

## North Pacific Research Board Core Research Program Semi-Annual Progress Report

Project number: 1801

Project title: Prevalence of Paralytic Shellfish Toxins in the Marine Food Webs of Prince William Sound and Kachemak Bay, Alaska

Principal Investigator(s): Xiuning Du, Rob Campbell and Steve Kibler

Reporting period: July 1, 2018 - Dec 31, 2019

Submission date: January 31, 2020

Please check all boxes that apply to the current reporting period. All forms and additional directions can be found at <u>http://www.nprb.org/core-program/annual-project-requirements/</u>

# **X** Project progress is on schedule.

**Project progress is delayed**. Please provide detail on impact to the project objectives and plan to resolve them. Major changes to the approach or objectives require NPRB approval.

**Re-budget request**. Required for reallocations over 10% of the total budget amount. Contact your Program Manager for a rebudget request form.

**No-cost extension request**. Submit at least 30 days prior to the end date of the project. Include the reason for the delay, the anticipated funds left at the original end date, and a revised end date.

**International travel**. All international travel must be approved by NOAA prior to booking, even if it was included in your approved project budget. Allow a minimum of three months processing time for NOAA approval. Contact your Program Manager for details.

Personnel changes. Provide CVs for proposed PIs and a description of the potential impact to the project.

Please answer the below questions with a level of detail proportional to the outcomes of the reporting period.

**1.** Summarize any accomplishments and significant results relating to your project objectives.

Summary of oceanography and biological sampling accomplished in PWS

PI Campbell conducted two more cruises in mid-Aug and early Nov, respectively in PWS for the year 2019. Hydrographic data (CTD profiles) and biological samples were collected at the scheduled 12 stations. Biological samples included:

- Samples for *Alexandrium* qPCR abundance quantification and phytoplankton community composition identification were filtered through a 20  $\mu$ m mesh plankton net from a total volume of 60 liter surface water. The total concentrated samples were preserved by neutral Lugol's solution and then split at a ratio of 4:1 for qPCR and species ID analysis, respectively.
- Samples for bulk water saxitoxin concentration quantification were collected from filtering 60 liter surface water through a 20 µm mesh plankton net. The concentrated water samples were then filtered through 47 mm diameter, 20 µm nylon mesh filters (one or several) before freezing at -20 °C pending for shipping and laboratory analysis.
- Zooplankton bulk toxin samples were collected by using a  $200 333 \mu m$  mesh zooplankton net towing at a low speed ( $\leq 2$  kt) at surface. The concentrated sample was then filtered through nylon mesh filters (47 mm diam, 20  $\mu m$  mesh) before freezing the filters at -20 °C pending for shipping and laboratory analysis.
- Zooplankton community composition samples were collected by vertical tow from 50 m (or bottom) to the surface using a 60 cm diameter, 2m long bongo net, 202µm mesh, Hydro-Bios flowmeter attached, Samples were preserved in 3-6% buffered formalin.

Meanwhile, partners at Simpson Bay oyster farm continued collecting *Alexandrium* qPCR and phytoplankton community ID samples biweekly from July to October. A dozen of oysters from the farm were also saved on a weekly to biweekly frequency for toxin analysis. In addition, mussels were collected from Cordova Harbor approximately biweekly from May through November. These shellfish samples were frozen at -20 °C immediately pending shipment and laboratory toxin analysis.

Herring were opportunistically sampled in June 2019, and a sample of 30 early run pink salmon were obtained from the Ocean Beauty cannery in late June.

## Summary of oceanography and biological sampling accomplished in KB

Shipboard oceanography and plankton offshore surveys in Kachemak Bay (including middle cross-bay transect 9 and along-bay transect KB) were conducted by Kris Holderied and Dominic Hondolero (NCCOS Kasitsna Bay Laboratory), with Kachemak Bay National Estuarine Research Reserve partners, as part of the Gulf Watch Alaska ecosystem monitoring program. Conductivity-temperature-vs-depth (CTD) casts were conducted at 10 cross-bay stations and between 8 to 13 along-bay stations each month. Phytoplankton and zooplankton samples were also collected at five of the CTD stations. During more intensive quarterly surveys in July and September 2019, an additional 10 CTD casts and 3 phytoplankton/zooplankton samples were collected along an outer cross-bay transect (Transect 4), with additional CTD casts made in southeastern Cook Inlet (transects 6, 7 and 3) and phytoplankton and zooplankton samples also collected at 3 of those stations.

Biological sampling on ship surveys included the following:

• Samples for *Alexandrium* qPCR abundance quantification and phytoplankton community composition identification were filtered through a 20 µm mesh plankton net from a total

volume of 40 liter surface water. The total concentrated sample was preserved by neutral Lugol's solution and then split for qPCR and species ID analysis.

- Zooplankton community composition samples were collected by vertical tow from 50 m (or bottom) to the surface using a 60 cm diameter, 2 m long bongo net with 333µm and 150 µm mesh and flowmeter attached, Samples were preserved in 3% buffered formalin and sent to Rob Campbell for identification and counting.
- Samples for bulk water saxitoxin concentration quantification were collected by filtering 60 L of surface water collected with a bucket through a 20  $\mu$ m mesh plankton net. The concentrate was then filtered through a 47 mm diameter, 20  $\mu$ m nylon mesh filter (one or several) before freezing at -20 °C pending shipping and laboratory analysis.
- Zooplankton bulk toxin samples were collected using plankton tows through a 333 μm net. The concentrated water samples were then filtered through 47 mm or 90 mm diameter, 20 μm nylon mesh filters (one or several) before freezing at -20 °C pending for shipping and laboratory analysis.

Biweekly to monthly phytoplankton sampling at the NCCOS Kasitsna Bay Laboratory dock continued from July through November 2019 using the same protocols as the offshore sampling by Dominic Hondolero.

Shellfish (oysters and mussels) were collected approximately biweekly by NCCOS Kasitsna Bay Laboratory and KBNERR partners in Homer Harbor and Kasitsna Bay. Forage fish and predatory fish samples were collected by NCCOS Kasitsna Bay Laboratory, NCCOS Beaufort Laboratory and KBNERR personnel at a variety of Kachemak Bay sites: China Poot Bay, Anchor Point, Tutka Bay and Halibut Cove, from July to October 2019. Sampling of predatory fish and forage fish were collected in KB in July 2019 from Pink salmon, Sockeye salmon, Chum salmon and Pacific Halibut at public fish cleaning tables in Homer Harbor and other sites, including digestive glands, liver, kidney, muscle, roe and stomach contents. Forage and predatory fish samples were also collected in August. In order to better define the practical detection limits for qPCR and toxin analyses in plankton samples, two focused experiments were completed in August. The first focused on phytoplankton in Kasitsna Bay, and the second was focused on zooplankton in Kasitsna and Tutka Bays.

Samples from other locations -- Project partners in University of Alaska Fairbanks and The Knik Tribe of Alaska collected a variety of comparative predatory and forage fish samples at sites in Kodiak, the Alaska Peninsula, the Aleutians and Pribilof Islands. Whole fish, carcasses, or tissue samples were frozen and shipped to NCCOS Beaufort Laboratory or the Alaska DEC Environmental Health Laboratory for toxin analysis. Shellfish toxicity data from concurrent projects were provided for comparative purposes.

## Progress in laboratory sample processing

Phytoplankton community identification samples collected in KB from April to December and samples collected in August and November in PWS were sent to PI Du at Hatfield Marine Science Center in Newport. By Dec 2019, all KB offshore and dock site microscopy analysis from April to August was completed. The rest of 2019 fall and winter samples from both KB and PWS laboratory processing are ongoing (expected to be done by the end of February). Zooplankton community identification have been undergoing at PI Campbell's lab at Prince William Sound Science Center. All zooplankton samples collected in 2018 from PWS and KB have been analyzed and the 2019 samples are being analyzed, with expected completion date of May 2020.

Phytoplankton and zooplankton community bulk toxin, shellfish, forage and predatory fish samples collected in PWS and KB through fall 2019 were shipped to the Beaufort Laboratory for analysis. As of January 2020, fish samples through June of 2019 have been analyzed for PSP toxins using ELISA. Pending analysis of samples from the August 2019 plankton detection limit experiments, the remaining phytoplankton and zooplankton samples will be analyzed by ELISA. Phytoplankton samples from qPCR have been analyzed through October 2019. These samples are expected to be completed by May 2020.

CTD oceanography data processing is being conducted by PI Campbell for PWS data and by Kris Holderied and KBNERR researchers (partners on Gulf Watch Alaska project) for KB data. CTD data are provided after each cruise on the Gulf Watch Alaska program Research Workspace, hosted by the Alaska Ocean Observing System (AOOS). In addition, water quality data are collected continuously from KBNERR water quality stations in Seldovia and Homer Harbors and at a mooring in Bear Cove. Water quality data are provided through the national NERR data portal and on the AOOS Research Workspace.

#### Results updates

*Oceanographic Data* -- Following the marine heat wave (aka "The Blob") of 2013-2015, temperatures in PWS were trending towards the climatological average in 2017 (Fig.1), but returned to positive anomalies in 2018/19, as has been observed elsewhere in the Gulf of Alaska and Bering Sea.



Figure 1: Temperature anomalies at four selected depths in central PWS. Anomalies were calculated as the residual to a second order cosine curve fit to all years data (to remove seasonality: Campbell (2018). Black points are observations, bars are monthly averages, and the green line indicates the linear trend. Slopes with text in black are significantly different from zero (p<0.05).

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Figure 2: Monthly water temperature anomalies for Sep 2001-Oct 2019, calculated from 15-minute observations at near-bottom (~8 m nominal depth) sensors at the Kachemak Bay NERR Seldovia harbor water quality station. Red bars are positive (warm) anomalies and blue bars are negative (cool) anomalies. Anomalies were calculated against 2004-2018 monthly means



Figure 3: Time series of vertical profiles of water column temperature (degrees C) from 2012-2019 collected from monthly CTD casts at a mid-Kachemak Bay station (Transect 9, station 6).



Figure 4: Time series of vertical profiles of water column salinity (PSU) from 2012-2019 collected from monthly CTD casts at a mid-Kachemak Bay station (Transect 9, station 6). Time series is from same casts as in Fig 3, but salinity contour plot is only for the upper 40 meters, to show more near-surface detail.

As shown with a time series of monthly water temperature anomalies from KBNERR sensors at the Seldovia harbor water quality station (Fig. 2), oceanographic conditions in Kachemak Bay were anomalously warm (up to 2 degrees C) for all of 2019, except for near normal conditions in October 2019. The increased warming from late 2018 through 2019 is similar to what was observed in PWS and across the northern Gulf of Alaska. The relatively warm conditions also extended throughout the water column, as shown from time series of vertical temperature profiles from a mid-Kachemak Bay CTD station (Fig. 3), with 2018-2019 winter temperatures remaining above 5 degree C throughout the water column and summer temperatures above 10 degree C reaching nearly to the bottom. Kachemak Bay and the Kenai Peninsula experienced unusually persistent sunny and dry weather conditions from June to August, with lack of rain producing a moderate drought. Interestingly, the lack of freshwater runoff from precipitation appears to have been balanced by increased glacial melt from warm temperatures, because observed salinities were near normal on the north side of Kachemak Bay (Fig. 4), which is influenced by glacial waters, while salinities at the Seldovia water quality station were higher than normal (not shown).

#### Phytoplankton community and Alexandrium abundance

PWS – The comparison of *Alexandrium* abundance and distribution (Fig. 5) between fall 2018 and spring 2019: *Alexandrium* cells were present more broadly in the fall than the spring although abundance levels were well under abundant levels in either season; relatively higher abundances were observed at Hinchinbrook entrance, Zaikof Bay and Montague Strait in either fall or spring; high abundance was also observed at head of Simpson Bay in fall 2018 while abundance was high at the outer Eaglek Bay in spring 2019. The higher abundance close to the Alaska inner shelf indicates the likely origin of *Alexandrium* through Alaska Coastal Current transport.



Figure 5. Seasonal patterns of *Alexandrium* cell abundance distribution in Sept/Oct 2018 and in May/June 2019 in Prince William Sound (12 stations). The crosses denote stations where the counts were zero.

KB – Microscopy showed phytoplankton cells were nearly absent in February in lower Cook Inlet and Kachemak Bay, and increased slightly in March. A diatom bloom occurred in outer and mid-bay in mid-April but only few *Alexandrium* cells were present at the time (Fig. 6 and 7). In May, moderate abundances of diatoms and dinoflagellates were observed in lower Cook Inlet. There were no phytoplankton samples from the bay in May but diatom blooms were expected to continue. In June, diatom abundance increased further throughout bay sites especially in the outer bay. The first high abundance of *Alexandrium* like cells was recorded in outer bay (160 cells/L) but nearly absent at other sites. In July, a large diatom bloom spread from inner to outer bay and dinoflagellate abundance was relatively high as well. The diatom bloom level was much lower in lower cook inlet. *Alexandrium* cells distributed more broadly in the bay in July with an abundance peak of 107 cells/L in outer bay while *Alexandrium* cells were absent in lower Cook Inlet. Similar diatom bloom magnitude and spatial distribution patterns continued in August in inner and mid-bay. The highest *Alexandrium* abundances (214 cells/L) were observed in both inner and outer bay. *Pseudo-nitzschia* was one of the dominants driving the total diatom abundance seasonal changes in July and August.

The dock site diatom and dinoflagellate abundance increased slightly earlier with more pronounced abundances from April through June, and again in August. *Alexandrium catenella* were few in late April and about 107 cells/L in early August. *Pseudo-nitzschia* cells gradually increased to blooms from April through July albeit to a lower magnitude to the offshore sites.

qPCR data indicated *Alexandrium* abundance was relatively low (<100 cell Eq. L<sup>-1</sup>) in KB and PWS during most of 2018 and 2019. Abundance exceeded 100 cell Eq. L<sup>-1</sup> in KB during the summer of 2019, but did not reach bloom levels (>1000). This pattern was also evident in shellfish toxicity, which remained below the FDA regulatory limit throughout, although toxicity was higher in 2019. This was consistent with the higher water temperatures observed in KB in 2019. In contrast, shellfish toxicity in Kodiak was substantially higher. Unfortunately, no cell abundance data are available from Kodiak sites.



Figure 6: Top panel: 2018-2019 Shellfish toxicity ( $\mu$ g STX Eq. 100 g<sup>-1</sup>) at sites in Prince William Sound (PWS), the Kachemak Bay area (Lower Cook Inlet, LCI), and Kodiak. The red line denotes FDA regulatory limit of 80  $\mu$ g STX Eq. 100 g<sup>-1</sup> for comparison. Lower panel: *Alexandrium* cell abundance measured by qPCR (Cell Eq. L<sup>-1</sup>) in PWS and LCI. No abundance data are available from Kodiak. The red line denotes a cell abundance level of concern, where shellfish toxicity may be increasing.



Figure 7. Microscopy-based cell abundance data showing seasonal changes of *Alexandrium* abundance from Feb to Aug 2019 in KB (subareas as outer, mid and inner bay) and lower Cook Inlet (LCI).

#### Zooplankton community

Following the 2013-2014 marine heat wave, the zooplankton community in PWS shifted towards a species assemblage more common in the California current (Fig. 8). In late 2018 and 2019 the community composition shifted back to a cool water assemblage of species more common to the subarctic north Pacific. Species shifts tend to lag temperature changes by a year or more, which means the return to warm conditions in 2019 may lead to another shift towards the warmwater assemblage.



Figure 8.: Time series of zooplankton anomalies in PWS, 2010-2018. Zooplankton were divided into "warm" (i.e. subtropical) and "cool" (i.e. subarctic) water copepod species per Peterson et al. (2017) and average anomalies calculated across groups per Fisher et al. (2015). Warm water species were Calanus pacificus, Clausocalanus sp., Corycaeus anglicus, Ctenocalanus vanus, Mesocalanus tenuicornis and Paracalanus parvus. Cool water species were Acartia longiremis, Calanus marshallae, Oithona similus, and Pseudocalanus sp. Abundances were log<sub>10</sub>+1 transformed prior to calculating anomalies. Note that the scaling of the ordinate varies among panels.



Figure 9 Toxin concentration in juvenile and forage fish samples collected during 2018 and 2019 at all study sites. The red line denotes FDA regulatory limit of 80  $\mu$ g STX Eq. 100 g<sup>-1</sup> for comparison.



Figure 10. Toxin concentrations in salmon tissue samples collected during 2018 and 2019 at all study sites. The red line denotes FDA regulatory limit of 80  $\mu$ g STX Eq. 100 g<sup>-1</sup> for comparison.

#### Toxicity in fish

As of January, 2020, all fish samples from PWS and KB have been analyzed via ELISA through October 2019. Additional samples collected by partners in Kodiak, the Aleutians, the Pribilof Islands, and Southeastern Alaska have also been analyzed, with the exception of ~20 remaining salmon samples from Kodiak.

Forage fish species collected in PWS and KB included Dolly Varden (*Salvelinus malma*), Pacific Sand Lance (*Ammodytes hexapterus*), Pacific Herring (*Clupea pallasii*), Capelin (*Mallotus villosus*), Surf Smelt (*Hypomesus pretiosus*), Longfin Smelt (*Spirinchus thaleichthys*), and juvenile Saffron Cod (*Eleginus gracilis*), Pacific Cod (*Gadus macrocephalus*), Ling Cod (*Ophiodon elongatus*), Walleye Pollock (*Gadus chalcogrammus*), Pink Salmon (*Oncorhynchus gorbuscha*), Coho Salmon (*O. kisutch*), Chum Salmon (*O. keta*) and Sockeye Salmon (*O. nerka*), as well as a small number of unidentified juvenile species. Toxicity results indicated 80-90% of forage fish samples were negative for PSP toxins or else contained trace amounts (<10 µg STX eq. 100 g<sup>-1</sup>). This was not surprising given the low regional shellfish toxicity and low *Alexandrium* abundances that were observed (see above). The remaining forge fish samples contained >10 µg STX eq. 100 g<sup>-1</sup>, with the highest toxin concentrations observed in Pacific Cod (66 µg STX eq. 100 g<sup>-1</sup>), Capelin (22 µg STX eq. 100 g<sup>-1</sup>), Sand Lance (51 µg STX eq. 100 g<sup>-1</sup>) and Pacific Herring (86 µg STX eq. 100 g<sup>-1</sup>), and other species. A sand lance sample from the KB during 2014 having 255 µg STX eq. 100 g<sup>-1</sup> was included in the graph, as this is the highest toxin level yet observed in the study area.

Predatory fish samples included Pink, Coho, Chum, Sockeye and Chinook Salmon (*O. shawytscha*), as well as Pacific Halibut (*Hippoglossus stenolepis*) from PWS, KB, Kodiak, and a variety of other sites in the Aleutians. Only four salmon species are shown in Figure 10 as the sample size for other species is still low (<10). Similar to the pattern in forage fish, most samples exhibited low toxin concentrations (>5  $\mu$ g STX eq. 100 g<sup>-1</sup>). Overall, toxin levels were highest in the digestive tissues (stomach, intestine, esophagus, pyloric caeca), the liver and kidney. The highest toxin levels to date observed in each tissue were: digestive organs (49.2  $\mu$ g STX eq. 100 g<sup>-1</sup>), liver (103  $\mu$ g STX eq. 100 g<sup>-1</sup>), kidney (142  $\mu$ g STX eq. 100 g<sup>-1</sup>) and muscle (10.5  $\mu$ g STX eq. 100 g<sup>-1</sup>). All toxicity data were measured by ELISA, with samples containing >10  $\mu$ g STX eq. 100 g<sup>-1</sup> also analyzed via HPLC for STX congener information.

# 2. List any products for this current report. This includes publications, presentations, technology, websites or other products as appropriate. Additional documents with images, tables, charts or other graphics may be included in this section.

July 2018 Beginning of project

PI meeting via telephone.

Forage fish, predatory fish, plankton and shellfish samples collected in KB. Samples received in Beaufort.

#### August 2018

Project website created as a common platform to share data files and research.

Testing of phytoplankton and zooplankton sampling protocols.

Forage fish, predatory fish, plankton and shellfish samples collected in KB. November 2018

PWS samples for toxin analysis received in Beaufort. PWS samples for species ID analysis received in Newport OR.

## December 2018

Data compilation for all 2018 samples.

Phytoplankton and zooplankton sampling protocols finalized.

Project summary presented at workshop in Kodiak with regional partners (Kibler). January 2019

Alaska Marine Science Symposium Poster Presentation (Xiuning Du, Campbell, Holderied). March 2019

Preliminary invertebrate samples collected by partners at sites in western Alaska for comparison with project data.

PI meeting via telephone.

# April 2019

Project overview presented to NOAA NCCOS leadership (Kibler).

PI meeting via conference call.

Project summary and preliminary results presented to NOAA HAB personnel (Kibler).

Project summary and preliminary results presented to KBNERR and Alaska Dept. Fish & Game personnel at half day HAB workshop in Homer (Kibler, Holderied, Hondolero).

# May 2019

Meeting via telephone with NOAA NMFS partners about establishing parallel sampling project to collect phytoplankton, zooplankton, shellfish, forage fish and predatory fish at a variety of cruises during July-September 2019. Regional samples and data will be shared with the current project.

June 2019

Analysis of all 2018 fish samples completed

Data compilation from all 2018 samples.

# July 2019

NOAA NCCOS leadership and communications personnel assisted with fish and plankton sampling in KB to learn about project.

Sampling cruise in the Bering Sea, Chukchi Sea begins to collect parallel food web samples. Project summary and initial findings provided during meeting with partners in AOOS,

Alaska DEC, NMFS, and the Alaska HAB Network (Kibler).

PWS phytoplankton samples for qPCR received in Beaufort.

Fish, shellfish, plankton samples from KB received in Beaufort.

Collection of predatory fish samples in Homer Harbor.

Meeting in Anchorage to discuss the project with interested HAB researchers in Alaska. August 2019

Forage fish, shellfish and plankton sample collection at study sites in KB.

Completed phytoplankton and zooplankton detection limit experiments in KB.

PI meeting call for project review and planning.

Online meeting with Alaska HAB Network. Provided project updates to collaborators and partners.

November 2019

PI Kibler oral presentation of project and results at the 10<sup>th</sup> National HAB Symposium in Orange Beach, Alabama.

January 2020

PIs Du, Campbell and Kibler attended 2020 Alaska Marine Science Symposium Poster presentation of project.

Meeting held with HAB partners in Alaska to build better collaboration, discuss method limitations, and plan future work.

PI Kibler attended metadata workshop at AMSS.

3. Describe outreach activities and list any media-related press during the reporting period. Include any hyperlinks to online resources such as news links, lesson plans, blogs, etc.



Infographic created for the project website, outreach documents, and presentations.



Screen capture of project website.

January 2020 - Project web page created for inclusion into NOAA NCCOS website.

## 4. Additional PI comments.

The project has been largely on schedule through summer and fall 2019. There were some delays in sample shipping from sampling sites to laboratories. The laboratory sample analysis delays are mainly due to the gradually accumulated samples which have been taking quite more time and labor effort.

Through close collaborations with other regional partners in Alaska, communication efforts for this project have led directly to three other projects aimed at characterization of HAB toxins in the Alaskan food web. The first of these is being led by the Aleutian and Pribilof Islands Association to characterize PSP toxins and domoic acid in shellfish, forage fish and benthic invertebrates that are used as subsistence resources by tribal communities in southwest and south central Alaska. Samples have been provided for inclusion in the current project and resulting data will be made available for comparison with project results. The second parallel project is being led by the USGS Alaska Science Center, with a focus on HAB toxins in zooplankton, forage fish and seabirds. This partnership has provided a number of fish samples from both PWS and KB for inclusion into the current project. The third effort is being led by Kathi Lefebvre the NOAA Northwest Fisheries Science Center, and is focused on all levels of the marine food web through joint sampling efforts with a variety of regional partners. The cruise-based sample collection effort is focused on phytoplankton, zooplankton, fish and marine mammals and will provide a number of such samples for inclusion into the current study. Funding to support sample collection and analysis is being supported by internal funds within

NCCOS in addition to three funded projects focused on other aspects of HABs in Alaska. Technical staff time will also be available to help support the current project starting in 2020.