Oysters and *Vibrio parahaemolyticus* Illness Prevention in Washington State: How data analysis could help improve current policy

by

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ABSTRACT

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Vibrio, a genus of natural bacteria found in coastal waters and estuaries around the world, can accumulate in shellfish, and can lead to severe illness if consumed raw or undercooked. The species known as *Vibrio parahaemolyticus* (Vp), is responsible for most of the illnesses associated with oyster consumption in the Pacific Northwest. Illness symptoms include gastrointestinal problems such as vomiting, diarrhea, and stomach cramping. Since warmer water temperatures have been associated with increases in Vp abundance, policy in Washington State controls for Vp from May through September. However, despite such efforts, illness cases have continued to increase, making it critical to understand if other factors contribute to higher illnesses. This study analyzed illness logs and sample data collected from 2015-2019 by the Washington State Department of Health (WDOH) and found that most illnesses between that time did indeed occur in July and August. The distribution of Vp increased over that time also, as seven of the growing areas involved in illnesses in 2019 were not implicated in illnesses in 2015. In Puget Sound, average internal tissue temperatures for each year collected by WDOH were higher than ambient air and water temperatures, demonstrating that oysters can hold onto heat and serve as an incubator for Vp. A short survey among oyster companies found that most believe the current Washington State Vp Control Plan is only somewhat effective in preventing illnesses. Based on the results of the analyses, I recommend that harvesters should be required to record both water and internal oyster tissue temperatures at time of harvest to provide more consistent data, as the current policy only requires one or the other.

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CHAPTER I – INTRODUCTION

Washington State is home to a multimillion-dollar shellfish industry, an industry susceptible to the whims of consumer demand and export vulnerabilities. A significant challenge currently facing the industry is the novel coronavirus, COVID-19, originating in Wuhan, China and spreading rapidly throughout Washington and the United States. Rural areas around the state have begun to feel some of the harshest economic hits because of ties to China (Bernton, 2020). Numerous shellfish companies export product to Chinese restaurants and markets; however, halted imports and the cancellation of air-traffic to China from Washington has suspended shipments. Domestic exports to San Francisco and Los Angeles have also been cut by more than half (King, 2020).

Without shipments, shellfish companies are making cuts to stay afloat. In February 2020, shellfish company giant and the state's leading shellfish producer, Taylor Shellfish Farms, temporarily let go 40 of its 700 workers, suspended its matching 401K program for the year and cut pay for upper management by 10-percent (Herzog, 2020). The Taylor family members who help with business operations took a 20-percent pay cut (Bernton, 2020). In Willapa Bay, Kenichi Wiegardt's company usually shucks oysters 40 to 45 hours a week during the winter months, but with the impacts of COVID-19, his company is down to 15 to 18 hours (King, 2020). Exporting product is the foundation of Wiegardt's company. Wiegardt typically ships about eight pallets of freshly jarred oysters to Hong Kong every Monday, but with COVID-19, he's sometimes shipping only one pallet (King, 2020). As a result, Wiegardt has cut hours for his employees or paid them less to keep his business operating (King, 2020). Things must make a turn for the better or shellfish companies will have to shut down operations and force people to find other jobs in rural areas that have few job opportunities and high unemployment rates ("Monthly Employment Report," 2020).

Along with employment, the shellfish industry contributes a significant amount to the state's economy. A study found that in 2010, the shellfish industry spent approximately $101.4 million in the state's economy, which turned to generate $184 million or 1.8 times the activity (Wellman et al., 2013). A 2013 industry study estimated that the shellfish industry generates an annual revenue of almost $150 million (Bernton, 2019a). These figures underline the importance of the shellfish industry to Washington's economy. Among the several shellfish species Washington produces, the oyster is regarded as the most valuable shellfish species in the country in terms of its economic contribution (National Oceanic and Atmospheric Administration, 2019). Apart from the impacts of COVID-19, Washington oyster growers face challenges every year.

One challenge the oyster industry faces each year is minimizing the potential for illnesses to arise. Summer months bring warmer temperatures, which increases the chance for bacteria to replicate in the water. The bacteria species known as *Vibrio parahaemolyticus* (Vp), is responsible for most illness cases in the Pacific Northwest (Nilsson, Paranjpye, Hamel, Hard, & Strom, 2019). Illness symptoms typically arise within 24 hours and last three days with most people recovering without treatment (Center for Disease Control, 2018). Illness symptoms include nausea, fever, and gastrointestinal problems such as vomiting, diarrhea, and stomach cramping (Center for Disease Control, 2018). Previous studies have led researchers to conclude that higher levels of Vp in oysters is associated with warmer water temperatures during harvests (Su & Liu, 2007). This correlation is the foundation of the current Vp Control Plan in

Washington and explains why monitoring occurs only from May 1st to September 30th every year, the months when temperatures may exceed safe harvest thresholds ("WAC 246-282-006," 2015). However, despite the Vp Control Plan, illnesses continue to occur every year.

Research questions

The research questions guiding this study are as follows: what effect do tidal elevations and temperatures have on the number of *Vibrio parahaemolyticus* (Vp) illness cases in Washington State? Do conclusions from data analysis point to a change in or maintenance of current policy?

Significance

The significance of this research is to protect public health in Washington State and other regions that Washington oysters are exported to. *Vibrio* peaks during the warm summer months of the year (Slayton, Newton, Depaola, Jones, & Mahon, 2014). With a changing climate, however, the peak season length for *Vibrio* abundance may increase (Trtanj et al., 2016). This illustrates the importance of studying current data to learn if anything can be improved in current policy to make it more efficient and to prepare for future conditions.

Roadmap

This thesis will begin by describing the oyster industry in Washington and the economic impact it has on the state. Then a literature review will discuss current scientific findings that draw relationships between Vp and environmental conditions. The methods chapter will outline the steps taken to narrow the focus of this study and how data was filtered for analysis. A results chapter will follow revealing what was found and what observations can be made. Afterward, a discussion chapter will explain the significance of the results, make recommendations to improve current Vp policy in Washington, and suggest areas where future research is needed. Finally, a conclusion chapter will summarize the key results from this study.

CHAPTER II: BACKGROUND

This chapter gives an overview of key components of the oyster industry. First, I will touch on the regions where oysters are grown to provide a spatial context to this project. A brief overview of the history of the oyster industry in Washington will follow to explain the development of the oyster industry. Then, I will build on the introduction to further discuss the economy the industry creates. Finally, I will review the different growing methods and harvest practices used to produce oysters.

Oyster geography

Washington is the United States' largest producer of Pacific oysters (Barton et al., 2015). The vast coastlines of Washington supply the ideal environment for wild and farmed (cultured) oysters. Numerous small- and large-scale oyster companies can be found from the southern coastline of Washington to the northern end of Hood Canal and the greater Puget Sound region. One of the most productive regions is Willapa Bay on the Pacific coast of Washington (Figure 1), producing 65 percent of the oysters in the state (Washington State Department of Ecology, 2014). North of Willapa Bay is Grays Harbor (Figure 1), which when combined with Willapa Bay represents approximately 25 percent of the total oyster landings in the United States (Washington State Department of Ecology, 2014).

Figure 1. Map of Willapa Bay and Grays Harbor (Bernton, 2019b).

Hood Canal (Figure 2) is another large region where oyster production is a staple and has been since 1989 (Washington Sea Grant & University of Washington, 2015). Pacific oyster production increased in this region from 711 thousand pounds to 1.3 million pounds in 2013, worth an estimated $5.3 million and accounting for 46 percent of the year's profit (Washington Sea Grant & University of Washington, 2015). Adjacent to Hood Canal is the Puget Sound region, which is divided into three sections: North Puget Sound, Central Puget Sound, and South Puget Sound (Figure 2). Each section produces oysters; however, South Puget Sound generally produces the most by weight (Washington Sea Grant & University of Washington, 2015). For example in 2013, South

Puget Sound produced 1.3 million pounds of Pacific oysters compared to 683 and 226,404 pounds of Pacific oysters in Central Puget Sound and North Puget Sound respectively (Washington Sea Grant & University of Washington, 2015).

Furthermore, each of the seven regions contains multiple shellfish "growing areas" that the Washington State Department of Health (WDOH) monitors for water quality. Identifying these growing areas helps the State regulate shellfish harvests ("Commercial Shellfish Map Viewer," 2020). For instance, if water quality from a southern section of Hood Canal was found to be compromised, WDOH can close harvests for the implicated growing area, instead of closing harvests for the entire Hood Canal region.

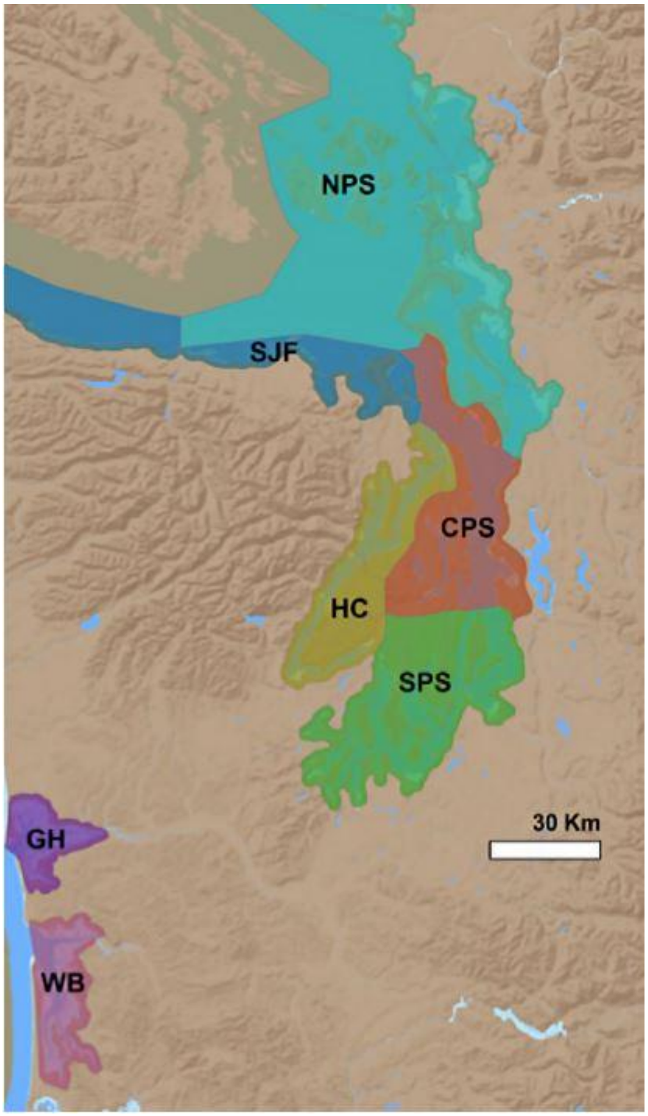

Figure 2. Map of categorized shellfish growing regions in Washington. North Puget Sound (NPS), Strait of Juan de Fuca (SJF), Central Puget Sound (CPS), South Puget Sound (SPS), Hood Canal (HC), Grays Harbor (GH), and Willapa Bay (WB) (Washington Sea Grant & University of Washington, 2015).

Washington tribes must also be recognized because they have a treaty right to half of the sustainable oyster harvests annually in each of their "usual and accustomed" harvest areas, reflecting historic regions where they collected shellfish ("Shellfish Treaty Rights FAQ," 2016; Toba, 2002). The Shellfish Treaty covers 15 western Washington tribes including: Jamestown S'Klallam, Lower Elwha Klallam, Lummi, Makah, Muckleshoot, Nisqually, Nooksack, Port Gamble S'Klallam, Puyallup, Skokomish, Squaxin Island, Suquamish, Swinomish, Tulalip, and Upper Skagit ("Shellfish Treaty Rights FAQ," 2016). Combined with private companies, these tribal stakeholders form the backbone of the Pacific Northwest shellfish industry.

Washington's oyster history

Washington has a long, but tumultuous history of harvesting and consuming oysters. Historically, locals harvested the native wild Olympia oyster, until over-harvesting and pollution, coupled with a slow growth rate of the species, reduced the population to a non-harvestable level (Camden, 2017; Toba, 2002). The current population status of the Olympia oyster in Puget Sound is less than four percent of the historic population (Horowitz & Hoberecht, 2016). To keep the oyster industry thriving, Washington transitioned to growing oyster species imported from other regions.

With the recommendation from the U.S. Commissioner of Fisheries along with biologists and scientists, the Eastern oyster was the first species that industry members tried to grow, importing seed (juvenile oysters) in the early 1900's from the East Coast because spawning naturally wasn't successful (Dumbauld, Kauffman, Trimble, & Ruesink, 2011). With no documented explanation, catastrophic mortality events started to deplete harvests in 1919, forcing the industry to search for another oyster species to grow (Dumbauld et al., 2011). Industry members then imported Pacific oyster seed from Japan from that time until the 1970's, when natural seed supplies in Willapa Bay and Hood Canal started to fully support the industry (Barton et al., 2015; Toba, 2002). This oyster species has a faster growth rate than the Olympia oyster (Toba, 2002), making it a more viable option to oyster farmers.

Although the Pacific oyster is now the most common oyster species grown in Washington, farmers grow a few other species as well. The Kumamoto oyster, native to Japan, is favored by numerous consumers but requires three to four years to become marketable (Taylor Shellfish Farms, 2019). Shellfish farm giant Taylor Shellfish Farms grows Totten Inlet Virginica oysters, which are East Coast oysters grown only in Totten Inlet, located between Olympia and Shelton, Washington. Taylor Shellfish Farms also created the unique Shigoku oyster (Leson & Spencer, 2009; Taylor Shellfish Farms, 2019). Shigoku oysters are essentially “tumbled” Pacific oysters grown in bags attached to floats that bob up and down with the tides, disturbing the oysters and coercing them to close their shells (Taylor Shellfish Farms, 2019). This tumbling process prevents shells from growing outward and creates a deep cup, meaning they grow faster than Pacific oysters (Leson & Spencer, 2009; Taylor Shellfish Farms, 2019). However, even though the Shigoku oysters grow faster, Pacific oysters remain the top oyster produced in the state because of high yields of shucked meat (oyster tissue removed from the shell) and their ability to tolerate a broad range of temperatures and salinity compared to other oysters (Harris, 2008; Ruesink et al., 2006; Taylor Shellfish Farms, 2019).

Economy

As previously mentioned, the Pacific Northwest shellfish industry generates millions of dollars in economic revenue and creates thousands of jobs. The authors of a 2012 study reported that the industry produced an estimated $270 million in economic revenue and created over 3,200 jobs, primarily in rural areas (*From the Tides of Puget Sound to Your Plate: Northwest Shellfish Industry Provides Important Ecological & Economic Value*, 2012). For example, in the rural counties of Pacific and Mason, shellfish growers constitute the largest and second largest private employers respectively ("Protect Willapa Bay," 2017; *Washington Shellfish Initiative*, 2011).

Washington also ranks as the top producer of farmed clams, mussels, and oysters in the nation (Washington Sea Grant & University of Washington, 2015). The state's shellfish industry accounts for 25 percent of domestic production by weight and is worth an estimated value of over $100 million ("Washington," 2013). In 2013, farmed bivalves and roughly 8.8 million pounds of Pacific oysters produced $150 million in revenue (Horowitz & Hoberecht, 2016). This underlines the importance of ensuring that the shellfish industry continues to thrive by maintaining the economically beneficial resource.

Socialization

Washington oyster harvests also provide economic revenue and socialization opportunities through recreation and tourism. Several public beaches allow recreational oyster harvesting including the Nahcotta tidelands in Willapa Bay, Dosewallips State Park in Brinnon, and Twanoh State Park in Union. However, harvesting oysters recreationally requires purchasing an annual "Shellfish/Seaweed license" that costs $17.40 from the Washington State Department of Fish and Wildlife (WDFW) ("Fishing license types and fees," 2019). Recreational harvesters purchase over 300,000 licenses annually to harvest clams and oysters from Washington, providing more than $3.3

million in revenue to the state (*Washington Shellfish Initiative*, 2011). While not the direct focus of this thesis, the high demand for recreational harvesting demonstrates the importance of reassuring tourists and consumers that consuming raw oysters is safe, whether collected as part of an outing on a beach or as part of commercial operation. Safety begins by making sure that everyone follows efficient harvest practices and uses the best equipment available to reduce illness risks.

Growing methods

Legal aspects of oyster farming must be completed before starting any operations. The most important is obtaining a lease from the Washington State Department of Natural Resources (DNR) for the aquatic lands that will be used ("RCW 79.135.110," n.d.). In addition, a lease must be acquired if a deep-water site will be used for floating shellfish culture (Washington State Department of Natural Resources, 2019). With oyster culture comprising about 80 percent of the commercial aquaculture in Washington, numerous lease applications and renewals from oyster companies pass through DNR's office each year (Washington State Department of Natural Resources, 2019).

Cultivating and growing oysters requires a delicate process involving hatcheries, nurseries, and farms. Hatchery production involves conditioning adult oysters to spawn and getting juveniles to settle to hard substrates (Toba, 2002). Oyster larvae undergo various changes as they mature into juveniles and settle (attach) to hard substrates such as oyster shells. Newly settled oysters are referred to as spat and the process of catching oysters onto the substrate is known as cultching and provides oysters a place to grow (Toba, 2002). Nursey tanks are then used to grow and prepare young oysters for their journey outdoors. Farmers can obtain seed naturally or from a hatchery to eventually be collected as spat and transported to a hatchery (Toba, 2002).

There are several different ways to grow oysters on tidelands to reach marketable size. Growing methods include bottom, stake, longline, and bag cultures as well as floating shellfish rafts (Toba, 2002; Washington State Department of Natural Resources, 2019). Bottom culture (Figure 3), the most common method due to its low maintenance, consists of placing cultched seed onto oyster beds and harvesting oysters when ideal size, 70 – 100 grams (shell on), is reached (Helm, 2005; Toba, 2002). For Pacific oysters, this process takes about 18 – 30 months (Helm, 2005). Off-bottom culture methods can be employed when the substrate can't support bottom culture.

Figure 3. Bottom culture. Photo credit: the author.

Off-bottom methods like stake culture have greater production costs than bottom culture because of the equipment needed, however, it reduces predation and yields can be higher than bottom culture (Toba, 2002). In one version of this method (Figure 4), oysters are nailed into stakes at a 45-degree angle, providing a place for larvae to attach, and the precut stakes are driven into the ground with the top of the stake no higher than the 3-foot tide level (Toba, 2002). Another off-bottom method is longline culture (Figure 5), which consists of spacing cultch equally on rope or wire (preferred because it doesn't degrade from the currents) that can be submerged from docks, anchored to the bottom, elevated in rows using polyvinyl chloride (PVC) pipe stakes, or hung from racks (Toba, 2002). Bag culture (Figure 6) is used for ground types that can't support beach culture and involves growing oysters in a series of different size polyethylene grow-out bags that are attached to rebar or sometimes anchored to the ground (Toba, 2002). The high maintenance growing method that requires a specific lease from the state is known as floating culture (Figure 7) (Toba, 2002). In this method, a sink float with one end tied to the anchor and the other free, is used to stack oyster grow-out trays or cages. The stack can also be suspended from rafts or floating longlines, which generally produces more per unit of surface area (Toba, 2002). These growing methods provide options for oyster companies to choose from; however, tideland conditions in different growing areas dictate the appropriate growing methods.

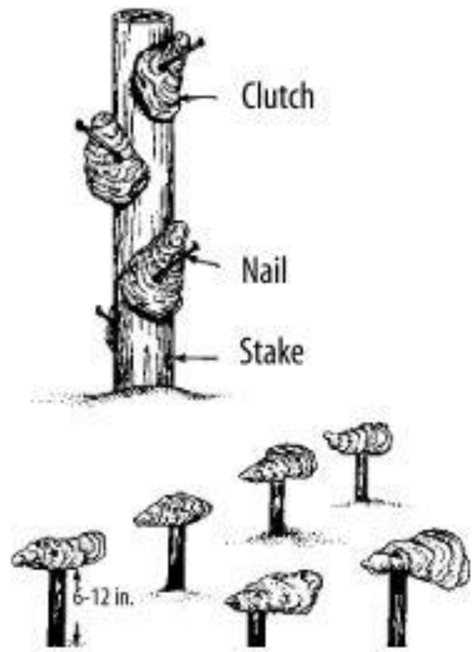

Figure 4. Stake culture (Toba, 2002).

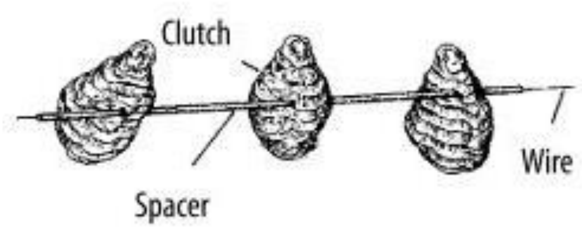

Figure 5. Longline culture (Toba, 2002).


Figure 6. Bag culture ("Oyster Bag Culture in Totten Inlet," n.d.).


Figure 7. Floating bag culture (Camden, 2017).

Oyster companies utilize different growing methods depending on elevation and ground/beach/sea floor type of their growing area. When farming for Pacific oysters, the optimal tidal elevation falls between 0- and +3-feet (Toba, 2002). Lower tides typically yield more growth potential and protection from damage during the warmer months, but may provide increased opportunities for predation (Toba, 2002). The substrate type of the ground must also be considered when determining which growing method to use.

Three common substrate types are found in the tidelands of Washington: firm mud-sand, sandy, and gravel/cobble beaches. In firm mud-sand areas, found in bays and heads of inlets, growers mostly utilize bed and off-bottom cultures (Toba, 2002). Sandy ground requires anchoring or using racks or trays to grow oysters on this unstable soil (Toba, 2002). Gravel to cobble beaches are indicative of heavy currents, therefore, growers typically use anchoring or the bag method to keep the oysters from moving off the tideland property or from damaging their shells (Toba, 2002).

These growing methods eventually result in oysters that have reached marketable size, determined by the weight and size of an oyster. These parameters vary among oyster species. As previously mentioned, with the shell on, the Pacific oyster should weigh about 70 – 100 grams to be considered marketable, which approximately happens when the oyster is four to six inches long (Helm, 2005; Toba, 2002). However, the Kumamoto oyster reaches marketable size when its two inches across (Toba, 2002). If harvests remove oysters before reaching marketable size, they can be sold as shucked meat.

Harvest practices

Just as they rely on different methods for growing oysters, farmers also utilize different practices for harvesting oysters. Selecting a harvest practice is largely determined by the substrate type of the tidelands and the conditions of the growing area. Some oyster companies may only use one type of harvest practice, while other companies may utilize several types. The three main types of harvest methods recognized by the WDOH include dredge, intertidal, and subtidal.

Commercial farmers may use dredges to harvest oysters in coastal bays of Washington. Mechanical oyster dredges use teeth to penetrate the bottom to remove partially buried oysters and a mesh basket that catches them (Mercaldo-Allen et al., 2011; Steele, n.d.). Suction dredges can also be used to collect oysters as well as transplanting oysters, relocating cultch, and cleaning leased grounds of predators (Mercaldo-Allen et al., 2011). This form of dredging pumps water from the seafloor into a hose to lift oysters off the bottom while simultaneously vacuuming up surface material to be washed and screened to capture oysters (Mercaldo-Allen et al., 2011). Oyster dredging is different from the navigational form of dredging because it redeposits sediment that is removed in the oyster harvesting process whereas navigational dredging removes significant quantities of sediment from the seafloor to deepen channels and harbors (Mercaldo-Allen et al., 2011).

If dredges aren't used, harvesting oysters by hand-picking them from either intertidal or subtidal zones can be done. The intertidal zone stretches from the highest to the lowest tide limits. Several oyster companies in Puget Sound harvest oysters intertidally by collecting oysters at low tide. The drawback to this method is that it allows oysters to be exposed to air temperatures when the tide is out, which can be extremely warm in the summer months. In contrast, oysters harvested in subtidal zones aren't exposed to air temperatures because they're continuously covered by water. Submerged oysters also feed more regularly since oysters can't feed out of water. As a result, oysters harvested from subtidal zones often grow faster than in intertidal zones (Scanes, Parker, O'connor, Stapp, & Ross, 2017). After harvesting, various techniques are employed to help keep oysters fresh and more importantly, to reduce illness risks which will discussed in the next chapter.

CHAPTER III: LITERATURE REVIEW

This chapter will review the history of how the *Vibrio parahaemolyticus* Control Plan was molded into what it is today. The chapter will begin by explaining what post-harvest processing methods growers use to help reduce illness risks. The most-documented environmental conditions that favor the growth of Vp in marine environments will be reviewed to understand how oysters accumulate this bacterium. The chapter will conclude with a history of how the Control Plan developed into what it is today and an explanation of how illnesses are reported.

Post-harvest processing methods

Some post-harvest processing methods help reduce the risk of illnesses from raw oysters. For example, the National Shellfish Sanitation Program (NSSP) recognizes the use of icing or mechanical refrigeration to control oyster temperatures (National Shellfish Sanitation Program, 2017). In addition, cold water baths and ice slurries may be used to bring the temperature of shellstock back down after being subjected to a dip in warm water for heat shock (National Shellfish Sanitation Program, 2017). Heat shock is a method used to help separate meat from the shell without significantly altering the characteristics of the oyster (National Shellfish Sanitation Program, 2017).

Irradiation also reduces the risk of illness because of the highly radiation-sensitive nature of *Vibrio* species (Drake, Depaola, & Jaykus, 2007; National Shellfish Sanitation Program, 2017). Specifically, exposure of oysters to low doses of gamma radiation can kill Vp; studies have found that most oysters can withstand this type of treatment without degrading quality (Drake et al., 2007). Moreover, consumers can’t distinguish irradiated from nonirradiated oysters (Drake et al., 2007). Other post-harvest processing methods include relaying oysters to open growing areas to purge contaminants, subjecting oysters to high hydrostatic pressure, or freezing them to eliminate pathogens (Depaola, Jones, Noe, Byars, & Bowers, 2009; Drake et al., 2007; Martinez-Urtaza, Bowers, Trinanes, & DePaola, 2010).

These methods all have the same goal of reducing pathogens because consumers often enjoy their oysters raw. A large risk to oyster consumers is *Vibrio*, a naturally occurring bacteria, and in Washington the most common species of concern is *Vibrio parahaemolyticus* (Nilsson et al., 2019).

Vibrio parahaemolyticus

Vibrio species thrive in coastal waters and estuaries around the world (Brett A Froelich & Noble, 2016; Iwamoto, Ayers, Mahon, & Swerdlow, 2010; Newton, Kendall, Vugia, Henao, & Mahon, 2012; Slayton et al., 2014; Wang, Li, & Li, 2015). Some *Vibrio* species are non-toxic, while others, such as *Vibrio parahaemolyticus* (Vp), can cause severe illness in humans and animals (Brett A Froelich & Noble, 2016). Most illnesses associated with Vp results from consuming raw or undercooked shellfish, typically oysters (Burge et al., 2014; Iwamoto et al., 2010; Paranjpye, Hamel, Stojanovski, & Liermann, 2012). Illness symptoms include gastroenteritis and can include diarrhea, abdominal cramps, headache, nausea, low fever, and vomiting (Su & Liu, 2007; WSDOH, 2017). The onset of symptoms can begin 4 – 96 hours after consumption and last 2 – 5 days (WSDOH, 2017).

Oysters accumulate Vp in their tissues through their natural filter feeding process. Their digestive systems filter seawater for oxygen and food, while simultaneously concentrating any microorganisms in the water column, including potentially toxic Vp strains (Drake et al., 2007; Nilsson et al., 2019). Through this process, oysters can accumulate Vp in higher concentrations than the surrounding water. For example, a study measuring Vp density in oyster tissue and seawater collected seasonally from nine states (Rhode Island, Virginia, South Carolina, Florida, Alabama, Louisiana, Texas, and Washington, and California) each with two locations from May 1984 through April 1985, found an average Vp density of more than 100 times greater in oysters than in the surrounding water (Depaola, Hopkins, Peeler, Wentz, & Mcphearson', 1990). Furthermore, Vp abundance can increase under specific environmental conditions, as discussed below.

Temperature and Vp growth

The most recognized environmental condition that favors the growth of Vp is warm water temperatures (Drake et al., 2007; C. L. Johnson, 2015; Martinez-Urtaza et al., 2010; J. B. McLaughlin et al., 2005; Miles, Ross, Olley, & Mcmeekin, 1997). *Vibrio* has been found to have one of the shortest generation times (time taken by bacteria to double in number) of any bacteria, about 10 minutes, and can multiply at room temperature (Martinez-Urtaza et al., 2010; Miles et al., 1997). The lowest temperature observed for Vp growth was 8.3°C (46.9°F), while the highest observed temperature was 45.3°C (110.3°F) (Miles et al., 1997). This study also found that optimum Vp growth occurred between 37 – 39°C (98.6 – 102.2°F) (Miles et al., 1997). Another study found that higher ambient temperatures increase Vp growth and yield higher Vp concentrations in oyster tissues (Shen, Su, Liu, Oscar, & DePaola, 2019). Together, these studies point to a preferred temperature range for Vp growth, approximately 8 – 45°C (46.4 – 113°F) , and emphasizes the importance of monitoring oysters when temperatures are higher.

Due to the strong association of temperature with Vp growth, a combination of water, ambient air, and oyster tissue temperatures are recorded in illness logs. It’s been found that the lowest seawater temperature related to Vp illnesses is 15°C (59°F) (J. B. McLaughlin et al., 2005). However, most Vp illnesses correspond to the warmer months of the year, underlining a seasonal distribution (Iwamoto et al., 2010). A factor that contributes to more illnesses in the warmer months is the impact that temperature has on the ability of oysters to depurate (filter/purge) bacteria efficiently. A study analyzing the impacts of heat shock on bacterial populations found that oysters who are exposed to both high temperatures and concentrations of Vp are potentially unable to depurate the bacteria to levels safe for consumers (Aagesen & Häse, 2014). Moreover, oysters can only depurate bacteria when submerged because their shells close when they are removed from the water (DePaola, 2019).

In a study analyzing Vp concentrations in oysters postharvest, researchers found that oysters held at 26°C (78.8°F) had a 50-fold increase in Vp after 10 hours and a 790-fold increase in Vp after 24 hours (Gooch, Depaola, Bowers, & Marshall, 2002a). These results indicate that Vp can multiply rapidly after harvests when live oysters are left in unrefrigerated environments (Gooch et al., 2002a). Therefore, temperature control of oysters during all stages of production (harvest, post-harvest processing, and throughout distribution) is vital to control Vp growth and to keep consumers safe (Gooch, Depaola, Bowers, & Marshall, 2002b; Love et al., 2020).

Salinity and Vp growth

Another environmental condition associated with Vp growth is salinity. This can be explained by the halophilic nature of Vp, thriving in environments with high salt concentrations (Martinez-Urtaza et al., 2010). Furthermore, the literature suggests a highly varied relationship between Vp and salinity, with studies reporting both significant and nonsignificant relationships between salinity and Vp abundance (Caburlotto, Haley, Lleò, Huq, & Colwell, 2010; C. N. Johnson et al., 2012). For example, Depaola et al. analyzed Vp densities after illness outbreaks in three states and found a statistically significant negative correlation ($r = -0.25$, $p < 0.05$) between Vp density and salinity (Depaola, Kaysner, Bowers, & Cook, 2000). Additionally, an 11-month study measuring Vp abundances in oysters and water collected from oyster harvest sites in North Carolina found a correlation between Vp concentrations and water temperatures, but no correlation with salinity (B. A. Froelich, Ayrapetyan, Fowler, Oliver, & Noble, 2015). However, a different study from Depaola et al. examined Vp densities in oysters and water from the Pacific, Atlantic, and Gulf coast waters and observed no significant correlation between Vp density and salinity (Depaola et al., 1990). To further complicate the issue, a study comparing Vp ecology in estuaries and offshore areas off the coast of Spain found that salinity influences Vp in estuaries but had no impact on Vp in offshore areas (Martinez-Urtaza et al., 2012). This suggests that salinity may impact Vp abundance differently for estuaries as compared to offshore areas, which would significantly impact Washington because Puget Sound is the second-largest estuary in the United States (United States Geological Survey, n.d.).

Only a few studies have examined the impact salinity has on the effectiveness of Pacific oysters to depurate Vp. These studies suggest that a minimum salinity of 20 parts per thousand (ppt) is needed for effective depuration (Centre for Environment Fisheries & Aquaculture Science, 2019; Phuvasate & Su, 2013). This 20 ppt figure emphasizes the importance of monitoring salinity in Washington during harvests to learn more about the influence it has on Vp densities and illnesses.

Intertidal exposure

Farmers typically harvest oysters at low tide, underlining the importance of understanding the impact tides have on Vp growth. During intertidal exposure, Vp has been shown to grow rapidly in oysters subjected to ambient air conditions, especially on sunny summer days (Gooch et al., 2002b; Jones et al., 2016; Nordstrom et al., 2004). A study of sites along Hood Canal, Washington examined Vp levels of oysters collected after they emerged from the receding tide (first exposure) and prior to submersion (maximum exposure) (Nordstrom et al., 2004). Overall, Vp densities in oysters increased significantly during exposure from receding tides with average densities of total Vp at first and maximum exposure of 51 and 280 CFU/g (colony forming unit per gram) respectively (Nordstrom et al., 2004). A similar increase was observed in a more recent study in Totten Inlet, Washington investigating the effects of intertidal harvest practices (using oyster bags and tubs) on Vp levels in oysters. Jones et al. found that the average level of Vp increased 1.38 log MPN/g (most probable number per gram) after approximately four hours of intertidal exposure (Jones et al., 2016).

The other factor of intertidal exposure is the effect it has on Vp levels in oysters after re-submersion, when the tide rises again. By examining Vp levels of samples taken throughout August 17-21, 2001, Nordstrom et al. determined that Vp levels returned to comparable levels of the previous day's first exposure counts, suggesting that a single tidal cycle is sufficient to allow oysters to purge Vp (Nordstrom et al., 2004). In a more recent study, Jones et al. found that Vp levels in oysters following re-submersion for one day returned to those not significantly different from first exposure (initial) Vp levels (Jones et al., 2016). This suggests that harvesting oysters while submerged in water for at least one day reduces Vp levels. However, temperatures are cooler at night, an unfavorable condition for Vp growth, suggesting other factors could be contributing to the drop in Vp as well.

Site location impacted Vp levels in the first study as South Hood Canal had an average total Vp count (first exposure) of 110 CFU/g, whereas the Dosewallips area and Eagle Creek had an average total Vp of only 20 and 52 CFU/g respectively for first exposure (Nordstrom et al., 2004). These findings imply varying levels of Vp among Washington oysters depending on the location.

The Jones et al. study compared different intertidal harvest practices at one site. They determined that the use of oyster bags or tubs did not increase the risk of illness (higher Vp levels) from Vp; both methods allowed for adequate purging (Jones et al., 2016). However, environmental conditions that harvesters cannot control such as climate change could have an impact on Vp levels in the coming years.

Climate change

Increased sea surface temperatures are highly recognized to be associated with climate change and as previously mentioned, warmer water temperatures are correlated with higher levels of Vp. Climate change is projected to further increase sea surface and air temperatures, posing a serious problem for oyster farmers and consumers in the future. Most researchers predict that the number of Vp illness cases will increase with climate change (Burge et al., 2014; Martinez-Urtaza et al., 2010; Vezzulli et al., 2012). This escalates the complexity of creating new and revising existing policy because officials will need to create new measures that haven't been previously implemented to keep people safe. This could mean implementing more stringent policy measures for time of harvest to cooling requirements (i.e. shorter time required to cool oysters to a specified temperature or reducing the temperature at which oysters need to be cooled to), which some oyster companies might not be able to follow because of financial or equipment reasons. Worst case scenario of creating new policy to prevent illnesses would be closing all harvests in the summer months, impacting oyster companies financially, reducing jobs, and negatively impacting the economy.

Furthermore, the geographic range and season length of Vp may expand, increasing the potential for more illnesses (DePaola, 2019; Levy, 2015). Therefore, areas like Washington who monitor for Vp primarily in the summer months, could be encouraged to lengthen their monitoring window to include additional months (i.e. monitor from March – November instead of May – September). These factors all need consideration when evaluating the efficacy of the current Control Plan.

Vp spreading to other areas

Climate change contributes to a larger geographic distribution of Vp illnesses (Martinez-Urtaza et al., 2010). For example, in 2004 Alaska experienced a Vp outbreak associated with oyster consumption that included 62 reported illnesses when warm waters traveled north most likely from the Pacific coast of the continental United States (Martinez-Urtaza et al., 2010; J. McLaughlin & Martinek, 2004). This outbreak extended the geographic distribution of Vp illnesses by over 1000 kilometers or approximately 620 miles (Martinez-Urtaza et al., 2010; J. McLaughlin & Martinek, 2004). To prevent illnesses from occurring the following year, Alaska changed their harvest practices by lowering oyster cages below the thermocline where the water temperatures fall below 10°C (50°) (Martinez-Urtaza et al., 2010). This method proved successful--only one illness case was reported in 2005 (Food and Agriculture Organization of the United Nations/World Health Organization, 2011; Martinez-Urtaza et al., 2010).

The geographic expansion and projected increase in Vp illnesses in future years present several problems for the oyster industry. For instance, increased water temperatures could prevent oyster companies from harvesting in the months of July and August if temperatures exceed the threshold for their risk category under the existing Control Plan, outlined in more detail below ("WAC 246-282-006," 2015). Furthermore, higher air temperatures require a faster time to temperature cooling period (the amount of time required to reach and maintain an internal oyster temperature of 50°F or below), which might not be feasible to continuously follow for all oyster companies. An increase in illnesses could also create a distrust among consumers, which would negatively impact the industry. The overlying question is, to what extent will these impacts have on the industry?

History of *Vibrio parahaemolyticus* illnesses and policy

The National Shellfish Sanitation Program (NSSP) was created in 1925 at a shellfish sanitation conference called by the Surgeon General of the United States Public Health Service (National Shellfish Sanitation Program, 2017). The major concern at this time was Salmonella typhi, which caused typhoid fever. This conference developed basic principles of the NSSP and adopted a set of resolutions for permanent oyster control to be followed by every state (Hackney & Pierson, 1994). Each state decided which agency would be responsible for issuing the required ordinances under the new resolutions. In addition, the conference created a committee to oversee proposed methods before becoming permanent, which sparked the committee to advise studies on water quality to gather more data to help inform their policy decisions (Hackney & Pierson, 1994).

The 1925, "Report of Committee on Sanitary Control of the Shellfish Industry in the United States," underwent revisions in 1937 and 1946 to incorporate knowledge from years of concentrated studies researching coliform organisms in oyster liquor, the liquid portion inside an oyster shell (Hackney & Pierson, 1994). This research also led to the development of state control programs. The 1946 revision became the *Manual of Recommended Practice for Sanitary Control of the Shellfish Industry*, which required examination of the entire oyster (including tissue meat), adopted a MPN (most probable number) of no more than 70 MPN per 100 ml of water, and highlighted the examination of both water and shellfish (Hackney & Pierson, 1994). Shellfish control officials and industry members have long desired an enforceable bacteriological standard for market shellfish; however, high natural variability in bacteriological quality, geographic and seasonal differences, and other uncontrolled factors have limited this goal (Hackney & Pierson, 1994). By 1959, this revision was later divided into two parts to separate sanitation of growing areas from sanitation of harvesting and processing (National Shellfish Sanitation Program, 2017). Then in 1965 another revision resulted in the Shellfish Sanitation Manual containing recommendations from state officials and industry members (National Shellfish Sanitation Program, 2017).

Subsequent revisions addressed public health concerns for paralytic shellfish poison, heavy metals, and pesticides throughout the 1940's to the 1970's (National Shellfish Sanitation Program, 2017). Additionally, from 1954 through 1977, several workshops with federal and state shellfish regulatory officials and industry representatives took place to review administrative and technical procedures of the NSSP (Hackney & Pierson, 1994). These workshops grew to become inadequate in improving revisions to the NSSP Manual because of their size and the increasing complexity of the issues they faced (Hackney & Pierson, 1994). Moreover, the NSSP was transferred to the Food and Drug Administration (FDA) under the Reorganization Act of 1968 that consolidated food control programs (Hackney & Pierson, 1994). The FDA reported in the early 1970's that several states had unsatisfactory program ratings, prompting them to publish NSSP regulations that would allow them to take authoritative actions such as withdrawing a state's endorsement from the federal government (Hackney & Pierson, 1994).

Bacterial pathogens continued to be the focus of the NSSP because they were more understood compared to naturally occurring marine pathogens. For example, the taxonomic position of Vp and its corresponding name weren't established until 1963 after studying 1,702 cultures (Sakazaki, Iwanami, & Fukumi, 1963). It wasn't until 1971 that the United States had its first Vp case from consuming undercooked crab in Maryland (Molenda et al., 1972). In a situation like this, an outbreak is defined as "the occurrence of two or more cases of a similar illness resulting from the ingestion of a common food, or if the food vehicle was undecided, sharing a common meal or food facility" (Wu, Wen, Ma, Ma, & Chen, 2014). The Maryland event involved three outbreaks and a total of 425 individuals with gastroenteritis cases and was later classified as the first foodborne epidemic due to Vp in the United States (Molenda et al., 1972).

Since the Maryland event, sporadic outbreaks of Vp have occurred throughout coastal regions as a result of consuming raw or undercooked shellfish/seafood. The Centers for Disease Control and Prevention (CDC) reported 40 Vp outbreaks in 15 states (including Washington) and the Guam Territories between 1973 and 1998 (Daniels et al., 2000). During this period, state shellfish control agencies started questioning the effectiveness of shellfish programs in other areas, prompting states and the FDA in 1982 to initiate the Interstate Shellfish Sanitation Conference (ISSC) (National Shellfish Sanitation Program, 2017). This organization "provides the forum for State regulatory officials to establish uniform National guidelines and to exchange information regarding sources of safe shellfish" (National Shellfish Sanitation Program, 2017). Members of the ISSC include officials from the shellfish industry, state regulatory agencies, FDA, National Marine Fisheries Service, and the United States Environmental Protection Agency (Interstate Shellfish Sanitation Conference, n.d.).

In 1981, just before the ISSC was founded, Washington and Oregon had a Vp outbreak that resulted from the consumption of raw oysters from Willapa Bay, Washington (Drake et al., 2007). At this point, the ISSC was just beginning to develop manuals for shellfish operations. At its first meeting in 1983 the organization adopted the 1965 NSSP Manuals of Operation, the most recent edition (National Shellfish Sanitation Program, 2017). In the following year, the FDA's relationship with the ISSC became formalized through a Memorandum of Understanding (MOU), which led to the FDA announcing in 1985 its recognition of the ISSC as an alternative to uphold principles of the NSSP Manual of Operations (National Shellfish Sanitation Program, 2017). After the initial meeting, the ISSC adopted updated issues of Part I: Sanitation of Shellfish Growing Areas and Part II: Sanitation of the Harvesting, Processing, and Distribution of Shellfish in 1986 and 1987 respectively (National Shellfish Sanitation Program, 2017).

> Part I of the Manual continued as a guide for preparing State shellfish laws and regulations pertaining to sanitary control of shellfish harvest area classification, laboratory procedures, relaying, patrol operations and marine biotoxin. Part II of the Manual continued as a guide for operating, inspecting and certifying shellfish shippers, processors and depuration facilities; and for controlling interstate shipments of shellfish. (National Shellfish Sanitation Program, 2017)

Following the adoption, several revisions were made to the manual by the FDA from 1986 – 1995 under the MOU to include knowledge of new available science (National Shellfish Sanitation Program, 2017). However, it wasn't until 1996 that the ISSC received funding from the FDA to start implementing a Vp Control Plan (DiStefano & Jones, 2015). Further modifications to the manual were made in 1999 to change the format into a Model Ordinance that allows ISSC participating states to use legal authority to implement regulations and establishes a standard for all states to follow, increasing public confidence and trust in shellfish products (National Shellfish Sanitation Program, 2017).

Developing Vp policy in Washington

Since the Vp outbreak in 1981, several reported Vp illnesses as well as outbreaks have occurred in Washington, with some of the largest occurring in 1997 and 2006 (Drake et al., 2007). The 2006 outbreak involved at least 110 residents and was the turning point for Washington to create its own Control Plan. The state enforced an emergency rule in 2007 to develop a Washington Administrative Code (WAC) with the goal to make more stringent standards than the Model Ordinance. A collaboration among stakeholders including the ISSC, WDOH, FDA, tribal representatives, policy makers, and the shellfish industry helped create the Control Plan (Washington State Legislature, 2007). These stakeholders reviewed historic illness data in Washington and determined that most illnesses occurred during the summer months with majority occurring in July and August (Washington State Legislature, 2007). Therefore, the Control Plan included more stringent time-to-temperature requirements for harvesters from the beginning of June through the end of September with different durations depending on the geographic location of the growing area and its illness history (Table 1) (Washington State Legislature, 2007). Shellfish dealers (individuals involved in manufacturing, processing, packing, or holding shellfish) and harvesters also had to maintain harvest records including the time of harvest to show compliance with the Control Plan regulations.

2007		
	Months of control	Time-to-temperature control
Puget Sound Growing Areas	June, July, August, September	10 hours
Coastal Growing Areas	June	24 hours
	July, August	10 hours
	September	24 hours

Table 1. Time-to-temperature requirements for Puget Sound and Pacific Coast growing areas that took effect in 2007 (Washington State Legislature, 2007).

A revision to the rule in 2008 expanded the duration of the control months to add the month of May in addition to June through September (Washington State Legislature, 2008a). A further reduction to time-to-temperature requirements was also made for Puget Sound growing areas (Table 2). Stipulations were formulated within the Control Plan to guide public health officials in the event of illness(es) such as reducing time-to-temperature control by an additional hour and/or closing a growing area (Washington State Legislature, 2008a).

2008		
	Months of control	Time-to-temperature control
Puget Sound Growing Areas	May	12 hours
	June	5 hours
	July, August	4 hours
	September	5 hours
Coastal Growing Areas	July, August	10 hours

Table 2. Time-to-temperature requirements for Puget Sound (including the Strait of Juan de Fuca) and Pacific Coast growing areas that took effect in 2008 (Washington State Legislature, 2008a).

The rule was revised again in 2009 to clarify the language and to determine if the time-to-temperature controls were enough because illnesses were higher than expected in the previous year (Washington State Legislature, 2008b). Despite the revisions, illnesses were continuing to increase. Policy makers made further revisions to the Control Plan in 2014 to clarify the rule language to be consistent with the FDA and the NSSP Guide for Molluscan Shellfish or the “Model Ordinance” (Washington State Legislature, 2014). In addition to clarification, policy makers considered including proactive measures such as harvest requirements based on environmental factors, but those ideas didn’t make it into the permanent rule (Washington State Legislature, 2014). This rule remained permanent until illnesses in coastal areas began increasing especially during the month of September (Washington State Legislature, 2015a).

Major changes to the Control Plan were made again in 2015 to help reduce illnesses. The new version of the Control Plan and the most current, implemented a new approach to reduce illnesses based on environmental conditions, considered during previous revisions. This version included more stringent harvest controls such as time-to-temperature requirements developed using relative risk (Washington State Legislature, 2015a). To keep harvesters and shellfish dealers in compliance, the updated Control Plan included new recordkeeping requirements (Washington State Legislature, 2015a). For example, during the control months every harvester must record air temperature at time and location of each harvest. In addition, the harvester must record either the water temperature at the depth in which oysters are harvested from or the internal oyster tissue temperature from a shucked oyster at time of harvest. For the purpose of the Control Plan, time of harvest begins after the first oyster becomes exposed to air (Washington State Legislature, 2015b). This data is primarily used if illness investigations occur to help rule out any post-harvest abuse (i.e. temperature abuse) made by growers.

Harvesters and dealers must also keep on file a current Vp harvest plan, completed and submitted to the WDOH by March 1st each year that: defines their methods of harvesting, temperature collection, cooling, and conveyance; includes an example of how they will record harvest temperatures; and identifies whether water or internal oyster tissue temperature will be used to meet Control Plan requirements (Washington State Legislature, 2015b).

The revised Control Plan also eliminated the controls that divided coastal and inland growing areas geographically, establishing the new controls based on historical illnesses (Washington State Legislature, 2015a). For example, growing area risk categories are currently based on a five-year average instead of a trend over time (Washington State Legislature, 2015b). The risk categories are classified as either a 1, 2, or 3 with 3 being the highest risk. A complete list of the most current classifications can be found on the Washington State Department of Health's website – Table 3 provides a list of the classifications for the growing areas used for this study ("Shellfish Growing Area Risk Categories," 2020).

The new Control Plan also made changes to procedures for closing a growing area as a result of illnesses. The WDOH only closes a growing area (i.e. shuts down all harvest operations) when there is an outbreak – when two or more people from different households have common exposure and the cases are confirmed by a laboratory or epidemiologist (U.S. Food and Drug Administration, 2020). Therefore, illnesses must meet these specific guidelines to close harvests at a growing area. This rule is what Washington currently follows despite reports of high illnesses. In April of 2019 it was determined that no changes should be made to the rule because they would have only been minor updates and clarifications.

Growing Area	Risk Category
Bay Center	2
Bruceport	1
Dabob Bay	2
Drayton Harbor	2
Dyes Inlet	1
Grays Harbor	2
Hammersley Inlet	3
Henderson Bay	3
Henderson Inlet	1
Hood Canal 1	3
Hood Canal 2	2
Hood Canal 4	1
Hood Canal 5	2
Hood Canal 6	3
Hood Canal 7	1
Hood Canal 8	1
Hood Canal 9	2
Nahcotta	3
Nisqually Reach	2
Oakland Bay	1
Peale Passage	2
Pickering Passage	2
Port Gamble	1
Port Madison	1
Reach Island	2
Rocky Bay	1
Samish Bay	3
Skookum Inlet	3
Stony Point	3
Stretch Island	1
Totten Inlet	3
Westcott Bay	1

Table 3. Risk categories for growing areas used for this thesis.

Reporting Vp illnesses

To report an illness, one should call their local health department to complete an investigative questionnaire. These illness records are subsequently reported to the state health department and other agencies. Numerous Vp illness cases spread across all states are then reported to the CDC every year. The CDC maintains the Cholera and Other Vibrio Illness Surveillance (COVIS) system to report human infections from vibriosis (human illnesses caused by *Vibrio* species) and cholera nationwide. Data from this system can be used to find that, for example in 2014, there were 1,252 reported vibriosis cases with 605 cases (48 percent) caused by Vp (Centers for Disease Control and Prevention, 2016). Analyzing data over time indicates that vibriosis and Vp cases have increased despite the development of more stringent policies (Centers for Disease Control and Prevention, n.d., 2016; DePaola, 2019; Newton et al., 2012).

Although illnesses are increasing, several factors could be contributing. For example, underreporting illnesses was a problem before more education and awareness spurred people to see their medical provider if they become ill after shellfish consumption. Signs posted at beaches that allow recreational oyster harvesting, inform the public of potential health impacts and what actions to take if illness symptoms arise. People generally know about illnesses related to shellfish, whether they have cooked them or have eaten shellfish in a restaurant. However, underreporting can also occur when individuals who become ill don’t see their medical provider because their symptoms are mild, or they don’t have insurance. The advancement in Vp testing technology allows medical providers to conduct testing in their office without having to culture or grow bacteria in a lab. For example, culture-independent diagnostic tests

(CIDTs) can identify the general type of bacteria causing illness within hours; however, this testing method doesn’t allow public health scientists to determine an organism’s strain to detect and prevent outbreaks, but it may mean that more illnesses get reported each year (Centers for Disease Control and Prevention, 2019).

Environmental factors previously discussed also are contributing to an increase in Vp illnesses and may continue to do so as the impacts of climate change intensify. This highlights the importance of analyzing current practices and data to determine if any changes to the Control Plan can be made to reduce illnesses and prepare for future conditions.

WDOH Vp monitoring

The Washington State Department of Health (WDOH) conducts oyster sampling and collects temperature and salinity data throughout each Vp control season for monitoring and research purposes. Samplers record ambient air and water temperatures as well as the internal tissue temperature of a shucked oyster at each sampling site to help identify environmental conditions that favor Vp growth. In addition, samplers collect oysters from each sampling site to be analyzed in the laboratory.

Although the Control Plan currently doesn’t incorporate a Vp concentration threshold, Vp concentration can be used to determine what the threshold would/should be and the efficiency of using a threshold in preventing illnesses. This process would be very costly for all involved because it would require significant testing from the laboratory as well as providing oysters to contribute to testing. Furthermore, harvesters could lose money under a concentration threshold policy, as it would most likely require them to delay their shipments until test results came back. To avoid this, more laboratories could be certified to test oysters for Vp to reduce the time spent waiting for results; however, this would also be costly. Procedures for gathering environmental data and laboratory testing will be explained in more detail in the following Methods chapter.

Conclusion

Regardless of policy efforts to reduce Vp illnesses, further improvements need to be made to achieve a preventive rather than reactive approach to keeping people safe. Policy officials should start developing this approach now to reduce illnesses and help set up Washington for success in future years if climate change does have its predicted impact. Even though certain environmental conditions have been identified to contribute to Vp growth in oysters, more research is needed to study the data WDOH has collected to determine if it can help improve the current Control Plan.

CHAPTER IV – METHODS

To analyze the effectiveness of the current Washington State Vp Control Plan, I collected a combination of quantitative and qualitative data. I obtained secondary data collected by WDOH, after submitting a Public Disclosure Request to EPHPublicDisclosure@doh.wa.gov requesting specific data files. The data files requested included: illness logs for Vp and *Vibrio* illnesses from 2010 to 2019, illness investigation follow-up results (temperatures, harvest record information, etc.), water temperature data (USB drive), tidal elevation data for growing areas that WDOH samples, WDOH Vp sampling data, and reported oyster production data for each company and growing area. In addition, I collected primary data in the form of a survey among Washington oyster companies to gain insight into their views of the current Vp Control Plan. This chapter describes these data categories in more detail in separate sections below.

Illness logs and investigation follow-up data

WDOH documents illness cases reported to healthcare professionals and local counties throughout the year in an Excel spreadsheet with various columns. Data includes information regarding how the oysters were consumed (i.e. raw, cooked, etc.), the venue where the individual was infected, traced back oyster harvest dates, the growing area where oysters were harvested from, and how many individuals became ill for each case. WDOH illness investigators also contact the oyster company that harvested the implicated bag of oysters and obtains harvest data (date of harvest, final harvest time, water and air temperatures, time of cooling, and cooling duration).

This thesis focused only on data from the years 2015 – 2019 because the current Vp Control Plan was last revised and took effect in 2015. Data organization was first utilized to filter records that would help answer various research questions. I carried out the following steps to curate the data:

1. Copied the entire illness log spreadsheet to new spreadsheet for each year (2015 – 2019), while saving copies of the original files

2. Filtered data to only include illness cases that were:
    a. Illness type = Vp
    b. Shellfish type = Oysters

3. Filtered data again to only include:
    a. Lab/Epi confirmed = YES
    b. Harvest type = C (commercial)

4. Filtered "harvest date" to delete any:
    a. Unknown
    b. Blanks
    c. Illegible
    d. Multiple
    e. Various
    f. "?"
    g. Typos
    h. "Hood Canal?" (2019)

5. Filtered "harvest date" to only include dates during the *control months* (May 1st – September 30th)

6. Filtered the "harvest in/out of state" to delete any records that were not harvested in Washington specifically – the following categories were deleted:
    a. B = Washington and out of state
    b. O = out of state only
    c. U = unknowns
    d. Blanks

7. Filtered the "source" to include only "single" source illnesses – "multi" source illnesses were deleted

8. Filtered "product type" to include only "ss" (shellstock) as this is what the Control Plan regulates – "sm" (shucked meat) and blanks were deleted. Shucked meat was deleted because its exempt from regulations under the Control Plan.

After data organization the total number of records for each year were as follows:

- 2015 – 23 records
- 2016 – 33 records
- 2017 – 24 records
- 2018 – 57 records
- 2019 – 30 records
- Total number of records for all five years combined – 167 records

One thing to note is that when WDOH reports the total number of Vp illnesses for the year, they omit any records that have “post-harvest abuse” – such as temperature abuse from retailers, dealers, and at the grower level. However for this study, the assumption was made that all illness cases were caused by oysters despite post-harvest abuse that occurred because Vp originates from coastal and estuarine environments; therefore, Vp had to be present before the restaurant or organization received the product and any potential post-harvest abuse would only increase Vp levels.

These 167 records were then compared with temperature and tidal data to determine if any correlations exist. The investigation follow-up data was pulled for each filtered illness record available to help provide data on the final time of harvest and temperatures (ambient air and water or tissue if available) that were recorded by oyster companies. In some instances, investigation follow-up reports didn’t yield any data from the oyster company because of missing records. In addition, it’s important to keep in mind that different strains of Vp are found in Puget Sound compared to the Pacific Coast, so these regions were analyzed separately.

USB temperature data

The WDOH collects a combination of air and water temperature data using universal serial bus (USB) data loggers. The USB data loggers are enclosed in small cages and placed at the two-foot tidal elevation zone for all WDOH Vp sample sites throughout the state (Figure 8). Placing data loggers at this elevation exposes them at low tide, capturing air temperatures and providing an ideal representation of the temperatures of oyster tissues at low tide (when companies typically harvest). When the tide rises and submerges the USB data logger, its then measuring water temperatures. The USB data loggers are connected to a computer at the end of the monitoring season to upload temperature data for analysis.

Using only the filtered illness records, I collected temperatures from the USB data files (data loggers) and transcribed them to the illness logs spreadsheet. Temperatures collected (when available) included:

- Temperature at final harvest time
- Temperature at low tide on harvest date
- Maximum temperature 3 days before harvest date
- Maximum temperature 2 days before harvest date
- Maximum temperature 1 day before harvest date

Figure 8. Vp sample site in Nahcotta, WA with orange flag marker and USB data logger enclosed in a small cage. Photo credit: the author.

WDOH Vp sampling data

WDOH collects field and laboratory data weekly during the Vp control months (May 1st – September 30th) for monitoring purposes. In the field, WDOH employees record the date, sampling time, time of low tide, site name, ambient air temperature, shore and surface water temperatures, salinity, and tissue temperature of a freshly shucked oyster for each sample site. Shore water temperature is taken where the water meets the shore, whereas surface water temperature and salinity measurements (using a refractometer) are taken where the water is about two feet deep. If a harvest date associated with an illness was within one week of when WDOH collected a Vp sample, the data was pulled to determine if any observations can be made regarding environmental conditions. The data was more abundant for Puget Sound rather than the coast because it takes extensive resources for WDOH employees to travel to the coast for sampling; therefore, only Puget Sound Vp sampling data was analyzed.

In addition, approximately 12 – 15 oysters are collected from each site and sent to the WDOH Public Health Laboratory in Shoreline, WA for analysis. Lab technicians shuck the oysters and combine the tissues to have enough weight to analyze genes correlated with pathogenicity. Both thermolabile hemolysin (TLH) which can identify Vp and for the virulence factors thermostable direct hemolysin (TDH) and thermostable direct hemolysin-related hemolysin (TRH) are measured (Klein, Gutierrez West, Mejia, & Lovell, 2014). The lab also performs an analysis to determine if the ORF8 gene (pandemic marker for Vp strains) or *Vibrio vulnificus* concentrations are present; however, this data was omitted for this study because ORF8 is generally not detected and *Vibrio vulnificus* is not monitored under the Control Plan.

Tidal elevation data

WDOH documents tidal elevations for each Vp sample site to help plan when they should sample, ideally at or very close to low tide. This represents the window of time when companies are most likely harvesting oysters from intertidal beaches. I transcribed the low tide elevations (the land above the water line at low tide) and corresponding times from the WDOH Excel files to the organized illness logs spreadsheet. This process was completed by filtering for each growing area and searching for the low tide and time of low tide for the harvest date that corresponded to the illness case. If low tide was close to 12:00 AM (midnight), I selected the lowest tidal elevation during daylight hours because this likely was more representative of when companies would be harvesting. Furthermore, if a harvest date had a low tide that corresponded to two different times (i.e. -2.1 at 10:30 AM and -2.1 and 11:00 AM), I selected the later time (11:00 AM) because the water would have receded from the beaches for a longer period. Some growing areas required using tidal data from nearby growing areas due to geographic proximity such as:

- Hood Canal 7 = Union tidal data (HC8)
- Peale Passage = Pickering Passage tidal data
- Reach Island/Stretch Island = Pickering Passage tidal data
- Rocky Bay = North Bay tidal data

Reported oyster production data

For each license they hold, oyster companies must record and report their production data to the WDOH at the end of each year for each species and size harvested. This data includes the amount produced in dozens for each oyster species harvested each month and the growing area from which they were harvested from. At the time this data was distributed to me, 2019 production data hadn’t been collected yet. Furthermore, only data from 2018 includes production data for every month out of the year, whereas production data from 2015 – 2017 only includes production data for Vp control months (May – September). Thus, production data from 2018 was the only year used for analysis. Production totals for each of the three size categories were added for each month to allow comparison between the Vp control months and non-control months.

Vp Control Plan Survey

I collected primary data using a paper survey distributed among representatives of oyster companies at the annual *Vibrio* Recap Meeting held at the WDOH in Tumwater, WA on December 12, 2019. A disclaimer was made prior to survey distribution that assured all participant names would be kept confidential and that responding to the survey was optional. The survey consisted of seven questions and was collected immediately after completion (a copy of the survey can be found in the Appendices). Survey questions included both Likert scale and open-ended questions. In addition, the last question on the survey asked for contact information from participants to allow for follow-up questions. I transcribed survey responses into an Excel spreadsheet for analysis.

The goal of the survey was to gain insight into how oyster growers view the current Vp Control Plan and to ask if they have any recommendations for improvement. Survey responses were analyzed for similarities and differences among participants.

CHAPTER V: RESULTS

This chapter will reveal results and observations from analyzing various datasets curated as outlined in the Methods chapter. I first describe illness counts to provide an overview of the amount of data used for analysis. I then analyze air, water, and tissue temperature data from Vp illness investigations separately to determine both spread and averages among the data. In addition, I review temperature data from USB data loggers to observe the conditions in the days leading up to the harvest deemed associated with an illness. Next, I examine Vp sampling data collected by WDOH to establish if any correlations exist with temperatures in the investigation follow-up reports. I investigate tidal elevation data in connection with illnesses to indicate if more illnesses were associated with extreme tides. Finally, I describe results from my survey among oyster company representatives.

Illness counts

In five years, from 2015 to 2019, Washington had a total of 167 Vp illnesses (Table 4). The highest illness count occurred during 2018, with 57 illnesses, and the lowest illness count was in 2015, with 23 illnesses. Illness counts also show that most cases occur in the summer months each year (Table 5).

Illness record counts by growing area (Table 6) show that six growing areas (Hammersley Inlet, Hood Canal 6, Nahcotta, Samish Bay, Stony Point, and Totten Inlet) had more than 10 illnesses from 2015 – 2019. These growing areas all have a risk category of “3” (Table 3), the highest category possible. Totten Inlet had the highest illness record count with 26 illnesses, followed by Samish Bay with 18 illnesses (Table

6). Three other growing areas also categorized as a "3" including Henderson Bay, Hood Canal 1, and Skookum Inlet (Table 3), had a total of nine, six, and five illnesses respectively from 2015 – 2019 (Table 6). The number of growing areas associated with Vp illnesses can be found in Table 7.

Strains of Vp differ in Puget Sound compared to the Pacific Coast; therefore, illnesses in these regions were separated for analysis. Puget Sound had a total of 135 Vp illnesses (Table 8), while the coastal growing areas only accounted for 32 Vp illnesses (Table 9).

Vp Illness Reports by Year						
	2015	2016	2017	2018	2019	Total
Number of Vp illnesses	23	33	24	57	30	167

Table 4. Vp illness totals by year.

Vp Illness Reports by Harvest Month						
	2015	2016	2017	2018	2019	Total count by month
May	0	1	0	6	5	12
June	2	1	1	13	5	22
July	10	17	6	20	11	64
August	9	10	13	15	7	54
September	2	4	4	3	2	15

Table 5. Vp illness counts by harvest month.

Vp Illness Records by Growing Area						
Growing Area	2015	2016	2017	2018	2019	Total
Bay Center	0	3	0	2	0	5
Bruceport	0	0	2	0	1	3
Dabob Bay	1	1	0	4	0	6
Drayton Harbor	0	0	1	1	1	3
Dyes Inlet	0	0	0	1	0	1
Grays Harbor	0	0	0	2	1	3
Hammersley Inlet	2	0	1	1	7	11
Henderson Bay	2	0	0	6	1	9
Henderson Inlet	1	0	0	1	0	2
Hood Canal 1	3	0	1	1	1	6
Hood Canal 2	1	0	1	0	1	3
Hood Canal 4	1	0	1	0	0	2
Hood Canal 5	0	0	2	3	0	5
Hood Canal 6	1	3	0	6	1	11
Hood Canal 7	0	0	0	0	1	1
Hood Canal 8	0	0	0	1	0	1
Hood Canal 9	0	0	0	2	0	2
Nahcotta	2	6	0	3	0	11
Nisqually Reach	0	3	0	1	0	4
Oakland Bay	0	0	0	1	0	1
Peale Passage	3	0	0	0	0	3
Pickering Passage	0	0	0	1	2	3
Port Gamble	0	0	0	1	0	1
Port Madison	0	0	1	0	0	1
Reach Island	0	0	0	1	6	7
Rocky Bay	0	0	0	1	0	1
Samish Bay	0	0	10	7	1	18
Skookum Inlet	1	2	2	0	0	5
Stony Point	1	5	0	4	0	10
Stretch Island	1	0	0	0	0	1
Totten Inlet	3	10	2	5	6	26
Westcott Bay	0	0	0	1	0	1

Table 6. Vp illness counts by growing area.

Growing Areas with Vp Illness Reports					
	2015	2016	2017	2018	2019
Number of growing areas with illnesses	14	8	11	24	13

Table 7. Number of growing areas with Vp illnesses.

Puget Sound Vp Illness Records						
Growing Area	2015	2016	2017	2018	2019	Total
Dabob Bay	1	1	0	4	0	6
Drayton Harbor	0	0	1	1	1	3
Dyes Inlet	0	0	0	1	0	1
Hammersley Inlet	2	0	1	1	7	11
Henderson Bay	2	0	0	6	1	9
Henderson Inlet	1	0	0	1	0	2
Hood Canal 1	3	0	1	1	1	6
Hood Canal 2	1	0	1	0	1	3
Hood Canal 4	1	0	1	0	0	2
Hood Canal 5	0	0	2	3	0	5
Hood Canal 6	1	3	0	6	1	11
Hood Canal 7	0	0	0	0	1	1
Hood Canal 8	0	0	0	1	0	1
Hood Canal 9	0	0	0	2	0	2
Nisqually Reach	0	3	0	1	0	4
Oakland Bay	0	0	0	1	0	1
Peale Passage	3	0	0	0	0	3
Pickering Passage	0	0	0	1	2	3
Port Gamble	0	0	0	1	0	1
Port Madison	0	0	1	0	0	1
Reach Island	0	0	0	1	6	7
Rocky Bay	0	0	0	1	0	1
Samish Bay	0	0	10	7	1	18
Skookum Inlet	1	2	2	0	0	5
Stretch Island	1	0	0	0	0	1
Totten Inlet	3	10	2	5	6	26
Westcott Bay	0	0	0	1	0	1
	20	19	22	46	28	135

Table 8. Puget Sound Vp illnesses.

Pacific Coast Vp Illness Records						
Growing Area	2015	2016	2017	2018	2019	Total
Bay Center	0	3	0	2	0	5
Bruceport	0	0	2	0	1	3
Grays Harbor	0	0	0	2	1	3
Nahcotta	2	6	0	3	0	11
Stony Point	1	5	0	4	0	10
	3	14	2	11	2	32

Table 9. Pacific Coast Vp illnesses.

Investigation follow-up and illnesses

It's important to note that some oyster companies couldn't report temperature data associated with harvests because of missing records. Reported air temperature data had the most available records, 145 out of 167 because companies are required to measure ambient air temperature under the Vp Control Plan. However, because oyster companies have the choice to record either water or internal tissue temperature, only 78 and 90 records, respectively were available out of the total 167 records.

Puget Sound air temperatures

Puget Sound harvest air temperature data comprised roughly 81 percent of the total number of available records, which is why most of the analysis will focus on this region. Graphs of harvest air temperatures for each year from 2015 – 2019 can be found in Figures 9 – 13. In 2015, a wide range of harvest air temperatures were associated with Vp illnesses (48 – 78°F). In the following year of 2016, the spread of harvest air temperatures decreased, but then proceeded to increase with each Vp season (Table 10). Average harvest air temperatures for each year and corresponding standard deviations can be found in Table 11. The year with the highest average harvest air temperature was 2017 (63.66°F), while the lowest was in 2015 (58.95°F). When examining standard deviations, the year with the highest was 2015 (7.36), while the lowest was 2016 (5.53).

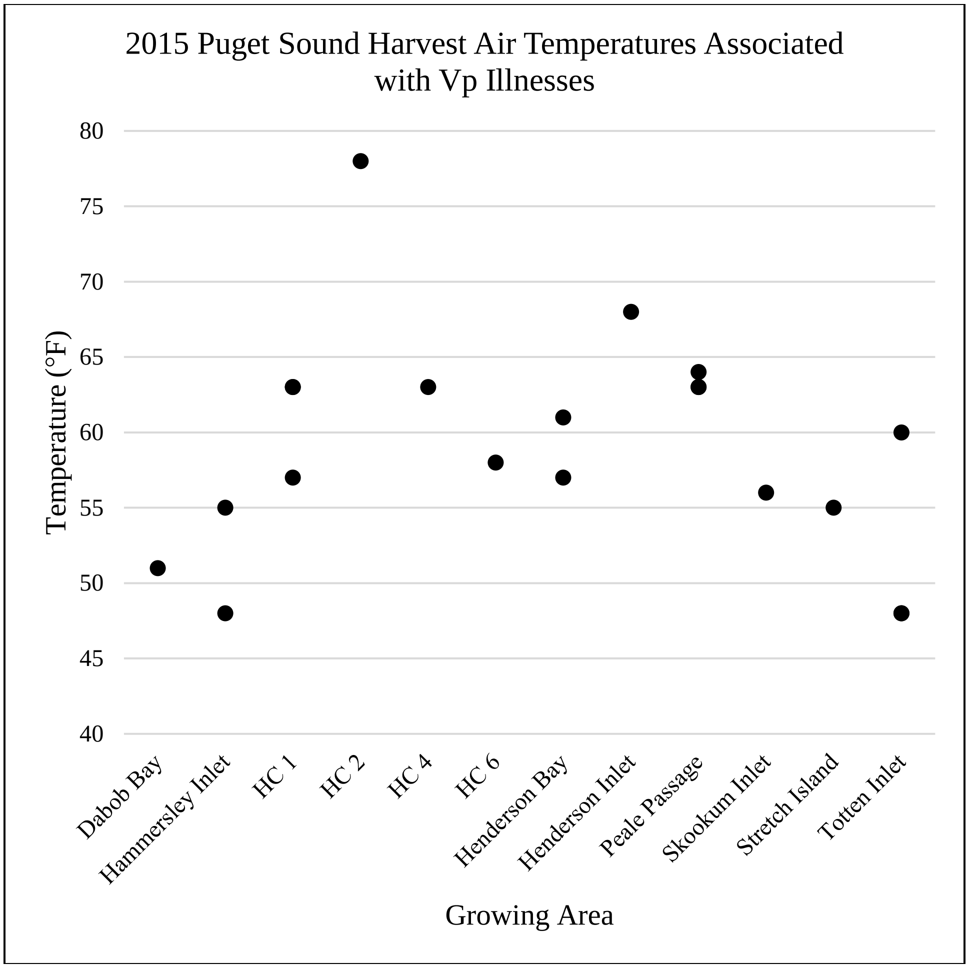

Figure 9. 2015 Puget Sound harvest air temperatures associated with Vp illnesses from investigation follow-up data.

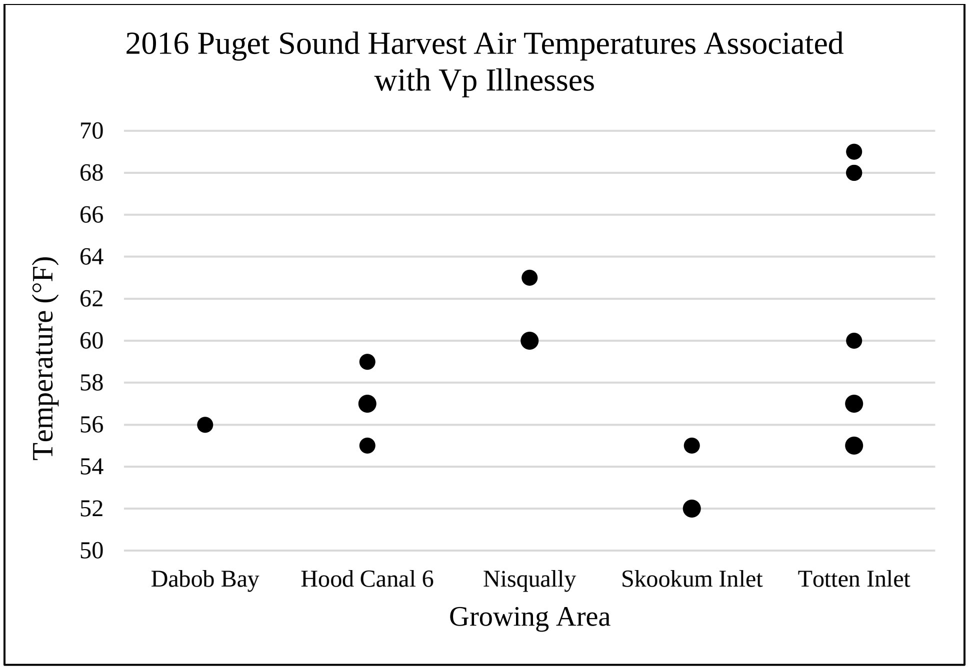

Figure 10. 2016 Puget Sound harvest air temperatures associated with Vp illnesses from investigation follow-up data.

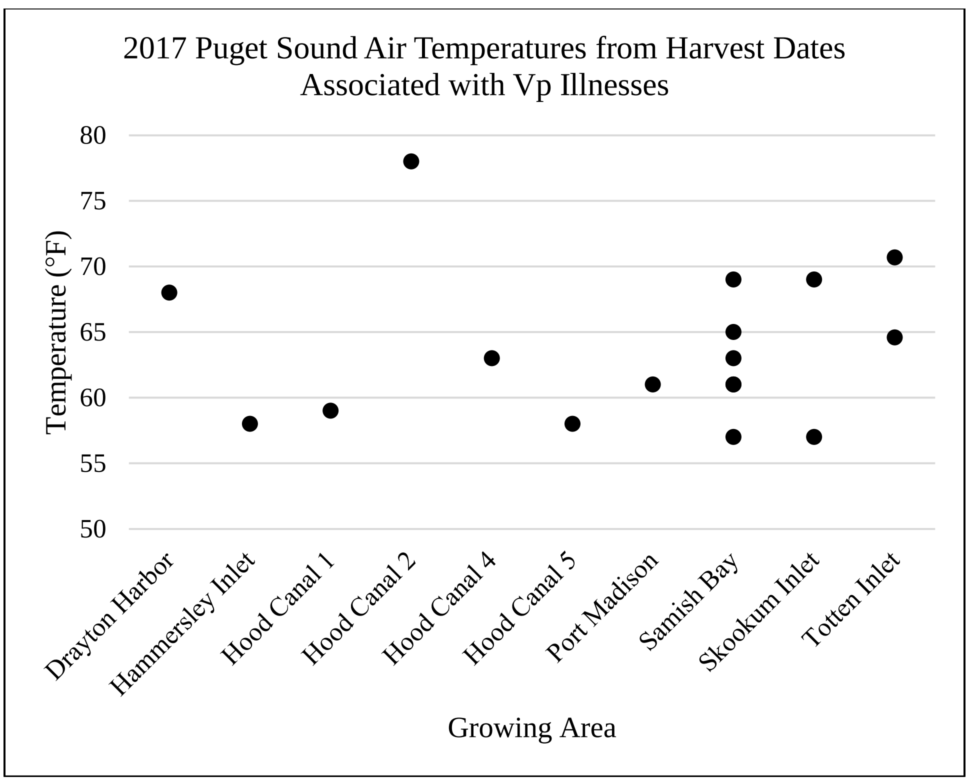

Figure 11. 2017 Puget Sound harvest air temperatures associated with Vp illnesses from investigation follow-up data.

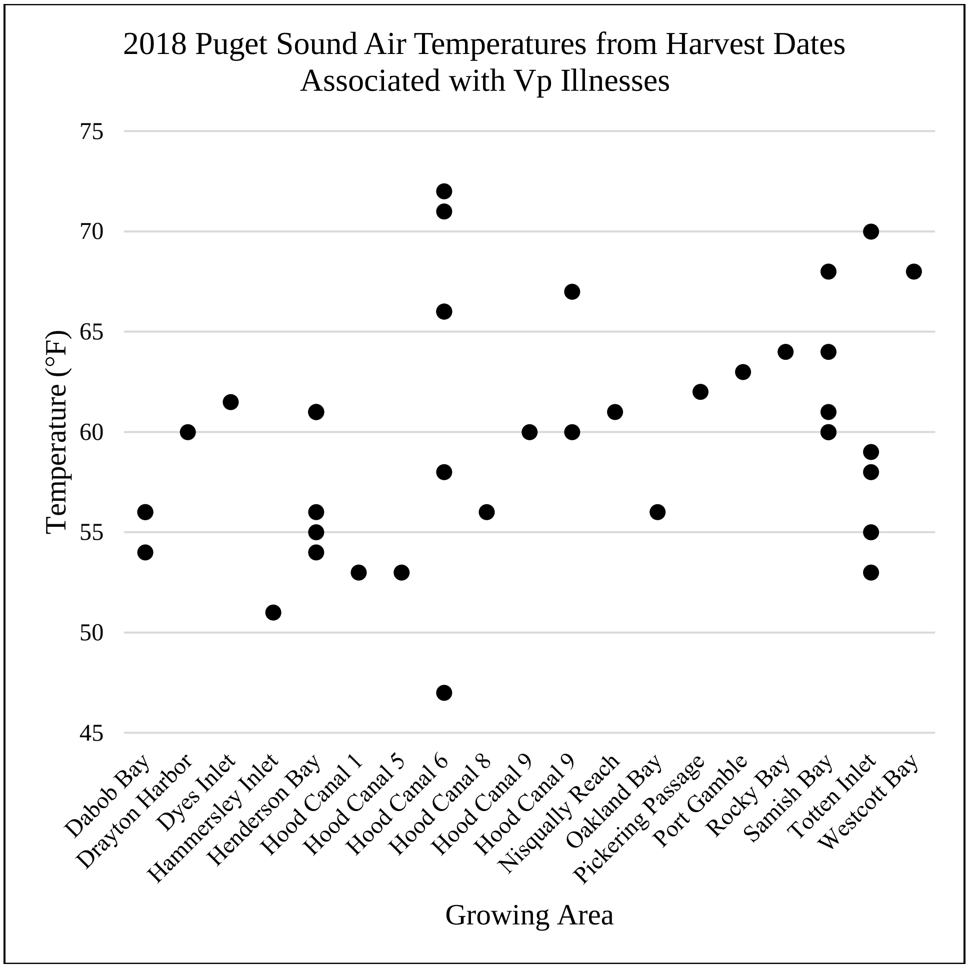

Figure 12. 2018 Puget Sound harvest air temperatures associated with Vp illnesses from investigation follow-up data.

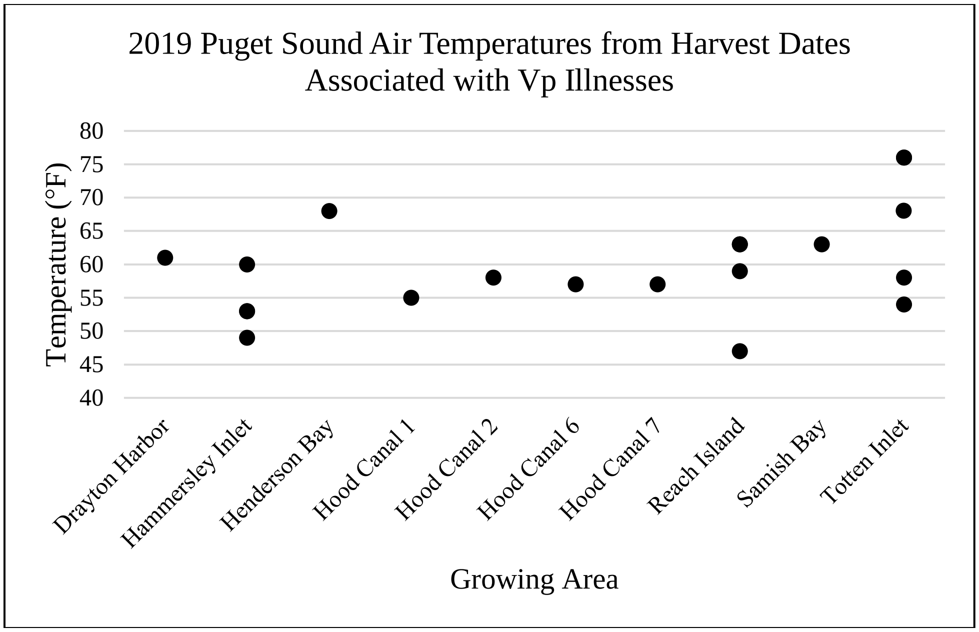

Figure 13. 2019 Puget Sound harvest air temperatures associated with Vp illnesses from investigation follow-up data.

	Puget Sound Harvest Air Temperature Spread		
Year	Low Temperature (°F)	High Temperature (°F)	Spread (°F)
2015	48	78	30
2016	52	69	17
2017	57	78	21
2018	47	72	25
2019	47	76	29

Table 10. Puget Sound harvest air temperature spread.

	Puget Sound Growing Areas	
Year	Harvest Air Temperature Average	Standard Deviation
2015	58.95	7.36
2016	59.61	5.53
2017	63.66	5.76
2018	59.94	5.72
2019	59.78	7.35

Table 11. Harvest air temperature averages and standard deviations from Puget Sound growing areas.

The only growing area associated with Vp illnesses each year from 2015 – 2019 was Totten Inlet (Figure 14). The average harvest air temperature was 61.09°F with a standard deviation of 7.99. When looking at the spread of harvest air temperatures for this area, it has generally increased since 2015 (Table 12). The only exception was in 2017; however, only two illness cases were reported for this year, lower than all other years analyzed.

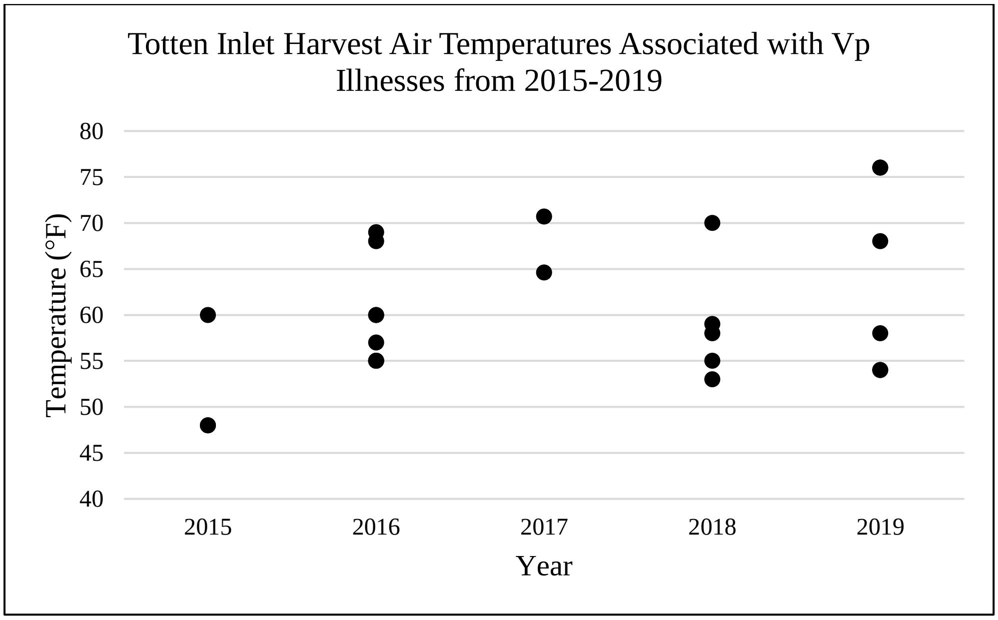

Figure 14. Totten Inlet harvest air temperatures associated with Vp illnesses from investigation follow-up data.

Totten Inlet Harvest Air Temperature Spread			
Year	Low Temperature (°F)	High Temperature (°F)	Spread (°F)
2015	48	60	12
2016	55	69	14
2017	64.6	70.7	6.1
2018	53	70	17
2019	54	76	22

Table 12. Totten Inlet harvest air temperature spread.

Coastal air temperatures

Coastal growing areas had significantly fewer Vp illnesses than Puget Sound. Unfortunately, only two out of the five years had data that could be used for analysis (2016 and 2018) and for those years, records were missing. Graphs of harvest air temperatures for these years can be found in Figures 15 and 16. The spread of air temperatures can be found in Table 13; however, I cannot reach a conclusion regarding a trend due to the lack of data. Both years analyzed had relatively similar harvest air temperature averages; however, in 2018 the standard deviation was less than that of 2016 (Table 14).

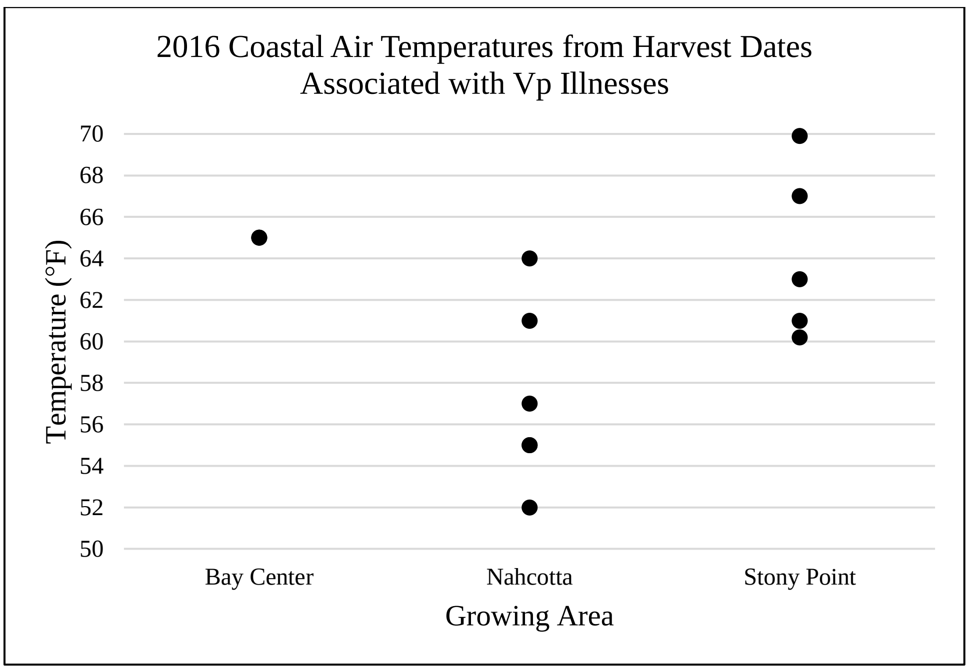

Figure 15. 2016 Coastal harvest air temperatures associated with Vp illnesses from investigation follow-up data.

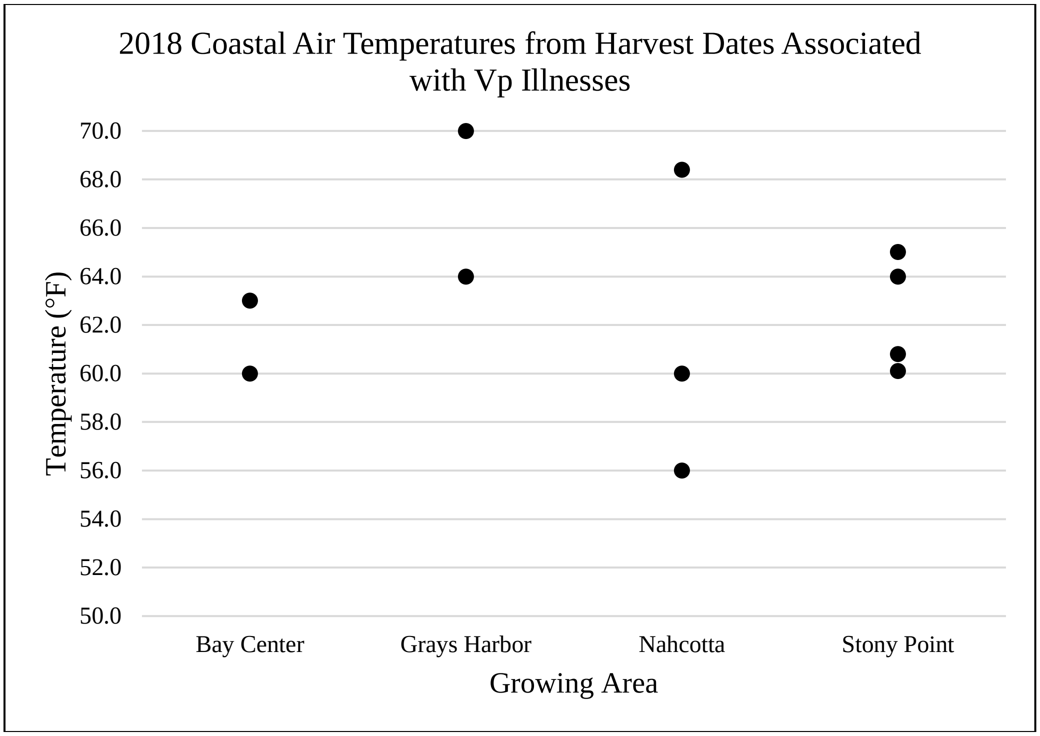

Figure 16. 2018 Coastal harvest air temperatures associated with Vp illnesses from investigation follow-up data.

Coastal Harvest Air Temperature Spread			
Year	Low Temperature (°F)	High Temperature (°F)	Spread (°F)
2015	NA	NA	NA
2016	52	69.9	17.9
2017	NA	NA	NA
2018	56	70	14
2019	NA	NA	NA

Table 13. Coastal harvest air temperature spread (NA = not applicable).

Coastal Growing Areas		
Year	Harvest Air Temperature Average	Standard Deviation
2015	NA	NA
2016	61.16	5.24
2017	NA	NA
2018	62.85	4.05
2019	NA	NA

Table 14. Harvest temperature averages and standard deviations from coastal growing areas (NA = not applicable).

Puget Sound water temperatures

Puget Sound growing areas accounted for roughly 83 percent of the available water temperature records. Graphs for each year from 2015 – 2019 can be found in Figures 17 – 21. Temperature spread for each year can be found in Table 15. Average harvest water temperatures ranged from 56.9 – 62.69°F with the lowest average in 2019 and the highest in 2017 (Table 16).

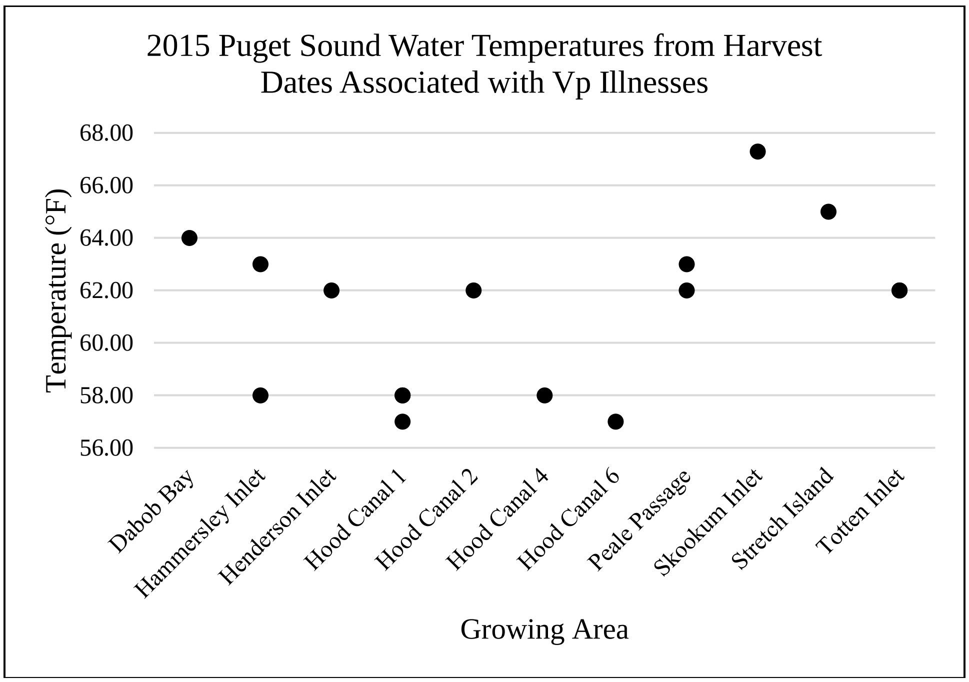

Figure 17. 2015 Puget Sound harvest water temperatures associated with Vp illnesses from investigation follow-up reports.

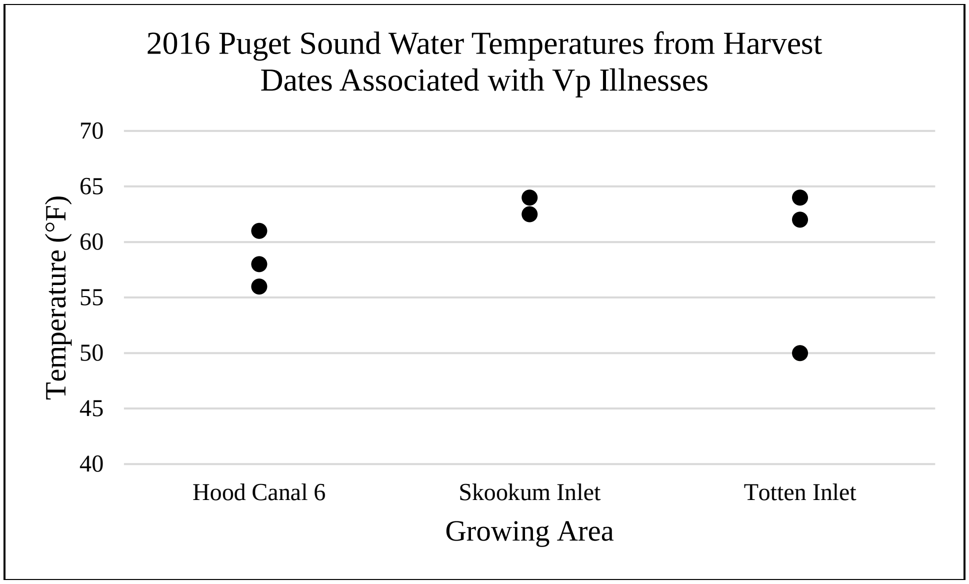

Figure 18. 2016 Puget Sound harvest water temperatures associated with Vp illnesses from investigation follow-up reports.

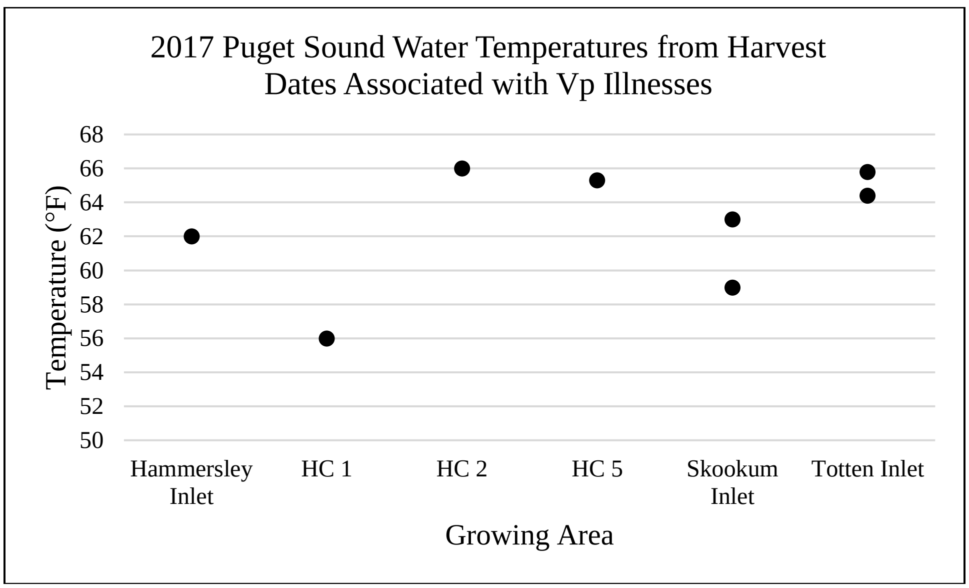

Figure 19. 2017 Puget Sound harvest water temperatures associated with Vp illnesses from investigation follow-up reports.

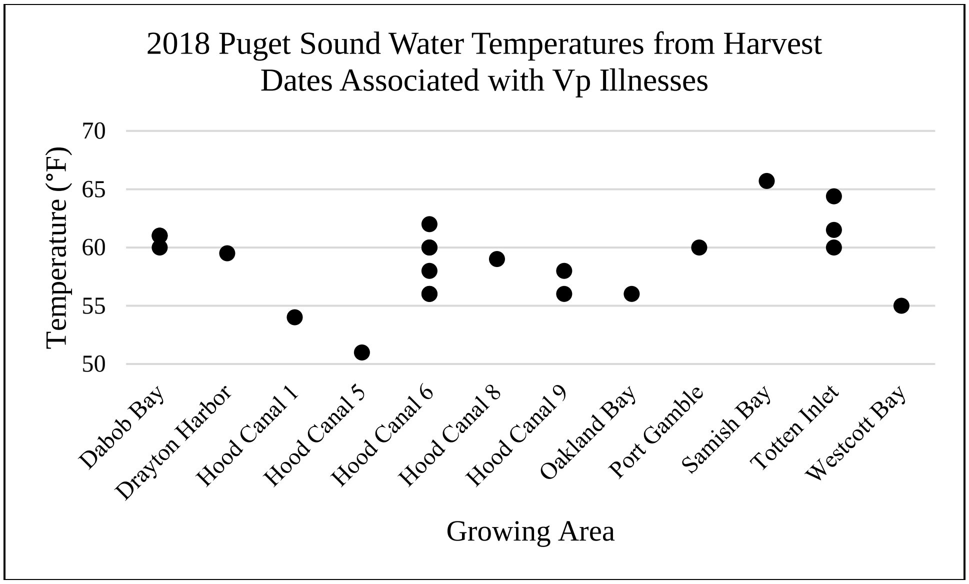

Figure 20. 2018 Puget Sound harvest water temperatures associated with Vp illnesses from investigation follow-up reports.

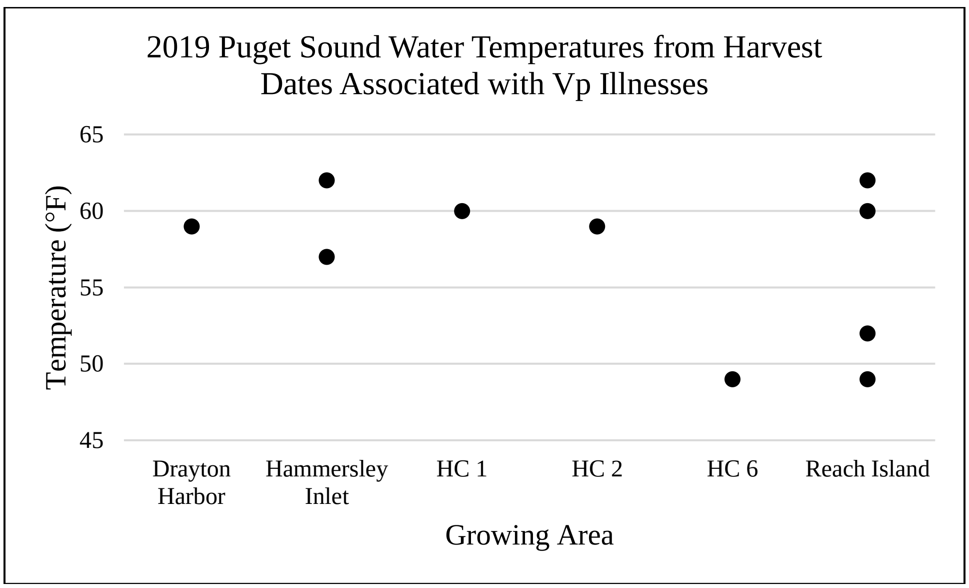

Figure 21. 2019 Puget Sound harvest water temperatures associated with Vp illnesses from investigation follow-up reports.

Puget Sound Harvest Water Temperature Spread			
Year	Low Temperature (°F)	High Temperature (°F)	Spread (°F)
2015	57	67.3	10.3
2016	50	64	14
2017	56	66	10
2018	51	65.7	14.7
2019	49	62	13

Table 15. Harvest water temperature spread from Puget Sound growing areas.

Puget Sound Growing Areas		
Year	Harvest Water Temperature Average	Standard Deviation
2015	61.14	3.11
2016	58.61	5.54
2017	62.69	3.57
2018	58.82	3.41
2019	56.9	5.04

Table 16. Harvest water temperature averages and standard deviations from Puget Sound growing areas.

Coastal water temperatures

The only years with water temperature data that could be used for analysis were 2016 (Figure 22) and 2018 (Figure 23), each containing six records. When studying the data, the spread in temperatures for 2016 was significantly greater than in 2018 (Table 17). A higher standard deviation was found for 2016 (7.94) than 2018 (2.63); however, the average harvest water temperature was higher for 2018 than 2016 (Table 18).

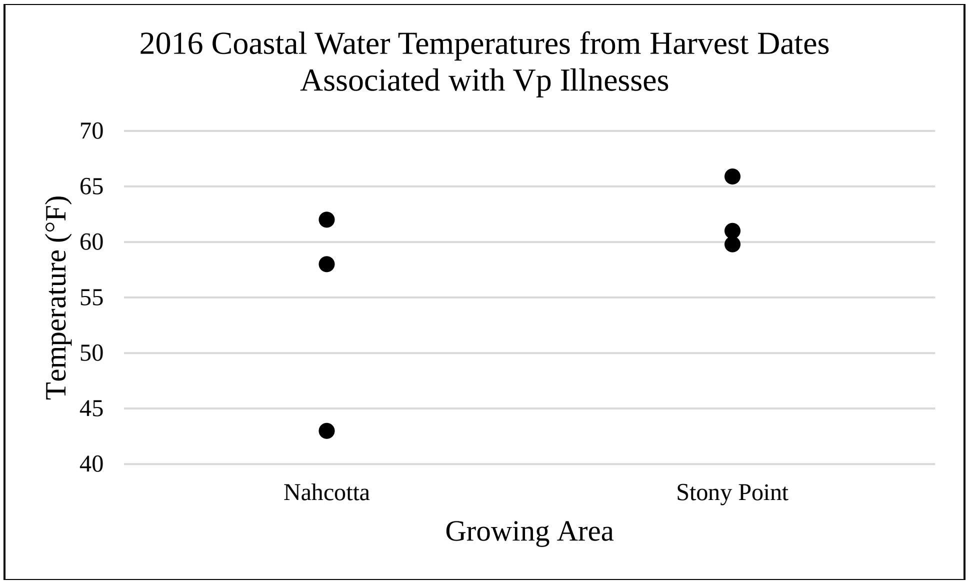

Figure 22. 2016 Coastal harvest water temperatures associated with Vp illnesses from investigation follow-up reports.

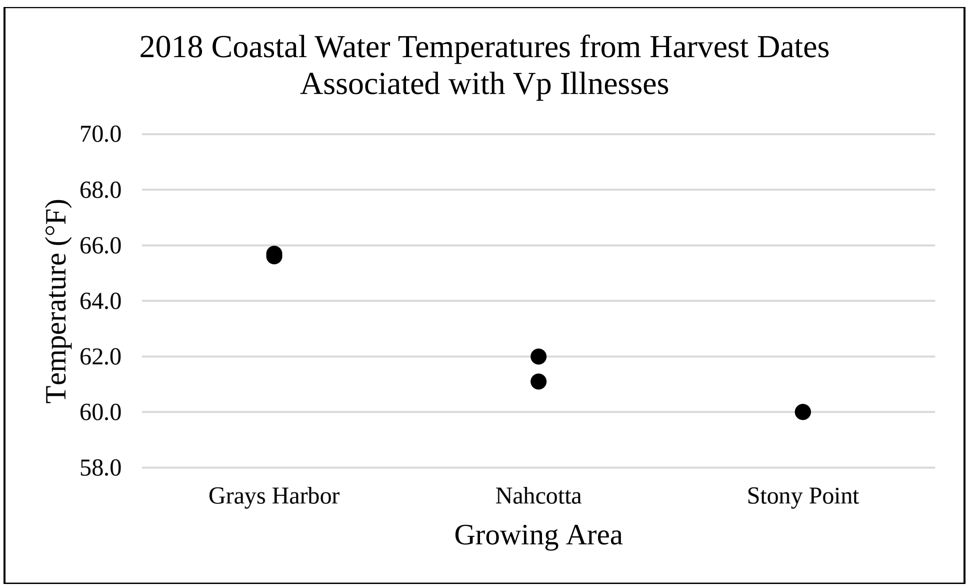

Figure 23. 2018 Coastal harvest water temperatures associated with Vp illnesses from investigation follow-up reports.

Coastal Harvest Water Temperature Spread			
Year	Low Temperature (°F)	High Temperature (°F)	Spread (°F)
2015	NA	NA	NA
2016	43	65.9	22.9
2017	NA	NA	NA
2018	60	65.7	5.7
2019	NA	NA	NA

Table 17. Coastal harvest water temperature spread.

Coastal Growing Areas		
Year	Harvest Water Temperature Average	Standard Deviation
2015	NA	NA
2016	58.28	7.94
2017	NA	NA
2018	62.4	2.63
2019	NA	NA

Table 18. Coastal harvest water temperature averages and standard deviations.

Puget Sound internal oyster tissue temperatures

Puget Sound growing areas comprised 83 percent of the available records for internal tissue temperatures. Graphs for each year can be found in Figures 24 – 28. The smallest temperature spread was in 2017, while the largest was in 2019 (Table 19). The lowest average tissue temperature was 57.25°F in 2019, which was the year with the highest standard deviation of 6.44 (Table 20). The highest average tissue temperature was 62.68°F in 2017, which had the lowest standard deviation of 2.65 (Table 20).

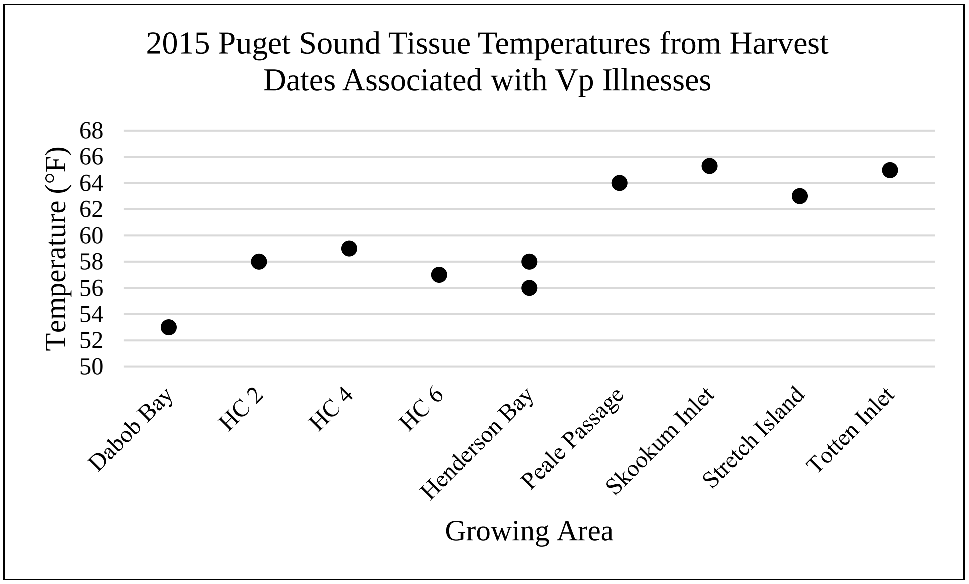

Figure 24. 2015 Puget Sound harvest tissue temperatures associated with Vp illnesses from investigation follow-up reports.

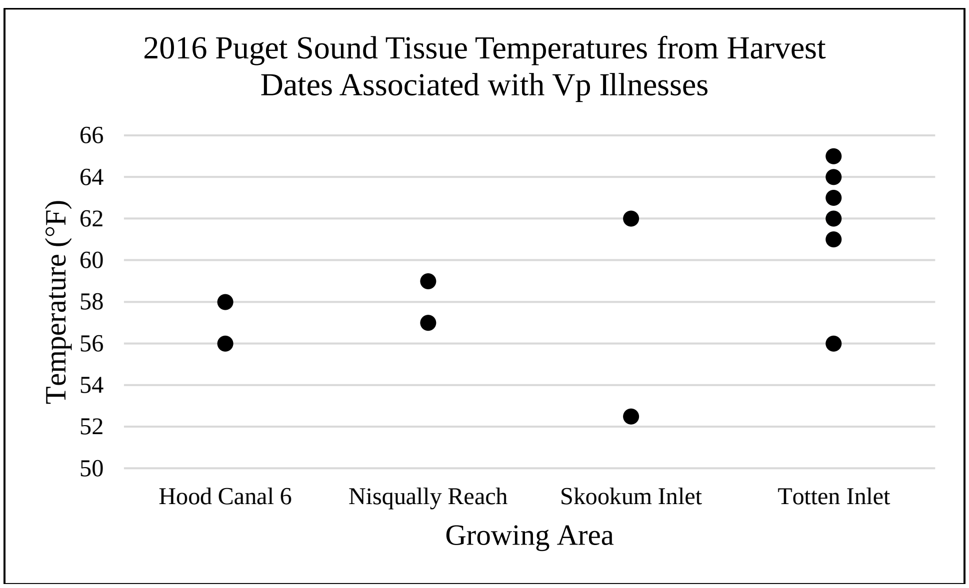

Figure 25. 2016 Puget Sound harvest tissue temperatures associated with Vp illnesses from investigation follow-up reports.

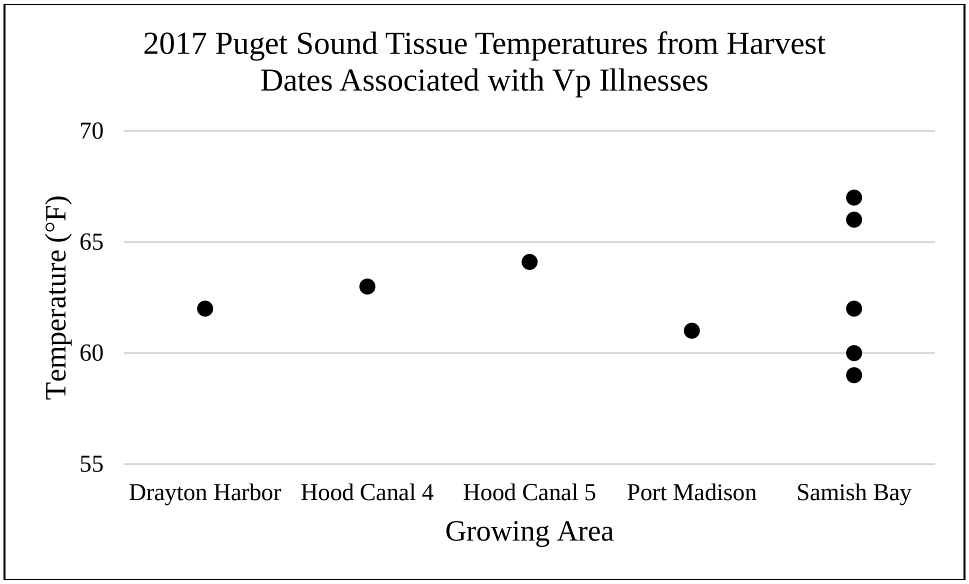

Figure 26. 2017 Puget Sound harvest tissue temperatures associated with Vp illnesses from investigation follow-up reports.

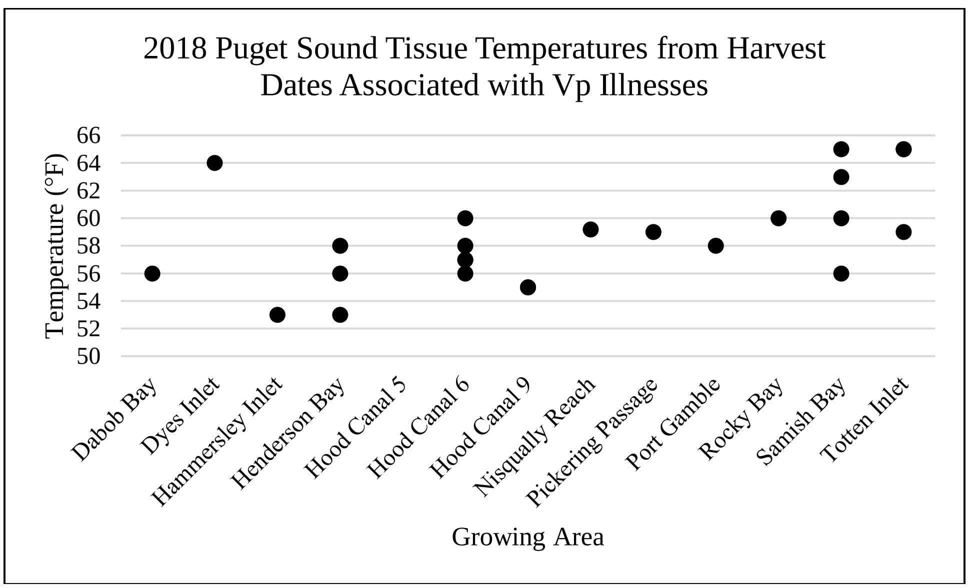

Figure 27. 2018 Puget Sound harvest tissue temperatures associated with Vp illnesses from investigation follow-up reports.

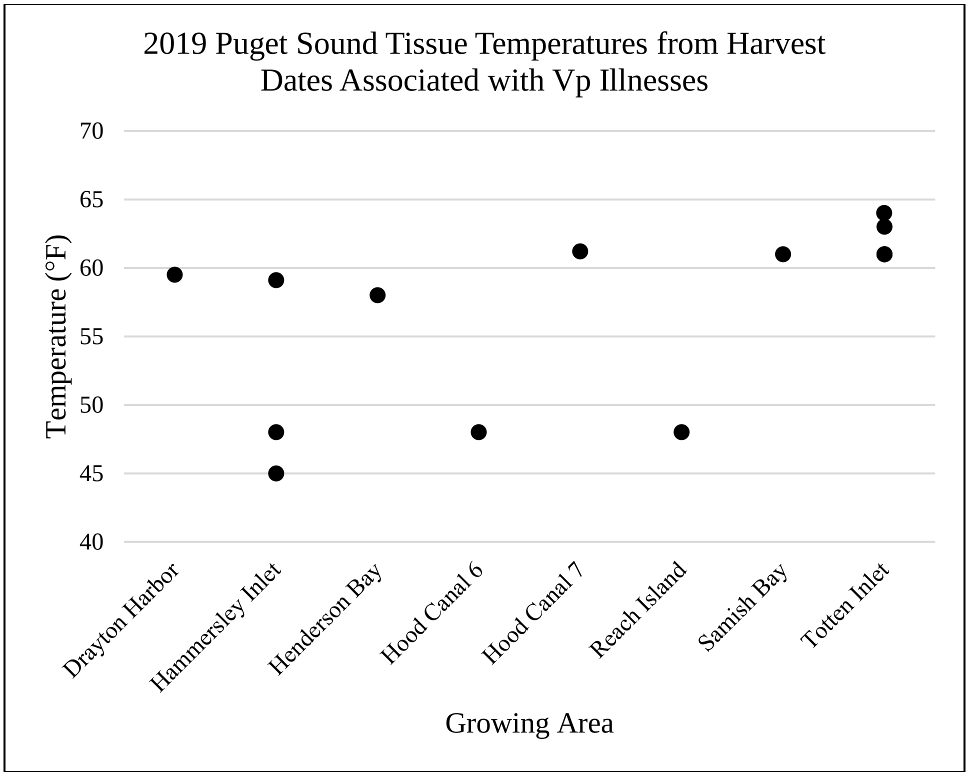

Figure 28. 2019 Puget Sound harvest tissue temperatures associated with Vp illnesses from investigation follow-up reports.

Puget Sound Harvest Tissue Temperature Spread			
Year	Low Temperature (°F)	High Temperature (°F)	Spread (°F)
2015	53	65.3	12.3
2016	52.5	65	12.5
2017	59	67	8
2018	48	65	17
2019	45	64	19

Table 19. Puget Sound harvest tissue temperature spread.

Puget Sound Growing Areas		
Year	Harvest Tissue Temperature Average	Standard Deviation
2015	59.83	4.23
2016	59.81	3.71
2017	62.68	2.65
2018	58.29	4.02
2019	57.25	6.44

Table 20. Puget Sound harvest tissue temperature averages and standard deviations.

Coastal internal oyster tissue temperatures

Only two years had internal tissue temperature data that could be used for analysis: 2016 (Figure 29) and 2018 (Figure 30), with seven and six records, respectively. Similar temperature spreads were found between 2016 and 2018 (Table 21). A higher average tissue temperature was observed in 2018 than 2016; however, 2018 had a lower standard deviation than 2016 (Table 22).

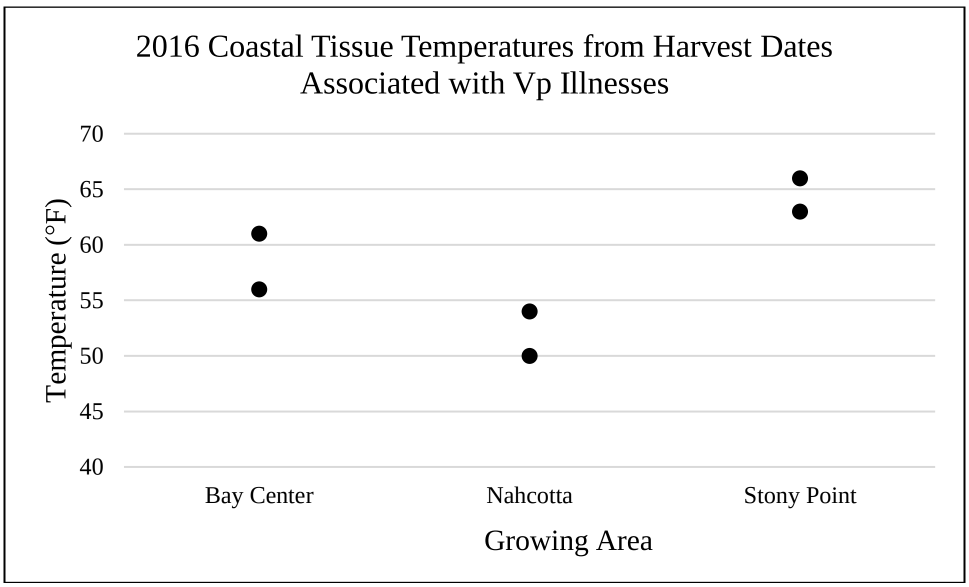

Figure 29. 2016 Coastal harvest tissue temperatures associated with Vp illnesses from investigation follow-up reports.

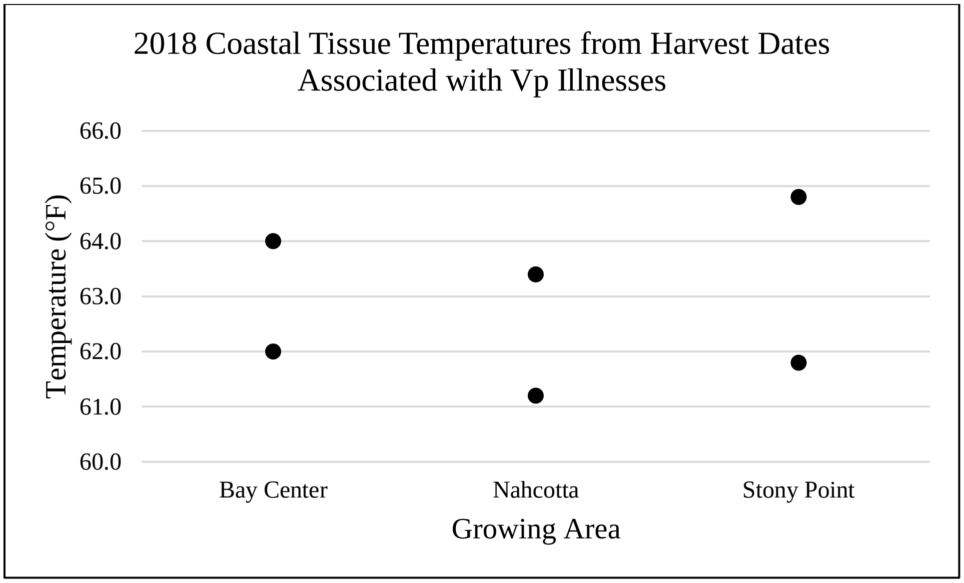

Figure 30. 2018 Coastal harvest tissue temperatures associated with Vp illnesses from investigation follow-up reports.

Coastal Harvest Tissue Temperature Spread			
Year	Low Temperature (°F)	High Temperature (°F)	Spread (°F)
2015	NA	NA	NA
2016	50	66	16
2017	NA	NA	NA
2018	48	65	17
2019	NA	NA	NA

Table 21. Coastal harvest tissue temperature spread.

Coastal Growing Areas		
Year	Harvest Tissue Temperature Average	Standard Deviation
2015	NA	NA
2016	57.71	5.74
2017	NA	NA
2018	62.9	1.41
2019	NA	NA

Table 22. Coastal harvest tissue temperature averages and standard deviations.

USB Temperatures prior to harvest data

If growing areas associated with illnesses had both universal serial bus (USB) data for temperatures leading up to the harvest date associated an illness (three days before) and more than two illness records, I included them in this analysis. Hammersley Inlet, Hood Canal 5, Samish Bay, and Totten Inlet were the only growing areas in Puget Sound that qualified for analysis. Maximum temperatures for each growing area are shown in each of the figures that follow to illustrate what conditions were like leading up to harvests (Figures 31 – 34). Unfortunately, coastal data wasn't sufficient for analysis because there were only two illnesses that had corresponding USB data.

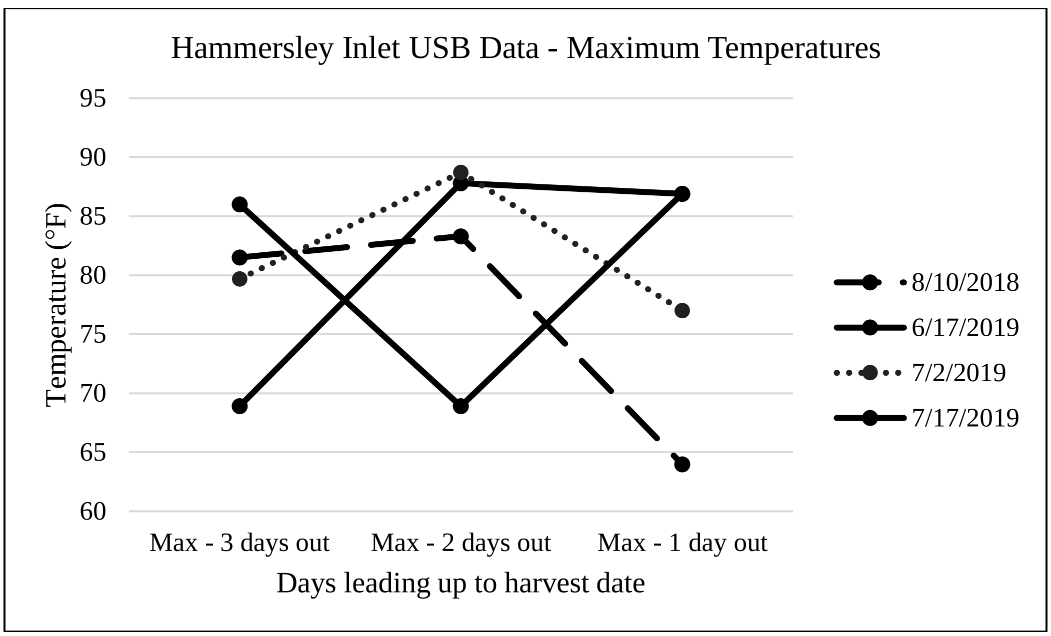

Figure 31. Maximum temperatures for Hammersley Inlet three days before harvest. The dates represent the harvest date associated with an illness.

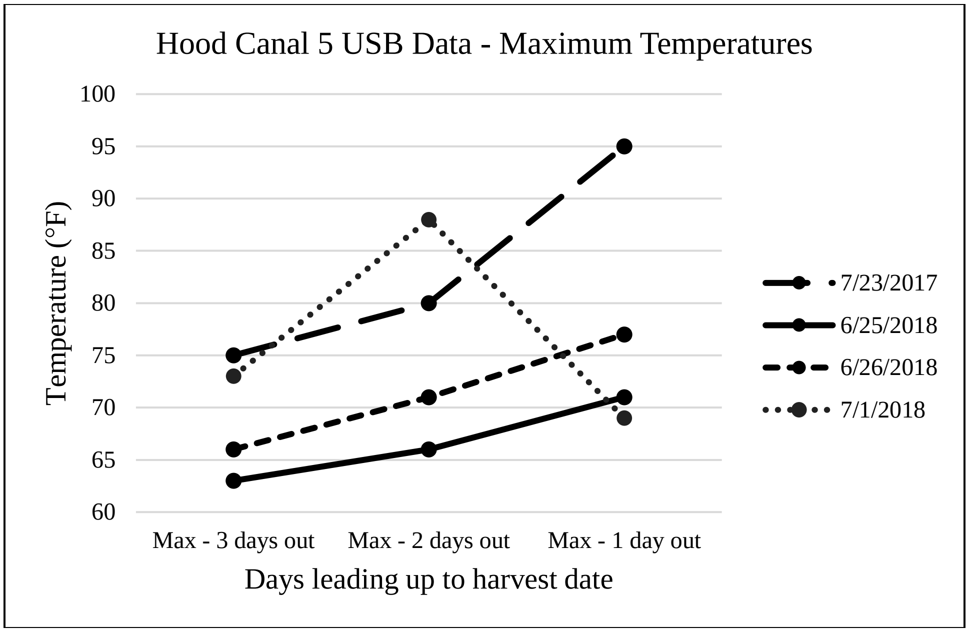

Figure 32. Maximum temperatures for Hood Canal 5 three days before harvest. The dates represent the harvest date associated with an illness.

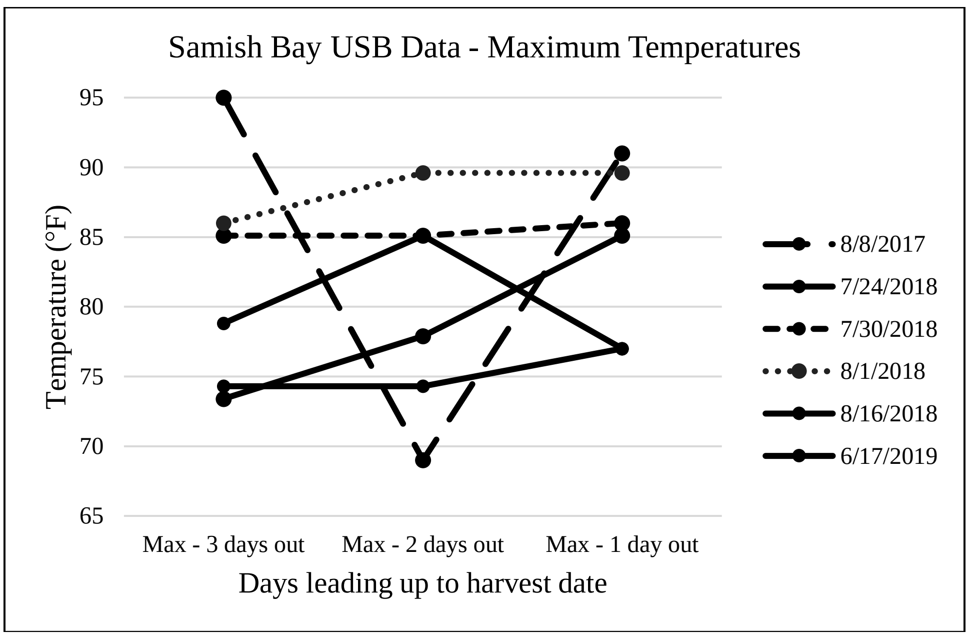

Figure 33. Maximum temperatures for Samish Bay three days before harvest. The dates represent the harvest date associated with an illness.

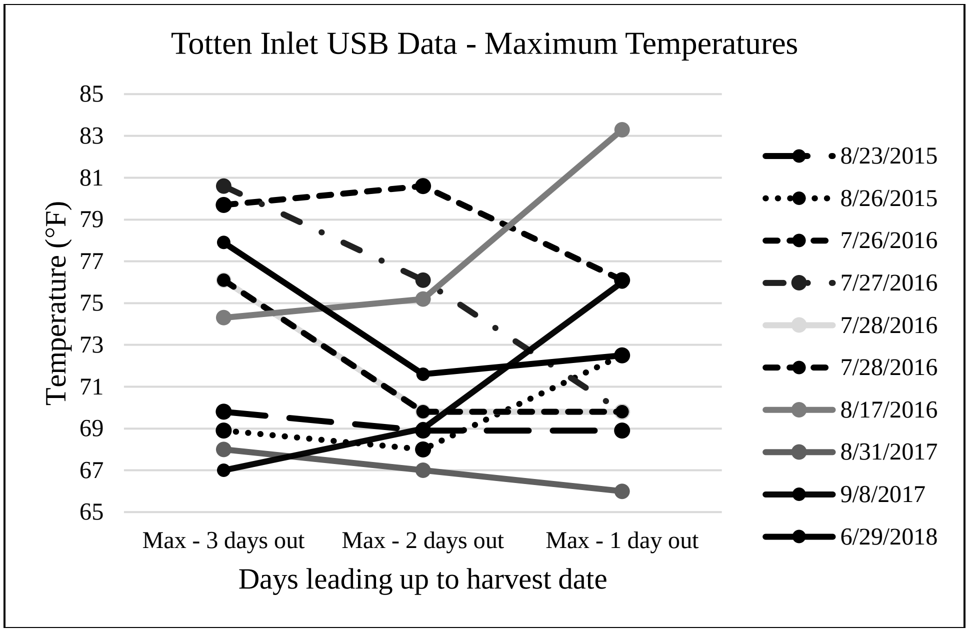

Figure 34. Maximum temperatures for Totten Inlet three days before harvest. The dates represent the harvest date associated with an illness.

WDOH Vp sampling – Puget Sound

All growing areas in Puget Sound (coastal data was insufficient) were combined to create graphs of the average, minimum, and maximum temperatures for ambient air, shore water, surface water, and internal oyster tissue (Figures 35 – 38). Ambient air temperatures from 2015 – 2019 show that 2015 had the highest maximum, although all years had maximum ambient air temperatures in the 80’s (Figure 35). Average ambient air temperatures for all five years were above 64°F. Average shore and surface water temperature were greater than 68 and 66°F respectively for all five years (Figures 36 and 37). Minimum shore and surface water temperatures never dove below 57.74 and 56.84°F, respectively. Average internal oyster tissue temperatures ranged from 69.88°F in 2017 to 76.88°F in 2015 (Figure 38). Maximum tissue temperatures were above 85°F for all five years and the minimum tissue temperature recorded among the five years was

53.6°F in 2017. Average, minimum, and maximum salinity measurements were also graphed (Figure 39). Salinity measurements in 2018 had the largest range, 5 – 33 ppt; however, the extremely low value of 5 ppt came from the growing area of Dabob Bay, which experiences an influx of fresh water from a nearby river.

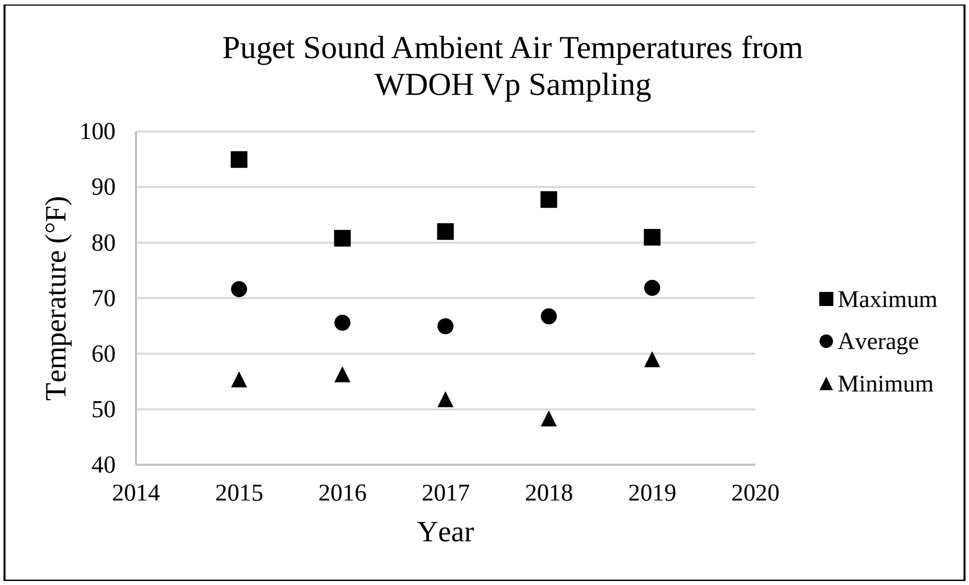

Figure 35. Maximum, average, and minimum ambient air temperatures from WDOH Puget Sound Vp sampling.

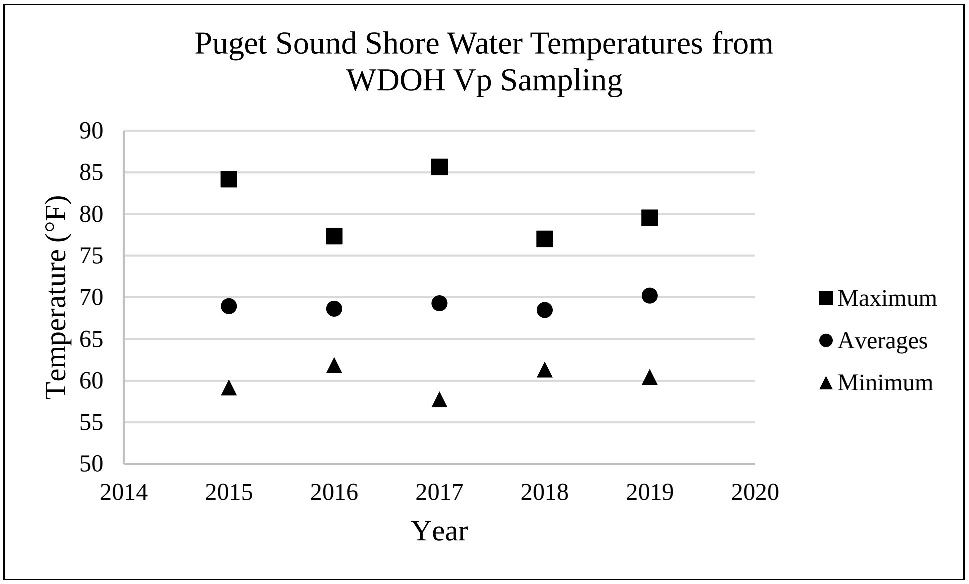

Figure 36. Maximum, average, and minimum shore water temperatures from WDOH Puget Sound Vp sampling.

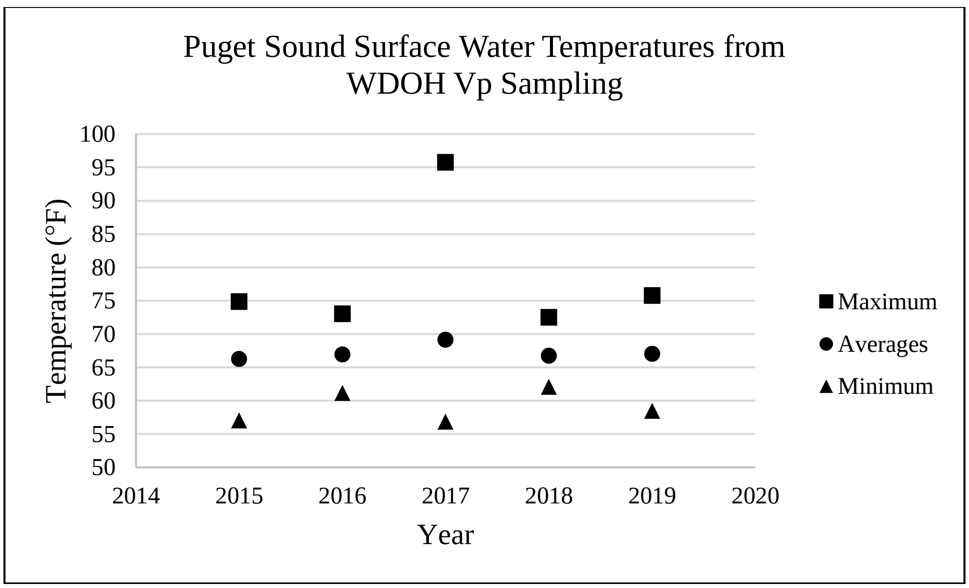

Figure 37. Maximum, average, and minimum surface water temperatures from WDOH Puget Sound Vp sampling.

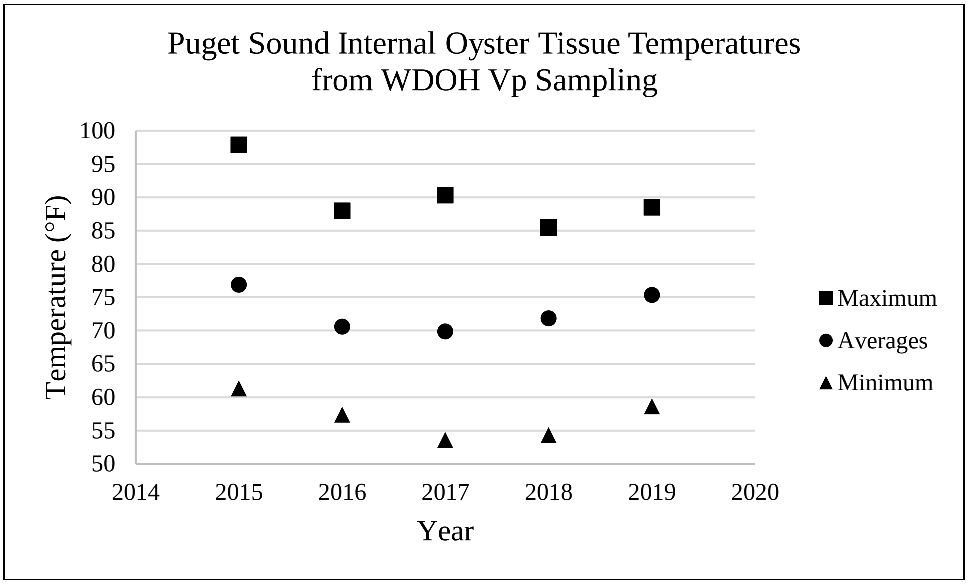

Figure 38. Maximum, average, and minimum internal oyster tissue temperatures from WDOH Puget Sound Vp sampling.

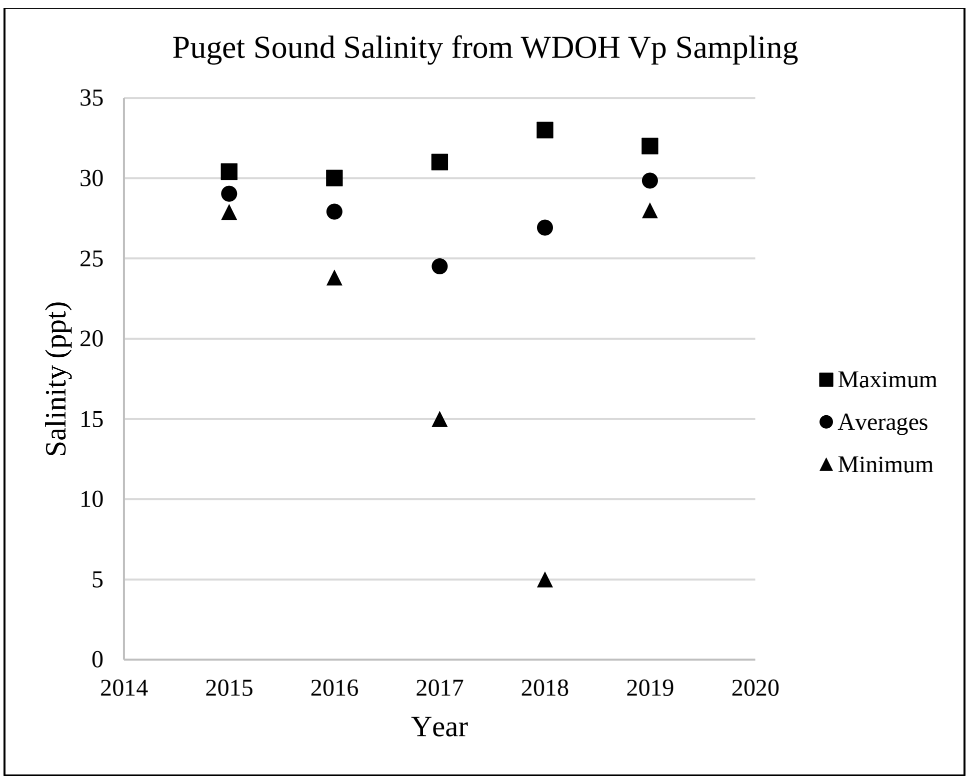

Figure 39. Maximum, average, and minimum salinity from WDOH Puget Sound Vp sampling.

Tidal elevations

Low tide elevations from harvest dates associated with Vp illnesses (Figure 40) show a wide distribution of tidal elevations. To break this down further, averages for each year were calculated (Figure 41) to illustrate whether more illnesses are associated with extreme low tides or high tides. Only in the year of 2015 were illnesses more associated with higher tides, the other four years (2016 – 2019) were more closely associated with low tides; however, the lowest average was -0.75 feet (2019), generally not considered an extreme low tide elevation.

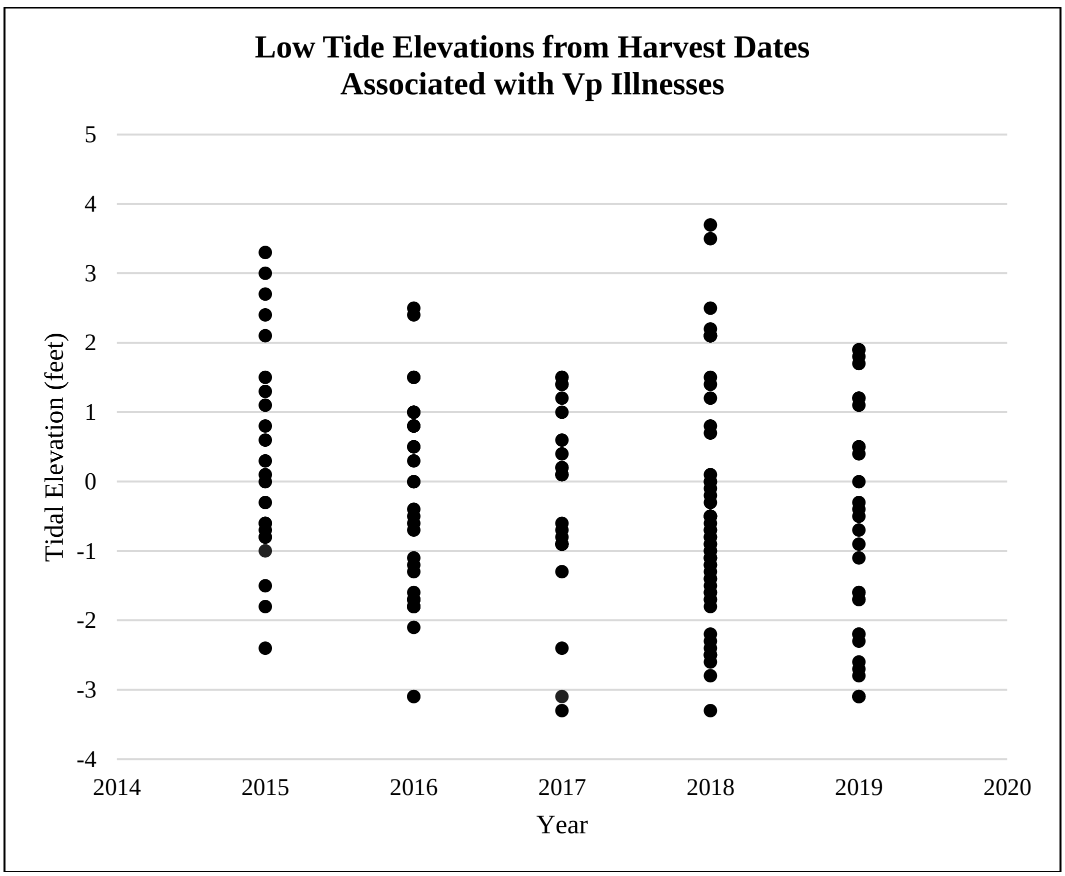

Figure 40. Low tide elevations from harvest dates associated with Vp illnesses by year.

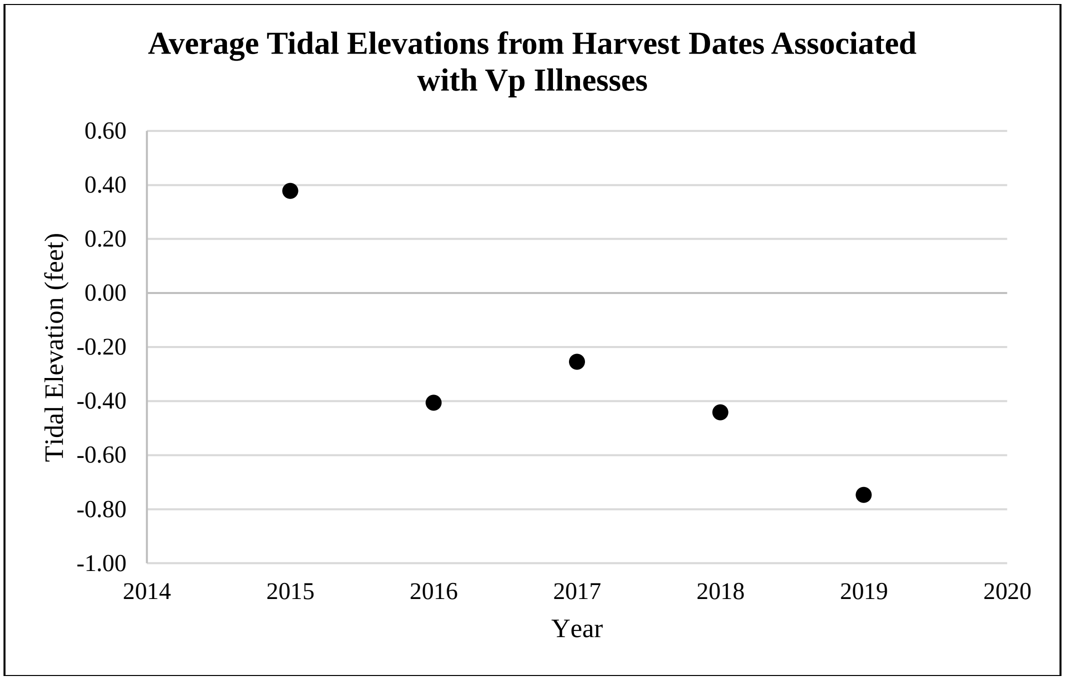

Figure 41. Average tidal elevations from harvest dates associated with Vp illnesses by year.

Reported oyster production data

Since all Vp illnesses used in this thesis were associated with Pacific oysters, only production data for Pacific oysters was included in the analysis. The total amount of the oysters produced (in dozens) for each month can be found in the Appendices. November produced the least amount (540637.5 dozen), while May produced the most (857369.5 dozen). Production totals for Vp control months (May – September) were greater than the non-control months.

Data summary

Data analyzed in this thesis included Vp illness counts broken down by year, month, and growing area. Analysis of ambient air, water, and internal oyster tissue temperatures collected by harvesters was made possible due to the documentation requirement under the current Vp Control Plan; however, some records were missing, which will be discussed in the next chapter. Analysis of USB temperatures from various growing areas was conducted to determine conditions leading up to harvests associated with illnesses. Environmental data collected by the WDOH Vp sampling program allowed for analysis of Puget Sound growing areas to assess air, water, and internal tissue temperatures, in addition to salinity measurements to identify if any trends are present. Tidal elevations from the harvest dates associated with illnesses were studied to determine if more illnesses are correlated with extreme low tides. Finally, reported oyster production data was analyzed to determine if production is greater in the control months compared to the non-control months.

Vp Control Plan Survey

I administered 18 surveys and had a response rate of 50-percent, yielding a sample size of nine. Survey questions can be found in the Appendices and responses can be found in Table 23 (grey filled boxes indicate the question was left blank). Eight of the nine respondents claimed that they utilize the bottom culture growing method. Other growing method responses included: tumble bag culture, bags, cages, off bottom, and suspended culture. Eight of the nine respondents also claimed that they primarily use the intertidal harvest method. Two-thirds of the respondents claimed to have a “very good” understanding of the current Vp Control Plan, the highest answer possible. Five respondents declared that the current Vp Control Plan is only “somewhat effective” at preventing Vp illnesses. Six respondents claim that extra measures are being taken beyond what is required to limit Vp growth, but only two respondents identified what those measures were. Finally, seven respondents made suggestions on how to improve the current Vp Control Plan, including further dividing growing area boundaries and adopting the British Columbia Vp Program. The suggestion of further dividing growing area boundaries surprised me because I consider the division already extensive, as seen in the map created by WDOH (“Commercial Shellfish Map Viewer,” 2020). Policy recommendations for the Vp Control Plan will be provided in the next chapter.

Question 1	Question 2	Explanation if more than one answer	Question 3	Question 4	Question 5	Question 6
singles on bottom	Intertidal		Very good	Somewhat effective	Yes	More field monitoring of growers for compliance
bottom culture and tumble bag culture	Intertidal & subtidal	Line and net. Oysters collected placed in nets and harvested at high tide	Good	Almost always effective	No	Devide large waterways "Grow Areas" in the lower sound. Reevaluate risk categories in those harvest areas.
on bottom, flip bags, cages	Dredge, intertidal, & subtidal	Harvest method depends on bed elevation although dredging is used for bottom culture only	Very good	Somewhat effective	No	Needs to be a provision in the plan to deal with problem companies. An entire growing area shouldn't be punished due to the actions of one or two companies.
bottom culture	Intertidal		Good	Undecided	Currently target to follow risk category 2 or 3 for all beaches we harvest	Containerizing shellfish at low tide during peak air/water temperatures but not removing from the growing area…
bottom & off bottom	Dredge & intertidal	Mainly intertidal harvest with high tide retreived; some dredging	Very good	Somewhat effective	Yes	Consider the BC program
beach & suspended culture	Intertidal & subtidal		Very good	Somewhat effective	Freshwater rinsing / post seawater cleaning. Chilled water temp control / pre-storage at temp control	
NA - all industry	NA	NA	Poor	Undecided	NA	Not at this time
on the ground	Intertidal		Very good	Not effective	Yes	Yes go after the outfits that are making people sick and see what they are up to.
all	Dredge, intertidal, & subtidal	Large volume of each	Very good	Somewhat effective	Yes	Yes. Canadian Standard of 100 TLH

Table 23: Vp Control Plan Survey responses (NA = not applicable).

CHAPTER VI: DISCUSSION

In this chapter I discuss the results and observations made in the previous chapter and make relations to current literature. I provide suggestions to help explain the data along with recommendations for future policy revisions. I also provide recommendations for improving the current WDOH Vp monitoring program. I offer suggestions for future studies to further understand how environmental conditions impact the growth of Vp. Finally, I discuss the results of the Vp Control Plan survey and make recommendations for this survey to be improved if used again in future years.

Vp Illnesses

The analysis of total Vp illnesses revealed that most occurred during either July or August of the control months (May – September), outlined in the Washington State Vp Control Plan, suggesting a strong correlation between temperature and Vp growth. This aligns with current literature on Vp (Drake et al., 2007; C. L. Johnson, 2015; Martinez-Urtaza et al., 2010). This thesis only focused on the control months; it would be worth examining the number of illnesses that occur in the non-control months to determine if the Vp season is lengthening.

One observation concerning all Vp illnesses is that all illnesses were associated with consuming Pacific oysters. As mentioned in the Literature Review, this is the most commonly grown oyster species in Washington. Pacific oysters have more resilience than other species and have higher population growth rates that allow them to spread rapidly across coastal and estuarine environments (Harris, 2008). Since Vp doesn't affect the health of oysters, when they move to other regions they could be bringing Vp to these waters as they depurate it out of their tissues, which could help explain why Vp is becoming more prevalent in other regions.

We need to keep oyster production data in mind because it would make sense that when more oysters are harvested, the number of illnesses increases. Oyster production in the Vp control months of 2018 was greater than the non-control months; however, a different conclusion might have been reached if production data were available for all five years. Future research should examine production data from 2019 and hereafter to determine if this same conclusion can be reached.

Studying illness count trends across the five-year period revealed two things. First, the highest illness count occurred during 2018, with 57 illnesses, and the lowest illness count was in 2015, with 23 illnesses – indicating illnesses have generally increased over time. However, when studying the number of illnesses over the five-year period, there appears to be a fluctuation in illnesses. For example, illnesses were low in 2015, then increased in 2016 before decreasing in 2017, followed by the highest number of illnesses in 2018, and another decrease in 2019 (Table 4). Recall that total illness counts for Puget Sound and Pacific Coast growing areas were studied separately to determine if this fluctuation occurs on a regional level. When examining illness counts each year for Puget Sound growing areas (Table 8), this fluctuation isn't observed; however, there is a fluctuation in illnesses that happens each year for coastal growing areas (Table 9). Future research should monitor the fluctuation in illnesses originating from oysters harvested along the Pacific Coast to determine if this pattern continues, which could lead to taking extra precautions in the years predicted to have more illnesses.

As discussed in the Literature Review and as illustrated by the results from this research, the geographic distribution of Vp illnesses is spreading. In 2015 and 2019, 14 and 13 growing areas respectively contributed to Vp illnesses; however, seven of the 13 growing areas in 2019 were not involved in the tally for 2015 illnesses. In 2015, Nahcotta and Stony Point in Willapa Bay as well as portions of Hood Canal and southern Puget Sound were implicated in illnesses. In 2019, WDOH tied two more growing areas to illnesses on the coast, Bruceport in Willapa Bay and Grays Harbor (Figure 42). In addition, a growing area in Hood Canal as well as two growing areas in southern Puget Sound that weren't associated with illnesses in 2015 were implicated in 2019 illnesses (Figure 43). Lastly, two northern Puget Sound growing areas were associated with illnesses in 2019, a region not tied to illnesses in 2015 (Figure 44).

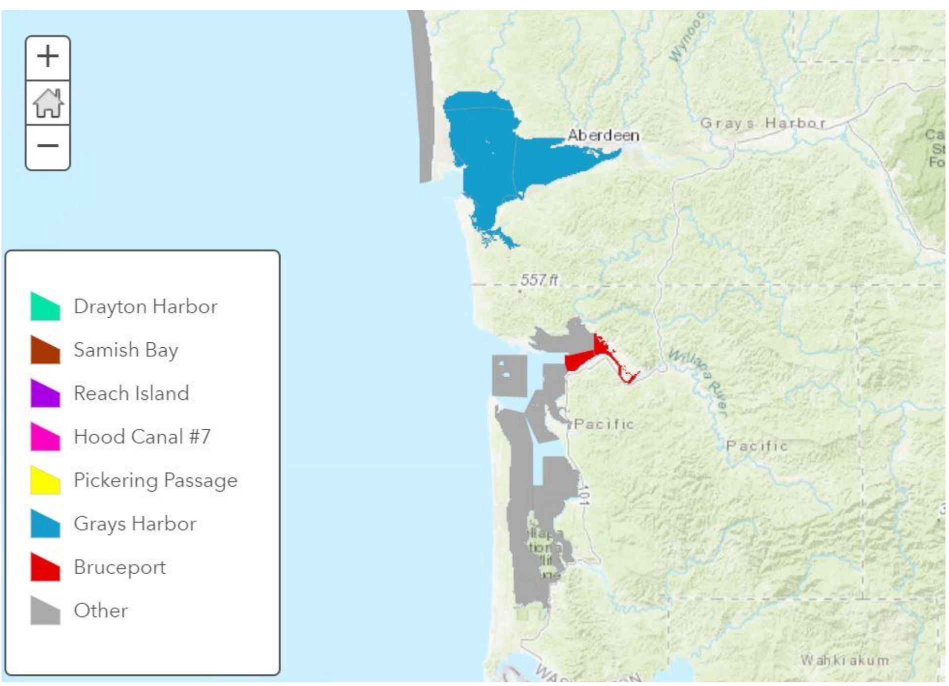

Figure 42. Growing areas along the coast implicated in Vp illnesses in 2019, Bruceport (red) and Grays Harbor (blue). The author created this map using ArcGis Online software.

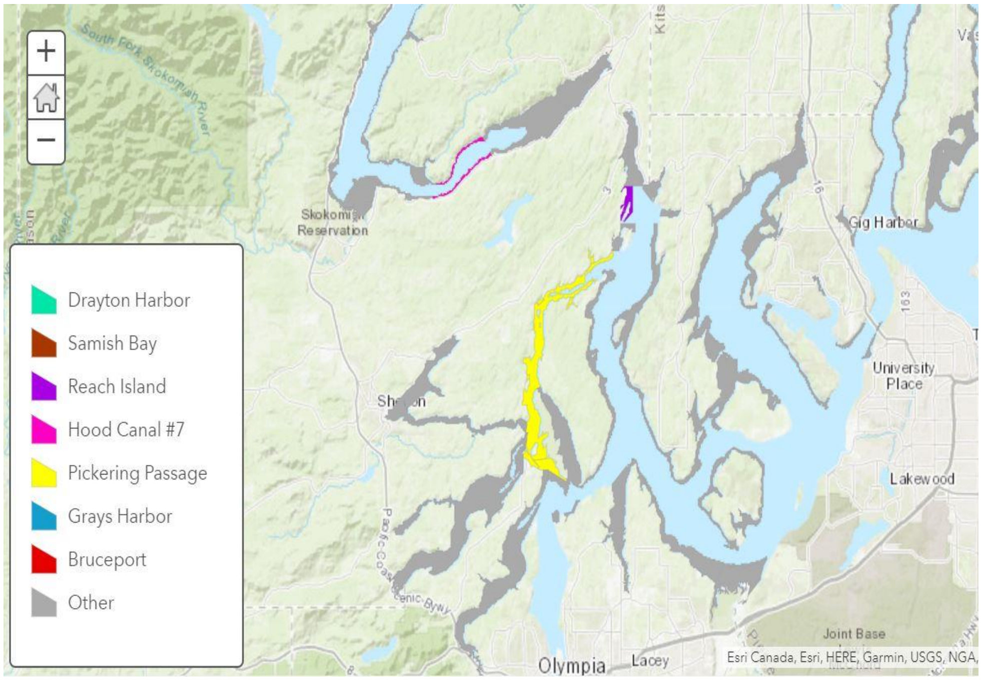

Figure 43. Growing areas in Hood Canal and southern Puget Sound implicated in Vp illnesses in 2019: Hood Canal 7 (magenta), Pickering Passage (yellow), and Reach Island (purple). The author created this map using ArcGis Online software.

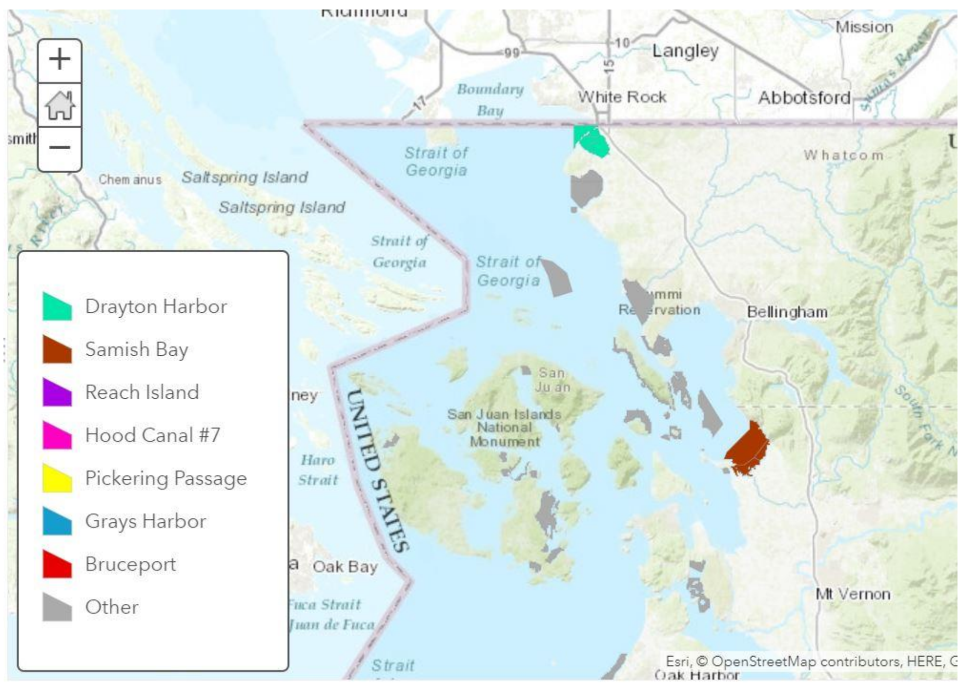

Figure 44. Growing areas in northern Puget Sound implicated in Vp illnesses in 2019, Drayton Harbor (turquoise) and Samish Bay (maroon). The author created this map using ArcGis Online software.

Future research might examine common denominators, aside from temperatures and salinity, among those growing areas to try to identify reasons for the spread. Since Vp originates in the water, studying circulation patterns may indicate how and where the water is traveling, thus distributing Vp.

Using the selected criteria outlined in the Methods chapter, significantly more Vp illnesses were found for Puget Sound (135 illnesses) compared to the Pacific Coast (32 illnesses); therefore, most of the analyses were carried out for Puget Sound growing areas. It could be that Puget Sound has more illnesses because air and water temperatures in the summer months are generally warmer in this region compared to the coast, and those conditions favor Vp growth; however, more research is needed in each of these regions to draw statistically based conclusions.

Investigation follow-up data

I discovered gaps in the air, water, and tissue temperature data from illness investigations. For some years, data for the coast didn't have enough illnesses for analysis (two or less), which led to only studying data from 2016 and 2018. Furthermore, studying growing areas individually provided a more detailed analysis; however, only one growing area, Totten Inlet, had air temperature data for all five years. This data showed that harvest air temperatures have generally increased over the last five years (Figure 14), as well as the number of illnesses in this growing area since 2017 (Table 6), suggesting a connection between harvests at higher temperatures and illnesses. Unfortunately, a lack of available records prevented a similar trend analysis for water and tissue temperatures in Totten Inlet. Harvesters probably chose to measure either water or internal tissue temperature but not both, as both are not required under the Vp Control Plan. To close that gap, harvesters should be required to measure both water at the depth the oysters are harvested from and internal tissue temperature at time of harvest.

Harvest air temperatures associated with illnesses covered a wide range, although all temperatures were above 47°F. No conclusion could be drawn from comparing temperatures with the time of day and month the harvest took place, high temperatures were associated with both morning and evening harvests, as well as every month during the control season (May – September). Some growing areas had relatively low harvest temperatures associated with illnesses when compared to others. For example, in 2019 Hammersley Inlet had harvest temperatures of 49, 53, and 60°F (May – July), whereas Totten Inlet had harvest temperatures as high as 76°F in July. Both growing areas harvested either early in the morning or later in the evening, suggesting that other factors such as bathymetry could be contributing to Vp growth.

When comparing Puget Sound water temperatures to illnesses, the year with the highest average temperature (2017), didn't correlate to the year with the highest number of illnesses (2018). However, 2018 had the largest temperature spread (Table 15) and had 24 different growing areas contributing to illnesses, the most of all five years, suggesting water temperatures across Puget Sound were warmer than prior years and consequently contributed to an increase in Vp growth.

When analyzing the follow-up data, I was most interested in tissue temperatures at time of harvest, as the internal tissue of an oyster can concentrate Vp much higher in their tissues compared to the surrounding water (Depaola et al., 1990). The spread of tissue temperatures has generally increased from 2015 to 2019, but the average tissue temperature hasn't increased.

Harvest air temperatures for coastal growing areas showed similar averages and spread. Air temperatures in Nahcotta were slightly warmer in 2018 (the year with the most illnesses) than 2016, aligning with current literature that higher temperatures increase the opportunity for Vp growth. Alternatively, air temperatures in Stony Point were slightly cooler in 2018 compared to 2016, suggesting other factors beyond temperature could have contributed to illnesses. Both average water and tissue temperatures for coastal growing areas in 2018 were higher than averages in 2016, which also supports current literature; however, this determination would carry more weight had I had more years of data to analyze. The investigation follow-up data provides insight into what conditions were like during harvests but analyzing temperature data in the days leading up to harvests illustrates if any conclusions should be made regarding adding more stringent measures to the current Control Plan.

Temperatures leading up to harvests

Studying harvest temperatures provided by oyster companies provides insight into the environmental conditions on that specific day, while USB dataloggers can provide temperatures that give insight into conditions on the days prior to harvests. This data can help illustrate if the temperatures were increasing leading up to harvests, which would have increased the opportunity for Vp growth.

There are a few things to keep in mind when analyzing the USB temperature data. First, the black canister that encases the device was painted white (Figure 8) at the beginning of the 2018 season to help mitigate inaccurate temperature extremes that resulted from the previously black canister absorbing heat from the sun. After the canister was painted white, extreme temperature readings no longer occurred; therefore, USB data from 2015 – 2017 may not represent conditions accurately. Secondly, when gathering data, I noticed an inconsistency in recorded temperatures. For example, not every day of each month had recorded data, preventing complete analyses. In addition, it costs WDOH valuable resources to place these dataloggers throughout the state, meaning not every growing area gets one, consequently precluding those growing areas from being included in this analysis. Furthermore, WDOH doesn't always have these dataloggers deployed for the entire duration of the Vp control months, so data may not be available for all illnesses that occurred during that time.

The growing areas with three days of temperature data prior to the harvest date deemed associated with an illness and more than two illnesses included: Hammersley Inlet, Hood Canal 5, Samish Bay, and Totten Inlet. These growing areas are categorized as a Risk Category 3, except for Hood Canal 5, which is categorized as a Risk Category 2. Maximum temperature graphs for each growing area showed no consistent pattern in air temperatures prior to harvests that resulted in illnesses; however, some observations can be made.

Hammersley Inlet had four harvest dates with enough data to analyze (Figure 31). Two of the four cases graphed had maximum temperatures of 86.9°F prior to the day harvests occurred. When studying the maximum temperatures for the remaining two cases, temperatures reached approximately 80°F or higher on both three and two days prior. These observations demonstrate that the water could have been warmer leading up to harvests, which may have increased Vp density in the water and subsequently in oyster tissues, leading to increased chance for illnesses. Harvests in the cooler days that followed may not have given oysters enough time to depurate.

Most cases that were analyzed for Hood Canal 5 (Figure 32) and Samish Bay (Figure 33) illustrated an increasing trend in temperatures leading up to harvests. Maximum temperatures on the day prior to harvests were all higher than 69 and 77°F for Hood Canal 5 and Samish Bay, respectively. Samish Bay had a case where temperatures were above 90°F prior to harvests; however, this was before the canister was painted white, meaning these records are most likely not accurate. Most of the cases that were analyzed for Totten Inlet also had data from 2017 or before, plus some temperature data from 2018 was missing, and no USB data logger was placed in this area in 2019, preventing any conclusions from being drawn.

Although conclusions can't be made confidently, these results demonstrate the importance for future research in air and water temperatures leading up to harvest dates associated with Vp illnesses. Future studies should place temperature collection devices in all growing areas, or at a minimum the high-risk growing areas (Risk Category 3), to examine conditions leading up to harvests. This research could reveal patterns that could ultimately lead to a change in the Vp Control Plan (i.e. preventing harvests if temperatures in the days prior were all above a specific threshold, as water temperatures need to be cooler for Vp density to decline).

WDOH Vp sample data

Several considerations need to be kept in mind when analyzing Vp sample data collected by WDOH. First, not all growing areas are sampled every year and not all are sampled as frequently as others. For example, growing areas with a higher risk category are sampled more frequently than others to monitor conditions in these areas. Some growing areas are also extremely far away from the WDOH office in Tumwater, Washington – preventing frequent sampling (or none) in areas such as Samish Bay (Risk Category 3), 141 miles one-way. Second, the environmental data is generally collected by new people each year, as WDOH hires interns, typically college students, to help collect samples each summer. The WDOH employee training the interns was not the same person over the five years used for analysis, which also could have impacted the consistency of the data. Third, a chance of human error enters when data is collected, especially when multiple people are collecting samples. Another source of potential error is the accuracy and reliability of the equipment being used, although the thermometers and refractometers used are calibrated at the beginning of each season and throughout if needed. Finally, sample collection generally doesn’t begin until June, possibly due to students having to wait until classes have ended for the summer; therefore, data was not available for harvests from May that were associated with illnesses, even though May 2018 had the highest production figures. I understand the circumstances why sampling starts when it does; however, conducting sampling earlier and more consistently would help improve the WDOH Vp monitoring program.

Overall, only Puget Sound samples were analyzed due to insufficient data available from coastal growing areas. Average shore water temperatures were higher than average surface water temperatures, indicating that oysters harvested intertidally on beaches are subjected to warmer water conditions, increasing the odds of Vp growth in oysters. Furthermore, average internal oyster tissue temperatures each year were higher than the ambient air, shore and surface water temperatures, demonstrating how oysters can hold onto heat and serve as an incubator for Vp.

Observations concerning salinity were difficult to make due to variation in data. For example, in 2018 an extreme low of 5 ppt was recorded; however, this was measured in Dabob Bay, which is fed by an influx of fresh water. Note: The average salinity of Puget Sound is 28.5 ppt, compared to approximately 34 ppt for the Pacific Ocean (MacCready, 2017). Salinity measurements may also be impacted by the ebb and flow of the tides and this should be considered when conducting future studies. In addition, future studies should examine this data by individual growing areas or smaller regions (i.e. Hood Canal and north, central, and south Puget Sound) to determine if the changes in salinity by growing area impact Vp density.

Tidal data

Low tidal elevations from harvest dates associated with illnesses demonstrate that oysters were harvested under all tidal conditions; oysters harvested during extreme tidal elevations were not associated with illnesses to a greater degree. Still, a few considerations need to be kept in mind when making this conclusion. For example, some oyster companies use dredges to harvest oysters, which generally wouldn’t be associated with an extreme low tide, contributing to difficulty when analyzing this dataset. Some companies in Puget Sound harvest oysters at high tide, place them in bags, and leave them in the water for one tidal cycle with the idea that the tidal cycle will help the oysters depurate any Vp concentrated in their tissues. This practice also contributes to difficulties when trying to analyze harvest tidal elevations related to illnesses. Future studies should analyze harvest tidal elevations based on the type of harvest method used, to help eliminate these discrepancies.

Survey

The Vp Control Plan survey distributed to oyster company representatives provided beneficial information and inspired another research question. The survey revealed that several oyster companies utilize more than one type of growing method and more than one type of harvest practice. Most respondents said they have a "very good" understanding of the current Vp Control Plan; however, the sample size for this survey was significantly smaller than the number of oyster companies in the state. Therefore, future surveys should aim to reach representatives of all oyster companies in the state to provide a more representative sample. This type of survey would also allow for more suggestions to help improve the current Vp Control Plan.

The same suggestion on how to improve the current Vp Control Plan was provided by two different respondents. Both recommended that Washington should consider adopting the British Columbia, Canadian Standard of 100 MPN/g TLH, as TLH identifies the presence of Vp ("Bacteriological guidelines for fish and fish products (end product)," 2019). As a result, I decided to determine how many of the illnesses used in this thesis would have been prevented if Washington had the same policy. Using the same procedures for selecting WDOH Vp sample data as outlined in the Methods chapter, only 82 samples, approximately half of the number of illnesses, qualified for analysis. I then filtered those 82 samples to determine how many were above the Canadian Standard of 100 MPN/g threshold for TLH. After filtering, 39 samples were determined to have a TLH presence greater than 100 MPN/g (Table 24). Thus, if commercial harvesters didn't harvest oysters on those dates or had found these oysters and had removed them from their shipments, almost 50 percent of these illnesses or approximately 23 percent of the total Vp illnesses used in this thesis, could have been prevented if Washington followed this policy. In the end, while implementing the Canadian Standard could have prevented some of the Vp illnesses, it would not have prevented all of them.

Year	Total Vp Illnesses	WDOH Vp samples used for anlaysis with similar dates	Samples >100 MPN/g TLH
2015	23	13	5
2016	33	18	11
2017	24	18	10
2018	57	21	11
2019	30	12	2

Table 24. Number of Vp illnesses prevented by implementing the Canadian Standard.

Future studies should aim to gather more data and thus enable an analysis of the effectiveness of the Canadian Standard in preventing illnesses in Washington. This would mean testing more oyster samples to provide data because some illnesses did not have a matching WDOH sample for comparison since the sample dates were not close enough to the harvest date implicated. In addition, illnesses in growing areas that are not sampled by WDOH were not included in this analysis; however, if data had been available from

WDOH for all 167 illnesses, the number of illnesses that would have been prevented by using the Canadian Standard may have been different. It should be noted that some samples sent to the lab may have been rejected if the oysters were too warm or too cold/frozen. There is also the possibility that some samples were never delivered to the lab due to courier errors.

Recommendations to improve the Vp Control Plan

This thesis has revealed several suggestions for improving the current Vp Control Plan. My strongest suggestion includes requiring harvesters to take both water and internal oyster tissue temperatures at time of harvest because only requiring one or the other provides inconsistent and inconclusive data for analyses. If this is not feasible, I would recommend requiring harvesters to take internal tissue temperatures because Vp density is typically greater in oyster tissues, providing a more accurate representation of the conditions under which Vp would be growing. Additionally, harvest temperatures (air, water, and tissue) associated with illnesses were missing records, preventing those data from being included in the analysis. Perhaps there needs to be a penalty for oyster companies not recording or misplacing temperature records, which may be in the form of a warning for the first offence and then if continued, a fine. This would not only give an incentive for companies to record and practice better record keeping of their temperatures but would also yield more data for researchers to conduct analyses.

A wide range of tidal elevations are needed for companies to grow and harvest oysters, yet this data is not included consistently in the illness investigation follow-up reports. Therefore, I suggest oyster companies disclose during illness investigations which type of growing method and harvest practice were used to produce the oysters associated with illnesses. Harvest tidal elevations when oysters are first harvested, even if they are put back in the water for a tidal cycle, should also be recorded because this information helps determine if extreme tides are more associated with illnesses.

I realize these recommendations are asking more from harvesters; however, this is the kind of data that provides insight into what environmental conditions were like from harvests that contributed to illnesses. If this data were collected, perhaps observations could be made to further understand what is contributing to higher levels of Vp in oysters to ultimately help mitigate illnesses.

Summary of future studies

This thesis has made several suggestions for future studies. First, a suggestion was made to analyze production data from 2019 to determine if production was higher in the Vp control months compared to the remainder of the year, as this was the case in 2018. Illnesses originating from oysters harvested in coastal growing areas should undergo further studies to identify if the fluctuation pattern in illnesses continues each year. The spread of Vp has increased in Washington; however, since Vp originates in the water, perhaps research needs to be done to study water circulation patterns to better understand Vp growth and distribution.

Future studies regarding air and water temperatures should aim to place devices in all growing areas or at least the high-risk growing areas to study conditions leading up to harvests implicated with Vp illnesses. This research is important because it could characterize what conditions are prominent leading up to harvests associated with illnesses, which could point to a change in current policy. Harvest tidal elevations should also be further studied based on the type of harvest method used, as studying all tidal elevations simultaneously doesn't provide an appropriate representation due to high variation in tidal elevations required for harvests.

Along with future studies focused on quantitative data, a survey among all oyster companies in the state should be conducted to provide a more well-rounded consensus of how the industry views the current Vp Control Plan. This would also provide a simple platform for industry members to express their policy improvement suggestions. It was from the survey in this thesis that the idea of adopting the Canadian Standard was conveyed; however, future studies should aim to analyze a larger dataset to determine how effective this policy would be in preventing illnesses in Washington.

Conclusion

Despite not being able to analyze all growing areas with illnesses because of unavailable data, the results found from this thesis provided both key insights and an evaluation of current data practices. Several environmental parameters were studied to determine their relationship with Vp illnesses, but more research is needed to better quantify these relationships. Additionally, more consistent record keeping is needed to allow for statistical analyses, which could ultimately help make improvements to the current Vp Control Plan. Nevertheless, the information gathered from this thesis provides a foundation against which future WDOH data can be compared and opened numerous doors for future data analyses.

CHAPTER VII: CONCLUSION

This thesis consisted of significant data management because studying how Vp interacts with oysters requires looking at environmental parameters from every corner (i.e. temperature, salinity, tidal elevations, etc.) and proved that current data practices need improvement to draw further conclusions. To coincide with this, future studies are needed to better understand specific conditions that favor Vp growth, such as analyzing water and air temperatures leading up to harvests associated with illnesses to determine if harvests should be delayed after a warming trend.

Recall one of my original research questions: “What effect do tidal elevations and temperatures have on the number of Vp illness cases in Washington State?” After analyzing low tidal elevations from the harvest dates associated with illnesses, I was unable to draw any conclusions due to high variation in the data; however, four out of the five years had average tidal elevations below zero. Going forward, I would recommend that harvesters either disclose the tidal elevation at time of harvest or disclose which harvest practice was used because different harvest practices require different tidal elevations (i.e. a higher tide is required for dredging compared to bottom-culture). I found temperatures had a direct association with higher illnesses, with most illnesses occurring in either July or August. Sample data collected by WDOH also proved that oysters can become warmer than their environment, which could consequently exacerbate Vp density.

My other research question was: “Do conclusions from data analysis point to a change in or maintenance of current policy?” Based on findings from this thesis, I recommend that the current Vp Control Plan undergo revisions to include additional requirements from harvesters so that we can improve our understanding of the relationship between oysters and Vp. The following policy recommendations are derived from my thesis research: 1. Require harvesters to record internal oyster tissue temperature at time of harvest and water temperature at the depth the oysters were harvested from, 2. Implement a penalty procedure for harvesters that can't provide appropriate temperature data during illness investigations, and 3. Require harvesters to disclose the type of growing method and harvest practice used during illness investigations. I understand that this requires extra tasks for oyster harvesters and unfortunately will end up wasting valuable oysters to take tissue temperatures, but we need the data to help further understand the association between Vp and oysters to mitigate illnesses.

Policy recommendations from this thesis can help set up the oyster industry with a solid monitoring plan that could prepare the industry for changes in future years. As discussed in the Introduction, the global pandemic COVID-19, has had significant impacts on the oyster industry, with numerous operations currently halted or reduced in size as I write this thesis. Three months later at the time this chapter is being written, COVID-19 has had further implications on the industry. For example, in February 2020, Taylor Shellfish Farms had laid off 40 of its 700 workers (about 5-percent), whereas of May 2020 they have had to lay off approximately 525 workers (75-percent), in addition to selling a parcel of timberland to help stay afloat (Newman & Wernau, 2020). This global pandemic has caused catastrophic economic impacts to the industry, which will consequently be felt at the state level too.

Possible threats to the industry similar to COVID-19, a different virus, or a Vp outbreak that forces a closure of all commercial oyster harvests could occur in the future.

These unchartered scenarios need appropriate protocols in place to help prevent or reduce severe economic losses, while keeping public health as the top priority. Hopefully, these situations will never happen, but if they do, lessons learned from COVID-19 along with research from future studies of Vp and environmental conditions can help the industry better prepare.

> As I ate the oysters with their strong taste of the sea and their faint metallic taste that the cold white wine washed away, leaving only the sea taste and the succulent texture, and as I drank their cold liquid from each shell and washed it down with the crisp taste of the wine, I lost the empty feeling and began to be happy and to make plans. ~ Ernest Hemingway, A Moveable Feast

BIBLIOGRAPHY

Aagesen, A. M., & Häse, C. C. (2014). Seasonal effects of heat shock on bacterial populations, including artificial Vibrio parahaemolyticus exposure, in the Pacific oyster, Crassostrea gigas. *Food Microbiology*, *38*, 93–103. https://doi.org/10.1016/j.fm.2013.08.008

Bacteriological guidelines for fish and fish products (end product). (2019). Retrieved May 14, 2020, from Canadian Food Inspection Agency website: https://www.inspection.gc.ca/food-safety-for-industry/food-safety-standards-guidelines/bacteriological-guidelines/eng/1558757049068/1558757132060

Barton, A., Waldbusser, G. G., Feely, R. A., Weisberg, S. B., Newton, J. A., Hales, B., … McLaughlin, K. (2015). Impacts of coastal acidification on the Pacific Northwest shellfish industry and adaptation strategies implemented in response. *Oceanography*, *28*(2), 146–159. https://doi.org/10.5670/oceanog.2015.38

Bernton, H. (2019a). Judge tosses federal permit for Washington shellfish industry, saying it doesn’t do enough to protect environment. Retrieved March 8, 2020, from The Seattle Times website: https://www.seattletimes.com/seattle-news/environment/judge-throws-out-federal-permit-for-washington-shellfish-industry-cites-lack-of-full-environmental-review/

Bernton, H. (2019b). Oyster growers agree to abandon quest to use controversial insecticide in Southwest Washington tidelands. Retrieved March 14, 2020, from The Seattle Times website: https://www.seattletimes.com/seattle-news/environment/in-settlement-with-state-oyster-growers-agree-to-abandon-quest-to-use-controversial-insecticide-in-southwest-washington-tidelands/

Bernton, H. (2020). In rural Washington, coronavirus outbreak takes a big bite out of pulp and shellfish exports to China. Retrieved March 8, 2020, from The Seattle Times website: https://www.seattletimes.com/seattle-news/in-rural-washington-coronavirus-outbreak-takes-a-big-bite-out-of-pulp-and-shellfish-exports-to-china/

Burge, C. A., Eakin, C. M., Friedman, C. S., Froelich, B., Hershberger, P. K., Hofmann, E. E., … Harvell, C. D. (2014). Climate Change Influences on Marine Infectious Diseases: Implications for Management and Society. *Annual Review of Marine Science*, *6*, 249–277. https://doi.org/10.1146/annurev-marine-010213-135029

Caburlotto, G., Haley, B. J., Lleò, M. M., Huq, A., & Colwell, R. R. (2010). Serodiversity and ecological distribution of Vibrio parahaemolyticus in the Venetian Lagoon, Northeast Italy. *Environmental Microbiology Reports*, *2*(1), 151–157. https://doi.org/10.1111/j.1758-2229.2009.00123.x

Camden, J. (2017). Shell games: Washington a leader in oyster farming | The Spokesman-Review. Retrieved November 9, 2019, from The Spokesman-Review website: https://www.spokesman.com/stories/2017/dec/17/shell-games-washington-a-leader-in-oyster-farming/

Center for Disease Control. (2018). Signs & Symptoms. Retrieved October 18, 2019, from CDC website: https://www.cdc.gov/vibrio/investigations/vibriop-07-18/signs-symptoms.html

Centers for Disease Control and Prevention. (n.d.). *Summary of human Vibrio isolates reported to CDC, 2006*. Atlanta.

Centers for Disease Control and Prevention. (2016). *National Enteric Disease Surveillance: COVIS Annual Summary, 2014*. Atlanta.

Centers for Disease Control and Prevention. (2019). Culture-independent Diagnostic Tests. Retrieved March 17, 2020, from CDC website: https://www.cdc.gov/foodsafety/challenges/cidt.html

Centre for Environment Fisheries & Aquaculture Science. (2019). *Cefas Protocol for Inspection and Approval of Purification (Depuration) Systems - England and Wales*. Retrieved from www.cefas.co.uk

Commercial Shellfish Map Viewer. (2020). Retrieved March 14, 2020, from Washington State Department of Health website: https://fortress.wa.gov/doh/oswpviewer/index.html

Daniels, N. A., Mackinnon, L., Bishop, R., Altekruse, S., Ray, B., Hammond, R. M., … Slutsker, L. (2000). Vibrio parahaemolyticus Infections in the United States. *Journal of Infectious Disease*, *181*, 1661–1666.

DePaola, A. (2019). Managing Vibrio Risk in Oysters. *Food Protection Trends*, *39*(4), 338–347. Retrieved from http://www.foodprotection.org/publications/food-protection-trends/archive/2019-07-managing-vibrio-risk-in-oysters/

Depaola, A., Hopkins, L. H., Peeler, J. T., Wentz, B., & Mcphearson', R. M. (1990). Incidence of Vibrio parahaemolyticus in U.S. Coastal Waters and Oysters. *Applied and Environmental Microbiology*, *56*(8), 2299–2302.

Depaola, A., Jones, J. L., Noe, K. E., Byars, R. H., & Bowers, J. C. (2009). Survey of

Postharvest-Processed Oysters in the United States for Levels of Vibrio vulnificus and Vibrio parahaemolyticus. *Journal of Food Protection*, *72*(10), 2110–2113.

Depaola, A., Kaysner, C. A., Bowers, J., & Cook, D. W. (2000). Environmental Investigations of Vibrio parahaemolyticus in Oysters after Outbreaks in Washington, Texas, and New York (1997 and 1998). *Applied and Environmental Microbiology*, *66*(11), 4649–4654. Retrieved from https://aem.asm.org/content/aem/66/11/4649.full.pdf

DiStefano, P. W., & Jones, B. (2015). *Department of Health and Human Services*.

Drake, S. L., Depaola, A., & Jaykus, L.-A. (2007). An Overview of Vibrio vulnificus and Vibrio parahaemolyticus. *Comprehensive Reviews in Food Science and Food Safety*, *6*, 120–144.

Dumbauld, B. R., Kauffman, B. E., Trimble, A. C., & Ruesink, J. L. (2011). The Willapa Bay Oyster Reserves in Washington State: Fishery Collapse, Creating a Sustainable Replacement, and the Potential for Habitat Conservation and Restoration. *Journal of Shellfish Research*, *30*(1), 71–83. https://doi.org/10.2983/035.030.0111

Fishing license types and fees. (2019). Retrieved November 13, 2019, from Washington Department of Fish & Wildlife website: https://wdfw.wa.gov/licenses/fishing/types-fees

Food and Agriculture Organization of the United Nations/World Health Organization. (2011). *Risk assessment of Vibrio parahaemolyticus in seafood: Interpretative summary and Technical report*. Retrieved from http://www.fao.org/3/a-i2225e.pdf

Froelich, B. A., Ayrapetyan, M., Fowler, P., Oliver, J. D., & Noble, R. T. (2015). Development of a Matrix Tool for the Prediction of Vibrio Species in Oysters Harvested from North Carolina. *Applied and Environmental Microbiology*, *81*(3), 1111–1119. https://doi.org/10.1128/AEM.03206-14

Froelich, Brett A, & Noble, R. T. (2016). Vibrio bacteria in raw oysters: managing risks to human health. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *371*, 1–6. https://doi.org/10.1098/rstb.2015.0209

From the Tides of Puget Sound to Your Plate: Northwest Shellfish Industry Provides Important Ecological & Economic Value. (2012). Retrieved from https://archive.fisheries.noaa.gov/wcr/publications/aquaculture/noaa_shellfish_initiative_f_sheet_011312.pdf

Gooch, J. A., Depaola, A., Bowers, J., & Marshall, A. D. L. (2002a). Growth and Survival of Vibrio parahaemolyticus in Postharvest American Oysters. *Journal of Food Protection*, *65*(6), 970–974. Retrieved from http://www.nwsmobile.

Gooch, J. A., Depaola, A., Bowers, J., & Marshall, A. D. L. (2002b). Growth and Survival of Vibrio parahaemolyticus in Postharvest American Oysters. In *Journal of Food Protection* (Vol. 65). Retrieved from http://www.nwsmobile.

Hackney, C. R., & Pierson, M. D. (1994). *Environmental Indicators and Shellfish Safety*. Dordrecht.

Harris, J. (2008). *Aquatic Invasive Species Profile Pacific oyster, Crassostrea gigas (Thunberg, 1793)*.

Helm, M. M. (2005). Cultured Aquatic Species Information Programme. Crassostrea gigas. Retrieved April 2, 2020, from FAO Fisheries and Aquaculture Department website: http://www.fao.org/fishery/culturedspecies/Crassostrea_gigas/en

Herzog, K. (2020). Is It Time to Start Panicking About Coronavirus? Retrieved March 8, 2020, from The Stranger website: https://www.thestranger.com/slog/2020/02/24/42957220/is-it-time-to-start-panicking-about-coronavirus

Horowitz, J., & Hoberecht, L. (2016). *Washington: A Shellfish State*. Retrieved from https://archive.fisheries.noaa.gov/wcr/publications/aquaculture/1.13.2016_wsi_factsheet.pdf

Interstate Shellfish Sanitation Conference. (n.d.). Conference Administration. Retrieved November 21, 2019, from ISSC website: http://www.issc.org/conference-administration

Iwamoto, M., Ayers, T., Mahon, B. E., & Swerdlow, D. L. (2010). Epidemiology of seafood-associated infections in the United States. *Clinical Microbiology Reviews*, *23*(2), 399–411. https://doi.org/10.1128/CMR.00059-09

Johnson, C. L. (2015). Influence of Environmental Factors on Vibrio spp. in Coastal Ecosystems. *Microbiology Spectrum*, *3*(3), 1–18.

Johnson, C. N., Bowers, J. C., Griffitt, K. J., Molina, V., Clostio, R. W., Pei, S., … Colwell, R. R. (2012). Ecology of Vibrio parahaemolyticus and Vibrio vulnificus in the Coastal and Estuarine Waters of Louisiana, Maryland, Mississippi, and Washington (United States). *Applied and Environmental Microbiology*, *78*(20), 7249–7257. https://doi.org/10.1128/AEM.01296-12

Jones, J. L., Kinsey, T. P., Johnson, L. W., Porso, R., Friedman, B., Curtis, M., … Bowers, J. C. (2016). Effects of intertidal harvest practices on levels of Vibrio Parahaemolyticus and Vibrio Vulnificus bacteria in oysters. *Applied and Environmental Microbiology*, *82*(15), 4517–4522. https://doi.org/10.1128/AEM.00721-16

King, A. (2020). Oysters, Cherries, Other Northwest Products Back Up As Coronavirus Quarantines Trade. Retrieved March 8, 2020, from Oregon Public Broadcasting website: https://www.opb.org/news/article/coronavirus-pacific-northwest-us-china-trade-exports-oregon-washington/

Klein, S. L., Gutierrez West, C. K., Mejia, D. M., & Lovell, C. R. (2014). Genes Similar to the Vibrio parahaemolyticus Virulence-Related Genes tdh, tlh, and vscC2 Occur in Other Vibrionaceae Species Isolated from a Pristine Estuary. *Applied and Environmental Microbiology*, *80*(2), 595–602. https://doi.org/10.1128/AEM.02895-13

Leson, N., & Spencer, A. (2009). Shigoku oysters. Hot stuff. Can’t get enough. Retrieved November 18, 2019, from The Seattle Times website: https://www.seattletimes.com/life/food-drink/shigoku-oysters-hot-stuff-cant-get-enough/

Levy, S. (2015). Warming Trend: how climate shapes Vibrio ecology. *Environmental Health Prospective*, *123*(4), A82–A89. https://doi.org/10.1289/ehp.123-A82

Love, D. C., Kuehl, L. M., Lane, R. M., Fry, J. P., Harding, J., Davis, B. J. K., …

Hudson, B. (2020). Performance of cold chains and modeled growth of Vibrio parahaemolyticus for farmed oysters distributed in the United States and internationally. *International Journal of Food Microbiology*, *313*(July 2019), 1–8. https://doi.org/10.1016/j.ijfoodmicro.2019.108378

MacCready, P. (2017). Puget Sound's Physical Environment. Retrieved May 16, 2020, from Encyclopedia of Puget Sound website: https://www.eopugetsound.org/articles/puget-sounds-physical-environment

Martinez-Urtaza, J., Blanco-Abad, V., Rodriguez-Castro, A., Ansede-Bermejo, J., Miranda, A., & Xose Rodriguez-Alvarez, M. (2012). Ecological determinants of the occurrence and dynamics of Vibrio parahaemolyticus in offshore areas. *The ISME Journal*, *6*, 994–1006. https://doi.org/10.1038/ismej.2011.156

Martinez-Urtaza, J., Bowers, J. C., Trinanes, J., & DePaola, A. (2010). Climate anomalies and the increasing risk of Vibrio parahaemolyticus and Vibrio vulnificus illnesses. *Food Research International*, *43*(7), 1780–1790. https://doi.org/10.1016/j.foodres.2010.04.001

McLaughlin, J. B., DePaola, A., Bopp, C. A., Martinek, K. A., Napolilli, N. P., Allison, C. G., … Middaugh, J. P. (2005). Outbreak of *Vibrio parahaemolyticus* Gastroenteritis Associated with Alaskan Oysters. *New England Journal of Medicine*, *353*(14), 1463–1470. https://doi.org/10.1056/NEJMoa051594

McLaughlin, J., & Martinek, K. (2004). *Outbreak of Vibrio parahaemolyticus Gastroenteritis Associated with Consumption of Alaskan Oysters-Part 2*. Retrieved from http://www.epi.alaska.gov/bulletins/docs/b2004_24.pdf

Mercaldo-Allen, R., Goldberg, R., Bryson, J. E., Lubchenco, J., Schwaab, E. C., & Administrator for Fisheries, A. (2011). *Review of the Ecological Effects of Dredging in the Cultivation and Harvest of Molluscan Shellfish*. Retrieved from http://www.nefsc.noaa.gov/nefsc/publications/.

Miles, D. W., Ross, T., Olley, J., & Mcmeekin, T. A. (1997). Development and evaluation of a predictive model for the effect of temperature and water activity on the growth rate of Vibrio parahaemolyticus. In *International Journal of Food Microbiology* (Vol. 38).

Molenda, J. R., Johnson, W. G., Fishbein, M., Wentz, B., Mehlman, I. J., & Dadisman, T. A. (1972). Vibrio parahaemolyticus Gastroenteritis in Maryland: Laboratory Aspects. *Applied Microbiology*, *24*(3), 444–448.

Monthly Employment Report. (2020). Retrieved May 29, 2020, from Washington State Employment Security Department website: https://esd.wa.gov/labormarketinfo/monthly-employment-report

National Oceanic and Atmospheric Administration. (2019). Aquaculture. Retrieved February 15, 2020, from Office for Coastal Management website: https://coast.noaa.gov/states/fast-facts/aquaculture.html

National Shellfish Sanitation Program. (2017). *Guide for the Control of Molluscan Shellfish 2017 Revision*. Retrieved from http://www.fda.gov/Food/GuidanceRegulation/FederalStateFoodPrograms/ucm2006754.htm

Newman, J., & Wernau, J. (2020). Coronavirus sees Americans cooking more seafood, but fishermen are struggling. Retrieved May 25, 2020, from Fox Business website: https://www.foxbusiness.com/economy/americans-are-cooking-more-seafood-but-fishermen-are-struggling

Newton, A., Kendall, M., Vugia, D. J., Henao, O. L., & Mahon, B. E. (2012). Increasing Rates of Vibriosis in the United States, 1996-2010: Review of surveillance data from 2 systems. *Clinical Infectious Diseases*, *54*(5), S391–S395. https://doi.org/10.1093/cid/cis243

Nilsson, W. B., Paranjpye, R. N., Hamel, O. S., Hard, C., & Strom, M. S. (2019). Vibrio parahaemolyticus risk assessment in the Pacific Northwest: it's not what's in the water. *FEMS Microbiology Ecology*, *95*, 1–8. https://doi.org/10.1093/femsec/fiz027

Nordstrom, J. L., Kaysner, C. A., Blackstone, G. M., Vickery, M. C. L., Bowers, J. C., & Depaola, A. A. (2004). Effect of Intertidal Exposure on Vibrio parahaemolyticus Levels in Pacific Northwest Oysters. *Journal of Food Protection*, *67*(10), 2178–2182. Retrieved from http://meridian.allenpress.com/jfp/article-pdf/67/10/2178/1676136/0362-028x-67_10_2178.pdf

Oyster Bag Culture in Totten Inlet. (n.d.). Retrieved April 2, 2020, from Protect Zangle Cove website: http://protectzanglecove.org/oyster-bag-culture.html

Paranjpye, R., Hamel, O. S., Stojanovski, A., & Liermann, M. (2012). Genetic Diversity of Clinical and Environmental Vibrio parahaemolyticus Strains from the Pacific Northwest. *Applied and Environmental Microbiology*, *78*(24), 8631–8638. https://doi.org/10.1128/AEM.01531-12

Phuvasate, S., & Su, Y. C. (2013). Impact of water salinity and types of oysters on depuration for reducing Vibrio parahaemolyticus in Pacific oysters (Crassostrea gigas). *Food Control*, *32*, 569–573. https://doi.org/10.1016/j.foodcont.2013.01.025

Protect Willapa Bay. (2017). Retrieved November 13, 2019, from https://protectwillapabay.org/

RCW 79.135.110. (n.d.). Retrieved April 3, 2020, from Washington State Legislature website: https://app.leg.wa.gov/rcw/default.aspx?cite=79.135.110

Ruesink, J. L., Feist, B. E., Harvey, C. J., Hong, J. S., Trimble, A. C., & Wisehart, L. M. (2006). Changes in productivity associated with four introduced species: Ecosystem transformation of a “pristine” estuary. *Marine Ecology Progress Series*, *311*(June 2014), 203–215. https://doi.org/10.3354/meps311203

Sakazaki, R., Iwanami, S., & Fukumi, H. (1963). Studies on the Enteropathogenic, Facultatively Halophilic Bacteria, Vibrio parahaemolyticus. *Japanese Journal of Medical Science and Biology*, *16*(4), 161–188. https://doi.org/10.7883/yoken1952.16.161

Scanes, E., Parker, L. M., O’connor, W. A., Stapp, L. S., & Ross, P. M. (2017). Intertidal oysters reach their physiological limit in a future high-CO 2 world. *Journal of Experimental Biologists*, *220*, 765–774. https://doi.org/10.1242/jeb.151365

Shellfish Growing Area Risk Categories. (2020). Retrieved April 6, 2020, from Washington State Department of Health website: https://www.doh.wa.gov/CommunityandEnvironment/Shellfish/CommercialShellfish/VibrioControlPlan/GrowingAreaRiskCategory

Shellfish Treaty Rights FAQ. (2016). Retrieved November 9, 2019, from Northwest Indian Fisheries Commission website: https://nwifc.org/about-us/shellfish/treaty-rights-faq/

Shen, X., Su, Y. C., Liu, C., Oscar, T., & DePaola, A. (2019). Efficacy of Vibrio parahaemolyticus depuration in oysters (Crassostrea gigas). *Food Microbiology*, *79*, 35–40. https://doi.org/10.1016/j.fm.2018.10.005

Slayton, R. B., Newton, A. E., Depaola, A., Jones, J. L., & Mahon, B. E. (2014). Clam-associated vibriosis, USA, 1988-2010. *Epidemiology and Infection*, *142*, 1083–1088. https://doi.org/10.1017/S0950268813001830

Steele, E. N. (n.d.). *The Immigrant Oyster*.

Su, Y. C., & Liu, C. (2007). Vibrio parahaemolyticus: A concern of seafood safety. *Food Microbiology*, *24*(6), 549–558. https://doi.org/10.1016/j.fm.2007.01.005

Taylor Shellfish Farms. (2019). Our Shellfish. Retrieved November 18, 2019, from https://www.taylorshellfishfarms.com/about-us/our-shellfish#

Toba, D. (2002). *Small-scale oyster farming for pleasure and profit in Washington*.

Trtanj, J., Jantarasami, L., Brunkard, J., Collier, T., Jacobs, J., Lipp, E., … Thurston, J. (2016). Climate Impacts on Water-Related Illness. In *The Impacts of Climate Change on Human Health in the United States: A Scientific Assessment* (pp. 157–188). https://doi.org/10.7930/J03F4MH4

U.S. Food and Drug Administration. (2020). Outbreaks of Foodborne Illness. Retrieved March 17, 2020, from FDA website: https://www.fda.gov/food/recalls-outbreaksemergencies/outbreaks-foodborne-illness

United States Geological Survey. (n.d.). Coastal Habitats in Puget Sound. Retrieved November 20, 2019, from USGS website: https://www.usgs.gov/centers/pcmsc/science/coastal-habitats-puget-sound?qt-science_center_objects=0#qt-science_center_objects

Vezzulli, L., Brettar, I., Pezzati, E., Reid, P. C., Colwell, R. R., Hö Fle, M. G., & Pruzzo, C. (2012). Long-term effects of ocean warming on the prokaryotic community: evidence from the vibrios. *The ISME Journal*, *6*, 21–30. https://doi.org/10.1038/ismej.2011.89

WAC 246-282-006. (2015). Retrieved October 7, 2019, from Washington State Legislature website: https://app.leg.wa.gov/wac/default.aspx?cite=246-282-006

Wang, W., Li, M., & Li, Y. (2015). Intervention Strategies for Reducing Vibrio Parahaemolyticus in Seafood: A Review. *Journal of Food Science*, *80*(1), R10–R19. https://doi.org/10.1111/1750-3841.12727

Washington. (2013). Retrieved November 11, 2019, from Pacific Shellfish Institute website: http://www.pacshell.org/washington.asp

Washington Sea Grant, & University of Washington. (2015). *Shellfish Aquaculture in Washington State*. Seattle.

Washington Shellfish Initiative. (2011).

Washington State Department of Ecology. (2014). *Fact sheet for Willapa Grays Harbor Oyster Grower's Association National Pollutant Discharge Elimination System*

(NPDES) Pemit NO. WA0039781.

Washington State Department of Natural Resources. (2019). Aquaculture. Retrieved November 19, 2019, from https://www.dnr.wa.gov/programs-and-services/aquatics/shellfish/aquaculture

Washington State Legislature. (2007). WSR 07-12-051 Emergency Rules. Retrieved November 22, 2019, from Washington State Code Reviser's Office website: http://lawfilesext.leg.wa.gov/law/wsr/2007/12/07-12-051.htm

Washington State Legislature. (2008a). WSR 08-11-051 Permanent Rules. Retrieved November 22, 2019, from Washington State Code Reviser's Office website: http://lawfilesext.leg.wa.gov/law/wsr/2008/11/08-11-051.htm

Washington State Legislature. (2008b). WSR 08-23-015 Preproposal Statement of Inquiry. Retrieved November 23, 2019, from Washington State Code Reviser's Office website: http://lawfilesext.leg.wa.gov/law/wsr/2008/23/08-23-015.htm

Washington State Legislature. (2014). WSR 14-09-003 Permanent Rules. Retrieved November 23, 2019, from Washington State Code Reviser's Office website: http://lawfilesext.leg.wa.gov/law/wsr/2014/09/14-09-003.htm

Washington State Legislature. (2015a). WSR 15-04-042 Proposed Rules. Retrieved November 23, 2019, from Washington State Code Reviser's Office website: http://lawfilesext.leg.wa.gov/law/wsr/2015/04/15-04-042.htm

Washington State Legislature. (2015b). WSR 15-08-083 Permanent Rules. Retrieved November 23, 2019, from Washington State Code Reviser's Office website:

http://lawfilesext.leg.wa.gov/law/wsr/2015/08/15-08-083.htm

Wellman, K., Cuyno, L., Schenken, W., Bond, A., Eaton, G., & Mccoy, T. (2013). *The Economic Impact of Shellfish Aquaculture in Washington, Oregon and California*.

WSDOH. (2017). Vibrio parahaemolyticus Training: Control Plan Requirements. Retrieved October 7, 2019, from https://www.doh.wa.gov/CommunityandEnvironment/Shellfish/CommercialShellfish/VibrioControlPlan

Wu, Y., Wen, J., Ma, Y., Ma, X., & Chen, Y. (2014). Epidemiology of foodborne disease outbreaks caused by Vibrio parahaemolyticus, China, 2003-2008. *Food Control*, *46*, 197–202. https://doi.org/10.1016/j.foodcont.2014.05.023

APPENDICES

Vp Survey

Survey on *Vibrio parahaemolyticus* Control Plan

If you are familiar with growing methods and harvest practices for the company you represent, please answer questions 1 and 2. If not, please skip to question 3.

1. What oyster growing method is primarily used by the company you represent?

2. What is the primary oyster harvest method utilized by the company you represent? Select all that apply. If more than one, please provide an explanation.

   Dredge ☐
   Intertidal ☐
   Subtidal ☐
   Unknown/other ☐ ____________________________

   Explanation if more than one:

3. How would you rate your knowledgeable and understanding of the Vp control plan? Please circle one.

   Very poor   Poor   Fair   Good   Very good

4. How effective do you feel the current Vp control plan is in preventing illnesses? Please circle one.

   Not effective   Somewhat effective   Undecided   Almost always effective   Very effective

5. Other than meeting the basic requirements of the control plan, has your company taken any additional steps or made changes to the operation to further limit Vp growth?

6. Do you have any suggestions to improve the effectiveness of the current Vp control plan?

7. If you are interested in assisting with any follow-up questions I may have, please provide the following information so I may contact you.

   Company name or organization:

   Your name and title:

   Phone number:

Reported oyster production data in dozens for 2018 Pacific oysters

Oyster Production Data for All Growing Areas in Washington State												
Pacific oyster size (inches)	January	February	March	April	May	June	July	August	September	October	November	December
Up to 3"	255523	244551	272758	280806	347847	349696	346820	346598	234363	300388.5	256977.5	262768
3 - 4"	167965.5	190443	159842	194932	231850	193849.5	190060	239355.5	148509.5	152787	149215	164876
More than 4"	199934	194230.5	196069.5	247661.5	277672.5	274239	270549	243054.5	165931.5	165894	134445	156821
	623422.5	629224.5	628669.5	723399.5	857369.5	817784.5	807429	829008	548804	619069.5	540637.5	584465