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Recent trends in paralytic shellfish toxins in Puget Sound, relationships to climate, and capacity for prediction of toxic events

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ABSTRACT

Temporal and spatial trends in paralytic shellfish toxins (PSTs) in Puget Sound shellfish and their relationships with climate are investigated using long-term monitoring data since 1957. Data are selected for trend analyses based on the sensitivity of shellfish species to PSTs and their depuration rates, and the frequency of sample collection at individual sites. These criteria limit the analyses to the shellfish species *Mytilus edulis* at 20 sites from 1993 to 2007. Blue mussel toxicity is highly variable, but typically exceeds the regulatory limit for human consumption from July to November annually, with most closures occurring early in fall. Using blue mussel data only, we find no robust evidence to suggest that the frequency, magnitude, duration, or geographic scope of PST events in Puget Sound increased between 1993 and 2007. However, there is a significant basin-wide trend for closures to occur earlier in the year. There are no significant correlations between annual indices of mussel toxicity and aspects of the local and large-scale climate. Case studies of daily variations in local environmental factors leading up to exceptionally toxic events identify a combination of conditions that generally precedes most closures from 1993 to 2007. These results suggest that periods of warm air and water temperatures and low streamflow on sub-seasonal timescales may facilitate toxin accumulation in mussels. No relationships were found between water residence times in the surface layer and either streamflow or mussel toxicity. Recommendations are made for future monitoring to improve forecasting of PST risks in Puget Sound, an important region for recreational, commercial, and tribal subsistence shellfish harvesting.

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1. Introduction

Paralytic shellfish toxins (PSTs) are a suite of toxins produced by species of harmful marine dinoflagellates of the genus *Alexandrium*. PSTs, the most potent being saxitoxin, block the conductance of nerve signals by interfering with the sodium channels of excitable cells, thus causing neuromuscular paralysis (Narahashi and Moore, 1968). Like many other dinoflagellates, *Alexandrium* is known to thrive when the water column is vertically stratified and when water temperatures are warm (Nishitani and Chew, 1984). During blooms of *Alexandrium*, shellfish concentrate the toxins in their tissues from the large volumes of water they filter when feeding. We use the word "bloom" here to describe an accumulation of cells at sufficiently high densities to cause concentrations of

PSTs in shellfish tissues to exceed the regulatory limit for human consumption. This could be as few as 10 cells ml⁻¹ (Nishitani and Chew, 1984). A "bloom" can therefore result from hydrographic processes retaining and concentrating *Alexandrium* cells in a particular location or from in situ growth.

Consumption of contaminated shellfish produces a condition known as paralytic shellfish poisoning (PSP; Gessner and Middaugh, 1995). The first documented cases of PSP in Washington State are from 1942 when the consumption of toxic clams and mussels from the Strait of Juan de Fuca resulted in three fatalities (Quayle, 1969). Nearby on the central coast of British Columbia, four cases of PSP and one fatality were documented in 1793 (Vancouver, 1793). It is generally accepted that blooms of *Alexandrium* in this region are not recent phenomena and that PSTs have been present in the waters off the northwest coast of North America for centuries (Horner et al., 1997).

The presence of PSTs in Washington State coastal waters, including Puget Sound, has been attributed to the species *Alexandrium catenella* (Whedon & Kofoid) Balech. Few direct observations of *A. catenella* cell concentrations exist from this

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region to examine trends in distribution and abundance. However, the Washington State Department of Health (WDoH) monitors for PSTs in shellfish tissues as part of their Biotoxin Monitoring Program. These records reliably date back to 1957, representing one of the longest historical records of PSTs in the United States. Observations of shellfish toxicity are useful because they integrate much of the spatial and temporal variations in PSTs in the water column and can indicate some aspects of *A. catenella* bloom dynamics (Bricelj and Shumway, 1998). However, care should be taken to avoid using shellfish toxicity (or PST event) interchangeably with *Alexandrium* cell density (or harmful algal bloom) as this relationship is complex, largely due to the differing rates of toxin accumulation and depuration by different species of shellfish.

Temporal trends in shellfish toxicity and relationships with climate have mostly been explored on a qualitative level in Puget Sound (e.g., Determan, 1998; Ebbesmeyer et al., 1995; Erickson and Nishitani, 1985; Nishitani and Chew, 1984; Norris-Nishitani et al., 1979; Postel et al., 2000; Rensel, 1993; Saunders et al., 1982; Trainer et al., 2003). A study of decadal patterns of shellfish toxicity indicated that the frequency, magnitude, and geographical scope of PSTs in Puget Sound have increased since the 1950s (Trainer et al., 2003), concomitant with an apparent worldwide increase in the occurrence of harmful algal blooms (Hallegraeff, 1993).

Other studies suggest relationships between shellfish toxicity and large-scale patterns of climate variability (Ebbesmeyer et al., 1995; Erickson and Nishitani, 1985). For example, a shift in the Pacific Decadal Oscillation (PDO) from cool to warm phase in 1977 was locally manifested by persistently warmer air and water temperatures and reduced precipitation and streamflow in the Puget Sound region (Ebbesmeyer et al., 1995; Mantua et al., 1997). This is thought to have contributed to the record levels of PSTs observed in mussels in 1978 and to the spread of *A. catenella* into Whidbey and Main basins (Erickson and Nishitani, 1985; Trainer et al., 2003). However, recent enumeration of cysts from sediment cores suggests *A. catenella* was present in Whidbey basin at least 2 decades prior to this incident (Cox et al., 2008). A relationship between warm phases of the El Niño Southern Oscillation (ENSO, hereafter El Niño) and exceptionally toxic events in Puget Sound, the outer coast of Washington State, and the inland waters of British Columbia has also been suggested (Erickson and Nishitani, 1985), and the retention of PSTs by butter clams, *Saxidomus giganteus*, in an embayment of Puget Sound has been linked to warm/dry climate regimes on decadal timescales (Ebbesmeyer et al., 1995). Rigorous and quantitative analyses are required to determine the statistical significance of these relationships and to develop predictive models of shellfish toxicity.

The mechanisms for these large-scale climate variations to increase shellfish toxicity in the Puget Sound region have not been clearly identified. One hypothesis was that warmer air temperatures and reduced winds that are generally associated with El Niño events lead to prolonged periods of increased water temperatures and thermal stratification of the water column (Erickson and Nishitani, 1985), favoring blooms of *A. catenella* over non-flagellated phytoplankton species. However, a recent assessment of climate influences on Puget Sound oceanographic properties found that El Niño events do not typically cause significant warming or stronger vertical stratification of the water column during summer and fall when shellfish toxicity from *A. catenella* is most commonly observed (Moore et al., 2008). Aspects of the local climate, such as air temperature and streamflow, were found to be more important in determining oceanographic variability in Puget Sound rather than large-scale climate variations like ENSO (Bos et al., 2005; Moore et al., 2008).

The skill and lead times for forecasting shellfish toxicity will be strongly determined by the robustness of relationships with local

environmental factors and certain aspects of the large-scale climate. In our analyses, we define local environmental factors to be specific to the Puget Sound basin and to typically vary on daily to seasonal timescales, whereas large-scale climate patterns are hemispheric-scale variations that typically occur on inter-annual to interdecadal timescales. If PST events are strongly driven by variations in local environmental factors, then advanced warning of shellfish toxicity will be limited by the same factors that limit the prediction of local weather conditions. In contrast, if large-scale climate variations are well correlated with PST events, then advanced warning of shellfish toxicity may be extended. For example, predictability of ENSO variations is possible at lead times up to at least 1 year (Cane et al., 1986).

This study was driven by the desire to better understand PST variations in Puget Sound shellfish and the influence of climate. Specific aims were to apply quantitative statistics to determine (1) potential increases in the frequency, magnitude, duration, and/or geographical scope of PST events, (2) possible relationships between shellfish toxicity, local environmental factors, and aspects of the large-scale climate, and (3) the capacity for predicting PST events. We use a subset of PST observations from a single shellfish species (the blue mussel, *Mytilus edulis*) with continuous and long-term records from the same locations to examine temporal and spatial patterns of variability at sub-seasonal to interannual timescales.

2. Study area

Puget Sound is a deep, fjord-type estuary covering an area of 2330 km² in the Pacific Northwest region of the United States (Fig. 1). A double sill at the entrance (i.e., Admiralty Inlet) separates it from the Strait of Juan de Fuca. Whidbey, Main, and Hood Canal basins are the three main branches of Puget Sound (Thomson, 1994). The shallower South Sound is highly branched with numerous finger inlets and is separated from Main basin by a sill at Tacoma Narrows. This study also examines PST variations to the north of Puget Sound in North and Northwest basins; regions encompassing the San Juan Islands and part of the Strait of Georgia, and the southeastern boundary of the Strait of Juan de Fuca, respectively.

Flow within Puget Sound is dominated by tidal currents reaching amplitudes of ~1 m s⁻¹ at Admiralty Inlet, and reducing to ~0.5 m s⁻¹ in the Main basin (Lavelle et al., 1988). The sub-tidal component of flow reaches ~0.1 m s⁻¹ and is driven by density gradients arising from the contrast in salty ocean water at the entrance to Puget Sound and freshwater from river inflows (Lavelle et al., 1988), and by surface winds (Matsuura and Cannon, 1997). Wind-driven flow is strongest in the upper 10 m of the water column, but under weakly stratified conditions can influence currents at depths to 100 m. Annual maxima in freshwater inflows result from periods of high precipitation and snowmelt, with the Skagit River accounting for the majority (Cannon, 1983). The sub-tidal circulation pattern mostly consists of a two-layered flow in Whidbey, Main, and Hood Canal basins, with fresher water flowing northward and exiting the basins at the surface and saltier water flowing southward and entering at depth (Ebbesmeyer and Cannon, 2001). Upwelling at the Tacoma Narrows sill and the absence of major river inflows results in lesser stratified waters in South Sound compared to the other basins, but surface waters generally continue to flow northward and deeper waters flow southward.

3. Shellfish toxicity data

A Biotoxin Monitoring Program for shellfish was first established by the WDoH in the early 1930s. Monitoring efforts initially focused on commercially important species such as the Pacific

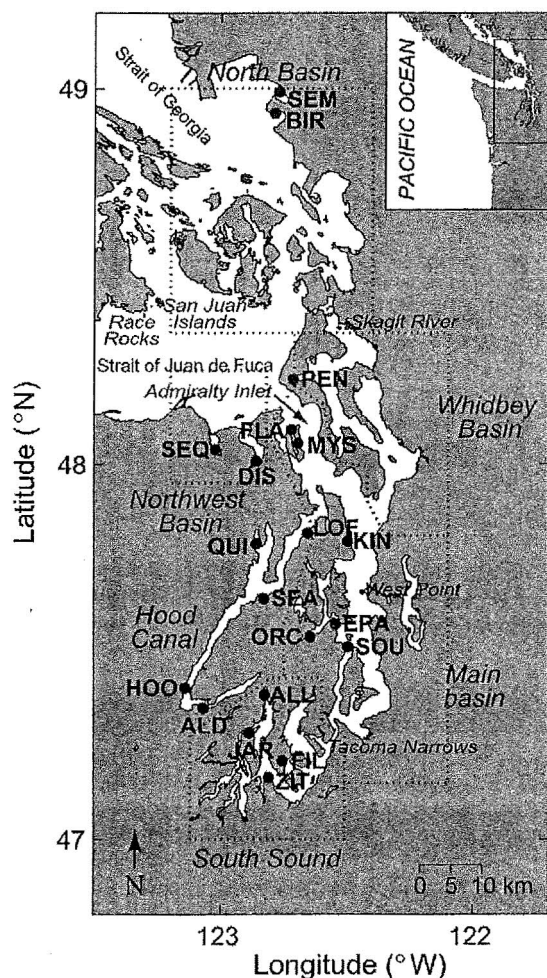


Fig. 1. Map of Puget Sound basins showing the locations of selected *M. edulis* sentinel monitoring sites. Sites are Semiahmoo Marina (SEM), Birch Bay (BIR), Sequim Bay (SEQ), Discovery Bay (DIS), Penn Cove (PEN), Fort Flagler (FLA), Mystery Bay (MYS), Kingston Marina (KIN), EPA Lab Dock (EPA), Port Orchard Marina (ORC), Southworth (SOU), Allyn (ALL), Jarrell Cove (JAR), Filcay Bay (FIL), Zittel's Marina (ZIT), Lofall (LOF), Quilcene Bay (QUI), Seabeck Marina (SEA), Hoodsport Marina (HOO), and Alderbrook (ALD).

oyster, *Crassostrea gigas*, and were highly concentrated in the North and Northwest basins of Puget Sound. In 1946, the program was temporarily terminated because an annual closure to shellfish harvesting in the Strait of Juan de Fuca and on the outer Washington State coast from April 1 to October 31 was deemed sufficient to protect public health. Monitoring resumed in 1957 in response to increasing PSTs detected in shellfish in British Columbia, and was expanded to include all species of commercial shellfish. In September of 1978, the highest levels of PSTs ever

Table 1

Distribution of water bodies, sites, and observations of PST concentrations in all shellfish species sampled in Puget Sound basins from the Washington Department of Health Biotoxin Program from 1957 to 2002.

Basin	Water bodies	Sites	Observations
North	29	217	11521
Northwest	5	61	6795
Whidbey	7	79	4952
Main	25	227	15945
South Sound	20	133	7442
Hood Canal	9	60	2846

recorded in Washington State were detected in mussels from Whidbey basin. This was the first time that PST concentrations in shellfish from inside Puget Sound exceeded the regulatory limit, prompting a substantial increase in monitoring efforts. In 1986, the WDoH became the official management body for recreational sport harvesting of shellfish and monitoring of these species increased. In 1988, trials of a Sentinel Monitoring Program began at several sites using caged mussels suspended ~1 m below the surface. By 1990, the Sentinel Monitoring Program was in place throughout Puget Sound and mussels were sampled at up to 70 sites at ~2 wk intervals. Fig. 2 shows the number of PST observations per year for the most commonly sampled shellfish species. Table 1 summarizes the distribution of sampling sites in Puget Sound basins.

Testing of PSTs in shellfish is conducted using the standardized mouse bioassay (Association of Official Analytical Chemists, 1990), and is expressed in saxitoxin equivalents (STXeq). This method has essentially remained unmodified since its development in the 1920s. The lower limit of detection of the mouse bioassay is 32 ug STXeq 100 g⁻¹ shellfish tissue (Bricelj and Shumway, 1998), and the regulatory limit for human consumption is 80 ug STXeq 100 g⁻¹.

Monitoring data from the WDoH Biotoxin Program were compiled for the period 1957–2002 by the Northwest Fisheries Science Center Marine Biotoxins Program. This database comprises 59151 observations of PSTs in the tissues of 27 identified species of shellfish at 882 sites in Puget Sound. The most commonly sampled species are *M. edulis*, *S. giganteus*, and *C. gigas* representing 31%, 18%, and 17%, respectively, of the total number of PST observations. Less commonly sampled species include littleneck clam (*Protothaca staminea*), manila clam (*Tapes japonica*), geoduck (*Panopea abrupta*), California mussel (*Mytilus californianus*) and pink scallop (*Chlamys hastata*).

4. Data selection

Differences in toxin sensitivity and detoxification rates among the most commonly sampled shellfish species determine their usefulness in trend analyses. Shellfish that are sensitive to PSTs, such as *C. gigas*, will exhibit mechanisms such as feeding inhibition to avoid or reduce exposure to the toxins. Because of this, these species do not generally acquire high levels of PSTs in their tissues

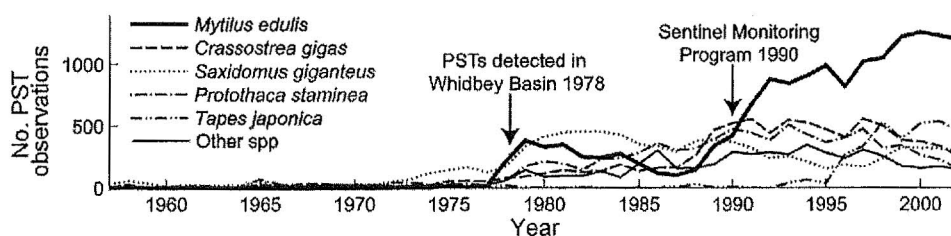


Fig. 2. Annual number of observations of PSTs in *M. edulis*, *C. gigas*, *S. giganteus*, *P. staminea*, *T. japonica*, and other shellfish species from 1957 to 2002 from the Washington Department of Health Biotoxin Program.

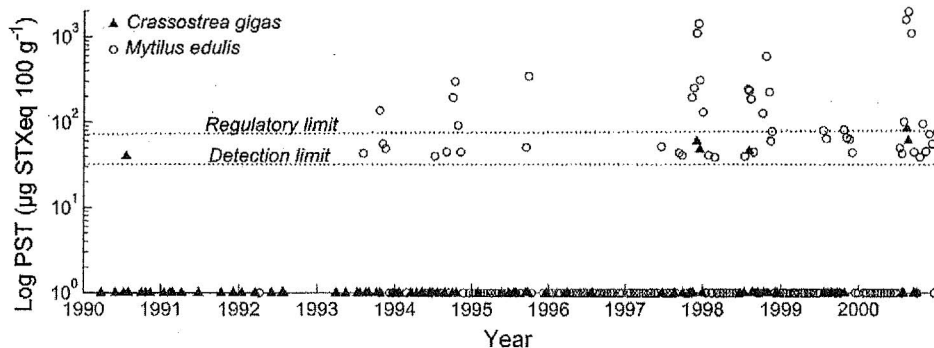


Fig. 3. PSTs in *C. gigas* from Filicly Bay from 1990 to 2000. The detection limit of the mouse bioassay ($32 \mu\text{g STXeq } 100 \text{ g}^{-1}$) and the regulatory limit ($80 \mu\text{g STXeq } 100 \text{ g}^{-1}$) are shown. PSTs in *M. edulis* also from Filicly Bay for the same period are shown for comparison.

(Bricelj and Shumway, 1998). For example, during the time period 1990–2000, only 6% of *C. gigas* PST observations from Filicly Bay (see Fig. 1 for location) were at or above the detection limit of the mouse bioassay, and only 1% exceeded the regulatory limit for human consumption (Fig. 3). In comparison, 27% of *M. edulis* PST observations from the same location for the same time period were at or above the detection limit, and 12% exceeded the regulatory limit. Six closures were apparent from examining PSTs in *M. edulis*. These closures would have gone undetected if *C. gigas* were the only species of shellfish used to monitor for PST events. Therefore, the already complicated relationship between the presence of *A. catenella* cells in the water column and shellfish toxicity is even more strongly decoupled for shellfish species that are sensitive to PSTs.

Detoxification rates of shellfish species also influence their usefulness to indicate certain aspects of *Alexandrium* bloom dynamics. For example, *S. giganteus* readily acquires high levels of PSTs from the water column, but is a slow detoxifier that may take several months to depurate toxins from its tissues (Bricelj and Shumway, 1998). This is much longer than the duration of a typical *Alexandrium* bloom. The detoxification timescale of *S. giganteus* from a site in Sequim Bay (see Fig. 1 for location) is shown in Fig. 4. This was calculated by applying a detoxification rate of $0.7\% \text{ d}^{-1}$ (Bricelj and Shumway, 1998) to *S. giganteus* PST concentrations from 1980 to 1990. If a toxin measurement was made after clams had sufficient time to detoxify to below the regulatory limit, the value was considered to reflect "new accumulation" of PSTs from the water column. From 1980 to 1990, only two observations can be considered to reflect new accumulation, and these two observations occurred after a long gap in the monitoring of the

clams at this site. The remaining observations were made while clams still had "residual" PSTs in their tissues and may not actually reflect the presence of *A. catenella* cells in the water column. The slow detoxification rate of *S. giganteus* introduces a "toxin memory" effect, such that PSTs may be detected in *S. giganteus* tissues many months following a bloom of *Alexandrium* even though cells may not be present in the water column. This toxin memory limits the usefulness of *S. giganteus* in examining PST trends on timescales shorter than a few years.

By comparison, *M. edulis* may take only a few weeks to detoxify in the absence of toxic *Alexandrium* cells (Bricelj and Shumway, 1998). Of the three most commonly sampled shellfish species from the WDoH Biotoxin Monitoring Program, *M. edulis* is the most suitable for examining PST trends on sub-seasonal to interannual timescales. *M. edulis* readily accumulates PSTs from the water column, and concentrations of PSTs in their tissues have been shown to exceed the regulatory limit in <1 h of exposure to a toxic strain of *Alexandrium* in the laboratory (Bricelj et al., 1990). *M. edulis* also rapidly detoxify in only a few weeks at a rate of $10.6\% \text{ d}^{-1}$ (Bricelj and Shumway, 1998). PST concentrations in *M. edulis* tissues are therefore a reasonable proxy for *A. catenella* blooms.

It was these traits that led *M. edulis* to be chosen as the sentinel species for monitoring PSTs in Puget Sound. However, unlike *C. gigas* and *S. giganteus*, which have observational records dating back to the 1950s, *M. edulis* was not sampled regularly until the WDoH Sentinel Monitoring Program began in the early 1990s. This increase in *M. edulis* sampling effort introduces a bias for a greater proportion of samples testing positive for toxin. The number of observations of *M. edulis* is significantly correlated with higher

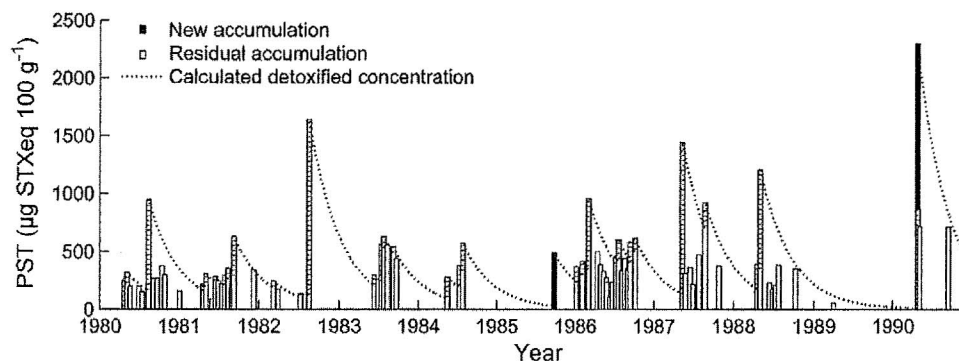


Fig. 4. Concentrations of PSTs in *S. giganteus* from Sequim Bay (bars), and associated PST levels derived from detoxification timescales (dotted line) from 1980 to 1990. Solid black bars are considered "new events", occurring after previously observed PST concentrations had depurated to below the regulatory limit. Gray bars are "residual events" that were observed before the shellfish had sufficient time to depurate PSTs acquired from previous events.

Table 2

Pearson's correlation coefficients (r) for the number of observations of *M. edulis* and the annual maximum PST values for each basin from 1957 to 2002. n is the number of years with observations for each basin. Probability values are not adjusted for autocorrelation.

Basin	n	r
North	29	.64**
Northwest	22	.77**
Whidbey	18	.41
Main	25	.43*
South Sound	22	.56**
Hood Canal	8	-.63

* $p \leq 0.05$.

** $p \leq 0.01$.

*** $p \leq 0.001$.

annual maximum PST values in North, Northwest, Main, and South Sound basins (Table 2). This relationship was only marginally insignificant in Whidbey basin ($p = 0.09$), but was not observed in Hood Canal; the only remaining basin in Puget Sound that experiences few or no closures. Increased sampling effort introduces a bias that can be overcome either by randomly selecting a consistent number of PST observations from the *M. edulis* database for each year examined, or only including sites with regular and consistent sampling frequency in trend analyses. The latter option was employed in this study because variability in PST levels from the same shellfish species can be high at spatial scales as low as 1–10 km (Nishitani and Chew, 1984; Prakash et al., 1971). Randomly selecting *M. edulis* observations from all available sites could therefore introduce variability that may mask temporal trends or potential relationships with climate. Sites with consistent sampling frequency were identified as those with at least one observation per month for at least 90% of months from 1993 to 2002. This approach reduced the scope of the data considered here to 20 sites, but it ensures an objective and quantitative assessment of PST trends. Sites meeting these selection criteria represent all basins of Puget Sound and comprise a subset of the sites sampled as part of the WDoH Sentinel Monitoring Program. Sampling frequency is generally biweekly for these sites, which is higher than the once per month criterion we specify. PST observations in mussels from these sites from

2003 to 2007 were obtained from the WDoH to extend their time series to 15 years for our analyses, producing the longest historical, most spatially comprehensive, and most consistent time series of PST observations for any shellfish species in Puget Sound.

5. Temporal and spatial variability

PST observations in mussels at the 20 selected sites were interpolated on a daily timescale. In cases where observations were missing for a period of 30 days or more, PST values were set to zero. This avoided interpolating from one closure to the next across data gaps and falsely lengthening periods of closures. Monthly means of the number of closure days for each selected site were calculated for 1993 to 2007. A closure day was defined as any day when the interpolated *M. edulis* PST concentrations exceeded the regulatory limit for human consumption. In general, three seasonal patterns are apparent (Fig. 5). The most common pattern is observed at stations in Northwest, Whidbey, and Main basins, and is for the maximum number of closure days per month to occur during fall. Other sites, particularly in North and South Sound basins, display a bimodal pattern where the number of closure days per month peak twice a year; once during mid to late summer and again during mid to late fall. The remaining sites rarely or never experienced closures from 1993 to 2007 and were mostly located in Hood Canal.

The basin-wide average number of closure days per month for all of the 20 selected sites in Puget Sound was most like the pattern observed for sites in Northwest basin, with PST events typically occurring annually from July to November (not shown). This is fairly consistent with the seasonal pattern documented for all of the western states of North America (i.e., from May to October annually; Homer et al., 1997). However, variability in the number of closure days per month is large among sites in Puget Sound. Sites in Northwest basin experienced closures for up to 20 d mo^{-1} approximately 10 d mo^{-1} more than the basin-wide average for the 20 selected sites for the same time of year. Months with the most number of closure days also have high variability. A snapshot comparison of monthly maximum PST concentrations in mussels from the 20 selected sites illustrates the vast interannual

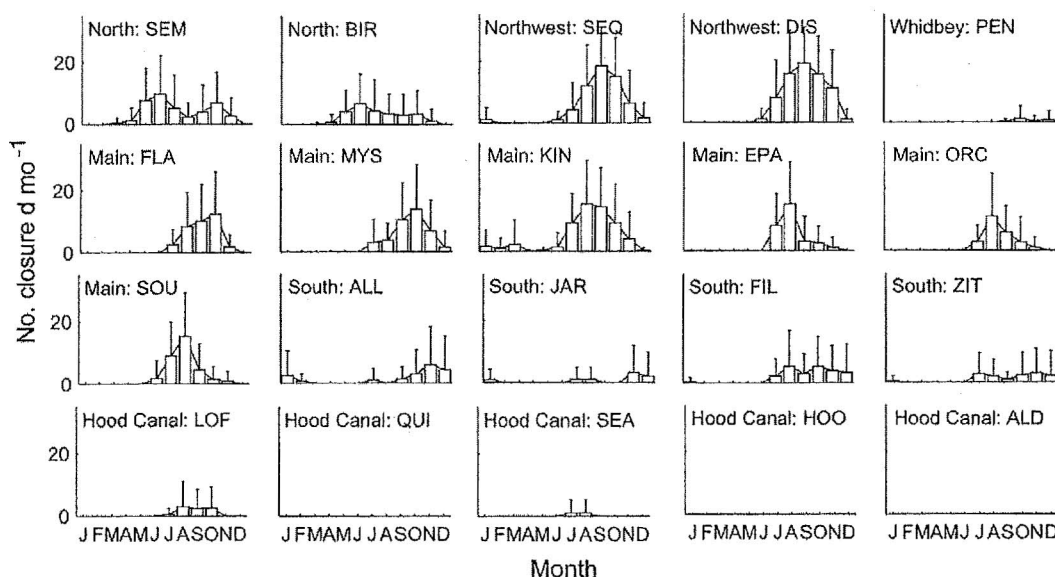


Fig. 5. Monthly means and standard deviations of the number of closure days per month when PSTs in *M. edulis* exceeded the regulatory limit of $80 \mu\text{g STXeq } 100 \text{ g}^{-1}$ for each selected site from 1993 to 2007.

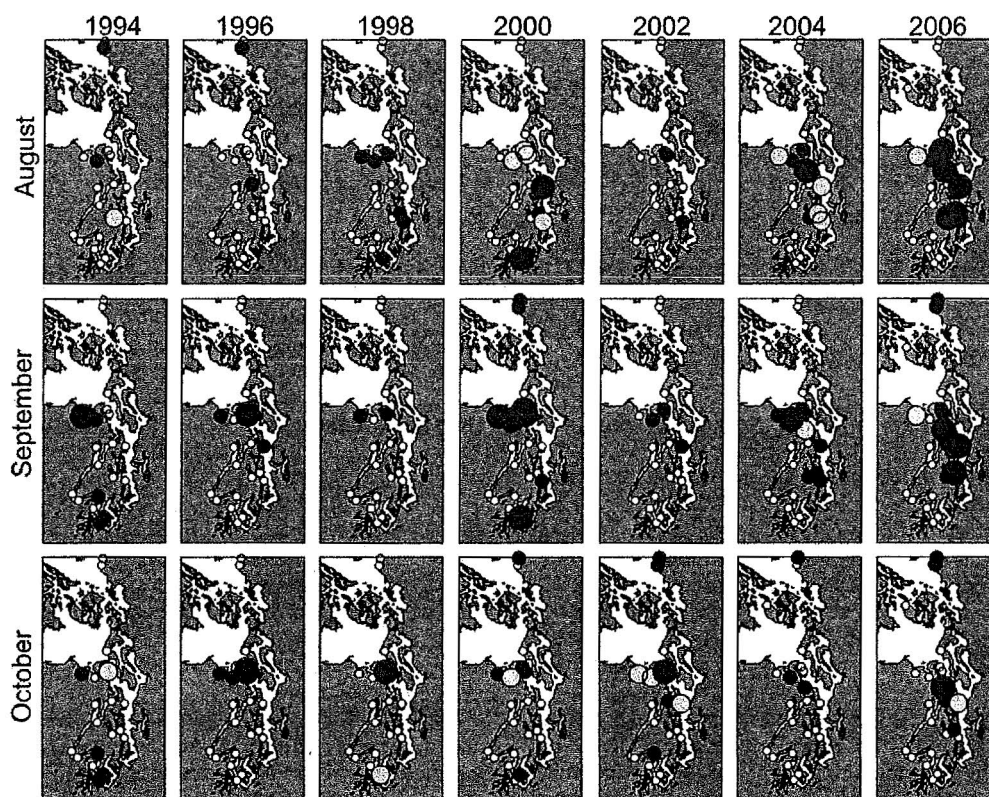


Fig. 6. Maximum values of PSTs in *M. edulis* at selected sites during the months of August, September, and October in 1994, 1996, 1998, 2000, 2002, 2004, and 2006. White, blue, yellow, and red circles represent sites with PST concentrations <32 , $32\text{--}80$, $80\text{--}500$, and >1000 $\mu\text{g STXeq } 100 \text{ g}^{-1}$, respectively. It is interesting to note that *M. edulis* toxicity peaked at 20.8×10^3 $\mu\text{g STXeq } 100 \text{ g}^{-1}$ in Main basin in November 2002, which is outside of the typical annual time period for toxin accumulation in mussels.

differences in the timing, location, and toxicity of events (Fig. 6). Monthly maximum PST concentrations were categorized as low, moderate, high, and very high corresponding to PST values <80 , $80\text{--}500$, $500\text{--}1000$, and >1000 $\mu\text{g STXeq } 100 \text{ g}^{-1}$, respectively, and mapped from August through October every other year from 1994 to 2006. Very high toxicity was observed in Northwest basin during September 1994, and in northern Main basin during September and October 1996 and October 1998. High toxicity was also observed in South Sound during October 1998. Widespread very high toxicity was observed in Northwest, Main, and South Sound basins during 2000, and began earlier in the year compared to other years. During 2002, very high toxicity was observed later in the year in Northwest and Main basins, with record levels of 20.8×10^3 $\mu\text{g STXeq } 100 \text{ g}^{-1}$ observed in November.

We used principal component analysis to test for coherent patterns of variability in the number of closure days per month at the 20 selected sites in Puget Sound from 1993 to 2007. The seasonal cycle was removed and anomalies were normalized such that they had a mean of zero and a standard deviation of 1. The first four principal components (PCs) represent 19%, 18%, 12%, and 10% of the total variability in the number of closure days per month, respectively. Loading vectors for each site show that variability represented by PC1 is greatest for sites in South Sound (i.e., Allyn, Filuey Bay, Jarrell Cove, and Zittell's Marina). PC2 loads most strongly on sites in North basin (i.e., Semiahmoo Marina and Birch Bay) and on Kingston Marina in Main basin; PC3 on Port Orchard Marina, EPA Lab Dock, and Southworth in Main basin; and PC4 on sites in Northwest basin (i.e., Sequim and Discovery Bays). However, eigenvalues for all of the principal components have overlapping 95% confidence intervals and are statistically indistinguishable (North et al., 1982). Therefore, the structures they

represent must be interpreted with caution and may in fact hold little meaning. The lack of statistically significant patterns of variability in the number of closure days further highlights the high degree of temporal and spatial variability in blue mussel PSTs in Puget Sound.

To facilitate the detection of temporal trends and possible relationships with climate, "hot spot" sites were identified by calculating cumulative indices of toxicity and the number of closure days for the 20 selected sites from 1993 to 2007. Mussels at Mystery, Discovery, and Sequim Bays, and at Kingston Marina reached distinctly higher cumulative indices of toxicity and closure days compared to all other sites (Fig. 7). In other words, these hot spot sites most frequently had the highest levels of mussel toxicity. Hot spot sites also display high variability of the monthly mean number of closure days from 1993 to 2007 (Fig. 8). We hypothesize that much of this variability is driven by changes in local environmental factors and large-scale climate patterns. As such, any possible relationships between PSTs and climate indices are most likely to emerge from examining hot spot sites.

6. Interannual trends

Daily interpolated blue mussel PST concentrations from 1993 to 2007 were used to develop a range of annual indices of toxicity and to examine interannual trends. In particular, we tested the hypothesis that the frequency, magnitude, duration, and geographic scope of PST events in Puget Sound have increased in recent years. Indices of toxicity were calculated for each site and include the first day of the year that a closure occurred; the number of days annually from the day of the first closure to the day of the last closure (including non-closure days in between); the total

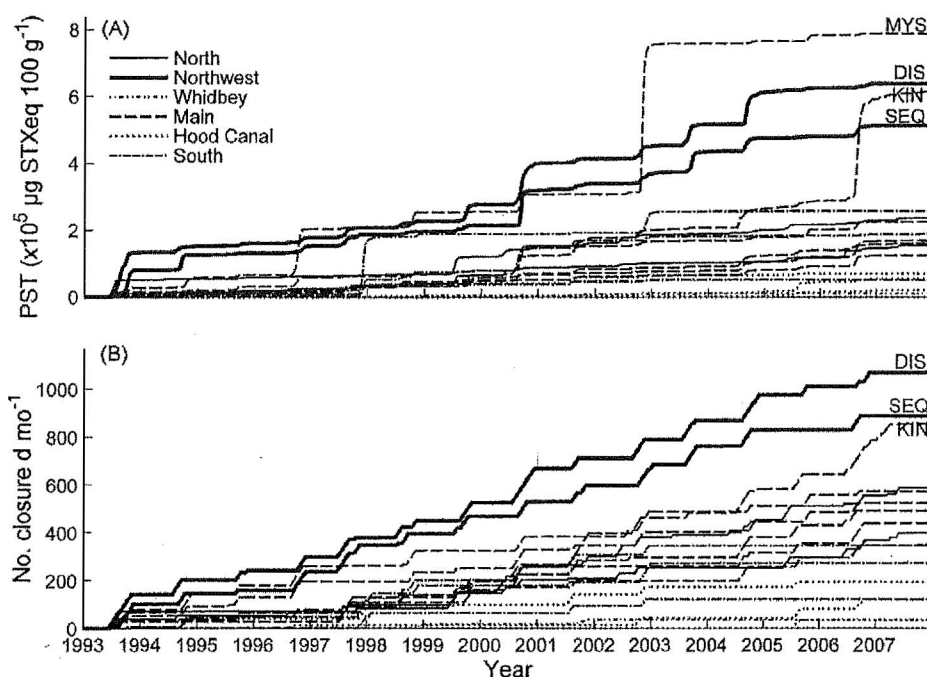


Fig. 7. Cumulative summation of (A) *M. edulis* toxicity values, and (B) days when PSTs in *M. edulis* exceeded the regulatory limit of $80 \mu\text{g STXeq } 100 \text{ g}^{-1}$. Sites at Mystery Bay (MYS), Kingston Marina (KIN), Sequim Bay (SEQ), and Discovery Bay (DIS) reached the highest cumulative toxicity values and cumulative number of closure days after the 15-year period from 1993 to 2007.

number of days annually that closures occurred (not including non-closure days); the day of the year that the maximum PST concentration was observed; the maximum annual PST concentration observed; the annual mean of PST concentrations; and the cumulative annual PST concentration. In some cases, PST events that occurred late in the season resulted in closures that persisted into the following year and the first day of the year that a closure occurred would be January 1. To avoid this carry over effect from the previous year, the first closure of any year had to occur after March 1. Mean values of each of the indices were calculated using hot spot sites only (i.e., Mystery, Discovery, and Sequim Bays, and Kingston Marina) and all of the selected sites and correlated with time (Table 3). For hot spot sites, the number of days each year from the first to the last closure and the total number of closure

days each year were negatively and significantly correlated with time, indicating that duration of PST events decreased at these sites from 1993 to 2007. For all of the selected sites, the day of the year when the first closure occurred and the day of the year that the maximum PST concentration occurred were negatively and significantly correlated over time, suggesting a trend for PST events to occur earlier in the year. No other correlations were statistically significant, indicating that neither the duration nor the magnitude of PST events in Puget Sound increased significantly between 1993 and 2007.

Temporal trends in PST events in Puget Sound were examined further by contouring the number of sites where closures occurred for each day of the year from 1993 to 2007 (Fig. 9A). Shaded areas represent periods of closures that typically occurred from July to November each year. If the duration and/or frequency of PST events had increased between 1993 and 2007, the shaded area would widen vertically toward the bottom of the plot. If the geographical

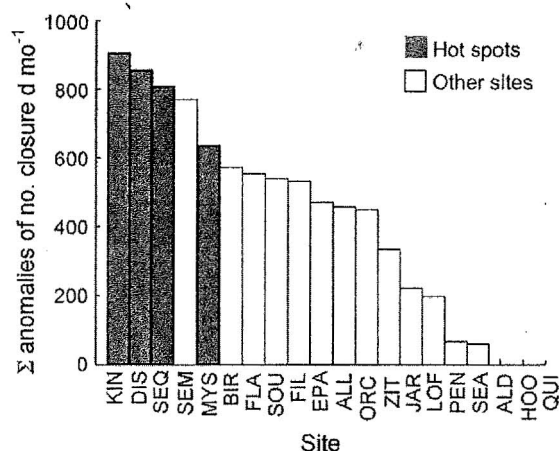


Fig. 8. Sums of the anomalies of the number of closure days per month from 1993 to 2007 for each selected site.

Table 3

Pearson's correlation coefficients for annual indices of toxicity at hot spot sites and for all sites with time from 1993 to 2007 ($n = 15$). Probability values are not adjusted for autocorrelation.

Annual indices of toxicity	Hot spots	All sites
Day of first closure [†]	.08	-.66**
Days from first to last closure	-.55*	.18
Total closure days	-.51*	.01
Day of max PST [†]	.10	-.60*
$\log_{10}(\text{max PST} + 1)$	-.42	-.03
$\log_{10}(\text{mean PST} + 1)$	-.44	-.14
$\log_{10}(\text{sum PST} + 1)$	-.45	-.08

* $p \leq 0.05$.

** $p \leq 0.01$.

[†] No PSTs were observed in mussels at hot spot sites in 2007; therefore, there are no values for the first day of the year that a closure occurred and the day of the year that the maximum PST level was observed at hot spot sites and $n = 14$ for correlations from 1993 to 2006.

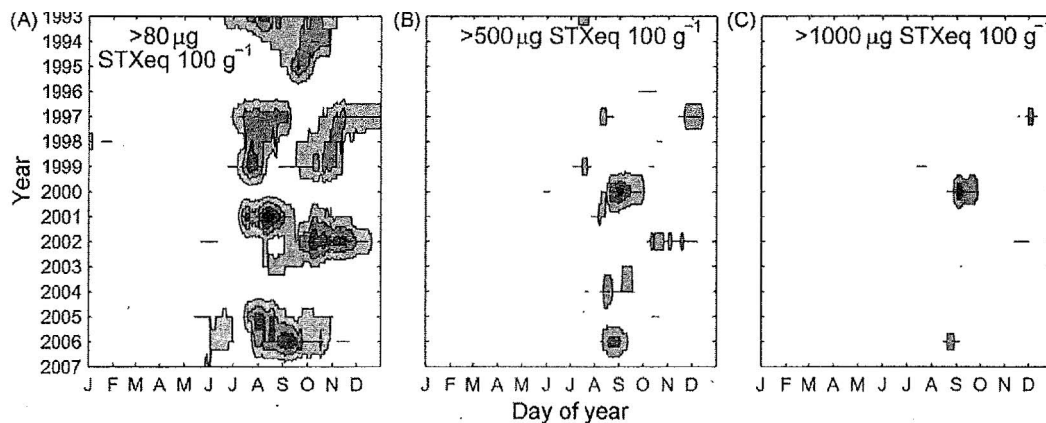


Fig. 9. Number of selected sites where PST concentrations in *M. edulis* exceeded (A) 80, (B) 500, and (C) 1000 $\mu\text{g STXeq } 100 \text{ g}^{-1}$ contoured on each day of the year from 1993 to 2007. Light gray, gray, dark gray, and black shading corresponds to 2-4, 4-6, 6-8, and 8-10 sites, respectively.

scope of blooms had increased, the shaded area would darken vertically toward the bottom of the plot, indicating an increase in the number of selected sites experiencing closures. Neither of these patterns is observed. Contour plots of the number of sites where mussel toxicity exceeds 500 and 1000 $\mu\text{g STXeq } 100 \text{ g}^{-1}$ (Fig. 9B and C, respectively) also show no darkening of the shaded area toward the bottom of the plot, indicating that PST events in Puget Sound have not become more toxic between 1993 and 2007. Similar contour plots using PST concentrations at hot spot sites show no

consistent darkening of the shaded area vertically toward the bottom of the plots (Fig. 10). Toxicity does appear to *have* increased at Kingston Marina and decreased at Mystery Bay between 2003 and 2007, but the interannual variability is high. In summary, a combination of quantitative and qualitative analyses suggest a trend toward earlier occurring PST events from 1993 to 2007, but we find no robust evidence for an increase in the frequency, magnitude, duration, and geographic scope of PST events in Puget Sound in this period based on blue mussel data.

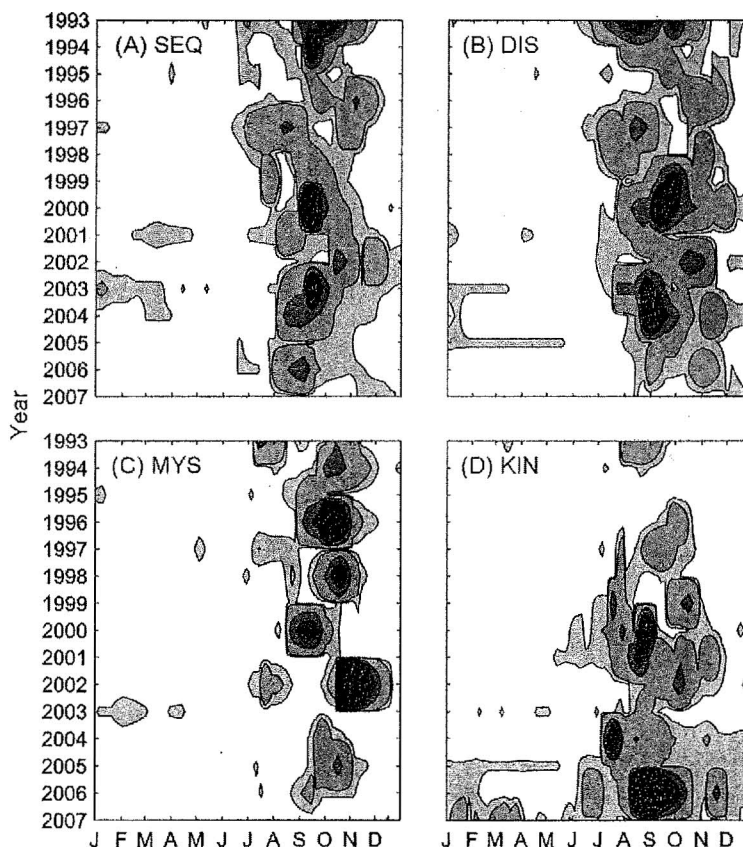


Fig. 10. PST concentrations in *M. edulis* at the hot spot sites (A) Sequim Bay, (B) Discovery Bay, (C) Mystery Bay, and (D) Kingston Marina contoured on each day of the year from 1993 to 2007. Light gray, gray, dark gray, and black shading corresponds to PST concentrations 32-80, 80-500, 500-1000, and $>1000 \mu\text{g STXeq } 100 \text{ g}^{-1}$, respectively.

7. Relationships with climate

7.1. Interannual timescale: indices of shellfish toxicity and climate

Correlations were conducted to determine any possible relationships between the annual indices of toxicity and annual means of local environmental factors and large-scale climate indices. Local environmental factors examined here are sea surface temperature and salinity, air temperature, precipitation, streamflow, wind-driven coastal upwelling, and local surface winds. Sea surface temperature (SST) and salinity (SSS) at Race Rocks lighthouse, British Columbia, Canada, were obtained from the Department of Fisheries and Oceans, Canada (http://www.pac.dfo-mpo.gc.ca/SCI/osap/data/SearchTools/Searchlighthouse_e.htm). Air temperature (AIR) and precipitation (PRCP) for Port Townsend (station 456678) were obtained from the United States Historical Climatology Network (Williams et al., 2006). Total freshwater inflow was approximated using the Skagit River (STRM), which accounts for over half of the total streamflow to Puget Sound (Cannon, 1983). Data were obtained for the Skagit River at Mount Vernon (site 12200500) from the U.S. Geological Survey Water Resources Division. The coastal upwelling index (UPWL) at 48°N and 125°W was obtained from the National Oceanic and Atmospheric Administration (NOAA) Environmental Research Division. Upwelling indices provide information on the strength of upwelling along the open coast. This is related to the seasonal upward movement of nutrients into the nearshore areas. In particular, nutrient-rich water is drawn into the Strait of Juan de Fuca with inflowing deeper water by estuarine and tidal processes (MacFadyen et al., 2008), and enters Puget Sound at Admiralty Inlet. Upwelling in the Pacific Northwest generally occurs during spring and summer and is indicated by persistent positive values of the upwelling index. In contrast, large negative values of the upwelling index indicate downwelling conditions that are driven by strong southerly winds. Local surface winds in Puget Sound (WIND) were approximated using observations at West Point obtained from the NOAA National Data Buoy Center. Unlike coastal upwelling, local winds do not introduce new sources of nutrients to the Puget Sound basin that could be utilized by *A. catenella* for growth. However, local winds generally do co-vary with the coastal upwelling index; they are weak with a more easterly component during upwelling conditions and strong with a more southerly component during downwelling conditions.

Large-scale interannual climate variations of interest to the Pacific Northwest are approximated using the North Pacific Index (NPI) and Nino 3.4 index. The NPI provides an index of the strength of the Aleutian Low Pressure System and is based on sea level pressure in the region 30°N–65°N, 160°E–140°W. Low values of the NPI indicate an intense Aleutian Low that contributes to a warmer coastal ocean and warmer and drier winter and spring conditions in the Pacific Northwest. The NPI was provided by the Climate Analysis Section, NCAR, Boulder, USA (Trenberth and Hurrell, 1994). Following Trenberth (1997), we used SST anomalies in the Nino 3.4 region (5°N–5°S, 120°W–170°W) as an index of ENSO. Nino 3.4 data were provided by the NOAA National Weather Service Climate Prediction Center. Annual values of the Nino 3.4 index were based on 12-month averages of July to June values because of high autocorrelation that rapidly declines from June through July. Essentially, this reflects the tendency for individual ENSO events to begin in one summer, peak the following winter, and then fade away in the subsequent spring (see Newman et al., 2003). The Pacific Decadal Oscillation is another large-scale climate variation important to the Pacific Northwest (Mantua et al., 1997), but the 15 years of blue mussel PST concentrations examined here is too short to assess any possible impacts of the PDO given that a typical PDO event lasts 20–30 years (Mantua and Hare, 2002).

We examined potential correlations between the mean values of the annual indices of toxicity (at hot spot sites only and at all selected sites) with climate indices from 1993 to 2007. The number of degrees of freedom used to determine the probability values was adjusted for autocorrelation following Bretherton et al. (1999). If the autocorrelation coefficient at a lag of 1 year was negative, the number of degrees of freedom was set to the default value of $n - 2$. No significant correlations were found between annual indices of toxicity and climate indices when examined on this timescale.

Previous qualitative studies suggest a strong relationship between El Niño and exceptionally toxic PST events in Puget Sound, the outer coast of Washington State, and the inland waters of British Columbia on interannual timescales (Erickson and Nishitani, 1985). This finding was based on the assumption that warmer than usual air and water temperatures and reduced winds during El Niño years create stable, vertically stratified water column conditions favorable for *A. catenella* bloom development. We found no significant correlations between annual indices of toxicity and ENSO from 1993 to 2007. This could be due to the fact that atmospheric and oceanic teleconnections of ENSO are strongest in the Pacific Northwest during winter and spring, with generally warmer (cooler) conditions experienced during El Niño (La Niña) winters (Rasmusson and Wallace, 1983; Ropelewski and Halpert, 1986, 1987). Warmer SSTs during El Niño events can persist into spring and summer, but by late summer and fall, when shellfish in Puget Sound typically become toxic, this SST response breaks down (Moore et al., 2008).

7.2. Daily timescale: local environmental factors preceding highly toxic events

Variations in local environmental factors preceding exceptionally toxic PST events were examined on shorter daily timescales. Mussel toxicity at hot spot sites was used to identify the five largest PST events from 1993 to 2007. These occurred on 7 October 1996, 14 September 2000, 3 November 2002, 2 September 2004, and 4 September 2006, with PST values well above the regulatory limit for human consumption and reaching 4.8×10^3 , 5.8×10^3 , 20.8×10^3 , 3.5×10^3 , and 8.4×10^3 $\mu\text{g STX}_{\text{eq}} 100 \text{ g}^{-1}$, respectively. Local environmental factors described above and tidal height variations were examined on a daily timescale before, during, and after each of the five largest PST events (Fig. 11). Tidal height data at Seattle (station 9447130) were obtained from the NOAA National Ocean Service Center for Operational Oceanographic Products and Services. Tidal height variability (TIDE) was calculated as the maximum height difference between high and low tides over the full tidal cycle for each day. Only a limited number of sites in the Puget Sound basin monitor environmental variations on a daily timescale and have a historical records dating back to 1993. Where there were multiple sites, sites were chosen that best reflect climate variations experienced in Puget Sound on a basin-wide scale, and are not specific to hot spot sites. For example, the Skagit River is the dominant source of freshwater inflow to the Puget Sound basin (Cannon, 1983), and Puget Sound temperature and salinity at all depths vary coherently and can be represented by observations at Race Rocks (Moore et al., 2008).

The mean values and standard deviations of the local environmental factors during the 20 days prior to the events are given in Table 4. We assume that conditions are most favorable for toxin accumulation in Puget Sound mussels when these local environmental factors occur in combination. Days when the local environmental factors were ± 1.5 standard deviations from the mean values observed during the 20 days prior to the five largest events and when they occurred in combination were determined from 1993 to 2007 (indicated by the solid gray bars in Fig. 12). These

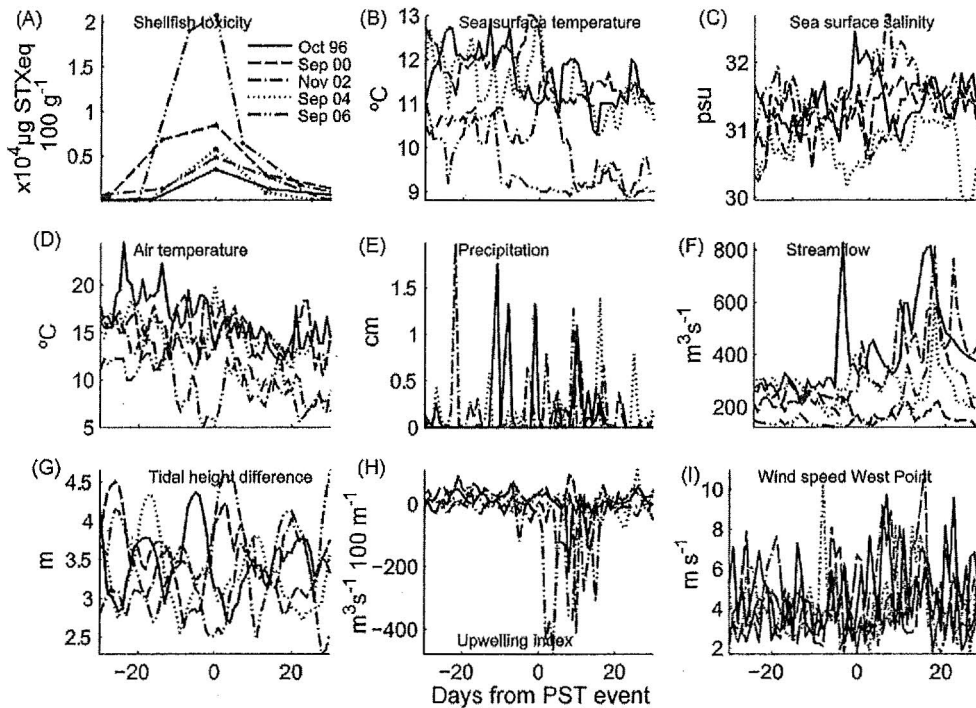


Fig. 11. (A) PST concentrations in *M. edulis* and (B–I) local environmental factors during the five most toxic events at hot spot sites from 1993 to 2007. Values are shown for 30 days prior to and 30 days following the peak of each event.

time periods precede the five largest PST events that were used to identify them; they also generally precede other closures at hot spot sites (Fig. 12A) and periods when the mean of mussel PST concentrations at all of the selected sites was increasing (Fig. 12B). It is particularly interesting to note that the window of favorable environmental factors for toxin accumulation in mussels was very narrow during 2007, and this was the only year in the 1993–2007 period when no closures were observed at hot spot sites.

An overlap index was developed to assess the performance of the hindcast model for mussel toxicity in Puget Sound. The overlap index was calculated as the number of days that favorable environmental factors coincided with toxin accumulation in blue mussels, divided by the total number of days with favorable environmental factors, and multiplied by 100 (Fig. 13). Days of toxin accumulation in mussels were defined as PST concentrations that were increasing and that exceeded the detection limit of the mouse bioassay. The overlap index therefore assesses the capacity for the combination of local environmental factors to coincide with the initial stages of PST events. The overlap index varied from year to year, but over the entire time period from 1993

to 2007, its mean value was 45 for hot spot sites and 31 for all of the selected sites (Table 5). Perfect overlap was not observed because Puget Sound mussels were not always toxic when environmental factors were favorable. This indicates that additional parameters and processes not considered here, such as macro or micro-nutrient availability (Rensel, 1993), also contribute to PST events in Puget Sound.

Time periods when individual environmental factors were favorable for toxin accumulation in mussels were determined

Table 5

Components of the overlap index to assess hindcasts of the number of days when the combination of favorable environmental conditions preceded mussel toxicity in Puget Sound from 1993 to 2007.

Year	No. days that environmental conditions overlap with PST accumulation in <i>M. edulis</i>		No. days of PST accumulation in <i>M. edulis</i>		Overlap index	
	Hot spots	All sites	Hot spots	All sites	Hot spots	All sites
1993	10	4	78	67	56	22
1994	17	18	37	43	50	53
1995	18	8	25	14	45	20
1996	19	19	41	35	58	58
1997	0	0	72	68	0	0
1998	31	8	75	38	61	16
1999	1	1	42	52	14	14
2000	9	9	61	66	27	27
2001	22	15	78	44	42	28
2002	34	25	87	54	79	58
2003	11	9	79	40	30	24
2004	17	15	60	49	40	36
2005	28	13	66	53	49	23
2006	11	10	75	52	26	24
2007	0	0	45	1	0	0
ALL	228	154	921	676	45	31

Table 4

Mean and standard deviations of the local environmental factors observed during the 20 days prior to the five largest PST events observed at hot spot sites from 1993 to 2007.

Local environmental factor	Mean	Standard deviation
SST (°C)	11.23	1.05
SSS (psu)	31.19	0.41
AIR (°C)	14.35	3.50
PRCP (cm d ⁻¹)	0.10	0.29
STRM (m ³ s ⁻¹)	245.75	104.63
TIDE (m)	3.38	0.44
UPWL (m ³ s ⁻¹ 100 m ⁻¹)	10.35	28.36
WIND (m s ⁻¹)	3.90	1.49

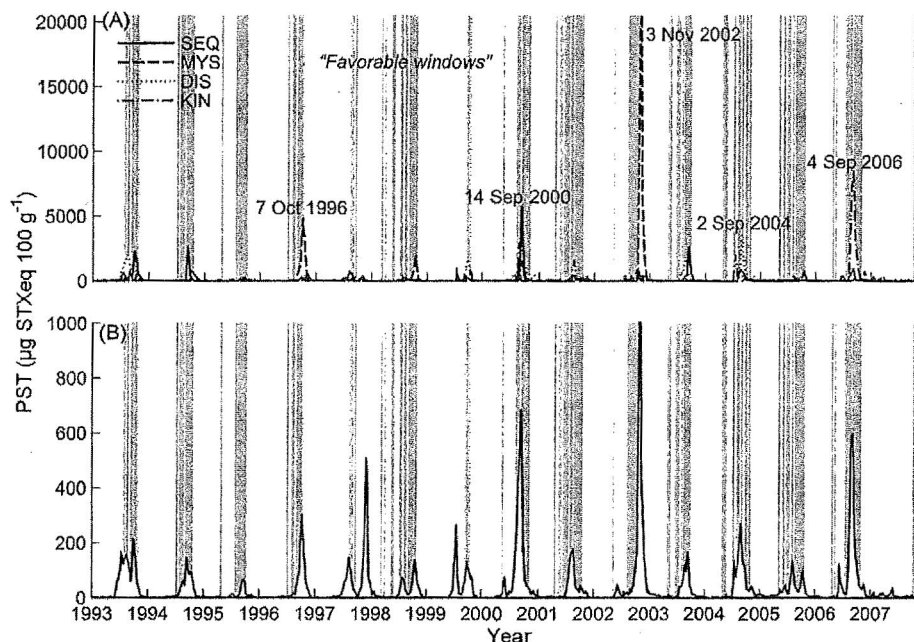


Fig. 12. Mean PST concentrations in *M. edulis* from (A) hot spot sites only and (B) all selected sites from 1993 to 2007. Shaded areas indicate time periods when the combination of environmental factors favorable for toxin accumulation occurs.

independently from the other factors from 1993 to 2007 (Fig. 14). SST and STRM had the highest individual overlap indices (i.e., 27 and 26, respectively) using periods of toxin accumulation in mussels at hot spot sites. The overlap index for AIR was also relatively high (i.e., 23). SSS, PRCP, TIDE, UPWL, and WIND were commonly within the range that was identified as being favorable for toxin accumulation in mussels (i.e., 74%, 89%, 71%, 63%, and 72% of days from 1993 to 2007, respectively). The overlap index for these environmental factors was <20. These factors may therefore contribute less to PST variability in Puget Sound compared to SST, AIR, and STRM. Nevertheless, the overlap index is greatly enhanced when all of the environmental factors are considered in combination, therefore they should not be discounted.

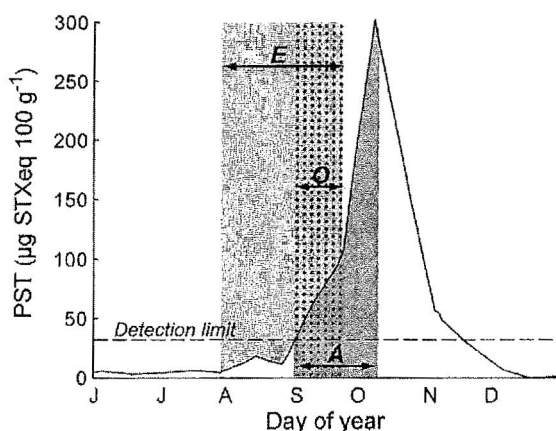


Fig. 13. Parameters used to calculate the overlap index; days of favorable environmental factors are shaded light gray (E), and days when *M. edulis* PST concentrations exceeded the detection limit of $32 \mu\text{g STXeq } 100 \text{ g}^{-1}$ and are increasing are shaded dark gray (A). The dotted area indicates the period of time when days of favorable environmental factors overlap with days of toxin accumulation in mussels (O).

Climatological monthly means of the number of days when individual environmental factors were favorable for toxin accumulation in mussels were calculated independently from the other factors and expressed as a percentage of the total number of days from 1993 to 2007 (Fig. 15). The percentages of days when SST, AIR, and STRM were favorable for toxin accumulation in mussels show strong seasonal patterns. Favorable SST and AIR conditions tend to occur during summer and fall, and favorable STRM conditions occur late in the fall. Favorable UPWL conditions also tend to occur during summer and fall, but the seasonal pattern is less pronounced compared to SST, AIR, and STRM. In contrast, the favorable conditions specified for SSS, PRCP, TIDE, and WIND did not show strong seasonal patterns. When all of the environmental factors were considered in combination, a very strong seasonal pattern is observed that closely matches the time period when PSTs in mussels typically exceeded the regulatory limit (i.e., from July to November annually; Fig. 15). The percentage of days when the environmental factors occurred in combination is highest in September, and the annual window appears to be strongly constrained by STRM.

8. A revised view of the possible causes of toxic events

It has been hypothesized that *A. catenella* growth in Puget Sound is favored by the presence of a stable and warm surface layer that forms when large precipitation events are followed by calm and sunny weather (Determan, 1998; Erickson and Nishitani, 1985; Nishitani and Chew, 1984). Large precipitation events facilitate the development of stable water column conditions by delivering large volumes of freshwater to Puget Sound and increasing salinity-stratification. If followed by calm and sunny weather, stratification might persist due to warming of the surface layer and reduced wind mixing. Our analysis finds that a large precipitation event with increased delivery of freshwater to Puget Sound (approximated by Skagit River streamflow) is not necessary to trigger mussel toxicity. Instead, the five largest PST events occurred when streamflow conditions were low, but no lower than

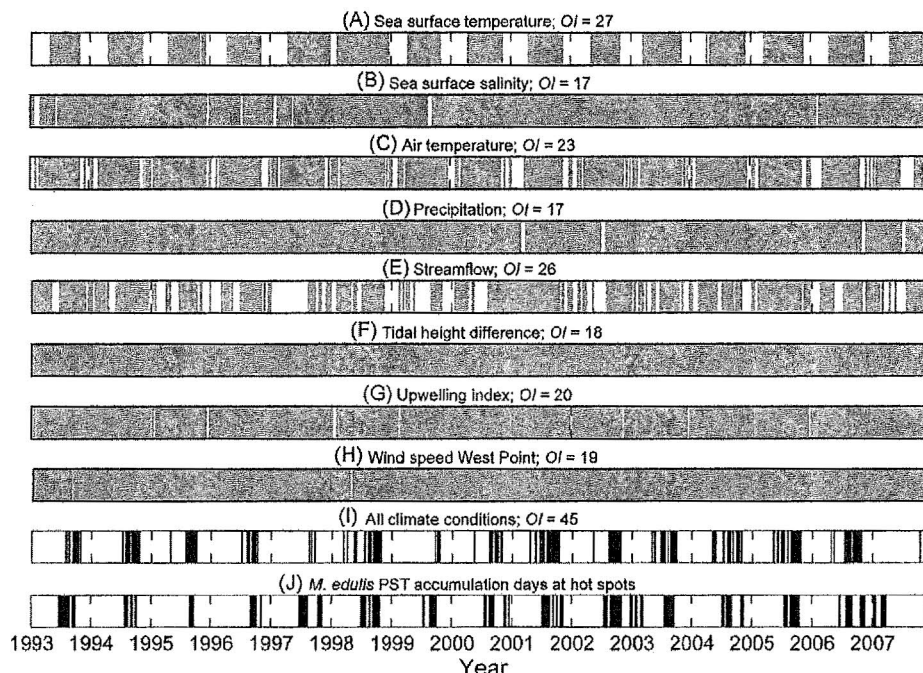


Fig. 14. Days when individual environmental factors were favorable for toxin accumulation in mussels from 1993 to 2007 (A–H; shaded gray), and when they occurred in combination (I; shaded black). The overlap index (OI), calculated using the mean of PST concentrations in *M. edulis* at hot spot sites, is given for each condition. Time periods when the mean of *M. edulis* PST concentrations at hot spot sites exceeded the detection limit of $32 \mu\text{g STXeq } 100 \text{ g}^{-1}$ and were increasing are indicated for comparison (J; shaded black).

usual for that time of year based on the climatological monthly mean values (Moore et al., 2008).

The sub-tidal circulation of Puget Sound is driven by horizontal density gradients (Lavelle et al., 1988), which are influenced by streamflow. We examined the possibility that PST events may be more likely when streamflow is low and residence times in the surface layer are high. Longer residence times could retain *A. catenella* cells within Puget Sound for longer time periods thereby allowing shellfish to acquire higher levels of toxin. Estimates of residence times in the surface layer of Puget Sound were derived from a box model by Babson et al. (2006). The model configuration divided Puget Sound into seven sub-basins, each represented by a surface and deep layer box (total of 14 boxes). We considered the surface layer only because *A. catenella* has been found to reside in the upper 7 m of the water column in Puget Sound during both the day and night (Nishitani and Chew, 1984), and because mussels sampled by the Sentinel Monitoring Program were collected from

cages situated in the surface layer. The depth of surface boxes differed for each sub-basin depending on the depth of no motion (where the sub-tidal velocity of exiting fresher water and entering ocean water crosses zero), and ranged from ~9 to 50 m. Residence times in the surface layer of the Main basin were calculated by dividing the volume of the surface box by the sum of the volume fluxes on a daily timescale from 1993 to 2003.

A negative relationship between surface layer residence times and Skagit River streamflow was expected. However, we found that high streamflow was not necessary for fast flushing of the surface layer in Malobasin, with short residence times also occurring when streamflow was low (Fig. 16A). This may reflect the contribution of deep oceanic water intrusions at Admiralty Inlet to residence times in Puget Sound (Cannon et al., 1990). The density of these deep water intrusions determines the displacement of water at depth, drives water exchange, and influences residence times (Leonov and Kawase, in press). We also find no significant

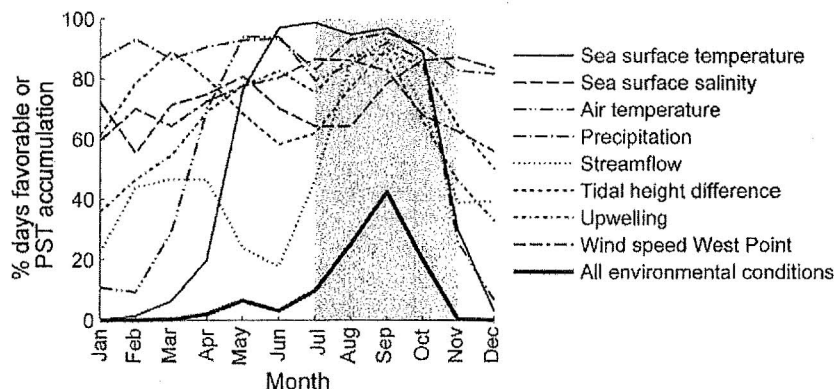


Fig. 15. Percentage of days each month when environmental factors were favorable for toxin accumulation in mussels, and when they occurred in combination, from 1993 to 2007. The time period when PST concentrations in mussels typically exceed the regulatory limit is shaded in gray (i.e., from July to November annually).

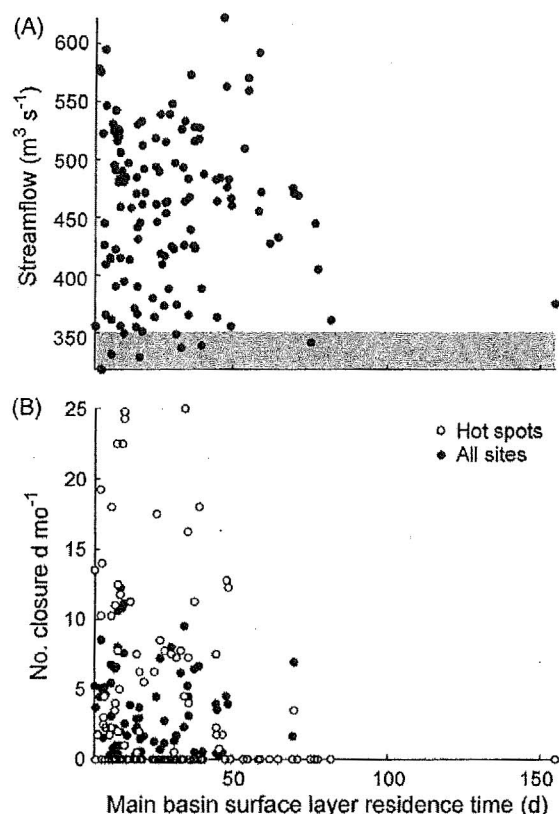


Fig. 16. Monthly mean values of water residence times in the upper layer of Puget Sound's Main basin plotted against Skagit River streamflow from 1993 to 2003. The range of streamflow conditions favorable for toxin accumulation in mussels is shaded gray.

relationships between residence times and the mean number of closures days per month at hot spot sites only or at all of the selected sites (Fig. 16B).

9. Capacity for prediction

Shellfish growers in Puget Sound have indicated that advanced warning of blooms of toxin-producing algae would be extremely valuable (F. Cox, WDoH, pers. comm.). Such forecasts would allow growers to prevent or mitigate economic losses by harvesting earlier before the shellfish acquire the toxins, or planning ahead for a later harvest after the bloom passes and shellfish have depurated toxins. For example, the long-term economic value of predictions of blooms of *A. fundyense* in the Gulf of Maine is estimated at between \$1 million and \$11 million (lin and Hoagland, 2008).

Advanced warning of PST events in Puget Sound will be limited by our ability to predict the combination of favorable local environmental factors that precede them. The prediction timescale of some of these conditions, such as air temperature and surface winds, is approximately 1–2 weeks. This is considerably less than the 1 year of advanced warning that would have been possible if the suggested relationships between PST events and large-scale climate variations, such as El Niño, were quantitatively confirmed.

In the Gulf of Maine, the concentration of *A. fundyense* resting cysts in sediments is a crucial component of the predictive model for blooms of this species and a good indicator of the risk of PST events (McGillicuddy et al., 2005). If we assume that the concentration of cysts in sediments is also a strong driver of blooms of *A. catenella*, there may be value in producing annual cyst

maps for Puget Sound to provide advanced warning of the risk of PST events. If this relationship holds, it may also be useful to explore conditions that influence encystment of *A. catenella* and the deposition of cysts to sediments in Puget Sound.

Understanding mechanisms for the termination of *A. catenella* blooms will also be an important component of any predictive model. Our daily analysis of the five largest PST events shows that air and water temperatures decreased, and streamflow and downwelling favorable winds at the coast (indicated by large negative values of the coastal upwelling index) increased in the 20 days following the events (Fig. 11). These changes are consistent with the passage of a storm. Storms may therefore be an important component of the local climate contributing to the termination of *A. catenella* blooms and PST events. Storms could disperse blooms of *A. catenella* by enhancing wind-driven turbulent mixing in the upper water column and disrupting warm and stable surface layers. Nutrient depletion may also contribute to the termination of *A. catenella* blooms, and biological factors may also be important; for example, infection by the parasite *Amoebophyra ceratii* coincided with the demise of a dense bloom of *A. catenella* in Puget Sound in 1981 (Nishitani and Chew, 1984).

10. Future monitoring

Future monitoring in Puget Sound should focus on identifying key ecological factors that influence *A. catenella* population dynamics and/or shellfish toxicity events, and tailoring programs to match the temporal and spatial scales over which these key factors occur. For example, a long-term and ongoing monitoring program conducted by the Washington State Department of Ecology provides monthly profiles of oceanographic properties from an extensive network of stations in Puget Sound (Janzen, 1992; Newton et al., 2002). The data are well suited for identifying environmental processes occurring on seasonal to interannual timescales (e.g., Bricker et al., 1999; Kawase, 2002; Moore et al., 2008; Newton, 1995; Newton et al., 2003). However, higher frequency processes, such as those identified here to precede PST events in Puget Sound, cannot be resolved using monthly sampling. In the Hood Canal basin of Puget Sound, automated observing systems are helping to bridge this gap. The Oceanic Remote Chemical-optical Analyzer (ORCA) is a moored buoy developed by the University of Washington that telemeters hourly profiles of atmospheric and oceanographic properties, including nutrients and chlorophyll concentrations, in near-real time (Dunne et al., 2002). We do not examine nutrients in this study due to the lack of long-term data on appropriate temporal and spatial scales, but concentrations of limiting nutrients are likely to be important for the development and termination of *A. catenella* blooms. To date, the data produced by the ORCA buoys have not elucidated possible mechanisms leading to PST events, primarily because Hood Canal rarely experiences closures due to unsafe levels of PSTs. Expansion of the ORCA and other buoy networks into other areas of Puget Sound, especially at hot spot sites, will considerably improve the ability to identify the high frequency variations in environmental factors that appear to be contributing to PST events on a more localized spatial scale.

Efforts to better understand the relationship between shellfish toxicity and *A. catenella* cell densities in Puget Sound should also be considered in the design of future monitoring programs. This relationship is complicated by biophysical factors, including the vertical migration of *A. catenella* cells making them difficult to monitor, and variable toxin composition of cells making it difficult to assign a standard cellular toxicity for the region. It will be important to decipher this relationship if bloom dynamics are to be inferred from shellfish monitoring programs. In addition to this,

monitoring programs should include automated devices to remotely detect PSTs and/or *A. catenella* in the water column. Combining platforms such as the ORCA buoys that measure basic physical parameters together with cell and/or toxin detection devices would considerably enhance efforts to provide early warning of PST events over the current approach of monitoring shellfish.

11. Conclusions

A 15-year continuous time series of PST observations in *M. edulis* at a network of 20 sites in Puget Sound was used to examine temporal trends in toxicity and possible relationships to climate from 1993 to 2007. Our analyses were limited to *M. edulis* because they continue to feed and rapidly acquire measurable PST levels in their tissues when toxic cells are present in the water column, and also rapidly detoxify over a period of a few weeks following the termination of a bloom. For these reasons, *M. edulis* is used as the sentinel species in monitoring programs and is the most appropriate shellfish proxy to examine PST variations on sub-seasonal to interannual timescales. Blue mussels typically become toxic between July and November annually, but even within this time period, variability in the timing and location of PST events remains high and little coherence exists among the 20 selected sites in Puget Sound. We find no robust evidence to suggest that the frequency, magnitude, duration, and geographic scope of PST events increased between 1993 and 2007. However, there is a significant basin-wide trend for closures to occur earlier in the year. On interannual timescales, no significant correlations exist between annual indices of mussel toxicity, local environmental factors, and indices tracking large-scale climate variations. PST hot spot sites in Mystery, Discovery, and Sequim Bays, and at Kingston Marina, were used to identify exceptional PST events from 1993 to 2007 and local environmental factors preceding these events were examined on a daily timescale. A combination of warm air and water temperatures and low streamflow appears to be favorable for toxin accumulation in mussels. Advanced warning of PST events may be constrained by the same factors as for weather prediction, and is therefore limited to approximately 1-2 weeks.

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