the aquatic environment is paramount in understanding how they affect the different life stage processes of *A. catenella.*

> "The complexities of *Alexandrium* blooms in dynamic coastal or estuarine systems are far from understood. One common characteristic of such phenomena is that physical forcings (environmental drivers) play a significant role in both bloom dynamics and the patterns of toxicity. The coupling between physics an biological "behavior" such as swimming, vertical migration, or physiological adaptation holds the key for understanding these phenomena, yet this is perhaps where our knowledge of the genus is weakest" (Anderson, 1998).

The best way to understand how different environmental drivers affect different stages of the life cycle of *A. catenella is* to look at how each environmental driver affects the processes associated with different stages in the life cycle of *A. catenella.* In order to do this we need to briefly describe the life cycle of *A. catenella* in a way that can easily be related to the physical properties of its environment. For the purpose of this paper I am going to break the life cycle of *A. catenella* into four stages: dormancy, germination, growth, and termination. These are just general categories and will be discussed more in depth, in relation to individual drivers, later.

Dormancy pertains to the stage when resting cysts of *A. catenella* lay dormant buried in sediments on the ocean floor. The duration of this interval, which is generally considered a time for physiological maturation, varies considerably among dinoflagellate species and can be anywhere from 12 hours to 6 months" (Anderson, 1998). They can remain this way for years unless disturbed by physical or natural forces. When oxygen is present and conditions are right germination may proceed. Germination typically occurs during spring and summer when warmer water temperatures and increased irradiance stimulate the cyst to break open. After the cyst breaks open a swimming cell emerges. Within a few days of emerging this cell will then reproduce by simple division. Growth will continue if favorable conditions persist and the cell will continue to divide, reproducing exponentially. A single cell of *A. catenella can* produce several hundred cells within a few weeks, add the fact that there are usually several hundred cysts and you have the makings of a bloom (Anderson, 2005). The termination of *A. catenellas* growth usually occurs when nutrients have been depleted in the water column, and or temperatures have dropped below an optimal range. At this time *A. catenella forms* a gamete, when two gametes join a zygote is formed which then turns into a resting cyst. The cyst then falls to the ocean floor where it awaits in the sediment for the return of more favorable growing conditions. We can now look at how the physical properties of its environment affect the various life stages of the dinoflagellate *A. catenella.*

2.2 Turbulence

It has long been noted that blooms of *A. catenella* typically occur during periods of weak winds, which creates less turbulence in the water column. Turbulence has been described as a “puzzling blend of order and disorder”, but really turbulence is a property of motion, not of the fluid, and two of its characteristics are randomness and diffusivity (Estrada, 1998). Not much is know about the affects of turbulence on cysts dormancy and germination. However, oxygen may help to stimulate germination and high levels of turbulence could supply that to the sediment. According to Smayda, (1997) turbulence can negatively influence dinoflagellate blooms by three mechanisms: physical damage; physiological impairment; and behavioral modification. Turbulence affects the growth of *A. catenella* in four important ways. First, essential nutrients are transported from below the thermocline to the euphotic zone by turbulence, negating the need for *A. catenella* to swim below the thermocline for food, thus modifying its behavior. Second, turbulence helps to suspend *A. catenella* in the euphotic zone which saves its energy for cell division because it does not have to expend energy to stay in the euphotic zone, which is another example of modified behavior. Third, turbulence may instead transport *A. catenella* from the surface to dark sterile waters where it may not survive, an important mechanism of loss thru physiological impairment. Fourth, strong turbulence can cause physically damage and destroy cells of *A. catenella,* sometimes terminating blooms all together.

Some laboratory experiments in which dinoflagellates were grown under strong agitation suggest, in general, a negative effect of turbulence on dinoflagellate cell division and growth (Lee Karp-Boss, 2000). Thus during periods of sustained strong winds, blooms of *A. catenella* are slower to develop and may even experience a decline in population. Sullivan et al. studied the affects of small scale turbulence on the division rate and morphology of *A. catenella.* After performing two replicate experiments Sullivan found the same results. The division rate of *A. catenella* was hardly affected by turbulence in the range of ε (ε = turbulence dissipation rate) approximately 10^{-8} to10^{-5} m_2 s^{-3}, but as ε increased above that value there was a 15–20% reduction in division (Sullivan et al., 2003). In the highest turbulence intensity treatment, the cross sectional area (CSA) of *A. catenella* increased approximately 20% (Sullivan et al., 2003). Sullivan also found that in his unstirred control treatments, 80–90% of the *A. catenella* population was found as single cells, indicating that during periods of low turbulence that *A. catenella* does not form chains. As the turbulence intensity increased, the number of cells in chains increased (up to 16 cells per chain), at the same time single cells decreased to less then 20% of the total *A. catenella* population (Sullivan et al., 2003). However, in the very highest turbulence intensity treatment, there was a reduction in the percentage of longer cell chains, and 4 cell chains became more common then 8 cell chains (Sullivan et al., 2003). Thus according to Sullivan the number of *A. catenella* cells per chain were markedly affected by turbulence. Lab experiments are often thought of as a poor replicate of natural conditions. But Sullivan compared his lab results with some in situ samples in the East Sound of Washington and found a similar pattern.

> "In 1997, *A. catenella* populations in East Sound, WA were present at very high cell concentrations (approx. 100 ml^{-1}), confined to a narrow depth range between 4 and 5m. The *A. catenella* layer was in a region of minimal current shear, and thus presumably in the depth interval associated with the lowest turbulence intensity in the vertical profile. The highest values of shear in the profile were in the layers above and below it. In this profile, there were marked changes in density, with the *A. catenella* layer in about the middle of the largest density gradient between approximately 3.5 and 6m depth. The next summer, 1998, *A. catenella* populations were again found in high cell concentrations (approximately 40 ml^{-1}) in a narrow depth range between approximately 8 and 10m (Fig. 8). Turbulence intensity in the profile was maximal near the surface (ε approx. 10^{-5} m2 s^{-3}), but decreased to its minimum values (less than ε approx. 10^{-7} m2 s^{-3}) in the *A. catenella* layer, before rising again toward the bottom" (Sullivan et al., 2003).

Blooms of *A. catenella* can be broken up by turbulence, due to excessive wind. Turbulence increases in the fall and winter and can be sustained for days to weeks on end. This may help to stimulate cells of *A. catenella* to begin forming gametes and zygotes which then turn into cysts and fall to the ocean floor as inoculums waiting for next year's bloom.

2.3 Precipitation and Runoff

Precipitation and runoff also have an important part in the bloom dynamics of *A. catenella.* Precipitation and runoff supply important nutrients that are the energy that fuels the cell division of *A. catenella.* This can be especially important during summer months when nutrients can become depleted in the surface mixed layer. Not only does precipitation and river runoff supply nutrients, but they also affect the physical environment. It has long been thought that the physical changes of a water column that are associated with heavy spring rains and the spring freshet (peak snowmelt) is an important stimulate for germination of toxic dinoflagellates cysts, such as those of *A. catenella* . A study done in Japan's Hiroshima Bay, using *Alexandrium tamarense* a dinoflagellate similar to *A. catenella,* demonstrated that runoff and precipitation also affect the salinity gradient of the water column.

> "In 1996, freshwater input had continued from 18 March to 7 May, followed by a short break 13-20 May, and then increased again between 20 and 28 of June. Although the river runoff affected the density distribution, it did not likely affect the temperature profile, particularly in the first half of the period. In 1997, the situation was basically the same as in 1996, showing that the density stratification was fundamentally formed by the salinity decrease in the surface water due to river runoff" (Yamamoto et al., 2002).

However Yamamoto et al. also found that if the rains are too heavy and runoff too great in terms of volume of flow blooms of *A. tamarense* can be dispersed (Yamamoto et al., 2002). To the contrary Weise et al. found that after analyzing a ten year data set on environmental conditions and populations of *A. tamarense* in the Gulf of St. Lawrence, Quebec, Canada, higher volumes of river runoff, such as during the spring freshet, were required for blooms to develop. "Nonetheless, our results indicate that other factors, such as higher then average river runoff and low wind speeds, that further intensify vertical stratification, are required for *A. tamarense* bloom to fully develop at Sept-Iles" (Weise et al., 2002). But what defines higher or too great in terms of rain and runoff. Each species of phytoplankton may have different thresholds, so these types of factors may need to be assessed on a species by species basis.

2.4 Salinity

Salinity directly influences the density of the water column and contributes to stratifying the water column. As precipitation and runoff increase the surface mixed layer becomes less saline. As the surface layer becomes less saline the thickness of the mixed layer will increase due to the separation of the more saline deeper waters. This change in water column stratification from salinity is important as it affects the biological processes of *A. catenella both* negatively and positively. However, not much is known about how salinity affects the dormancy and germination periods of *A. catenella.* Nor has any data been compiled on how salinity affects the cell division rate or growth rate of *A. catenella.*

Salinity is generally used as an indicator of the water column stability; by looking at changes in salinity over depth and time it has been suggested that salinity can be used as indicator of the initiation time of blooms of dinoflagellates. Yamamoto et al. found that in Japan's Hiroshima Bay salinity induced stratification may be a good indicator of the timing of blooms of *A. tamarense*.

> "At the beginning of the observations in 1996, the volume of river discharge may have been too high to sustain the population, while the bloom coincided with the less stratified period after the flushing, the bloom also coincided with the less stratified period developing between the two marked flushing episodes on 31st of March to the 14th of April and the 6th thru the 19th of May. These results imply that river water runoff does not support *A. tamarense* blooms despite the formation of salinity induced stratification. Water column stability is considered an important parameter determining species dominancy: dinoflagellates usually dominate in stratified conditions, and diatoms in mixed conditions. However, these general criteria do not seem to be the case for spring blooms of *A. tamarense* in Hiroshima Bay" (Yamamoto et al., 2002).

Weise et al. found that blooms of *A. tamarense* in the Gulf St. Lawrence had a strong negative correlation with surface salinity. "Salinity can be considered an indication of freshwater input and water column stability and the importance of both these factors is inferred by the strongly significant negative correlation between surface salinity and the occurrence of A. tamarense cells at Sept-Iles in the Gulf of St. Lawrence, during the study period" (Weise et al., 2002). However this may be region specific, and needs further investigation.

2.5 Water Temperature

Water Temperature also affects water column stability and stratification. This in turn has direct affects on the biological process of *A. catenella.* The time it takes a cyst of *Alexandrium* species to mature during dormancy may be regulated by water temperatures. “For a single species, this dormancy can vary with soil storage temperature and the duration of this process can have significant effect on the timing of recurrent blooms, as cysts with a long maturation requirement may only seed on or two blooms per year, whereas those that can germinate in less time may cycle repeatedly between the plankton and the benthos and contribute to multiple blooms in a single season” (Anderson, 1998). Field studies of *A. tamarense* have shown that cysts that are subject to cold water temperatures remain dormant until water temperatures increase above a certain level, the exact value may be geographically linked and is undetermined at this time. However, the same pattern was found to be true for cysts in high water temperatures, which stay dormant until water temperatures decrease (Anderson, 1998). This indicates that a specific temperature window may be required for dinoflagellates cysts of the species *Alexandrium* to germination. This may help to explain why we see such a seasonal pattern in the initiation and timing of blooms of *A. catenella.*

“Various authors have discussed the importance of water temperature in the dynamics of HAB’s, with general agreement that this parameter plays a fundamental role both in the germination of cysts, and in vegetative growth of the cells. Temperature can affect division rates, photosynthesis and respiration, cell size, and other factors in species participating in blooms” (Navarro et al., 2006).

Temperature was one of the main environmental factors which affected the development of dinoflagellate blooms, with optimal values of 5-8 degrees Celsius observed at high latitudes" (Navarro et al., 2006).

Cell growth and division can be heavily influenced by water temperature and the subsequent layering of the water column that is associated with it. It has long been thought that water temperature may be one of the key factors driving fluctuations in blooms of toxic dinoflagellates. Navarro et al. (2006) found optimal cell concentrations of the Chilean species *A. catenella* at experimental water temperatures of 12 degrees Celsius, and significantly lower cell concentrations and growth rates at16 degrees Celsius. This is indicative of the fact that optimal water temperature requirements may be necessary for extensive blooms of *A. catenella* to perpetuate. Not only does water temperature influence the initiation of blooms as discussed above, but it may also have significant affects on the toxin content of individual cells. Navarro et al. also found, during his experiment, an inverse relationship between toxicity and water temperature in a laboratory experiment, which was reflected in observations of *A. catenella in* the field*.* This suggests that during the spring when water temperatures are warm but not too hot yet, growth is optimal and as summer progresses toward fall and water temperatures start to cool down at this time cell growth slows down and toxin production goes up. Water temperature most likely is also an important trigger in the termination of blooms of *A. catenella.* As water temperatures In the Puget Sound decrease in the fall *A. catenella*s cell growth and division may go up at first, but eventually slow down and this may trigger the formation of zygotes and gametes.

In order to understand how environmental drivers and environmental conditions such as turbulence, precipitation/runoff, salinity and water temperature set the stage for the initiation of blooms of *A. catenella,* it is pertinent to understand how these processes affect different aspects of the life cycle of *A. catenella.* By understanding how the physical properties of the aquatic environment affect *A. catenella s* life cycle processes we can extrapolate how large scale oceanic circulation patterns such as ENSO affect the different physical and biological process associated with the life cycle stages of this unique toxic dinoflagellate.

Chapter 3

Large Scale Oceanic Circulation Patterns Such as ENSO Affect Regional Weather, and Drive Small Scale Physical Processes

The term El Niño is Spanish for “the boy Christ-Child” and was originally used by fisherman to refer to the Pacific Ocean warm currents near the coast of Peru and Ecuador that appeared periodically around Christmas time and lasted for a few months. Only later, when it was linked to its atmospheric component the “Southern Oscillation”, was the phenomenon give the name “El Niño Southern Oscillation” (ENSO). Hanley et al. described the ENSO as a natural, coupled atmospheric-oceanic cycle that occurs in the tropical Pacific Ocean on an approximate timescale of 2-7 years. Which has three phases: warm tropical Pacific Ocean sea surface temperatures (El Niño), cold tropical Pacific Ocean sea surface temperatures (La Niña), and near neutral conditions (ordinary periods). ENSO is a complex ocean atmospheric circulation phenomenon and many aspects of it are still not well understood.

3.1 The El Niño Southern Oscillation (ENSO)

The Southern Oscillation is a seesaw of atmospheric pressure between the eastern equatorial Pacific and Indo-Australian areas, and is closely linked with El Niño (Glantz et al, 1991). The Southern Oscillation is the result of winds blowing over the equatorial Pacific, commonly referred to as the trade winds, which blow from east to west and are driven by an area of average high pressure in the eastern part of the Pacific Ocean and a low pressure area over Indonesia. The southern Oscillation consists of irregular intervals of strong and weak trade winds, which are related to changing sea surface pressures.

> "A physical explanation for the existence of the Southern Oscillation is provided at least in part by the "Walker Circulation", a large-scale atmospheric circulation consisting of sinking air in the eastern Pacific and rising air in the western Pacific and caused feedback between trade winds and ocean temperatures" (Katz, 2002).

In General, the tropical Pacific Ocean is characterized by warm surface water (29-30ºC) in the west and much cooler sea surface water temperatures in the east (22-24ºC) (Webster et al., 1997). This is due to the fact that during ordinary (non El Niño/La Niña) periods, eastern trade winds produce cool surface water in the eastern Pacific Ocean and at the same time they pool, or corral, warm surface waters in the far western Pacific Ocean. The cooler sea surface temperatures in the eastern Pacific Ocean are the result of evaporation and the upwelling of colder sea water from below the surface by the trade winds converging with the warm pool. The warmer waters in the west create what is known as the Pacific warm pool. The warm pool is relatively deep, with the temperature decreasing slowly with depth to the thermocline (see chapter 2) before dropping off more rapidly.

Approximately every two to seven years the state of equilibrium that exists during ordinary (non El Niño/La Niña) periods breaks down.

> “The onset of an El Niño is often marked by a series of prolonged westerly wind bursts in the western Pacific, which persist for one to three weeks and replace the normally weak easterly winds over the Pacific warm pool. At which time during an El Niño event, warming of sea surface temperatures occurs across the entire Pacific Ocean basin, which can last for a year or occasionally longer” (Webster et al., 1997).

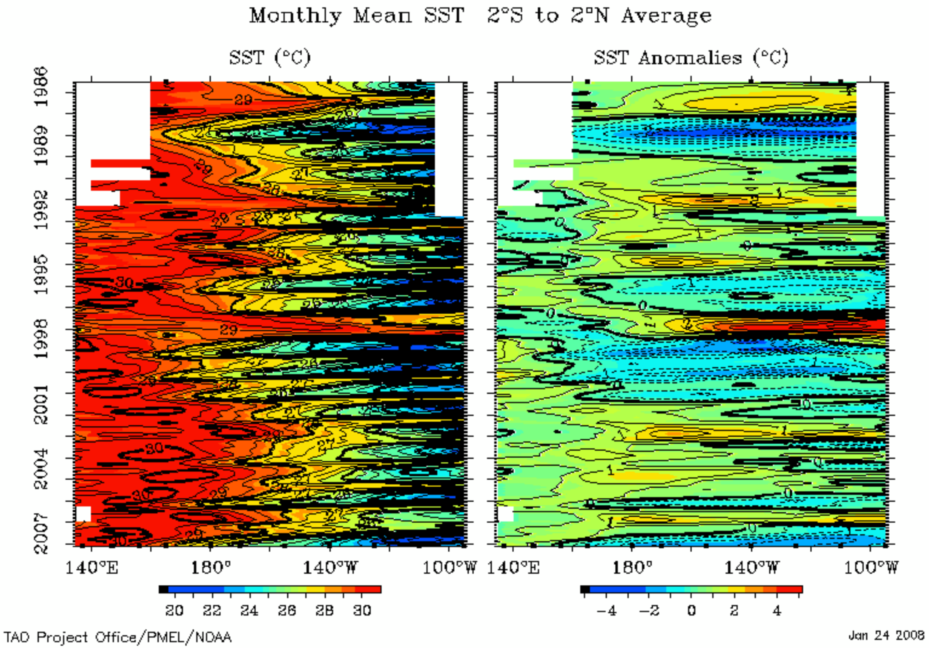


Figure 4: Mean and anomalies of sea surface temperature (SST) from 1986 to present, showing El Niño events left 1986-1987, 1991-1992, 1993, 1994 and 1997 and La Niña events right in 1985 and 1995 (www.pmel.noaa.gov/tao/elNiño/la-Niña-story.html).

During periods of El Niño the easterly trade winds weaken, causing the upwelling of deep water to cease or slow down in the eastern Pacific. The consequent increase in sea surface temperatures further weakens winds and strengthens the El Niño affect. As the trade winds weaken, so does the containment of the warm water in the west and the maintenance of the cooler waters in the east. As a result the relatively warmer water of the Pacific pool becomes ubiquitous all across the Pacific Ocean basin (Figure 4, left). This is commonly known as an El Niño event; typically the Pacific warm pool is displaced to the east, causing a shift in the major precipitation regions of the tropics and the disruption of normal climate patterns at higher latitudes (Webster et al., 1997).

3.2 La Niña

Typically an opposite and cooler state of the tropical Pacific follows a year or so after El Niño. This phenomenon is El Niño's twin sister and is known as La Niña ("the little girl" in Spanish). La Niña is characterized by unusually cold ocean temperatures in the eastern equatorial Pacific (Figure 4, right), as compared to El Niño, which is characterized by unusually warm ocean temperatures in the eastern Equatorial Pacific (Figure 4, left). This cooling of the Pacific Ocean is brought about partly by increases in the intensity of the eastern trade winds. The result of the eastern trade winds strengthening is more intense periods of upwelling in the eastern Pacific Ocean. The upwelling brings more nutrient rich deep cold water to the surface, and this colder water further cools the eastern Pacific. At the same time the Pacific warm pool is pushed farther west due to the stronger easterly winds. These warming (El Niño) and cooling phases (La Niña) are interspersed with the more common, normal, or quasi-equilibrium state, which for the sake of this paper we will call ordinary events or years.

3.3 ENSO Influence on Pacific Northwest Climate and Weather

The ENSO has global impacts on regional weather patterns, however, for the purpose of this study; we will concentrate on the impacts to Pacific Northwest weather and climate. The Climate Impacts Group examined monthly average temperature and precipitation values for El Niño versus La Niña years (1931-1999). They found that that in the Pacific Northwest El Niño winters tend to be warmer and drier then average, versus La Niña winters which tend to be cooler and wetter then average (C.I.G., 2008).

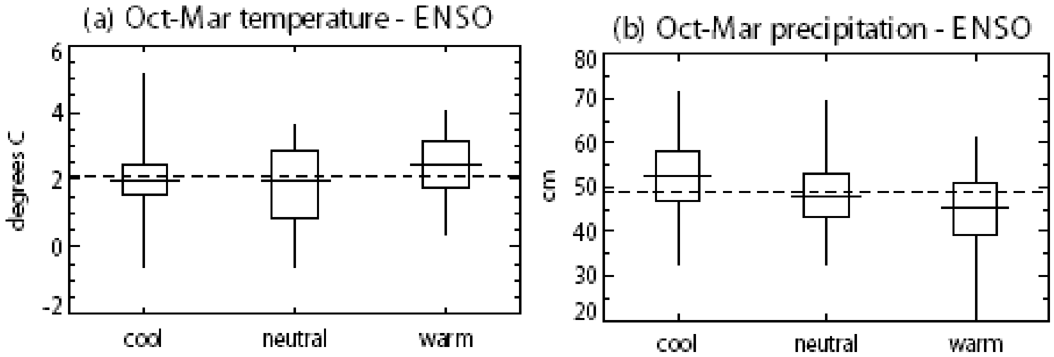


Figure 5: a (left)-b (right): Box-and-whisker plots showing the influence of ENSO on October-March (a) temperature and (b) precipitation (1899-2000). For each plot, years are categorized as cool (La Niña), neutral (ENSO neutral), or warm (El Niño). For each climate category, the distribution of the variable is indicated as follows: range of values (whiskers); median value for the phase category (solid horizontal line); regional mean for all categories combined (dashed horizontal line); 75th and 25th percentiles (top and bottom of box). Area-averaged Climate Division data are used for temperature and precipitation (C.I.G., 2008).

More specifically they found that October through March temperature is approximately .7 to 1.3 degrees Fahrenheit higher on average, during El Niño vs. La Niña years (Figure 5a)(C.I.G., 2008). The also found the October through

March precipitation is 14% less, on average, during El Niño vs. La Niña years (Figure 5b)(C.I.G., 2008). Reductions in winter time precipitation amounts during El Niño years reduce the amount of annual Snowpack in the mountains.

Less snow pack in the mountains means less than average stream flow during the spring, which when combined with lower precipitation amounts from El Niño can drastically reduce river flows in the Pacific Northwest. Ropelewski *et al* found that areas of Alaska and Canada experienced positive temperature anomalies in 17 out of 21 (81%) ENSO episodes from 1890 to 1984, during the "season" defined by December of the ENSO year through the following March. During El Niño, the northern part of the country experiences a positive shift in winter mean temperature anomalies central tendency, agreeing with the common understanding of the El Niño signal (Gershunov et al., 1998). Gershunov et al. also found the opposite during La Niña years, where the patterns for cold extreme frequency anomalies are roughly inverses of those for warm extremes with more cold outbreaks in the north western & western part of the country.

Not only does El Niño affect temperature and precipitation patterns in the Pacific Northwest, it also has dramatic effects on wind patterns. The strongest most, persistent wind gust patterns occur during the fall and winter for both El Niño and La Niña. This is not uncommon as both the warm and cold phases of ENSO reach maturity during the fall. Enloe et al. found that after examining peak wind gust from 1948 to 31st of August 1998, that during La Niña events for the months of November to March, the Pacific Northwest experiences an overall increase in the gustiness of the winds. While La Niña is associated largely with positive differences (greater than 20%) in monthly means of the peak wind gust and the frequency of gale force wind gusts El Niño is often associated with reduced peak wind gust in the Pacific Northwest (Enloe et al., 2004).

> "Though strong differences in means are not observed, there is a general reduction in peak wind gusts over the entire Contiguous United States during El Niño, as well as a reduction in occurrences of gale force wind gusts. One persistent warm phase signal, though weak, is observed in the Northwest. From the beginning of the El Niño year (October) through February, a general weak reduction n the monthly mean peak wind gust (0 to -5%) spans most of the country. However, percent changes of -5% to -10% are more common in the Northwest, with some magnitudes as great as -15% to -20% occurring during these months. In a warm phase November, Seattle averages a daily peak wind gut of 10.3% (19.2kt (knots) or 9.9 meters per second) smaller then the average neutral (ordinal event) phase wind gust of 21.4kt or 11 meters per second" (Enloe et al., 2004).

Now that we know how different the affects that El Niño and La Niña have on weather patterns in the Pacific Northwest we can begin to see how they would have different effects on the different life cycle stages of *A. catenella.*

Chapter 4

Climate Changes Impacts to Pacific Northwest Weather and ENSO

Twentieth century climate shows evidence of change. We see headlines in the newspapers on an almost daily basis, but what does it mean for P.S.P. levels in Washington? According to the projections of a recent study, the Pacific Northwest will experience warmer, wetter winters and hotter, drier summers (UW CIG, 2004). While natural climate variability has caused (and will continue to cause) fluctuations in Pacific Northwest climate on seasonal and decadal scales, analysis of observed twentieth century conditions shows evidence of longer term trends that are consistent with modeled projections of twenty-first century climate change (UW CIG, 2004). These trends include region-wide warming, increased precipitation, declining snow pack, earlier spring runoff, and declining trends in summer stream flow.

> “To understand the implications of rising temperatures and potentially increased winter precipitation on the PNW water cycle it may be helpful to consider 1998 and 1999 water years. In 1998 one of the strongest El Niño's on record created unusually warm winter temperatures in the PNW. While snow-pack was only a little below normal for the winter due to near normal precipitation, the snow began melting approximately one month earlier then normal and very rapidly due to unusually warm spring conditions. A warm, dry summer followed and the lengthened summer season caused by the early melt created water supply problems in some areas in the late summer. These effects are similar to what we think would be experienced under climate change on average in the summer.

> Some years would be dryer, some wetter then the average (Hamlet, 1997)".

Climate change affects Pacific Northwest weather in two ways, first by changing the temperature, and second by changing precipitation patterns. With relation to harmful algal blooms the first would have the most impact, though the second does play an important role as well. Climate models simulate average winter temperature increases ranging from about 3.2-3.9 degrees F by the 2020s and 4.8-6.1 degrees F by the 2050s (Hamlet, 1997). In snowmelt-dominated systems, these higher winter temperatures would cause more of the dominate precipitation to fall as rain in the winter, leaving less water stored as snow to supply summer stream flow. Higher spring and summer temperatures melt the snow earlier, increasing the length of the growing season, and increasing summer evapotranspiration, which results in less spring, summer, and fall stream flow. The earlier melt also effectively lengthens the period between the end of snowmelt and the onset of fall rains. In hydrologic terms this is like making summer several months longer then it is now.

This may have drastic affects on the duration and initiation of harmful algal blooms. As we know *A. catenella* thrives in a stratified environment (chapter 2). In the Puget Sound Basin during the summer when stream flow is lowest (low flow period), this is also the period when the basin becomes heavily stratified. With warmer climate trends this period of low flow will be arriving sooner and lasting longer, according to a recent analysis done on streamflow data, spring streamflow during the last five decades has shifted so that the major April-July streamflow peak now arrives one or more weeks earlier, resulting in declining fractions of spring and early summer river discharge (Stewart, 2005). This could have implications for the monitoring and prediction of when blooms of *A. catenella* may occur and how long (duration) they may last. If low flow events are occurring earlier then it follows suit that blooms of *A. catenella* may occur earlier in response to environmental conditions. If low flow events will occur over a longer temporal period than it seems likely that blooms of *A. catenella* may occur over a longer temporal period (duration). With this in mind it is important that we understand how physical processes influence the life history of *A. catenella,* before changing hydrological and weather patterns make it even harder to predict and monitor blooms of this species. This in turn may make it easier in the future to separate out the anthropogenic influences (nutrient inputs) from the influences of natural climate drivers such as ENSO.

The International Panel on Climate Change recently published a new report about the impacts of climate change on ENSO. They found that the 1997-1998 El Niño event was the largest on record in terms of sea surface temperatures anomalies and they also found that the global mean temperature in 1998 was the highest on record, at least until 2005 (IPCC 4th assessment, 2007). It was estimated that the global mean surface air temperatures were 0.17º Celsius higher for the year centered on March 1998 due to the influence of an El Niño phase of ENSO. Since the El Niño phase of ENSO is associated with warming sea surface temperatures it makes sense that if sea surface temperatures continue to rise, then there will likely be more frequent and more persistent El Niño phases of ENSO if global warming persist. However, the exact influence of global warming, whether it is anthropogenic or natural, on the phases of ENSO is not understood at this moment so this is merely speculation. At the same time whether it is natural or anthropogenic, understanding how changing climate patterns will affect P.S.P. levels may be crucial to understanding the year to year fluctuations in P.S.P. levels.

Chapter 5

Data Sources

This study involves the analysis of ENSO influence on Paralytic Shellfish Poisoning (P.S.P) levels present in shellfish throughout Washington States marine waters from January of 1977 to December of 2007. Statistical analysis was performed to determine if there is a relationship between P.S.P. levels and the phases of ENSO.

5.1 ENSO Index

Until recently there has been no consensus within the scientific community as to which index best defines ENSO years, including the strength, timing, and duration of events. However, during 2005 the National Weather Service, the Meteorological Service of Canada, and the National Meteorological Service of Mexico have reached a consensus on an index (Anonymous, 2005). The index is defined as a 3-month average of sea surface temperature departures from the normal average temperatures as defined by the period of time 1971-2000, for a critical region of the equatorial Pacific (Niño 3.4 region; 5ºN-5ºS, 170º-120ºW). “Departures from average sea surface temperatures in this region are critically important in determining major shifts in the pattern of tropical rainfall, which influence jet streams and patterns of temperature and precipitation around the world" (Anonymous, 2005). For this study the Niño 3.4 region was used to calculate shifts from ordinary weather events to El Niño and La Niña phases. The Niño 3.4 region overlaps portions of the Niño-3 and Niño-4 regions covering an area between 5ºN-5ºS latitudes and 170º-120ºW longitudes. Hanley *et al* found the Niño-3.4 and the Niño-4 indices to be the most sensitive for predicting ENSO events. The Climate Impacts Group found the Niño-3.4 index to be the best indicator of weather pattern anomalies in the Pacific Northwest associated with the ENSO (C.I.G., 2008).

The data for this study was acquired from the National Weather Service Climate Prediction Center (http://www.cpc.noaa.gov). Each phase of ENSO, whether it be El Niño(+) or La Niña (-), is determined based on a threshold of +/- 0.5ºC, which is calculated from a 3-month running mean of sea surface temperature anomalies, in the Niño 3.4 region, departures from normal. An El Niño or La Niña event occurs when the threshold of +/- 0.5ºC is met or exceeded for a minimum of 5 consecutive months.

5.2 WDOH Database

Paralytic Shellfish Poisoning (P.S.P.) data were provided by the Washington State Department of Health (WDOH) Office of Shellfish and Water Protection. The WDOH has been routinely monitoring P.S.P. throughout Washington State in both commercial and recreation shellfish since the 1930's

(Cox, 2001). Though *A. catenella* produces an entire family of toxins, the best known and the one thought to be the cause of P.S.P. is Saxitoxin, and thus is the toxin the WDOH test for (Cox, 2001). The minimum Lethal Dose of Saxitoxin (P.S.P.) for humans is 9µg/Kg of (human) body weight (Halstead, 1965). Figure 6 shows the chemical structure of saxitoxin.

Testing in Washington State began in 1930 after a large outbreak of P.S.P. related illnesses and deaths that occurred in California, and was expanded in 1957 to include the northern inland waters of the state after a severe outbreak of P.S.P. in nearby British Columbia (Cox, 2001). Monitoring was expanded again in 1978 to include most of Puget Sound after a widespread outbreak, with P.S.P. levels as high as 30,000 µg per 100g of mouse, caused 10 serious illnesses in the Whidbey Island area. The monitoring program was expanded again in 1988 when oysters in Carr Inlet had detectable levels of P.S.P. as high as 2,200 µg each, causing the first shellfish area closure, due to P.S.P. levels, south of the Tacoma Narrows Bridge (Cox, 2001). Monitoring is now conducted uniformly throughout all marine waters in Washington State, regardless of past P.S.P. history.

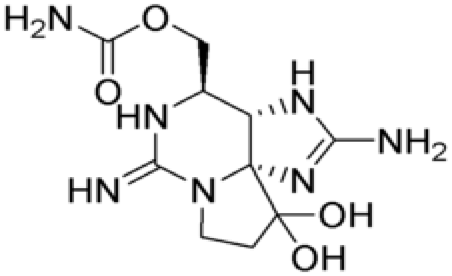


Figure 6: Chemical structure of Saxitoxin.

The WDOH maintains two biotoxin monitoring programs; one for P.S.P, and one for domiac acid poisoning. The larger of the two is designed to monitor P.S.P. biotoxin in numerous species of shellfish, including clams geoducks and oysters. These samples are collected from hundreds of locations throughout Puget Sound by volunteers, WDOH employees, other state employees, and industry representatives and then delivered to the Washington State Department of Health's Food Safety lab for testing. A 100 grams of shellfish tissue are collected (needed) for the toxin analysis, this is about a 100 average size (1-2 inches in length) mussels, and then placed in one-gallon size baggies, packed in Styrofoam containers with frozen gel packs and shipped to the Washington State Department of Health's Food Safety lab for testing. Once an area has tested positive for P.S.P., it will be tested until it has had no positive results for three consecutive years. Up until 1990 this was generally how samples were collected and disseminated for analysis. In 1990 the Sentinel Monitoring Program was started to assess if the WDOH can become more predictive then reactive to P.S.P. levels.

The Sentinel Monitoring Program is designed to act as an early warning system for the onset of P.S.P. activity. The Sentinel Monitoring Program helps guide regional monitoring under the larger general sampling program. A single species of shellfish is sampled, typically the blue mussel *Mytilus edulis* however *M. galloprovincialis* (a possible subspecies of *m. edulis*) and the oyster *M. californianus* are used at a few Puget Sound sites, from about 40 fixed locations throughout Washington's marine waters (Determan, 2003). At most sites, wire mesh cages are periodically stocked with the blue mussel and suspended about one to two meters deep. Sampling occurs on the frequency of every 2 weeks during the year, except following an event of high detection levels when samples are found to have detectable levels of P.S.P. (>38 µg STX/100g). “In other words, there was increased sampling during a toxic event, to characterize the extent and severity of the event, resulting in a greater proportion of tests that are positive for toxin” (Trainer et al., 2003).

The shellfish samples are analyzed using the mouse bioassay procedure. The mouse bioassay is the most commonly used method for routine analysis of P.S.P. in shellfish throughout the world and is the accepted method for regulatory purposes in the United States. During its initial use the mouse bioassay results were typically expressed as mouse units. However, the mouse bioassay has been modified since it was first used in the 1920’s. Since then, the US Food and Drug Administration (FDA) has added a saxitoxin standard, the results are now expressed in saxitoxin equivalents, STXeq (Trainer et al., 2003). Now, results are given as micrograms of saxitoxin equivalents per 100 grams of shellfish meats (µg STX/100g).

The results are then reported to the WDOH office of Shellfish and Water Protection for entry in their database. Test results are coded and entered into the database using the following classification system: A -1 indicates that no toxins were detected; A -2 indicates that no test was preformed, which can occur for several reasons ranging from not enough meat submitted to the shellfish spoiling before it reaches the lab; A -3 indicates that only trace amounts were detected, but not enough to determine the amount; A -4 indicates that the level of P.S.P. present in the shellfish was less than 38µg STX/100g of shellfish; A -5 indicates that the test was unsatisfactory. Results that are greater than 38µg STX/100g of shellfish are simply reported as the amount of saxitoxin present in the shellfish (#µg STX/100g of shellfish). Molluscan shellfish with a P.S.P. content of less than 80 µg/100g meats are permitted to be harvested, processed and sold.

Chapter 6

Analysis and Results

Using the WDOH data set on P.S.P. levels in Washington States marine waters and the ENSO index from NOAA, statistical analysis can be performed to determine if a relationship exists between the two. In order to see if there is any relationship between the P.S.P. levels and ENSO index, the available data were examined over time. By visually comparing the patterns of distribution between the ENSO 3-month moving averages departures from normal over time with P.S.P. levels over time we can see if any similar patterns exist. Time plots of the ENSO 3.4 region index and the P.S.P. levels over the period January 1st of 1977 through December 31st of 2007 are shown in Figures 7 & 8.

As we can see from Figure 7 there is a cyclical pattern to the phases of ENSO but what drives the length of each phase is still being determined by climatologists (chapter 3). Similar descriptive statistics were used in an attempt to examine potential seasonal patterns of P.S.P. levels over time. And as we can see from Figure 8, there is a seasonal pattern of fluctuation to the P.S.P. levels in Washington’s marine waters; however due to some extreme events of unusually high P.S.P. levels this seasonal pattern is difficult to discern.

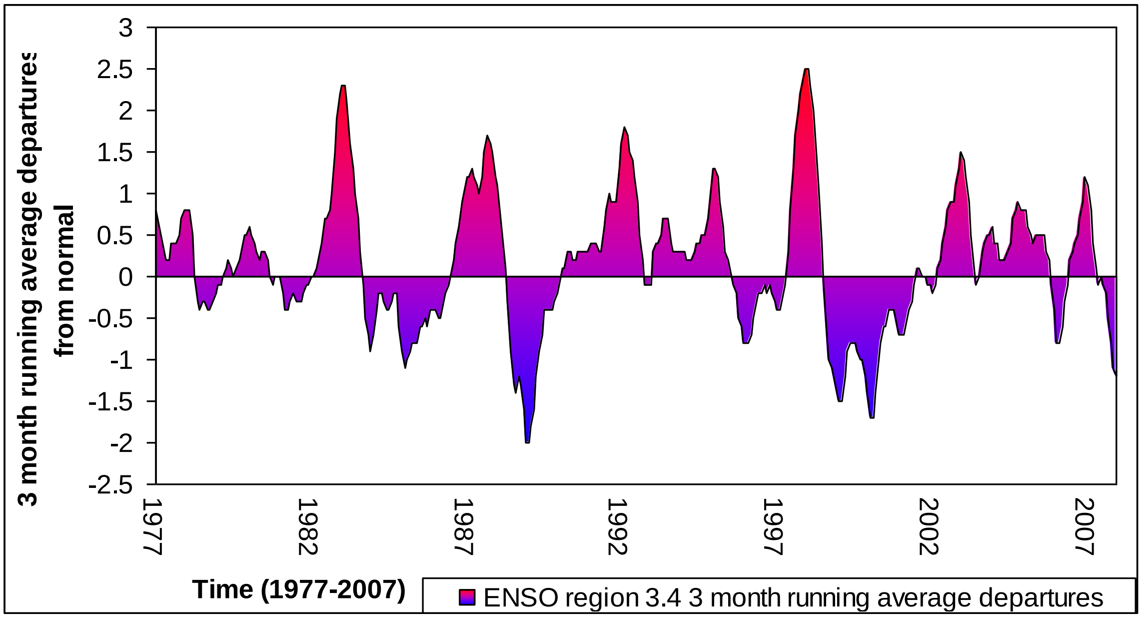


Figure 7: ENSO over time based on a threshold of +/- 0.5ºC which is calculated from a 3 month running mean of sea surface temperature anomalies or "departures from normal" in the Niño 3.4 region which is based on the 1971-2000 base period. An El Niño or La Niña event occurs when the threshold of +/- 0.5ºC is met or exceeded for a minimum of 5 consecutive months.

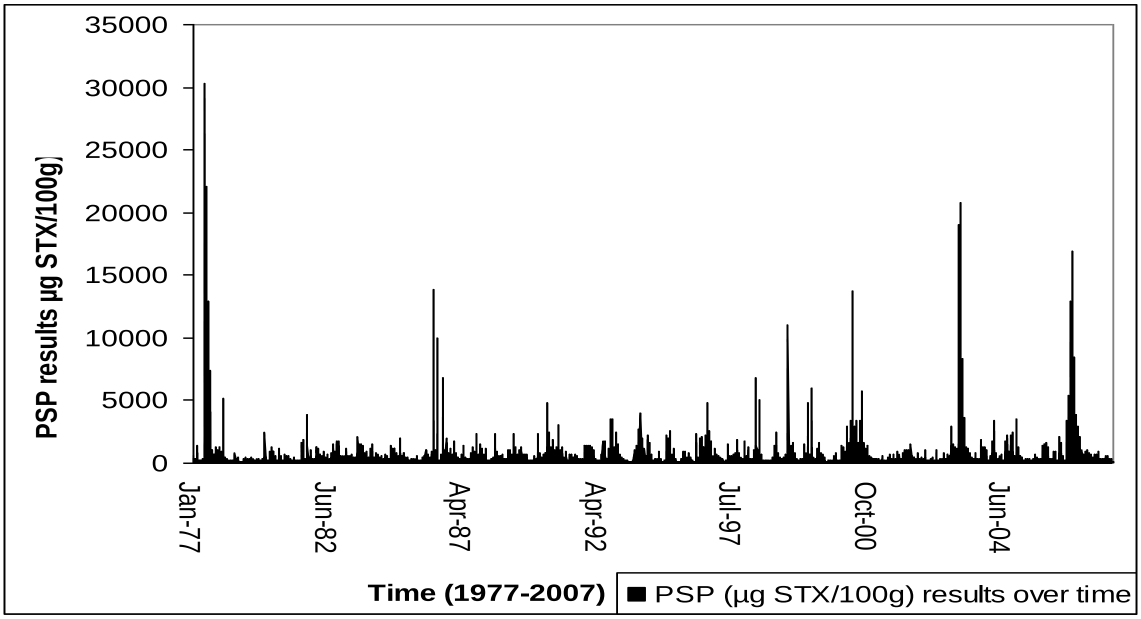


Figure 8: P.S.P. levels for the period 1/1/1977-12/31/2007, over time.

No obvious visual similarities immediately stand out between the ENSO 3.4 region index and the P.S.P. data when visually comparing Figures 7 & 8. However, as we can see from Figure 8, there are a few outliers (>5,000µg STX/100g) that are dominating the data and thus masking any regular seasonal or cyclical pattern. These outliers are likely the result of a localized phenomenon and not indicative of the overall pattern of P.S.P. seen in Washington's marine waters. Removing these extreme events may reveal patterns in the P.S.P data over time and allow us to see whether they are similar to the patterns of the ENSO index over time. Further investigation of these outliers should be done to determine the conditions associated with them, but this is not within the scope of this thesis.

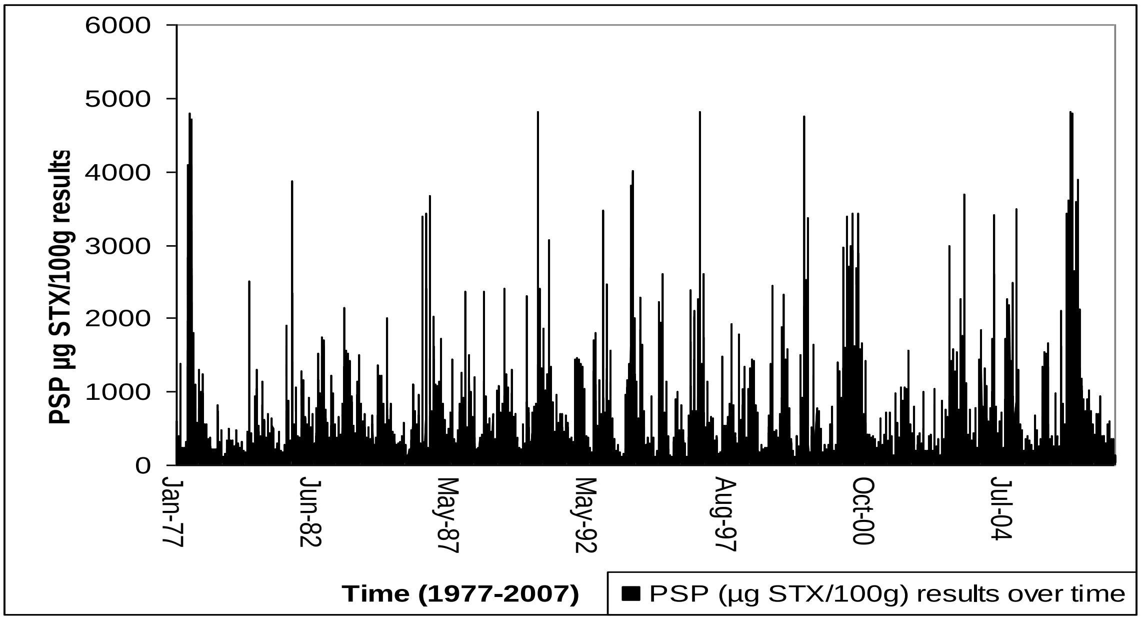


Figure 9: P.S.P. levels equal to or less than 5,000µg STX/100g over time.

From the distribution of the P.S.P. levels in Figure 9 we can see the seasonal fluctuations more clearly, but strong variation in the pattern exist from year to year. What causes this variation is still unknown at this time; it could be weather related, which may become evident through this analysis, or it could be due to other parameters of *A. catenella's* environment, such as trophic structures.

In order to more appropriately assess whether there is a cyclical pattern to P.S.P. levels in Washington State marine waters, the monthly P.S.P. values were converted to a three-month moving average. The three-month moving average of the P.S.P. data will smooth out the daily variations and show the overall trend of the P.S.P. data. The three-month moving average transformation was chosen to match the averaging time of the ENSO index, so that the P.S.P. levels and ENSO index can be compared, and to provide a better visual graphic of the distributions of the phases of ENSO and P.S.P. levels over time. From Figure 10 we can see that there is still a great deal of variation but now we can see that during certain years there are larger peaks in P.S.P. levels.

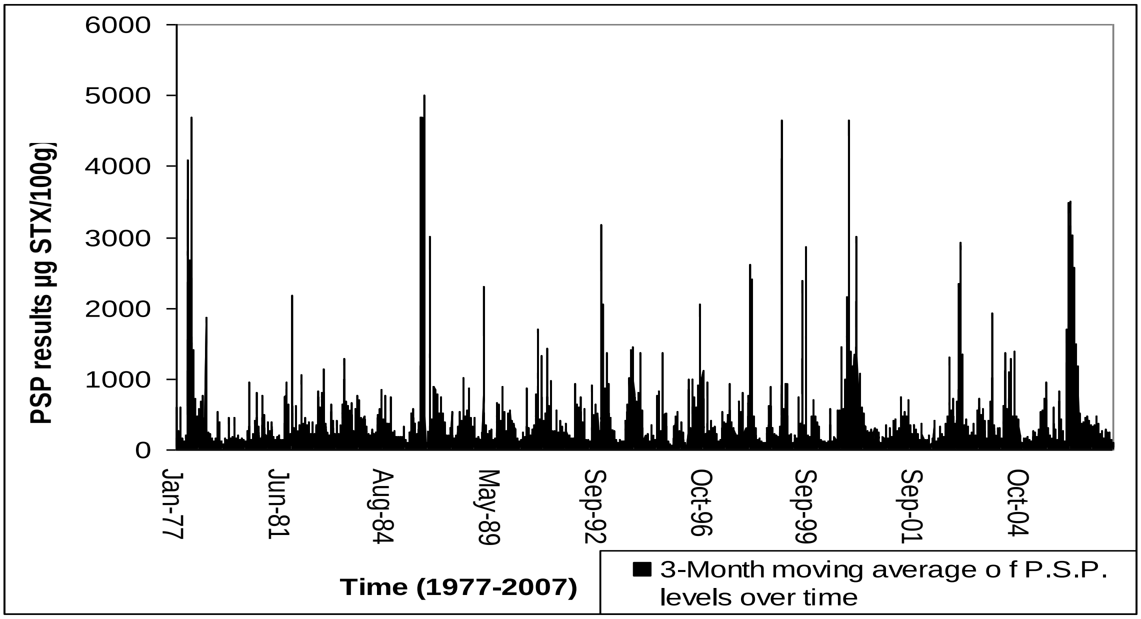


Figure 10: 3 month moving average of P.S.P. µg STX/100g levels over time.

However, the ENSO index is expressed as moving average *departures* from a baseline and reveals both increases and decreases from it. In order to determine whether the three-month moving average of P.S.P. levels over time coincide with the phases of ENSO the P.S.P levels were converted to moving average *departures* from the all time average (1977-2007) and the two parameters were graphically displayed superimposed on the same graph. As we can see from Figure 11, qualitatively it appears that there are similarities in the phases of ENSO and P.S.P. levels in Washington States marine waters. This graphic display suggests that there may be a relationship between the phases of ENSO and P.S.P. levels.

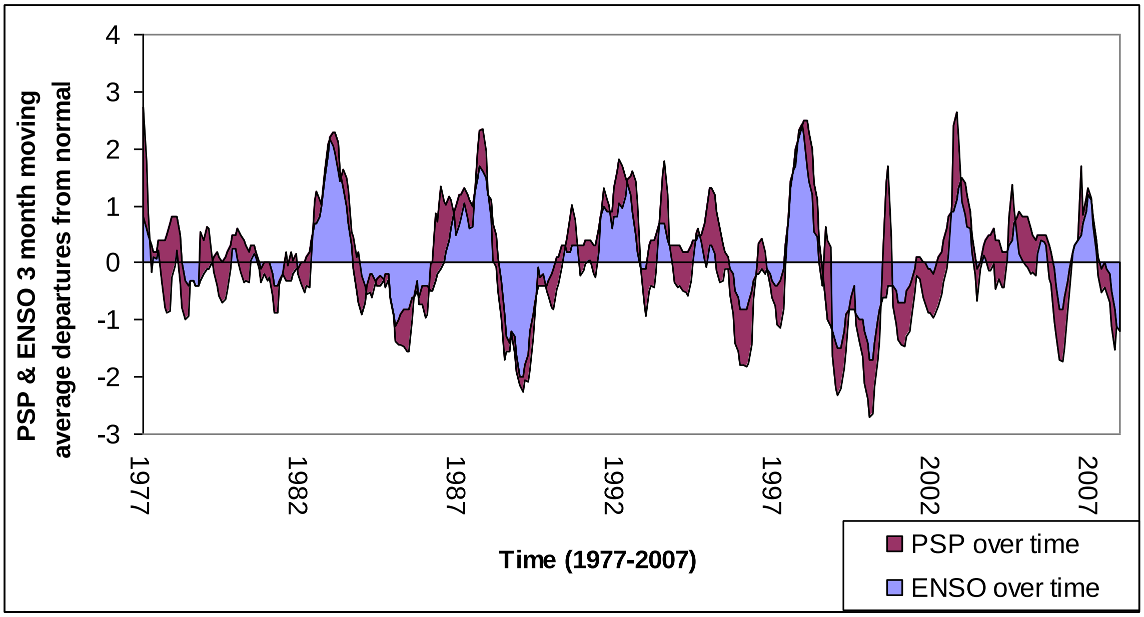


Figure 11: Area plot of 3 month running means departures from normal for both ENSO and P.S.P. levels in Washington States marine waters.

Despite being suggestive, this evidence is not adequate to show that there is a significant relationship between the two variables. In order to determine if ENSO has a direct affect on P.S.P. levels in Washington States marine waters, further analysis is needed to determine if there is a relationship between the two.

A simple linear regression was chosen to test the significance of the relationship between the two parameters. However, because the distribution of the P.S.P. data and the 3-month moving average showed significant departures from a normal distribution, the 3-month moving average P.S.P. data were log-transformed to be converted to a normal distribution. Therefore, a simple linear regression analysis was performed using the ENSO 3.4 region index as the explanatory variable and the log of the 3-month moving average of P.S.P. levels as the response variable to see if a linear relationship exists between the two variables.

The regression showed that there was a significant linear relationship between these parameters with an F=0.0000 and p =4.75^{E-10}, although the slope of the line was very low, b_1=0.0250 (95% CI=0.0171, 0.0328). Indeed, a very low R^2 value of 0.0000 indicates that the variability of the log of the 3-month moving average of P.S.P. levels is not adequately explained by the variability of the ENSO index, and suggests that ENSO alone is not enough to explain the pattern of P.S.P. levels in Washington States marine waters (Figure 12).

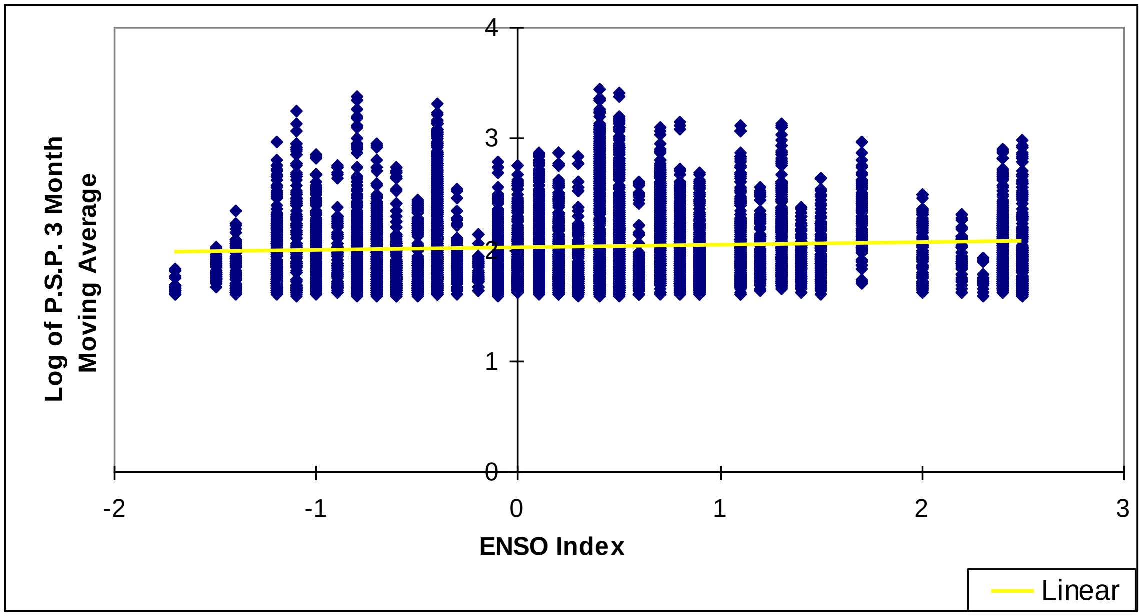


Figure 12: Linear Regression of Log of 3 month moving average vs. ENSO Index.

To see if there is any relationship between extreme weather events of both the cold and warm phases of ENSO and higher P.S.P. levels, linear regression was performed between the 3-month moving average of P.S.P. levels and the absolute ENSO index values to indicate only the degree of departure from baseline. This is the variation of P.S.P. levels related to *extreme* ENSO events regardless of the direction away from the baseline, in other words, extreme departures from normal regardless of whether they are El Nino or La Nina. The regression showed that there was a significant linear relationship between these parameters with an F=0.0084 and p =0.0084, although the slope of the line was very low, b_1=0.0163 (95% CI=0.0042, 0.0284). Indeed, a very low R^2 value of .0009 indicates that the variability of the log of the 3-month moving average of P.S.P. levels is not explained by the variability of the absolute ENSO index, and suggests that absolute ENSO index alone is not enough to explain the pattern of P.S.P. levels in Washington States marine waters.

As discussed in chapter 3, the phases of the ENSO have different affects on local weather patterns, and from chapter 2 we know that different weather parameters have different effects on the life cycle of *A. catenella*. As such we would expect to see some influence on the levels of P.S.P. by localized weather patterns. Ten years (1997-2006) worth of weather data was collected from a weather station at Seattle Tacoma international airport and entered into Excel (http://www.wunderground. com/history/airport/KSEA.html). The parameters that more directly influence the life cycle of *A. catenella* include salinity, water temperature, Turbulence, and precipitation. Unfortunately, despite the close relationship between the *A. catenella* biotic cycle and these parameters, no related data was available for this study. These are not measured on a routine basis. Therefore, other local weather parameters were chosen as proxy indicators for them. Data available for this study are mean daily air temperature (C^o), max daily wind speed (mph), average daily wind speed (mph), and daily precipitation (inches). Initially a simple linear regression was performed for each of the available parameters.

6.1 Air temperature

Water Temperature affects water column stability and stratification. This in turn has direct affects on the biological process of *A. catenella.* Cell growth and division can be heavily influenced by water temperature and the subsequent layering of the water column that is associated with it. Air temperature was chosen as a good indicator of water temperature. A linear regression analysis of the log of the 3-month moving average of P.S.P. levels and mean daily air temperature was performed to see if linear relationships exist between the two variables.

The regression showed that there was a significant linear relationship between these parameters with an F=0.0000 and p =7.87^{E-55}, although the slope of the line was very low, b_1=0.0107 (95% CI=0.0093, 0.0120). Indeed, a very low R^2 value of 0.0300 indicates that the variability of the log of the 3-month moving average of P.S.P. levels is not explained by the variability of mean daily air temperature, and suggests that mean daily air temperature alone is not enough to explain the pattern of P.S.P. levels in Washington States marine waters (Figure 13).

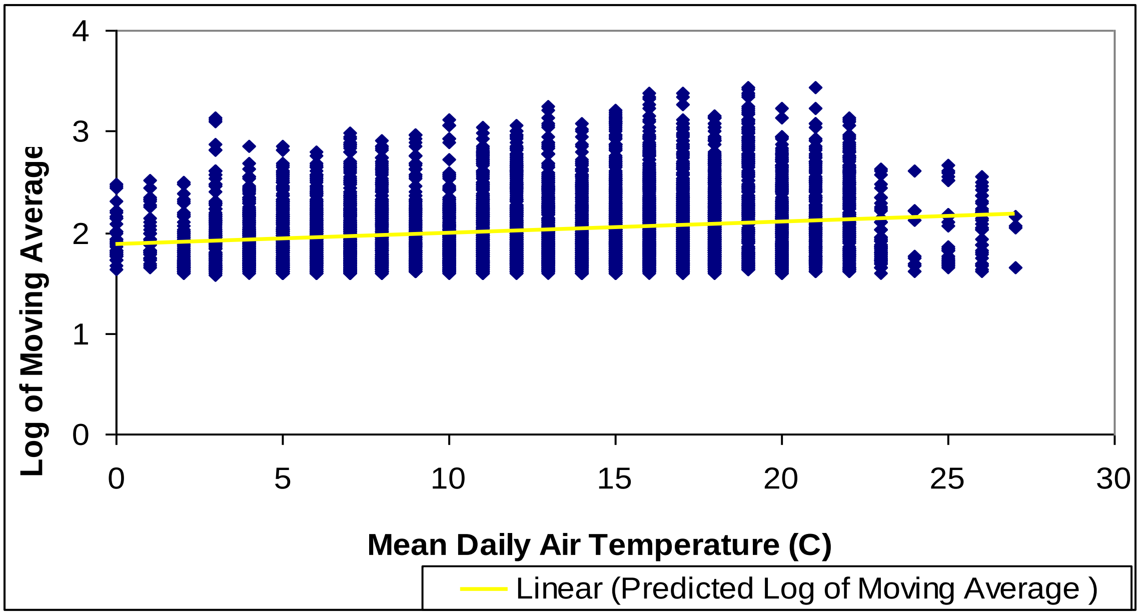


Figure 13: Linear Regression of Log of 3 month moving average vs. mean daily air temperature (C).

6.2 Wind

According to Smayda, (1997) turbulence can negatively influence dinoflagellate blooms by three mechanisms: physical damage; physiological impairment; and behavioral modification. It has long been noted that blooms of *A. catenella* typically occur during periods of weak winds, which creates less turbulence in the water column thus inducing stratification.

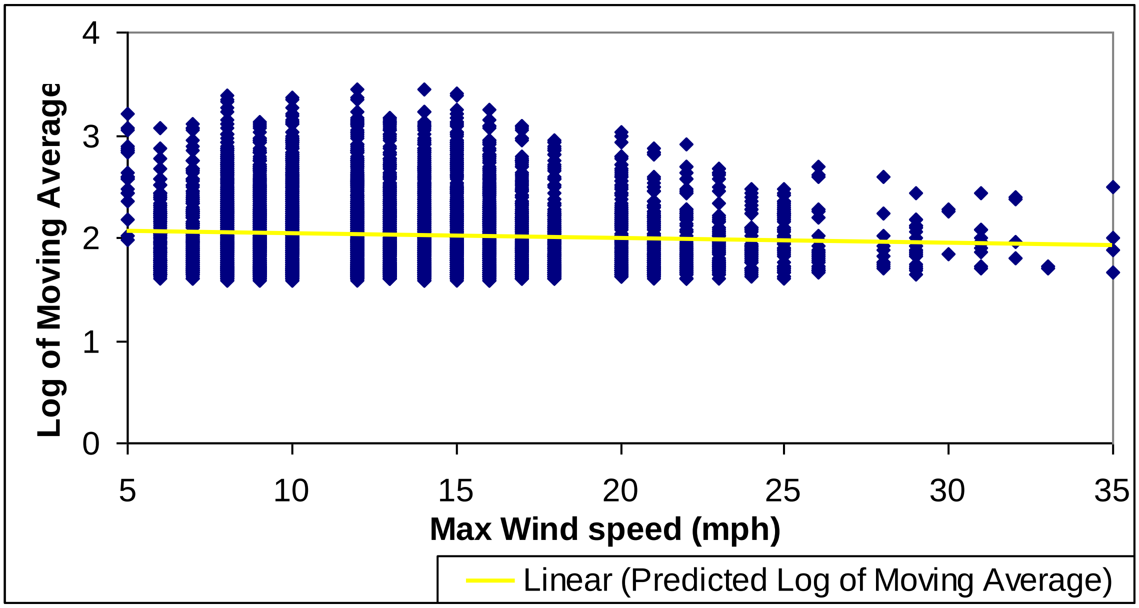


Figure 14: Linear Regression of Log of 3 month moving average vs. max daily wind speed.

Two indicators of wind were used for this study, max daily wind speed and average daily wind speed. Max daily wind speed is representative of peak wind gust for the day while average daily wind speed is representative of the overall wind speed for the day. A linear regression analysis of the log of the three-month moving average of P.S.P. levels and max daily wind speed (mph) was performed to see if linear relationships exist between the two variables.

The regression showed that there was a significant linear relationship between these parameters with an F=0.0000 and p =5.21^{E-07}, although the slope of the line was very low, b_1= -0.0043 (95% CI= -0.0059, -0.0026). Indeed, a very low R^2 value of 0.0031 indicates that the variability of the log of the 3-month moving average of P.S.P. levels is not explained by the variability of the max daily wind speed, and suggests that max daily wind speed alone is not enough to explain the pattern of P.S.P. levels in Washington States marine waters (Figure 14).

A linear regression analysis of the log of the 3-month moving average of P.S.P. levels and the average daily wind speed (mph) was performed to see if linear relationships exist between the two variables. The regression showed that there was a significant linear relationship between these parameters with an F=0.0000 and p =3.77^{E-07}, although the slope of the line was very low, b_1= -0.0054 (95% CI= -0.0074, -0.0033). Indeed, a very low R^2 value of 0.0032 indicates that the variability of the log of the 3-month moving average of P.S.P. levels is not explained by the variability of the average daily wind speed, and suggests that average daily wind speed alone is also not enough to explain the pattern of P.S.P. levels in Washington States marine waters (Figure 15). However it should be noted that the slope is negative for both wind variables indicating a decrease in P.S.P. with higher wind speeds and by extension supporting the reasoning about higher winds speeds breaking up or dissipating blooms of *A. catenella*.

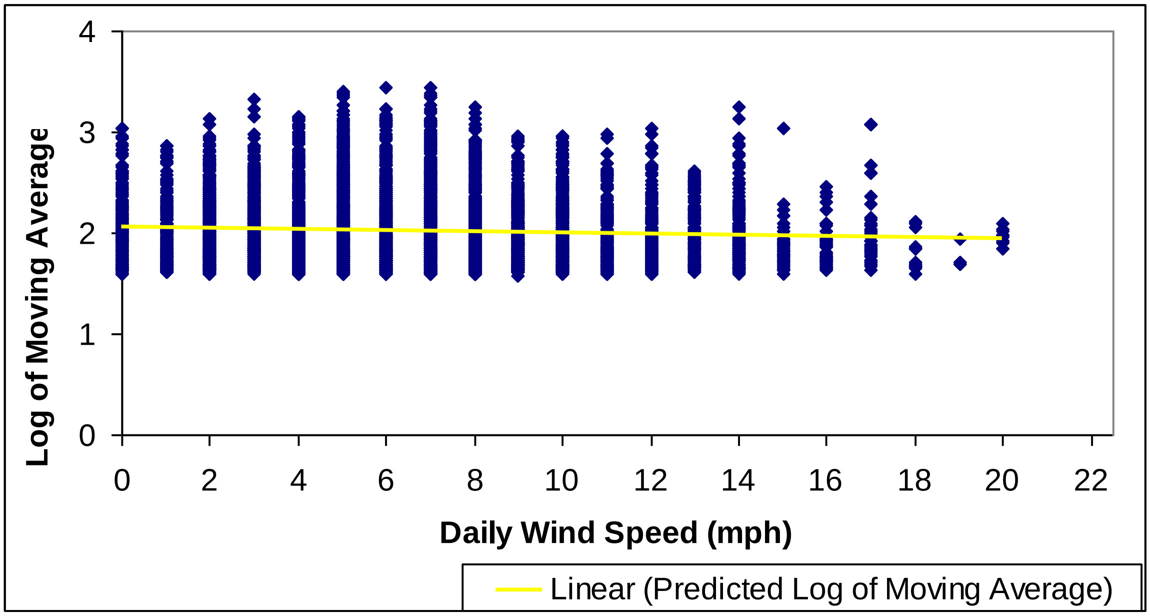


Figure 15: Linear Regression of Log of 3 month moving average vs. average daily wind speed.

6.3 Precipitation

Precipitation supplies important nutrients that are the energy that fuels the cell division of *A. catenella.* This can be especially important during summer months when nutrients can become depleted in the surface mixed layer. A linear regression analysis of the log of the 3-month moving average of P.S.P. levels and the daily precipitation amount (inches) was performed to see if linear relationships exist between the two variables.

The regression showed that there was a significant linear relationship between these parameters with an F=0.0000 and p=7.64^{E-06}, although the slope of the line was very low, b_1= -0.0622 (95% CI= -0.0895, -0.035). Indeed, a very low R^2 value of 0.0025 indicates that the variability of the log of the 3-month moving average of P.S.P. levels is not explained by the variability of the daily precipitation amount, and suggests that daily precipitation alone is not enough to explain the pattern of P.S.P. levels in Washington States marine waters (Figure 16).

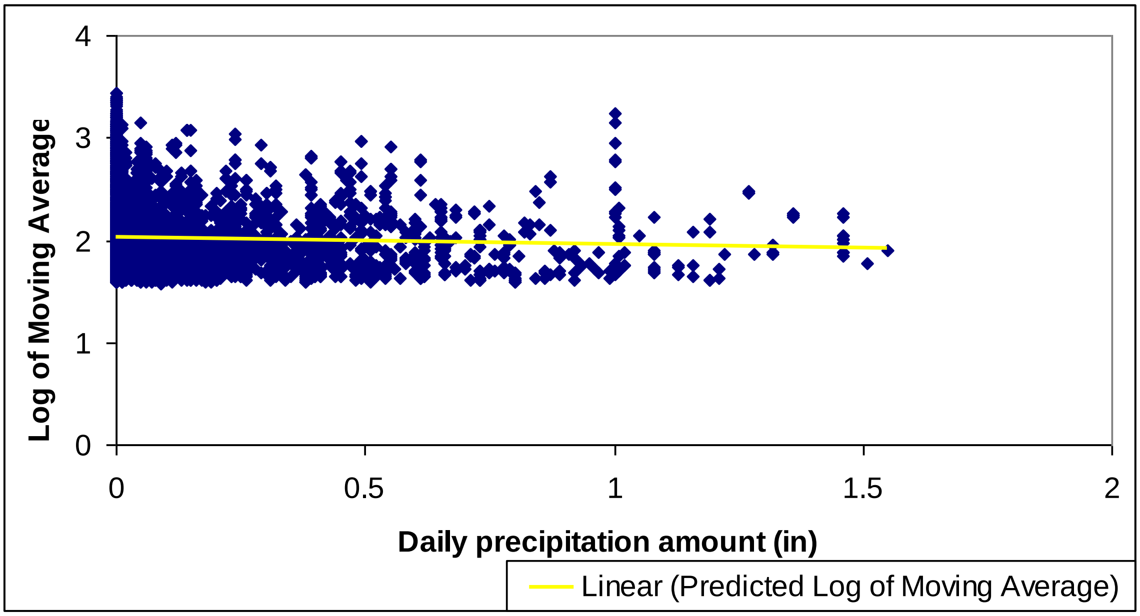


Figure 16: Linear Regression of Log of 3 month moving average vs. daily precipitation amount (inches).

6.4 Multiple liner regression analysis

Though each of the above variables alone do not explain the variation in P.S.P. levels seen in Washington States marine waters, perhaps their combination does allow a more complex relationship to become evident. A multiple linear regression analysis was performed using the log of the 3-month moving average of P.S.P. levels as the response variable and a number of explanatory variables including the ENSO index, daily ambient air temperature (Cº), daily max wind speed (mph), average daily wind speed (mph), and daily precipitation amount (inches).

Table 1: Multiple regression of the log of the 3 month moving average vs. the ENSO index, daily air temperature, max daily wind speed, daily wind speed, and daily precipitation.

Adjusted R Square	0.0383			
F	64.6784			
Significance F	0.0000			
	slope	***Confidence Interval Lower 95%, Upper 95%***	***t Stat***	***P-value***
ENSO Index	0.0314	0.0236, 0.0393	7.8599	4.35^{E-15}
Mean Daily Air Temperature (C)	0.0106	0.0092, 0.0119	15.1930	2.05^{E-51}
Max Wind speed (mph)	-0.0009	-0.0031, 0.0013	-0.7939	0.4273
Daily Wind Speed (mph)	-0.0026	-0.0052, 0.0000	-1.9441	0.0519
Daily precipitation amount (in)	-0.0236	-0.0516, 0.0044	-1.6509	0.0988

As we can see from the significance of F (0.0000) in Table 1 the model is significant. Also from Table 1 we see that, despite low slope values, the first two variables, ENSO index and Mean Daily Air Temperature, show a significant linear relationship with P.S.P. levels. However the last three variables t-stat and p-values indicate that these variables are not significant. The adjusted R^2 indicates that only 3.8% of the variability of P.S.P. levels is explained by the variability of all these five variables, indicating the model is incomplete. However, the model including all five explanatory variables has helped increase R^2 (from <0.3% to 3.8%) compared to the simple linear models with each variable alone.

However some of the independent variables may be correlated to each other and that may affect the outcome of the regression. A correlation analysis shows that only two variables are correlated (Table 2), Max wind speed (mph) is correlated with average daily wind speed (mph). This is not surprising, since a higher average daily wind speed is likely associated with a higher max wind speed for the same day. It also happens to be that the least significant variable in the first multiple regression output (Table 1) is max wind speed. Thus removing max daily wind speed from the regression may improve the model.

Table 2: Correlation output among the Independent variables.

	ENSO Index	***Mean Daily Air Temperature (C)***	***Max Wind speed (mph)***	***Daily precipitation amount (in)***
ENSO Index	1			
Mean Daily Air Temperature (C)	-0.0824	1		
Max Wind speed (mph)	0.1513	-0.2053	1	
Daily precipitation amount (in)	0.0234	-0.1468	0.2804	1
Daily Wind Speed (mph)	0.0577	-0.1394	0.6264	0.1709

The results of the updated multiple regression model (Table 3) without max daily wind speed (mph) are described in Table 3. The significance of F (0.0000) in table 3 indicates that this model is significant. In addition, three of the four explanatory variables, ENSO index, daily ambient air temperature (Cº), and average daily wind speed (mph), have a significant linear relationship with P.S.P. levels as indicated by their t-stats and p-values. However, daily precipitation (inches) has a low t-stat, and the p-value of 0.0627 indicates that this variable is not significant. The adjusted R^2 has not been impacted by the removal of max wind speed from the model, indicating this second model is improved compared to the previous model. However, only 3.8% of the variability of P.S.P. levels is explained by these four variables, indicating the model is still poor.

Table 3: Multiple regression of the log of the 3 month moving average vs. the ENSO index, daily air temperature, daily wind speed, and daily precipitation amounts.

Adjusted R Square	0.0384
F	80.6941
Significance F	0.0000

	slope	***Confidence Interval Lower 95%, Upper 95%***	***t Stat***	***P-value***
ENSO Index	0.0310	0.0232, 0.0387	7.8262	5.67^{E-15}
Mean Daily Air Temperature (C)	0.0106	0.0093, 0.0120	15.3982	9.60^{E-53}
Daily precipitation amount (in)	-0.0260	-0.0534, 0.0014	-1.8616	0.0627
Daily Wind Speed (mph)	-0.0032	-0.0053, -0.0011	-3.0441	0.0023

Daily precipitation is not significant in model 2 and a new multiple regression (Table 4) was performed to see whether removing this variable has any impact on the overall fit of the model. The results of model 3 are described in Table 4. The significance of F (0.00) in Table 4 indicates that this model is significant, and all the remaining variables are significant as indicated by each variables t-stat and p-values. However, this final model with only three of the five explanatory variables available is simpler without compromising the overall fit: the adjusted R^2 remains at the same level. Overall, given the low values of the coefficients and the low R^2, only 3.8% of the variability of P.S.P. levels is explained by the variability of these three variables, indicating this model is also poor. In conclusion, all these explanatory variables combined explain little of the P.S.P. variability in Washington States marine waters and they are probably poor predictors of high P.S.P. events.

Table 4: Multiple regression of the log of the 3 month moving average vs. the ENSO index, daily air temperature, and daily wind speed.

Adjusted R Square	0.0381
F	106.4042
Significance F	0.0000

	slope	***Confidence Interval Lower 95%, Upper 95%***	***t Stat***	***P-value***
ENSO Index	0.0309	0.0232, 0.0387	7.8171	6.10^{E-15}
Mean Daily Air Temperature (C)	0.0108	0.0095, 0.0121	15.7536	4.36^{E-55}
Daily Wind Speed (mph)	-0.0035	-0.0056, -0.0015	-3.3687	0.0008

Chapter 7

Implications of This Study

Before we can speculate how this study may be used we need to discuss who may want to use it. There are four groups of individuals who would benefit the most from this study. The first group would be the regulators and would consist of groups or entities who are involved in the regulation of shellfish to protect public health, such as the United States Food and Drug Administration and the WDOH who provided the P.S.P. monitoring data as is mentioned earlier in chapter 5. These are only a few local examples of what a regulator is; however there are many different types of regulatory agencies worldwide who would benefit from this information and who may be able to apply a similar study to other toxic algal species.

The second group would consist of those who harvest shellfish to sell. This would include shellfish harvesters, growers, and distributors (not equal to processors). Washington's shellfish industry is multi-billion dollar industry, and is economically a very important part of Washington State's economy. Washington is the leading producer of farmed bivalve shellfish in the United States, generating an estimated $77 million in sales (Figure 17) and accounting for 86 percent of the West Coast's production in the year 2000 (http://www .psat.wa.gov/Programs/shellfish/fact_sheets/economy_web1.pdf). The cost of

P.S.P. to the commercial fishery, recreational harvesters, and the aquaculture industry is believed to exceed $10 million annually (http://www. nwfsc.noaa.gov/hab/outreach /pdffiles /RedTides2000.pdf).

The third group would be comprised of consumers and individuals who harvest shellfish for recreation, as well as Native American tribes in Washington State who harvest shellfish for subsistence, ceremonial harvesting, or for market. The fourth group would be fellow scientists who may find this study useful in their own endeavors to better understand why toxic species of phytoplankton exhibit such variability in between years as well as within year.

Figure 17: Estimated West Coast production of farmed oysters, clams, mussels and geoduck, 2000 http://www.psat.wa.gov/Programs/shellfish/fact_sheets/economy_web1.pdf**.**

The remainder of this chapter is divided into three sections to discuss the implications this study may have for those that are impacted by P.S.P. in Washington’s marine waters. The first section will cover the regulatory implications this study may have. The second section will look at the benefits this study may have for shellfish harvesters, growers, and distributors, whom for the purpose of this study will be called the shellfish industry, this includes tribal harvest for commercial sale. The third section will cover how this information may be important to consumers of shellfish including recreational harvesters, and the Native American tribes around Puget Sound and on Washington States Coastal areas.

7.1 Regulatory Agencies and/or Groups

“The Washington State Department of Health (WDOH) monitors biotoxin levels in shellfish from sites throughout Western Washington to protect consumers from poisoning by naturally occurring biotoxin’s that if present can accumulate in shellfish tissue” (Determan, 2003). Better understanding how and why levels of P.S.P. in Washington State’s marine waters fluctuate the way they do would be a great advantage in trying to predict when a bloom of P.S.P. may occur and prevent illness outbreaks before they happen.

The WDOH has been very successful to date with their P.S.P. monitoring program in preventing illness. However, sometimes there are questions about how to treat rising levels of P.S.P. that have not gone above the 80µg STX/100g of shellfish tissue needed to close a beach. When shellfish areas are open for harvesting the WDOH samples biweekly unless a sample has detectable levels of P.S.P. which is equal to or greater than 38µg STX/100g, at which time they start sampling weekly. Samples are also submitted for testing prior to any tribal harvest for commercial, ceremonial and subsistence harvesting (Cox, 2001).

One final recommendation would be to collect more local environmental data such as water temperature, salinity, air temperature, wind speed and direction, and rain data and look for relationships that may help to predict blooms of P.S.P. A complete data set of environmental parameters taken in conjunction with P.S.P. samples would greatly facilitate the effort to find the most reliable environmental indicator and predictors of rising P.S.P. levels.

7.2 Shellfish Industry

The Shellfish industry primarily grows, harvests, and distributes the many diverse and abundant species of commercial shellfish in Washington State. However, many of the industry are involved in one way or another with the regulatory aspects and decision making processes of the government. The shellfish industry is also involved in projects to enhance shellfish beds through environmental enhancement of the surrounding areas. Since there are many species of shellfish harvested for commercial purposes in Washington State and just as many ways to harvest them, this study may need to be applied on a case by case basis as needed. However, this study may prove useful to the shellfish industry in a variety of ways such as the ones listed below.

If a relationship can be found between the phases of ENSO and local P.S.P. levels, then the shellfish industry may want to plan to stop or reduce their harvesting of shellfish during times and phases of ENSO that may be more likely to have higher levels of P.S.P. present. In order to limit the amount of product recalled or destroyed, because it gets harvested but does not get sold. This may be especially beneficial to those companies that harvest species of commercial shellfish that retain P.S.P. longer in their systems then other shellfish thus prolonging the unsafe harvest period. For example "Blue mussels (*Mytilus edulis*) are quick to pick up P.S.P. toxin and also quick to purge the toxin, once the mussels stop feeding on the toxic algae. At the other extreme are shellfish such as Butter clams (*Saxidomus nuttalli*), that are slow to pick up P.S.P. toxin and are also slow to purge the toxin" (Cox, 2001).

The shellfish Industry may want to plan for periods when ENSO has the possibility to impact their business negatively such as mentioned above. They can potentially avoid the costs of P.S.P. related closures to their businesses by choosing to not harvest or test more rigorously/frequently during times when P.S.P. may have the potential to be higher, thus ensuring that the product will not get recalled and result in immediate loss of money as well as keeping the shellfish for future sales.

An outbreak of P.S.P. during November and December in 1997 (this apparently is not the rule: it just happened to be an El Nino phase: it doesn't mean that the same should be expected in future El Nino phases, there is not a good correlation or relationship between the two, and therefore it has no predictive value) is a good example of the economic hardships P.S.P. has had on the Shellfish industry that can potentially be mitigated, if a relationship between

ENSO and P.S.P. levels exist and can be used to predict when levels of P.S.P. will be higher. The 1997 P.S.P. blooms severely impacted the oyster harvest in Puget Sound and in the coastal estuaries of Willapa Bay and Grays Harbor. Many of the small farms in these areas were closed and suffered great financial losses.

"A Puget Sound-area farmer of clams and oysters said that he was forced to close for eight weeks, causing him to miss out on Thanksgiving, Christmas, and New Year's sales, and estimated his losses to be $5,000 per week. The P.S.P. bloom in Willapa Bay and Grays Harbor was felt just before Thanksgiving Day, which in the oyster industries is the busiest time of the year, accounting for 40% of the year's business. Although the coastal bays were reopened by mid-December, sales during the Christmas season were also lost because out-of-state competitors had moved into the market. About 34 coastal shellfish farms lost approximately 50% of their sales, reducing average sales by about $8 million. Over 100 workers were laid off and many more had hours reduced" (http://www.nwfsc.noaa.gov/hab/outreach/pdffiles/RedTides 2000.pdf). Not only did the small oyster farms suffer in 1997, the entire shellfish industry felt the economic impacts of the P.S.P. bloom. Large companies had to scale back shellfish farming in the coastal estuaries as well as in Puget Sound resulting in the loss of, oyster diggers and shuckers, jobs throughout the state.

One of the processes of shellfish enhancement is to seed the actual shellfish beds with oysters, clams, and geoduck seeds or inoculums. Different environmental parameters work better for seeding shellfish beds. Though it is beyond the scope of this paper, enhancing shellfish beds during one phase of ENSO may prove to have more productive results then the other phases.

7.3 Recreational Harvest and Tribal Ceremonial/Subsistence Harvesting

Thousands of recreational shellfish harvesters participate in the extremely popular razor clam fishery in Washington State each year. When recreational shellfish harvest is closed due to the presence of toxic algae, recreational harvesters are deprived from their favorite activity and food, but it is not only them that bear the impact. Recreational shellfish harvesting provides a significant economic support to local coastal communities especially for service businesses. Therefore, it is also the hundreds of business owners, and the thousands employed by them, who greatly suffer from the loss of money spent by clam diggers that stay overnight or pass through Washington's many small coastal communities. The annual value of the coastal razor clam fishery is estimated at $12 million recreationally and another $7 million commercially (http:// www.psat.wa.gov/ Programs/shellfish/factsheets/economyweb1.pdf).

Washington's Puget Sound basin and coastal regions are a unique area where treaty Indian tribes reside. For ceremonies, subsistence, and commercial sales, these tribes depend on the harvest of marine species such as oysters, razor clams, California mussels, littleneck clams, horse clams, butter clams, gooseneck barnacles and Dungeness crabs. Unfortunately, all of these species can accumulate toxins by filtering seawater. When toxins reach levels too dangerous for human consumption, tribes can face tremendous economic and quality-of-life losses. Because of declining fish stocks in the Pacific Northwest, including rockfish and salmon, tribes are relying more heavily on shellfish then ever before. Shellfish and crustaceans are a primary source of income to many tribal members.

The commercial harvest of shellfish by Washington's Indian tribes would benefit from this study in the same way as indicated above for the rest of the shellfish industry. The current WDOH monitoring program will ensure the continued safety and health of the tribe's subsistence and ceremonial harvests by continuing to have samples submitted and tested prior to their consumption or use. However if one phase of ENSO tends to have higher average P.S.P. levels, perhaps the tribes may want to be prepared to offset the harvest of shellfish with alternative food sources and income, though this may not be a viable solution for all tribes. It is beyond the scope of this paper to provide alternatives, but this is an important subject to look into in the future so that the Tribes can continue to sustain their way of life and cultural integrity during those times when P.S.P. makes the harvest of shellfish unsafe.

Chapter 8

Conclusion

Knowing how *A. catenella* and other harmful algal booms are affected by the physical processes of their environment may lead us to an answer about why they are becoming more widespread globally. It may also provide some insight into why we are seeing the annual patterns of harmful algal blooms, in Washington State's marine waters and other coastal areas, that we are and why they are becoming more widespread globally. From a policy and health perspective understanding how and why *A. catenella* is affected by its environment, and the physical processes associated with it, may lead policy makers and local health officials to a better understanding of how to predict and monitor outbreaks of harmful algal species such as *A. catenella.*

Understanding why P.S.P. levels in Washington State's marine waters have such variability inter-annually and seasonally will help in mitigating the impacts to Washington's economy and health. This understanding will also enable regulatory officials to predict when levels of P.S.P. are more likely going to be higher then average. This will also help shellfish growers and harvesters to attempt to mitigate the impacts of P.S.P. to their businesses and in turn Washington's economy and health as stated in chapter 7. However, understanding how the physical properties of *A. catenellas* environment influences its production of P.S.P. is still something that needs to be determined more accurately. One direction to take would be to attempt to understand how levels of P.S.P. in Washington change with regional and global weather patterns such as ENSO.

Even though no relationship was found between ENSO and P.S.P. levels in this study it does not mean that one does not exist. It may be a more complex relationship involving other phenomena and combined with variations in local parameters. The limitations of the data availability at this time limit this study's ability to determine what is the relationship between large scale climate phenomena's such as ENSO and localized P.S.P. levels, if one does exist. Given that the global scale ENSO index is not adequate by itself in explaining variations of P.S.P. levels, and neither ENSO nor three local weather parameters combined explain more then 3.8% of P.S.P. variability, more detailed information about local parameters is needed. It would be important to continue to monitor *A. catenellas* response to localized weather patterns and relate these to large scale climate indicators such as ENSO for future studies.

Due to the long-term variability (centennial) of the phases of ENSO, this study will be more complete as more data are gathered, thus as time goes on we will have a longer timescale beyond just a few decades to compare P.S.P. levels to the phases of ENSO. At the same time the monitoring program at the WDOH, will hopefully continue to collect more P.S.P. samples and add a environmental data collection aspect to their monitoring program, though more funding may be required for this. This will also help to ensure a more robust analysis in the future. If more toxic algae monitoring programs continue to gather and maintain long term databases, in other states and countries, then perhaps others groups will find that the phases of ENSO, or similar large scale climate phenomena's, do have an influence on the levels of toxins present in their areas. The more we know about toxic species of algae as a whole the more we can begin to understand the patterns of toxicity (present in space and time) associated with them.

Determining how regional climate and weather affect P.S.P. levels will not help us to completely stop the impact of P.S.P. to Washington's economy and health; however, if used properly they should help to reduce the impact. **And if more local environmental data are collected in conjunction with P.S.P. samples over time, perhaps we will be able to determine a relationship between a physical property of *A. catenellas* environment and its P.S.P. production.** If a relationship can be determined then we can try to correlate the physical property responsible with the different phases of ENSO in an attempt to become more precise in predicting P.S.P. outbreaks. The more we understand the better we can mitigate the impacts of P.S.P.

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