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Learning from Traditional Ecological Knowledge: Paralytic Shellfish Toxins in Butter Clams

Jackelyn Garcia
Western Washington University, garcia89@wwu.edu

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Paralytic Shellfish Toxins in Butter Clams

By

Jackelyn Tolentino Garcia

Accepted in Partial Completion
of the Requirements for the Degree
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ADVISORY COMMITTEE

Dr. Marco B.A. Hatch

Dr. Misty Peacock

Dr. Ruth Sofield

GRADUATE SCHOOL

David L. Patrick, Dean

Master's Thesis

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Jackelyn Tolentino Garcia

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A Thesis
Presented to
The Faculty of
Western Washington University

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Abstract

Anthropogenic forcing of marine ecosystems is disproportionately impacting Indigenous food systems and the health of coastal Indigenous communities. With increasing harmful algal events, there is rising concern for access and health of coastal communities who rely on shellfish for commercial, food, subsistence, and ceremonial harvest. In the U.S West Coast, the dinoflagellate *Alexandrium* spp. may produce paralytic shellfish toxins, which can cause shellfish to become toxic and is of especial concern. While recent research has led to greater awareness of the risks associated with paralytic shellfish toxins (PSTs), the concern for harmful algae is not new. Coastal Indigenous communities have long been aware of harmful algae and have relied on traditional ecological knowledge to reduce impacts from harmful algae since time immemorial. In particular, traditional ecological knowledge relevant to the preparation of clams has been used to reduce risk from harmful algae. To analyze paralytic shellfish toxins in butter clams (*Saxidomus gigantea*), *S. gigantea* were collected between April 2022-November 2022 in Bellingham Bay, WA. Clams were partitioned into five distinct tissue groups, and enzyme-linked immunosorbent assay (ELISA) was used to quantify paralytic shellfish toxins within the siphon, siphon tip, gills, digestive tract, and rest of body. Results from this study show that the concentration of paralytic shellfish toxins is temporally variable in *S. gigantea*. We also quantified that the toxin contribution of different tissues varied over the course of two *Alexandrium* spp. blooms, with disproportionately higher contributions from the siphon, relative to other tissues studied. The selective removal of tissues can significantly reduce exposure to PSTs, however this may not mitigate PSTs completely. The benefit of selectively removing *S. gigantea* tissues and the cost of sacrificing tissue for consumption to reduce exposure also varied throughout the study period, though discarding the siphon tip, and siphon, and at times the digestive tract commonly showed potential to reduce exposure while optimizing the mass available for consumption.

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1. Introduction

The extent to which anthropogenic forcing, human-caused changes to climate, is impacting marine ecosystems and is influencing harmful algal events worldwide is not clear due to the confounding factors of increased monitoring. Increased monitoring efforts for harmful algae has improved detection, both in time and space, and would lead to more harmful algae events recorded, independent of anthropogenic forcing (Hallegraeff et al. 2021). However, changes in sea surface temperature, eutrophication, and increased stratification of the ocean are a few factors subject to anthropogenic forcing understood to influence the dynamics of harmful algae (Moore et al. 2008, Wells et al. 2015). Anthropogenically driven factors have been expected to impact the frequency, magnitude, and phenology of harmful algal blooms (Moore et al. 2008, Hallegraeff 2010, Wells et al. 2015, Ralston and Moore 2020). While the global trends are more difficult to attribute primarily to human related causes, there is strong evidence for changes in the occurrence of harmful algae at regional scales, where coastal areas have experienced increased frequency and geographic expansion of harmful algal events since the 1980's (Gobler 2020, Hallegraeff et al. 2021).

1.1. Harmful algae

Harmful algal blooms (HABs) or harmful algae events refer to the proliferation of potentially toxic and non-toxic microscopic phytoplankton that are associated with negative impacts to the environment and humans (Hallegraeff et al. 2003). Phytoplankton blooms, and associated biomass, can cause hypoxia or anoxia in waters, resulting in death and dispersal of marine species and altered ecology (Hallegraeff et al. 2003). Certain phytoplankton can also naturally produce toxins (marine biotoxins) that can cause several known shellfish poisoning illnesses (Visciano et al. 2016). For instance, dinoflagellates of the genus *Alexandrium* spp. can produce a suite of toxins known as paralytic shellfish toxins (PSTs), which shellfish can accumulate naturally by filter feeding (Visciano et al. 2016). This suite of toxins can cause an acute illness in consumers called paralytic shellfish poisoning (PSP) from ingesting shellfish contaminated with PSTs (Moore et al. 2011, Visciano et al. 2016). PSTs are neurotoxins that block voltage-gated sodium channels of excitable cells and can cause mild to severe illness and

even death (Visciano et al. 2016). Saxitoxin (STX), one of the chemical congeners of PSTs, is regarded to be the most toxic among other structures (Etheridge 2010). A mild case of PSP due to the ingestion of PSTs can cause symptoms of numbness in the lips and face, tingling sensation in the extremities, headache, and nausea (Visciano et al. 2016). In more critical cases, PSP can cause respiratory difficulty, muscular paralysis, and lead to death (Visciano et al. 2016). The detection and understanding of HABs is a major public health concern. Since HABs can have serious consequences to human health, monitoring programs for HABs exist across the globe.

1.2. Paralytic shellfish poisoning in Washington state

PSP is prevalent in the U.S West Coast (Lewitus et al. 2012). The first written record of PSP related illness in the U.S West Coast is from 1793 when members of Captain George Vancouver's Royal Navy crew became ill after consuming shellfish harvested from Poison Cove in southeast Alaska, USA (Lewitus et al. 2012). In Washington State, PSP has been monitored since the early 1930's by the Washington State Department of Health (Trainer et al. 2003). Washington State uses a regulatory limit of 80 µg PSTs/100 g shellfish tissue to protect shellfish consumers from PSP, consistent with national and international standards (Wekell et al. 2004, Finch et al. 2020). Since the start of its formal monitoring, the geographic distribution of PSTs has increased (Trainer et al. 2003, Lewitus et al. 2012). In the 1950's PSTs were mainly present in the northern basins of Puget Sound, but by the 1970's, had spread to the southern basins of Puget Sound which were previously unaffected by these toxins (Quayle 1969 and Nishitani and Chew 1988 as cited in Trainer et al. 2003, Lewitus et al. 2012). While HABs can occur all year-round globally, *Alexandrium* spp. blooms and shellfish closures in the U.S West Coast historically occurred from late spring through summer or early fall (Horner et al. 1997, Lewitus et al. 2012). In recent decades, not only has the spatial extent of *Alexandrium* spp. blooms increased, but the seasonal timing for *Alexandrium* spp. blooms has expanded in Puget Sound (Moore et al. 2009). In Puget Sound, *Alexandrium* spp. blooms are causing shellfish harvest closures earlier in the year compared to in the past, and this shift in the phenology of toxic *Alexandrium* spp. is expected to further impact access to shellfish (Moore et al. 2009).

1.3. Impacts of paralytic shellfish toxins on Indigenous community health

Paralytic shellfish toxins disproportionately impact Indigenous food systems and the health of coastal Indigenous communities who rely on shellfish for commercial, recreational, subsistence, and ceremonial purposes (Donatuto et al. 2011, Lewitus et al. 2012). In Indigenous communities, the ability to access, harvest, prepare, eat, and share traditional foods such as shellfish is important to maintaining community health (Donatuto et al. 2011, Lynn et al. 2013). Shellfish harvest closures due to PSTs prevent access to traditional foods. The Swinomish Indian Tribal Community in Washington, defines health in a multi-dimensional way, recognizing that eating traditional foods not only feeds the physical body but also feeds the soul and fulfills the spiritual 'hunger' for traditional foods (Donatuto et al. 2011). Though there is risk associated with consuming shellfish that may be contaminated with paralytic shellfish toxins, the harvest of traditional foods can be integral to the way of life of Indigenous community members. In the context of anthropogenic contaminants, a Swinomish elder states, 'Like we say, it's our spiritual food so it feeds our soul; so it might poison our body, but then we'd rather nourish our soul' (Donatuto et al. 2011).

1.4. Indigenous knowledge and Western science understandings of harmful algae toxins

Coastal Indigenous communities in the U.S West Coast have been long aware of harmful algae and have developed traditional ecological knowledge-based strategies to mitigate marine toxins since time immemorial. In the U.S West Coast, many Indigenous communities would use preparation methods to reduce exposure to PSP. Traditional practices vary across communities, but the practice of selectively removing and discarding certain tissues of butter clams (*Saxidomus gigantea*) that are believed to hold elevated levels of toxin prior to consumption is a method that has been used to reduce the impacts of PSP (Batdorf 1990, Moss 1993, Williams 2006, Lummi Seafood Consumption Study 2012). The tissues most referred to be removed are the siphon tip and siphon, though the viscera and gills are also cited as tissues that may be discarded.

'You know our old people were conscious of pollutants, like for example, the Soxwe the butter clams. You had to cut that black nose off because the pollutants are all in the black part of the nose, you have to cut it off, every time they are all cut off, you don't eat that part. If you cut the noses off and clean the bellies out of the clams you can eat them.'

-Tsi'li'xw Bill James, the Lummi Nation's Hereditary Chief

The late Bill James says to remove the 'black nose,' which is also known as the siphon tip in *S. gigantea*, because the 'pollutants,' which are presumably PSTs are held in the siphon tip. The 'bellies,' of the clams are likely the viscera. From a dialogue on local ecological knowledge revolving around harvesting clams, the whole siphon and gills may be removed to reduce exposure to toxins:

"Billy's wife Yvonne removes siphons and gills from harvested clams since they can hold red tide for some time…he says local Native people followed these harvesting guidelines in the summers (Williams 2006, page 83)*."*

Despite the body of knowledge that certain tissues in clams can vary in toxin contribution, the influence of the selective removal of specific tissues on total toxin exposure has shown varying results. In *S. gigantea* collected from Kodiak Archipelago, Alaska*,* the toxin contribution of the siphon tip, siphon, gut, and rest of body (remaining muscular tissues) varied, and the removal of these tissues contributed from little to significant change in toxin concentration (Kibler et al. 2022). For instance, the removal of the siphon tip was seen to reduce toxin concentration but its removal did not always reduce concentrations enough to levels that are safe for humans to ingest (Kibler et al. 2022). It is noted that the toxin concentration and contribution of tissues in clams is not constant overtime (Bricelj and Shumway 1998). Studies demonstrate a relationship between the occurrence of *Alexandrium* spp. and PSTs in shellfish. However, the degree to which various tissues accumulate toxin when they ingest toxic *Alexandrium* spp. cells, and how the selective removal of tissues influences toxin exposure is

not fully understood. Further study into exposure to toxic *Alexandrium* spp. cells and seasonal progression of PSTs in tissues would be valuable in understanding how partitioning and selectively discarding tissues may influence total toxin concentration and potential for exposure.

Traditional ecological knowledge can provide insight that Western Science is unable to (Kimmerer 2002, Berkes 2009, Jessen et al. 2021). Using traditional ecological knowledge to guide this study's questions, we investigated (1) seasonal variability of PSTs in five tissues of *S. gigantea* (2) seasonal variability of *Alexandrium* spp. cells, and (3) how the selective removal of tissues of *S. gigantea* would impact human exposure to PSTs.

2. Methods

2.1. Study area

Bellingham Bay is a semi-enclosed embayment located in northern Puget Sound, WA (Wang and Yang 2015) (Figure 1). It is bordered by the Lummi peninsula and Portage Island on the west, and by the City of Bellingham and the Chuckanut mountains on the east (Wang and Yang 2015). The Nooksack River is the major freshwater source that feeds into the northern portion of the bay (Shull 2021). The southern end of the bay connects to Samish Bay and the rest of northern Puget Sound.

Long-standing environmental and biological monitoring primarily led by Northwest Indian College (NWIC) made Bellingham Bay a favorable site for our studies on PSTs in *S. gigantea*. Phytoplankton species, dissolved PSTs, and water quality parameters (temperature, pH, and salinity) have been monitored at Taylor Dock in Bellingham Bay since 2018. The desire to analyze this long-term data set with the toxin data collected from nearby *S. gigantea* shaped our decision to collect *S. gigantea* at Boulevard Park and Marine Park, which lie on either side of Taylor dock, along the east side of Bellingham Bay (Figure 1).

2.2 Sample collection

Ten 50 x 30 m sampling sites were established within Boulevard Park and Marine Park as areas to collect *S. gigantea* for this study. Starting April 2022 and ending in November 2022, one of these sampling sites was randomly selected biweekly for the collection of 10 *S. gigantea*. Within the randomly selected sampling site, a transect perpendicular to the shore was stretched from -0.2 to Mean Lower Low Water (MLLW) to collect clams. At randomly chosen locations along the transect, five 25 x 25 cm quadrats were laid on alternate sides of the transect. A column sample (or pit) the size 25 x 25 x 25 cm were removed for each quadrat, and only *S. gigantea* larger than 3.81 cm wide were collected (Groesbeck et al. 2014). Retaining *S. gigantea* greater than 3.81 cm is to reflect what is legally allowed for harvest in Washington state, and it also ensures enough tissue material for toxin analysis (Washington Department of Fish & Wildlife 2023).

2.3. Clam partitioning

Within five hours of collection, each of the 10 clams were rinsed in freshwater to remove excess sediment. Clams were measured for width, maximum growth axis, length, and height using a caliper (0.05mm). Then, each clam was opened and the whole clam was partitioned into five tissue groups: (1) siphon, (2) siphon tip (tip of the siphon), (3) gills, (4) digestive tract and (5) rest of the body (Figure 2). For the purposes of this study, the “visceral mass” which contains reproductive and digestive parts of the clam is referred to as the digestive tract. The “rest of the body” are the remaining tissues of the clam that include the adductor muscles, mantle, and foot.

The total mass (g) of the whole clam (without the shell), and the mass of each of the five tissues of the clam, were recorded with a Melter Toledo ML3002T/00 precision balance (±0.01 g). The individual partitioned samples were stored separately in 15 mL and 50 mL (for the rest of the body) GenClone centrifuge tubes and frozen at -25°C until ready to be prepared for toxin analysis.

2.4. Preparing clams for toxin analysis

Each tissue was individually homogenized and mixed with 50% methanol to extract PSTs from the clam tissue. To do this, an IKA T25 digital ULTRA-TURRAX homogenizer fitted with an IKA S 25 N - 8 G dispersing tool was used to blend each clam tissue into a smooth consistency. Then, 0.5 g of each homogenized tissue was mixed with 1 mL of 50% methanol and thoroughly vortexed. A Fisherbrand Model 120 Sonic Dismembrator with a 1/8 in probe was used to further break down the tissue at the cellular level. Each homogenate sample was placed in an Eppendorf Centrifuge 5430 R at 5000 rpm for 10 minutes. Once complete, the supernatant of each sample was collected and stored separately into 2 mL Fisherbrand Threaded Cryogenic Vials at -80°C.

2.5. Analysis of paralytic shellfish toxins

For every sampling event, three of ten clams were randomly selected for toxin analysis. To measure total paralytic shellfish toxin, enzyme-linked immunosorbent assay (ELISA) kits were used. ELISA is a competitive enzyme-linked immunoassay that measures total paralytic shellfish toxin, and can detect paralytic shellfish toxin congeners: saxitoxin, neosaxitoxin, decarbamoyl saxitoxin, gonyautoxin 2 & 3 (GTX), GTX 1 & 4, decarbamoyl GTX 2 & 3, decarbamoyl neosaxitoxin, and GTX-5, lyngbyatoxin, and sulfo GTX 1 & 2 (Table 1). These kits are designed to have the highest cross-reaction with saxitoxin and cross-react with other PST congeners to varying degrees, reported in the kit manufacturer's manuals (Table 1).

PST concentrations of 48 clams, representing 240 individual partitioned samples quantified using three PSP ELISA kit manufacturers. PerkinElmer MaxSignal Saxitoxin (PSP) ELISA Kits (limit of detection: 3 ng/g STX equivalent) was initially used to analyze samples (n=115) but permanent discontinuation of the product resulted in using Eurofins Abraxis Saxitoxin (PSP) ELISA kits (limit of detection: 0.015 ng/g STX equivalent) to continue quantifying PSTs in samples (n=34) (Table 2). However, some of the Eurofins Abraxis kits resulted in high variability with the samples when compared to standards, and SeaTox Research Inc Saxitoxin/Paralytic

Shellfish Poisoning ELISA Kits (limit of detection: 0.03 ng/g STX equivalent) were used to complete toxin analysis (n=91) (Table 2).

Instructions for each ELISA kit were followed using the provided reagents, and supernatant collected from each partitioned sample. Solutions in the wells of each microplate were mixed using a Thermo Scientific Compact Digital Microplate Shaker at 260 x speed. A BioTek Model ELX508 Microplate Washer was used to thoroughly wash the microplate using provided wash buffer solutions, and following an incubation period, the microplate was read immediately on a BioTek ELX800 Microplate reader at 450 nm.

If the sample was read as below the detection limit, samples were assigned 3 ng/g STX equivalent, which is the highest detection limit among the PSP ELISA kit manufacturers used. If the sample was read as above the max detection limit, and outside of the standard curve, the sample was diluted with the provided diluent and re-analyzed on a new microplate. If the sample continued to read above the max detection limit, the sample was diluted by a larger dilution factor, and re-ran on a separate microplate until the sample reading was determined to be within the standard curve.

2.6. Phytoplankton sampling and Alexandrium spp. counts

Vertical net tows for phytoplankton were completed by NWIC staff to determine and quantify the presence of *Alexandrium* spp. in Bellingham Bay. Vertical net tows took place at Taylor Dock on a weekly basis. Each week, 20 µm net measuring 30 cm in diameter, and 1.5 m in length was vertically submerged and pulled through the water column for a total distance of 12.91 m at approximately 2.5 m depth. Attached to the bottom of the net was a cod end that collected the phytoplankton. After completing a net tow, the phytoplankton collected in the cod end was transferred into a 500 mL Thermo Scientific Nalgene Bottle to estimate relative abundance and *Alexandrium* spp. counts.

To estimate relative abundance of phytoplankton species, the 500 mL bottle was gently inverted to mix the sample evenly. Then, a subsample of 25 mL from the bottle was aliquoted onto a Petri dish. Using a Leica M125 C dissecting scope, phytoplankton were identified to the species level. If *Alexandrium* spp. was observed, a 0.5 mL sample from the 500 mL bottle was

placed on a Model 1801-G20 Gridded Sedgewick Rafer 1 mm^2, and an Olympus 1X71 Inverted microscope was used to count *Alexandrium* spp. The magnifications 10x, 20x and 40x were used as necessary to count phytoplankton cells.

2.7. Statistical analysis

Linear mixed effect models (LMM) using R and the function 'lme' was used to determine statistical significance of mass variation in tissues of *S. gigantea*, PST concentrations, percent contribution, and percent contribution relative to mass ratios of the siphon, siphon tip, digestive tract, gills, and rest of body (Peng and Lu 2012). LMM was also used to test statistical significance in difference in total PST between three clam preparations involving the removal of the siphon tip, or the siphon and siphon tip, and no tissue removal. To account for the random effect of individual clams and repeated sampling of clam tissues from the same individual, the function 'lme' was used to fix the best model. A LMM with fixed effects, random effects, and interactions were fitted (Peng and Lu 2012). Computed p-values through an ANOVA determined which variables were not significant in explaining variance between the predictor and response variables, and the most parsimonious model was determined through Akaike's Information Criterion (AIC) (Theobald 2018). The LMM was followed by a post-hoc test, using expected marginal means and the function 'emmeans' to compare significant differences between means of groups (Lenth et al. 2018).

2.7.1. Calculations for analysis

Percent mass (%) of each tissue was calculated by dividing the mass contribution of the tissue by the mass of the whole clam (mass of all tissues without the shell).

$$Percent\ Mass\ (\%) = \frac{tissue\ mass\ (g)}{total\ tissue\ mass\ (g)} * 100 \quad \text{(eq 1)}$$

The total concentration of PST (µg/100 g shellfish tissue) in each tissue was calculated by multiplying the individual tissue mass with mass PST in the tissue and converting to µg/100 g shellfish tissue by multiplying the value by 100. Percent contribution (%) of PST defined as the

relative contribution of the tissue to total PST concentration was calculated for each tissue by dividing the concentration of PST in each tissue (eq1) by the total mass PST (µg) in the whole body (eq3).

$$Total\ PST\ of\ tissue\ \left(\frac{\mu g}{100\ g}\right) = \left(\frac{\mu g}{g} mass\ PST\ in\ 0.5\ g\ tissue\ subsample * tissue\ mass\ (g)\right) * 100$$

(eq 2)

$$Percent\ Contribution\ (\%)\ of\ PST = \frac{mass\ PST\ in\ tissue\ (\mu g)}{total\ mass\ PST\ (\mu g)} * 100$$

(eq 3)

The percent contribution relative to mass ratios, which is a ratio of percent contribution of PST to percent mass was quantified for all tissues to describe the concentration of PSTs accumulated per unit of mass for all the tissues (eq4). A ratio greater than 100% means the amount of PST in the clam tissue is disproportionately higher relative to the mass of the tissue. A ratio less than 100% indicates that the concentration of PSTs in the tissue is disproportionately less relative to the mass of the tissue.

$$Percent\ Contribution\ Relative\ to\ Mass\ Ratio\ (\%) = \frac{percent\ contribution\ (\%)\ of\ PST}{percent\ mass\ (\%)}$$

(eq 4)

Total PST was calculated for the analysis of how the selective removal of tissues (the siphon and/or siphon tip, and no tissue removal) would reduce total PST concentration (eq 5-eq 7). Total PST was calculated for the whole body (no tissue removal) by summing tissue PST (µg) within each individual clam, dividing this value by the total mass of the whole clam, and converting the value to µg/100 g shellfish tissue by multiplying by 100 (eq5). For calculating total PST for siphon and/or siphon tip removal, the summed tissue PST (µg) within each

individual clam was divided by the total mass of the whole clam with the mass of the siphon and/or siphon tip subtracted (eq6, eq7).

For whole body (no tissue removal):

$$Total\ PST\ whole\ body\ \left(\frac{\mu g}{100\ g}\right) = \frac{\sum mass\ PST\ in\ tissue\ (\mu g)\ of\ individual}{total\ mass\ of\ whole\ clam\ (g)} * 100$$

(eq 5)

For siphon removal:

$$Total\ PST\ siphon\ removed\ \left(\frac{\mu g}{100\ g}\right) = \frac{\sum mass\ PST\ in\ tissue\ (\mu g)\ of\ individual}{total\ mass\ of\ whole\ clam\ (g) - (\sum mass\ of\ siphon\ tip, digestive\ tract, gills, rest\ of\ body\ (g))} * 100$$

(eq 6)

For siphon and siphon tip removal:

$$Total\ PST\ siphon\ and\ siphon\ tip\ removed \left(\frac{\mu g}{100\ g}\right) = \frac{\sum mass\ PST\ in\ tissue\ (\mu g)\ of\ individual}{total\ mass\ of\ whole\ clam\ (g) - (\sum mass\ of\ digestive\ tract, gills, rest\ of\ body\ (g))} * 100$$

(eq 7)

For visualizing the total mass PST contributions (µg/100 g shellfish tissue) of all tissues (siphon, siphon tip, digestive tract, gills, and rest of body), total PST of the whole body was multiplied by the fraction contribution (PST) of each tissue (eq8).

$$Total\ mass\ PST\ of\ tissue\ \left(\frac{\mu g}{100\ g}\right) = Total\ mass\ PST\ whole\ body \left(\frac{\mu g}{100\ g}\right) * PST\ Fraction\ contribution\ of\ tissue\ (unitless)$$

(eq 8)

3. Results

3.1. Mass variation across clam tissues

The mass of the five tissues of the clam analyzed for this study varied significantly from each other ($p<0.0001$) (Tables 1 and 6, Figure 7A). The "rest of the body" composed 57.5% ± 5.1 of the total mass of the total tissue. The siphon tip contributed the least amount of mass making up 2.9% ± 1.0 of the whole clam (minus the shell). The rest of the body (57.5% ± 5.1), digestive tract (21.4% ± 6.0), siphon (12.1%± 3.0), gills (6.1%± 1.6), and siphon tip (2.9± 1.0) are ordered heaviest to lightest, respectively. The digestive tract had the highest variability in mass (21.4% ± 6.0) compared to other clam tissues.

3.2. Paralytic shellfish toxin concentration variability in clam tissues

The average concentration of PSTs in the five clam tissues studied were statistically different from each other ($p<0.0001$) (Tables 2 and 7, Figure 8). The concentration of PSTs in the siphon ranged from below the detection limit of 0.30 to 1400.4 µg/100 g shellfish tissue ± 652.6, the siphon tip 1.5 to 618.0 µg/100 g shellfish tissue ± 261.5, digestive tract 0.30 to 390.9 µg/100 g shellfish tissue ± 117.5, rest of body 0.30 to 246.0 µg/100 g shellfish tissue ± 101.1, and gills 0.30 to 88.0 µg/100 g shellfish tissue ± 20.7. Ordered from highest to lowest average concentration of PSTs quantified, the mean concentration of PSTs detected in the siphon is 216.9 µg/100 g shellfish tissue ± 280.6, siphon tip 167.5 µg/100 g shellfish tissue ± 176.0, digestive tract 59.6 µg/100 g shellfish tissue ± 88.6, rest of body 40.0 µg/100 g shellfish tissue ± 58.0, and gills 18.6 µg/100 g shellfish tissue ± 24.6, respectively. The mean concentration of PSTs in the siphon (216.9 µg/100 g shellfish tissue ± 280.6) are not significantly different to the mean concentration found in the siphon tip (167.5 µg/100 g shellfish tissue ± 176.0), but are significantly different from the digestive tract, rest of body, and gills. The mean concentration of PSTs in the digestive tract (59.6 µg/100 g shellfish tissue ± 88.6), and rest of body (40.0 µg/100 g shellfish tissue ± 58.0) are not significantly different from one another, are significantly different from the gills (18.6 µg/100 g shellfish tissue ± 24.6).

3.3. Temporal variability of paralytic shellfish toxin concentrations

The mean PST concentrations of all five clam tissues temporally varied throughout the study period (Figure 9, Figure A1-A5). The siphon accumulated mean PST concentrations above the regulatory limit of 80 µg/100 g shellfish tissue on 11 of the 16 sampling events. The siphon tip was detected to have accumulated average PST concentrations above the regulatory limit 12 of the 16 sampling events. The digestive tract was detected to have accumulated average PST concentrations above the regulatory limit four of the 16 sampling events. The rest of body was detected to have accumulated average PST concentrations above the regulatory limit in four of the 16 sampling events. The rest of body was also detected to have a mean of 0.30 µg/100 g shellfish tissue (below the detection limit) on six of the 16 sampling weeks. The gills were not detected to accumulate average PST concentrations above the regulatory limit throughout the 16 sampling weeks.

The siphon and siphon tip accumulated PST concentrations above the regulatory limit by 6/13/22. The siphon increased in PST concentration by 9 times the amount, and the siphon tip 21 times the amount compared to their mean concentrations on the prior sampling event on 6/1/22. All five of the clam tissues increased in mean PST concentrations between 6/30/22 and 7/11/22, but only the siphon, siphon tip, and digestive tract remain above the regulatory limit for the entire period. There is a gradual decrease in the PST concentrations in all clam tissues between 7/25/22 and 8/26/22, but the siphon and siphon tip maintain PST concentrations above the regulatory limit with the exception for the siphon on 8/26/22. On 7/11/2022, the siphon reached a max of 1400.4 µg/100 g shellfish tissue ± 652.6, the siphon tip 618.0 µg/100 g shellfish tissue ± 261.5, and digestive tract 390.9 µg/100 g shellfish tissue ± 117.5. On 5/17/2022, the rest of body reached a max of 246.0 µg/100 g shellfish tissue ± 101.1 and gills 88.0 µg/100 g shellfish tissue ± 20.7.

3.4. Percent contribution of paralytic shellfish toxin variability

The variability in percent contribution of PSTs accumulated by the five clam tissues studied were statistically significant from each other ($p<0.0001$) (Tables 3 and 8, Figure 7B). The siphon accumulated the highest percentage of PSTs compared to the other tissues of the clam,

and had the greatest variability in percent contribution (44.6% ± 22.2). The gills accumulated the least percentage of PSTs compared to the other tissues of the clam (2.5% ± 3.5). The rest of body, digestive tract, and siphon tip on average accumulated similar percentages of PSTs, ranging between 11.2% (± 11.3) to 22.5% (± 19.6). However, the siphon tip (12.1% ± 12.3) also shared similar percentages of PST as the gills (2.2% ± 3.7). The average difference in percent contribution between the siphon to the digestive tract, rest of body and siphon tip is 27.0%.

3.5. Temporal variability in percent contribution of paralytic shellfish toxins

The percent contribution of PSTs of each clam tissue varied throughout the study period (Figure 10). The mean percent contribution in the siphon varied from 4.4-96.0% ± 18.6, rest of body below detection limit to 65.2% ± 17.8, digestive tract below detection limit to 90.8% ± 44.9, the siphon tip 0.5-57.6% ± 26.2, and gills below detection limit to 22.3% ± 10.3. The siphon displayed the highest mean percent contribution (44.6% ± 22.2), and the gills displayed the lowest mean percent contribution 2.5% ± 3.5. On 4/19/22, the max percent contribution of the siphon was 96.0% ± 18.6, on 6/1/22 the digestive tract was 90.8% ± 44.9, on 10/1/22 the rest of body was 65.2% ± 17.8, on 10/28/22 the siphon tip was 57.6% ± 26.2, and on 5/3/22 the gills was 22.3% ± 10.3. The percent contribution in the siphon remained above 25% throughout the entire period that clams were collected, and was at its highest at the beginning of the sampling period. The mean percent contribution detected in the rest of body was between 1.2 to 10% on six of the 16 total sampling events. The rest of body had steep decreases in percent contribution between 5/17/22 and 6/1/22, where the mean percent contribution in rest of body decreased from 43.2% ± 11.2 to 7.6% ± 6.3, and between 7/25/22 and 8/8/22, where the mean percent contribution in rest of body decreased from 46.8% ± 20.4 to 1.2% ± 1.5. The mean percent contribution in the rest of body also showed sharp increases in percent contribution from 4.4 ± 7.5 to 43.2% ± 11.2 between 5/3/22 and 5/17/22, and between 6/13 and 6/30, where the mean percent contribution in rest of body increased from 11.4 ± 12.9 to 37.4% ± 2.3. Between 7/25/22 and 10/28/22, the digestive tract and siphon tip gradually increase and decrease in percent contribution. By 10/1/22, the siphon tip, and siphon also

increase and decrease in percent contribution while the digestive tract decreases in percent contribution.

3.6. Percent contribution of paralytic shellfish toxins relative to percent mass

The percent contribution relative to mass varied significantly across all five clam tissues studied ($p<0.0001$) (Tables 4 and 9, Figure 7C). The siphon tip (457.2% ± 631.8), siphon (391.0% ± 230.1), digestive tract (92.6%± 84.1), gills (40.9%± 54.7), and rest of body (38.3% ± 32.9) are ordered highest to lowest mean percent contribution relative to mass, respectively (Table 4, Figure 7C). The percent contribution relative to mass of the siphon tip ranged between 20.4 to 3811.2% ± 1896.5, the siphon between 36.4 to 1343.2% ± 462.2, digestive tract between 1.2 to 481.8% ± 237.5, gills between 0.8 to 348.8% ± 166.3, and rest of body between 0.6 to 111.2% ± 31.9. The mean percent contribution relative to mass ratios of the siphon tip and siphon are not significantly different from each other, but are significantly different from the mean percent contribution relative to mass ratios of the digestive tract, gills, and rest of body. The mean percent contribution relative to mass of the siphon tip average 457.2% ± 631.8 and siphon 391.0% ± 230.1, though the siphon tip has the highest percent contribution relative to mass, with 4.6 times the amount of PSTs per unit of mass. The mean percent contribution relative to mass of the digestive tract is significantly different from the mean percent contribution relative to mass of all other tissues. The percent contribution relative to mass of the gills and rest of do not significantly differ, and range between 38.3% ± 32.9 to 40.9% ± 54.7.

3.7. Temporal variability of percent contribution of paralytic shellfish toxins relative to percent mass

The percent contribution relative to mass of each clam tissue varied across the study period (Figure 11). On 10/28/22, the siphon tip reached a maximum percent contribution relative to mass of 3811.2% ± 1896.5, on 4/19/22 the siphon had a maximum of 1343.2% ± 462.2, on 6/1/22 the digestive tract had a maximum of 481.8% ± 237.5, on 5/3/22 the gills had a maximum of 348.8% ± 166.3, and on 10/1/22 the rest of body displayed a maximum of 111.2% ± 31.9.

Siphon and the siphon tip on average maintained higher percent contribution relative to mass throughout the course of the sampling period compared to other tissues (siphon tip 20.4 to 3811.2% ± 1896.5, siphon 36.4 to 1343.2% ± 462.2). The digestive tract, gills, and rest of body vary in percent contribution relative to mass but maintain lower ranges than the siphon tip and siphon in across the sampling period. However, the mean percent contribution relative to mass of the digestive tract (1.2 to 481.8% ± 237.5) were significantly higher than the gills (0.8 to 348.8% ± 166.3) and rest of body (0.6 to 111.2% ± 31.9). The mean percent contribution relative to mass in the rest of body was between 2.3 – 8.0% on four of the 16 total sampling events. The siphon tip shows three distinct events of high mean percent contribution relative to mass with a high of 1602.5% ± 642.2 on 6/13/22, a high of 926.5% ± 1036.1 on 8/26/22, and a high of 3811.2% ± 1896.5 on 10/28/22. The siphon tip also shows three repeating patterns of increasing and decreasing in percent contribution relative to mass throughout the study period.

3.8. Selective removal of tissues

Total PST of three clam preparations were statistically different ($p<0.0001$) and varied throughout the study (Table 10, Figure 3 and 4). Total PST in the clam was lowest with the removal of the siphon and siphon tip (mean total PST 42.5 µg/100 g shellfish tissue ± 54.6), and highest with no tissue removal (mean total PST 64.4 µg/100 g shellfish tissue ± 74.0) (Table 5). The removal of the siphon and siphon tip resulted in an average reduction in PST concentration by 21.9 µg/100 g shellfish tissue compared to when no tissue was removed (Table 10, Figure 3 and 4). The removal of the siphon tip resulted in an average reduction in PST concentration by 2.7 µg/100 g shellfish tissue compared to when no tissue was removed. Total PST in the clam was above the regulatory limit for five of the 16 sampling events. The removal of the siphon and siphon tip resulted in lower total PST concentrations throughout the study period relative to other preparations. Higher values of total PST in clams showed greater variance in total PST regardless of tissue removal within individual sampling events.

Total mass PST of different tissues varied throughout the study period (Figure 5). Higher mass PST contributions relative to other tissues were from the siphon and rest of body mostly

during times when total PST of the clam was higher than the regulatory limit. During four of the five sampling events that total PST of the clam was higher than the regulatory limit, the removal of certain tissues was able to reduce total PST below the regulatory limit (Figure 6). Total mass PST was reduced below the regulatory limit when the siphon, siphon tip, and digestive tract was removed on 6/30/2022 and 11/23/2022 (Figure 6). Total mass PST was reduced below the regulatory limit when the siphon and digestive tract was discarded on 7/11/2022, and was reduced below the regulatory limit with the removal of the siphon on 7/25/2022. The removal of tissues required discarding between 12 to 36% of the total mass of the clam to bring total mass PST of the clam below the regulatory limit (Figure 6).

3.9. Temporal variation of Alexandrium spp. concentrations

Alexandrium spp. cell concentration varied temporally throughout the study period (Figure 12). Two *Alexandrium* spp. blooms occurred throughout the study period, with the first bloom in June and the second bloom in September. Prior to the blooms, there were 13 *Alexandrium* spp. cells/L on 4/14/2023. From 4/22/2022 to 6/14/2022, *Alexandrium* spp. cells were not detected (0 cells/L). By 6/15/2022, *Alexandrium* spp. concentrations were 333 cells/L. The following week, 6/23/2022, *Alexandrium* spp. concentrations increased 530 cells/L. From 6/30/2022 to 8/24/2022, *Alexandrium* spp. concentrations were no longer detected (0 cells/L). However, *Alexandrium* spp. concentrations gradually increased from 0 cells/L to 3 cells/L (9/2/2202), 36 cells/ L (9/8/2022), to 919 cells/L (9/15/2022), marking the second *Alexandrium* spp. event of the study period. By 9/29/2022, concentrations decreased to 16 cells/L and remained 0 cells/L from 11/10/22 until 12/1/2022.

4. Discussion

The purpose of the study is to understand how the selective removal of *S. gigantea* tissues influence human exposure to PSTs. This study focused on tissue groups guided by traditional ecological knowledge that reflect how *S. gigantea* may be prepared before consuming. While traditional practices vary regionally, the siphon, siphon tip, digestive tract, and gills have been selectively removed to reduce biotoxin exposure. For instance, the late Bill

James, Hereditary Chief of Lummi Nation whose traditional territory this study took place in has said, 'You had to cut that black nose off [siphon tip] because the pollutants are all in the black part of the nose,' and that 'if you cut the noses off and clean the bellies [digestive tract] out of the clams you can eat them' (Lummi Seafood Consumption Study 2012). In this quote, the late Bill James refers to the removal of the' black nose,' known as the siphon tip, because the 'pollutants,' which are likely PSTs, are being held in the tissue. The 'bellies,' which is presumably the viscera, the digestive tract in our study, is also said to be discarded. Further north, in southeast Alaska, Indigenous communities removed the tip of the siphon and gills in clams and as a safety measure (Batdorf 1990, Moss 1993). In northeast Vancouver Island, Billy Proctor, a non-native local removed the siphon of butter clams, citing "[they] hold red tide for some time," a practice he learned from local Native people who "followed these harvesting guidelines in the summers" (Williams 2006).

Our study shows similar insight as traditional ecological knowledge with the siphon tip and the siphon disproportionately holding more PSTs relative to its mass compared to other tissues. The removal of the siphon and the siphon tip resulted in the highest reduction of PSTs. Our study also highlights considerable intra variability in PST between individual clams. During the study, individual clam PST concentrations ranged from below detection limit to at least 3.6 times above the regulatory limit. The selective removal of the siphon tip, or the siphon and siphon tip reduced total toxin exposure to PST, but the removal of these tissues did not always reduce PST exposure to levels safe for consumption. Especially when clams have accumulated PST levels above the regulatory limit, the removal of the siphon and siphon tip may not reduce PST to below the regulatory limit. Due to the high intra-sample PST variability, the ability of siphon and siphon tip removal to result in the rest of the tissues below the regulatory limit was mixed within the sampling date. The largest PST concentration reduction from removal of the siphon and siphon tip occurred in samples with the highest overall concentration of PST (i.e. 6/30/22), while samples with lower starting concentrations showed a more modest reduction (i.e. 4/19/22). During the study period, from April - November, clams were above the regulatory limit in five of the 16 collection events. The seasonal pattern for samples above the regulatory limit was temporally variable, with unsafe levels recorded in May, June, July, and November.

Traditional practices also refer to removing the digestive tract and gills to mitigate marine biotoxins. The removal of the digestive tract did not always reduce exposure substantially, but its removal had potential to lower overall concentration of PST to safe levels when discarded with the siphon and siphon tip. The capacity for the digestive tract to reduce PST concentration when removed with other tissues could be influenced by the fact that the digestive tract is where harmful algae is initially deposited when ingested (Bricelj and Shumway 1998). The gills in our study did not contribute considerably to PST reduction, and overall held lower PST concentrations. If *Alexandrium* spp. cells were more abundant and cells were producing more toxin, PST levels could be influenced by these conditions and PST concentration in the gills could be different than what was found in this study.

Variability in PST between individual clams was evident in our study, and is consistent with other studies that also find that shellfish collected from the same location or from proximity with one another can have considerably different PST levels (Quayle 1969, Gibble 2016, Peacock et al. 2018). Varying PST concentrations detected in shellfish can also be attributed to differences in feeding behavior, the toxicity of the *Alexandrium* spp. cells being ingested by the shellfish (Marsden and Shumway 1993), and the timing of harvest, how long it has been since the toxin was initially uptake by the clam. In a similar study, PST concentrations were analyzed in the same tissues as our study in *S. gigantea* collected from Kodiak Archipelago, Alaska. Kibler et al. 2022 quantified that the rest of body pooled with the gills contributed the most to PST, and the highest average PST concentration was found in the gut (digestive tract in our study). In contrast, in our study, the siphon contributed the most to PST, and had the highest average PST concentration compared to other issues. *S. gigantea* collected from British Columbia showed that when clams were in the toxification stage, tissue groups aside from the siphon (presumably the rest of body, gills, and especially the digestive tract) held the majority of the toxin (Quayle 1969, Bricelj and Shumway 1998). When *S. gigantea* were in the detoxification stage, most of the toxin was found in the siphon and what is assumed to be the siphon tip (Quayle 1969). A number of the clams in our study were likely in the detoxification stage since the siphon and siphon tip accounted for most of the toxin throughout our study period. It is likely that toxin was already accumulated in clams for potentially ≤ 6

weeks, the duration of time most of the toxin may be distributed from other tissues to the siphon (Bricelj and Shumway 1998). It is possible clams in Kibler et al. 2022 were mostly in the toxification stage since the rest of body pooled with the gills, and the digestive tract accounted for most of the PST exposure. The amount of time it has been since PSTs were initially accumulated, and individual variability in the uptake of PSTs may explain variability in PST concentration of *S. gigantea*, and contrasting results between our study and Kibler et al. 2022. In addition, Kibler et al. 2022 used high performance liquid chromatography (HPLC) with pre column oxidation to analyze PST in pooled *S. gigantea* samples. Our study utilized enzyme-linked immunosorbent assay (ELISA) as the main method to analyze PST in *S. gigantea*. Both HPLC and ELISA methods can quantify PST congeners differently. Additionally, our study required the use of three different ELISA kit manufacturers, which also quantify PST congeners differently. All three PSP ELISA kits used for our study provide results in STX equivalent, which adds cross-reacted congeners of STX to the total concentration. Total PSTs in *S. gigantea* is complex, as it can be composed of varying PST congeners, though STX is the main congener found in *S. gigantea* and is the most potent of all PST congeners (Etheridge 2010, Raposo et al. 2020). PSP ELISA kits that are highly sensitive to STX but can additionally target other PST congeners is normal within the industry and comparable to other literature (Harrison et al. 2016). High performance liquid chromatography (HPLC) or Liquid chromatography with tandem mass spectrometry (LC-MS/MS) to analyze PST composition in *S. gigantea* samples would reduce the differences we see across ELISA kits, but was beyond the scope of this project.

S. gigantea are not the only bivalve species known to have variability in PST concentration. PST contribution of different tissues is understood to vary between the visceral mass/digestive gland, adductor muscles, foot, gills, gonad, mantle, and siphon in several clam species such as the Atlantic deep-sea scallop (*Placopecten magellanicus*) (Cembella et al. 1994), hard clam (*Mercenaria mercenaria*), soft-shell clam (*Mya arenaria*) (Bricelj and Shumway 1998), surf clams (*Spisula solidissima* and *Mesodesma donacium*) (Shumway et al. 1994, Álvarez et al. 2019). In *M. mercenaria, M. arenaria, S. solidissima,* and in the blue mussel *(Mytilus edulis)*, muscular tissues (adductor muscles, foot) disproportionately contribute lower proportions of toxin relative to its weight contribution compared to the viscera, which contribute 80-90% of

toxin relative to its much smaller weight contribution (Bricelj and Shumway 1998). *S. gigantea* has been found to retain PSTs for over two years, and sequester PSTs in the siphon accounting for 60-80% of the total toxicity (Price and Lee 1972, Kvitek 1991a). The tendency for *S. gigantea* to sequester PSTs into the siphon is hypothesized to have evolved as a chemical defense adaptation likely due to siphon nipping fish (Kvitek 1991b) though, this defense has also shown effective against other predators such as sea otters and gulls which frequently prey on *S. gigantea* (Kvitek 1991c,d). Sea otters and glaucous-winged gulls have been observed to develop an aversion to toxic *S. gigantea* and discard siphons prior to consumption when exposed to highly toxic *S. gigantea* (Kvitek 1991c,d). When fed highly toxic *S. gigantea*, captive sea otters increasingly discarded siphons along with the kidney and pericardial glands, which fundamentally reduced their exposure to saxitoxin by 21-86% compared to their expected toxin exposure (Kvitek 1991b). Glaucous-winged gulls often regurgitated toxic *S. gigantea* and were observed to discard siphons of *S. gigantea* at a study site known for chronic toxicity for PSTs for over 14 years (Kvitek 1991d) though, this it is unclear whether the behavior to avoid siphons at toxic sites was a socially transmitted behavior (Kvitek 1991d).

The regulatory limit of 80 ug/100 g shellfish tissue has served to protect communities from PSP sufficiently (Trainer et al. 2003), but there are implications for populations who consume more shellfish than the general population. The regulatory limit of 80 ug/100 g shellfish tissue for PSTs is set on the basis that ingesting more than 80 ug STX equivalents could cause symptoms of PSP, with a built-in 2.5 x safety factor as a cautionary measure (Wekell et al. 2004). The regulatory limit also assumes that 100 g of shellfish tissue is a reasonable amount of shellfish that would be consumed (Wekell et al. 2004). In Washington, the daily legal limit for the harvest of *S. gigantea* is 40 clams, with 3-4 moderate sized clams equaling to about 100 g of shellfish tissue (based on our data) (WDFW 2023). Populations such as Indigenous communities who regularly subsist on shellfish may have more frequent and constant exposure to PST, thus be at higher risk for PSP. Access to traditional foods, such as shellfish, is integral to the way of life of many Indigenous community members. The current regulatory limit of 80 ug/100 g shellfish tissue may not adequately protect communities who consume greater amounts of shellfish. The implications of constant exposure to PST, even at low levels may have

consequences not yet well understood. Changing ocean conditions make understanding how PST will continue to impact human health even more complex.

Changing ocean conditions such as warming ocean temperatures, increased stratification of the ocean, and nutrient pollution are predicted to influence the frequency, magnitude, and phenology of HAB dynamics (Trainer et al. 2003, Lewitus et al. 2012, Wells et al. 2015). Many tribal nations used seasonal timing to avoid HABs and to determine when shellfish were safe for harvest (Newton and Moss 2005). For example, in southeast Alaska, Tlingit elder, George Davis expressed to avoid harvesting shellfish in certain times of the year to avoiding illness from PSTs stating, "All I can tell you is that when the grouse hoots, it is time to stop eating clams. In Tlingit, this is called *Núkt*, grouse–clams, the month of April" (Newton and Moss 2005). Tlingit member, Henry Katasse similarly said that "According to the southeastern Tlingit, whenever the herring spawn, it is time to stop eating all shellfish. The month is called *Héen taanáx kayaaní dísi*, plant month in the sea. Everyone just accepts it and leaves all seafood alone. Clams are mostly dug in the winter months…" (Newton and Moss 2005). The seasonal timing used by various tribal nations coincide with the typical timing of HABs in the U.S West Coast. Across the U.S West Coast, the window of time that marine HABs typically occur is May – October (Horner at al 1997). In the Puget Sound, HABs usually occur between July – November (Horner at al 1997, Moore et al. 2009). However, the window of time that HABs occur has been expanding (Moore et al. 2009). In the Puget Sound, shellfish harvest closures due to marine biotoxins are being detected earlier and later in the year compared to years past (Moore et al. 2009). The persistence of marine biotoxins have impacted the local Lummi Nation, who have experienced shellfish harvest closures as early as March until even February of the following year (Hintz 2020). In the last three decades, the total number of days that shellfish is closed for harvest has increased by 2.4 days each year in Whatcom County, and by 4.4 days each year in Bellingham Bay, which is in proximity to Portage Bay, an important area for shellfish harvest by community members (Hintz 2020). Shellfish are also becoming increasingly toxic with PSTs in the region (Hintz 2020). Shellfish are regularly accumulating PST concentrations unsafe for consumption, and have held maximum PST concentrations up to three times the amount than in the past in recent years (Hintz 2020).

Changes in HAB dynamics, and that current HAB conditions are likely more severe than in the past make it even more difficult to mitigate marine biotoxins, and emphasizes the need for enhanced monitoring of HABs (Trainer at al. 2020). Shifts in the phenology of HABs due to climate change implicate that the seasonal timing used for shellfish harvesting practices are changing. The increasing severity of HABs are already limiting opportunities for shellfish harvest, and is expected to continue to impact the recreational, commercial, and subsistence harvest of shellfish. Monitoring programs for HABs across the U.S West Coast such as SoundToxins (Trainer and King 2023), Southeast Alaska Tribal Ocean Research (SEATOR) (Harley et al. 2020), and Communities Advancing the Studies of Tribal Nations Across the Lifespan (CoASTAL cohort) (Tracy et al. 2016) increase our understanding of how HABs will impact human health by monitoring phytoplankton and providing early warning of HAB events.

Aligning monitoring efforts with the ways that human communities interact with HABs would improve its relevance to communities heavily impacted by HABs, and increase reception to environmental monitoring and management decisions by government entities. The reluctance to trust information shared by government bodies has historically been pervasive in Indigenous communities (Goetze 2005). Long-standing distrust between Indigenous peoples and government bodies makes the acceptance of information shared by the government challenging (Goetze 2005, Simms 2014). In a study on Indigenous water governance, Indigenous community members of Lower Similkameen Indian Band in British Columbia expressed their disbelief of data shared by the government, and need for their own community monitoring for their waters. A member said that “The government has data, the mining companies have data, the corporations have data, but we don’t have any, yet we own the resource. Scientists are muzzled; you can’t necessarily trust the information they have” (Simms 2014). Another member expressed that “I don’t really trust their water sampling reports, because you can type whatever you want in those little spreadsheets, and who is going to utilize it? And there’s a reason we don’t press these things, because it’s only because we’ve experienced it over and over again. So unless our own people are doing that work and documenting it, I don’t personally trust it. They send it [that data] every week but I don’t necessarily trust or believe in it” (Simms 2014). Integrating traditional ecological knowledge into the environmental

monitoring could serve more relevant information to human communities. As seen with Heiltsuk Nation, traditional preparation of shellfish could be incorporated into management decisions. At the request of Heiltsuk Nation, Fisheries and Oceans Canada (DFO) in British Columbia has permitted the harvest of *S. gigantea* with the siphon removed for Heiltsuk FSC (food, social, ceremonial) harvest. Our study is one example of integrating traditional ecological knowledge of HABs in the way that human communities consume shellfish in a western science framework. Aligning monitoring efforts of HABs with the ways that food and/or shellfish are consumed by human communities can provide pertinent information on risks of HABs to human health, strengthen confidence in the data shared through government monitoring, and build capacity for stronger relationships between Indigenous communities and the government.

Table 1. Mean, maximum, minimum, median, and standard deviation (Std) of percent mass (%) in the five clam tissues, listed in decreasing order, including sample size for the analysis.

Tissue	Mean (%)	Max (%)	Min (%)	Median (%)	Std (%)	N
Rest of body	57.5	70.8	46.8	57.4	5.1	48
Digestive tract	21.4	34.6	8.5	20.8	6.0	48
Siphon	12.1	21.3	5.7	11.6	3.0	48
Gills	6.1	11.4	3.6	5.7	1.6	48
Siphon tip	2.9	6.1	0.5	2.8	1.1	48

Table 2. Mean, maximum, minimum, median, and standard deviation (Std) of PST concentrations (µg/100 g shellfish tissue) in the five clam tissues listed in decreasing order, including sample size for the analysis. Samples determined below detection limit are assigned "bdl."

Tissue	Mean (µg/100 g)	Max (µg/100 g)	Min (µg/100 g)	Median (µg/100 g)	Std (µg/100 g)	N
Siphon	216.9	1400.4	bdl	108.1	280.6	48
Siphon tip	167.5	618.0	1.5	104.3	176.0	48
Digestive tract	59.6	390.9	bdl	17.6	88.6	48
Rest of body	40.2	246.0	bdl	1.9	57.9	48
Gills	18.6	88.0	bdl	8.4	24.6	48

Table 3. Mean, maximum, minimum, median, and standard deviation (Std) of percent contribution of PST (%) in the five clam tissues, listed in decreasing order, including sample size for the analysis.

Tissue	Mean (%)	Max (%)	Min (%)	Median (%)	Std (%)	N
Siphon	44.6	96.0	4.4	40.5	22.2	48
Rest of body	22.5	65.2	0.3	20.2	19.6	48
Digestive tract	19.2	90.8	0.2	16.0	16.6	48
Siphon tip	11.2	57.6	0.5	7.2	11.3	48
Gills	2.5	22.3	0.1	1.3	3.5	48

Table 4. Mean, maximum, minimum, median, and standard deviation (Std) of percent contribution relative to mass (%) in the five clam tissues, listed in decreasing order, including sample size for the analysis.

Tissue	Mean (%)	Max (%)	Min (%)	Median (%)	Std (%)	N
Siphon tip	457.2	3811.2	20.4	266.0	631.8	48
Siphon	391.0	1343.2	36.4	340.4	230.1	48
Digestive tract	92.6	481.8	1.2	76.0	84.1	48
Gills	40.9	348.8	0.8	25.2	54.7	48
Rest of body	38.3	111.2	0.6	36.5	32.9	48

Table 5. Mean, maximum, minimum, median, and standard deviation (Std) of total PST (µg/100 g shellfish tissue) of three clam preparations in the five clam tissues listed in increasing order, including sample size for the analysis. Samples determined below detection limit are assigned "bdl."

Preparation	Mean (µg/100 g)	Max (µg/100 g)	Min (µg/100 g)	Median (µg/100 g)	Std (µg/100 g)	N
Siphon and Siphon Tip Removed	42.5	206.6	bdl	12.0	54.6	48
Siphon Tip Removed	61.7	291.8	0.5	33.4	72.4	48
Whole Body	64.4	293.8	0.5	33.5	74.0	48

Table 6. Summary of post-hoc contrasts of percent mass (%) of all five clam tissues using expected marginal means and Tukey's HSD method to compare significant differences between means. S=siphon, ST=siphon tip, DT=digestive tract, G=gills, ROB=rest of body.

Pairwise comparison	estimate	SE	df	t ratio	p-value
DT - G	0.1533	0.00942	188	16.270	<.0001
DT - ROB	-0.3603	0.01219	188	-29.547	<.0001
DT - S	0.0937	0.01012	188	9.260	<.0001
DT - ST	0.1850	0.00929	188	19.906	<.0001
G - ROB	-0.5136	0.00805	188	-63.799	<.0001
G - S	-0.0595	0.00431	188	-13.807	<.0001
G - ST	0.0318	0.00158	188	20.081	<.0001
ROB - S	0.4540	0.00886	188	51.234	<.0001
ROB - ST	0.5453	0.00790	188	69.007	<.0001
S - ST	0.0913	0.00403	188	22.653	<.0001

Table 7. Summary of post-hoc contrasts of PST concentrations (µg/100 g shellfish tissue) of all five clam tissues using expected marginal means and Tukey's HSD method to compare significant differences between means. S=siphon, ST=siphon tip, DT=digestive tract, G=gills, ROB=rest of body.

Pairwise comparison	estimate	SE	df	t ratio	p-value
DT - G	41.0	11.81	188	3.468	0.0058
DT - ROB	19.4	13.60	188	1.430	0.6092
DT - S	-157.3	39.91	188	-3.940	0.0011
DT - ST	-107.9	26.10	188	-4.135	0.0005
G - ROB	-21.5	6.74	188	-3.192	0.0142
G - S	-198.2	38.13	188	-5.199	<.0001
G - ST	-148.9	23.28	188	-6.395	<.0001
ROB - S	-176.7	38.72	188	-4.564	0.0001
ROB - ST	-127.4	24.23	188	-5.255	<.0001
S - ST	49.3	44.67	188	1.105	0.8040

Table 8. Summary of post-hoc contrasts of percent contribution (%) of PST of all five clam tissues using expected marginal means and Tukey's HSD method to compare significant differences between means. S=siphon, ST=siphon tip, DT=digestive tract, G=gills, ROB=rest of body.

Pairwise comparison	estimate	SE	df	t ratio	p-value
DT - G	0.1679	0.0245	188	6.857	<.0001
DT - ROB	-0.0330	0.0370	188	-0.892	0.8997
DT - S	-0.2531	0.0400	188	-6.327	<.0001
DT - ST	0.0805	0.0290	188	2.779	0.0468
G - ROB	-0.2009	0.0287	188	-7.003	<.0001
G - S	-0.4210	0.0324	188	-12.977	<.0001
G - ST	-0.0874	0.0170	188	-5.126	<.0001
ROB - S	-0.2201	0.0427	188	-5.154	<.0001
ROB - ST	0.1135	0.0326	188	3.483	0.0055
S - ST	0.3336	0.0359	188	9.282	<.0001

Table 9. Summary of post-hoc contrasts of percent contribution relative to mass (%) of all five clam tissues using expected marginal means and Tukey's HSD method to compare significant differences between means. S=siphon, ST=siphon tip, DT=digestive tract, G=gills, ROB=rest of body.

Pairwise comparison	estimate	SE	df	t ratio	p-value
DT - G	51.70	14.48	188	3.570	0.0041
DT - ROB	54.33	13.04	188	4.166	0.0004
DT - S	-298.33	35.37	188	-8.435	<.0001
DT - ST	-364.53	92.00	188	-3.962	0.0010
G - ROB	2.63	9.21	188	0.286	0.9985
G - S	-350.03	34.14	188	-10.252	<.0001
G - ST	-416.23	91.53	188	-4.547	0.0001
ROB - S	-352.66	33.56	188	-10.510	<.0001
ROB - ST	-418.87	91.32	188	-4.587	0.0001
S - ST	-66.20	97.05	188	-0.682	0.9601

Table 10. Summary of post-hoc contrasts of total PST (µg/100 g shellfish tissue) of three clam preparations using expected marginal means and Tukey's HSD method to compare significant differences between means.

Pairwise comparison	estimate	SE	df	t ratio	p-value
Siphon and Siphon Tip Removed - Siphon Tip Removed	-19.19	3.372	94	-5.693	<.0001
Siphon and Siphon Tip Removed - Whole Body	-21.87	3.414	94	-6.407	<.0001
Siphon Tip Removed - Whole Body	-2.68	0.537	94	-4.988	<.0001

Figure 1. (A) Study area in Bellingham, WA. (B) Sites in Marine Park and Boulevard Park that *S. gigantea* were collected between April – November 2022, and sediment temperature loggers deployed. (C) Taylor dock located at Boulevard Park where sampling for dissolved toxins and phytoplankton occurred. Light gray patterned marking indicates most southern end of sampling area for *S. gigantea* in Boulevard Park.

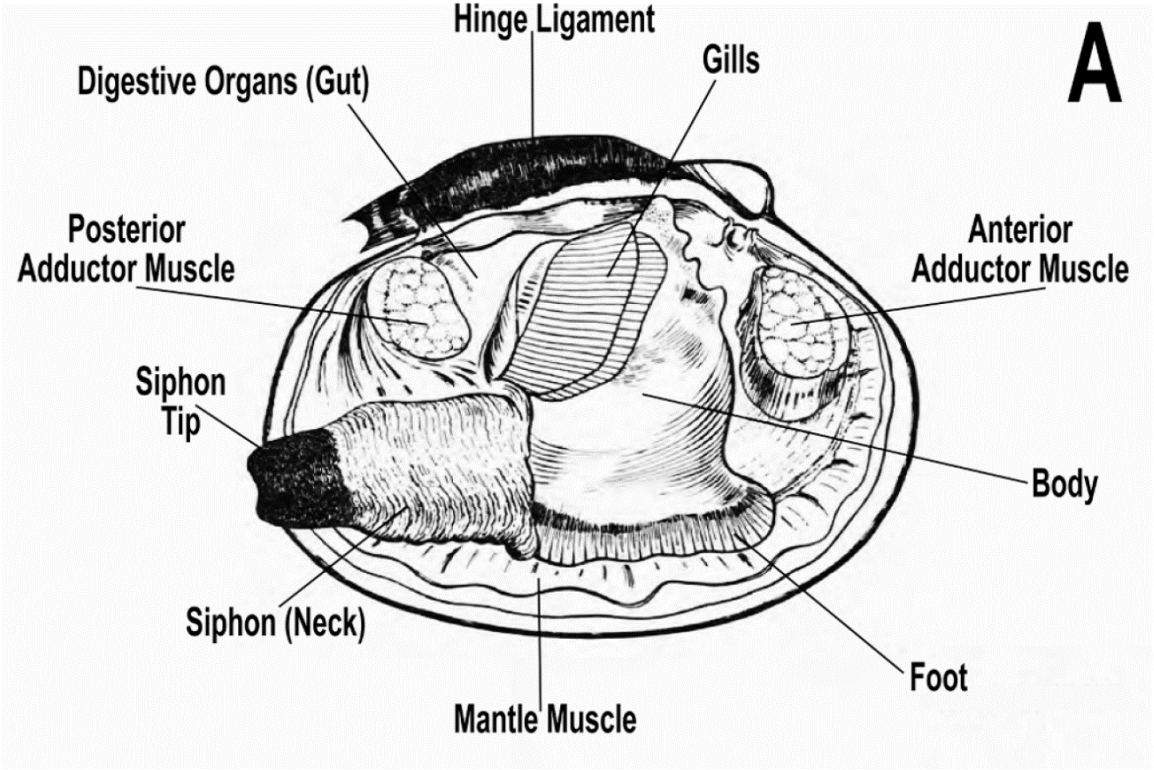

Figure 2. Diagram of the anatomy of *S. gigantea* (Diagram from Quayle and Bourne (1972) and cited in Kibler et al. 2022).

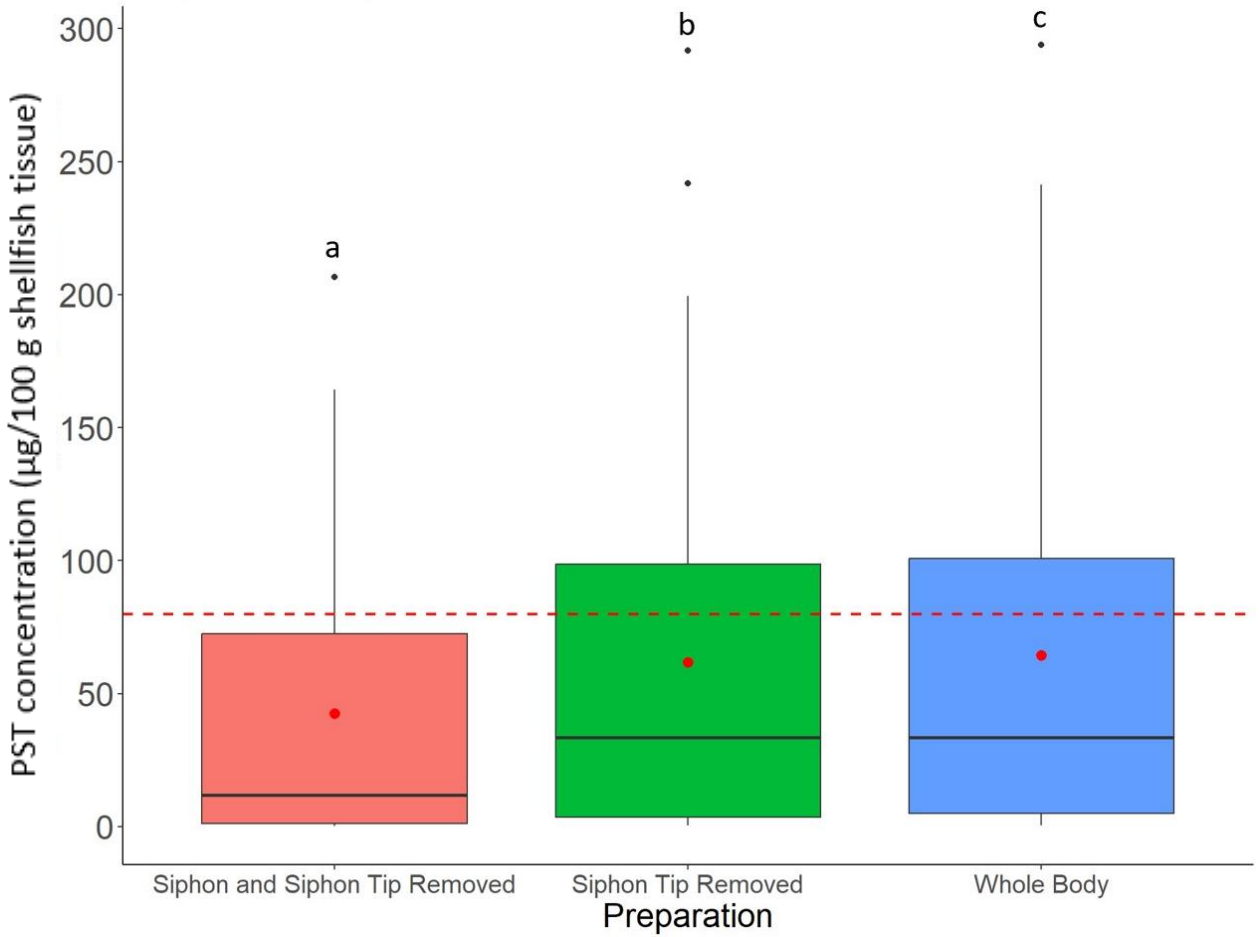

Figure 3. Boxplot showing mean (red dot), median, minimum, maximum, and quartiles of total PST concentrations (µg/100 g shellfish tissue) in the whole body, and in remaining tissues with the removal of the siphon tip, and removal of siphon and siphon tip. Significant differences from post-hoc comparisons indicated by different letters. Red dashed line marks the regulatory limit for PST (80 µg/100 g shellfish tissue).

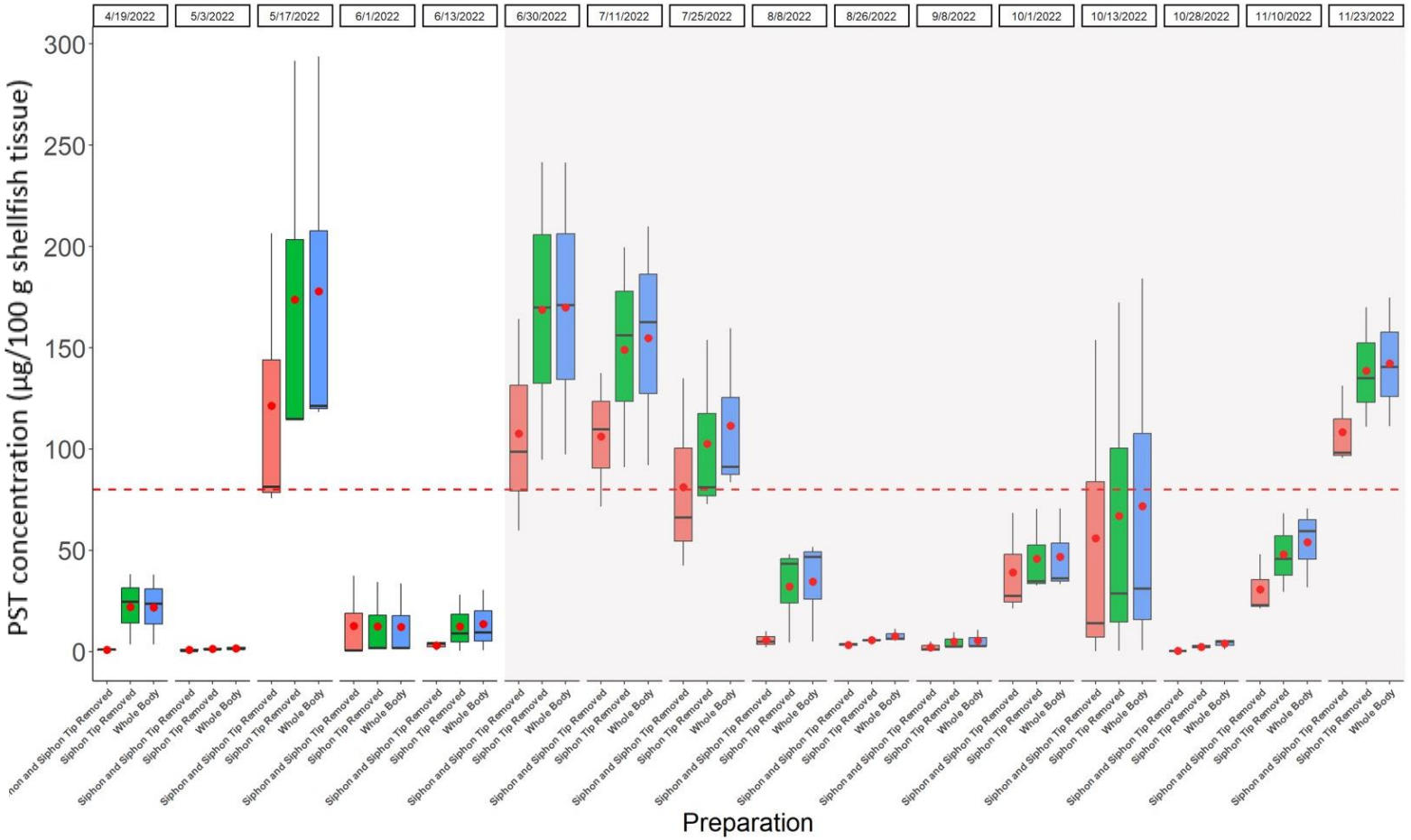

Figure 4. Boxplot showing mean (red dot), median, minimum, maximum, and quartiles of total PST concentrations (µg/100 g shellfish tissue) in the whole body, and in remaining tissues with the removal of the siphon tip, and removal of siphon and siphon tip throughout study period. Red dashed line marks the regulatory limit for PST (80 µg/100 g shellfish tissue). Gray shading indicates the time period of harvest closure for all species in Bellingham Bay due to PST concentrations above the regulatory limit, determined by WA Department of Health.

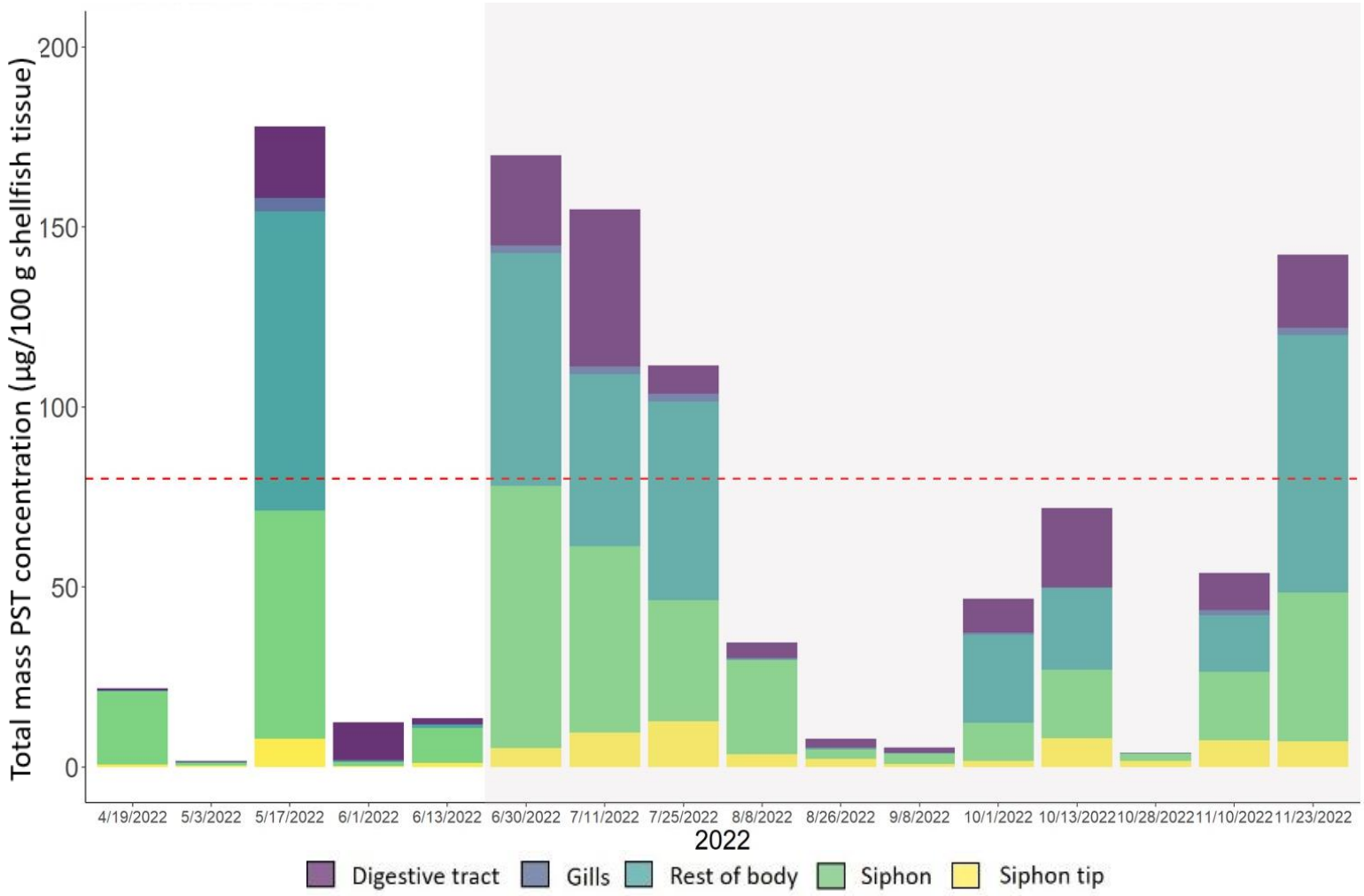

Figure 5. Stacked barplot of the total mass PST concentrations (µg/100 g shellfish tissue) in the whole body and relative contributions of all five tissues. Red dashed line marks the regulatory limit for PST (80 µg/100 g shellfish tissue). Gray shading indicates the time period of harvest closure for all species in Bellingham Bay due to PST concentrations above the regulatory limit, determined by WA Department of Health.

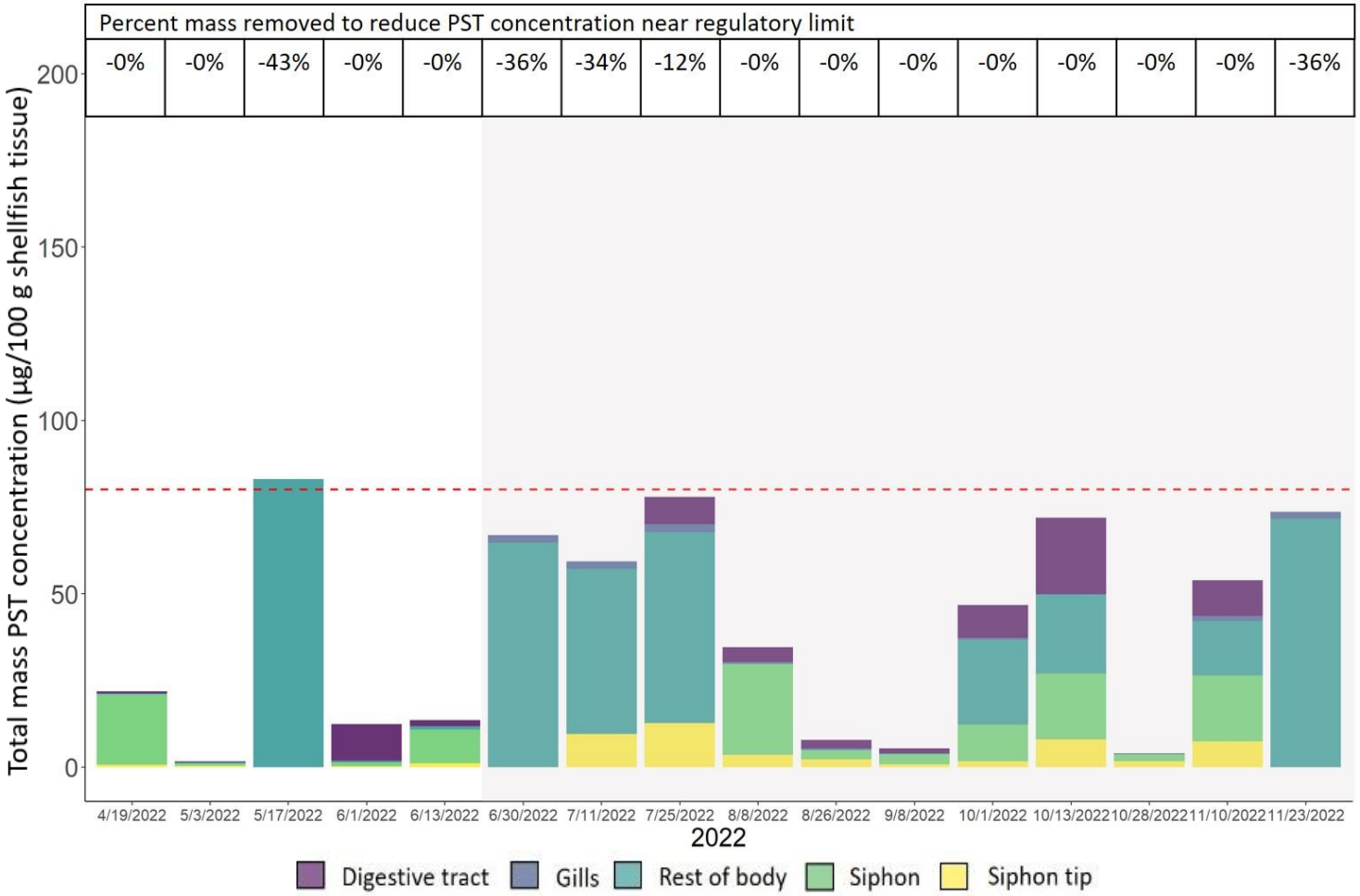

Figure 6. Stacked bar plot of the total mass PST concentrations (µg/100 g shellfish tissue) in remaining tissues left for consumption when tissues are selectively removed until total mass PST concentration in remaining tissues are near or below the regulatory limit for PST (red dashed line) (80 µg/100 g shellfish tissue). Percentages are the percent mass (%) needed to be discarded for total mass PST concentration in remaining tissues to total near or below the regulatory limit. Gray shading indicates the time period of harvest closure for all species in Bellingham Bay due to PST concentrations above the regulatory limit, determined by WA Department of Health.

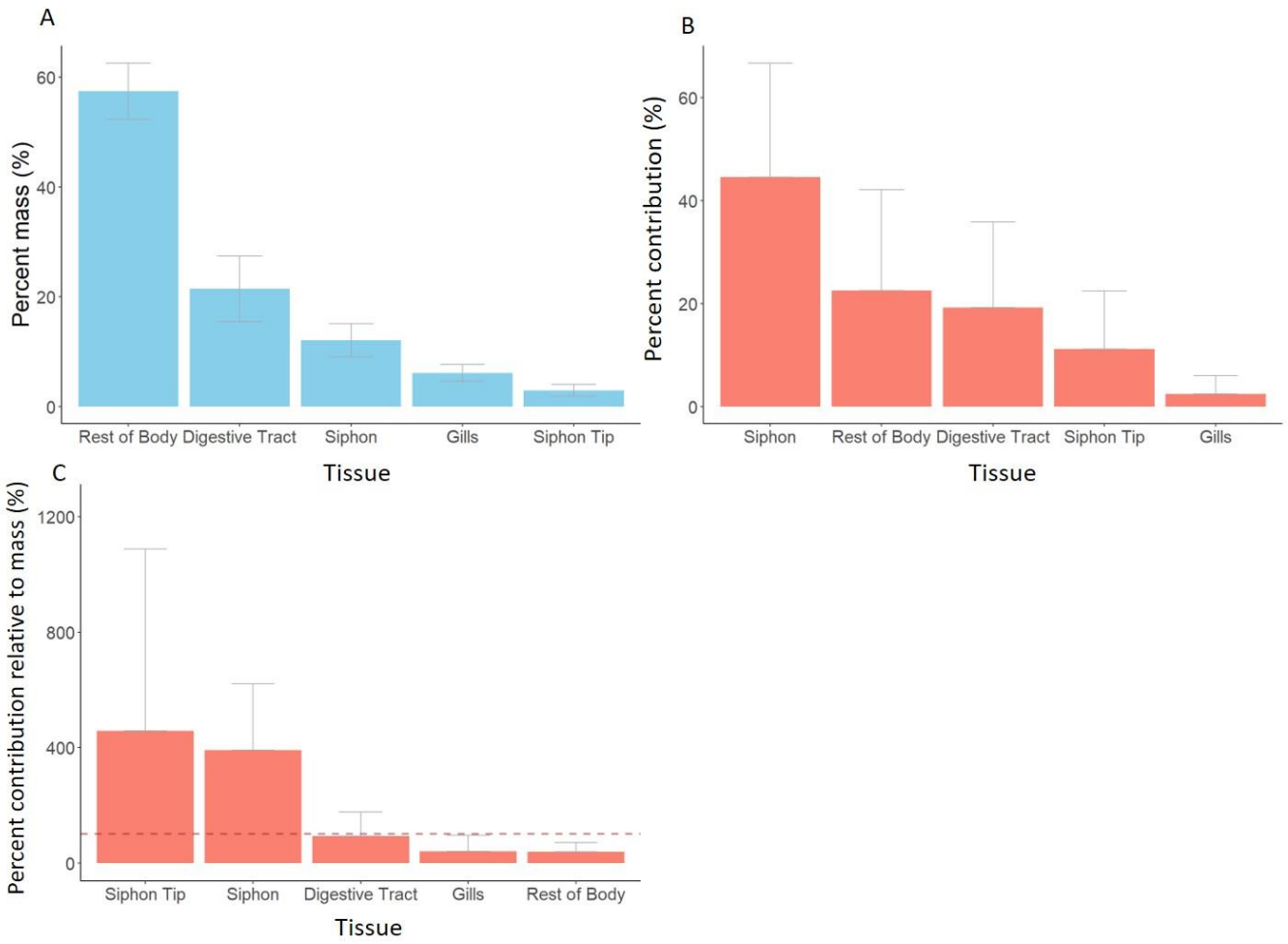

Figure 7. Mean and post-hoc comparisons of percent mass (%), percent contribution (%) and percent contribution relative to mass (%) of *S. gigantea*. (A). Mean percent mass of all five clam tissues plotted in decreasing order with post-hoc groups. (B) Mean percent contributions of all five clam tissues plotted in decreasing order with post-hoc groups. (C) Mean percent contribution relative to mass of all five clam tissues plotted in decreasing order with post-hoc groups. Red dashed line marks a percent contribution relative to mass ratio of 100%.

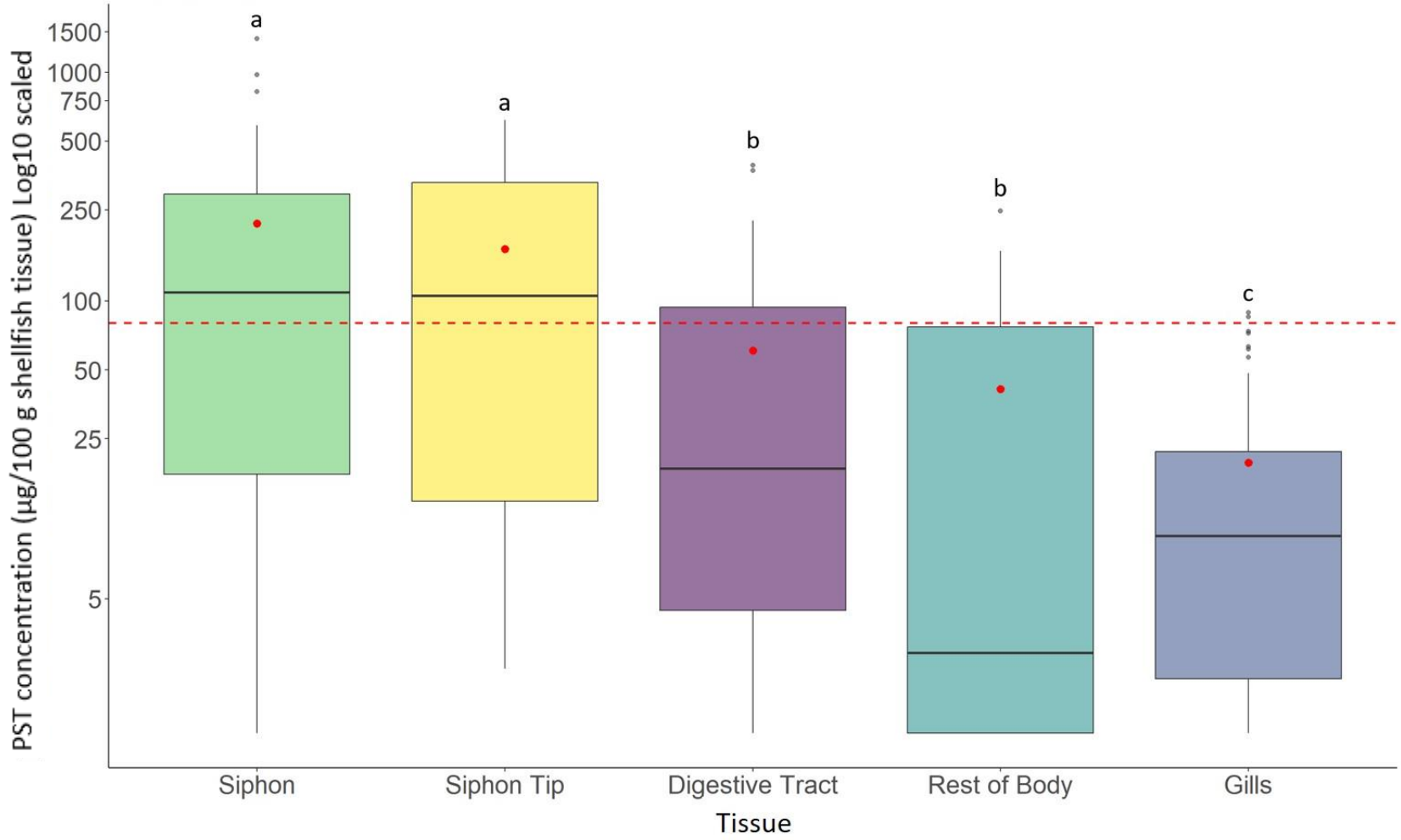

Figure 8. Boxplot showing mean (red dot), median, minimum, maximum, and quartiles of PST concentrations (µg/100 g shellfish tissue) of all five clam tissues. Significant differences from post-hoc comparisons indicated by different letters. Red dashed line marks the regulatory limit for PST (80 µg/100 g shellfish tissue).

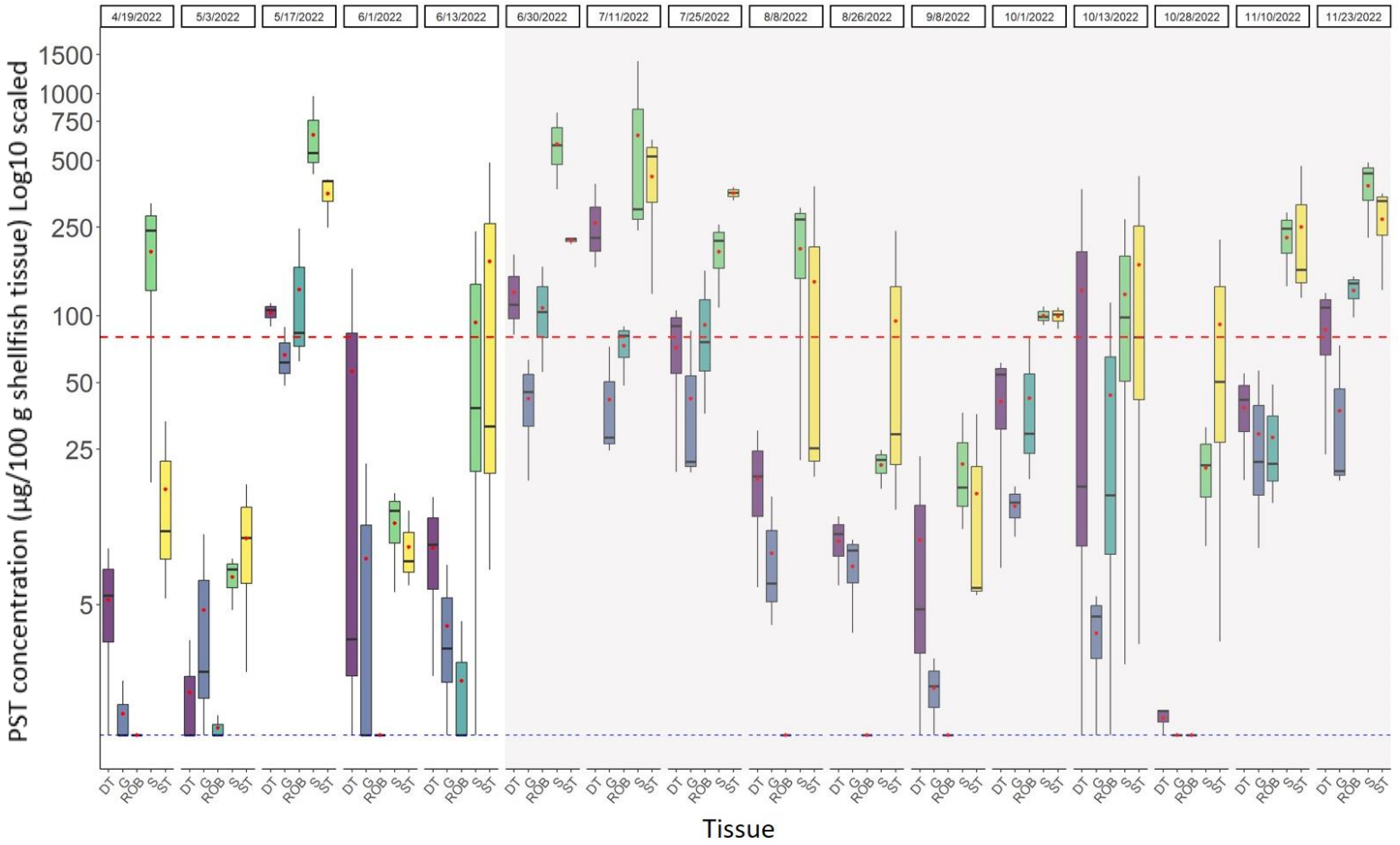

Figure 9. Boxplot showing mean (red dot), median, minimum, maximum, and quartiles of PST concentrations (µg/100 g shellfish tissue) of all five clam tissue types throughout the study period on a log scale. S=siphon, ST=siphon tip, DT=digestive tract, G=gills, ROB=rest of body. Red dashed line marks the regulatory limit for PST (80 µg/100 g shellfish tissue). Blue dashed line marks detection limit (0.30 µg/100 g shellfish tissue). Gray shading indicates the time period of harvest closure for all species in Bellingham Bay due to PST concentrations above the regulatory limit, determined by WA Department of Health.

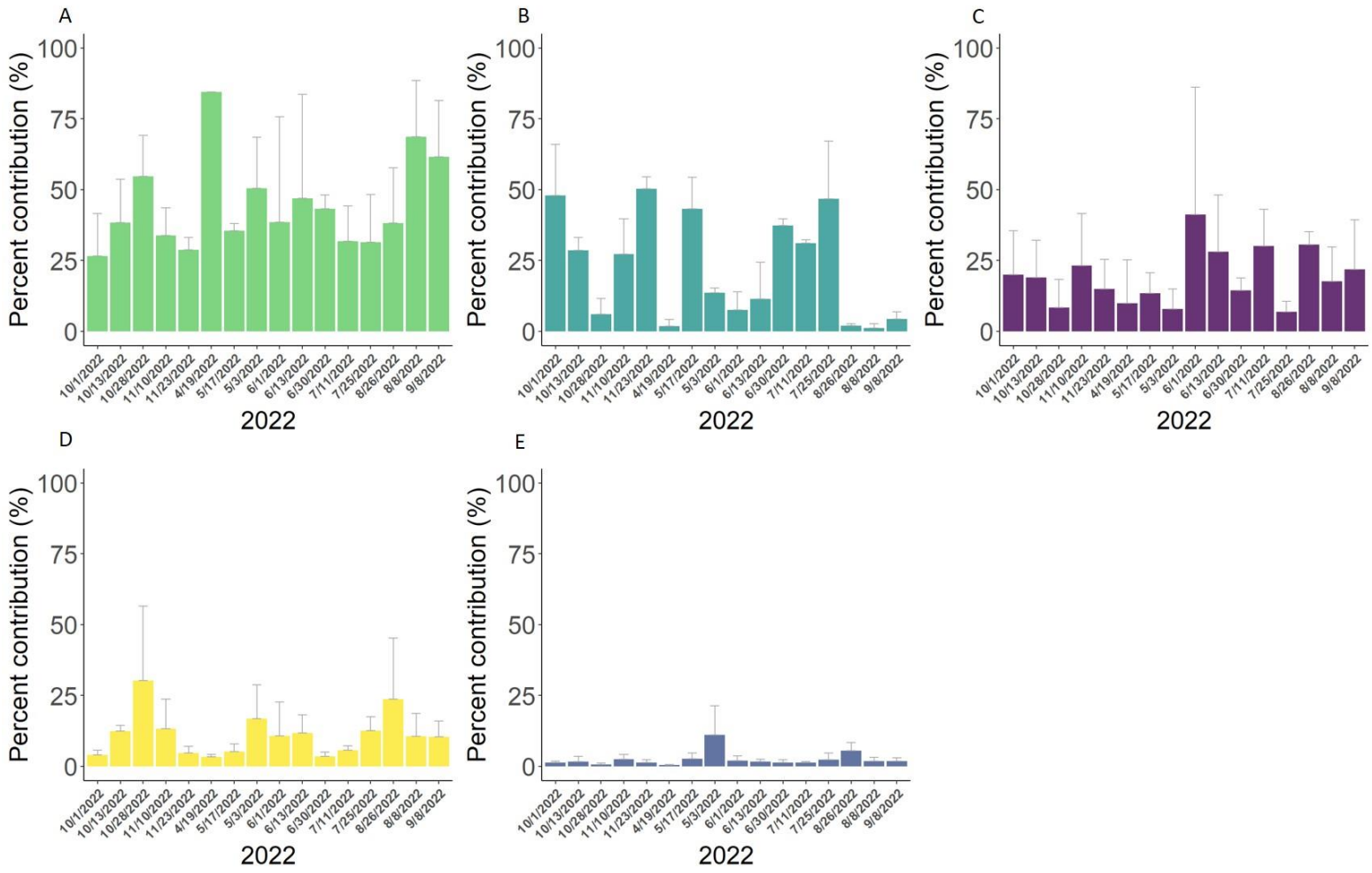

Figure 10. Percent contribution (%) of PST of all five clam tissues between April-November 2022, ordered A-E based on highest to lowest mean Percent contribution. (A) Percent contribution in the siphon. (B) Percent contribution in the rest of body. (C) Percent contribution in the digestive tract. (D) Percent contribution in the siphon tip. (E) Percent contribution in the gills.

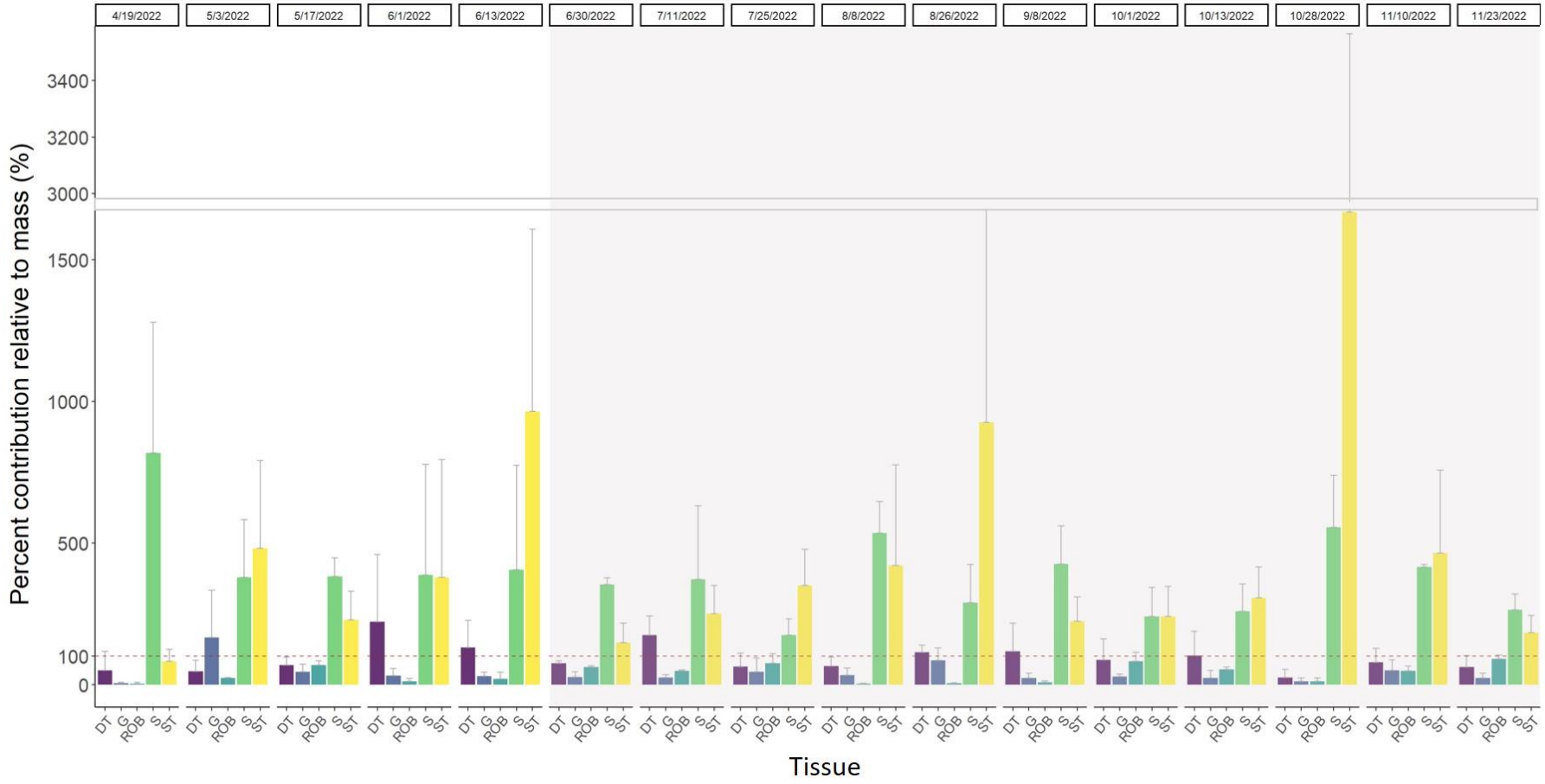

Figure 11. Mean percent contribution relative to mass (%) of all five clam tissues throughout study period. S=siphon, ST=siphon tip, DT=digestive tract, G=gills, ROB=rest of body. Red dashed line marks a percent contribution relative to mass of 100%. Y-axis break between 1600-3000% for lower data values to be viewed more closely. Gray shading indicates the time period of harvest closure for all species in Bellingham Bay due to PST concentrations above the regulatory limit, determined by WA Department of Health.

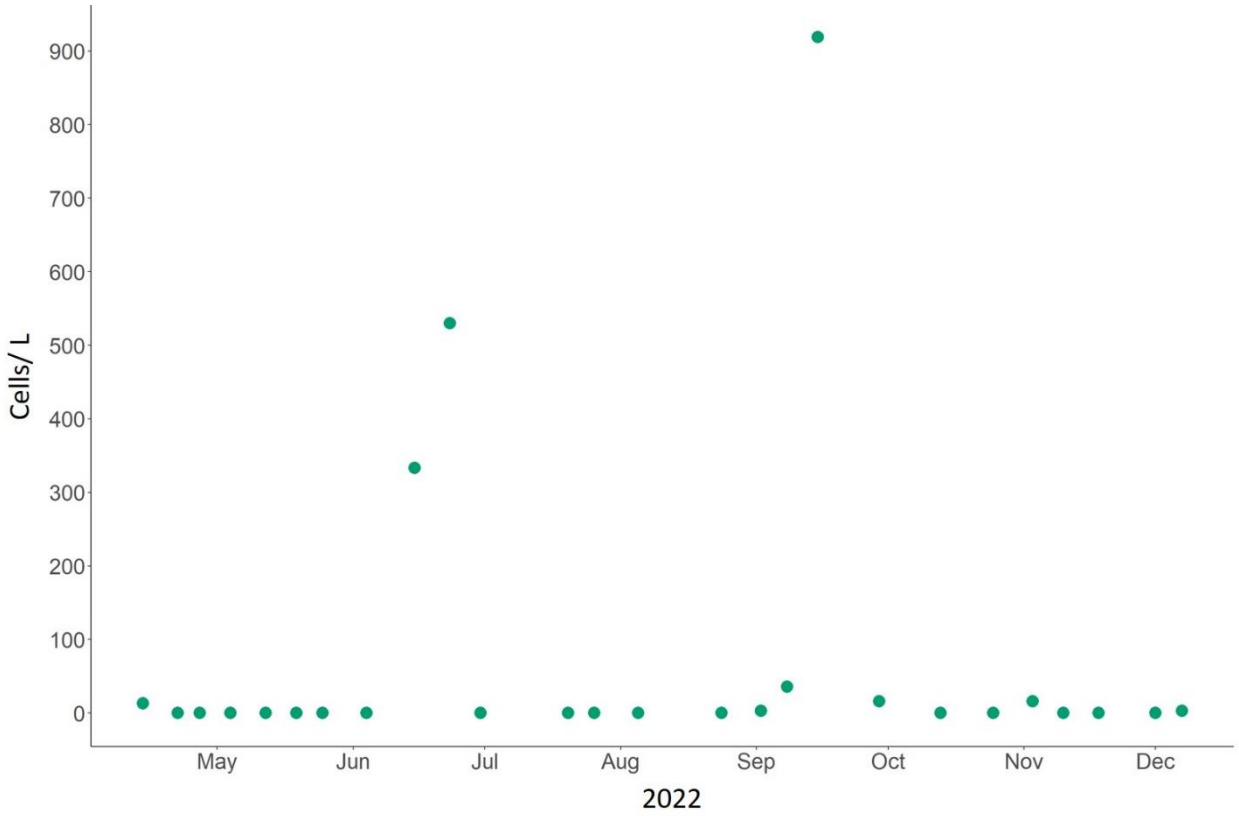

Figure 12. *Alexandrium* spp. concentration (cells/ L) in Bellingham Bay from April – November 2022. Peaks are the two *Alexandrium* spp. blooms that occurred during study period, with the first bloom in June 2022 and the second bloom in September 2022.

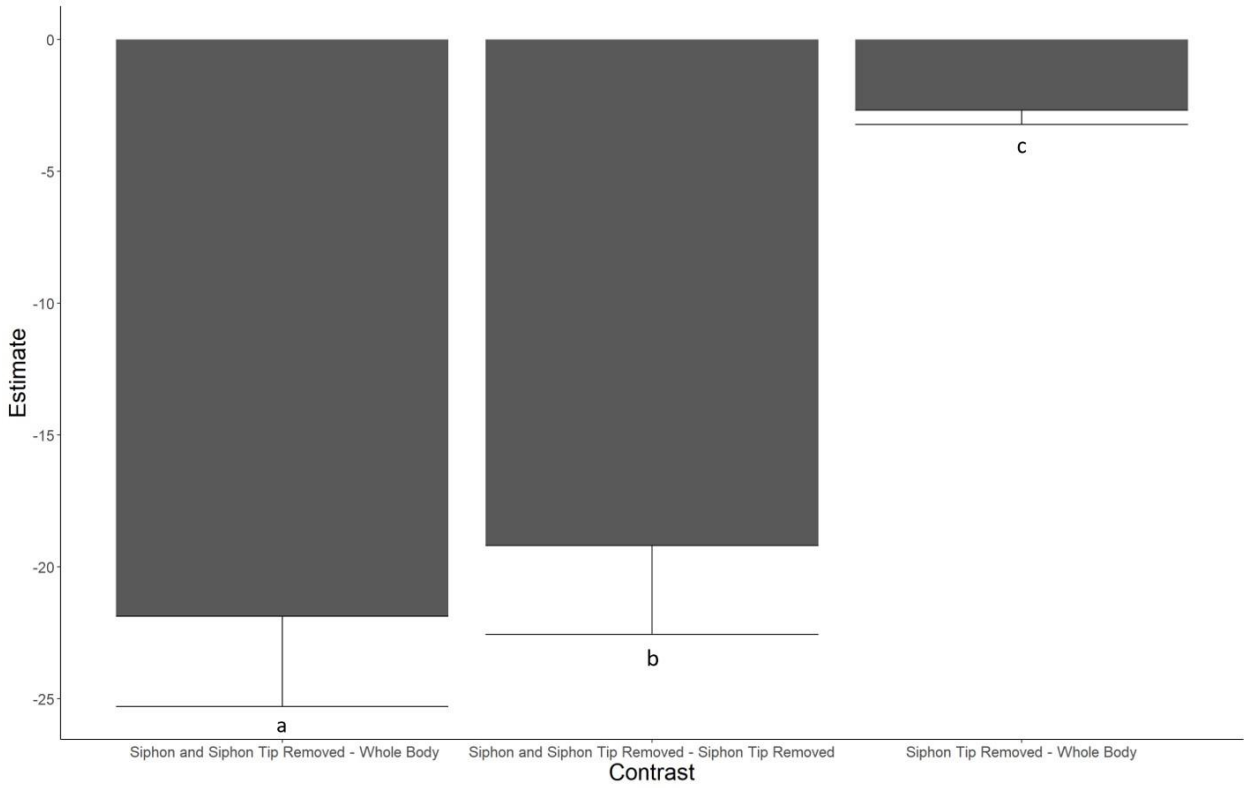

Figure 13. Post-hoc estimates from comparing differences between means of total PST concentrations (µg/100 g shellfish tissue) in the whole body, and in remaining tissues with the removal of the siphon tip, and removal of siphon and siphon tip.

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Appendix

Table A1. Cross-reactivities of PST congeners in PerkinElmer MaxSignal Saxitoxin (PSP), Eurofins Abraxis Saxitoxin (PSP), and SeaTox Research Inc Saxitoxin/Paralytic Shellfish Poisoning ELISA kits (McCall et al. 2019, Li and Persson 2021, Eurofins Abraxis Saxitoxin (PSP) ELISA Protocol 2022).

PST congener	MaxSignal Cross-Reactivity	Abraxis Cross-Reactivity	SeaTox Research Inc Cross-Reactivity
Saxitoxin (STX)	100%	100%	100%
Neosaxitoxin (neoSTX)	20%	1.3%	80.4%
Decarbamoyl Saxitoxin (dcSTX)	100%	29%	9.9%
GTX 2 & 3	43%	23%	14.8%
GTX 1 & 4	2%	<0.2%	13.2%
Decarbamoyl GTX 2 & 3	10%	1.4%	NA
Decarbamoyl Neosaxitoxin (dcNeoSTX)	4%	0.6%	NA
GTX-5	61%	23%	NA
Lyngbyatoxin	NA	13%	NA
Sulfo GTX 1 & 2	NA	2.0%	NA

Table A2. Saxitoxin standards of PerkinElmer MaxSignal Saxitoxin (PSP), Eurofins Abraxis Saxitoxin (PSP), and SeaTox Research Inc Saxitoxin/Paralytic Shellfish Poisoning ELISA kits.

Saxitoxin standards		
MaxSignal	Abraxis	SeaTox Research Inc
0 ng/g	0 ng/g	0 ng/g
0.05 ng/g	0.02 ng/g	0.125 ng/g
0.15 ng/g	0.05 ng/g	0.25 ng/g
0.5 ng/g	0.10 ng/g	0.5 ng/g
1.5 ng/g	0.20 ng/g	1 ng/g
4.5 ng/g	0.40 ng/g	2ng/g

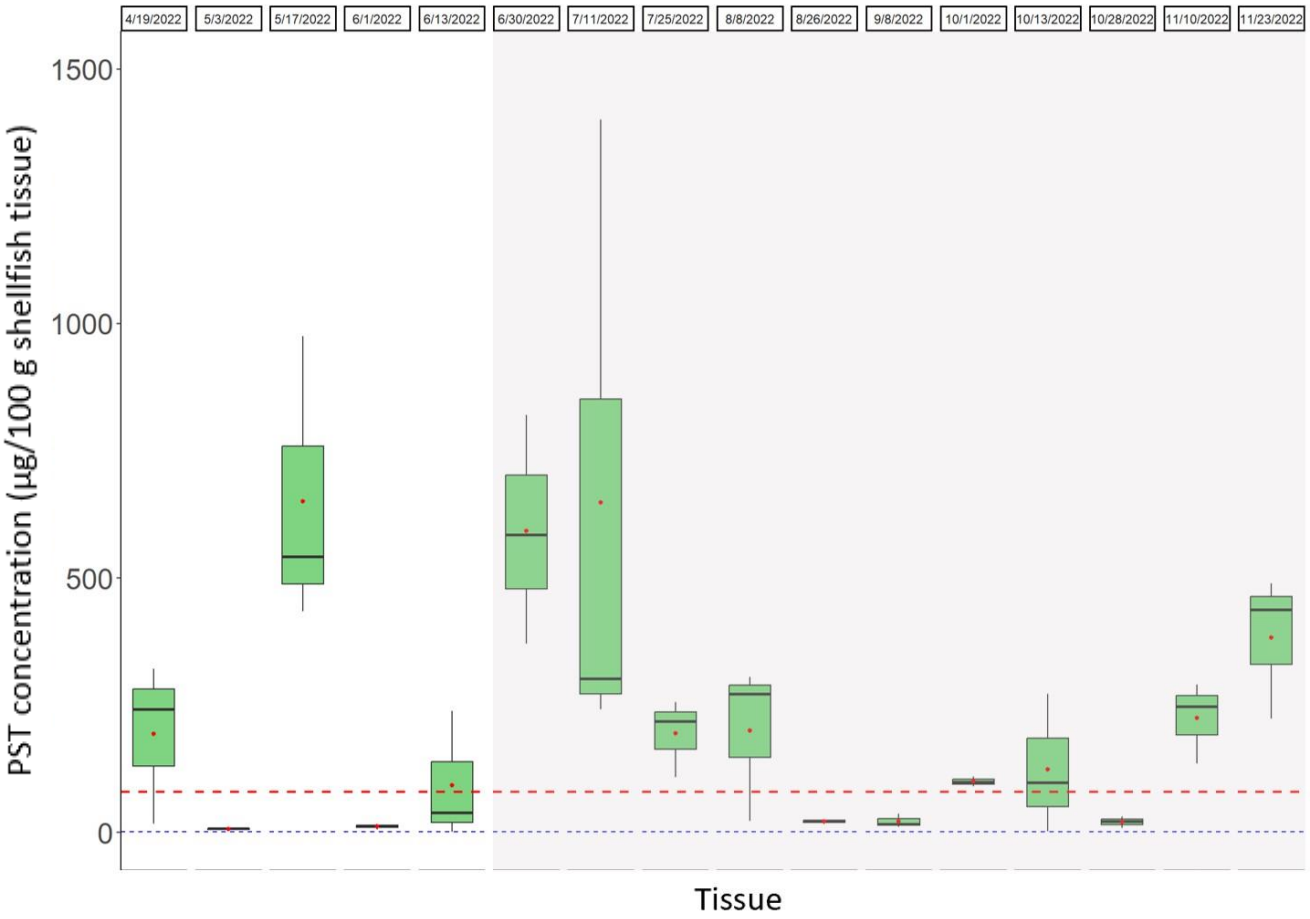

Figure A1. Boxplot showing mean (red dot), median, minimum, maximum, and quartiles of PST concentrations (µg/100 g shellfish tissue) of siphon throughout study period. Red dashed line marks the regulatory limit for PST (80 µg/100 g shellfish tissue). Blue dashed line marks detection limit (0.30 µg/100 g shellfish tissue). Gray shading indicates the time period of harvest closure for all species in Bellingham Bay due to PST concentrations above the regulatory limit, determined by WA Department of Health.

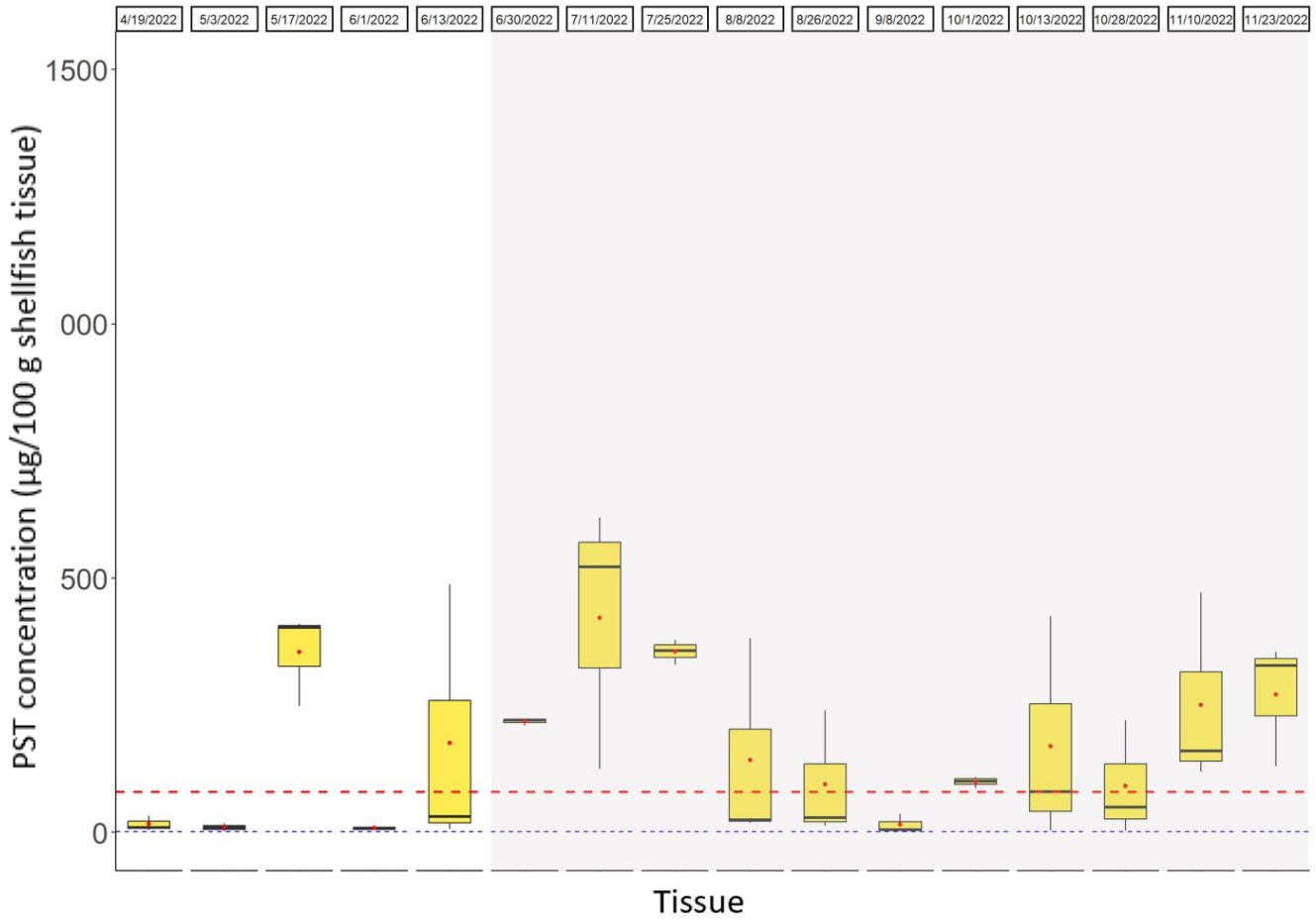

Figure A2. Boxplot showing mean (red dot), median, minimum, maximum, and quartiles of PST concentrations (µg/100 g shellfish tissue) of siphon tip throughout study period. Red dashed line marks the regulatory limit for PST (80 µg/100 g shellfish tissue). Blue dashed line marks detection limit (0.30 µg/100 g shellfish tissue). Gray shading indicates the time period of harvest closure for all species in Bellingham Bay due to PST concentrations above the regulatory limit, determined by WA Department of Health.

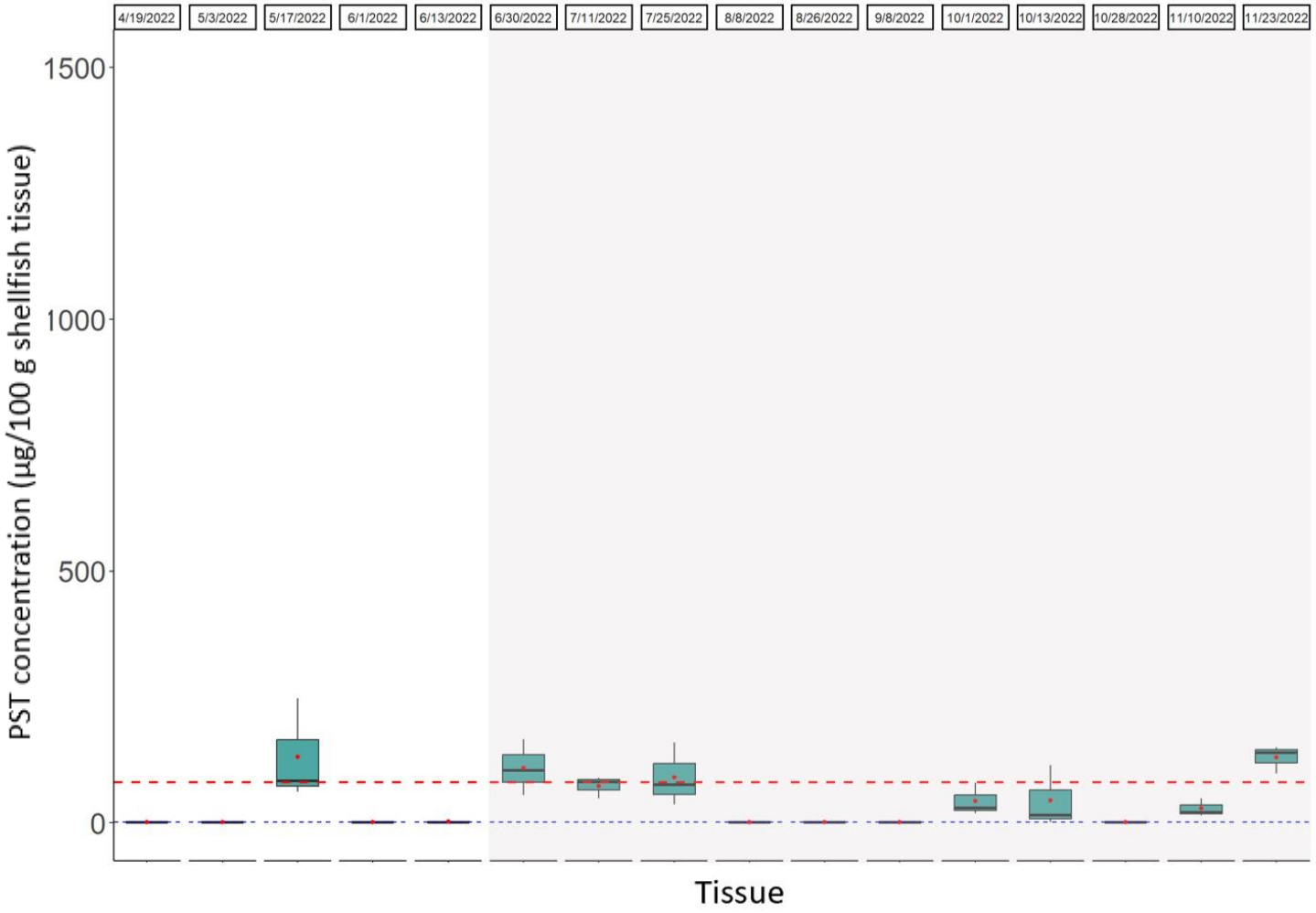

Figure A3. Boxplot showing mean (red dot), median, minimum, maximum, and quartiles of PST concentrations (µg/100 g shellfish tissue) of rest of body throughout study period. Red dashed line marks the regulatory limit for PST (80 µg/100 g shellfish tissue). Blue dashed line marks detection limit (0.30 µg/100 g shellfish tissue). Gray shading indicates the time period of harvest closure for all species in Bellingham Bay due to PST concentrations above the regulatory limit, determined by WA Department of Health.

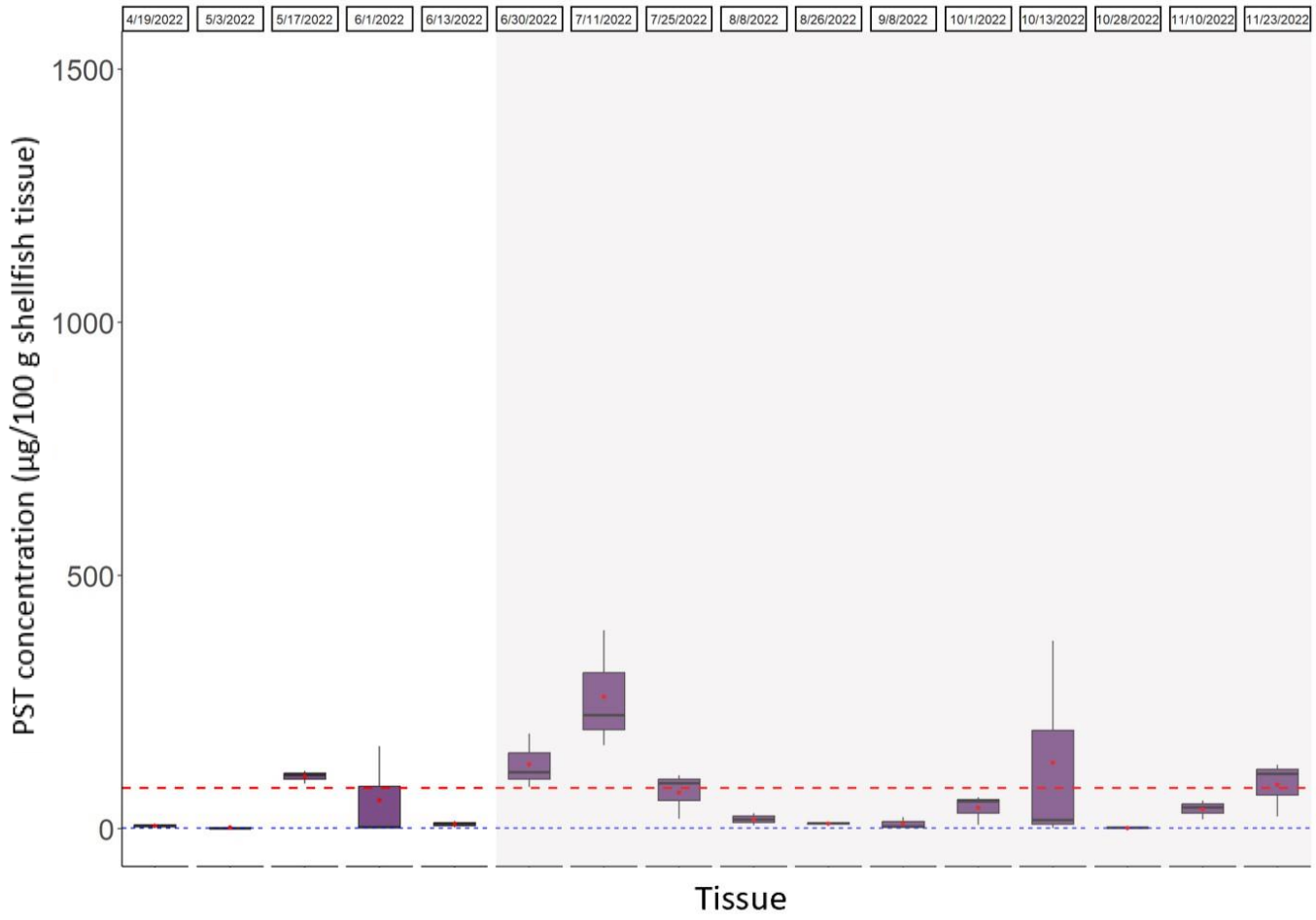

Figure A4. Boxplot showing mean (red dot), median, minimum, maximum, and quartiles of PST concentrations (µg/100 g shellfish tissue) of digestive tract throughout study period. Red dashed line marks the regulatory limit for PST (80 µg/100 g shellfish tissue). Blue dashed line marks detection limit (0.30 µg/100 g shellfish tissue). Gray shading indicates the time period of harvest closure for all species in Bellingham Bay due to PST concentrations above the regulatory limit, determined by WA Department of Health.

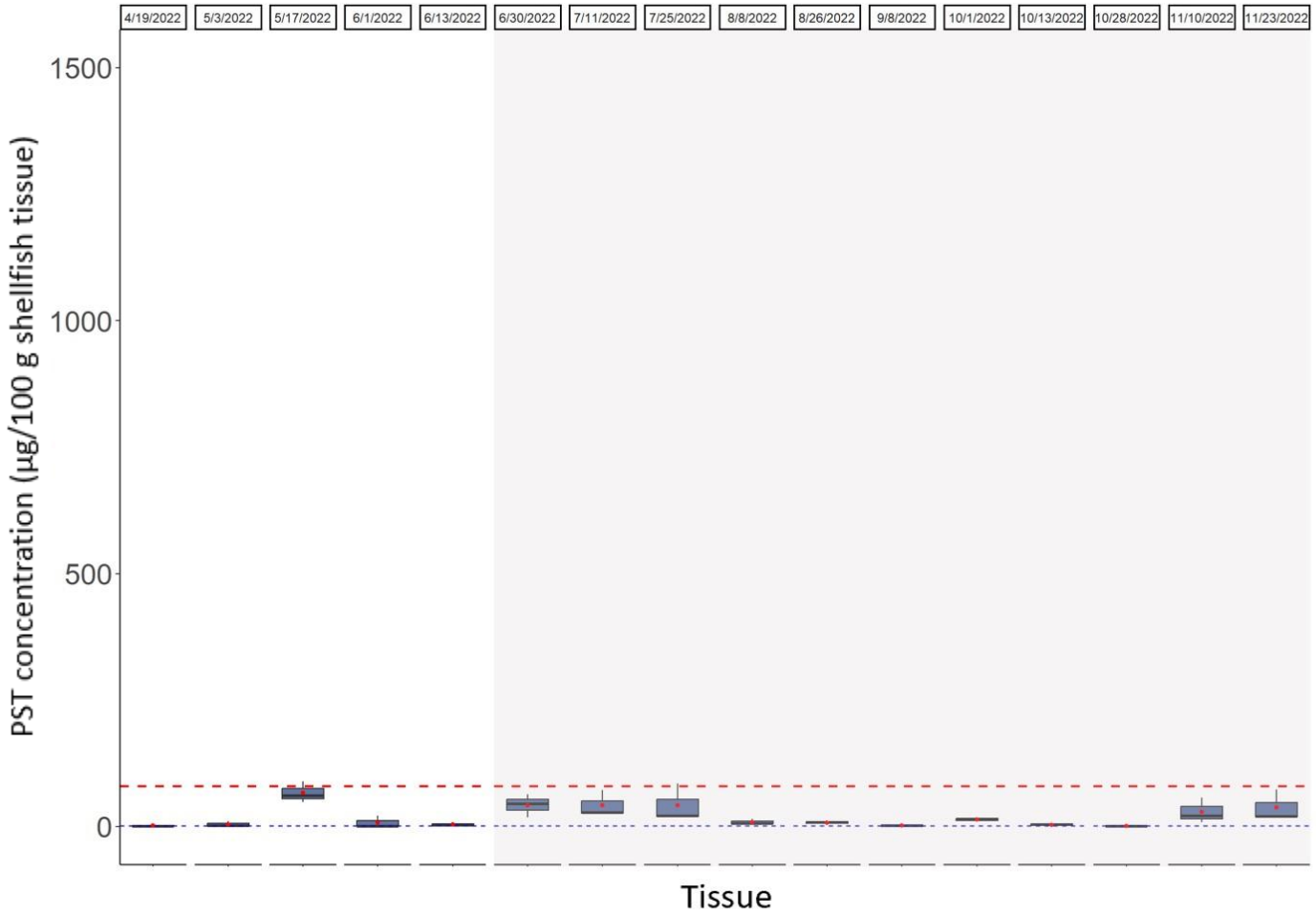

Figure A5. Boxplot showing mean (red dot), median, minimum, maximum, and quartiles of PST concentrations (µg/100 g shellfish tissue) of gills throughout study period. Red dashed line marks the regulatory limit for PST (80 µg/100 g shellfish tissue). Blue dashed line marks detection limit (0.30 µg/100 g shellfish tissue). Gray shading indicates the time period of harvest closure for all species in Bellingham Bay due to PST concentrations above the regulatory limit, determined by WA Department of Health.