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Spatial and temporal trends of the annual first detections of Paralytic Shellfish Toxin in Puget Sound, WA

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Spatial and temporal trends of the annual first detections of Paralytic Shellfish Toxin in Puget Sound, WA

By

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of the Requirements for the Degree
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Master's Thesis

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Margaret Lee Taylor

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A Thesis
Presented to
The Faculty of
Western Washington University

In Partial Fulfillment
Of the Requirements for the Degree
Master of Science

Margaret L. Taylor
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Abstract

Since the 1950s, the Washington State Department of Health has routinely monitored the suite of toxins in shellfish associated with Paralytic Shellfish Poisoning. These toxins, known collectively as Paralytic Shellfish Toxins, are produced by species of the marine dinoflagellate in the genus *Alexandrium*. The role of the monitoring program is primarily to protect public health and safety; and therefore, use of these data for long-term statistical analysis has been limited due to opportunistic and irregular sampling of various shellfish species in space and time. However, some studies suggest that initiation of these toxic events have recently shifted to earlier in the year (Hanein and Borchert, 2015). To test this hypothesis, I extracted a subset of these data to analyze for trends in timing of bloom initiation and location of Paralytic Shellfish Toxins after the first annual appearance. I did not find that bloom initiation to be occurring earlier in the year in any subbasins or regulatory closure zones which may indicate that a shift is either absent or undetectable. This contrasts with the finding of others that Paralytic Shellfish Toxins are being detected earlier in the year and indicate that endogenous conditions are a stronger driving factor in bloom initiation than larger climatic shifts. Additionally, although PST has been observed to be more widespread in recent years, I observed no clear progression of shellfish toxicity from one basin to another within any particular year. There was also no clear spatial relationship between the locations of cyst beds (i.e., areas with high concentrations of dormant cysts in sediments) and locations where PST is first detected in shellfish.

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Chapter 1. Introduction to Harmful Algal Blooms in Puget Sound.

The dangers of eating shellfish contaminated with Paralytic Shellfish Toxins (PST) has been recognized by native peoples of the Salish Sea since long before written records (Horner, et al. 1997). So entrenched was this knowledge that First Nations were known to consume tree bark or their own dogs rather than risk eating shellfish from areas known to harbor toxins. Legends say a group of Russian settlers were killed when coastal Alaskan tribes invited them to a feast of toxic shellfish (Dale and Yentsch, 1978). In 1793, a seaman under the command of Captain George Vancouver died from eating contaminated mussels off the coast of British Columbia (Vancouver, 1793). Since that time, the potentially lethal effects of PST have been well documented. PST is now known to be produced by a suite of organisms throughout the world; but in Puget Sound, PST is most commonly produced by the dinoflagellate *Alexandrium catenella.* In an effort to prevent Paralytic Shellfish Poisoning (PSP), the disease that results from consuming shellfish contaminated with PST, the Washington State Department of Health (WDOH) monitors shellfish for PST on both commercial and public beaches. Since monitoring began in the 1950s, very few fatalities have been recorded, demonstrating the health and economic success of monitoring programs; however, the economic cost of monitoring and management of PST is also considerable. Economic impacts include: (1) reduced shellfish production and export; (2) costs associated with illness caused by harmful algal blooms (HABs); and (3) reduced consumption of seafood due to perceived threats of HABs (Lewitus et al., 2012). Therefore, there is great potential value in finding ways to more efficiently monitor for PST and better protect public health.

Monitoring conducted by WDOH and other agencies is primarily for the protection of public health. Shellfish samples from a variety of shellfish species are sometimes collected at different sites and at somewhat irregular time intervals, resulting in a dataset that makes it difficult to develop predictive models. This creates challenges to researchers attempting to use these data to link HAB dynamics to climatic patterns and other environmental data. It has been suggested that PST has been increasing in magnitude, geographic scope, and duration in recent years likely as a result of changes in temperature, eutrophication, stratification, and climate-related shifts (Feifel, et al. 2012; Moore, et al. 2009; Trainer, et al. 2003). Additionally, several local health jurisdictions have reported PST closures earlier in the year (Hanein and Borchert,

2015). However, there has been limited research on whether the timing of when PST first appears in Puget Sound shellfish is occurring earlier and whether any clear spatial patterns are developing after toxins are first detected in shellfish. Thus, my primary goal was to use an existing dataset to determine whether *A. catenella* bloom initiation, as indicated by shellfish toxin detection, is occurring earlier in the year, as suggested by Moore et al. (2009) among others.

1.1.Puget Sound Hydrography

It has been suggested that the movement of dinoflagellates is limited by shallow sills within complex basins like Puget Sound (Anderson, 1997). Therefore, the data used for this research were divided into six subbasins delimited by sills in Puget Sound (Trainer, et al., 2003; Moore, et al., 2009; Figure 1). The North basin is separated by two sills at Rosario Strait and a partial barrier at the southern end of the San Juan Islands. It extends to the Canadian border and includes Bellingham Bay, the Strait of Georgia, the San Juan Islands, and Samish Bay. The Northwest basin has one of the longer histories of PSTs in the Pacific Northwest and has been the focus of multiple studies of *A. catenella* (Cox et al., 2008; Feifel et al., 2012; Fernandez et al., 2008). The Strait of Juan de Fuca borders this basin's two semi-enclosed bays, Sequim and Discovery Bays. Two sills at Admiralty Inlet bound the Central, Whidbey, and South basins from the Northern basins and the Strait of Juan de Fuca. The Whidbey basin has significant freshwater influence from the Skagit River and is home to the oldest and largest commercial mussel farm in the United States (Washington Sea Grant, 2015). The Central basin includes the population hub of Seattle and King County with lower freshwater inputs than the Whidbey basin. The sill at the Tacoma Narrows separates the Central Basin from the narrow embayments of the Southern Basin which experiences high tidal influence and complex circulation patterns due to its numerous inlets (Albertson et al., 2007; Ebbesmeyer et al., 1998). Finally, Hood Canal is separated by a sill at its northern end that limits the influx of deep ocean waters from the Strait of Georgia and into Puget Sound. This limited transport of water and minimal freshwater input means Hood Canal is the most poorly flushed of all six basins (Babson et al., 2006; Strickland, 1983). However, substantial water is exchanged between all basins in Puget Sound, and any differences are small

compared to the hydrological similarities (Babson et al., 2006; Strickland, 1983). In general, the magnitude of marine water exchange, freshwater inputs, and inlet topography may have the potential to create unique environments that influence the timing and rate of phytoplankton growth. However, note that aside from the sill at Admiralty Inlet separating Puget Sound from the waters to the north, and the sill separating the waters of Hood Canal, none of the sills described above truly perform a hydraulic function of slowing exchange, vertically mixing water, or significantly altering circulation patterns (e.g. Sutherland et al., 2011).

1.2.Washington State Department of Health (WDOH) Shellfish Toxin Sampling Program

The Biotoxin Monitoring Program under the WDOH collects and analyzes shellfish samples throughout the Puget Sound for biotoxins that impair the health of both humans and marine mammals. Without this monitoring, consumers risk eating shellfish containing potentially lethal toxins. If concentrations of biotoxins are detected in shellfish above the regulatory limits for human consumption, temporary harvest moratoriums may be enacted, thereby helping to ensure safe harvest at both commercial and recreational beaches.

Initially, sampling only occurred seasonally since summertime closures were assumed to be sufficient for public health protection. This sporadic sampling continued until the early 1970s when detections of dangerous levels of PST in Bellingham Bay were measured. A study in the late 1980s prompted a shift to a preemptive, regular sampling regime throughout Puget Sound that continues to this day (Nishitani & Chew, 1988). Figure 2 is a map of WDOH sampling sites.

1.3. *A. catenella* Cyst Bed Mapping

Like most dinoflagellates, *A. catenella* has a life cycle consisting of both a motile state and a dormant resting state. During the warmer summer months, cells emerge from the sediment to grow and proliferate in the water column all while producing toxins. If not consumed by filter feeders, cells undergo sexual or asexual reproduction and return to the sediment once again, forming a resting cyst that will remain dormant until the cyst matures and optimal conditions for vegetative growth return, usually in mid- to late-spring (Anderson, 1998; Moore et al., 2015a;

Tobin and Horner, 2011; Figure 3). The dormant resting state allows cells to survive growth limiting conditions such as extreme temperatures. By limiting mortality due to adverse conditions, a broader range of habitats can be exploited than if the cells were strictly planktonic. Oxygen and light can have a profound effect on the emergence of cysts, thus cysts buried deep in the sediment can remain dormant for years (Anderson et al., 1997; Shull et al., 2014). Areas with high concentrations of cysts also function as "seed beds" that can act as source populations for *Alexandrium* blooms in surrounding areas (Anderson, 1998).

The identification of locations where there is a consistent connection between cysts and shellfish toxicity may serve to help shellfish growers and regulatory agencies to anticipate regions of potential PST events. When cysts are non-motile and embedded in the sediment, researchers can more easily measure the population of *Alexandrium* compared to sampling active cells dispersed throughout the water column. In 2005 and 2006 and again from 2011 through 2013, researchers at the National Oceanic and Atmospheric Administration (NOAA) and University of Washington Tacoma collected sediment to map the concentrations of dormant cysts of *A. catenella* throughout Puget Sound (Horner et al., 2011; Greengrove et al., 2015). From 2014 through 2017, this work has focused on Hood Canal due to an increasing presence of PST in that subbasin. Recent analyses of these data found no significant relationship between cyst abundance and the severity of the following year's PST events but did find a positive correlation between PST events and the following winter's cyst concentrations (Greengrove et al., 2015). Numerous other studies have attempted to determine the relationship between cysts and cells in the water column, and between cysts and shellfish toxicity, with variable results depending on location (for example: Cox, et al. 2008; Nishitani and Chew 1984).

1.4. Research Questions

Numerous studies have used the extensive sampling data collected by WDOH to answer questions regarding bloom intensity and timing (e.g. Moore et al., 2009; Trainer et al., 2003), but limited work has been done to determine whether *A. catenella* blooms are occurring earlier in the year and whether there is consistency in where they initiate. Preliminary analyses had been conducted by Hanein and Borchert in 2015 but neither the results nor methods were formally documented. Additional work (Moore et al., 2009) found that in four selected "hot spots" both shellfish bed closures and the maximum PST concentration appear to be occurring earlier in the

year, but this does not necessarily correlate to earlier bloom initiation, as measured by shellfish toxicity, nor is it clear whether this is a widespread phenomenon. Thus, the question of bloom timing remains, and an increased understanding could impact the future of monitoring programs and shellfish harvest in the Salish Sea.

In this study I used an extensive set of pre-existing data sets provided by the Washington Department of Health and extracted a subset of these data to analyze for trends in timing of bloom initiation and location of Paralytic Shellfish Toxins.

This thesis will address the following questions:

1) Does the first annual appearance of PST in shellfish occur at similar *times* every year? Or has there been a shift to earlier occurrence of the first annual appearance of PST in shellfish within the span of the available data set?
2) Does the first annual appearance of PST in shellfish occur at similar *locations* every year? Does PST subsequently appear in locations near to the location of first annual appearance?
3) Does the first annual appearance of PST in shellfish occur at, or near to, locations known to have high concentrations of *A. catenella* cysts in sediments?
4) Is there a relationship between environmental conditions (e.g. temperature and salinity) and any of the above observations?

Chapter 2. Methods

Two data sources were used in this study: (1) shellfish toxicity data from the WDOH, and (2) *A. catenella* cyst abundance data from the University of Washington Tacoma and NOAA. WDOH has been testing for toxins in shellfish for many decades; however, the sampling protocol has changed over time. A seminal paper in the 1980s (Nishitani, 1984) prompted a shift in sampling regime beginning in 1990 to use mussels as the shellfish species for monitoring PST as well as to establish "sentinel sites" that would be monitored throughout the year. Multiple species of shellfish are still collected as a part of the WDOH biotoxin monitoring program, but *Mytilus edulis* (blue mussels) are the most frequently and consistently sampled. *M. edulis* have also been shown to exceed regulatory limits within 1 hour after exposure to PST in a laboratory setting and to rapidly detoxify in just a few weeks (Bricelj et al., 1990; Bricelj and Shumway, 1998). Other sampled shellfish species have different rates of accumulation and retention and were thus omitted from this study in order to reduce variability and most accurately identify the first shellfish closure related to PST. This provides both a consistent and first indication of the first detection of PST. The result of these changes in sampling strategy was greater sampling frequency and more consistency in the shellfish species sampled which allows more robust data analysis. Thus, although monitoring began in the 1950s, I selected a subset of data collected from *M. edulis* samples from 1990 through 2017 to reduce variability associated with increased sampling frequency over time and different rates of PST uptake and depuration by different shellfish species.

The University of Washington Tacoma and NOAA have mapped the distribution and abundance of *A. catenella* cysts throughout Puget Sound in 2005 (Horner et al., 2011) and from 2011 through 2013 (Greengrove et al., 2015). Sediment samples were collected using a Craib corer and subsamples were subsequently diluted, sieved, and enumerated using the standard microscopy method of Yamaguchi and others (1995). The locations of WDOH sentinel sites are typically nearshore where staff can easily access shellfish for testing, whereas the sites for cyst mapping were offshore and are accessed by boat. Further, cyst mapping was conducted only once a year during the winter, whereas shellfish are monitored much more frequently (every 2 weeks). Therefore, there is little overlap in space and time for these two datasets (the University of Washington Tacoma surface sediment cysts and the WDOH shellfish PST data). Therefore, the

surface sediment cyst data are primarily used to examine if the first annual occurrence of PST in shellfish occurs at or near cyst bed locations.

Temporal consolidation of the data was required to enable trend analysis, particularly for the WDOH records which include well over a hundred sampling sites throughout Puget Sound with variable frequency of sampling rates. These shellfish samples are collected by WDOH, Tribes, and County Health Departments at roughly two-week intervals from locations (sentinel sites) located throughout Puget Sound. If biotoxins are detected, then then sampling increases to weekly until the threat diminishes. Additional samples are collected from non-sentinel locations prior to commercial harvest or if there is a suspicion that biotoxins might be present. Biotoxins may be suspected if (a) biotoxins were detected in adjacent monitoring sites, (b) high concentrations of phytoplankton known to produce biotoxins are observed, or (c) a person or marine mammal presents symptoms consistent with biotoxin exposure. While this is useful for management purposes, the result is an inconsistent sampling regime that I condensed into a two-week time step for trend analysis.

2.1. Identifying initiation date prior to examining trends

I assumed that the first annual detection of PST in shellfish marks the timing of *A. catenella* bloom initiation. Before determining whether bloom initiation has changed with time, I first had to define bloom initiation and develop a method, consistent at all sites, to identify when it occurs at each site for each year. This section describes the approach I developed.

The WDOH sample shellfish at sentinel sites every other week with an increase to weekly sampling if biotoxin is detected. I therefore used a two-week time step for trend analysis and condensed data into biweekly periods. Table 1 shows how each shellfish sampling event was converted to the biweekly period in which it falls. If more than one sample was collected during a biweekly interval, the highest PST value was used in the analysis.

Sample sites were also spatially grouped by both closure zone and subbasin (i.e. North, Northwest, Whidbey, Central, Hood Canal, and South) (Figures 1, 2). Closure zones are defined and utilized by WDOH and are used to delineate regions of shellfish harvest closures after toxins exceed regulatory limits. I chose to conduct analysis on closure zones since it reflects the way

samples are used to enforce harvest rules. I also analyzed for trends in subbasins to see if there was a noticeable large-scale geographic component.

Using ArcGIS, I created a point shapefile from the GPS points listed in the raw PST data provided by WDOH. A second polygon shapefile representing regulatory closure zones was downloaded from the WDOH website. I then binned site locations into the larger closure zones using the Spatial Join tool. A similar process was used to bin sites into subbasins. Combining sites into closure zones also increased the number of observations within a given time interval which allowed more robust analysis. However, since some closure zones still had very few data points, I also reduced the number of closure zones included in this analysis to those with more than five PST detections during the 27-year sampling period. Although a statistically rigorous analysis would require more points, the limited dataset led to my determination that five datapoints was a reasonable number to determine whether a trend likely existed.

Finally, in order to better visualize patterns, the dataset was formatted better suited for descriptive analysis. Data from each sampling site are given in units of μg saxitoxin equivalents (STXeq/100 g shellfish meat). Toxin concentrations at the time of initiation were binned into three categories: (1) below the detection limit of <38 μg STXeq/100 g shellfish meat (as determined by mouse bioassay), (2) above the detection limit but below the regulatory limit of 80 μg STXeq/100 g shellfish meat, (3) above the regulatory limit (Table 2). This also allowed the data to be treated as binary where 1 is the toxin is absent and 2-3 the toxin is present (above 80 μg STXeq/100 g shellfish meat). The resulting dataset consisted of closure zone, subbasin, year, biweek sample period, toxin concentration, and binned toxin level.

2.2. Determining temporal patterns of bloom initiation

Annual bloom initiation is defined as the first time PST is detected at a sample location at or above the detection limit of 38 μg STXeq/100 g shellfish tissue. It is possible that *A. catenella* cells were present in the water column but not at levels high enough for PST to be detected in shellfish samples; however, this is the most sensitive indicator of bloom initiation possible with these data.

Although *A. catenella* blooms typically occur from April through October, in some cases toxins remained high from one year to the next. In that case, bloom initiation would be biweek 1 of the calendar year. However, this does not give an appropriate representation for modeling purposes of the beginning of the bloom *season* and instead shows a "carry over effect" (Moore, et al., 2009). Therefore, I did not consider toxin detections occurring at the very beginning of the year if they were preceded by toxin detections at the end of the previous year in the same location. This adjustment identifies bloom initiation as the proliferation of new cells rather than residual toxic cells from the previous season. Additionally, PST will sometimes appear in one sample and then rapidly dissipate, and not reappear for several weeks. I determined that these events were not a good representation of the beginning of the PST season, and thus I did not consider data points if they were not followed by another positive detection when the next sample was collected. These conditions used to define bloom initiation are summarized as a flowchart in Figure 4.

After the timing of bloom initiation was determined for each closure zone, Pearson's correlation was used to find significant trends ($p < 0.05$) in each closure zone with five or more years of PST detection. I also used empirical orthogonal function (EOF) to examine any spatial patterns of variance of initiation bloom timing among closure zones. These shifts could then be compared with environmental data to find whether there were any predominant factors that may be driving the timing of bloom initiation (climate patterns, temperature, dissolved oxygen, etc.).

2.3. Determining spatial patterns of bloom progression

I used the same dataset described in the above section to determine the spatial progression of PST first detections each year. I developed maps for each biweekly interval in the 27-year period with closure zones marked with PST status. Visual inspection of these maps was used to examine the progression of PST first detections. Following methods from previous studies (Trainer et al, 2003, Moore et al, 2009), data locations were divided into six subbasins delimited by major sills in Puget Sound for comparison between subbasins (Trainer, et al., 2003; Moore, et al., 2009) (Figures 1, 2).

2.4. Spatial Relationship to Cyst Beds

Cyst data were classified by presence or absence of *A. catenella* cysts. All sediment sampling locations and bloom initiation sites were mapped for each year and subbasin. Given knowledge of *Alexandrium* life history and the results of previous studies in other regions, I hypothesized that there would be a positive relationship between the locations of cyst bed and where *A. catenella* blooms initiate within short distances (<5 km). Proximity of bloom initiation to cyst beds mapped the previous year would indicate rapid onset of toxicity after excystment. Additionally, if cysts were found near bloom initiation sites it would indicate *A. catenella* cells encysted at locations close to where blooms initiated.

2.5. Environmental factors (primarily water temperature) influencing excystment using other publicly available data sets

A. catenella cyst germination is dependent primarily on fulfillment of a obligate dormancy period and secondarily on environmental conditions including, but not limited to, temperature, temperature history, salinity, stratification, nutrients, oxygen, and benthic mixing/deposition (Anderson et al., 1987; Fischer et al., 2018; Leftwich, 2014; Moore et al., 2015; Tobin and Horner, 2011). There are a limited number of long-term datasets for these environmental factors in Puget Sound; however, the vast majority of this monitoring occurred at the sea surface where conditions can be significantly different than the benthic conditions where these cysts overwinter.

I selected four long-term sea surface temperature datasets from locations in Bellingham Bay (Washington State Department of Ecology), Quartermaster Harbor (King County), Seattle (NOAA – Station ID 9447130), and Race Rocks (racerocks.com). However, Bellingham Bay records only consisted of 11 years of data, and these monthly grab samples were too coarse to draw conclusions. Quartermaster Harbor records also included only 11 out of the 27 years of my study period. Only Race Rocks and Seattle contained continuous temperature data for the entire period of study. Data collected from the Race Rocks Ecological Preserve in British Columbia represent one of the only long term (>80 years) water temperature data sets in the Salish Sea.

While other temperature datasets exist, they are either relatively short-term (<10 years), were not sufficiently frequent (ex: grab samples) or did not have a nearby sentinel site where shellfish were tested for PST. A list of datasets considered is given in Table 3. For the four selected sites, I plotted temperatures over time and indicated the date of first PST detection (as defined in Figure 4) for corresponding closure zones (Figure 16). I then visually analyzed the plots for any distinct temperature changes prior to the first appearance of PST.

I was unable to locate any long term (>10 years) datasets for nutrients, salinity, benthic mixing/deposition and thus surface temperature was the only parameter included.

Chapter 3. Analysis Results

3.1. Determining temporal patterns of bloom initiation

Out of the 152 closure zones sampled by WDOH in Puget Sound since 1990, I identified 74 closure zones with at least five qualifying data points. In other words, I identified five years in which bloom initiation was defined according to the conditions stated above. These are shown in Figure 5. Of these 74, eight closure zones had a highly significant consistent trend ($p < 0.05$) for bloom initiation to occur earlier in the year over the period of study (Table 4; Appendix A). Three out of these eight closure zones contained a sampling site that is monitored by WDOH year-round (aka sentinel sites – specifically Birch Bay, Mystery Bay, and Sequim Bay). When I included moderately significant locations ($p < 0.10$), an additional two closure zones show a trend toward earlier in the year and three show a trend of blooms occurring later in the year. However, in contrast to closure zones with highly significant trends, none of these sites were sampled year-round. Nevertheless, all significant trends were derived from at least 11 years of PST detection.

Ten of the closure zones with significant trends were located in the Central Basin (10 of 13). The remaining three were located in the Northwest, North, and South Basins. These four basins also contain the majority of locations included in the analysis, with the Central Basin

containing the greatest number (Figure 7). Trends of all sites with greater than 5 years of sampling are shown in Figure 8.

The empirical orthogonal function I used to determine if annual shifts in timing corresponded among closure zones showed that none of the first five modes explained more than 12% of the data thus there is no dominant mode of variability (Figures 9 & 10). If predominant variable was present there would be a dominant mode of variability that explained a much greater percent of variance and was followed by modes with significantly lower percentages.

3.2. Determining spatial patterns of bloom initiation

After condensing the PST data according to the methods above, I was left with a total of 74 closure zones throughout Puget Sound. Unfortunately, this excluded all closures in the Hood Canal basin. This is likely because PST detections have occurred only relatively recently in that area, so there are fewer than 5 years with blooms that enabled me to calculate the timing of bloom initiation. Of the 74 closure zones, 89% were located in the North, Central, or South subbasins (Figure 5). Although the 74 closure zones are not evenly distributed among subbasins, they are generally proportional to subbasin size and frequency of historical PST events. In order to visualize north to south trends, Figure 11 shows closure zones in the North and Central subbasins sorted by latitude. Other subbasins are excluded because there are few sites, and the geography is not oriented in the same north to south pattern. PST generally first appears in the far North closure zones at Drayton Harbor and Birch Bay, but a cluster of early sites can also be observed in the mid-Central subbasin.

Variability of bloom initiation was high both within and among sites. Figure 12 shows the ranges of bloom initiation for all sites in the North and Central basins ranked from earliest to latest by mean. Although both subbasins show large variability within sites (spanning over 10 months at some locations) the range of mean initiation time is much narrower in the North basin (Mar-Jun) than in the Central basin (Jan-Aug).

Sporadic appearances and progression of PST year-to-year make it difficult to describe overall patterns with much certainty, but I was able to extract a few general trends. In the North

subbasin I observed movement of bloom initiation from North to South through the season. Biweekly maps show detections in the San Juan Islands, located in the center of the North subbasin, almost never precede detections in the northernmost closure zones, but typically follow soon after. The pattern along the mainland coast of the North subbasin is less clear. Aside from a cluster of sites in the mid-Central subbasin that tend to be the first detected in the bloom season (Eglon, Edmonds, Kingston, Rolling Bay), there is not a clear spatial pattern in this subbasin. Other subbasins were not included because either there were too few sites (Northwest, Whidbey, and Hood Canal) or the orientation was such that it wouldn't be informative (South).

3.3. Spatial Relationship between PST detection and *A. catenella* Cyst Beds.

In winters of 2011 through 2013 sediment cores were collected in all subbasins throughout Puget Sound. Sites with high cyst concentrations were located in patches throughout Puget Sound with the highest counts located consistently in Bellingham Bay, Liberty Bay, and Quartermaster Harbor (Greengrove, et al., 2015). Additional high cyst counts were found in the very North of Puget Sound near Drayton Harbor and in Birch Bay in 2011. By mapping both the location of surface sediments (0-1 cm from cores) with high cyst counts (>100 cysts/cm^3 wet sediment) and annual locations of first PST detection, I determined that this was not a strong spatial relationship (Figures 13-15). In both 2011 and 2012 Drayton Harbor and Birch Bay were two of the first sites with detectable PST in Puget Sound. In 2013, PST was detected at Drayton Harbor within a month of its first appearance in Puget Sound. Despite consistently higher cyst counts in the Central sites, toxins in these areas were preceded by the Northern-most locations by several months in all years studied. This is despite consistently higher cyst counts in Central basin areas of Quartermaster Harbor and Liberty Bay. Thus, these analyses do not indicate a consistent relation between the location of seed beds and sites where *A. catenella* blooms first initiate.

3.4. Environmental factors (primarily water temperature) influencing excystment using other publicly available data sets

I plotted the four longer-term temperature datasets that I was able to acquire and compared them to the biweek of first PST detection. However, I was unable to detect any large or sudden change in environmental conditions prior to the first PST detection at these locations (Figure 16).

Chapter 4. Discussion

Using WDOH shellfish PST data as a proxy for *A. catenella* blooms combined with data describing the distribution and abundance of *A. catenella* cysts, I set out to describe the onset of these blooms in space and time to look for evidence of earlier onset of bloom initiation over the 27-year time series and identify any relationships with the locations of seed beds. I did not find a consistent, significant trend of blooms occurring earlier in the year at the subbasin level; however, at the finer spatial scale of closure zones, a limited number of locations appeared to have blooms occurring earlier. There also appeared to be a general spatial trend of blooms initially appearing in the north of Puget Sound before appearing elsewhere in the region. I did not find a spatial correlation between locations where blooms initiate each year and locations of cyst seed beds.

4.1. Determining temporal patterns of bloom initiation

Forecasts of HABs in Puget Sound predict earlier and longer lasting toxic events due to a widespread increase in the length of favorable growing conditions, specifically increased temperature and stratification (Moore et al., 2008; Moore et al., 2015b). There is limited evidence in this study to support this idea. Those eight sites with highly significant annual trends pointed toward earlier bloom initiation. Out of the five moderately significant sites, two showed a trend towards blooms beginning earlier in the year and three beginning later. Five of the highly significant sites were sentinel sites and therefore regularly sampled year-round. None of the

moderately significant sites were sampled year-round and thus may not accurately represent when toxins first occur.

Spatially, the majority of closure zones with a significant ($p < 0.10$) trend occurred in the Central Basin; however, this is relatively proportionate to the total number of sites included in the analysis (Figure 6) and thus likely is more reflective of sample size than proclivity. However, the number of locations with significant trends represents a small percentage of overall closure zones. This likely indicates that either the driving forces causing this trend are not occurring uniformly across Puget Sound and/or that some subbasins are not responding to these changes and thus there is not an overall shift toward blooms earlier in the year.

It is unclear what might drive such trends since many physical and biological factors influence the growth responses of harmful algal species. It is also possible that shifts are occurring, but the data are not robust enough to indicate a trend at some sites. Evidence of significant trends may emerge in the future at these sites as more incidences are added to the dataset.

The lack of a trend in the remaining closure zones also potentially contrasts with previous findings that described a significant increase in both the frequency and duration of PST-related shellfish closures (Trainer et al., 2003). However, the discrepancy may have resulted from differences in methods. In my study I chose the PST detection limit as our threshold for inclusion in the dataset in an effort to include more data points and to better capture when excystment actually occurs, while other research focused on shellfish harvest closures and used more than one species of shellfish in their analyses (Hanein and Borchert, 2015; Trainer et al., 2003). Since cells of *A. catenlla* can be present at a detectable level without progressing to a bloom, the timing of the detection and closure may be different and thus it is difficult to contrast the findings of this study with those listed above.

4.2. Determining spatial patterns of bloom initiation

It has been suggested that PST is first detected in the far north of Puget Sound each year (J. Borchert, personal communication; Moore et al., 2009). My results support those observations with PST typically first appearing near the Canadian border in Drayton Harbor

and/or Birch Bay. There has been speculation that the seed source for *A. catenella* may be located across the border in British Columbia (B.C), Canada (Trainer et al., 2003). Historical records show many PST events off the B.C. coast and prevailing winds and currents from the north during the summer months, when growing conditions are ideal, may promote cell movement into those early detection areas (Vancouver, 1798; Hickey, 1989). Given the complex shorelines and numerous inlets with localized environmental conditions in Puget Sound, I am cautious to draw too many conclusions based on latitude. However, shifts to early bloom initiation at sites near the Canadian border (ex: Birch Bay) and in Central Puget Sound (ex: Quartermaster Harbor) are consistent with the institutional knowledge of people with many years of experience sampling in that region.

High variability in the biweek of bloom initiation at several sites, such as Kingston and Poulsbo among others, makes it very difficult to describe spatial trends. The wide range of timing in bloom initiation may be due to natural variation in environmental conditions. Some closure zones likely experience a wider range of conditions as well as more varied flow regimes that may introduce cells from neighboring embayments. Additionally, these are embayments that experience regular boat traffic year-round which may contribute to sediment disturbance and subsequent resuspension of cysts that may stimulate excystment.

Beyond these broad generalizations, it is difficult to establish from one location to another, despite the extensive sampling conducted by WDOH. In contrast to the geography of the outer coast, Puget Sound has a more extensive shoreline (>2,100 km) and more complex circulation patterns. Furthermore, without genetic analysis it is impossible to conclusively determine if cells observed in one location originated from a bloom in a neighboring location. However, it is still potentially informative to the monitoring program to describe the progression of blooms, regardless of the underlying cause. Additionally, identifying the areas that do appear to predictably follow a pattern has the potential to be beneficial for WDOH monitoring programs in designing when and where they should target their limited sampling resources. It may also be possible for shellfish growers to anticipate when their beds are likely to experience PST closures by observing the status of closure zones that typically precede it.

4.3. Spatial Relationship to Cyst Beds

I hypothesized that locations where toxins are first detected in shellfish during a given year would correlate with the locations of cyst seed beds mapped the winter before; however, cyst locations and first detected shellfish toxicity show no pattern. A strong spatial relationship would indicate that the first annual detections of shellfish toxicity are caused by cysts that germinated locally, and would require several conditions to be met: (1) temperature (and other factors) must be adequate to trigger excystment, (2) conditions governing growth, competition, and grazing by zooplankton must be sufficient for newly germinated cells to proliferate, and (3) cells must be retained in the system long enough to be consumed by nearby shellfish. However, high concentrations of cysts do not necessarily result in a higher probability of more toxic shellfish. A number of factors can influence this relationship, including cyst dormancy patterns and physiological requirements for excystment (Cox et al., 2008; Hallegraeff et al.,1998; Tobin and Horner, 2011). Researchers have also occasionally observed cells in the water column without corresponding PSTs in shellfish (Dyhrman et al., 2010). Additionally, research on bloom initiation of a similar species, *A. fundyense*, in shallow inlets on the coast of Maine, USA showed low concentrations of cysts in shallow waters relative to concentrations off the outer coast, but PST events regularly occurred sooner in the inland sites indicating a disconnect between cyst counts and the timing of when shellfish become toxic with high PST concentrations (McGillicuddy et al., 2014). This is in contrast to studies that found a relationship between cyst concentration and bloom magnitude (Li et al., 2009). Similarly, research on the same species in Massachusetts, USA, did not find a relationship between cell abundance and cyst concentration, but rather blooms were a factor of cell retention and nutrient conditions within each site (Crespo et al., 2011). It is also possible that the correlation between cysts and toxicity is weakened because cells suspended in the water are subject to hydrodynamic transport via tides and currents. Thus, excystment at one location does not mean the toxic cells remain at that same location. Since the relationship between cyst counts and PST concentrations has not been well established by other studies, it is not surprising that I found similar results.

The presence of a source population of cysts does not necessarily mean that shellfish in that location will become toxic. Environmental conditions may not be appropriate for germination and growth and/or cells may be flushed into another water body before detectable

levels of toxins accumulate in shellfish. Unlike similar studies off the coast of Maine, the complex hydrogeography of Puget Sound with its numerous inlets and tidal mixing make it difficult to track motile cells of *A. catenella* to their source (Anderson et al., 2005). Future research could apply existing flow models, such as the ones developed by Pacific Northwest National Laboratory or the Coastal Monitoring Group at the University of Washington, to search for hydrodynamic linkages between cysts and PST events (Moore et al., 2015b).

4.4. Environmental factors (primarily water temperature) influencing excystment using other publicly available data sets

The seawater temperature records examined here did not show any relationship with the timing of bloom initiation. However, the lack of local data may have limited this analysis (Moore et al., 2008). It is difficult to accurately interpret trends without knowing whether the local environment near a sentinel monitoring site has changed, due to dredging or restoration for example, resulting in a shift in the ideal conditions for *A. catenella* growth. Endogenous factors, rather than environmental factors like temperature, and interactions between endogenous and environmental factors (e.g., "chilling units") also play a role in excystment (Fischer et al., 2018). Excystment is only possible after a cyst maturation period has been completed (Moore et al., 2015a). After this mandatory dormancy requirement is met, cysts can modulate their dormancy cycle based on their temperature history (Fischer et al., 2018). Additionally, a more detailed analysis would have required more consistent datasets at all locations covering the entire time series and with fewer temporal gaps.

Chapter 5. Conclusions

The idea that PST events are occurring earlier in the year is consistent with predictions that increasing temperatures will increase the window of opportunity for *A. catenella*. However, aside from a few locations, this examination of over two decades of PST data did not reveal a significant Sound-wide trend. The degree of temporal resolution (samples collected <2 weeks

apart) increases the threshold for detecting any significant change, which was not surpassed here. However, by observing the locations of blooms over many years it appears that there is a spatial pattern of initiation in the north before appearing elsewhere in Puget Sound. Shellfish growers and consumers depend on this robust monitoring to maintain their health and livelihood, and, if shifts in bloom initiation and frequency do occur in the future as is predicted, this work will become even more important.

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Chapter 7. Tables

Table 1. Biweekly intervals and corresponding months.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
Jan		Feb		Mar		Apr		May		June		July		Aug		Sep		Oct		Nov		Dec			

Table 2. Classification of shellfish toxicity by concentration of saxotoxin.

Non-detectable	<38 μg STXeq/100 g shellfish meat
Toxin detected	38-79 μg STXeq/100 g shellfish meat
Shellfish harvest closed	>79 μg STXeq/100 g shellfish meat

Table 3. Links to long-term monitoring datasets from Puget Sound explored for comparison with timing of PST initiation. Selected datasets include consistent monitoring over at least ten years of the study period.

Eyes Over Puget Sound (EOPS)	https://ecology.wa.gov/Research-Data/Monitoring-assessment/Puget-Sound-and-marine-monitoring/Eyes-over-Puget-Sound
King County	https://green2.kingcounty.gov/marine-buoy/
National Data Buoy Center (NDBC)	https://www.ndbc.noaa.gov/maps/NW_Straits_Sound.shtml
Northwest Association of Networked Ocean Observing Systems (NANOOS)	https://ecology.wa.gov/Research-Data/Monitoring-assessment/Puget-Sound-and-marine-monitoring/Eyes-over-Puget-Sound
Northwest Environmental Moorings (ORCA buoys)	http://orca.ocean.washington.edu/
Race Rocks	http://www.racerocks.com/racerock/abiotic/temperature/seatemperature.htm

Table 4. Closure zones with highly significant ($p < 0.05$) and moderately significant ($p < 0.10$; lower portion of table) correlation coefficient (r) of PST bloom initiation date by year from 1990-2017. Probability values are not adjusted for autocorrelation. Locations in Figure 7.

Site Name	Number of Years	R	P-value	Mean Biweek	Biweek SD	Slope	Subbasin
Sequim Bay	24	-0.655	0.0005	7.17	5.5377	-0.476	NW
Edmonds	7	-0.958	0.0007	5.00	4.6188	-0.725	C
Mystery Bay	16	-0.745	0.0009	10.13	8.1639	-0.693	C
Quartermaster Harbor	22	-0.588	0.0040	9.68	3.4893	-0.379	C
Des Moines	17	-0.634	0.0062	11.53	4.7053	-0.508	C
North Kilisut	21	-0.507	0.0191	13.24	6.0738	-0.366	C
Birch Bay	18	-0.523	0.0261	9.72	5.6235	-0.323	N
Rich Passage	16	-0.531	0.0343	12.50	7.2296	-0.511	C
East Vashon Island	21	0.408	0.0666	5.52	5.18	0.361	C
Eglon	18	-0.428	0.0768	2.89	2.00	-0.149	C
Rolling Bay	14	-0.488	0.0768	2.57	2.56	-0.299	C
Raft Island	11	0.535	0.0898	13.64	5.73	0.483	S
Gig Harbor	19	0.398	0.0919	9.32	1.89	0.133	C

Chapter 8. Figures

Figure 1. Map of regional subbasins separated by shallow sills in the lower Salish Sea.

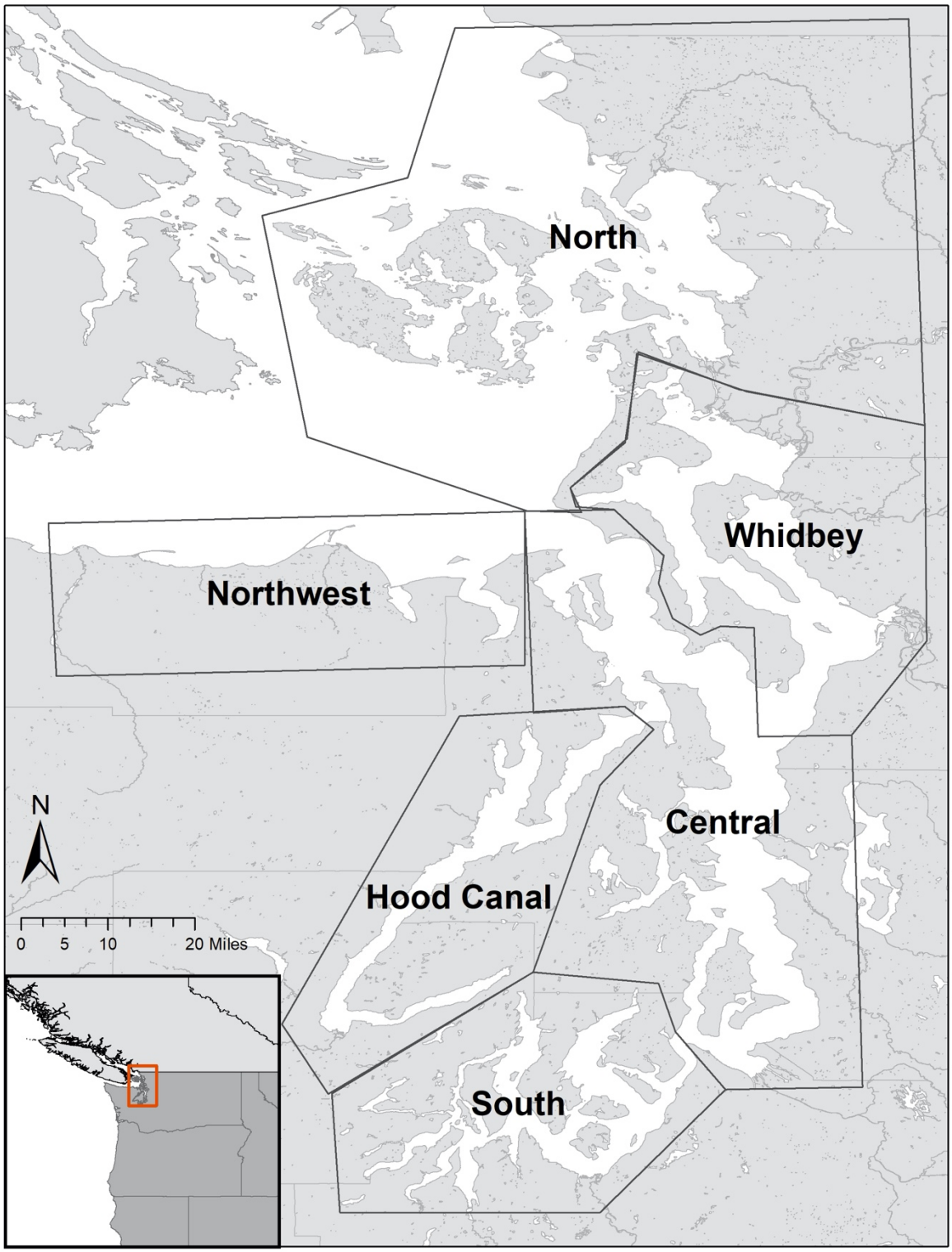


Figure 2. Map of sample sites and the associated closure zones in the lower Salish Sea.

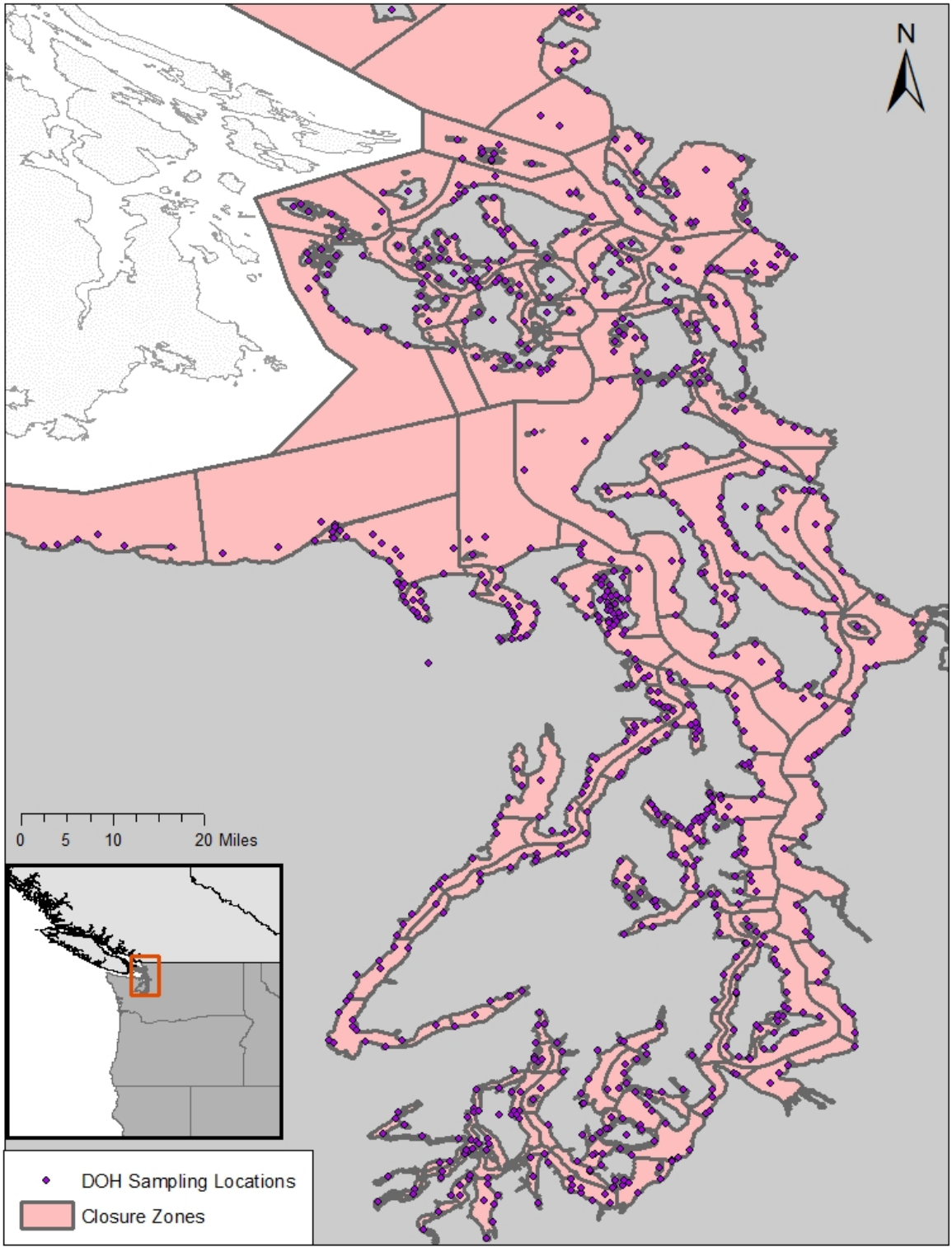


Figure 3. Lifecycle of *Alexandrium catenella*.

(1, 2) Dormant cysts emerge from the sediments when environmental conditions are right, and a mandatory dormancy period has been completed. (3) These motile cells can then reproduce via simple division. (4) This will continue until nutrients are exhausted. (5) At this point, growth stops, and cells form gametes that combine to form a zygote that will settle back down to the sediment as a cyst.

Illustration by Jack Cook, Woods Hole Oceanographic Institution.

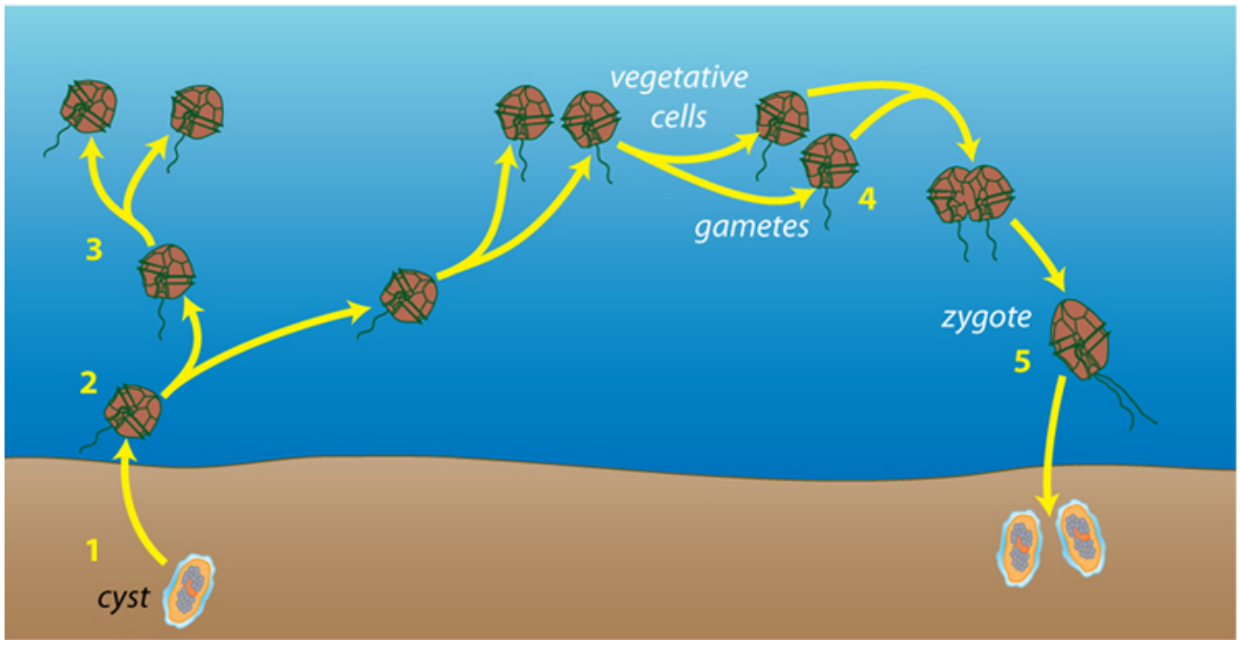
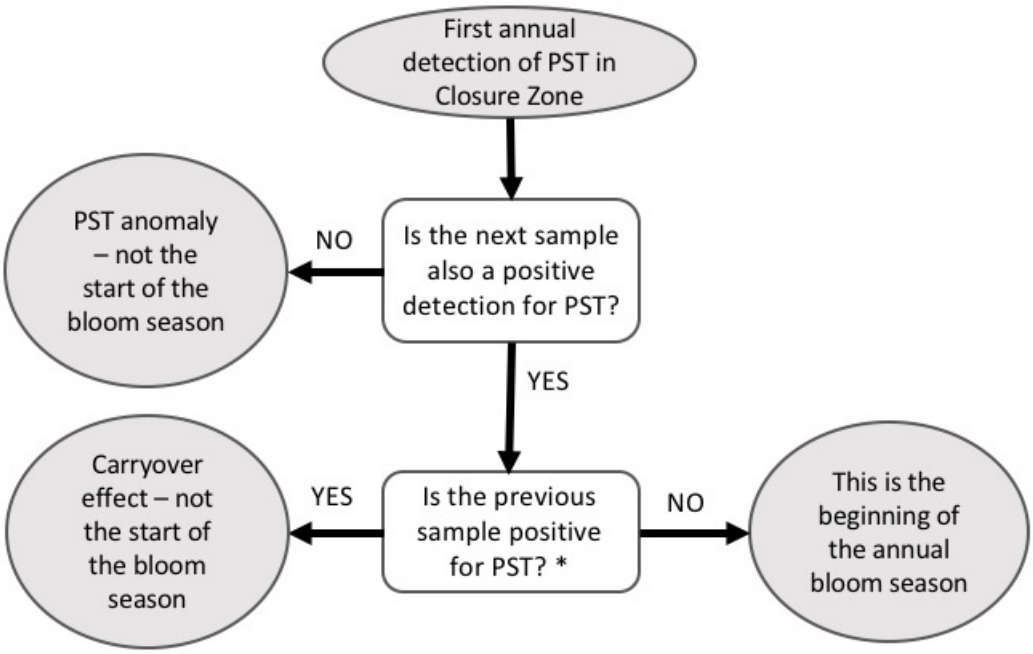


Figure 4. Decision tree for determining the beginning of the bloom season.



* If first detection occurs at the beginning of the year, then the "previous sample" may be from the end of the preceding year

Figure 5. Map of closure zones with at least five years of toxins detected.

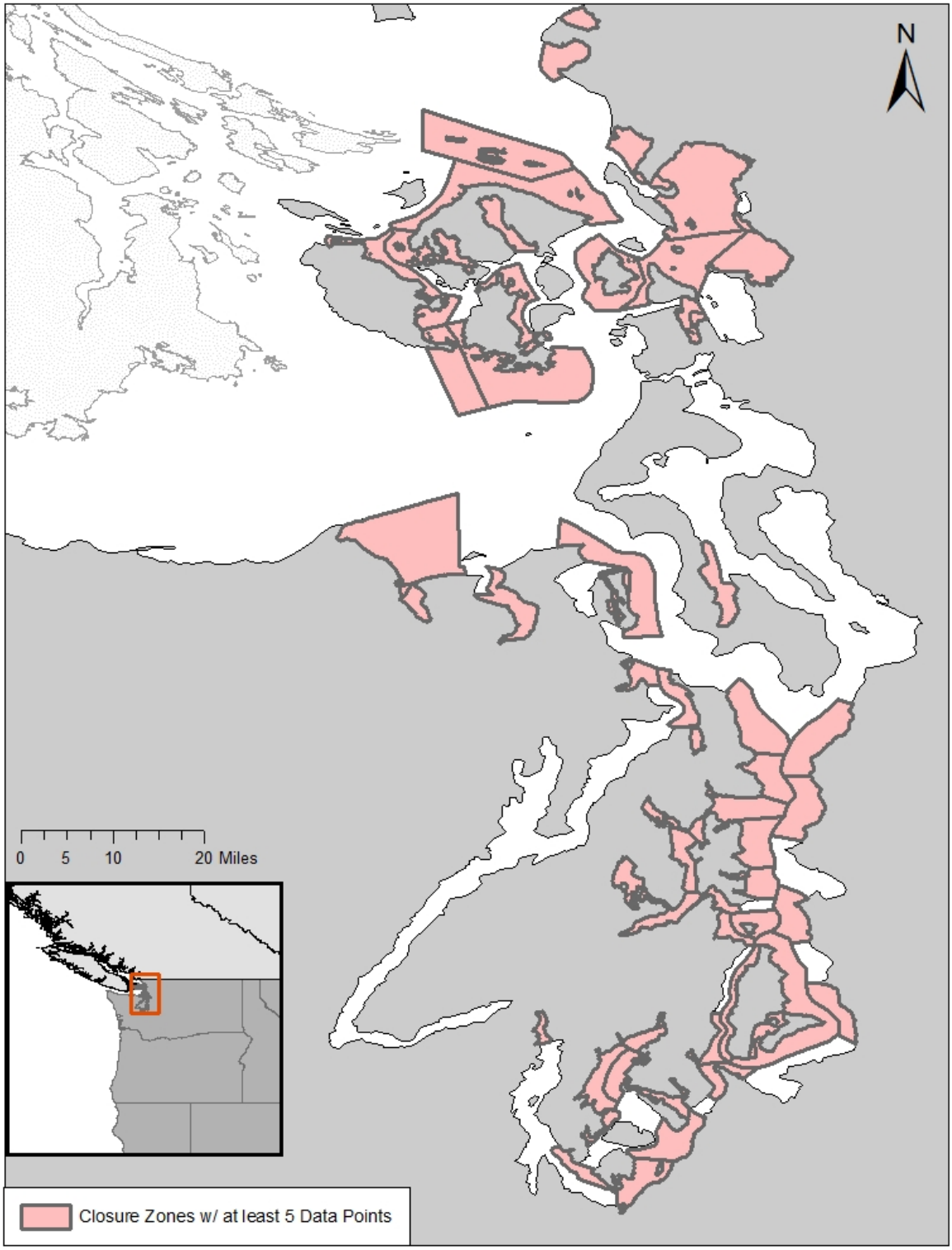


Figure 6. Number of closure zones with at least five years of toxin detection sorted by subbasin.

Figure 6a. The greatest number of qualifying closure zones were located in the largest and most populated subbasin, the Central Basin (33 closure zones). Hood Canal did not have any qualifying closure zones likely because it has the most recent appearance of PST.

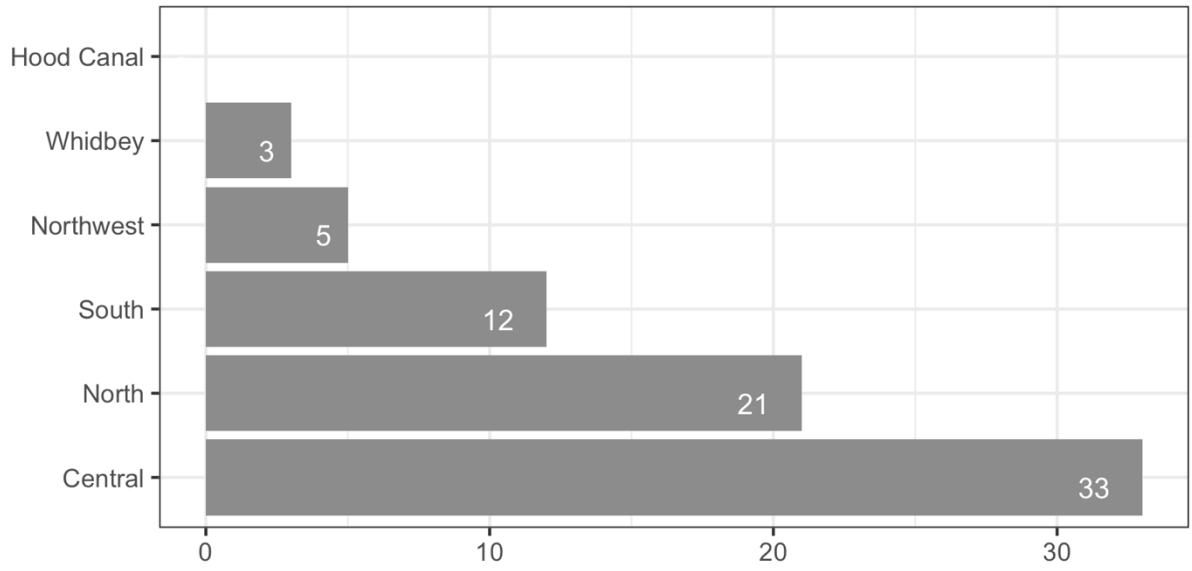


Figure 6b. The number of closure zones with sites showing a significant trend toward earlier bloom initiation was generally proportional to the total number of closure zones within a subbasin.

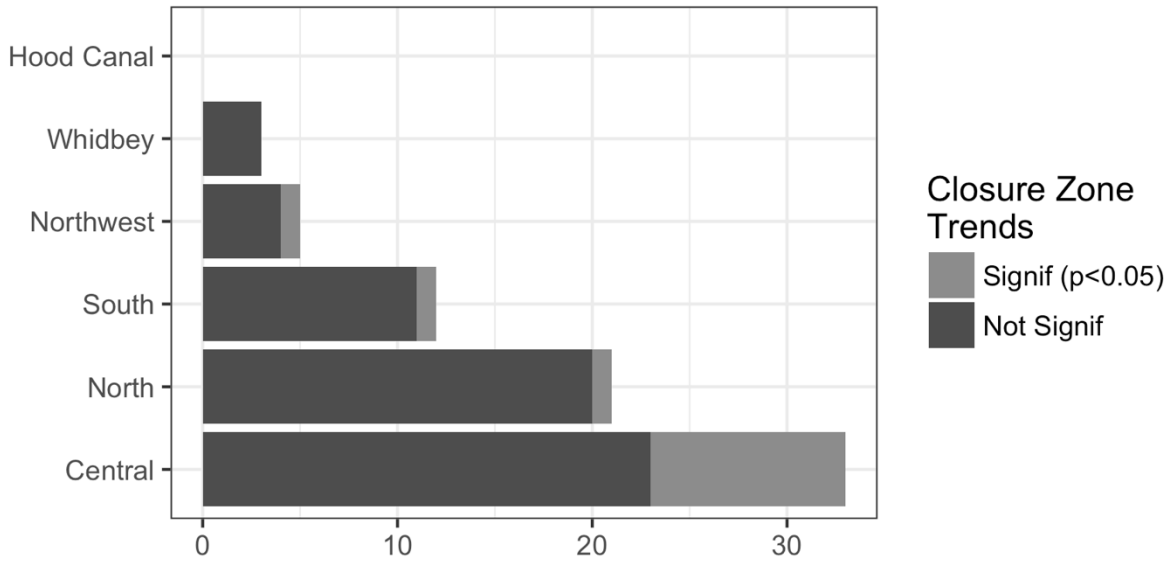


Figure 7. Map of closure zones with significant trends ($p < 0.10$ and $p < 0.05$).
Most sites had a trend toward later in the year. The trend at the last site, Edmonds, was determined to be invalid due to outlying data and a large data gap.

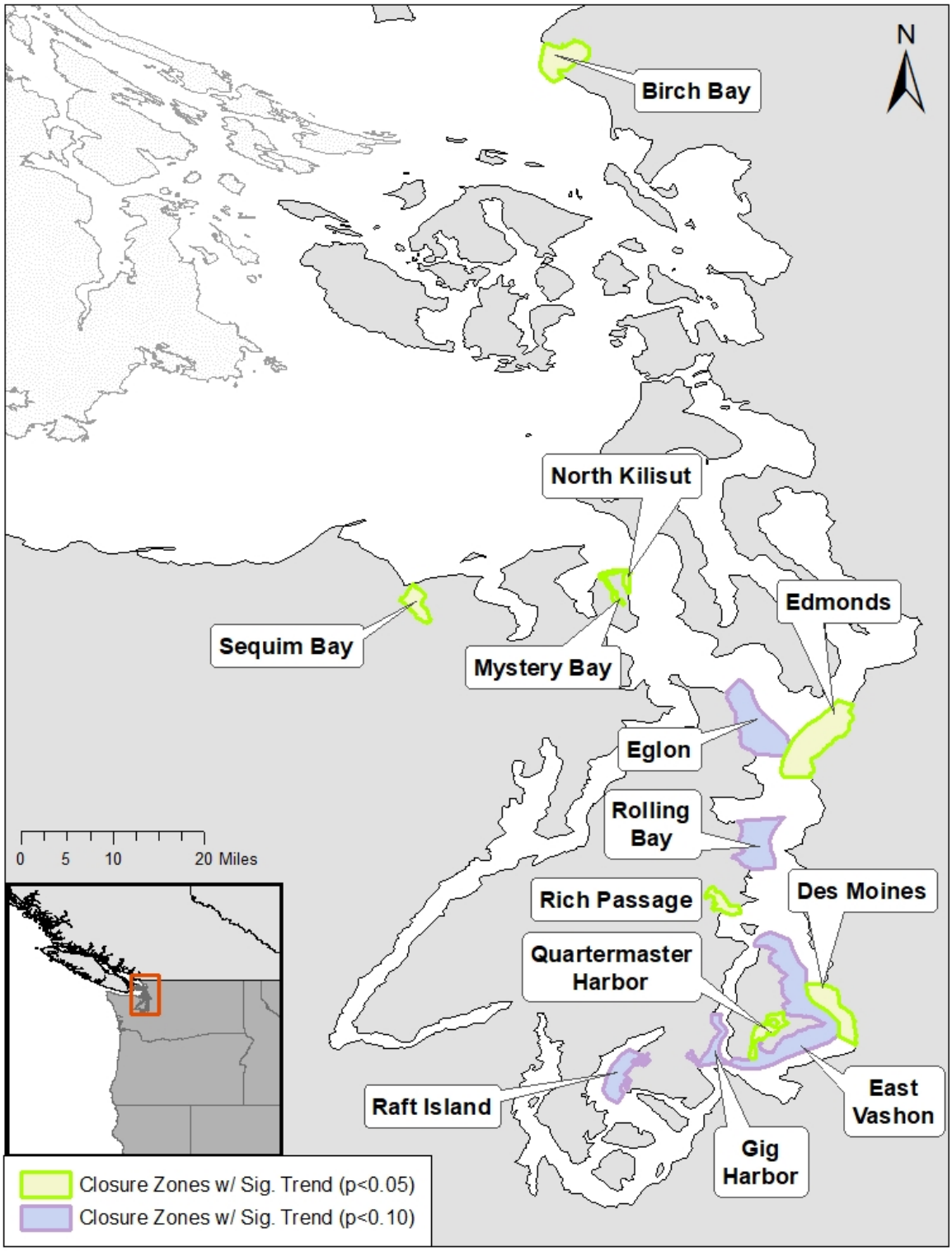


Figure 8. Trends of biweek of bloom initiation by year.

Figure 8a. Significant trends of biweek of bloom initiation by year ($p < 0.05$) toward earlier in the year.

All other sites did not have a significant trend earlier or later. Although statistically significant, Edmonds is not considered to have a meaningful trend due to an outlier and a large span with no toxins detected.

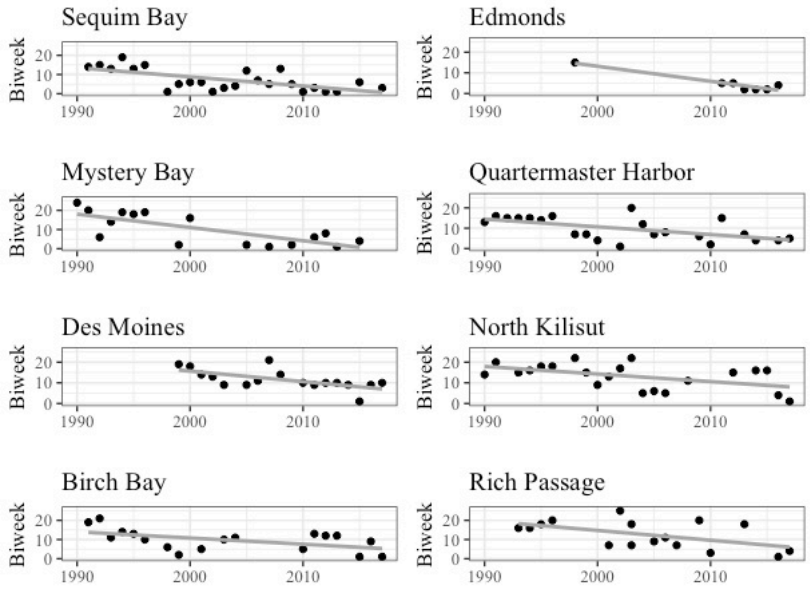


Figure 8b. Trends of biweek of bloom initiation by year ($p > 0.05$). These sites were not considered to have a significant trend earlier or later. Sites are ordered by p-value. Specific p-values are listed in Appendix A. See also Figures 8c and 8d.

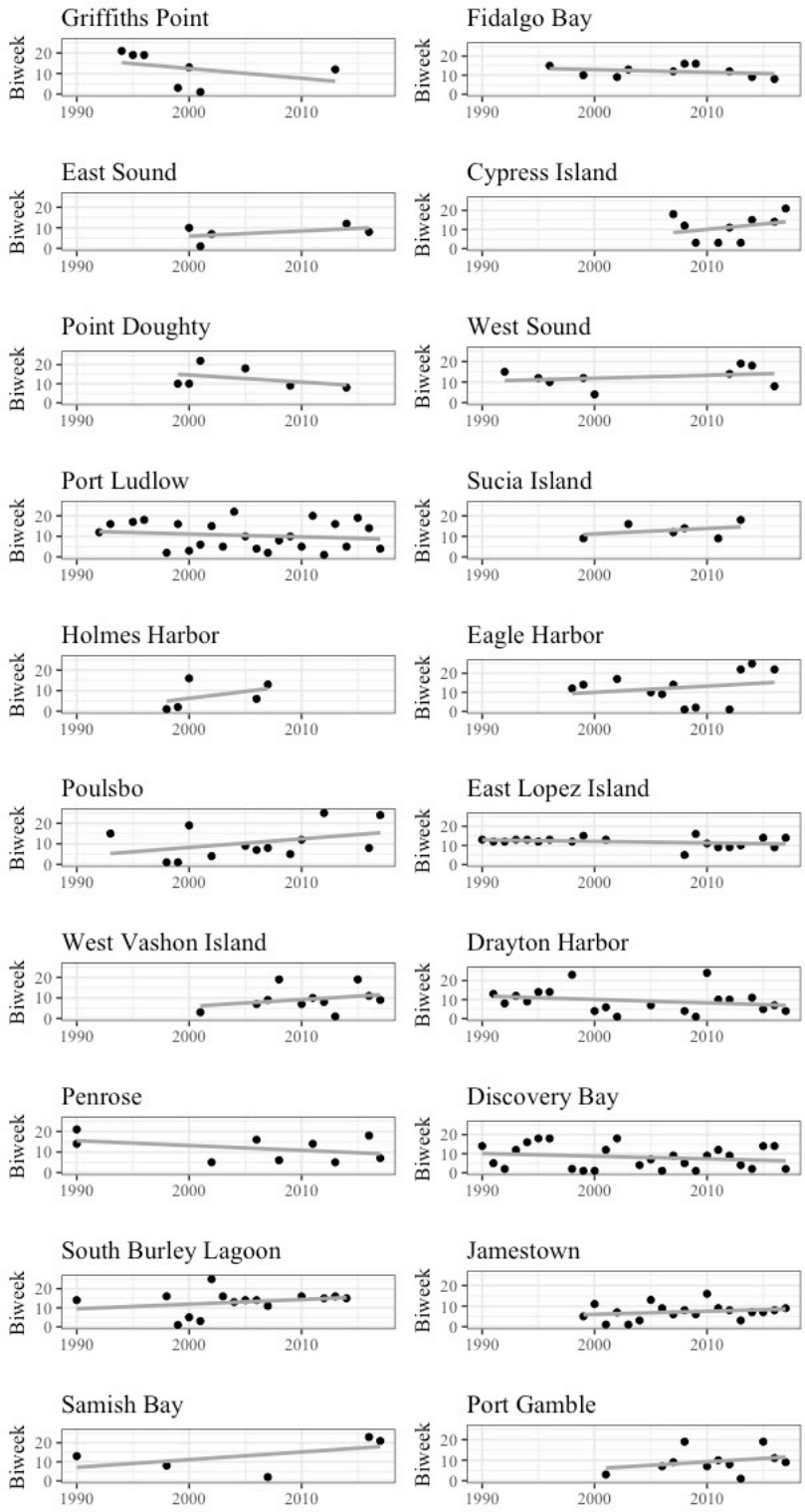


Figure 8c. Trends of biweek of bloom initiation by year ($p > 0.05$). These sites were not considered to have a significant trend earlier or later. Sites are ordered by p-value. Specific p-values are listed in Appendix A. See also Figures 8b and 8d.

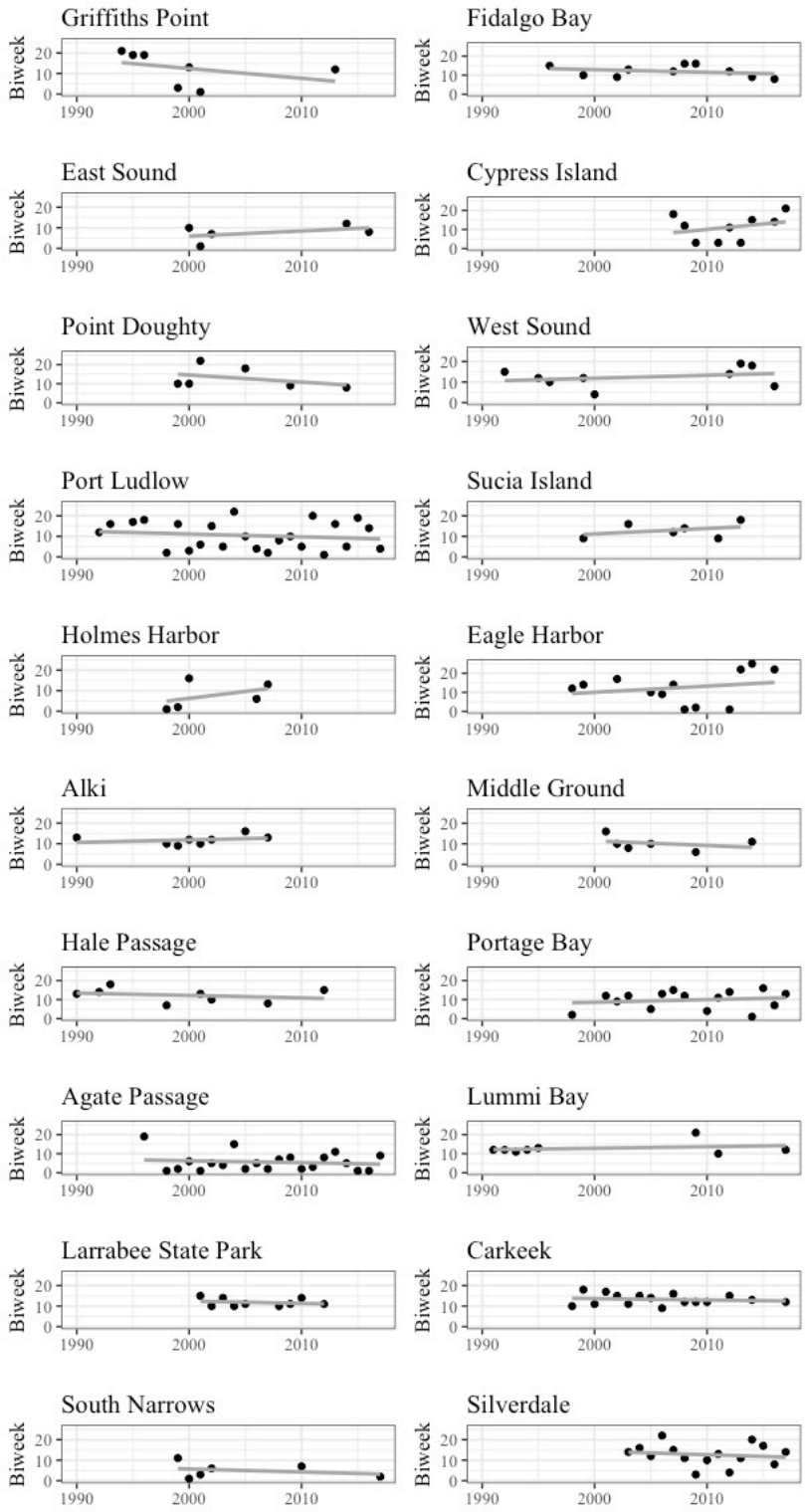


Figure 8d. Trends of biweek of bloom initiation by year ($p > 0.05$). These sites were not considered to have a significant trend earlier or later. Sites are ordered by p-value. Specific p-values are listed in Appendix A. See also Figures 8b and 8c.

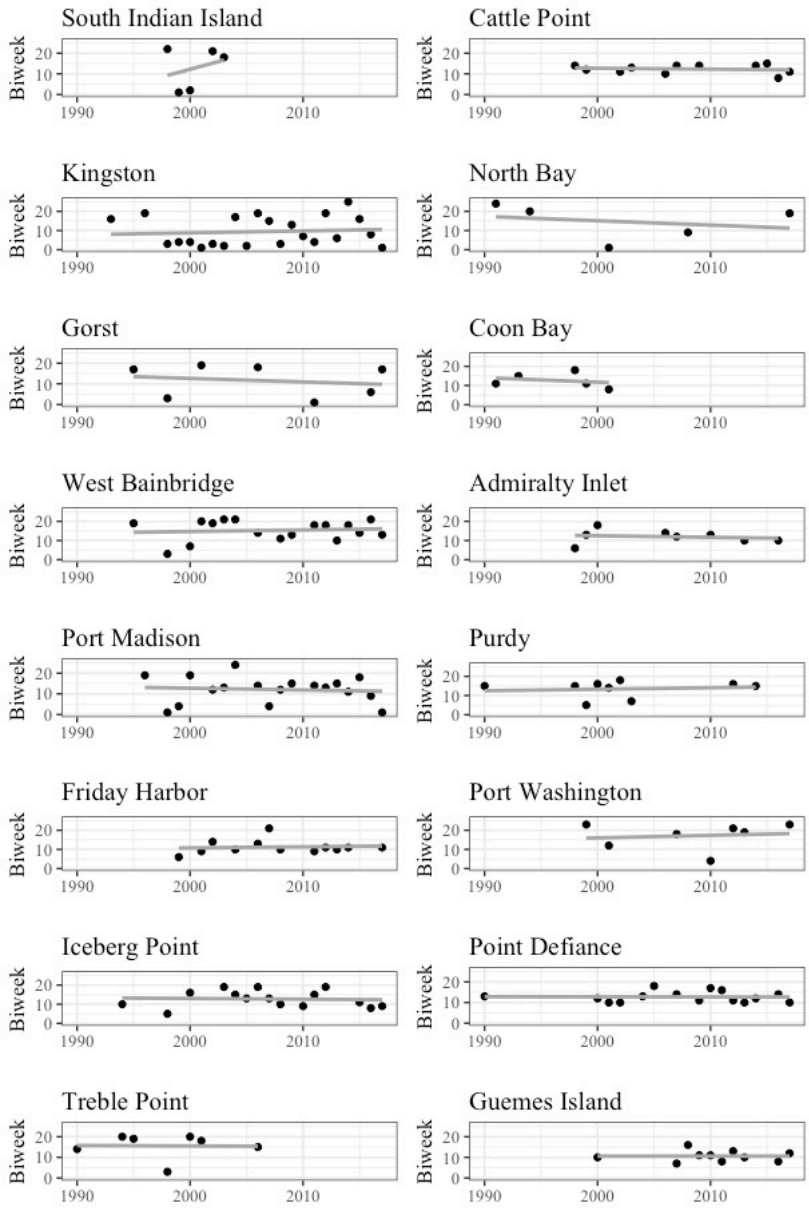


Figure 9. Annual variations in the first biweek of PST detection were compared using an empirical orthogonal function.

Below are the loadings of the first five principal components (PCs). All of the first five PCs are less than 15% demonstrating low coherence. The error bars for also overlap for all first five PCs indicating that none has a unique ability to explain variation in the data.

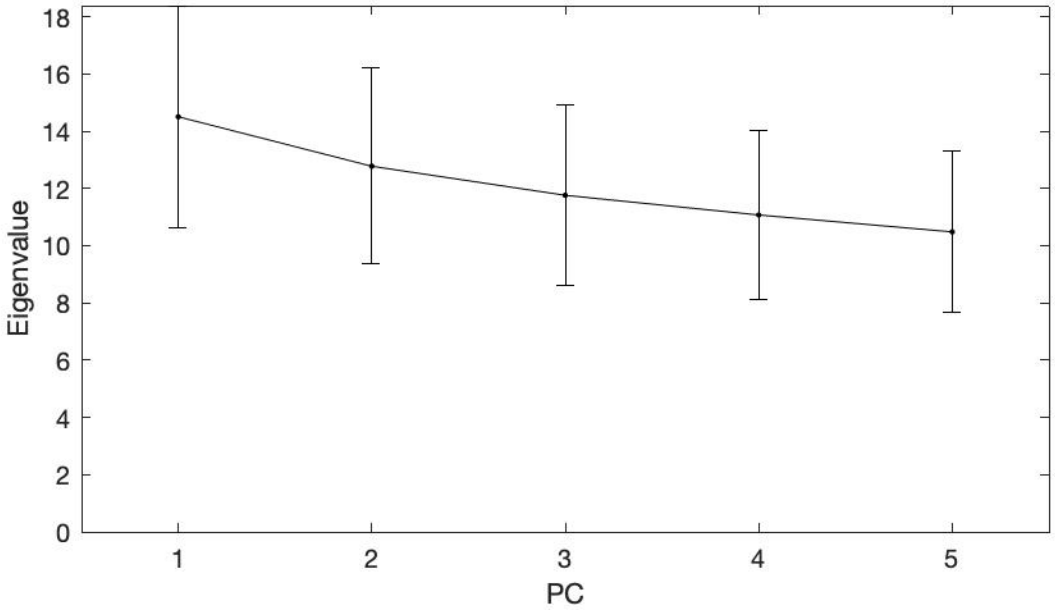


Figure 10. Annual variations in the first biweek of PST detection were compared using an empirical orthogonal function.

None of the first five principal components explain more than 12% of the variation.

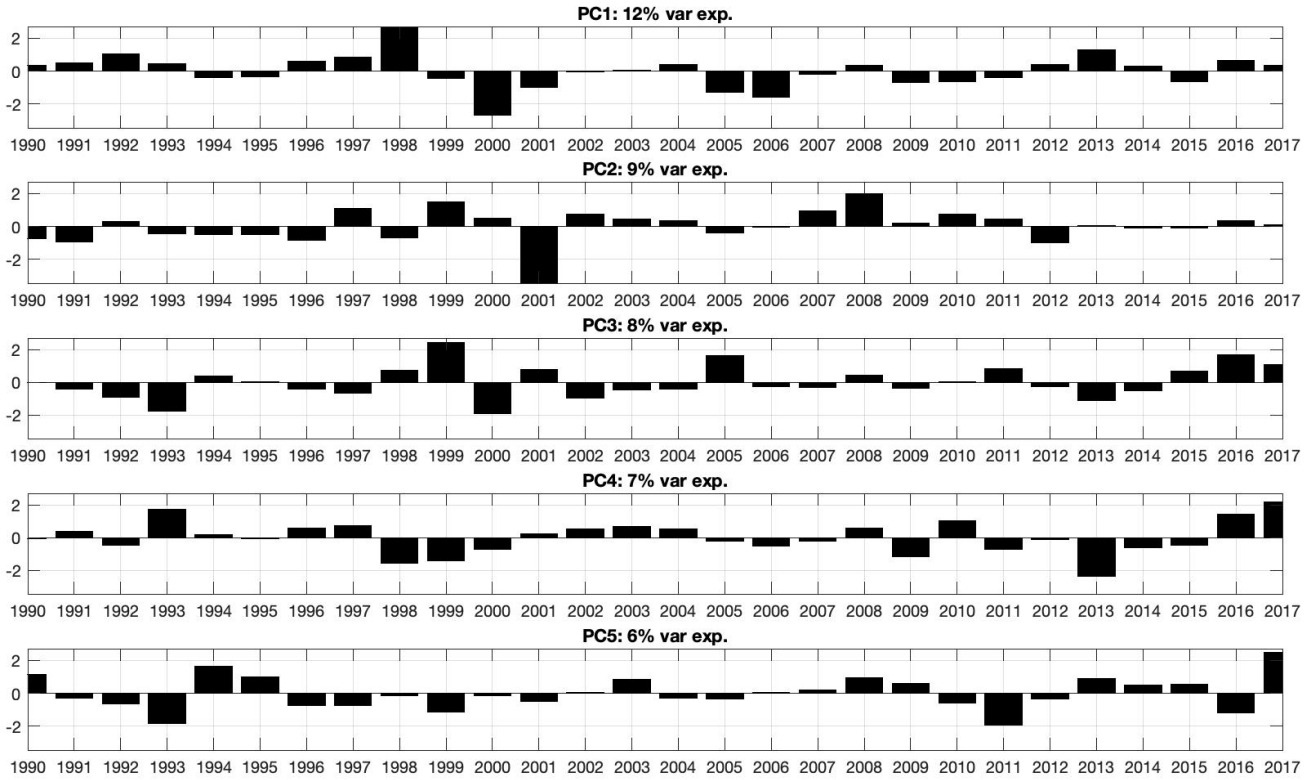


Figure 11. Timing of annual first detection of PST by Closure Zone organized from earliest to latest.

This figure also demonstrates the variable ranges in the timing of first PST detection in different closure zones.

Figure 11a. North Basin.

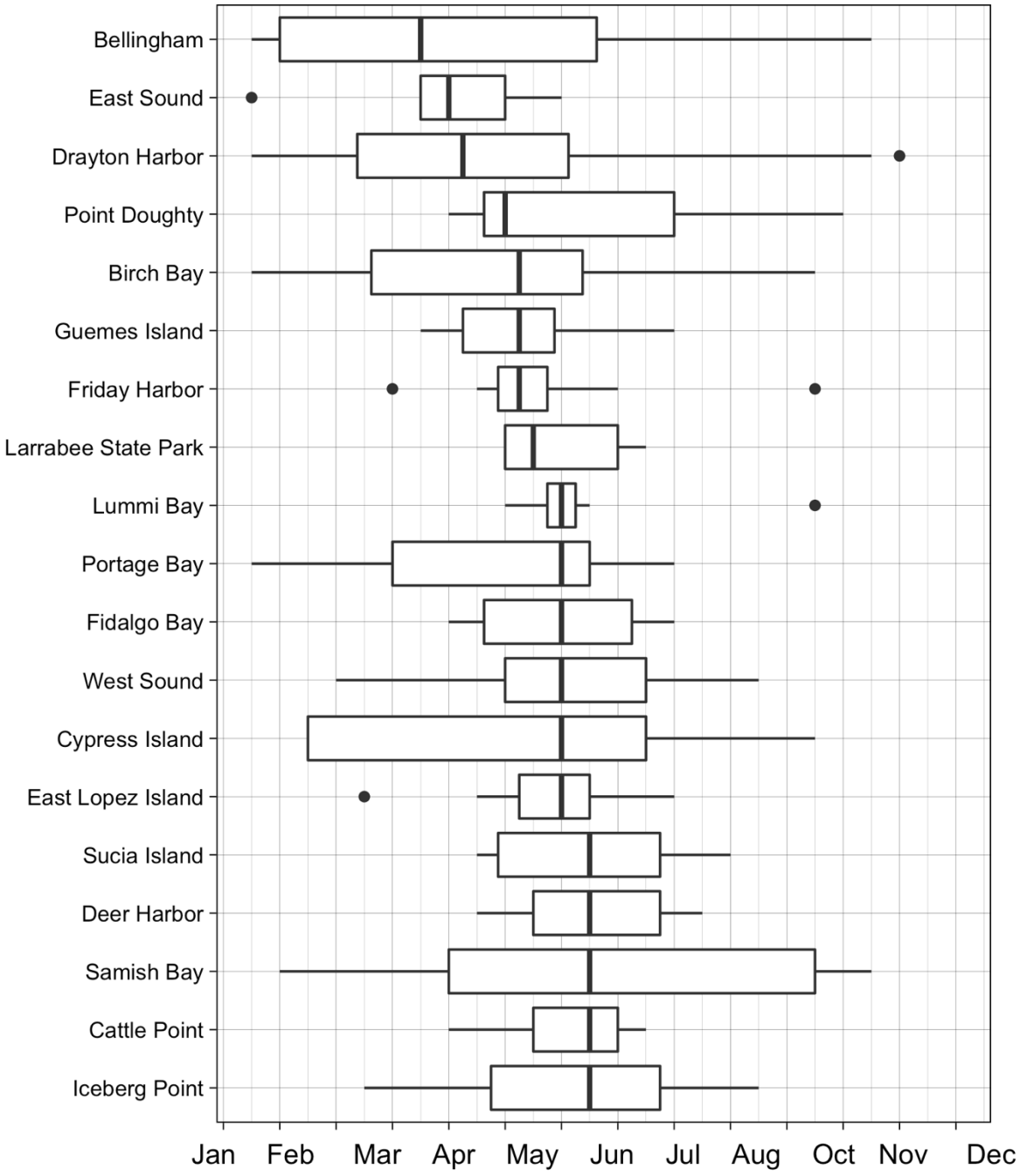


Figure 11b. Central Basin.

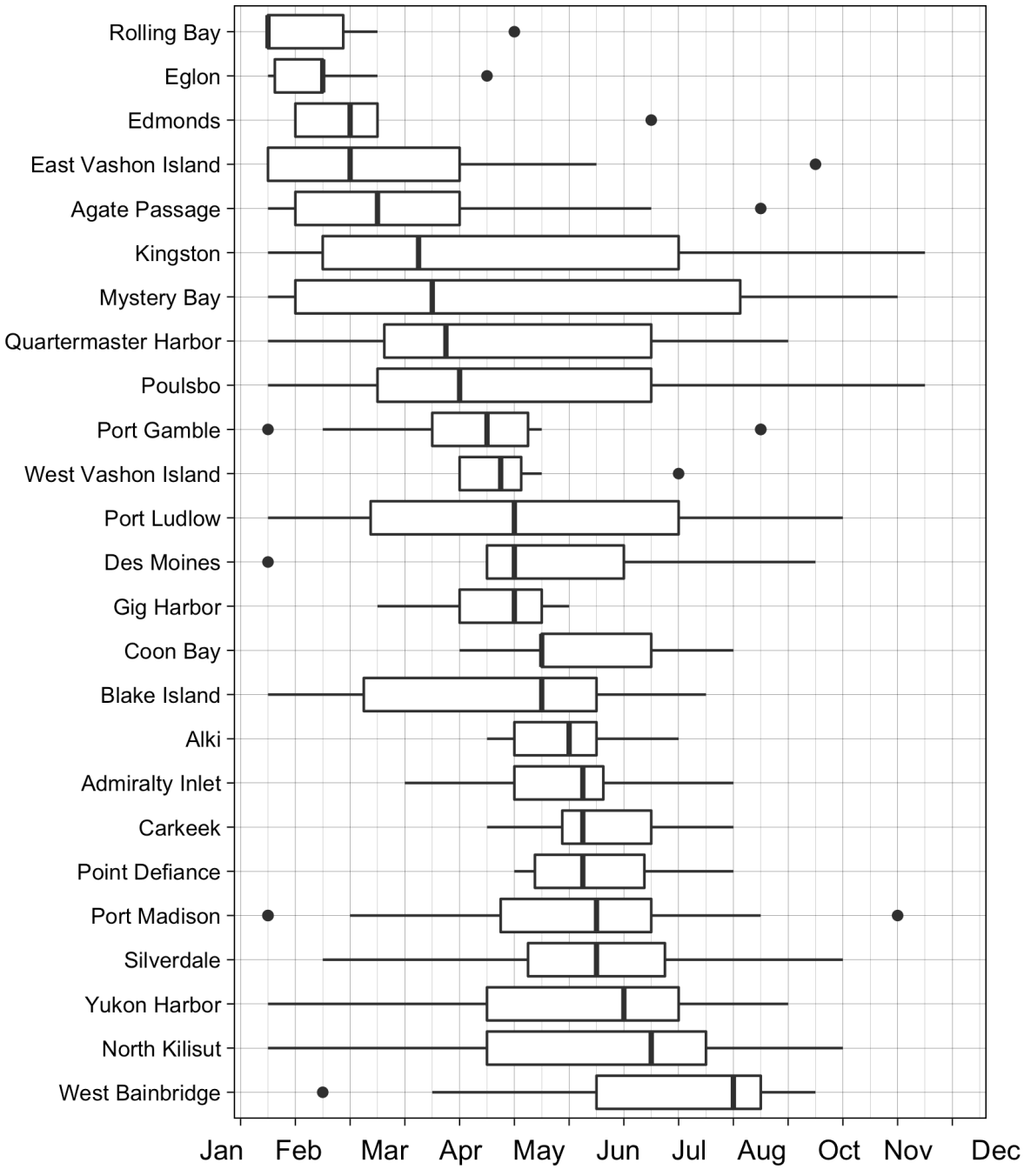


Figure 12. Timing of annual first detection of PST by Closure Zone sorted from North to South by Latitude.

Only North and Central subbasins are included since other subbasins Salish Sea are either much more geographically complex (South Basin) or had too few sites (Northwest, Hood Canal, Whidbey). This figure also demonstrates the variable ranges in the timing of first PST detection in different closure zones.

Figure 12a. North Basin.

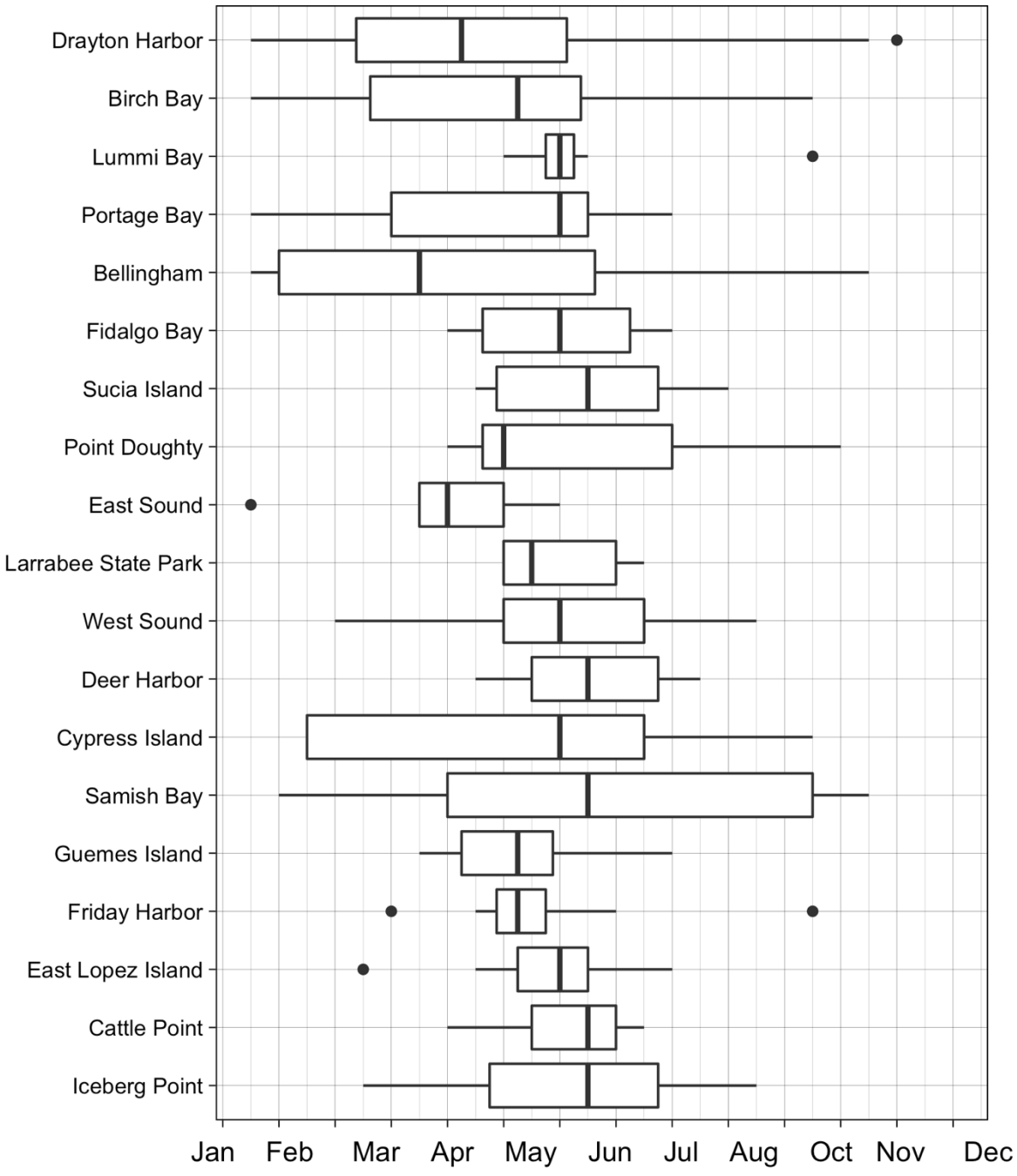


Figure 12b. Central Basin.

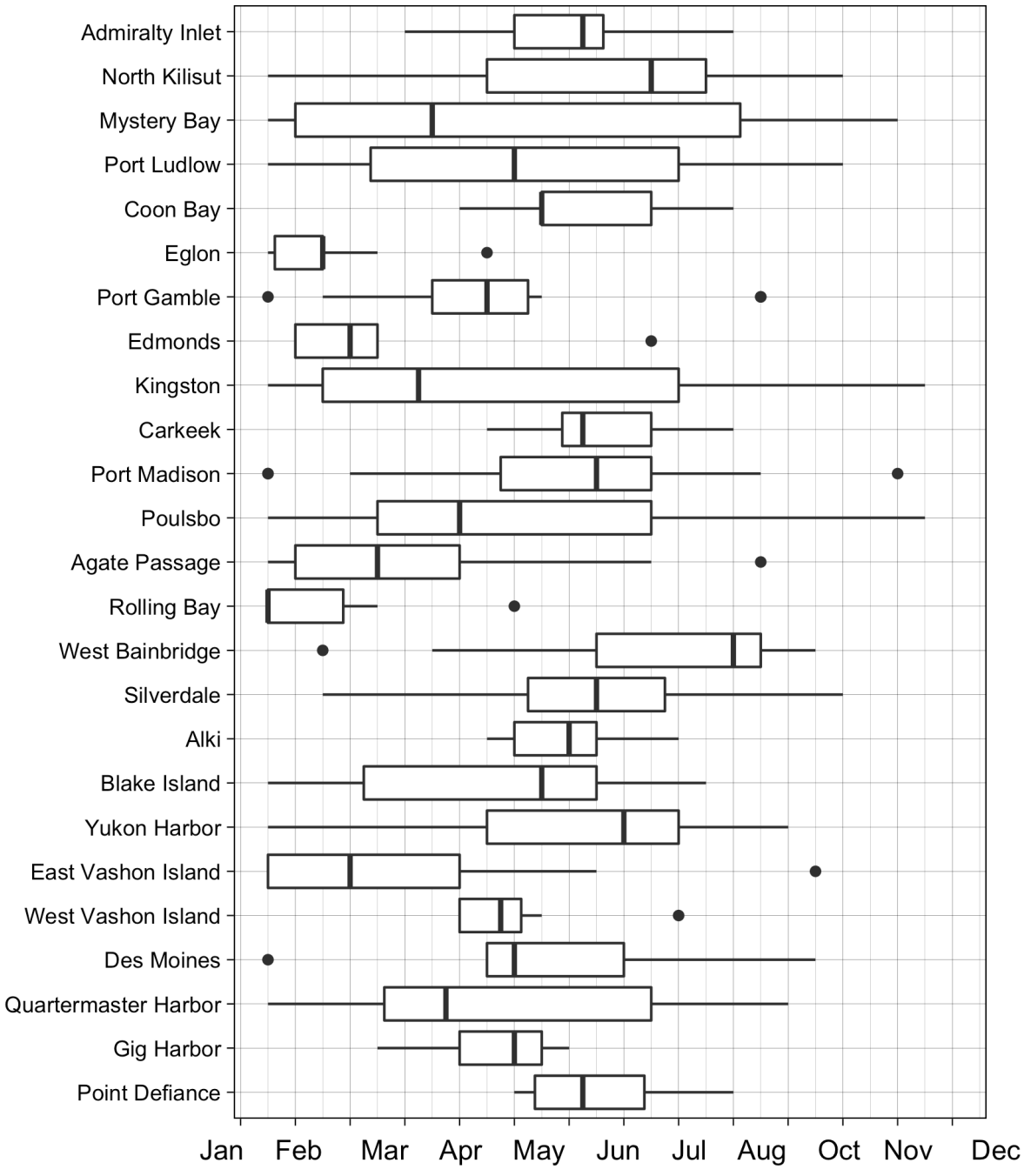


Figure 13. Cyst beds with more than 100 cysts/cm^3 measured in early 2011 and closure zones with the first detections of PST in spring 2011.

Stars indicate cysts and salmon color indicates PST first detections. All three closure zones with early PST detection had elevated cyst concentrations spatially overlapping or adjacent.

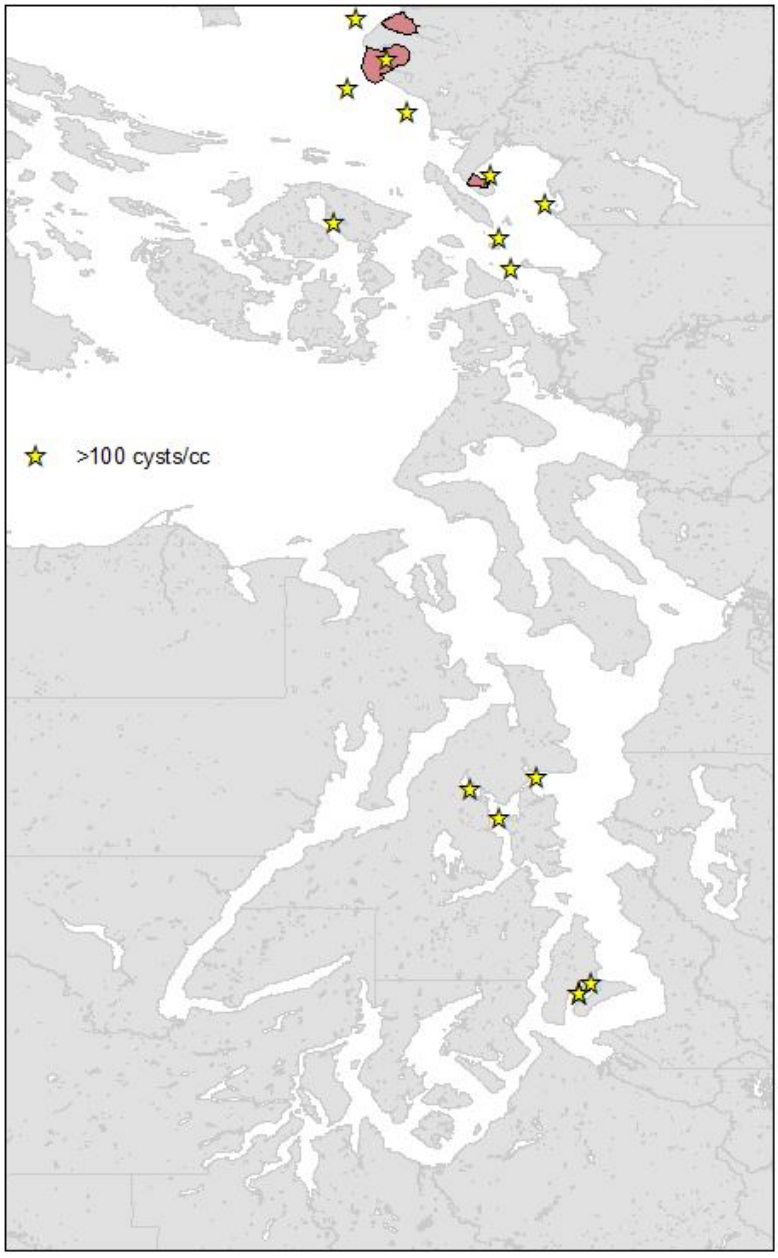


Figure 14. Cyst beds with more than 100 cysts/cm^3 measured in early 2012 and closure zones with the first detections of PST in spring 2012.

Stars indicate cysts and salmon color indicates PST first detections. Only one closure zone with early PST detection had an elevated cyst concentration nearby. Northern sites, Drayton Harbor and Birch Bay, did not have corresponding cyst beds although, like the previous year, they were among the first closure zones with detectable PST.

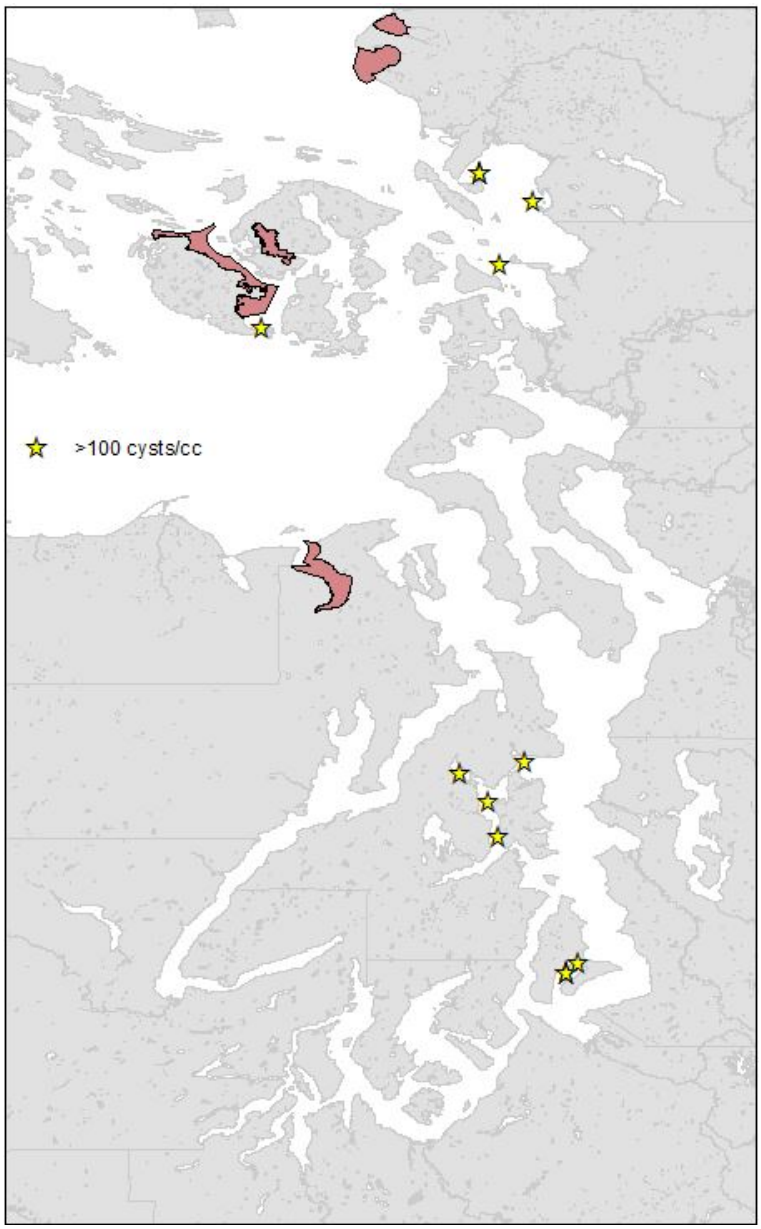


Figure 15. Cyst beds with more than 100 cysts/cm^3 measured in early 2013 and closure zones with the first detections of PST in spring 2013.

Stars indicate cysts and salmon color indicates PST first detections. Discovery Bay was the only closure zone with an elevated concentration of cysts nearby. This site was also one of the earliest detections in 2012, despite cysts not being measured in high abundance that spring.

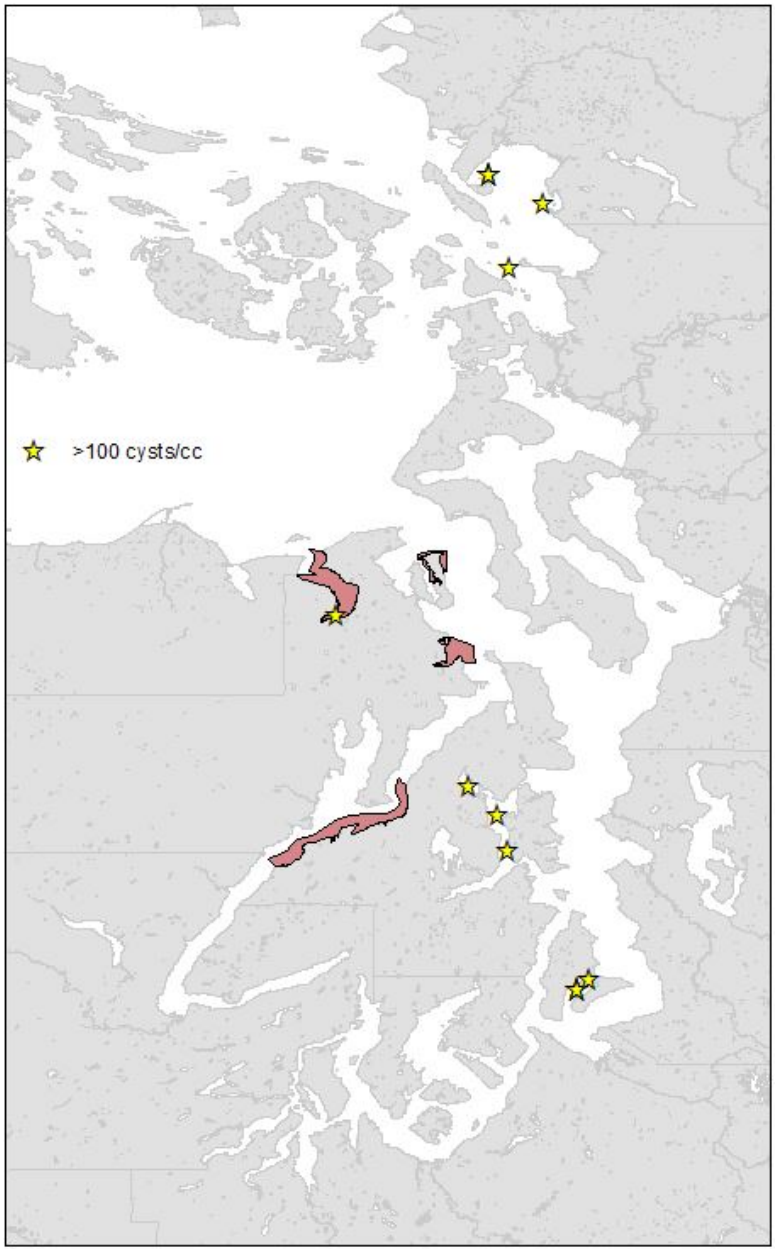
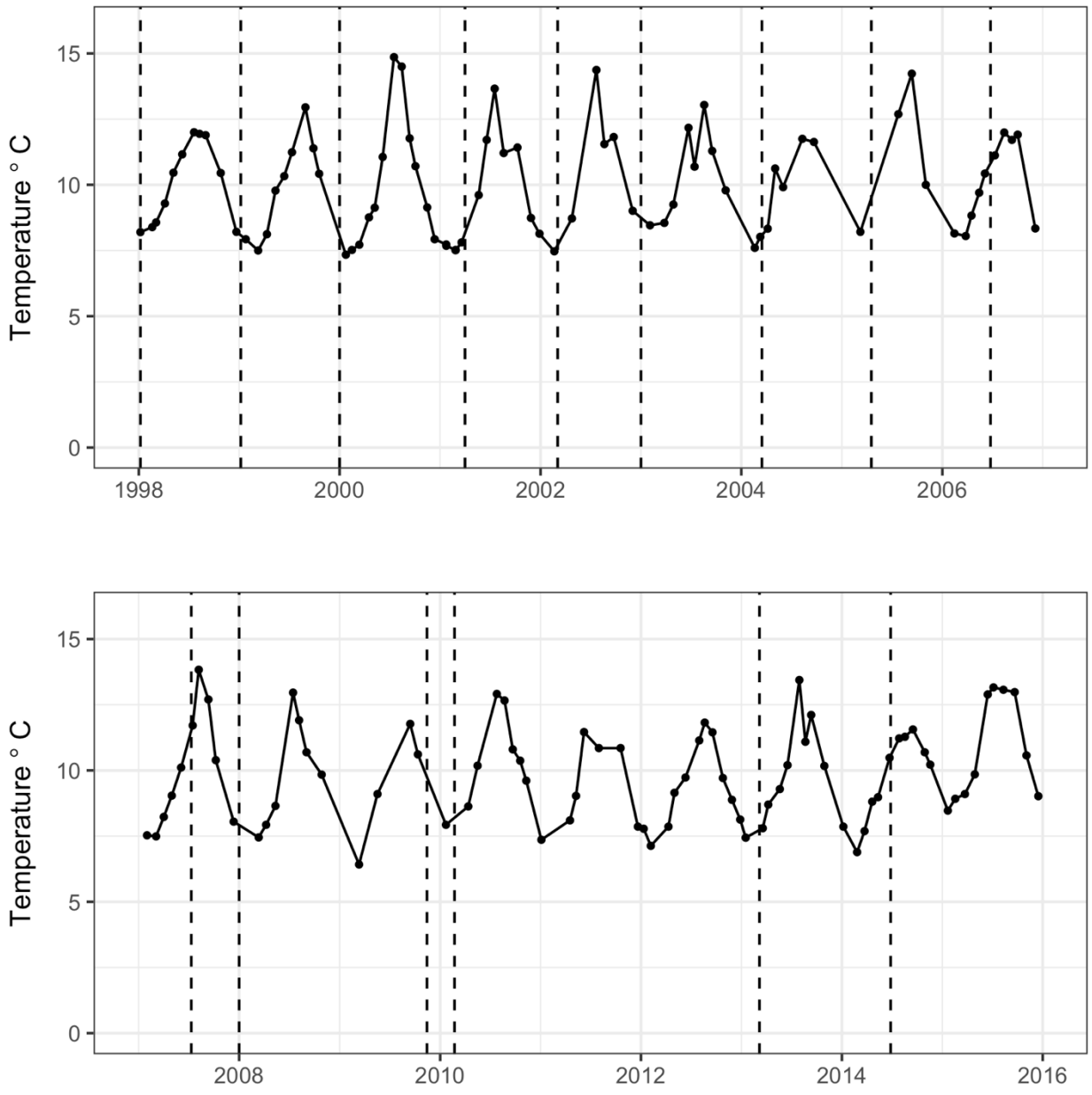


Figure 16. Monthly grab sample temperatures in Bellingham Bay (points and solid line) compared to date of bloom initiation (vertical dashed lines).

There was no clear relationship between either absolute temperature or bloom timing, nor was there a relationship with seasonal minimum/maximum temperature. However, temperature data was insufficient for a statistical analysis.



Appendix A. All closure zone initiation date trends.

CLOSURE ZONE	R-value	P-value	Mean	Stad. Dev.	# Years Incl.
Sequim Bay	-0.6547509	0.00051715	7.16666667	5.53774924	24
Edmonds	-0.9579452	0.00068098	5	4.61880215	7
Mystery Bay	-0.7449366	0.00092946	10.125	8.16394513	16
Quartermaster Harbor	-0.5879148	0.00400797	9.68181818	5.4892654	22
Des Moines	-0.6342624	0.0062465	11.5294118	4.70528489	17
North Kilisut	-0.5067086	0.01906961	13.2380952	6.07375306	21
Birch Bay	-0.522574	0.02608437	9.72222222	5.6235383	18
Rich Passage	-0.531124	0.03425932	12.5	7.22956891	16
East Vashon Island	0.40761103	0.06663568	5.52380952	5.18284717	21
Eglon	-0.4275504	0.07675485	2.88888889	1.99672935	18
Rolling Bay	-0.4877717	0.07683483	2.57142857	2.56347978	14
Raft Island	0.53523709	0.08975586	13.6363636	5.73188847	11
Gig Harbor	0.39758439	0.09185496	9.31578947	1.88716812	19
Yukon Harbor	-0.4573461	0.1161074	12.8461539	5.87148695	13
Deer Harbor	-0.4603497	0.15420404	13.0909091	2.73695252	11
Bellingham	0.37137607	0.15670017	8.1875	6.52399418	16
Pitt Passage	-0.4190964	0.17506849	14.1666667	8.67423912	12
Blake Island	-0.4141216	0.20542878	8.90909091	5.48551812	11
Poulsbo	0.37576395	0.2057548	10.6153846	7.99519086	13
East Lopez Island	-0.2918255	0.22540239	11.8421053	2.5660856	19
West Vashon Island	0.33038048	0.22909613	10.0666667	2.34419242	15
Drayton Harbor	-0.2563522	0.27528032	9.35	6.22621791	20
Penrose	-0.3902444	0.29910621	11.7777778	6.11918658	9
Discovery Bay	-0.1975502	0.33336923	8.15384615	6.04445073	26
South Burley Lagoon	0.2613493	0.34676387	12.9333333	5.99364743	15
Jamestown	0.22462655	0.35520274	7.21052632	3.77975524	19
Samish Bay	0.53242598	0.35564525	13.4	8.79204186	5
Port Gamble	0.28606055	0.39378522	9.36363636	5.59057649	11
Griffiths Point	-0.3845055	0.39440669	12.5714286	7.95523188	7
Fidalgo Bay	-0.2908262	0.41495029	12	2.98142397	10
East Sound	0.46791057	0.42675373	7.6	4.15932687	5
Cypress Island	0.2923041	0.44530833	11.1111111	6.77208322	9
Point Doughty	-0.3806032	0.456662	12.8333333	5.74166062	6
West Sound	0.28492079	0.45741009	12.4444444	4.74634362	9
Port Ludlow	-0.1575728	0.46212665	10.4166667	6.63270334	24
Sucia Island	0.36826467	0.47257482	13	3.68781778	6

CLOSURE ZONE	R-value	P-value	Mean	Stad. Dev.	# Years Incl.
Holmes Harbor	0.4220044	0.47909118	7.6	6.65582452	5
Eagle Harbor	0.22533522	0.48133232	12.4166667	8.28424939	12
Alki	0.27813451	0.50476882	11.875	2.23207143	8
Middle Ground	-0.3304571	0.52235767	10.1666667	3.37144875	6
Hale Passage	-0.2609312	0.53250738	12.25	3.69362385	8
Portage Bay	0.16571255	0.55503789	9.73333333	4.80277697	15
Agate Passage	-0.1353163	0.55866399	5.57142857	4.84325746	21
Lummi Bay	0.24130003	0.56481799	12.875	3.39905449	8
Larrabee State Park	-0.2112588	0.58531374	11.7777778	1.98606255	9
Carkeek	-0.1448944	0.59237124	13.25	2.56904652	16
South Narrows	-0.2770286	0.59508738	5	3.74165739	6
Silverdale	-0.1434599	0.61000429	12.6666667	5.23268119	15
South Indian Island	0.29371405	0.63148038	12.8	10.4259292	5
Cattle Point	-0.1493778	0.66112059	12.3636364	2.15743956	11
Kingston	0.09290648	0.6809078	9.40909091	7.52556969	22
North Bay	-0.2532393	0.68104598	14.6	9.39680797	5
Gorst	-0.1874912	0.68726988	11.5714286	7.87098348	7
Coon Bay	-0.245414	0.69069476	12.6	3.91152144	5
West Bainbridge	0.10352673	0.69254491	15.2941177	5.30052717	17
Admiralty Inlet	-0.1519224	0.71949827	12	3.50509833	8
Port Madison	-0.0903966	0.72130252	12.1111111	6.35136751	18
Purdy	0.12808616	0.74260616	13.4444444	4.39064662	9
Friday Harbor	0.08952812	0.78201458	11.25	3.67114052	12
Port Washington	0.1239537	0.79118251	17.1428571	6.91444313	7
Iceberg Point	-0.0581086	0.83702385	12.7333333	4.36653944	15
Point Defiance	-0.0165453	0.9533315	12.7333333	2.63131327	15
Treble Point	-0.0203244	0.96550341	15.5714286	6.02376247	7
Guemes Island	0.00171126	0.99625662	10.6	2.67498702	10

Appendix B. R code.

B.1. Figure 4: Barplot of number of closure zones with at least five datapoints.

```
### SETUP & DATA CLEANING ###################################

setwd("~/Documents/_GradSchool/Thesis_Research/Thesis_data")

psp_first <- read.csv("final_final_attempt/pspdata_max_cz.csv")
psp_first <- psp_first[,c("C_ZNAME", "Subbasin")]
psp_sites <- unique(psp_sites)

psp_5data <- read.csv("final_final_attempt/trends_cz_closure.csv")

psp_5data <- merge(psp_5data, psp_sites, by.x="C_ZNAME")

table(psp_5data$Subbasin)

subbasin <- c("NW", "N", "W", "C", "HC", "S")
freq <- c(5,21,3,33,0,12)
subbasin_freq <- data.frame(subbasin, freq)


### PLOTTING ################################################

library(ggplot2)

ggplot(data.frame(subbasin_freq), aes(x=reorder(subbasin, -freq), y=freq)) +
  geom_bar(stat="identity", fill="darkgray") + theme_bw() +
  coord_flip() + ylab("") + xlab("") +
  geom_text(aes(label=freq), vjust=1, hjust=2, color="white", size=3.5) +
  scale_x_discrete(labels = rev(c("Hood Canal", "Whidbey", "Northwest",
                                  "South", "North", "Central")))
```

B.2. Trends analysis using simple first closure (not subject to conditions in Figure 4).

```
### Set Working Directory & Upload files ####################

setwd("~/Documents/_GradSchool/Thesis_Research/Thesis_data")

psp_first <- read.csv("final_attempt/pspdata_max_cz.csv")
# data grouped by closure zone
# in the case of multiple closure zone data points in a biweek, the maximum
 toxicity was selected


### First Closures Only #####################################

psp_first <- psp_first[psp_first$PSP_Result > 79,]
# excludes data points below the regulatory closure limit
# change to 37 to exclude all non-detects

psp_first <- aggregate(bi_week ~ C_ZNAME + Year, data = psp_first, FUN = min)
# selects first appearance of PST in tissue by closure zone and year


### Exclude <5 datapoints ###################################

CZ_freq <- as.data.frame(sort(table(psp_first$C_ZNAME)))
CZ_freq <- CZ_freq[CZ_freq$Freq > 4,]
write.csv(CZ_freq, "final_attempt/simple_first_closure/CZ_freq.csv")
# manual formatting in Excel

CZ_freq <- read.csv("final_attempt/simple_first_closure/CZ_freq.csv")
CZ_names <- CZ_freq$Var1

#psp_first <- psp_first[psp_first$C_ZNAME == CZ_freq$Var1,]


### Determine Trends ########################################

summ_CZ <- function(l){
  site<-psp_first[psp_first$C_ZNAME==l,]
  x <- cor.test(site$bi_week, site$Year, method = "pearson")
  y <- mean(site$bi_week)
  z <- sd(site$bi_week)
  site_results <- data.frame(x$estimate, x$p.value, y, z)
  names(site_results) <- c("r.value", "p.value", "mean", "sd")
  write.table(site_results,
 "final_attempt/simple_first_closure/trends_cz_closure.csv", sep = ",",
              col.names= FALSE, append = TRUE)
}

for(i in CZ_names){
  summ_CZ(i)
}

dev.off()
```

B.3. Trends analysis and regression plots

```
### Set Working Directory and Upload Files ###############

setwd("~/Documents/_GradSchool/Thesis_Research/Thesis_data")

psp_first <- read.csv("final_attempt/init_trend/pspdata_max_cz_nosite.csv")

psp_first <- psp_first[psp_first$Init == "y",] #Initiation Weeks only


### List of Closure Zones w/ at least 5 datapoints ######

CZ_freq <- as.data.frame(sort(table(psp_first$C_ZNAME)))
CZ_freq <- CZ_freq[CZ_freq$Freq > 4,]
write.csv(CZ_freq, "final_attempt/init_trend/CZ_freq.csv")
CZ_freq <- read.csv("final_attempt/init_trend/CZ_freq.csv")
CZ_names <- CZ_freq$Var1


### Determine Trends ###################################

summ_CZ <- function(l){
  site<-psp_first[psp_first$C_ZNAME==l,]
  x <- cor.test(site$bi_week, site$Year, method = "pearson")
  y <- mean(site$bi_week)
  z <- sd(site$bi_week)
  site_results <- data.frame(x$estimate, x$p.value, y, z)
  names(site_results) <- c("r.value", "p.value", "mean", "sd")
  write.table(site_results, "final_attempt/init_trend/trends_cz_detect2.csv",
 sep = ",",
              col.names= FALSE, append = TRUE)
}

for(i in CZ_names){
  summ_CZ(i)
}

dev.off()



### Plot Closure Zones with Significant Trends ###############

library("ggplot2")

d <- psp_first[psp_first$C_ZNAME == "Edmonds",]
p1 <- ggplot(data = d, aes(x=Year, y=bi_week)) + geom_point(size=2) +
  ylim(0,26) + theme_bw() + labs(x="", y="Biweek", title = "Edmonds") +
  geom_smooth(method = "lm", se=F, colour="darkgray") +
  annotate("text", x = 2000, y = 24, label = "p = 0.0007")

d <- psp_first[psp_first$C_ZNAME == "Raft Island",]
p2 <- ggplot(data = d, aes(x=Year, y=bi_week)) + geom_point(size=2) +
  ylim(0,26) + theme_bw() + labs(x="", y="Biweek", title = "Raft Island") +
  geom_smooth(method = "lm", se=F, colour="darkgray") +
  annotate("text", x = 2000, y = 24, label = "p = 0.0247")
```

```
d <- psp_first[psp_first$C_ZNAME == "Samish Bay",]
p3 <- ggplot(data = d, aes(x=Year, y=bi_week)) + geom_point(size=2) +
  ylim(0,26) + xlim(1998,2017) +
  theme_bw() + labs(x="", y="Biweek", title = "Samish Bay") +
  geom_smooth(method = "lm", se=F, colour="darkgray") +
  annotate("text", x = 2000, y = 24, label = "p = 0.0293")

d <- psp_first[psp_first$C_ZNAME == "Agate Passage",]
p4 <- ggplot(data = d, aes(x=Year, y=bi_week)) + geom_point(size=2) +
  ylim(0,26) + theme_bw() + labs(x="", y="Biweek", title = "Agate Passage") +
  geom_smooth(method = "lm", se=F, colour="darkgray") +
  annotate("text", x = 2000, y = 24, label = "p = 0.0524")

d <- psp_first[psp_first$C_ZNAME == "Discovery Bay",]
p5 <- ggplot(data = d, aes(x=Year, y=bi_week)) + geom_point(size=2) +
  ylim(0,26) + theme_bw() + labs(x="", y="Biweek", title = "Discovery Bay") +
  geom_smooth(method = "lm", se=F, colour="darkgray") +
  annotate("text", x = 2000, y = 24, label = "p = 0.0533")

d <- psp_first[psp_first$C_ZNAME == "Bellingham",]
p6 <- ggplot(data = d, aes(x=Year, y=bi_week)) + geom_point(size=2) +
  ylim(0,26) + theme_bw() + labs(x="", y="Biweek", title = "Bellingham") +
  geom_smooth(method = "lm", se=F, colour="darkgray") +
  annotate("text", x = 2000, y = 24, label = "p = 0.0657")


grid.arrange(p1,p2,p3,p4,p5,p6, ncol=3)
```

B.4. Temperature plots.

```
setwd("~/Documents/_GradSchool/Thesis_Research/Thesis_data")

library("ggplot2")

psp_first <- read.csv("final_attempt/init_trend/pspdata_max_cz_nosite.csv")
psp_first <- psp_first[psp_first$Init == "y",]


### Plot Bham Bay Temps ############################

bham_psp <- psp_first[psp_first$C_ZNAME == "Bellingham",]

bham <-
 read.csv("final_attempt/EnvData/Bellingham_raw/Bellingham_Bay_EOPS.csv")

bham <- bham[c(2,4,6)]
bham$date <- as.Date(bham$date, "%m/%d/%y")

bham_deep <- aggregate(depth_m ~ date, data=bham, FUN = max) #select deepest
 pts
bham_deep <- merge(bham_deep, bham) #add temp back in

bham_deep$year <- lubridate::year(bham_deep$date)
bham_deep <- bham_deep[bham_deep$year > 1997,]
bham_deep <- bham_deep[bham_deep$temp_c > 0,]

bham_plot <- ggplot(bham_deep, aes(x=date, y=temp_c, group=1)) + geom_line()
 +
  geom_point(pch=20) + theme_bw() +
  xlim(as.Date(c("1998-01-01", "2002-12-31"))) + ylim(0,16) +
  xlab("") + ylab("Temp C") + ggtitle("Bellingham Bay Temp & PSP Initiation")

bham_plot + geom_vline(xintercept=as.Date("1998-01-07"), lty=2) +
  geom_vline(xintercept=as.Date("1999-01-07"), lty=2) +
  geom_vline(xintercept=as.Date("2000-01-01"), lty=2) +
  geom_vline(xintercept=as.Date("2001-04-01"), lty=2) +
  geom_vline(xintercept=as.Date("2002-03-04"), lty=2)



### Plot Dockton Temps ############################

dockton <- read.csv("final_attempt/EnvData/dockton.csv")

dockton <- dockton[,c(1,4)]
dockton <- dockton[dockton$Water_Temperature_degC <16,]
dockton$Date <- as.Date(dockton$Date, "%m/%d/%y")
dockton <- aggregate(dockton, by=list(dockton$Date),
                     FUN = mean, na.action = na.omit)
dockton <- dockton[,c(2,3)]
names(dockton) <- c("Date", "Temp")

ggplot(dockton, aes(x=Date, y=Temp, group=1)) + geom_line() +
  ylim(0,16) + xlab("") + theme_bw()
```

```
### Calc Dockton Temps ########################

dockton$Week <- lubridate::week(dockton$Date)

dockton_agg <- aggregate(Temp ~ Week, dockton, mean)

write.csv(dockton_agg,
 "final_attempt/EnvData/dockton_temp_week_aggregate.csv")

ggplot(dockton_agg, aes(x=Week, y=Temp, group=1)) + geom_line() +
  xlab("Week") #aggregated weekly temp plot



### Calc Dockton Temp Anomolies ###############################

dockton_agg <-
 read.csv("final_attempt/EnvData/dockton_temp_week_aggregate.csv")

dockton <- dockton[complete.cases(dockton),]

dockton <- merge(dockton, dockton_agg, by="Week")
names(dockton) <- c("Week", "Date", "Temp", "X", "Avg_Temp")
dockton$Date <- as.Date(dockton$Date, "%Y-%m-%d")
dockton$Year <- lubridate::year(dockton$Date)
dockton <- dockton[order(dockton$Date),]

dockton$Temp_Var <- (dockton$Temp - dockton$Avg_Temp) / dockton$Avg_Temp
 #calc variance

dockton <- aggregate(dockton, by=list(dockton$Date),
                     FUN = mean, na.action = na.omit) #aggregate daily
 variance

ggplot(dockton, aes(x=Date, y=Temp_Var, group=1)) + geom_line() +
  ylim(-.5,.5) + xlab("") + ylab("Temp Variance") +
  ggtitle("Water Temperature Variance - Dockton") +
  theme_bw() #temp anomolies


### Plot Dockton Temps by Year ###################

library("gridExtra")

quartermaster <- psp_first[psp_first$C_ZNAME == "Quartermaster Harbor",]

dockton_byyear <- dockton[dockton$Year == "2009",]
dockton_byyear <- dockton_byyear[dockton_byyear$Week < 18,]

ggplot(dockton_byyear, aes(x=Date, y=Temp_Var, group=1)) + geom_line() +
  ylim(-.5,.5) + xlab("") + ylab("Temp Variance") +
  ggtitle("Water Temperature Variance - Dockton") +
  theme_bw() #temp anomolies


#2009
dockton_byyear <- dockton[dockton$Year == "2009",]
dockton_byyear <- dockton_byyear[dockton_byyear$Week < 18,]
```

```
dockton_2009 <- ggplot(dockton_byyear, aes(x=Date, y=Temp, group=1)) +
  geom_line() + theme_bw() +
  ylim(0,12) + xlim(as.Date(c("2009-01-01", "2009-04-30"))) +
  xlab("") + ggtitle("Water Temp @ Dockton - 2009") +
  geom_vline(xintercept=as.Date("2009-02-04"), lty=2)

#2010
dockton_byyear <- dockton[dockton$Year == "2010",]
dockton_byyear <- dockton_byyear[dockton_byyear$Week < 18,]
dockton_2010 <- ggplot(dockton_byyear, aes(x=Date, y=Temp, group=1)) +
  geom_line() + theme_bw() +
  ylim(0,12) + xlim(as.Date(c("2010-01-01", "2010-04-30"))) +
  xlab("") + ggtitle("Water Temp @ Dockton - 2010") +
  geom_vline(xintercept=as.Date("2010-01-07"), lty=2)

#2011
dockton_byyear <- dockton[dockton$Year == "2011",]
dockton_byyear <- dockton_byyear[dockton_byyear$Week < 18,]
dockton_2011 <- ggplot(dockton_byyear, aes(x=Date, y=Temp, group=1)) +
  geom_line() + theme_bw() +
  ylim(0,12) + xlim(as.Date(c("2011-01-01", "2011-04-30"))) +
  xlab("") + ggtitle("Water Temp @ Dockton - 2011") +
  geom_vline(xintercept=as.Date("2011-04-08"), lty=2)

#2012
dockton_byyear <- dockton[dockton$Year == "2012",]
dockton_byyear <- dockton_byyear[dockton_byyear$Week < 18,]
dockton_2012 <- ggplot(dockton_byyear, aes(x=Date, y=Temp, group=1)) +
  geom_line() + theme_bw() +
  ylim(0,12) + xlim(as.Date(c("2012-01-01", "2012-04-30"))) +
  xlab("") + ggtitle("Water Temp @ Dockton - 2012")

#2013
dockton_byyear <- dockton[dockton$Year == "2013",]
dockton_byyear <- dockton_byyear[dockton_byyear$Week < 18,]
dockton_2013 <- ggplot(dockton_byyear, aes(x=Date, y=Temp, group=1)) +
  geom_line() + theme_bw() +
  ylim(0,12) + xlim(as.Date(c("2013-01-01", "2013-04-30"))) +
  xlab("") + ggtitle("Water Temp @ Dockton - 2013") +
  geom_vline(xintercept=as.Date("2013-02-11"), lty=2)

#2014
dockton_byyear <- dockton[dockton$Year == "2014",]
dockton_byyear <- dockton_byyear[dockton_byyear$Week < 18,]
dockton_2014 <- ggplot(dockton_byyear, aes(x=Date, y=Temp, group=1)) +
  geom_line() + theme_bw() +
  ylim(0,12) + xlim(as.Date(c("2014-01-01", "2014-04-30"))) +
  xlab("") + ggtitle("Water Temp @ Dockton - 2014") +
  geom_vline(xintercept=as.Date("2014-01-21"), lty=2)

#2015
dockton_byyear <- dockton[dockton$Year == "2015",]
dockton_byyear <- dockton_byyear[dockton_byyear$Week < 18,]
dockton_2015 <- ggplot(dockton_byyear, aes(x=Date, y=Temp, group=1)) +
  geom_line() + theme_bw() +
  ylim(0,12) + xlim(as.Date(c("2015-01-01", "2015-04-30"))) +
  xlab("") + ggtitle("Water Temp @ Dockton - 2015")
```

```
#2016
dockton_byyear <- dockton[dockton$Year == "2016",]
dockton_byyear <- dockton_byyear[dockton_byyear$Week < 18,]
dockton_2016 <- ggplot(dockton_byyear, aes(x=Date, y=Temp, group=1)) +
  geom_line() + theme_bw() +
  ylim(0,12) + xlim(as.Date(c("2016-01-01", "2016-04-30"))) +
  xlab("") + ggtitle("Water Temp @ Dockton - 2016") +
  geom_vline(xintercept=as.Date("2016-01-21"), lty=2)

#2017
dockton_byyear <- dockton[dockton$Year == "2017",]
dockton_byyear <- dockton_byyear[dockton_byyear$Week < 18,]
dockton_2017 <- ggplot(dockton_byyear, aes(x=Date, y=Temp, group=1)) +
  geom_line() + theme_bw() +
  ylim(0,12) + xlim(as.Date(c("2017-01-01", "2017-04-30"))) +
  xlab("") + ggtitle("Water Temp @ Dockton - 2017") +
  geom_vline(xintercept=as.Date("2017-01-28"), lty=2)


grid.arrange(dockton_2009, dockton_2010, dockton_2011, ncol=1)
grid.arrange(dockton_2012, dockton_2013, dockton_2014, ncol=1)
grid.arrange(dockton_2015, dockton_2016, dockton_2017, ncol=1)


quartermaster <- psp_first[psp_first$C_ZNAME == "Quartermaster Harbor",]



################################################
### SEATTLE ####################################

### Plot Seattle Temps ######################

seattle <- read.csv("final_attempt/EnvData/Seattle_temp.csv")

seattle$Date <- as.Date(seattle$Date, "%m/%d/%y")
seattle <- aggregate(seattle, by=list(seattle$Date),
                     FUN = mean, na.action = na.omit)
seattle <- seattle[,c(3,4)]
seattle$Year <- lubridate::year(seattle$Date)
seattle <- seattle[seattle$Year > 1997,]


ggplot(seattle, aes(x=Date, y=Temp, group=1)) + geom_line() +
  ylim(40,60) + xlab("")



### Calc Seattle Temps ########################

seattle <- read.csv("final_attempt/EnvData/Seattle_temp.csv")

seattle$Date <- as.Date(seattle$Date, "%m/%d/%y")
seattle$Week <- lubridate::week(seattle$Date)
seattle <- seattle[,c(3,4)]

seattle <- aggregate(Temp ~ Week, seattle, mean)
write.csv(seattle, "final_attempt/EnvData/Seattle_temp_week_aggregate.csv")
```

```
ggplot(seattle, aes(x=Week, y=Temp, group=1)) + geom_line() +
  ylim(40,60) + xlab("Week") #aggregated 20-year temp plot


### Calc Seattle Temp Anomolies ##################

seattle <- read.csv("final_attempt/EnvData/Seattle_temp.csv")
seattle_aggreg <-
 read.csv("final_attempt/EnvData/Seattle_temp_week_aggregate.csv")

seattle <- seattle[complete.cases(seattle),]

seattle <- merge(seattle, seattle_aggreg, by="Week")
names(seattle) <- c("Week", "DateTime", "Date", "Temp", "Avg_Temp")
seattle$Date <- as.Date(seattle$Date, "%m/%d/%y")
seattle$Year <- lubridate::year(seattle$Date)
seattle <- seattle[seattle$Year > 1997,]
seattle <- seattle[order(seattle$Date),]

seattle$Temp_Var <- (seattle$Temp - seattle$Avg_Temp) / seattle$Avg_Temp
 #calc variance

seattle <- aggregate(seattle, by=list(seattle$Date),
                     FUN = mean, na.action = na.omit) #aggregate daily
 variance

ggplot(seattle, aes(x=Date, y=Temp_Var, group=1)) + geom_line() +
  ylim(-.1,.1) + xlab("") + ylab("Temp Variance") +
  ggtitle("Water Temperature Variance - Seattle") +
  theme_bw() #temp anomolies



### Plot Anomolies by Year ########################

seattle_byyear <- seattle[seattle$Year == "1998",]

seattle_byyear <- aggregate(seattle_byyear, by=list(seattle_byyear$Week),
                     FUN = mean, na.action = na.omit) #aggregate weekly
 variance

ggplot(seattle_byyear, aes(x=Week, y=Temp_Var, group=1)) + geom_line() +
  ylim(-.1,.1) + xlab("") + ylab("Temp Variance") +
  ggtitle("Water Temperature Variance - Seattle") + theme_bw() #temp
 anomolies



### Dockton plot part 2

library(lubridate)

dockton <- read.csv("final_attempt/EnvData/dockton.csv")

dockton$Date_num <- c(1:268800)

dockton <- dockton[dockton$Water_Temperature_degC <20,]
```

```
dockton <- dockton[dockton$Water_Temperature_degC >0,]

dockton$Date <- as.Date(dockton$Date, "%m/%d/%y")
dockton$Year <- lubridate::year(dockton$Date)

dockton_2014 <- dockton[dockton$Year == 2014,]

dockton_2014 <- subset(dockton, Year==2014)

ggplot(dockton_2014, aes(x=Date_num, y=Water_Temperature_degC)) + geom_line()
 + theme_bw() +
  theme(axis.text.x = element_blank()) +
  labs(title="2014 Temp @ Dockton", x="Time", y="Temp")


#write.csv(dockton_2014, "final_attempt/EnvData/dockton_2014.csv")
dockton_2014 <- read.csv("final_attempt/EnvData/dockton_2014.csv")


ggplot(dockton_2014, aes(x=Date_num, y=MA25)) + geom_line() + theme_bw() +
  theme(axis.text.x = element_blank()) + ylim(10.3,11) +
  labs(title="2014 Temp @ Dockton", x="Time",
       y="25 Day Moving Average (Tidal Signal Removed)") +
  geom_smooth()


### Race Rocks / Sequim Bay

sequim <- read.csv("final_attempt/EnvData/racerocks.csv")
sequim$Date <- as.Date(sequim$Date, "%m/%d/%y")
sequim$Temp_Sequim <- as.numeric(as.character(sequim$Temp_Sequim))

ggplot(sequim, aes(x=Date, y=Temp_Sequim)) + geom_line() + theme_bw() +
  geom_hline(yintercept=13) + ylim(13,18) +
  xlim(as.Date(c('1998-01-01', '2003-12-31')))
```

B.5. Figures 11 & 12: Boxplots of bloom initiation timings.

```
### Set Working Directory & Upload Files #################

setwd("~/Documents/_GradSchool/Thesis_Research/Thesis_data")

psp_first <- read.csv("final_attempt/init_trend/pspdata_max_cz_nosite.csv")


### Format Data ########################################

psp_first <- psp_first[psp_first$Init == "y",] # Init Weeks only

# Exclude <5 datapoints
CZ_freq <- read.csv("final_attempt/init_trend/CZ_freq.csv")
CZ_names <- CZ_freq$Var1

psp_first <- psp_first[psp_first$C_ZNAME %in% CZ_names,]
```

```
### CZ by subbasin

library(ggplot2)

#psp_CZ_names <- unique(psp_first[,c("C_ZNAME", "Subbasin")])
subbasin_table <- as.data.frame(table(psp_CZ_names$Subbasin))
subbasins <- data.frame(c("Central", "Hood Canal", "North",
               "Northwest", "South", "Whidbey"))
subbasin_table <- cbind(subbasin_table, subbasins)
names(subbasin_table) <- c("Subbasin1", "Freq", "Subbasin2")


ggplot(subbasin_table, aes(x=Subbasin2, y=Freq)) + geom_bar(stat="identity")
 +
  theme_minimal() + coord_flip() +
  xlab("") + ylab("Number of Closure Zones") +
  ggtitle("Number of Closure Zones included by Subbasin") +
  scale_x_discrete(limits = rev(levels(subbasin_table$Subbasin2)))



### Boxplots ##############################################

#Early to Late Closures

library(ggplot2)

month.labels <- c("Jan       Feb      Mar     Apr     May      Jun      Jul
 Aug      Oct      Nov       Dec")

psp_box <- ggplot(psp_first, aes(x=reorder(C_ZNAME, -bi_week, FUN=median),
 y=bi_week)) +
  geom_boxplot() + coord_flip() + xlab("") + ylab(month.labels) +
  theme_linedraw() + theme(plot.margin = unit(c(.5,.5,1,.5), "cm"),
 axis.text.x = element_blank()) +
  scale_y_continuous(breaks=seq(0, 26, 2),limits=c(1, 26)) +
  ggtitle("PSP Closures from Earliest to Latest")
psp_box


#NorthtoSouth

psp_first <- psp_first[order(-psp_first$LAT),]

psp_box <- ggplot(psp_first, aes(x=reorder(C_ZNAME, LAT), y=bi_week)) +
  geom_boxplot() + coord_flip() + xlab("") + ylab(month.labels) +
  theme_linedraw() + theme(plot.margin = unit(c(.5,.5,1,.5), "cm"),
                           axis.text.x = element_blank()) +
  scale_y_continuous(breaks=seq(0, 26, 2),limits=c(1, 26)) +
  ggtitle("PSP Closures from North to South")
psp_box


#NorthtoSouth C & N Subbasins only

northcentral <- c("n", "c")
```

```
psp_first_nc <- psp_first[psp_first$Subbasin %in% northcentral,]

psp_first_nc <- psp_first_nc[order(-psp_first_nc$LAT),]

psp_box <- ggplot(psp_first_nc, aes(x=reorder(C_ZNAME, LAT), y=bi_week)) +
  geom_boxplot() + coord_flip() + xlab("") + ylab(month.labels) +
  theme_linedraw() + theme(plot.margin = unit(c(.5,.5,1,.5), "cm"),
                           axis.text.x = element_blank()) +
  scale_y_continuous(breaks=seq(0, 26, 2),limits=c(1, 26)) +
  ggtitle("PSP Closures from North to South - North/Central Subbasins")
psp_box
```

Appendix C. Matlab code.

C.1. Principle Component Analysis and associated plots (Figures 9 & 10)

Note: The input excel document was produced in Excel using a spreadsheet of bloom initiation biweeks by year and site for those locations with at least five years of data (the same used for the trends analysis). Bloom initiation "anomalies" were determined by subtracting the mean initiation point for each site. Data were then normalized by subtracting the mean initiation biweek for each site from each data point and dividing by the mean initiation biweek for that site.

```
% PCA (using svd function)

[sites, values] = xlsread('psp_pca2_sm.xls');

[ev,lam,pc]=svd(anoms);

% pc = principal components
% lam = eigenvalues
% ev = eigenvectors

% Compute the percent variance explained by the first 5 PCs.
evals=diag(lam);
tot=sum(evals.^2);
for i=1:5
    vf(i)=evals(i)*evals(i)/tot;
end

for n=1:length(sites)
    a(n,:)=auto(anoms(n,:),1);
end
a1=sum(a(:,2))/length(sites);
Neff=dt(end)*(1-a1)/(1+a1);
for m=1:length(evals)
    lam_err(m)=evals(m)*sqrt(2/Neff);
end

% Plot the eigenvalues for the first 5 PCs and the 95% confidence error
figure
for l=1:5
    errorbar(l,evals(l),lam_err(l),'.k'); hold on;
end
plot([1:1:5],evals(1:5),'k');
xlabel('PC')
ylabel('Eigenvalue')
axis([0.5 5.5 0 max(evals)+max(lam_err)]);
set(gca,'xtick',[1:1:5]);

% Compute normalized PC's and rescaled eigenvectors.
nk=5; % just look at the first 5 PCs.
for im=1:nk
    evn(:,im)=ev(:,im)*evals(im)/sqrt(dt(end)-1);
    pcn(:,im)=(pc(:,im)-mean(pc(:,im)))/std(pc(:,im));
```

```
end

% Plot the time series of the first 5 normalized PCs
figure
for ip=1:nk
    subplot(nk,1,ip),bar(dt,pcn(:,ip),'k'); grid;
    title(['PC',num2str(ip),': ',num2str(fix(100*(vf(ip)))),'% var exp.']);
    axis([dt(1) dt(end) min(min(pcn)) max(max(pcn))]);
    set(gca,'XTick',[dt(1):1:dt(end)]);
    set(gca,'XTickLabel',[year(1):1:year(end)]);
end

% Plot loading vectors for PC1
figure
b=barh(evn(:,1),'k'); hold on; set(b,'BarWidth',[1]); set(gca,'YDir','rev');
title('Loading vectors for PC1'); axis([-1 1 0 length(sites)+1]); grid;
set(gca,'ytick',[1:1:length(sites)],'yticklabel',[sites],'xtick',[-1:0.5:1]);
```