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PARALYTIC SHELLFISH TOXINS IN PUGET SOUND, WASHINGTON STATE

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ABSTRACT The first illnesses and only deaths in Washington State resulting from paralytic shellfish poisoning were documented in the 1940s, resulting in the establishment of one of the longest monitoring programs for paralytic shellfish toxins in commercial and recreational shellfish in the United States. An analysis of the Washington Department of Health's monitoring data for the Puget Sound area has allowed us to examine temporal changes in shellfish toxin levels and geographical distribution of shellfish harvesting closures. The values of toxins in shellfish were normalized to control for variable levels of toxin accumulation in different shellfish species by dividing individual values by the yearly average for a given species. These normalized values increased significantly over the past five decades, indicating that the observed increase in paralytic shellfish toxin levels in Puget Sound shellfish was not caused by the shift in species monitored. A geospatial map of the first shellfish closures or paralytic shellfish-poisoning event in each Puget Sound basin suggests that over time, toxigenic *Alexandrium* cells have been transported from northern to southern Puget Sound. Shallow sills that restrict the exchange of water between adjacent basins have hindered the transport of toxic dinoflagellates, especially because these cells generally do not prosper in mixing conditions that are characteristically found at sills. Large-scale events, such as the bloom that occurred in the Whidbey and Central basins in 1978, may have been induced by global climate changes or shifts, such as the Pacific Decadal Oscillation. Although greater numbers of closures have been observed over time in basins of Puget Sound, closures as a percentage of total samples analyzed have decreased or remained constant in all basins, indicating that the Washington Department of Health has established an effective monitoring program to protect public health while allowing for maximum harvest potential.

KEY WORDS: paralytic shellfish poisoning, saxitoxin, Puget Sound

INTRODUCTION

Background

Paralytic shellfish poisoning (PSP) is an acute illness in humans caused by eating bivalve shellfish (e.g., mussels and clams) that have ingested dinoflagellates that produce neurotoxic compounds. The dinoflagellate, *Alexandrium catenella* (Whedon and Kofoid) Balech, previously described as belonging to the genus *Gonyaulax* Whedon and Kofoid or *Protogonyaulax* Taylor, has been identified as the primary causative organism on the west coast of North America, but recent evidence indicates that at least five known species of *Alexandrium* can produce PSP toxins (PSTs) in Northwest waters (Horner et al. 1997). These dinoflagellates occur either as single cells or as chains of cells. Their two flagella enable them to vertically migrate to the surface during the day and to depth at night, giving them advantages over nonflagellated phytoplankton. Generally, dinoflagellates thrive in stratified water because of their motility and ability to move to nutrient-rich areas within the water column. When conditions for growth become less favorable, *A. catenella* cells form resting cysts that settle to the sediments, where they await the return of favorable growth conditions (Anderson 1980).

Historically, PSP has been known in the Pacific Northwest and Alaska for centuries. Records of PSP events date back as early as June 15, 1793 (Vancouver 1798), when a member of Captain George Vancouver's exploration team died after eating contaminated mussels harvested in the uncharted coastline of what is now known as British Columbia. In 1799, 100 Russian hunters died after consuming toxic mussels near Sitka, Alaska (Halstead 1965). The first recorded outbreak of PSP on the eastern shore of Van-

couver Island, Canada, in October 1957 caused serious illness in a number of people (Waldichuk 1958) and resulted in a mandatory monitoring program for PSTs in Washington State.

The PSTs include saxitoxin and at least 12 structurally related chemical compounds (see, for example, Baden 1983). The record level of PSTs in shellfish ever measured along the Pacific coast of North America was 31,000 µg saxitoxin equivalents (STXeq)/100 g shellfish in October 1989 in the inside passage of British Columbia, just north of the US and Canadian border (Bricelj & Shumway 1998).

PSP in Washington State

The Washington State Department of Health (WDOH) initiated a shellfish toxicity surveillance program in the early 1930s (Lilja 1978) as a joint effort between WDOH and the George Williams Hooper Foundation for Medical Research in San Francisco. This initial monitoring by WDOH focused on commercial shellfish and was expanded to include recreational shellfish in the early 1990s when the Puget Sound Water Quality Authority gave WDOH the authority to monitor recreational species. Washington State's only three fatalities due to PSP were recorded in 1942 (Quayle 1969) near the entrance to the Strait of Juan de Fuca (Fig. 1). Since then, the Washington Department of Fisheries has imposed annual harvesting closures for all shellfish except razor clams from April 1 to October 31 in the area west of Dungeness Spit (near Port Angeles, WA; Fig. 1) along the Strait of Juan de Fuca and southward along the coast to the Columbia River (Nishitani & Chew 1988). In general, razor clams do not retain high levels of PSTs but are now known to accumulate domoic acid (Wekell et al. 1994). The shellfish surveillance program in Washington State was terminated in 1946 when it was believed that this seasonal closure was effectively protecting public health. In June 1957, PST monitoring was reestablished to include all species of commercial shellfish in areas of north Puget Sound and the outer coast after WDOH was advised

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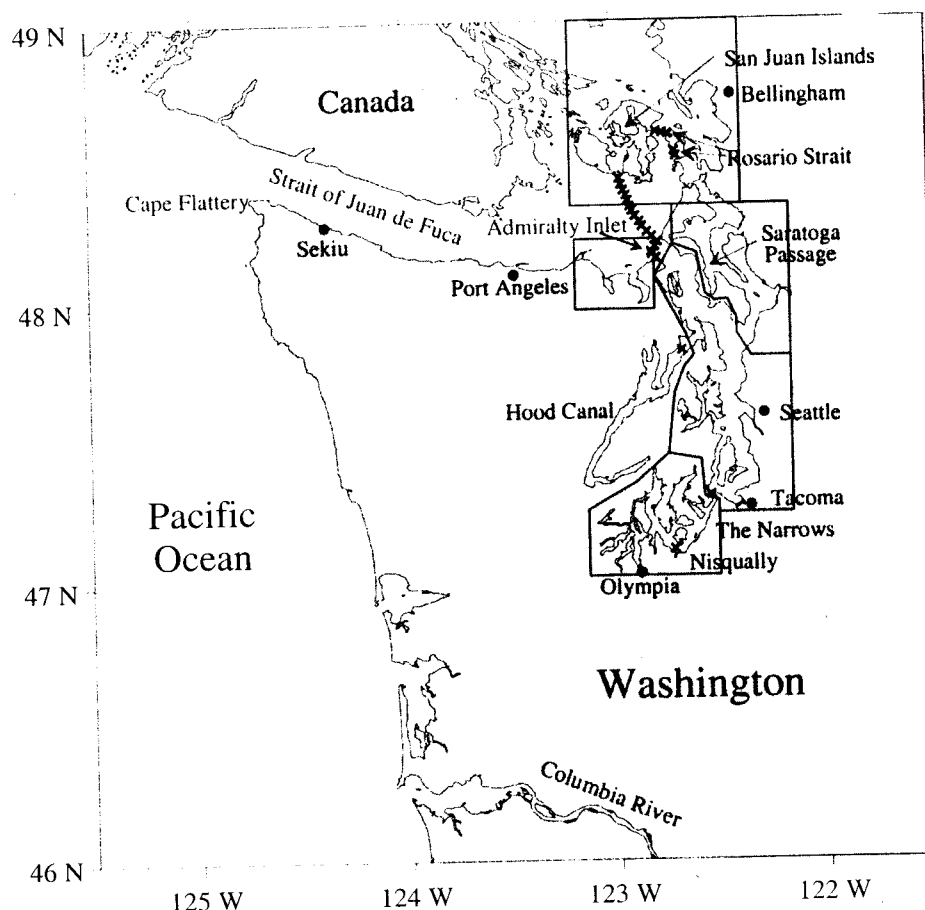


Figure 1. Map of western Washington. Puget Sound basins described in the text are outlined. Locations of sills less than 70 m deep are noted by Xs.

of the prevalence of PSTs in British Columbia shellfish. The monitoring of recreation and sport harvesting on the outer coast and in Puget Sound was sporadic until the early 1970s, when closures caused by PSTs in shellfish above the FDA regulatory limit of 80 μg STXeq/100 g shellfish occurred in the Bellingham area (Fig. 1) for the first time.

Physical Oceanography

Puget Sound is a complex fjord made of several distinct environments that are each influenced by different forces and conditions, including river runoff controlled by dams, free flowing rivers that undergo flooding due to snow-melt or heavy mountain rain and tidal flushing (Strickland 1983). Because of these distinguishing environmental factors, Puget Sound can be partitioned into a series of basins or environments using the descriptions and chart developed by Strickland (1983). The North basin extends from the Canadian border and includes the Strait of Georgia, San Juan Islands and Samish bay. In the North basin, the San Juan Islands are partially bounded from the Northwest basin by a sill at their southern edge (Fig. 1). In addition, the waters in Bellingham Bay are partially separated from the San Juan Islands by sills in the Rosario Strait. The Northwest basin is comprised of two semi-enclosed bays, Sequim and Discovery bays, with oceanic influence from the Strait of Juan de Fuca. This basin has the longest recorded history of PSTs in the Puget Sound with frequent blooms of varied intensity and duration. The Central, Whidbey, and South basins

are partially bounded from the Strait of Juan de Fuca by a sill at Admiralty Inlet to the north and west (Fig. 1). The Whidbey basin is relatively shallow and strongly influenced by high volumes of fresh water from the Skagit River, controlled by a series of hydroelectric dams on its upper reaches. The Central basin fronts the high population center of Seattle and contains the deepest waters of Puget Sound. While the Central basin receives fresh water inputs from a number of rivers to the north and south, the volume of its salt water mass is enormous compared with the other basins. Its circulation is influenced by sills at both the northern (Admiralty Inlet) and southern (Tacoma Narrows) ends. The sill at Tacoma Narrows also borders the South basin that extends to the southernmost reach of Puget Sound as a series of small, finger-like shallow fjords. The eastern and western finger inlets of south Puget Sound are believed to be two dynamically distinct water bodies with separate circulation (Ebbesmeyer et al. 1998). The primary freshwater influence in the South basin is the Nisqually River, fed by melting snows from Mt. Rainier and the surrounding mountain ranges. Currents in the South basin are strongly influenced by tides, due largely to the shallowness of this area. Finally, Hood Canal is partially isolated by a sill near its entrance that limits the transport of deep marine waters in and out of the canal (Burns 1985). Currents in Hood Canal are slow, perhaps because the basin is a closed-ended fjord without large volume rivers. It is the most poorly flushed of all inlets in Puget Sound (Strickland 1983), but the strongest currents tend to occur near the entrance at the north.

In summary, all Puget Sound basins are strongly influenced by fresh water input, resulting in density-dependent stratification, especially in the summer months.

The spread of PSTs into previously unaffected areas, such as south Puget Sound (Nishitani & Chew 1988) has raised an awareness of the significant and expanding threat to human health and economics of some of the most productive recreational and commercial shellfish regions on the US west coast. An analysis of PST data for the Puget Sound areas collected over the past five decades has allowed us to examine changes in PST levels and geographical distribution over the past five decades. This assessment will allow us to evaluate whether modifications of the current monitoring program or additional preventive measures are needed to effectively protect seafood consumers as well as assist aquaculturists.

METHODS

WDOH Database

Shellfish toxin data were provided by the WDOH Office of Food Safety and Shellfish Programs that routinely monitors PSTs throughout the state in both commercial and recreational shellfish. The data have been collected over a period of more than 40 y from samples submitted by commercial growers and local health agencies as required by federal and state regulations. In some cases, local health agencies have collected samples directly from beaches in their jurisdictions but have also relied on samples submitted by volunteers.

In the last 20 y, mussels have been selected as a sentinel species for PSTs because they bioaccumulate the toxins at a faster rate than other shellfish. However, in the early years of monitoring (1960–1980), Pacific oysters (*Crassostrea gigas*) and butter clams (*Saxidomus giganteus*) constituted the major species sampled for PSTs (Table 1). Since 1989, WDOH established a sentinel mussel-monitoring program (Nishitani 1990) in which the blue mussel, *Mytilus edulis*, generally was sampled; however, *M. galloprovincialis* and *M. californianus* were collected at a few Puget Sound sites (Determan 2000). At most sites, mussels were sampled every 2 wk during the year from wire mesh cages suspended about one meter deep below floats and docks. These cages were periodically restocked with mussels. About 100 mussels provided the 100 g of

tissue needed for toxin analysis. Mussels were collected, packed with frozen gel packs, and shipped to WDOH for analysis.

WDOH performed all testing for PSTs using the standardized mouse bioassay. The procedure has been modified since its inception in the 1920s by the inclusion of a saxitoxin standard provided by the US Food and Drug Administration (FDA), and expression of results in saxitoxin equivalents, STXeq (AOAC 1990). Early data from the 1950s and 1960s expressed as "mouse units," were converted to the newer designation by multiplying the mouse units (MU) by the factor 0.2. Thus 400 MU/100 g shellfish tissue is equivalent to 80 µg STXeq/100 g, the current "action level" specified by the FDA (AOAC 1990).

Data collected over the years by WDOH were not intended for establishing trends but rather were collected solely to protect the health of shellfish consumers. In other words, there was increased sampling during a toxic event, to characterize the extent and severity of the event, resulting in a greater proportion of tests that are positive for toxin. For the purpose of this study, we included all data for shellfish collected from 1957 through 1999. Blue mussels, butter clams, littleneck clams (*Protothaca staminea*), and Pacific oysters make up the largest number of samples analyzed (Table 1). During the 20-y period from 1957 to 1977, sampling by WDOH was relatively constant, averaging about 145 samples per year. After the record-breaking PST level measured in 1978 (30,360 µg STXeq/100 g), the agency increased its sampling.

Data Analysis

A shellfish toxin database was constructed from individual PST test data sets from the WDOH for each year from 1957 through 2000. These data sets were formatted and imported into a data table in Microsoft Access (Microsoft Corp, Bellevue, WA). The sample numbers that were assigned by WDOH were used as unique identifiers for each record. A table containing latitude and longitude coordinates along with sampling site descriptions was linked to the PST data table through a field containing a code that uniquely identified each sampling site. Similar methods were used to link tables containing common names for the samples and the names of the counties in which the sampling sites were located. Queries were constructed that allowed fields in any of the tables in the database to be searched.

RESULTS

Data Reduction for Trend Analysis

Sampling intensity throughout Puget Sound has been variable over the past 40 y, primarily because of budgetary constraints of the WDOH monitoring program. A variety of edible shellfish species with different toxin accumulation and retention capabilities was selected for monitoring purposes primarily because of availability. The major species used for monitoring in each basin since 1957 were oysters (North basin), littleneck clams (Northwest basin), blue mussels (Whidbey basin), littleneck clams (Central basin), and blue mussels (South basin; Table 2). These shellfish have different rates of accumulation and depuration of PSTs. For example, butter clams are known to retain high levels of toxin for months, whereas mussels are known to depurate toxins over a period of days (Bricelj & Shumway 1998). Additional variability in the data is caused by a disproportionate increase in sample size over time in certain basins relative to other basins. During recent decades, more reports of PSP illness, especially in south Puget

TABLE 1.

Number of shellfish samples collected by the Washington State Department of Health during each decade.

Decade	<i>Mytilus edulis</i>	<i>Saxidomus giganteus</i>	<i>Protothaca staminea</i>	<i>Crassostrea gigas</i>	Other*
1950s	4	127	20	169	69
1960s	0	208	146	362	157
1970s	649	1248	471	684	422
1980s	2361	3977	2712	2327	1773
1990s	9246	2498	4237	7078	779

*Other species (not all shellfish) include: *Cancer magister*, *Chione* sp., *Chlamys rubida*, *Clinocardium nuttallii*, *Crassadoma gigantea*, *Crassostrea sikamea*, *Ensis americanus*, *Fusitriton oregonensis*, *Haliotis kamtschaticana*, *Macoma nasuta*, *Macoma secta*, *Modiolus modiolus*, *Mya arenaria*, *Mytilus californianus*, *Mytilus galloprovincialis*, *Ostrea edulis*, *Ostrea lurida*, *Panopea abrupta*, *Parastichopus californicus*, *Patinopecten caurinus*, *Polinices lewisii*, *Tapes philippinarum*, *Tresus nuttallii*.

TABLE 2.
Shellfish collected by the Washington State Department of Health (1957–1999).

Basin	Total	<i>Mytilus edulis</i> (%)	<i>Saxidomus giganteus</i> (%)	<i>Protothaca staminea</i> (%)	<i>Crassostrea gigas</i> (%)	Other*
North	10,175	18	19	11	31	21
Northwest	5,961	12	22	38	13	15
Whidbey	3,696	55	29	7	1	8
Central	13,673	25	25	26	8	16
South	5,644	43	4	4	33	16

* Other species (not all shellfish) include: *Cancer magister*, *Chione* sp., *Chlamys rubida*, *Clinocardium nuttallii*, *Crassadoma gigantea*, *Crassostrea sikamea*, *Ensis americanus*, *Fusitriton oregonensis*, *Haliotis kamtschatkana*, *Macoma nasuta*, *Macoma secta*, *Modiolus modiolus*, *Mya arenaria*, *Mytilus californianus*, *Mytilus galloprovincialis*, *Ostrea edulis*, *Ostrea lurida*, *Panopea abrupta*, *Parastichopus californicus*, *Patinopecten caurinus*, *Polinices lewisii*, *Tapes philippinarum*, *Tresus nuttallii*.

Sound, have required an increase in PST testing. The different sampling intensity as well as the shift in shellfish species collected over time has necessitated data reduction for the purpose of trend analysis. Because we examined the data for trends in PST activity, only samples having quantifiable levels ($\geq 32 \mu\text{g STXeq}/100 \text{ g}$) of PST by mouse bioassay were included. All the quantifiable PST data for San Juan Island shellfish are shown in Figure 2A. San Juan Island was chosen because one of the longest historical records in Puget Sound is available from this site. Data were simplified by showing only the highest annual level of PST (Fig. 2B). Averages per decade (Fig. 2C) of those maximum annual levels were calculated in all shellfish from the San Juan area from the 1950s to the 1990s. Finally, data were normalized to control for different rates of uptake and depuration of PSTs in all shellfish tested by dividing individual PST values by the average for that species. The maximum normalized PST values were determined for each year then averaged for the decade (Fig. 2D). When the normalized maxima per decade for the 1950s through 1970s were compared with the past two decades (1980s and 1990s), the more recent two decades were significantly higher (*t*-test, $P < 0.001$). The rise in PST values over the past several decades is clearly seen in Figures 2C and D.

PST in Basins of Puget Sound

A series of environmental factors such as the presence of bounding sills, river input, and unique bathymetry were used to divide Puget Sound into distinct basins (Strickland 1983; Fig. 3). Sites that show typical PST levels within a given basin were selected for this study upon recommendation by WDOH. Because central and south Hood Canal shellfish have remained essentially free of PSTs, this arm of water west of the Central basin was not included as part of this analysis. A summary of averages by decade of maximum PSTs in all defined basins in Puget Sound showed increasing magnitude of toxins in all shellfish monitored at all sites with the exception of Whidbey and Central basins (Fig. 3). In the North basin, Samish Bay had relatively low levels of PSTs during the past three decades, whereas San Juan Island and Georgia Strait had more intense toxic events with the average by decade of annual maximum levels increasing from the 1970s to the 1990s. In the Northwest basin we observed obvious increases in levels of PSTs in both Sequim and Discovery bays over several decades. In the Whidbey basin, PST levels remained relatively low, except for an anomalously high level of toxin ($30,360 \mu\text{g STXeq}/100 \text{ g}$) in 1978 at Holmes Harbor. Levels of this magnitude had never before (and have not yet again) been observed in Washington State. Rec-

ord levels of shellfish toxin measured from Whidbey Island south to central Puget Sound in 1978 were responsible for the anomalous peaks seen in Holmes Harbor and Agate Pass in the 1970s (Fig. 3). In the Central basin, Quartermaster and Kilisnoe harbors, as well as

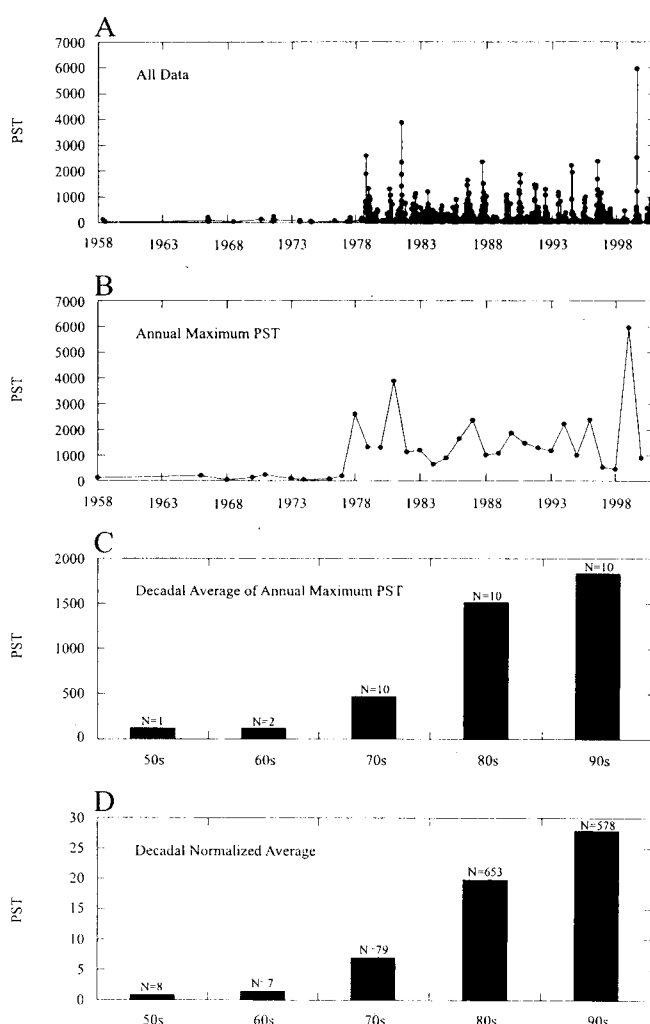


Figure 2. PST levels ($\mu\text{g STXeq}/100 \text{ g}$) in all shellfish from San Juan Island, collected from Jan 1958 to Nov 1999. All data (A), maximum annual PST levels (B), and average per decade of annual maximum PST levels (C), and normalized average per decade of annual maximum PSTs (D) are shown.

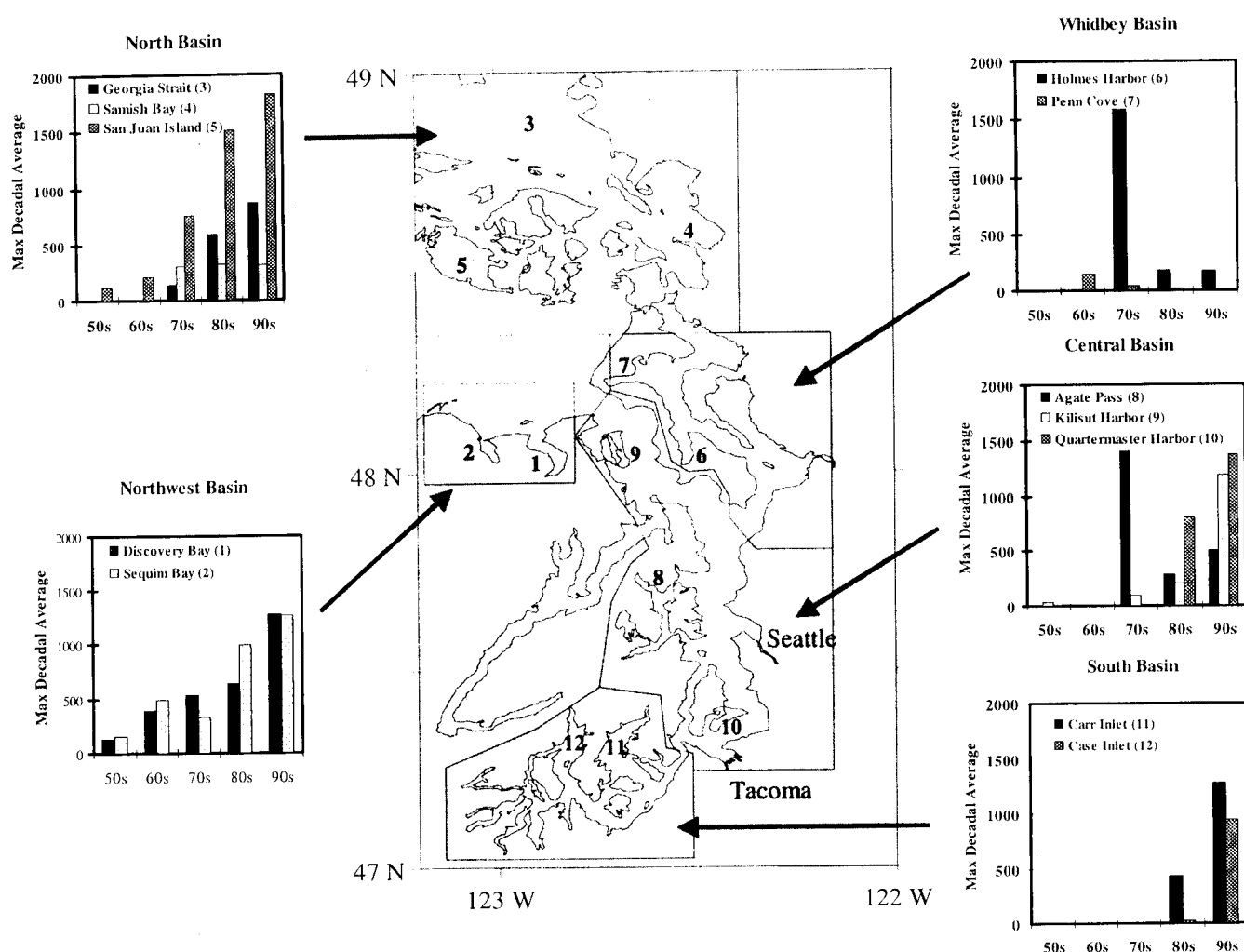


Figure 3. Averages per decade of maximum PSTs ($\mu\text{g STXeq}/100\text{ g}$) in each Puget Sound basin. The locations of representative sites in each basin are numbered.

Agate Pass, showed clear increases in average of annual maximum levels over the past two decades. In the South basin, PST levels have recently reached record highs. Carr Inlet had its first shellfish harvesting closures in 1988, although monitoring had been done at this site since 1957. Before 1988, PSTs had only occasionally been measured in the South basin but at levels below regulatory limit. Nearby Case Inlet had its first closure in 1991. Since the 1991 event, this area has experienced more frequent toxic events and

higher levels of PSTs, reaching a maximum of $13,769\text{ }\mu\text{g STXeq}/100\text{ g}$ in blue mussels in 2000.

Frequency of PST Closures

The frequency of PST closures over time in each Puget Sound basin is shown in Table 3. Although the number of samples collected over time has increased, closures as a percentage of total

TABLE 3.
Number of closures in Puget Sound basins, also as a percentage of total samples analyzed during each decade.

Decade	Northwest		North		Whidbey		Central		South	
	Closures	%	Closures	%	Closures	%	Closures	%	Closures	%
1950s	32	25	1	2	0	0	0	0	0	0
1960s	195	45	2	1	0	0	0	0	ND*	ND
1970s	227	27	260	20	165	39	109	18	0	0
1980s	610	34	827	22	119	7	912	23	238	22
1990s	387	14	486	10	31	2	1088	12	998	22

* ND = No data

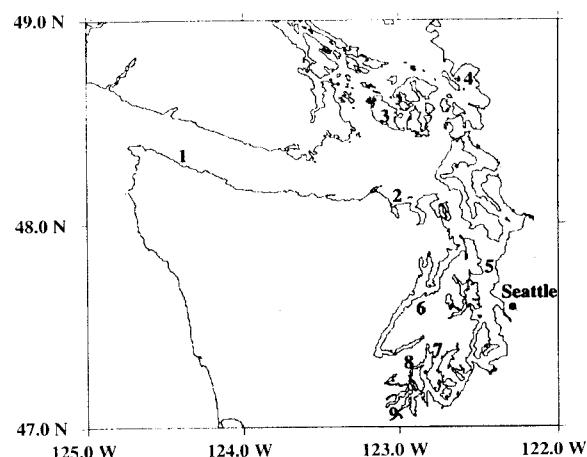
samples analyzed in each basin were variable. However, in general a decrease in percentage of closures in each basin during the 1990s relative to previous decades was evident, except in the South basin, where 22% of the samples analyzed resulted in closures in both the 1980s and 1990s.

Seasonal Duration of Closures

The greatest number of closures during each decade occurred from July through November with 81% of all closures occurring during these months in the 1950s, 69% in the 1960s, 63% in the 1970s, 65% in the 1980s, and 73% in the 1990s (Table 4).

Spread of PSTs

The historical record of PSP events causing illness and death in humans and initial shellfish closures in the different regions of Puget Sound is shown in Figure 4. The death of three people and illness of two others after their consumption of mussels and butter clams from the beach in Sekiu in 1942 was the first evidence of high levels of PSTs in Washington State. The death of three members of the Ucluelet Tribe after eating mussels containing PSTs on the west coast of Vancouver Island, British Columbia, Canada, was recorded three days prior to the mortalities in Sekiu (L. Hanson, pers. comm.), indicating that this event was probably widespread in the Pacific Northwest. From 1942 to 1957, Washington State monitoring was sporadic and was actually temporarily stopped in 1946 because of blanket closures that were in effect at this time (Lilja 1978). Monitoring for PSTs in Washington became formalized in 1957 after a large outbreak of PSTs occurred in British Columbia, Canada (Waldichuk 1958). During this year, the first shellfish closure occurred in Sequim Bay when a level of 162 μg STXeq/100 g was measured in butter clams. The first shellfish closure in the San Juan Islands occurred in 1958 when a level of 122 μg STXeq/100 g was measured in butter clams. In the early 1970s, when WDOH monitoring efforts increased, shellfish containing PSTs were found further east in Lummi Bay (Fig. 4) when 465 μg STXeq/100 g was measured in Pacific oyster in 1973. In 1978, anomalously high PST levels (up to 30,360 μg STXeq/100 g) caused the first shellfish closures in both Whidbey and Central Puget Sound basins. Over a period of several weeks, the contamination spread southward in Puget Sound to an area between Seattle and Tacoma in south-central Puget Sound. In 1987, levels of PSTs



First Shellfish Harvesting Closures and PSP Event in Each Region

Record	Year	Region	Location of first closure and/or PSP event
1	1942	NW	Five cases of PSP in Sekiu, three deaths
2	1957	NW	Sequim Bay/Discovery Bay
3	1958	N	San Juan Islands
4	1973	N	Lummi Bay
5	1978	C	Whidbey Basin/Central Basin, 9 cases of PSP
6	1987	C	Northern Hood Canal
7	1988	S	Carr Inlet, 1 case of PSP
8	1991	S	Case Inlet
9	1997	SW	Totten and Eld Inlets

Figure 4. First recorded PSP events and shellfish harvesting closures in each Puget Sound basin. Locations of each event are numbered on the map of Puget Sound.

in northern Hood Canal were measured above the closure limit for the first time since WDOH sampling began (234 μg STXeq/100 g in Pacific oyster). The first closures of shellfish harvesting in south Puget Sound in 1988 were due to PST levels up to 10,982 μg STXeq/100 g in Carr Inlet. One person was hospitalized after ingesting oysters from Minter Bay, Carr Inlet in September 1988 (F. Cox, pers. comm.). In 1991, the first incidence of shellfish

TABLE 4.

Number of monthly closures, also as a percentage of total closures during each decade.

Month	1950s		1960s		1970s		1980s		1990s	
	Closures	%	Closures	%	Closures	%	Closures	%	Closures	%
January	1	2	10	5	40	5	80	3	82	4
February	0	0	11	5	30	4	49	2	66	3
March	0	0	8	4	35	5	97	4	53	2
April	4	9	14	7	65	8	173	7	60	3
May	2	4	1	0	33	4	155	6	70	3
June	2	4	2	1	42	5	238	9	147	7
July	3	7	24	12	101	13	442	17	353	16
August	13	28	20	10	109	14	449	18	337	15
September	5	11	27	13	98	13	408	16	321	14
October	6	13	41	20	115	15	260	10	393	18
November	10	22	29	14	65	8	111	4	204	9
December	0	0	15	7	37	5	94	4	150	7

closures occurred in Case Inlet, with levels of 779 $\mu\text{g STXeq}/100\text{ g}$ in blue mussels. In the fall of 1997, PST levels up to 6799 $\mu\text{g STXeq}/100\text{ g}$ were measured in Eld and Totten inlets, causing the first shellfish closures in these small southwestern finger inlets of south Puget Sound. Previous routine monitoring, necessitated by the presence of commercial shellfish operations at these sites, detected only low levels of PSTs that were below the regulatory limit of 80 $\mu\text{g STXeq}/100\text{ g}$ (Saunders et al. 1982, Determan 2000). For example, the first measurement of PST in Carr Inlet was in 1981 at a level of 57 $\mu\text{g STXeq}/100\text{ g}$ in blue mussels.

When the highest annual PST levels exceeded 80 $\mu\text{g}/100\text{ g}$ even once at a particular monitoring site during a given decade, that site was shown to have a closure during that decade (Fig. 5). Although samples were tested in several areas throughout Puget Sound, in the 1950s and 1960s the only areas with shellfish clo-

sure were in the Northwest and North basins. In the 1970s, the number of sampling sites increased substantially, and closures were seen in central Puget Sound. During the 1980s, the first closures were seen in the eastern inlets of the South basin; shellfish closures occurred throughout much of south Puget Sound in the 1990s. An increase in the number of monitoring sites sampled over the decades is evident. Data from the 1970s indicated the high number of closures in 1978 in the Whidbey basin, however by the 1990s, few closures were observed here. The actual numbers of samples tested for toxins and closures in each basin as a percent of the total closures in all of Puget Sound are shown in Table 5. The greatest number of closures occurred in the Northwest basin in the 1950s (97% of all closures) and 1960s (99% of all closures), in the North basin in the 1970s (34% of all closures), in the Central basin in the 1980s (34% of all closures), and in the Central (36% of all

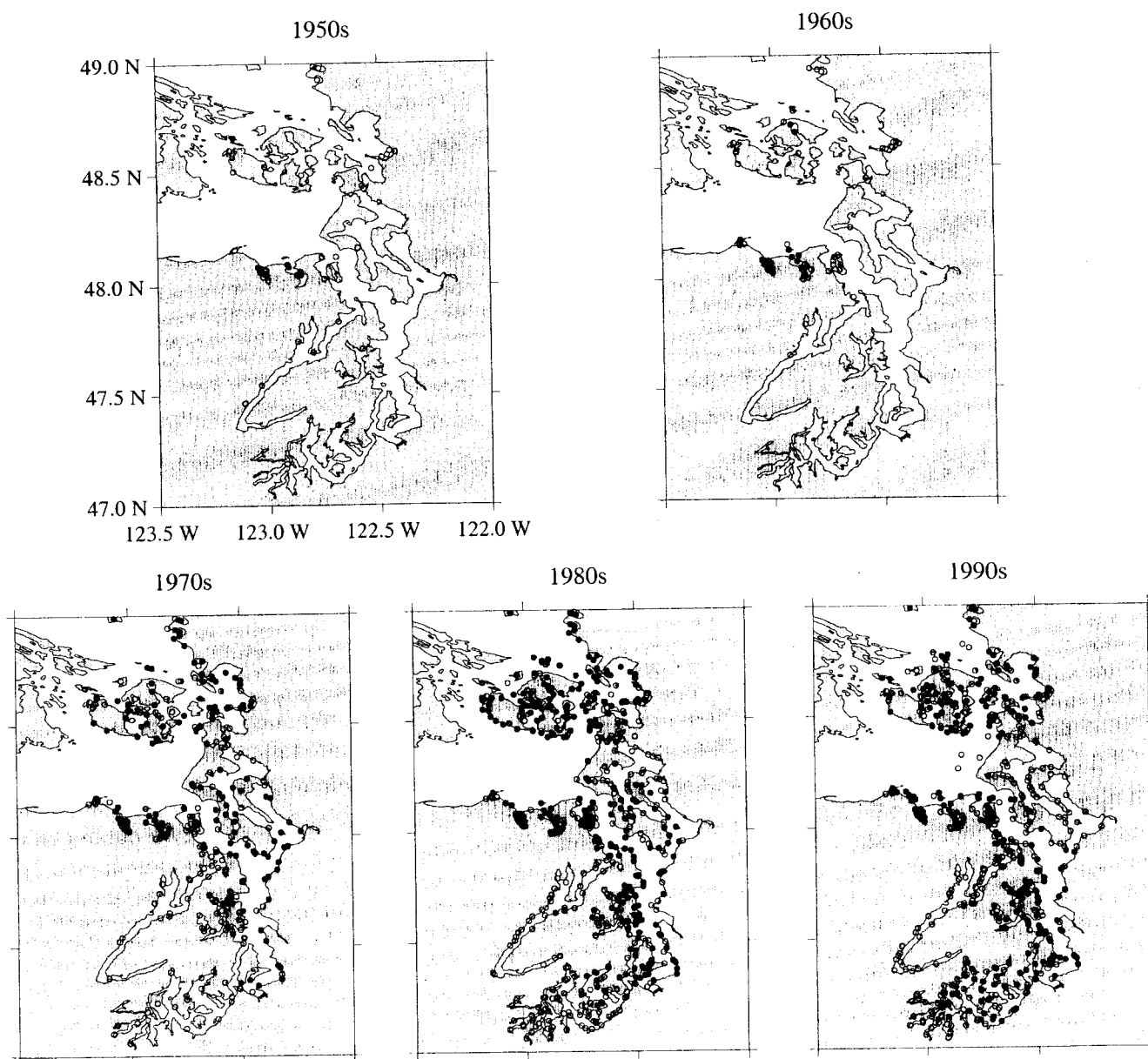


Figure 5. Closures because of PST in shellfish at all Puget Sound monitoring sites for each decade. Symbols represent maximum values for each decade shown as open circles (below 80 $\mu\text{g STXeq}/100\text{ g}$) or solid circles (greater than or equal to 80 $\mu\text{g STXeq}/100\text{ g}$). Data for the 1950s include only 1957–1959.

TABLE 5.

Number of samples analyzed for PSP toxins in each basin and closures in each basin as a percentage to total closures in Puget Sound.

Decade	Northwest		North		Whidbey		Central		South		Total Measurements	Total Closures	Closures as a % of Total Measurements
	Number	(%)	Number	(%)	Number	(%)	Number	(%)	Number	(%)			
1950s	130	97	53	3	1	0	13	0	4	0	201	33	16
1960s	433	99	202	1	1	0	17	0	ND [#]	ND	653	197	30
1970s	841	30	1302	34	423	22	607	14	24	0	3197	761	24
1980s	1793	23	3759	31	1704	4	3966	34	1080	9	12302	2706	22
1990s	2764	13	4859	16	1566	1	9070	36	4536	33	22795	2990	13
Highest level* (date)	3074 (9/17/90)		5968 (7/27/99)		30360 (9/28/78)		4822 (7/11/90)		10982 (10/22/98)				

* Highest level = $\mu\text{g STXeq } 100\text{g}^{-1}$, all in blue mussels.

[#] ND = no data.

closures) and South basins (33% of all closures) in the 1990s. Whereas the highest percentage of total closures occurred in northern Puget Sound in the 1950s, the greatest percentage has more recently occurred in the central and south Puget Sound regions. Closures as a percent of total measurements made have decreased since the 1960s.

DISCUSSION

There is speculation that harmful algal bloom events are increasing in intensity, frequency, duration, and geographical location; however, the long-term monitoring data needed to support these ideas are often insufficient for trend analysis. Because of documented illnesses and deaths due to PSP beginning in the 1940s, Washington State has one of the longest monitoring histories for PSTs in the United States with the State of Maine having the next oldest monitoring program, established in 1958 (Shumway et al. 1988). Data collected in Washington State are compilations of PST measurements at shellfish harvest sites designated by the WDOH to have the greatest risk for human exposure to PSP. Although the location and frequency of monitoring at these sites have changed substantially over the years, we were able to use the data to establish trends for Puget Sound shellfish closures due to PSTs.

Spread of PSTs into Central and Southern Puget Sound

Until the last decade, the only Puget Sound basins with no measured PSTs were southern Hood Canal and the southernmost inlets of Puget Sound (Rensel 1993, Determan 2000). Since the 1980s, the frequency of PST detection has increased in southern basins of Puget Sound, an area that contains the region's most productive shellfish-growing beaches. Shallow sills that restrict the exchange of water between adjacent basins (Strickland 1983; see also Fig. 1) have likely hindered the movement of toxic dinoflagellates, especially because these cells generally do not prosper in mixing conditions that are characteristically found at sills. *Alexandrium* cells thrive in stratified environments, presumably due to the supply of nutrients, trace minerals, and natural humic substances that may serve as growth stimulants at the density interface (see, for example, Anderson 1997). Therefore, sills, which are found at several sites in Puget Sound (Fig. 1), have likely delayed the spread of *Alexandrium* cells to the South basin.

A geospatial map showing the first accounts of shellfish closures or PSP in each region of Puget Sound (Fig. 4) suggests that over time, toxigenic *Alexandrium* cells, cysts or both have made a

slow progression from northern Puget Sound to the south. The numbers of cysts and cells likely have increased over the decades in the areas near sills, eventually reaching a critical mass that enabled their survival during transport over these natural barriers. Conditions for *Alexandrium* cell growth are ideal in south Puget Sound because of the many shallow, poorly flushed bays and inlets where thermally-caused stratification occurs during summer months, allowing ideal growth conditions for dinoflagellate cells to persist for weeks (Rensel 1993). However, the initial population of *Alexandrium* cells or cysts probably entered south Puget Sound only in recent years. The first detectable PSTs in south Puget Sound were noted in Carr Inlet ($57 \mu\text{g STXeq}/100 \text{ g}$ in blue mussels) in 1981. Some anecdotal evidence from the epidemiologic record also supports the gradual spread of toxigenic *Alexandrium* cells into south Puget Sound. Of the nine people who became ill after eating mussels from Carr Inlet in the summer 2000, one woman who was sick during that event previously had eaten shellfish from the same beach in Carr Inlet for more than 50 y with no PSP symptoms (Cox 2000).

A possible pathway of cells into Puget Sound was through the Strait of Juan de Fuca, into the Northwest basin and western San Juan Islands, then past the sill to the south of the San Juan Islands and Rosario Strait into Bellingham Bay. From the Northwest basin, cells may have been transported southward to the Whidbey basin, past the sill at Admiralty Inlet to central Puget Sound, and also past the sill at the entrance to Hood Canal to northern Hood Canal. Finally, from the Central basin, cells spread into south Puget Sound past the sills at Tacoma Narrows and the Nisqually (Fig. 1). The hydrographic separation of the eastern and western inlets of south Puget Sound (Ebbesmeyer et al. 1998) can explain the temporal lag in the first documented shellfish harvesting closures in Case Inlet in 1991 compared with Totten and Eld inlets in 1997 (Fig. 4).

The Initial Population of *Alexandrium* in Washington State

The first recorded PSP event in Washington State, at Sekiu in 1942 (Fig. 4), coincided with three deaths on the western coast of Vancouver Island, Canada. The next PSP episode in British Columbia was in the inland waters of the Strait of Georgia in 1961 when 61 people fell ill (Taylor & Horner 1994). It is possible that the source of the "seed" population of toxigenic *A. catenella* cells in Washington State originated from the inland or coastal waters of Canada. Indeed, the first documented PSP event in all of North America dates back to 1793, when four members of Captain

George Vancouver's crew became sick and one died of PSP during exploration of present day British Columbia (Quayle 1969). Unlike its neighbor to the north, Washington State had no recorded illnesses or deaths of humans with descriptions of PSP symptoms before 1942. *Alexandrium catenella* is the chief source of PSP off the west coast of British Columbia and eastern Vancouver Island (Taylor & Harrison, 2002) and evidence suggests that the earliest recorded PSP outbreaks were at least partially because of blooms of this dinoflagellate species (Quayle 1969). Because prevailing winds and currents are from the north during the summer months (Hickey 1989), when growth conditions for *Alexandrium* are optimal, and because the inlet to Puget Sound is at the north end of this fjord, a north to south transport would support the natural dispersal of algal cells from Canada. The routes of toxigenic cell dispersal in the Pacific Northwest could be defined in the future by a study of population genetics of *A. catenella* isolates from both British Columbia and Washington State.

Increased PST Levels

Because of increases in aquaculture activity as well as the measurement of PSTs in new areas of Puget Sound, the number of samples taken annually for PST testing has increased steadily from 1988 to the present time (Table 5). However, increased sampling frequency has not resulted in a higher percentage of closures during the latter decades (Table 3). The majority of closures during each decade was in July through November; a shift to more closures in earlier or later months has not been observed in recent years. In addition, no correlation between the highest toxin levels and total number of samples collected annually was observed (Table 5), suggesting that apparent increases in PST intensity are not due to increased sampling. Because mussels can accumulate higher levels of PSTs, the shift of reliance on oyster and clam samples in the monitoring program in the 1960s to mussel samples in the 1990s (Table 1) may account for some of the observed increase in toxin intensity. However, the normalized maximum values of PSTs in all shellfish have also increased over the past five decades (Fig. 2D), showing a statistically significant increase during the 1980s and 1990s compared with the three previous decades, supporting the fact that the increase in PST levels in Puget Sound shellfish was not due to the change in shellfish species monitored over the years.

PST Intensity Versus Human Population Growth

Over the last four decades, modern human development has extensively altered the shoreline habitats of Puget Sound (see the Department of Ecology, Water Quality Monitoring web page, http://www.ecy.wa.gov/programs/cap/mar_wat.html). A comparison of maximum PST averages per decade and population estimates (of all counties bordering Puget Sound) over the last 40 y shows a high level of correlation ($r^2 = 0.987$; Fig. 6). Although statistical correlation does not establish a causal link, it does suggest that some factor(s) associated with population growth may influence the magnitude of PSTs at any given site. Increased nutrients to our coastal environment may provide more favorable growth conditions for *Alexandrium* cells that populate a given basin. It has been speculated that the lack of nitrogen in surface and subsurface waters of Puget Sound has been a major factor limiting the further spread of PSTs into bays and inlets otherwise suitable for *A. catenella* (Rensel 1993). Land clearing, logging, aerial forest fertilizing by timber companies, direct sewage out-

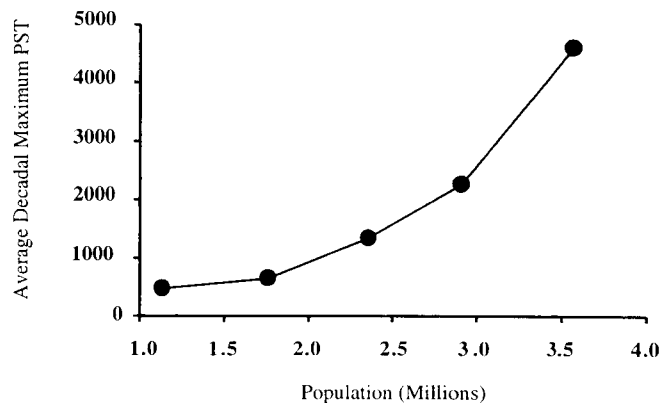


Figure 6. Maximum PST average per decade versus population estimates. Census data for counties bordering Puget Sound were obtained from the following site: <http://www.census.gov/population/cencounts/wa190090.txt>

falls, agricultural runoff, and even aquaculture operations have increased the amounts of nutrients, including nitrogen, that are supplied to the coastal ecosystems of Puget Sound (Howarth 2001). Inlets and fjords with low flushing rates that adjoin urbanized shorelines have the greatest sensitivity to nutrient addition (Mackay & Harrison 1997). The increased levels of PSTs in the semi-enclosed bays of south Puget Sound in recent years may, at least partially, be explained by increased eutrophication and generally poor circulation. Indeed, south Puget Sound is described by the Washington State Department of Ecology as one of the areas most susceptible to impacts of eutrophication (Cusimano 2002). Because the depth of south Puget Sound inlets is much shallower and flushing time is slower, nutrient inputs to surface waters provide ideal growth conditions for *A. catenella*.

Natural Events

Although the intensity of PSTs in shellfish has increased with time (Fig. 2), toxic events do not occur in each basin in every year. For example, shellfish closures have occurred in northern Hood Canal only in 1991, 1996, and 1997–1999. What sets those years apart from all other years? Environmental conditions such as water temperature, mixed layer depth, sunlight, and nutrients all work together to increase the chance of a toxic event in a particular basin and in any given year (Rensel 1993, Nishitani et al. 1988). In addition to microscale, basin-specific environmental factors that result in a periodicity of *Alexandrium* blooms, large-scale occurrences, such as the bloom that occurred in the Whidbey and Central basins in 1978, may have been motivated by global climatic events or shifts. In 1977, a large shift to a positive Pacific Decadal Oscillation occurred, with a resulting ecological response to the environmental changes. This period was marked by an enhancement of overall productivity that appeared to be closely related to changes in upper ocean mixed-layer depths and temperatures (Mantua et al. 1997). Indeed, an exceptionally deep surface layer of warm water was believed to have exacerbated the 1978 Whidbey basin bloom (Erickson & Nishitani 1985). Toxin levels of that magnitude have not been measured since that year in Whidbey basin, giving credence to the possibility that some unique, large-scale environmental factors influenced the occurrence of this event. The linkage of harmful algal bloom magnitude and frequency to climatic regime shifts has been suggested in recent

studies (Epstein et al. 1998, Hayes et al. 2001). The specific covariance of levels of PSP toxins in shellfish with strong El Niño/Southern Oscillation events (Erickson & Nishitani 1985) and other environmental parameters such as the condition of oysters in Willapa Bay (Ebbesmeyer et al. 1995) has been suggested.

Effective Monitoring

Although greater numbers of closures have been observed over time in many of the basins of Puget Sound, the percentage of closures relative to the total sites monitored in a given basin has decreased in all but south Puget Sound (Table 3). Although PSP toxins pose a serious threat to commercial and recreational shellfishing operations, the large number of sites monitored by WDOH allows the agency to pinpoint areas within a basin that are safe for harvest. This rigorous monitoring has resulted in a greater proportion of open than closed sites for shellfishing in the Puget Sound region where the risk for PSP is extreme. Increased HAB events and interest in commercial shellfish operations in all regions of Puget Sound and wide-scale, year-round recreational harvest opportunities will likely result in a mandate for the WDOH to sustain its rigorous sampling efforts. In the future, improved monitoring methods (e.g., molecular probes for cells and rapid analytical assays for toxins) will be essential for cost-effective and timely management of the fishery in Puget Sound.

CONCLUSIONS

The following conclusions can be obtained from our study. 1) There has been a significant increase in the magnitude of PSTs in

Puget Sound shellfish with time. 2) The geographical scope of shellfish closures caused by high levels of PSTs in Puget Sound has increased over the past four decades. The first recorded shellfish closures in the Northwest basin in the 1950s, the Central basin in the 1970s, and the South basin in the 1980s are likely due to the spread of *A. catenella* cysts and/or cells from north to south. 3) Shellfish closures in south Puget Sound may have been delayed until recent years by the physical blockage of cell movement by sills to the north. Hydrographic blockage may also explain the delayed appearance of PSTs in the southwestern finger inlets of south Puget Sound. 4) Increased shellfish closures caused by PSTs over the past few decades are not just the result of greater numbers of samples collected over time. 5) Global climate changes, such as the Pacific Decadal Oscillation and increased eutrophication in nearshore areas, are possible explanations for the increased magnitude of PSTs in shellfish today.

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