North Pacific Research Board
Core Research Program
Final Progress Report

Project number: 1616

Project title: Implementation of Community Based PSP Testing for Subsistence and Recreational Shellfish Harvesting in Southwestern Alaska.

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Reporting period: Final Report June 30, 2020

Submission date: June 30, 2020

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Abstract

Project objectives were (1) develop a paralytic shellfish poisoning (PSP) field test method, (2) determine PSP toxins in butter clams from Kodiak and the Aleutian Islands' sites, (3) examine climate change and PSP risk and (4) validate butter clam cleaning methods to reduce risk of PSP. An improved ELISA test using a chemical reduction step was developed but is not practical for a field test at this time. The laboratory-based assay has been commercialized and is currently being used in three major ongoing studies to document the flux of saxitoxins into Alaskan coastal food webs. Community-based monitoring of butter clams revealed PSP toxin levels were above regulatory limits most of the time at the study sites. Results were provided to each community to better inform harvesting decisions. Locally collected shellfish should be held until they can be tested to ensure they are safe to eat. Comparison of sea surface temperature anomalies and shellfish toxicity demonstrated that increasing water temperatures associated with changing climate will substantially increase the risk of PSP events in coming years. This increased risk will adversely impact human and animal health in the coming decades and have significant implications for how resource managers and public health officials document and communicate this risk to the public. The experiment on how various cleaning methods affect toxicity unambiguously demonstrated no cleaning methods reliably reduced the PSP risk of butter clams. This information is being communicated to communities throughout Alaska.

Key Words

Alaska, , paralytic shellfish poisoning, PSP, butter clams, *Saxidomus gigantea*, shellfish cleaning methods, climate change, HPLC, ELISA, saxitoxin

Citation

Litaker R.W, Matweyou J., Kibler S, Tester, P. 2020. Implementation of Community Based PSP Testing for Subsistence and Recreational Shellfish Harvesting in Southwestern Alaska. NPRB Project 1616 Final report. 20 pp.

Chronology

Related Projects:

NPRB Project 1118 – Improved Detection Kit for the Toxins Which Cause Paralytic Shellfish Poisoning (PIs: Litaker, R.W. and Stewart, T.N.) – completed in July 2014

This project developed new and effective anti-saxitoxin antibodies to detect a larger array of saxitoxin variants (congeners) than previous assays. The newly produced antibodies were used to create an assay that correlated well with HPLC, the regulatory standard analysis. This assay was converted to a rapid detection format (spot test) featuring a visual color change and an improved shellfish extraction kit was developed. The assay became commercially available through Mercury Science, Inc. and efforts were underway to convert the assay to a digital readout format for determining saxitoxin concentrations in shellfish. The prototype potentiostat meter and disposable sensor cartridges was the basis for the current project. The improved detection technology from project 1118 focused on the development of monoclonal antibodies for saxitoxin and neosaxitoxin, testing for cross reactivity, determining the dilution

series necessary to report STX levels of regulatory interest, comparisons with the Abraxis ELISA and HPLC. Project#1118 did not have a field testing or end user component.

NPRB # 1215. (Himelbloom, RaLonde, Matweyou) Evaluation of the Abraxis Saxitoxin Enzyme-Linked Immunosorbent Assay (ELISA) for Testing Subsistence Alaska Shellfish.

The project involved testing of an ELISA for Paralytic Shellfish Toxins developed by Abraxis, a biotech company specializing in rapid tests for algal toxins and other environmental contaminants. The Abraxis commercially produced ELISA was evaluated using three commonly harvested Alaskan bivalve species in direct comparison with HPLC results. An important outcome of the project was capacity building for ELISA testing at the Kodiak Seafood and Marine Science Center, with the long-range goal to develop a center for marine toxin testing for research and monitoring. Community-based testing was not an objective of the project.

NPRB #0821. (RaLonde, Wright) Using blue mussels as an indicator species for testing domoic acid toxicity in subsistence bivalve harvest.

The project involved development of a shellfish testing program for domoic acid (DA, the cause of amnesic shellfish poisoning) at geographically disbursed sites, from southeast Alaska and along the Alaska coastline to Dutch Harbor. Using trained citizen monitors, weekly sampling of mussels from ten sites occurred from July 1 through September 30, 2009 and samples were tested using a spot test for domoic acid manufactured by Mercury Science, Inc. This early technology used a single antibody, colorimetric test for domoic acid. Because there are numerous forms or congeners of saxitoxin, multiple antibodies and more sophisticated technology was used for basis of the ECtest being proposed for use in this study for saxitoxin (not domoic) detection.

Alaska Department of Environmental Conservation, Division of Environmental Health Recreational Shellfish – Pilot Program. 2012-2015. http://dec.alaska.gov/eh/RecSHell/index.html
This pilot study supported community-based shellfish monitoring at four selected areas in Alaska are shipped monthly to the ADEC-EHL for analysis using HPLC. The study established monitoring in the Kodiak region and continued monitoring at one of the former APIA sites, Sand Point. EHL HPLC results were combined for Western Alaska under the present project and archived shellfish homogenates from the Recreational Shellfish program were shared to standardize analysis at the NOAA Beaufort, NC lab.

<u>Project Report Timeline:</u>
August 1, 2016 – NPRB Project 1616 began

October 21, 2016 – Reimbursable Service Agreement established between UAF and State of Alaska ADEC for shellfish homogenates

July 31, 2017 – Annual report submitted; Project progress is on schedule

Jan 26, 2018 – Six-month no-cost extension request submitted and accepted. The request to extend the project six months to June 30, 2020 was based on the following justifications: *Unforeseen delay in field test kit development because of sudden departure of study partner.*

July 17, 2018 – Annual report submitted; Project progress is delayed.

July 31, 2019 – Annual report submitted; Project progress is on schedule and Re-budget request submitted for Ocean Tester, LLC. to redirect funds to purchase laboratory equipment to establish the capacity to analyze samples for STX using the newly developed ELISA at the Kodiak Seafood and Marine Science Center.

July 31, 2019 – Requested to move funds from travel to equipment purchase. Approved August 2019.

October 30, 2019 – Re-budget request submitted for UAF by request of NPRB to rebalance grant for commodities charges.

June 1, 2020 – Re-budget request submitted by Ocean Tester, LLC to reallocate remaining travel funds that could not be used due to COVID-19 to pay for additional samples. Approved June. 2020.

April 9, 2020 – Re-budget request submitted for UAF to reallocate travel funds that could not be used due to COVID-19.

June 30, 2020 – Project ended.

Introduction and Objectives

1. Project overview and objectives

This project was conceived in direct response to community input provided by subsistence harvesters on Kodiak Island and the Alaska Peninsula (Matweyou & Bartz 2015) and addressed NPRB interests in Community Involvement. In 2012-2015 an Alaskan Department of Environmental Conservation (ADEC) pilot study employed community groups in four regions to collect shellfish samples for paralytic shellfish poisoning (PSP) screening by Alaska's Environmental Health Laboratory (EHL). The communities of Kodiak, Sand Point, South Kenai Peninsula and Haines were selected to be part of study. This was the first effort by the State of Alaska to direct state funds toward testing recreational and subsistence shellfish. Previous efforts were organized by the Aleutian Pribilof Islands Association and community-based monitoring were recognized as an effective strategy in reducing PSP risk. Beginning with this pilot study, the EHL began analyzing shellfish samples by high performance liquid chromatography (HPLC), an analytical technique that provides detail on the saxitoxin congeners. These data provide powerful insight into the seasonality of PSP in Alaska and can inform development of new toxin testing methods. This project (NPRB Project #1616) used the EHL results from Kodiak and the Aleutian Islands to design a community-based project to address the needs of subsistence shellfish harvesters in understanding the seasonality of PSP and to harvest butter clams safely. The objectives were:

- To develop a rapid PSP test kit that could be used in the field to assess saxitoxin concentrations in shellfish, to validate the assay against standard HPLC methods and to train community members to perform the assay. When the grant was submitted there was no organized method in Alaska for testing samples collected by recreational or subsistence harvesters. Our goal was to empower subsistence harvesters in these communities with a rapid, low cost means of reducing PSP risks and increasing seafood security.

- To employ community members from Kodiak, Old Harbor and Ouzinkie to conduct regular sampling of butter clams in order to characterize seasonal patterns of toxicity, to build on their initial dataset, and to evaluate the extent to which preparation methods reduced clam toxicity.

Butter clams are the primary shellfish consumed in these communities. Community members wanted to know if the prevailing wisdom/tradition among residents removing specific tissues, thought to be more toxic, substantially reduced PSP risk.

- To continue to provide HPLC saxitoxin analysis for shellfish collected by Aleutian Pribilof Islands Association (APIA) samplers in the Aleutian Islands and Alaska Peninsula, so those communities could continue the data series initiated under the ADEC pilot study (2012-2015). The goal was to develop long-term databases documenting how saxitoxin concentrations in shellfish varied in different communities over time. These data are critical for informing harvesting and testing activities.

- To conduct community outreach in order to inform community members about the research project and its results.

2. Accomplishments and significant results relating to project objectives

Shellfish testing methodology – An initial test kit for saxitoxins in shellfish was based on preliminary data showing a linear relationship between reactivity of anti-saxitoxin antibodies and saxitoxin levels in Alaskan shellfish determined by HPLC (Fig. 1A). The project began with development of a spot test kit and PSP meter (potentiostat) that would measure saxitoxin concentrations in shellfish extracts (Figs. 1B-D). In conjunction, a method for rapidly extracting STX from shellfish samples using a battery operated Dremel tool was also successfully developed.

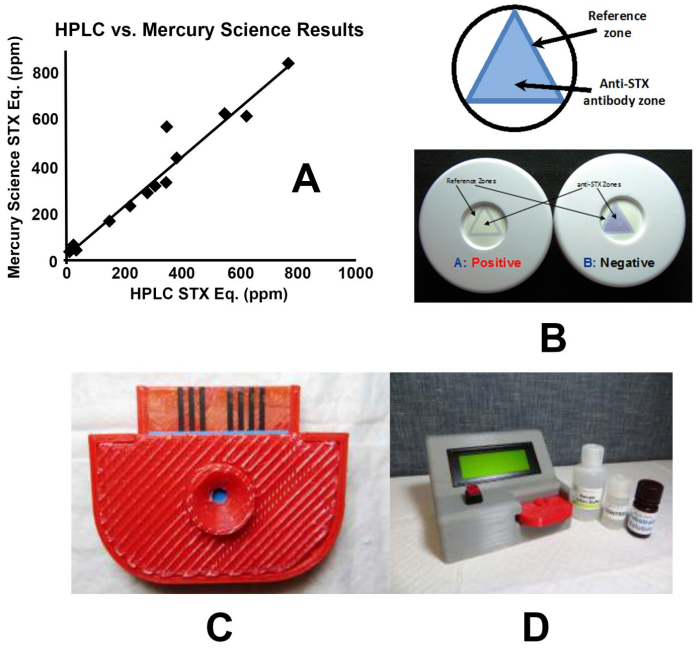


Figure 1. Mercury Science, Inc. immuno-tests for Paralytic Shellfish Toxins (PSTs) in shellfish. (A) ELISA vs HPLC comparison. (B) Spot field test kit layout with negative and positive test results. (C) Electrochemical flow-through sensor for PSTs. (D) Low cost potentiostat with digital readout used to read samples.

To ensure the accuracy of HPLC laboratory results, we obtained archived shellfish homogenates from the ADEC Recreational Shellfish Pilot Project that had been analyzed previously by the Environmental Health Laboratory (EHL). These samples were analyzed using the HPLC system at the NOAA Beaufort laboratory. There was a good agreement between the STX estimates obtained in both laboratories (Fig. 2).

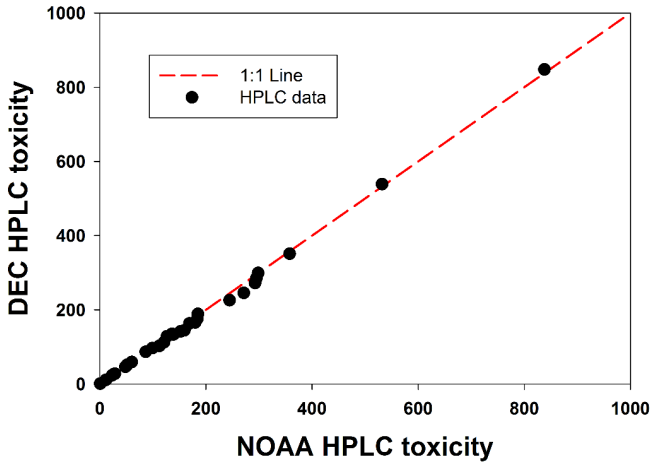


Fig. 2. Comparison of ADEC vs. NOAA HPLC results from butter clam and mussel samples collected in the Kodiak and Aleutian Islands (December 2016, n = 74; June 2017, n = 20 and July 2018, n = 16). Shown are total relative toxicity (µg STX Equivalents 100 g^{-1}) measured at the two facilities and a 1:1 reference line. The data indicate highly significant overall agreement between HPLC data from both facilities. This was demonstrated even though NOAA does not utilize the more advanced post-column oxidation method used by the ADEC Environmental Health Laboratory.

In early 2017, the chief scientist at Mercury Science abruptly pulled out of the project, sold his company (Mercury Science) and moved to Thailand. The manufacturing of the flow-through STX meter and electrodes was not sufficiently developed at this time to transfer to another company. As an alternative to developing a test kit, in early 2018 PIs, Litaker and Tester worked with Mercury Science to transfer the antibodies and the rights to use them to SeaTox Research, Inc. (Wilmington, NC). Rance Hardison and Chris Holland (NOAA staff members), in collaboration with researchers at SeaTox Research, successfully established an improved ELISA for detecting STXs in shellfish in June of 2018. Preliminary testing showed the new ELISA had high affinity to both STX and neoSTX. None of the kits on the market had a sensitivity this broad. During 2017 and 2018, analysis of shellfish samples collected from the Aleutians and Kodiak were shown to contain higher levels of gonyautoxins (GTX1, 2, 3 and 4) than found in the initial samples used to test the antibodies. The antibodies used in the assay were not highly sensitive to these GTXs and remained underestimated. Because STX, neoSTX and the GTXs cumulatively account for most of the toxicity found in shellfish samples, it was necessary to find a way to deal with this underestimation to improve assay accuracy. This was accomplished by incubating the shellfish samples with L-cysteine at 80 °C for 30 minutes. This treatment chemically converted most of the GTXs in the sample to STX and neoSTX, congeners which are accurately measured by the Seatox Research ELISA. The assay methodology was published in McCall et al. (2019) (attached) and the ELISA is now commercially available (https://www.seatoxresearch.com/testing-kits/).

The PSP ELISA developed through this project is currently the most accurate STX ELISA on the market, but includes a necessary incubation step. This makes it unsuitable for use in a rapid field test kit. Despite this, the laboratory-based test kit developed during our joint research has already achieved wide application in Alaska. The assay is currently being used as a the screening tool for assaying all the samples in the NPRB 1801 project aimed at determining saxitoxin concentrations in components of the Alaskan food web (https://coastalscience.noaa.gov/project/prevalence-of-paralytic-shellfish-toxins-in-marine-food-webs-of-prince-william-sound-and-kachemak-bay-alaska/). This approach allows samples to be screened rapidly at low cost and can detect STX levels at much lower concentrations than is possible using standard HPLC methods. As a result, it is possible to track low levels of STX entering the food chain. To date, over 825 samples have been screened using the new ELISA test kit. Extracts from any screened samples registering ≥10 µg STX-equivalents/100 g tissue are subsequently analyzed using more time consuming and expensive HPLC methods. This tiered approach allows a far greater number of samples to be analyzed with low toxicity samples identified and eliminated from further analysis.

The ELISA method is also being used by researchers at the USGS Alaska Science Center (John Piatt, Caroline Van Hemmert) to screen STX levels in bird mortality events. The assay has been included as the primary tool in assessing saxitoxin levels in phytoplankton, zooplankton and shellfish in the recently funded five-year NOAA Ecology and Oceanography of Harmful Algal Blooms project on Trophic Transfer & Effects of HAB Toxins in Alaskan Food Webs.

To broaden the potential applicability of the assay, project funds were used to equip PI Matweyou's laboratory at the Kodiak Seafood and Marine Science Center so the new assay could be used locally to better understand how environmental factors govern shellfish toxicity. STX concentrations in Kodiak shellfish tend to be above regulatory levels most of the time (see sections below), and it is intended that the new laboratory capacity will promote greater collaboration for HAB research in southwest Alaska and lead to safer shellfish consumption by local communities.

SeaTox is currently testing a PSP field test produced by Neogen, Inc. as a marketing pathway for a rapid test kit that can meet this project's goals. If successful, the new ELISA technology may be incorporated into the Neogen field testing platform for rapid testing if the rapid toxin

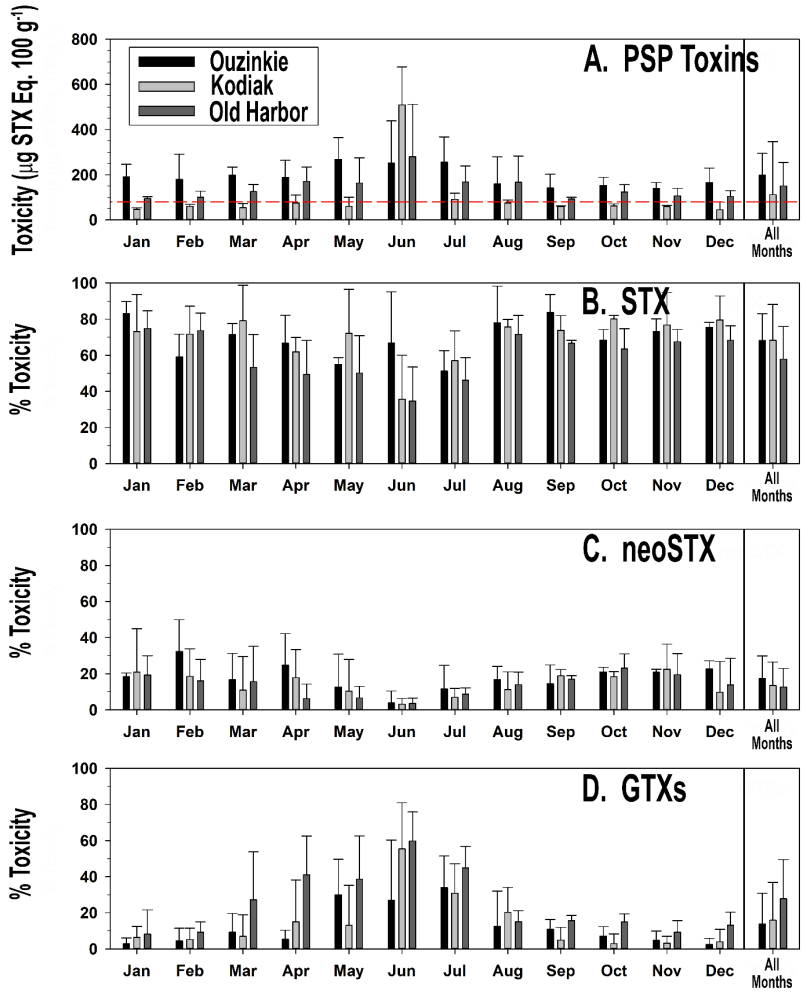


Figure. 3. Average 2013 - 2018 monthly butter clam toxicity (µg STX Eq. 100 g^{-1}) ± Std and contributions from STX, neoSTX and GTX (% toxicity) in butter clams from Sourdough Flats, Ouzinkie; Near Island, Kodiak and Shipwreck Beach, Old Harbor. (A) Monthly butter clam toxicity from all PSP toxins (µg STX Eq. 100 g^{-1}). (B) Percent toxicity due to saxitoxin (STX). (C) Percent toxicity due to neosaxitoxin (neoSTX). (D) Percent toxicity due to gonyautoxins (GTX1, 2, 3, 4, 5). The red dashed line in panel A denotes the action limit for saxitoxins in shellfish established by the U.S. Food and Drug Administration (80 µg STX Eq. 100 g $tissue^{-1}$).

conversion step can be resolved.

Assessing distribution of toxins in butter clam tissues and how preparation methods affect toxicity – This portion of the project objectively evaluated the distribution of saxitoxins in various sections of butter clams over time and the extent to which preparation methods used by Kodiak subsistence harvesters reduced PSP toxin levels. For this study, butter clam samples were collected in 2015-2018 by residents of Ouzinkie, Old Harbor and Kodiak. Butter clam monitoring data showed 2013-2018 toxicity in Ouzinkie butter clams averaged 191 ± 93 µg STX Eq. 100 g^{-1}, compared to 75 ± 59 µg STX Eq. 100 g^{-1} in Kodiak and 149 ± 108 µg STX Eq. 100 g^{-1} in Old Harbor. Saxitoxin averaged 36-95% of clam toxicity at Ouzinkie, Kodiak, and Old Harbor, while neoSTX accounted for 14-17%. GTXs varied seasonally, accounting for 27-55% of the clam toxicity during May-July, but only 3-8% of toxicity during the winter (Fig. 3). Distribution of toxins in clam tissues varied as well, where ≤65% of the toxicity was located in the gut during June-July (mainly GTXs) before toxins were re-distributed to the siphon during the winter as STX & neoSTX. These data are consistent with accumulation of GTXs in the gut when butter clams are actively feeding on toxic *Alexandrium* cells in May – July (Figs. 3, 4). By winter STX is the predominate congener in butter clam tissue and is concentrated in the siphon.

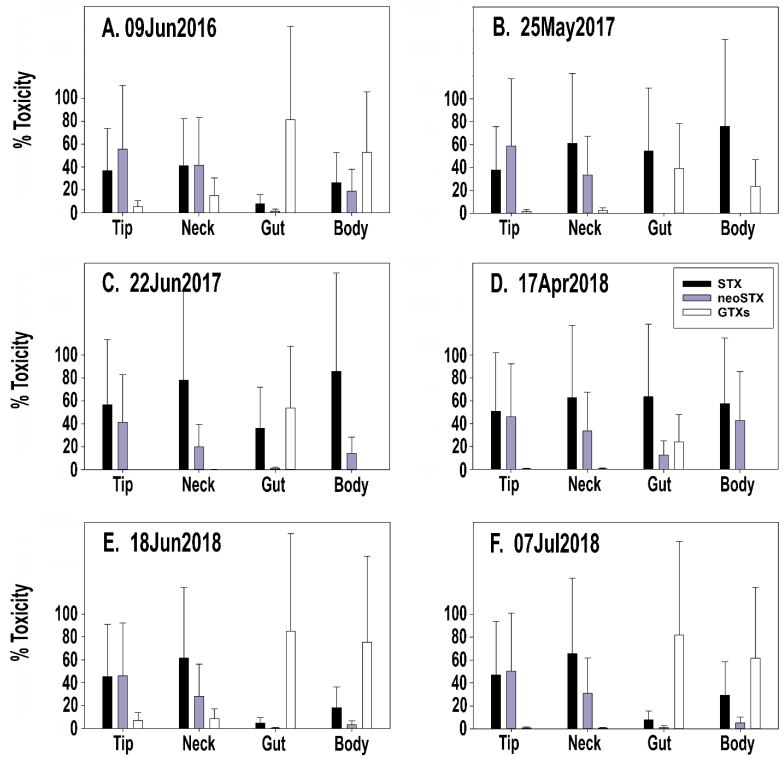


Figure. 4. Contribution of saxitoxin congeners (% by weight) within four butter clam tissue components collected at Mission Beach, Kodiak in 2015 - 2018: the black tip of the siphon (Tip), the remainder of the siphon (Neck), the gut and contents (Gut), and the remaining tissue (Body). Bars represent mean percentages of saxitoxin (STX, black bars), neosaxitoxin (neoSTX, gray bars) and total gonyautoxins (GTXs, white bars) among 3-6 groups of butter clams (n = 12 per group) collected on each date.

The percent of total toxicity contributed by the siphon tip (2-29%), the neck (3-56%), the gut (3-65%) and the body (6-85%) varied widely (Fig. 4). These calculations were based not only on the concentration of toxin found in the tissue, but also on the total weight of each individual tissues relative to the total weight of the clam. Removal of the siphon tip reduced clam toxicity substantially in some instances but had little effect on others. For instance, removal of the siphon tip from butter clams collected at Kodiak in May 2017 lowered total toxicity relative to consuming the entire clam by 18-29%, but by only 2-3% in June 2018. Because toxicity varied so greatly in butter clam tissues, even among groups of clams harvested on the same beach at the same time, the study **results show that tissue removal is not a reliable strategy for reducing PSP risk in Kodiak butter clams.**

A draft manuscript titled "Occurrence of Paralytic Shellfish Poisoning Toxins in the Tissues of Butter Clams (*Saxidomus gigantea*) from Three Communities in the Kodiak Islands, Alaska" describing this work is scheduled for submission to the journal Environmental Health Perspectives in July 2020 (confidential draft MS is attached to this report).

In another component of this project, we examine traditional preparation methods used by Kodiak subsistence harvesters and the effect on PSP risks. Three residents informed PI Matweyou and demonstrated their preferred butter clam cleaning techniques. Matweyou repeated two of the methods, and an Old Harbor resident performed the third method monthly for five months. For these comparisons, butter clam tissues were separated into "edible" and "non-edible" fractions according to the three methods. In one method for instance, edible tissues were deemed to include the body, neck, mantle and adductor muscles, while the non-edible fraction included the gills, tip of the siphon, gut contents, and other tissues with a dark or black appearance. These data are being analyzed and prepared for publication. In the meantime, the results were summarized in an informational pamphlet for distribution to harvesters (see attached document). The primary information contained in the pamphlet includes the following:

- PSP toxins increase dramatically during the summer months due to the seasonal increase of the toxic *Alexandrium* cells. In Kodiak, toxins begin to increase in April and can remain elevated through September.
- Risk of PSP exists year-round because toxins can be retained in some bivalve shellfish.
- Butter clams can accumulate very high levels of toxins and retain toxins for long periods.
- PSP toxin distribution in butter clam tissues changes seasonally, as do the forms of toxins (STX, neoSTX, GTXs).
- Removing the black siphon tip, at least half of the siphon neck and the gut content in butter clams is recommended if harvesters choose to eat untested clams; a percentage of toxin is reduced. However, the effectiveness of the toxin removal varies and is not predictable.
- **Removal of these tissues does not guarantee processed butter clams will be toxin-free. Toxin levels can remain above recommended limits in the "cleaned" meat.**
- Where extreme toxin concentrations are common, such as Kodiak, it can be assumed that the STXs will be present in all edible tissues.
- **The practice of harvest and hold is recommended.** Harvest the clams, sacrifice some of the clams for testing, and wait for test results before consuming. Only testing ensures safe consumption.

These results were consistent with the more in-depth study on the distribution of PSP toxins in different butter clam tissues. This informational document will be distributed by Sea Grant and by Bruce Wright (Knik Tribe of Alaska) to their respective distribution lists, effectively reaching over a thousand individuals throughout Alaska. The document is also being translated into Tagalog for distribution to the Filipino community in Kodiak and the Aleutians. Fishing communities in Alaska often contain a number of Filipino immigrants who regularly harvest shellfish, but who are often unaware of the risks of PSP. This document is intended to provide actionable information for this group of shellfish harvesters in Alaskan communities. The last bullet above is particularly important for Kodiak residents, since the ongoing collaboration between the Kodiak Area Native Association (KANA) and SEATOR has made shellfish testing more available for subsistence and recreational harvesters.

To better understand how selection of individual clams and mussels may affect toxicity monitoring, butter clams from Mission Beach, Kodiak and King Cove Lagoon were sampled individually. Composite toxicity was determined using the mathematical average of all individual shellfish in the sample, but the variability among individuals was also determined. Preliminary results indicated that a group of 17 clams collected at Mission Beach varied in toxicity between 71.1 and 317.8 µg STX Eq. 100 g^{-1}. If 12 individuals were randomly selected to measure average toxicity (i.e., DEC sampling protocol), the mean would have been 29.3 to 95.7 µg STX Eq. 100 g^{-1}, depending on which clams were selected for a composite sample. In comparison, if only 6 clams were selected, then average toxicity ranged from 13.4 to 153.6 µg STX Eq. 100 g^{-1}. This analysis was conducted in response to suggestions by community harvesters that fewer clams might be sampled. Overall, this extreme level of variability means that a typical 12-clam sample used to monitor toxicity, may not be an adequate measure of PSP risk. In fact, a statistical analysis showed the sample size of clams needed to calculate average toxicity with 95% confidence was 329 clams! Clearly, this level of sampling is impossible, but it does indicate 12 clams is still better for assessing toxicity than if only 6 clams were used. This information clearly indicates that current sampling methods can significantly

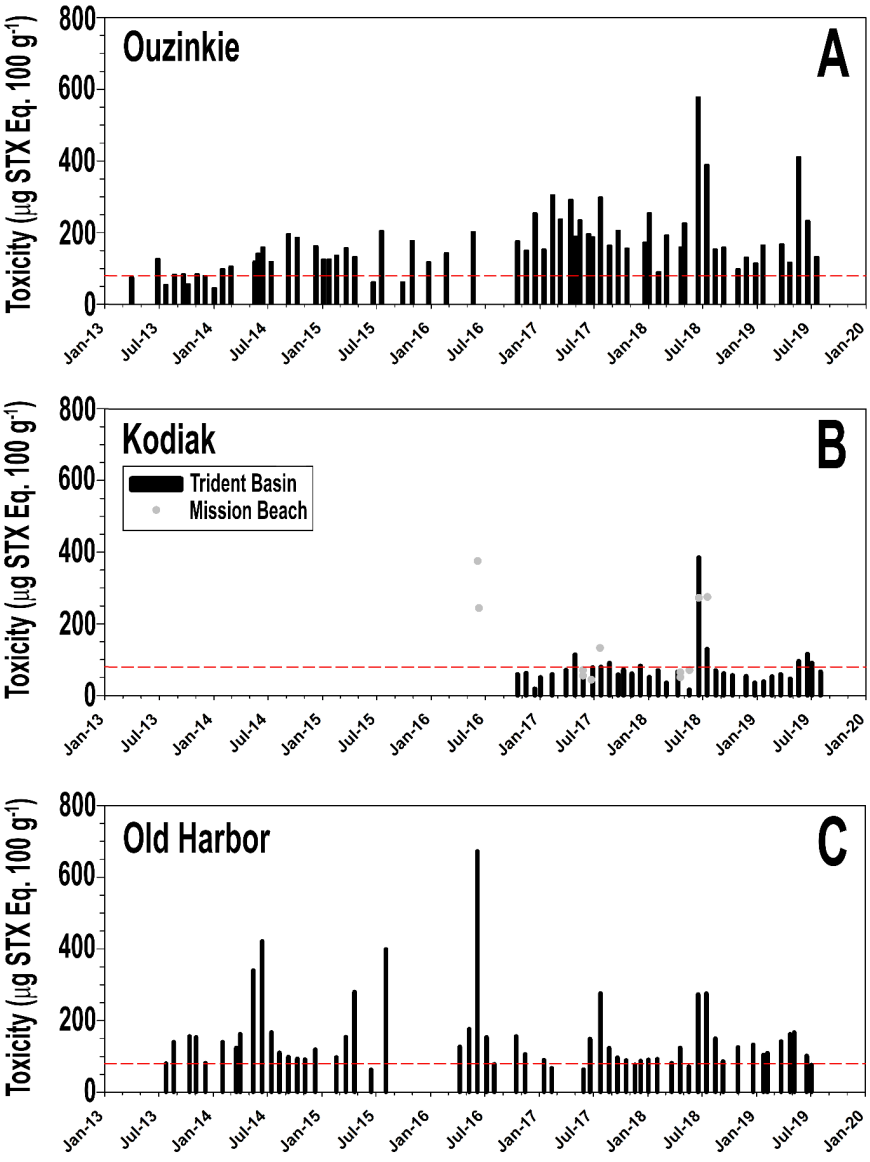


Figure. 5. Butter clam toxicity during 2013 - 2020 at A. Sourdough Flats, Ouzinkie, B. Trident Basin (bars) & Mission Beach (gray dots), Kodiak, and C. Shipwreck Beach, Old Harbor. The red dashed line denotes the action limit for saxitoxins in shellfish established by the U.S. Food and Drug Administration (80 µg STX Eq. 100 g^{-1} tissue).

underestimate the potential toxicity for harvesting even in a small area, a consideration for assessing PSP risk.

Samples for butter clam monitoring were collected monthly from October 2016 to February 2020, allowing extension of the original dataset collected for the 2013 - 2015 DEC community testing pilot project (Fig. 5). The results showed that butter clam toxicity peaks in June-July but remains high throughout the year. These data were provided back to the communities in Kodiak, Old Harbor and Ouzinkie. Community members were involved in gathering these data, and it is hoped that community involvement will reinforce knowledge and acceptance of the high levels of PSP toxins in shellfish. It will also underscore that even in the cooler months when toxicity is generally less, significant risk still exists if harvesters consume untested shellfish. A few of the final samples were slated for analysis but were not completed due to COVID-19 closing the laboratory for the past 4 months. These samples will be analyzed as soon as the Beaufort Laboratory once again begins normal operations.

Continuation of long-term community sampling in the Aleutians – The Aleutian villages of King Cove and Sand Point have worked to maintain continuous collection and analysis of PSP toxins in shellfish under the program established by the Aleutian Pribilof Islands Association (APIA) lead scientist B. Wright, and maintained by the communities. The goal has been to understand the long-term annual and inter annual variation in toxicity and how it affects the safety of consuming locally collected shellfish. Funding for this project lapsed at about the time the current project started, and we agreed to support continuation of this program as part of NPRB project 1616. The data obtained were directly relevant to informing the goals of the current project. Recently, new funding has been received to carry out sampling in these two communities for the next couple of years so the time series will be continued. The resulting time series

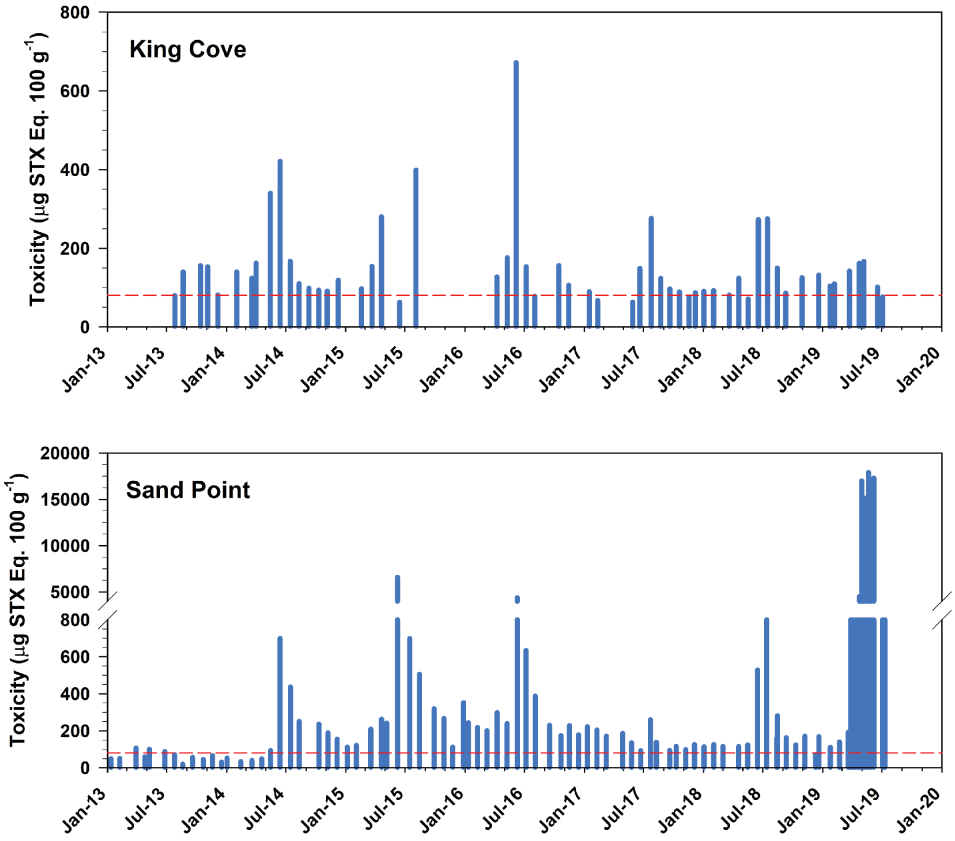


Fig. 6. Time series of PSP toxicity in butter clams and blue mussels collected by local communities of King Cove and Sand Point in the Aleutians. The dashed red line indicates 80 µg per 100 g clam tissue, the regulatory limit for STX in safe shellfish.

are shown in Figure 6. The data indicate levels of toxicity are typically above safety limits with highest toxin concentrations in June through August. These data have been provided to each of the communities to inform their harvesting practices and to emphasize the potential risk of PSP throughout the year.

Relationship of toxicity with water temperature. In a manuscript published in 2018, Vandersea et al. showed that even slight increases in temperature above 12 °C in Alaskan coastal waters will increase the chances that intense bloom of the microalgae *Alexandrium* will form. These microalgae naturally produce STXs. As shellfish filter feed, they consume the *Alexandrium* cells and bioaccumulate STXs. Consequently, temperature-driven increases in *Alexandrium* blooms result in more severe PSP events. We expanded upon that work in this project by examining the relationship between toxicity observed at the Kodiak and Aleutian sampling sites versus water temperature anomalies (Figs. 7 and 8).

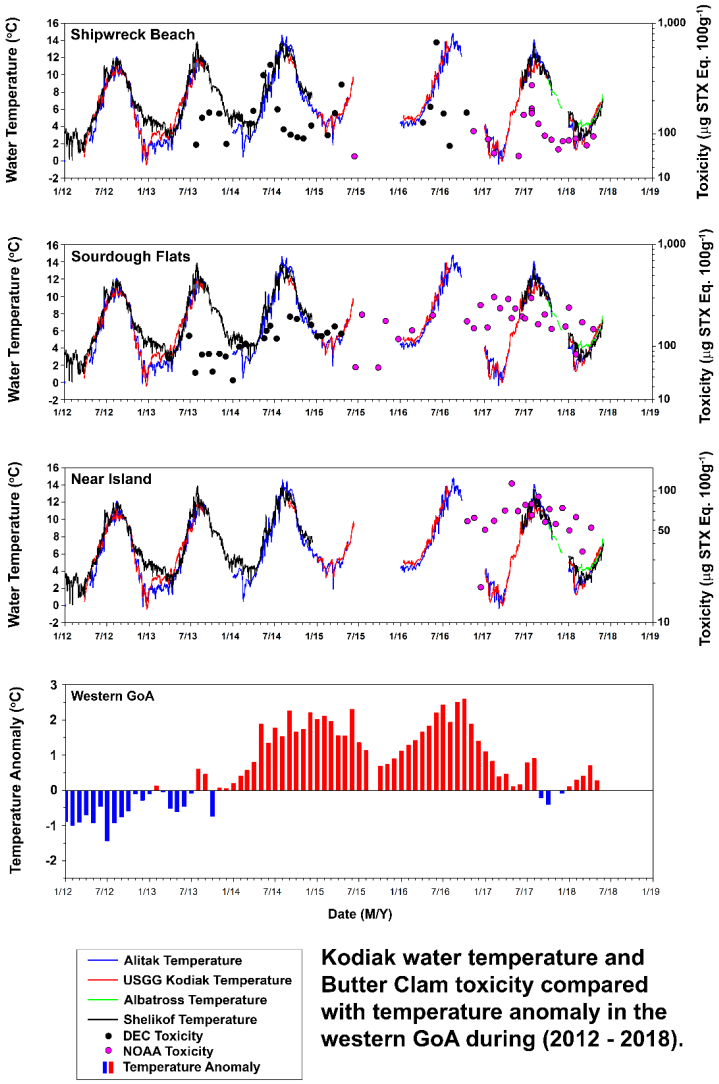
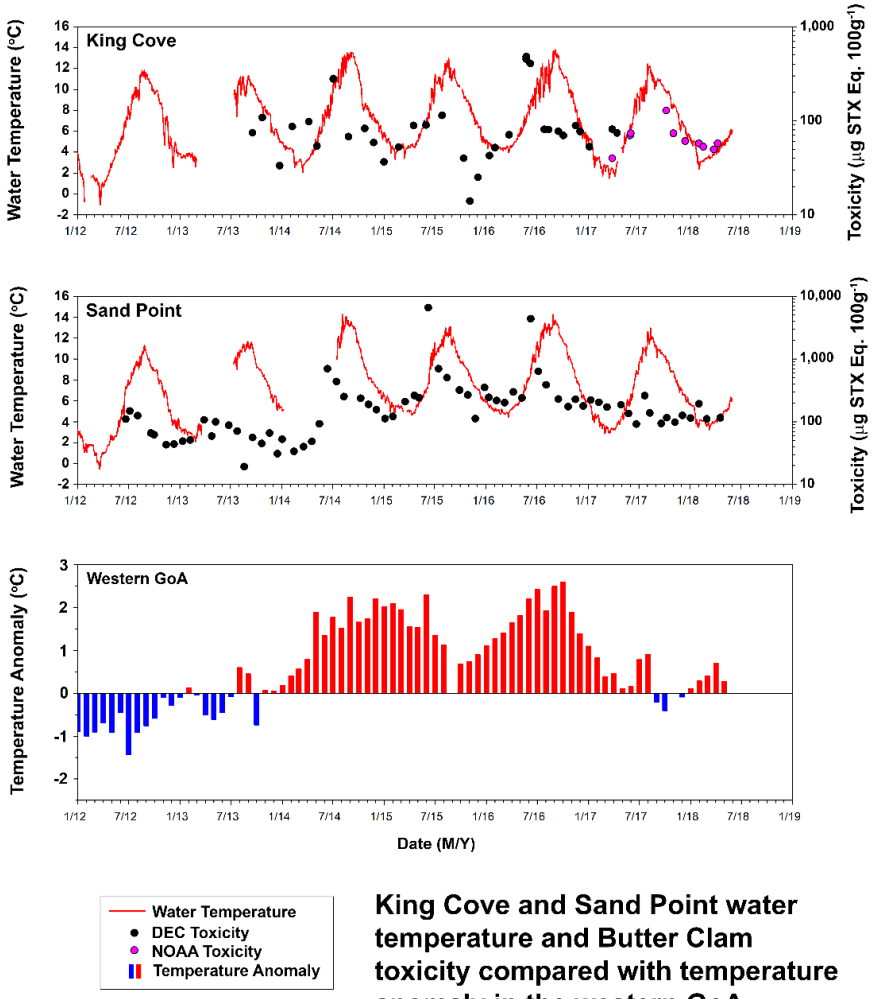


Fig. 7. Butter clam toxicity at Kodiak sites: Relationship between water temperature and butter clam toxicity at Shipwreck Beach (Old Harbor), Sourdough Flats (Ouzinkie) and Near Island (City of Kodiak) during 2012-2018. Water temperature data from the National Data Buoy Center (www.ndbc.noaa.gov) are shown from Alitak (western Kodiak), USCG station Kodiak (Womens Bay), Albatross Bank (SE of Kodiak Island) and the Shelikof Strait (data from the National Data Buoy Center). Butter clam toxicity in µg STX Equivalents per 100 g shellfish as measured by Alaska DEC Environmental Health Lab (●) and the NOAA Beaufort Lab (●). The regional water temperature anomaly data from the western Gulf of Alaska (GoA) are shown in the lower panel.



King Cove and Sand Point water temperature and Butter Clam toxicity compared with temperature anomaly in the western GoA during (2012 - 2018).

Fig. 8. Butter clam toxicity at Aleutian Islands' sites: Relationship between water temperature and butter clam toxicity at King Cove and Sand Point during 2012-2018. Water temperature data from the National Data Buoy Center (www.ndbc.noaa.gov) are shown for each location. Butter clam toxicity in µg STX Equivalents per 100 g shellfish as measured by Alaska DEC Environmental Health Lab (●) and the NOAA Beaufort Lab (●). For comparison, the regional water temperature anomaly data from the western GoA are shown in the lower panel.

The results were consistent with warmer years having a higher risk of significant PSP events. **The data also indicate the rapid increase in water temperatures, particularly in the northern Gulf of Alaska and the Arctic, will greatly increase the chances of more intense *Alexandrium* blooms and associated PSP toxicity in the near term. This has significant implications for adverse impacts on wildlife populations as well as human health.**

Documenting STX entering the Alaskan food web

Though not included in the original proposal, the data gathered in this study are being collated with other existing data sets to document the amounts of STX found in organisms throughout Alaska. The data sets are still being assembled but will be included in a manuscript scheduled for completion in late August or

early September 2020. Figure 9 shows a preliminary example of the STX data accumulated to date. The goal is to provide an up to date inventory of how extensively STXs permeate the Alaskan coastal food web.

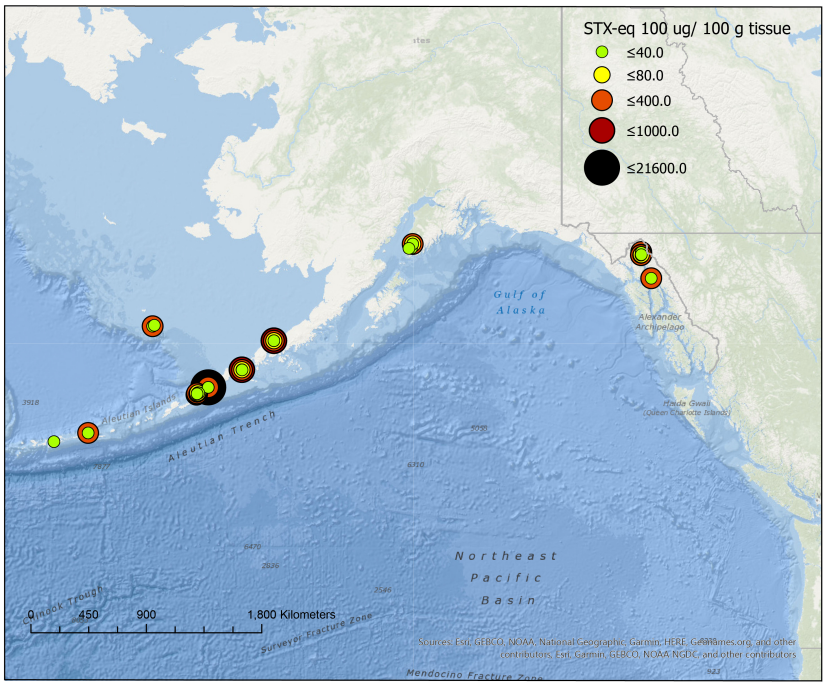


Figure. 9. Preliminary graphic showing distribution of STX in blue mussels found at various locations in the Gulf of Alaska and Bering Sea.

Chapters

1) McCall JR, Holland WC, Keeler DM, Hardision DR, Litaker, RW. 2019. Improved Accuracy of Saxitoxin Measurement Using an Optimized Enzyme-Linked Immunosorbent Assay. Toxins 11(11):632. DOI: 10.3390/toxins11110632
2) Kibler SR, Matweyou JA, Hardison DR , R Litaker RW , Wright BA, Tester PA. 2020. Occurrence of Paralytic Shellfish Poisoning Toxins in the Tissues of Butter Clams (*Saxidomus gigantea*) from Three Communities in the Kodiak Islands, Alaska. Confidential draft manuscript was uploaded to the workspace and will be submitted to the journal Harmful Algae.

Conclusions

The key findings and accomplishments for this project include the following:

- Development of accurate laboratory-based test kit for Saxitoxin that is being used by three major ongoing studies to assess toxin concentrations in Alaskan marine food webs.
- Showed unambiguously that various butter clam cleaning methods **do not reliably reduce the risk of PSP**. This information is being communicated to communities throughout Alaska.
- Extended the long-term record of shellfish toxicity in five different communities so shellfish harvesters will better understand the risk posed by PSP and how that risk can vary over time.
- Demonstrated that increasing water temperatures associated with changing climate will substantially increase the risk of PSP events in coming years.
- Carried out extensive outreach activities including distribution of the cleaning study and time series findings to thousands of individuals and multiple communities throughout the state.

Management or Policy Implications

There are three major management or policy implications for this study.

1) The concentrations of paralytic shellfish toxins in shellfish collected by samplers located in three communities on Kodiak Island and two in the Aleutian Islands were analyzed. The results showed that saxitoxin levels in recreationally or subsistence harvested shellfish were often above recommended safety levels. This information was conveyed to the communities so they could see the results for their locally harvested shellfish and better understand the recommendation that the only shellfish safe to eat are those which have been tested. Another issue addressed was the general belief that cleaning shellfish in a particular manner significantly lowers the risk of paralytic shellfish poisoning (PSP). Different cleaning methods were tested. The data conclusively showed no cleaning method reliably reduced the risk of PSP. This information was provided to local communities throughout Alaska to inform people of the potential danger from assuming shellfish cleaned a certain way are safe to eat.

2) An analysis showed an extreme level of variability with a typical 12-clam sample used to monitor toxicity and may not be an adequate measure of PSP risk. In fact, a statistical analysis showed the sample number of clams needed to calculate average toxicity with 95% confidence was 329! This information clearly indicates that current sampling methods can significantly underestimate the potential toxicity for harvesting even in a small area, a consideration for assessing PSP risk. This information may help inform monitoring efforts in Alaska.

3) The changing climate will increase the risk of PSP in Alaska coastal waters in coming decades with adverse impacts on human and animal health. Given this increasing threat, it is critical for managers and public health officials to increase testing capacity to understand how toxins are entering and vectoring through the food chain and to communicate to local communities where risk is highest.

Outreach activities

Project team members Matweyou, Tester, Kibler and Wright, met in Kodiak in August 2016 at the onset of the project and planned as series of outreach projects to communicate the goals and finding of the project. These outreach activities included the following activities that have advanced information about the risk of PSP toxins in shellfish in local communities.

August 2016
- Project presentation at the Old Harbor City Council meeting [Weather cancelation].
- Community of Old Harbor Project Kickoff Workshop [Weather cancelation].
- Project presentation at the Ouzinkie City Council meeting [Weather cancelation].
- Community of Ouzinkie Project Kickoff Workshop [Weather cancelation].
- Project presentation and discussion with the Sun'aq Tribe of Kodiak. PIs presenting.
- Community of Kodiak Project Kickoff Workshop, UAF KSMSC. PIs presenting.
- PI Matweyou interviewed by Kodiak public radio station KMXT.
  - Media – local: http://kmxt.org/2016/08/researchers-developing-cheaper-faster-monitoring-method-psp/
  - Media – state: http://www.adn.com/alaska-news/health/2016/08/28/researchers-developing-a-cheaper-at-home-test-for-shellfish-poisoning/

September 2016
- Project presentation at the Old Harbor Leadership meeting. PI Matweyou presenting.

November 2016

PSP and project overview. UAF FISH F261-Introduction to Fisheries. PI Matweyou presenting.

December 2016

Project overview at the Alaska HAB workshop held in Anchorage. PI Matweyou presenting.

January 2017

Project overview [poster] presented at the 2017 AMSS in Anchorage. Matweyou presenting.

February 2017

PI Kibler and Matweyou presented overview of NPRB 1616 at the Alaska HAB Network Meeting.

PI Matweyou delivered lesson on PSP to 100+ 8th grade students at Kodiak Middle School.

Project presentation at Ouzinkie City Council meeting [Weather cancelation].

Project presentation delivered to the City of Ouzinkie via telephone, with abbreviated presentation content sent via email. PI Matweyou presenting.

PI Matweyou co-led multi-day PSP lab through the UAA Kodiak College Microbiology class.

PI Matweyou delivered lesson on PSP to Kodiak High School students.

April 2017

PI Matweyou gave PSP and project overview presentation at Kodiak Area Marine Science Symposium.

PI Matweyou interviewed by Kodiak Daily Mirror newspaper.

Media – local: http://www.kodiakdailymirror.com/news/article_2dc863a6-312c-11e7-9a0d-d326054a523d.html?mode=print; abbreviated coverage picked up by state news.

Media – National Magazine: http://www.foodandwine.com/news/alaska-steps-create-better-testing-methods-shellfish-toxins; Note Media correction composed by ASG and ADEC to respond to inaccurate press in Food & Wine online publication.

Media – local: http://kmxt.org/2017/04/old-harbor-student-contributes-psp-studies-island/

May-Aug 2017

Under the direction of PI Matweyou, Sun'aq Tribe of Kodiak Natural Resource intern Mandi Cox, assisted with development and delivery of PSP educational activities; sample collection, preparation and inventory; furthering contact personnel list for the rural villages.

May 2017

PI Meeting to discuss project milestones, products and upcoming plans.

PI Matweyou delivered lesson on PSP to approximately 46 middle and high school students in 7 rural schools via AKTeach (distance delivery through video technology).

PI Matweyou delivered PSP lesson to Kodiak Middle School students during their field trip to KSMSC.

Media – Kodiak Daily Mirror "Toxic shellfish monitoring gets boost from local groups" http://www.kodiakdailymirror.com/news/article_2dc863a6-312c-11e7-9a0d-d326054a523d.html?mode=print PI Matweyou.

June 2017

PI Matweyou provided instruction and oversight to ALMA youth intern. Duties included assistance on NPRB1616 sample inventory and archive.

PI Matweyou delivered PSP presentation to the Filipino-American Association.

July 2017

PI Matweyou delivered educational activities on butter clam dissection and HAB monitoring to Old Harbor youth during Nuniaq Culture Camp. The site visit also focused on bivalve harvest and handling practices by local residents.

August 2017

PI Kibler meeting with Mark Monaco, director of NOAA/NCCOS Marine Spatial Ecology Division. Presented project overview and spatial data products available for incorporation into project.

PI meeting (Matweyou, Kibler) Decision to include samples from oyster farm in Larsen Bay, Kodiak for comparison with Butter clam and mussel toxicities.

October 2017

PI meeting (Matweyou, Kibler, Wright, Hardison) to discuss project milestones, results, planning over the next several months.

PI Matweyou gave oral presentation by invitation at the Alutiiq Museum entitled "The Deadliest Dig – Kodiak Shellfish and PSP".

November 2017

Alaska HAB Network meeting to review HAB sampling methods. PI Kibler presented NPRB 1616 methodology for comparison with projects in southeast Alaska.

January 2018

AMSS Poster presentation "Implementation of community-based PSP testing for subsistence and recreational shellfish harvesting In Southwestern Alaska – year 1 update." Matweyou presenting.

February 2018

Oral presentation featuring Alaska PSP and Community shellfish testing, subsistence harvesting and PSP mitigation in Kodiak given to NOAA Beaufort Laboratory staff.

PI Matweyou gave oral presentation at the Pacific Fisheries Technologist Conference entitled "An Electrochemical Field Test for Detection of Saxitoxin".

Media - Alaska Sea Grant news article by Paula Dobyn "Alaskan's Try to Combat Death by Marine Toxins" http://news.uaf.edu/alaskans-try-to-combat-death-by-marine-toxins/ PI Matweyou

March 2018

PI Kibler gave oral presentation entitled "HAB Forecasting and PSP Risk in Alaska" featuring Community Shellfish testing project given to NOAA NCCOS Stressor Detection and Impacts Division.

April 2018

PI Matweyou co-led multi-day PSP lab through the UAA Kodiak College Microbiology class.

PI Matweyou delivered educational activity on clam dissection during the Old Harbor Earth Day event.

PI Matweyou provided training to new field technician in Old Harbor and initiated of tissue study based on Old Harbor clam cleaning techniques.

May 2018

Seminar to Univ. North Carolina college students on Alaska HABs and the community monitoring project entitled "Alexandrium Blooms and Paralytic Shellfish Poisoning." PI Kibler presenting.

June 2018

PIs Litaker and Kibler meeting with NOAA NCCOS social scientists about communication, outreach, and interaction with Alaskan tribal groups in Kodiak and the Aleutians.

PIs Litaker and Kibler meeting with NCCOS communication team about messaging results from NPRB 1616 to public, stakeholders, NOAA, and other audiences.

Aug 2018

PIs Matweyou and Kibler delivered youth educational activity in Old Harbor (phytoplankton and clam lesson).

October 2018

PSP and project overview. UAF FISH F261-Introduction to Fisheries. PI Matweyou presenting.

December 2018

PI Matweyou presented Harmful Algal Blooms overview at the KANA Water Quality Workshop to rural environmental samplers.

PI Matweyou presented Harmful Algal Blooms overview at the Environmental Forum at KSMSC to public audience.

PI Matweyou presented "Paralytic Shellfish Toxins on Kodiak Island" with two-way dialogue at the KANA Water Quality Workshop to rural environmental samplers.

January 2019

PI Matweyou presented "Paralytic Shellfish Toxins in Butter Clam Tissues and Effect of Cleaning Methods Used by Kodiak Harvesters" at AMSS oral session.

AMSS Poster presentation "Implementation of community-based PSP testing for subsistence and recreational shellfish harvesting In Southwestern Alaska – year 2 update." Matweyou presenting.

February 2019

PI Matweyou presented "Paralytic Shellfish Poisoning on Kodiak Island" at the Alaska Forum on the Environment.

PI Matweyou presented "Distribution of Saxitoxin in Butter Clams and Cleaning Methods (used by Kodiak Harvesters)" at the Alaska Forum on the Environment.

March 2019

Media - Alaska Sea Grant news article by Paula Dobyn – interview with PI Matweyou https://alaskaseagrant.org/2019/03/06/kodiak-agent-works-with-tribes-to-keep-residents-safe-from-psp/

April 2019

PI Matweyou and Kodiak partners deliver overview of PSP collaboration for the Alaska Sea Gran site review.
PI Matweyou co-led multi-day PSP lab through the UAA Kodiak College Microbiology class.
Media – Kodiak Daily Mirror article "New Grant Expands Toxic Shellfish Monitoring" – interview with PI Matweyou http://www.kodiakdailymirror.com/news/article_0f2953d6-57bf-11e9-95d9-7fad9b934f39.html

July 2019
PI Matweyou participated in KMXT Talk of the Rock – talk show focused on HABs. PI Matweyou co-presented.
PI Matweyou co-delivered HAB and clam dissection educational activity with KANA environmental technician at the Old Harbor Nunia Camp.
PI Matweyou delivered "Effects of Paralytic Shellfish Toxins on Shellfish Resources" at the Bering Strait Algal Toxin Workshop in Nome.

September 2019
PI Matweyou delivered Paralytic Shellfish Poisoning (PSP) and its Impacts on Establishing Viable Shellfish Farms at the KALI mariculture workshop in Kodiak.

October 2019
PI Matweyou co-presented a PSP presentation to the Kodiak community at the Aluttiq Museum. The event was organized by KANA and included Kodiak partners working together to address PSP.
PSP and project overview. UAF FISH F261-Introduction to Fisheries. PI Matweyou presenting.
PSP and project overview. CFOS Fisheries and Ocean Sciences Seminar. PI Matweyou presenting.

December 2019
PI Matweyou presented "Addressing Paralytic Shellfish Toxins in the Kodiak Region" at the year 2 KANA Water Quality Workshop to rural environmental samplers.

January 2020
AMSS Poster presentation "Community-based PSP testing for subsistence and recreational shellfish harvesting In Southwestern Alaska (NPRB #1616)". Kibler presenting.

February 2020
Media - Grist article by Zoya Teirstein. Alaskan Roulette:As warming waters make shellfish toxic, a way of life becomes deadly for Native Alaskans. Interview with PI Matweyou https://grist.org/food/climate-change-is-turning-shellfish-toxic-and-threatening-alaska-natives/

April 2020
Planned oral and poster presentations at Kodiak Area Marine Science Symposium– canceled due to COVID19.
Planned butter clam cleaning workshop at Kodiak Area Marine Science Symposium– canceled due to COVID19.
Planned PSP lesson at the Kodiak College - canceled due to COVID19.
PI Matweyou provided telephone update and Butter clam PPT with project sampler in Ouzinkie.

June 2020
PI Matweyou participated in KMXT Talk of the Rock – talk show focused on HABs. PI Matweyou co-presented.
Alaska Sea Grant outreach publication ASG MAB-78 "Paralytic Shellfish Toxins in Butter Clam Tissues" produced, printed and shared digitally. PI Matweyou as lead author.
Project community poster entitled Community-based PSP Testing for Shellfish - Kodiak Region Summary prepared and printed for distribution to participating organizations. PI Matweyou lead author.
Project summary report prepared for distribution to participating organizations. PI Matweyou lead.
Project summary of results will be shared with participating organizations via video (in progress) and at next available venue (outreach to the Kodiak community on PSP is ongoing).

Literature cited

Matweyou J., Bartz, K. 2015. Recreational shellfish project final report. Anchorage, AK: Recreational Shellfish Beach Monitoring Pilot Program, Alaska Dept. of Environmental Conservation.

McCall, J.R., W.C. Holland, Keeler, D.M., D.R. Hardision, R.W. Litaker. 2019. Improved Accuracy of Saxitoxin Measurement Using an Optimized Enzyme-Linked Immunosorbent Assay. Toxins 11(11):632. DOI: 10.3390/toxins11110632

Vandersea, M., Tester, P., Holderied, K., Hondolero, D., Kibler, S., Powell, K., Baird, S., Doroff, A., Dugan, D., Litaker, W. 2018. Environmental factors influencing the distribution and abundance of *Alexandrium catenella* in Kachemak bay and lower Cook Inlet, Alaska. Harmful Algae 77:81-92.

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We thank the North Pacific Research Board for funding this study and the NOAA program funds for partial support of this project. We thank our community partners including the Alutiiq Tribe of Old Harbor, the City of Ouzinkie, the Sun'aq Tribe of Kodiak, the Agdaagux Tribe of King Cove, and the Qagan Tayagungin Tribe of Sand Point for the sustained sampling effort. We recognize the contributions by the Alaska Department of Environmental Conservation Environmental Health Lab for sharing tissue homogenates and the SeaTox Research group for advancing improved testing capacity.

Synopsis

In the absence of a state-run toxin monitoring program, Alaskan recreational and subsistence shellfish harvesters are looking for tools to guide safe harvest. Paralytic Shellfish Poisoning remains a very real problem for our coastal residents and this study addressed community concerns in SW Alaska. Project objectives were (1) develop a paralytic shellfish poisoning (PSP) field test method, (2) determine PSP toxins in butter clams from Kodiak and the Aleutian Islands sites, (3) examine climate change and PSP risk and (4) validate butter clam cleaning methods to reduce risk of PSP. We succeed in developing a laboratory-based test that uses improved antibodies, combined with a reduction step, to detect the most toxic saxitoxin congeners commonly found in Alaska shellfish (saxitoxin, neosaxitoxin and gonyautoxins). However, some of the components of the test are not applicable to a field test and work is still ongoing to develop as easy-to-use test for harvesters. The improved ELISA was commercialized by SeaTox, Inc. and is being used in three major ongoing studies to document the flux of saxitoxins into Alaskan coastal food webs. Community-based monitoring of butter clams revealed PSP toxin levels were above regulatory limits most of the time at the study sites. These locally determined results were provided to each community to allow local residents to gain a better understanding of the risk involved in consuming untested, locally collected shellfish. Accompanying outreach materials were developed and distributed emphasizing shellfish should be held until they are tested to ensure safety. A companion study was conducted to evaluate what sample size would be needed to accurately measure PSP risk in butter clams given the known variation in toxin accumulation between shellfish collected in close proximity to one another. Calculating average toxicity with 95% confidence, it was shown that 329 clams would be required. This indicates the typical 12-clam sample used to monitor toxicity may not be an adequate measure of PSP risk. Comparison of sea surface temperature anomalies and shellfish toxicity demonstrated that increasing water temperatures associated with changing climate will substantially increase the risk of PSP events in Alaska in the coming years. This increased risk will adversely impact human and animal health in the coming decades and have significant implications for how resource managers and public health officials document and communicate this risk to the public. The experiment on how various cleaning methods affect toxicity unambiguously demonstrated **NO** cleaning methods reliably reduced the PSP risk of butter clams. This information is being communicated to communities throughout Alaska.

Data and Metadata

The metadata have been uploaded to NPRB project 1616 workspace and are available as required.