2023 Analysis of Harmful Algae in Bed Sediments of the Puget Sound in the Salish Sea

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Major:	Biomedical Science
Abstract:	*Alexandrium catenella* is a toxic dinoflagellate that has two life stages, a dormant cyst form within bed sediment, and a vegetative form that swims freely in the water column. Both produce a saxitoxin that can bioaccumulate within shellfish and when ingested by mammals, could potentially lead to paralytic shellfish poisoning (PSP). This project looked at the distribution of cysts of *A. catenella* in the Salish Sea to inform shellfish harvesters of the potential for harmful algal blooms in their region. In order to analyze for cysts, sediment was collected at 50 stations throughout the Salish Sea. These samples were processed and stained with Primulin, then viewed under a fluorescence microscope to identify and quantify the cysts present. The average cyst counts for all stations was 8 cysts/cc(wet) and 3 cysts/cc(dry). The highest cyst counts came from Central Basin-North, and Hood Canal-Central. Both locations shared 40 cysts/cc(wet) and 12 cysts/cc(dry). The low was 0, which was shared across 20 stations. Compared to 2022, there was an increase in cysts in areas around the south Puget Sound, more specifically Hood Canal, Dyes Inlet, and the Central Basin. For 2023, there was a decrease in cysts in Bellingham Bay, which is known to be a hotspot/seedbed for *A. catenella*. This data was shared with stakeholders to be used to determine if increased monitoring would be needed in locations where cysts were found.
Key Words:	*Alexandrium catenella,* cyst, algae, Puget Sound, Salish Sea, sediment
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Recommended citation:

Scheschy P. 2024. 2023 Analysis of Harmful Algae in Bed Sediments of the Puget Sound in the Salish Sea. Environmental Science Undergraduate Thesis. University of Washington Tacoma.

This research paper used samples collected by the PSEMP Project from the Washington State Department of Ecology at the 50 long-term stations in the Salish Sea's Puget Sound. In addition to the *Alexandrium* cysts analysis, other student researchers were able to analyze the sediments on particle size, total organic content, and microplastics. I would like to thank Julie Masura and Cheryl Greengrove for their continued mentorship and guidance throughout my undergraduate studies and allowing me to participate in this project. I would also like to thank Savanna Thayer for her particle size and total organic content data, as well my classmate Mia Robbins for her continued support throughout this project.

Please contact Julie Masura at jmasura@uw.edu with any questions concerning this work.

Introduction

Alexandrium catenella is a dinoflagellate that has two life stages, a dormant cyst form within bed sediment, and a vegetative form that can freely move through the water column. Both forms are responsible for producing the paralytic shellfish toxin (PST), saxitoxin, which bioaccumulate in shellfish and when ingested by mammals such as humans, can lead to paralytic shellfish poisoning (PSP). An approach to monitor these organisms is to analyze bed sediment for dormant *A. catenella* cysts.

The Puget Sound Partnership's Puget Sound Ecosystem Monitoring Program (PSEMP) is a consortium of researchers partnered with the University of Washington Tacoma (UWT) with the goal of monitoring and improving the health of the Puget Sound (PSP). To understand the state of the Sound, members of PSEMP in partnership with the Department of Ecology (DOE) collaborate on multiple projects such as particle size analysis (PSA), microplastics, total organic content (TOC), and cyst monitoring. UWT undergraduate students have been analyzing bed sediments since 2014, which allows for data to be collected and archived to help get a better understanding of the Puget Sound's ecosystems. Many locations throughout Puget Sound have been known to contain cysts and are regularly monitored and reported to stakeholders to increase tracking in areas that present cysts and to ensure the harvesting of shellfish is safe. Sharing this information with stakeholders can contribute to policy change, enhance monitoring for locations containing cysts, and notification be given so that the harvesting of shellfish in areas can be limited to protect human health. Since this is an annual project, comparisons to past years may give us an understanding of the movement and distribution of this organism, possibly seeing a trend due to environmental factors that may be attributing to blooms or an increase in cyst abundance.

Review of Literature

The first illnesses and deaths relating to PSP were in the 1940s, resulting in the Washington State Department of Health (WDOH) Office of Food Safety and Shellfish Programs to start routinely monitoring PSTs in both commercial and recreational shellfish (Trainer *et al.* 2003). This group applied data on the PSTs in mussels, oysters, and clams collected by the WDOH over a period of more than 40 years and analyzed it to establish a trend. The results of this study showed an increase in both the magnitude of PSTs in shellfish and in closures of shellfish harvesting areas over time. Five conclusions resulted from this work: 1) there had been an increase in PSTs in Puget Sound shellfish over time; 2) the geographical scope of shellfish closures had increased, likely due to the spread of *A. catenella* from north to south; 3) shellfish closures in south Puget Sound may had been delayed by the physical blockage of cells by sills, which also attributed to the delay of PSTs in the south Puget Sound inlets; 4) increased shellfish closures and PSTs were not just the result of increased sampling; and 5) global climate changes were possible explanations for increased PSTs in shellfish (Trainer *et al.* 2003). These trends provide ample evidence that increased monitoring throughout the Puget Sound was needed in order to track the movement of *A. catenella* and protect shellfish harvesting.

The National Oceanic and Atmospheric Administration's (NOAA) Ecology and Oceanography of Harmful Algal Blooms (ECOHAB) Program has been funding and leading research in understanding the impacts of HABs and their toxins. These projects have been prevalent since 1998, being authorized by the Harmful Algal Bloom and Hypoxia Research and Control Act (HABHRCA)(ECOHAB). In 2011, Horner et al. described the relationship between PSTs in shellfish and distribution of *A. catenella* cysts/vegetative cells. Funded by ECOHAB, 32 sites were sampled throughout the Puget Sound in 2005. A higher resolution sampling in

Quartermaster Harbor and Dyes Inlet was conducted in 2006, due to Quartermaster Harbor's high cyst density. Cyst enumeration was determined using the Yamaguchi *et al.* (1995) method, and sediment characteristics were conducted on all samples including PSA, TOC, and metal concentration. The results of this study showed cysts concentration ranging from 0 in sites of the southern Hood Canal, to a high of over 12,000 cysts/cc in Quartermaster Harbor. Cyst abundance was positively correlated with cadmium concentration, but no correlation was seen with sediment grain size, TOC, or any other metals tested (Horner *et al*. 2011). However, the higher resolution study showed cysts abundance to be correlated with finer grain and higher TOC sediment (Horner *et al*. 2011). Overall, this study found that any area in the Puget Sound where PSP had been reported had the potential for recurring blooms when conditions support growth, but more intensive cyst surveys, bloom dynamics, and local environment conditions were needed to determine possible cyst production areas (Horner *et al*. 2011).

Waters of the Salish Sea's Puget Sound are continuously sampled for *A. catenella* to get a better understanding of predicting HABs. While cyst enumeration is one of the leading analyses in trying to predict a possible HAB, germination of *A. catenella* may also be dependent on endogenous and environmental factors. In 2011, Tobin and Horner (2014) focused on these characteristics with sediment samples from Quartermaster Harbor, WA. They started by preserving the sample for an original cyst abundance, 48 wells were then set up and incubated at 13°C on a 12-hour dark: 12-hour light cycle (Tobin and Horner 2014). They found that germination occurred rapidly, in less that 24 hours, with no evidence of a synchronous endogenous clock. This meant that *A. catenella* could germinate year-round. Given this data, the mapping of cyst concentrations continues to be an important factor in forecasting potential HABs, with yearly station sampling and cyst enumeration.

The Puget Sound Alexandrium Harmful Algal Blooms (PS-AHAB) Group was a component of the NOAA ECOHAB program, focused on modeling *A. catenella* in the Puget Sound to evaluate the effects of climate change on habitat areas. Moore *et al.* (2015) experimentally determined *A. catenella* responses to temperature and salinity to investigate the future changes in timing, duration, and the extent of blooms. Using climate models incorporating coastal winds, orographic precipitation, and air-sea interactions over the Puget Sound, as well as including land surface characteristics established by the National Center for Atmospheric Research (NCAR), a realistic model of *Alexandrium* blooms was produced. The results for future *Alexandrium* bloom maps in this model included longer than normal HAB-favorable conditions, which support higher growth rates and longer bloom seasons. The largest increases were up to 30 more days of HAB-favorable conditions, which were projected in areas such as the North basin and in the finger inlets of South basin, with moderate increases in the Main Basin and Hood Canal (Moore *et al.* 2015). This was a simulation and assessment of *Alexandrium* bloom risk, with bloom conditions depending on many other factors including location of cyst "seed beds", timing of germination, transport of cells, nutrient availability, competition with other phytoplankton, and infection by parasites (Moore *et al.* 2015). However, temperature is strong determinant of biological rates, and with this model we can pull insight into the potential risks and development of *Alexandrium* blooms in the Puget Sound.

In 2015, Feifel analyzed *A. catenella* cysts within Sequim Bay. Sequim Bay is located at the northwest entrance of Puget Sound. Sequim Bay is almost entirely closed off by Travis Spit, with only about 10% of water being exchanged between the bay and the Strait of Juan de Fuca (EDPU 2005). This leads to Sequim Bay functioning as a controlled environment for *A. catenella* blooms. Using the Yamaguchi et al. (1995) method, Feifel processed a 197-cm long sediment core, cut into 2 cm intervals. She found that cysts were identifiable from the top down to about 100 cm, indicating that *A. catenella* cysts were present since the late 1800s. This technique showed a more widespread timescale of *A. catenella* cysts within a bay, giving data that could be used to track cyst numbers as they migrate and move within the Puget Sound waters (Feifel 2015).

NOAA's Monitoring and Event Response for Harmful Algal Blooms (MERHAB) Research program is tasked with enhanced routine water quality and shellfish monitoring to contribute better HAB detection methods, training, and strategies (MERHAB). Continued monitoring in the Puget Sound, Alaska, and the Gulf of Maine has been established since 2019, and in recent years, new methods are being developed to help track *A. catenella.* These include the use of quantitative polymerase chain reaction (qPCR) and Fluorescent In Situ Hybridization (FISH) techniques. UWT, NOAA, and the University of Alaska Fairbanks (UAF) have been leading a project on this topic with hopes of a more rapid and accurate detection of *A. catenella* cysts in sediment from the Gulf of Maine, Puget Sound, and waters around Kodiak Island and Kachemak Bay, AK (Greengrove *et al.* 2022). While the paper and results of the study remain unpublished, the idea of a faster and more reliable *A. catenella* cyst detection method shows promise in the prediction and monitoring of HABs.

This project is a continuation of the PSEMP conglomerate and cyst mapping, which has been going on since 2014. Research from 2023 allows for past data to be interpreted and for the movement of *A. catenella* through the Salish Sea's Puget Sound to be documented.

Methods

Sampling

Samples were collected at 50 locations throughout the Salish Sea (fig. 1), spanning from Budd Inlet up to Bellingham Bay, by Washington State Ecology's Marine Sediment Monitoring

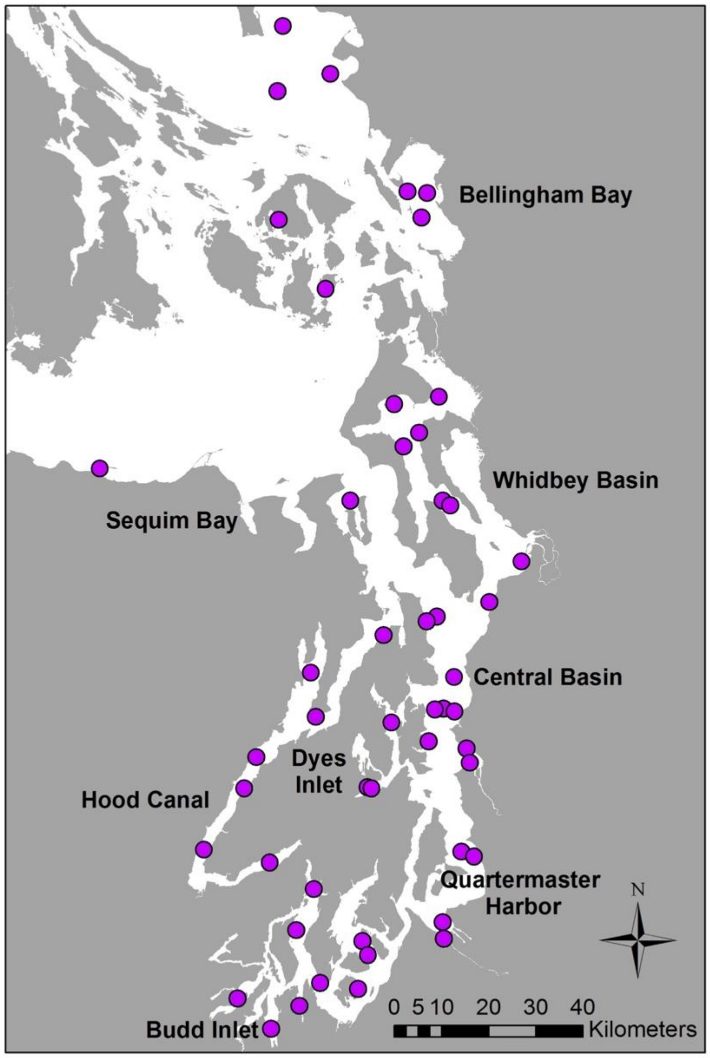

Figure 1. Station map of all 50 stations sampled throughout the Salish Sea's Puget Sound.

Group (WADOE 2019). Sediment was collected from the seafloor using a van Veen sediment grabber at 0-1 cm deep. Samples were placed in Ziploc bags and stored in dark and cold conditions, around 4°C. They were then transferred to the University of Washington Tacoma for use in PSA, TOC, harmful algal bloom and microplastic analysis. This project focuses on harmful algal blooms and *A. catenella* cysts.

Laboratory Analysis

Preparation of the samples came from the modified Yamaguchi *et al.* (1995) method. Five milliliters of sample sediment were diluted 1:5 with filtered sea water (FSW). Ten milliliters were subsampled with an autopipette and put into a transfer tube and diluted once more. The solution was sonicated to remove any excess sediment and the mucous layer surrounding the cysts. The sample was sieved using 90 µm and 20 µm sieves, stacked respectively, to narrow down sediment size. The solution was continuously rinsed with at least 1000 mL of FSW. The remaining sediment in the 20 µm sieve was transferred into a 15 mL centrifuge tube. It was again diluted with FSW to the 14 mL mark and topped off with 0.75 mL of formalin to preserve the cysts. After 2 hours, the tube was centrifuged, aspirated, and 10 mL of methanol was added to etch the cysts for staining. After two days, the material was centrifuged, aspirated, and then rinsed with 10 mL of FSW twice and stained with 2 mL of Primulin to allow for fluorescence. After 2 hours, the sample was centrifuged, aspirated, and rinsed with FSW three times. The solution was then ready to be counted after the addition of FSW to get a total volume of 10 mL.

The counting process involved loading a Sedgewick-Rafter counting chamber with 1 mL of vortexed or mixed solution and viewing under an Olympus BX41 fluorescent microscope. An FITC filter was used at a wavelength of 204 nm. *A. catenella* was visually identified by size, shape, wall structure, and color (Hallegraeff *et al.* 1995), which these cysts range from 38 to 56 µm long, "jellybean" or oblong shaped, contain a well-defined wall, and appear colorless with a red dot (fig.

2). Under fluorescence cysts appear green. Finally, using the series of calculations seen below, the cyst/cc wet and dry was found. The wet and dry masses were acquired through TOC analysis.

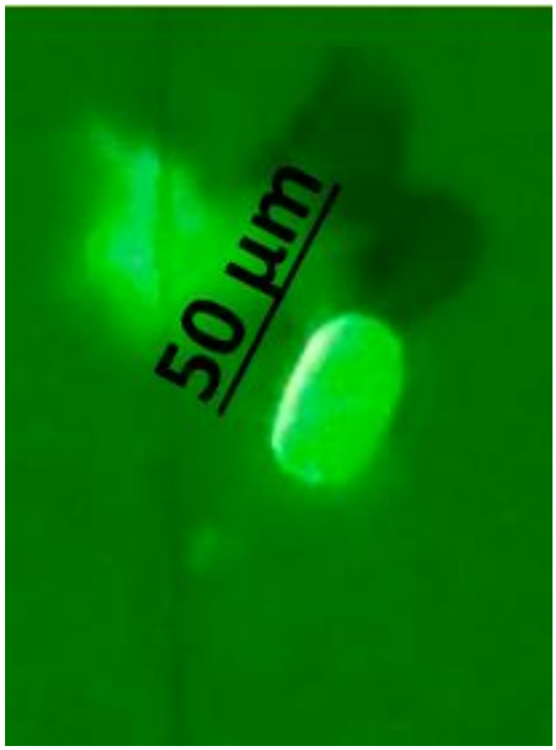

Figure 2. *A. catenella* epifluorescent microscope image. (Photo by C. McFarland 2019).

$$\text{cysts/cc(wet)} = \text{final count} * (V_{initial} / V_{sub}) *(V_{final} / V_{sediment})$$

$$\text{cysts/cc(dry)} = (\text{cysts/cc (wet)}) * (\text{mass (g)/cc (dry)}) / (\text{wet/dry (mass)})$$

Cysts/cc(wet) indicates the distribution of cysts in wet sediment, to give an understanding of the spatial distribution of cysts. Cysts/cc(dry) indicates the cyst distribution in dry sediment, or in solids with no water fraction.

The samples were collected for this analysis during the spring to summer season, when the weather was warmer and there were more resources available in the ecosystem. These conditions could allow for the germination of *A. catenella* cysts and when HABs could occur, so the amount of *A. catenella* cysts in the sediment samples may be lower. While this data is still valuable in understanding the distribution of this organism, collection of samples in the winter may show more cysts, making it easier to identify if a potential harmful algal bloom may occur in the spring/summer.

Results

A. catenella cyst enumeration was recorded and used to calculate cysts/cc(wet) and cysts/cc(dry) as well as the average across all stations. The average cyst counts for all stations was 8 cysts/cc(wet) and 3 cysts/cc(dry). The highest cyst count came from Central Basin-North, with 40 cysts/cc(wet), and 12 cysts/cc(dry). Hood Canal-Central, shared similar numbers with 40 cysts/cc(wet) and 11 cysts/cc(dry). The low was 0, which was shared across 20 stations. All counts can be seen in the Appendix. Comparisons between cyst counts (wet and dry) to percent TOC (wet and dry) were made to see if there was a relationship. TOC was acquired through the loss on ignition method. When cysts/cc(wet) counts were plotted against the percent TOC(wet), a R^2 value of 0.0685 was observed (fig. 3). Cysts/cc(dry) and percent TOC(dry) presented an R^2 value of 0.0007 (fig. 4). Stations with no cysts were removed from graphs. No significance was seen for either TOC plot. Cysts/cc (wet and dry) were also plotted against median grain size of the sediment collected. Grain size was acquired by using a Beckman-Coulter LS 200 Laser Diffraction Particle Size Analyzer at each station. The cyst/cc(wet) vs. median grain size provided an R^2 value of 0.0743 (fig. 5). Cysts/cc(dry) vs. median grain size proposed an R^2 value of 0.0007, (fig. 6). No correlation between cysts/cc and median grain size was observed and locations with no cyst abundance were excluded from data. Percent carbon and median grain size data can be seen in the Appendix.

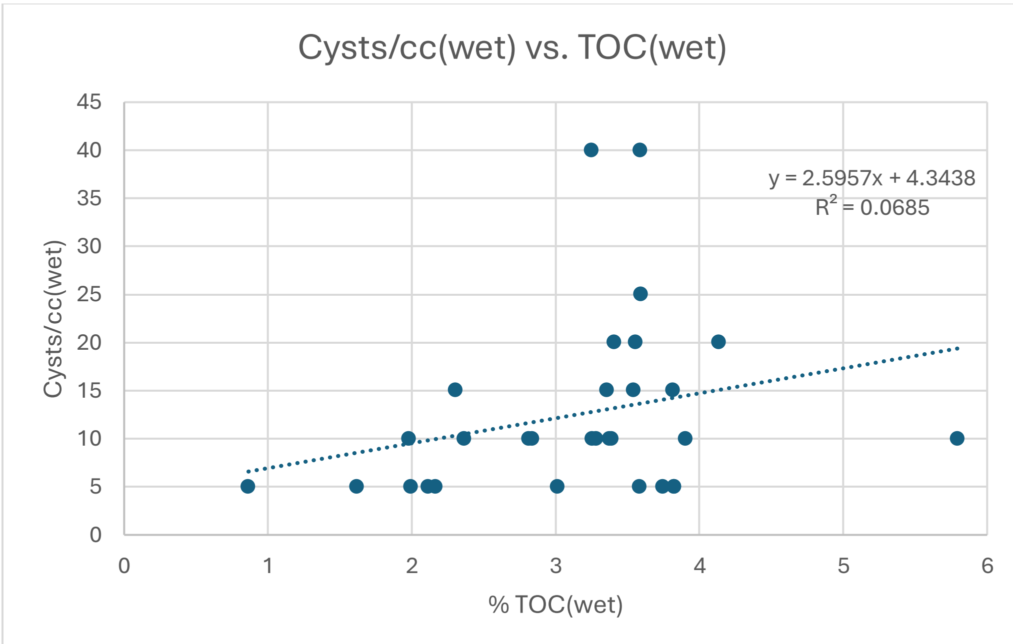

Figure 3. Cysts/cc(wet) vs. percent TOC(wet). An R2 value of 0.0685 was recorded, indicating no correlation between cysts/cc(wet) and percent TOC(wet).

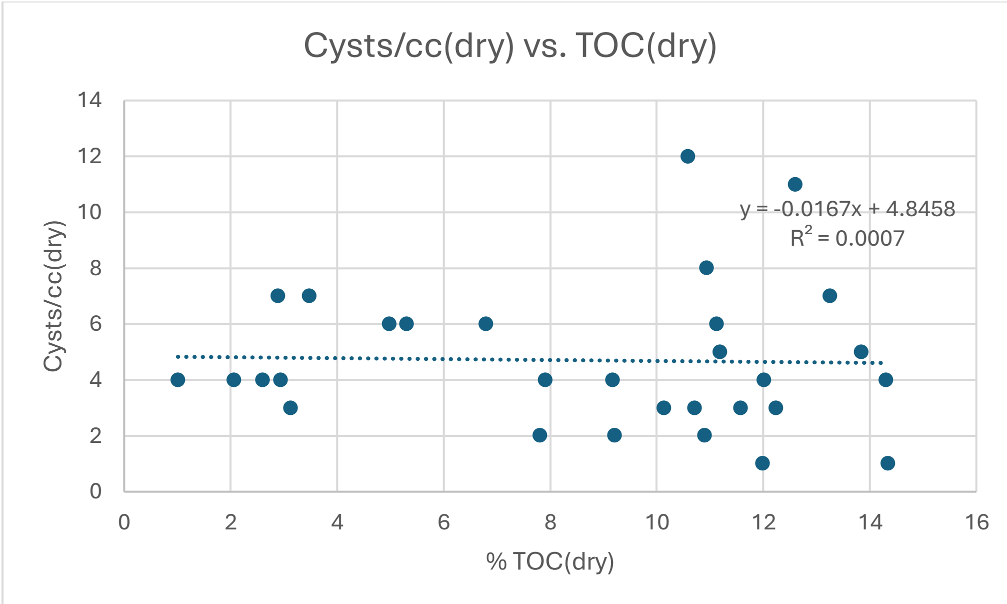

Figure 4. Cysts/cc(dry) vs. percent TOC(dry). An R2 value of 0.0007 was recorded, indicating no correlation between cysts/cc(dry) and percent TOC(dry).

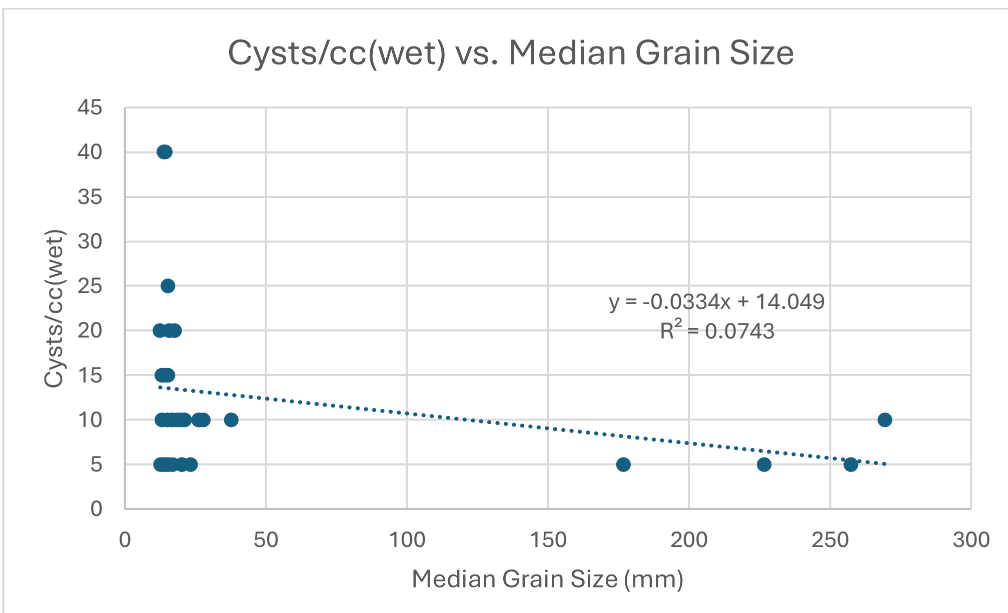

Figure 5. Cysts/cc(wet) vs. percent median grain size. An R2 value of 0.0743 was recorded, indicating no correlation between cysts/cc(wet) and median grain size.

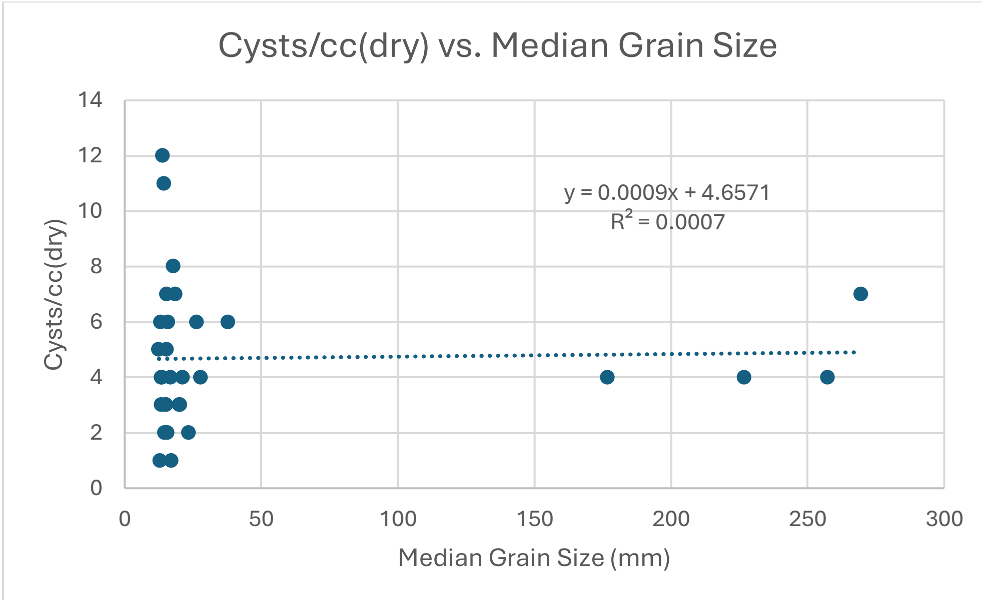

Figure 6. Cysts/cc(dry) vs. percent median grain size. An R2 value of 0.0007 was recorded, indicating no correlation between cysts/cc(dry) and median grain size.

Discussion

Choropleth maps of both cysts/cc(wet) and cysts/cc(dry) were made, seen in Figures 7 and 8. The highest cyst count came from Central Basin-North, with 40 cysts/cc(wet) and 12 cysts/cc(dry). Hood Canal-Central contained 40 cysts/cc(wet) and 11 cysts/cc(dry). The highest count of cysts in Hood Canal-Central, which was not previously known to contain high or any concentrations of PSP (Horner *et al.* 2011), shows the movement of *A. catenella* into the semi-isolated fjord. The changes in HAB-favorable conditions in Puget Sound can be due to changes in climate, which was described by Moore *et al.* 2015 and the model of sea surface temperature. A comparison to the 2022 samples of the same stations can be seen in the bar map of Figure 9. We see an increase in cyst enumeration in the Hood Canal, south Puget Sound, and Whidbey Basin, but also a decrease in areas of the north, like Bellingham Bay. The northern Puget Sound is consistently higher in PSP concentrations according to historical records (Trainer *et al*. 2003), but this shift shows the higher cyst concentration to the south, which may attribute to higher PSTs.

No correlation between cyst concentration, TOC and median grain size were observed. The R^2 values of 0.0685 for TOC(wet) and 0.0007 for TOC(dry) indicate that organic content does not play a factor in cyst enumeration. Organic content may play a role in excystment of *A. catenella*, with the nutrient availability being associated with their rapid germination, but the amount of organic content does not correspond to encystment. Median grain size against cysts/cc(wet and dry) showed no relationship. Cysts act as fine particles, settling in deeper and calmer parts of bays (Horner *et al*. 2011). This observation would imply that clay to silt-rich sediments should be most abundant, but with no correlation, this is not apparent. The highest cyst concentrations were however found to be in Hood Canal and the south Puget Sound, which are clay and silt dominant (Horner *et al*. 2011).

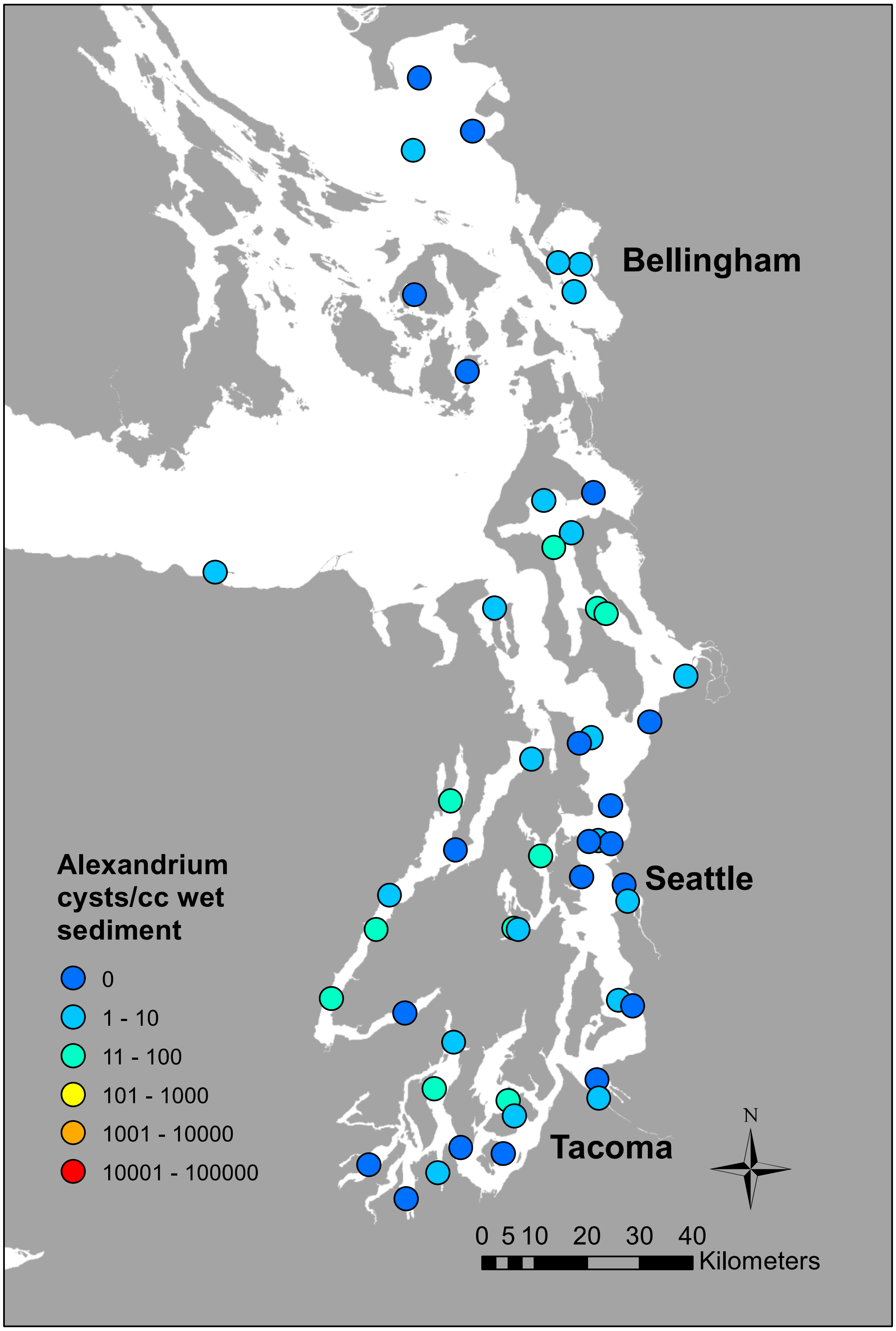

Figure 7. Cysts/cc(wet) choropleth maps of 50 stations sampled. Cysts/cc(wet) describes the spatial distribution with water fraction.

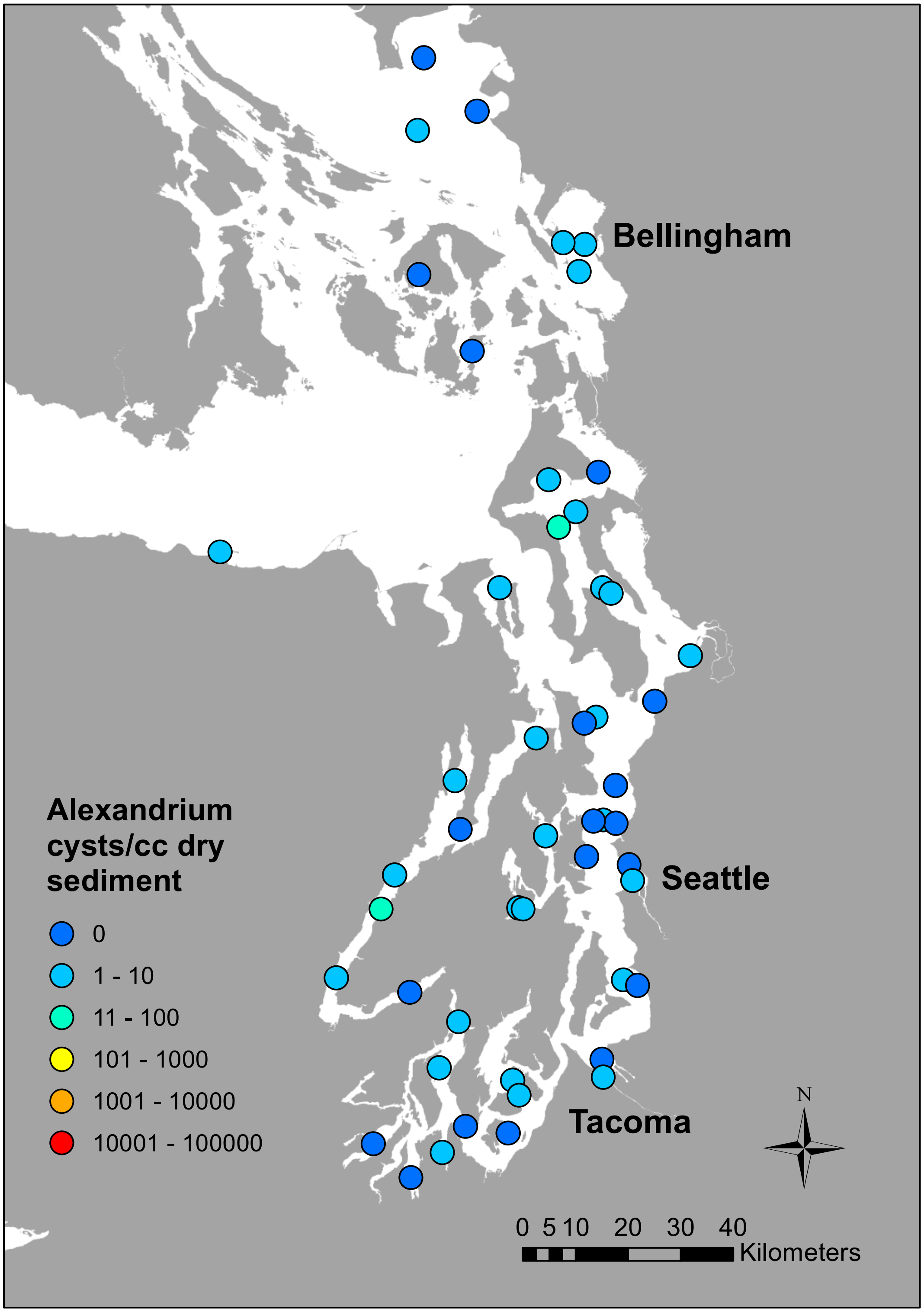

Figure 8. Cysts/cc(dry) choropleth maps of 50 stations sampled. Cysts/cc(dry) describes the distribution of cysts with water fraction removed.

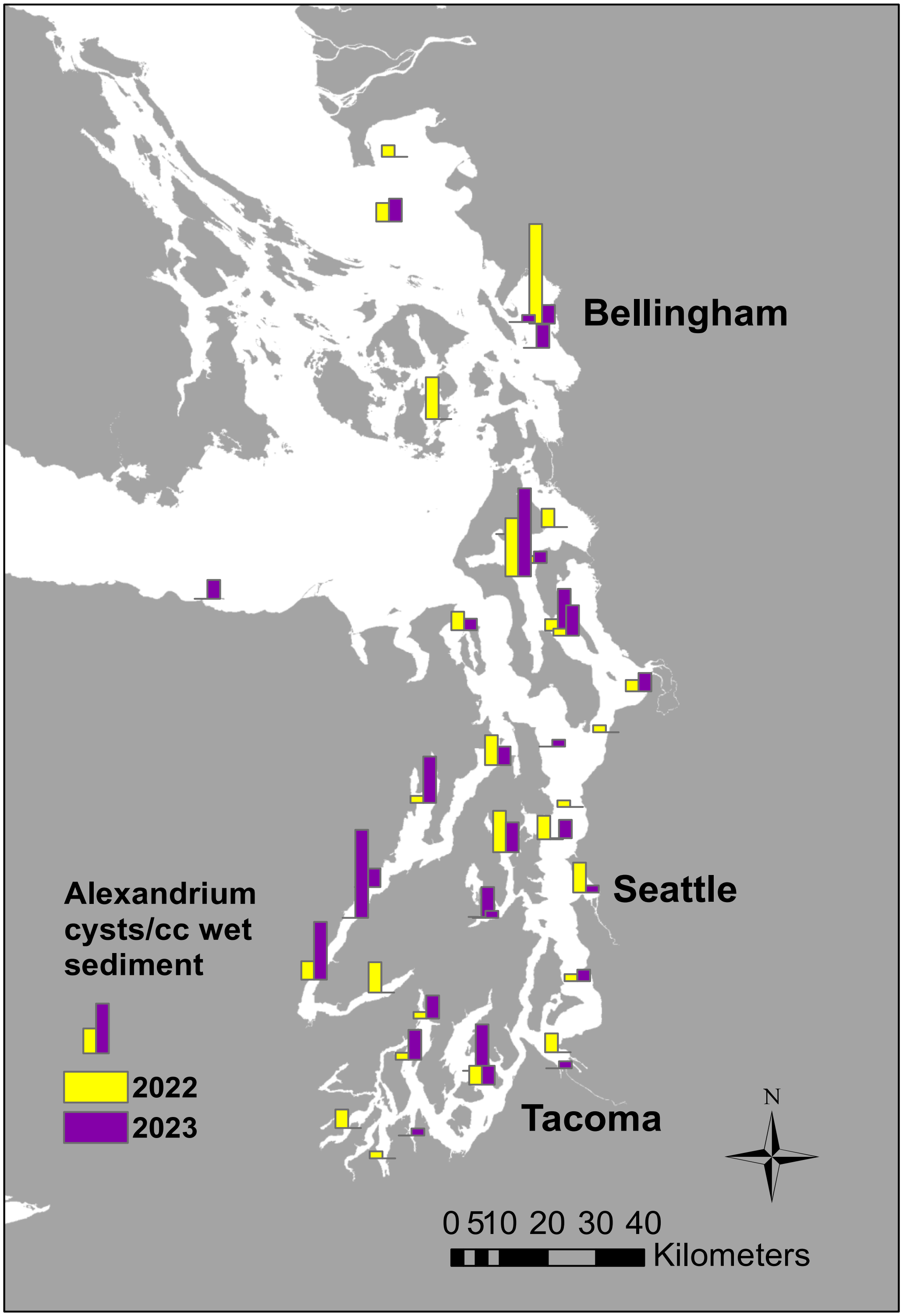

Figure 9. Bar graph comparison of cysts/cc(wet) of 50 stations sampled. Gold represents 2022 enumeration, purple represents 2023. An increase in Hood Canal and Southern basin can be seen, with a decrease in the north and Bellingham Bay.

Conclusion

Leading into the future of studies involving *A. catenella*, the main goal is the continuation of monitoring within the Puget Sound. The University of Washington Tacoma has been partnered with the DOE since 2014 and through the years there has been yearly data collected. This allows for present and future analysis to identify trends within the *A. catenella* population. These trends can give new light to other environmental factors that may be attributing to the movement and blooming of *A. catenella*. Through MERHAB, the use of qPCR and FISH techniques are also promising in both prediction and monitoring of *A. catenella*. While the process itself is still in development, the ability to rapidly and reliably detect cysts proves useful for in-field detection and laboratory analysis. Standard and “analog” cyst counting methods are labor intensive, so a rapid field-ready tool gives faster and more precise data.

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Appendix: Cyst/cc counts, TOC, and Median Grain Size

Station Name	Cysts/cc (wet)	Cysts/cc (dry)	Percent Carbon (wet)	Percent Carbon (dry)	Median Grain Size
Admiralty Inlet- south	5	4	0.864	1.009	226.750
North Hood Canal (South of Bridge)	10	7	1.979	2.887	269.650
Saratoga Passage	20	6	3.406	11.122	15.950
Skagit Bay	0	0	2.109	3.033	102.410
Port Gardner (Everett)	10	6	3.254	5.312	37.915
Hood Canal- N of Seabeck	0	0	3.146	7.451	27.095
Case Inlet	15	5	3.539	11.190	15.430
Carr Inlet	20	8	4.136	10.938	17.720
Commencement Bay	0	0	3.119	6.829	13.285
Shilshole	10	4	3.376	9.171	21.295
Lynch Cove	0	0	4.490	18.016	23.500
Sinclair Inlet	15	3	2.304	10.134	15.015
Point Pully (3-Tree Point)	5	1	3.583	11.986	12.835
Strait of Georgia (North of Patos Island)	10	7	2.362	3.474	18.475
Inner Port Angeles Harbor	10	4	5.796	14.313	27.845
Murden Cove	0	0	1.494	2.127	169.500
Saratoga Passage- north- Camano Island	5	4	1.616	2.068	176.850
Carr Inlet- NE of Gertrude Island	10	3	3.282	12.244	19.930
Strait of Georgia- outer Birch Bay	0	0	1.912	2.690	25.875
Central Hood Canal- S of Triton Cove	10	6	3.902	6.801	13.235
Central Basin- N of Shilshole	0	0	3.616	7.818	33.010
Elliott Bay- Smith Cove	0	0	1.465	1.940	92.655
Reads Bay	0	0	2.910	5.638	21.405
Saratoga Passage- South	15	4	3.356	12.014	13.745
Henderson Inlet	5	2	3.824	10.906	15.670
Boundary Bay	0	0	1.690	2.484	78.075
Hood Canal- Hoodsport	25	7	3.590	13.260	15.410
South Possession Sound	0	0	4.150	10.882	20.075
Shilshole Bay	0	0	1.805	2.404	15.000

Crescent Harbor	5	2	3.012	9.210	14.540
Brownsville	15	3	3.814	11.576	13.310
West Sound	0	0	3.482	8.371	19.015
Dabob Bay	20	5	3.553	13.847	12.500
Admiralty Inlet- N of Rose Point	0	0	1.791	2.424	206.650
Totten Inlet	0	0	3.507	13.240	13.500
North Samish Bay	10	4	2.835	7.916	16.775
Sinclair Inlet	5	1	3.825	14.341	17.085
Inner Case Inlet- Rocky Bay	10	6	2.814	4.985	26.325
Port Townsend- mouth of Kilisut Harbor	5	4	1.993	2.603	257.450
Des Moines	0	0	2.880	4.729	12.725
Central Basin- North	40	12	3.250	10.591	13.960
North Central Basin	0	0	3.453	12.034	23.305
City Waterway (Commencement Bay)	5	3	2.166	3.135	20.290
East Anderson Island	0	0	2.548	4.320	158.700
Inner Budd Inlet	0	0	4.386	13.554	19.000
Bellingham Bay	10	3	3.388	10.709	15.150
W of Devils Head- E end Nisqually Reach	0	0	2.343	3.704	17.465
Bellingham Bay- Pt. Frances (Portage Is.) Voice 50	5	4	2.113	2.940	13.490
Hood Canal- Central	40	11	3.588	12.610	14.375
Central Elliot Bay	5	2	3.746	7.811	23.345
Average	8	3			