FY 22-31 *PROGRAM* PROPOSAL
LONG-TERM RESEARCH AND MONITORING PROGRAM

Proposals requesting FY22 - 31 funding are due to shiway.wang@alaska.gov and linda.kilbourne@alaska.gov by March 29, 2021. Please note that the information in your proposal and budget form will be used for funding review. Please refer to the Invitation for the specific proposal requirements for each Focus Area. The information requested in this form is in addition to the information requested in each Focus Area and by the Invitation. We may make inquiries regarding the project and proposer(s), including consulting with agencies or other parties. Project proposals may be submitted in response to only one current Invitation (FY 22-31 or FY 22-26). A project that is submitted under both Invitations may be disqualified from consideration. Please indicate below if your proposal contains confidential information.

Does this proposal contain confidential information? ☐Yes ☒No

Program Number* and Title

22220201 Chugach Regional Ocean Monitoring Program: A Tribally led initiative to monitor baseline oceanic conditions and phytoplankton dynamics for safe shellfish harvest in Prince William Sound and Lower Cook Inlet, Alaska

Primary Investigator(s) and Affiliation(s)

Maile Branson, Science Director, Alutiiq Pride Marine Institute | Chugach Regional Resources Commission

Willow Hetrick-Price, Executive Director, Alutiiq Pride Marine Institute | Chugach Regional Resources Commission

Date Proposal Submitted

August 13, 2021

Program Abstract (maximum 300 words)

The abstract should provide a brief and concise overview of the overall goals and hypotheses of the project and provide sufficient information for a summary review as this is the text that will be used in the public work plan and may be relied upon by the EVOSTC Public Advisory Committee and other parties.

Harmful Algal Blooms (HABs) are becoming a topic of increasing concern in coastal Alaskan ecosystems. These blooms produce dangerous biotoxins, which primarily accumulate in shellfish. Subsistence, recreational, and mariculture-based shellfish harvests are a significant source of both economic and food security across the spill-affected region, and there are currently no State operated HAB or biotoxin monitoring programs for shellfish safety in Alaska. Chugach Regional Resources Commission (CRRC) is a consortium representing seven Tribes in the prince William Sound and Lower Cook Inlet regions of Alaska. CRRC operates the Alutiiq Pride Marine Institute (APMI), located in Seward. CRRC/APMI presently conduct monitoring for harmful algae, shellfish biotoxins, and seawater carbonate chemistry across the spill-affected region through our Chugach Regional Ocean Monitoring (CROM) program. The current CROM program works with Tribal members in each of the seven communities to conduct ecological and biochemical sampling on a weekly basis. Samples are sent to APMI for analyses and data dissemination to the public, with the ultimate goal of informing safe shellfish harvest in the region.

The proposed project will expand and improve CRRC/APMI's existing CROM program to include more comprehensive and quantitative methods of monitoring for harmful phytoplankton species, shellfish toxins, and total seawater chemistry through a long-term monitoring effort. Final data will be utilized to provide 1) weekly reports of these findings to both Tribal members and the general public to inform safe harvest opportunities, and 2) baseline phytoplankton abundance, shellfish biotoxin, and seawater chemistry profiles across the costal Gulf of Alaska region to understand precipitating factors for HAB events. The overall goal of this project is to build regional Tribal capacity through CRRC/APMI to monitor and study harmful algae and biotoxins in native shellfish in order to support safe and sustainable harvest opportunities for both local communities and the shellfish industry in southcentral Alaska.

EVOSTC Funding Requested (round to the nearest hundred, must include 9% GA)

FY22	FY23	FY24	FY25	FY26	FY22-26 Total
$672,345	$529,451	$517,738	$558,054	$589,310	$2,866,899
FY27	**FY28**	**FY29**	**FY30**	**FY31**	**FY27-31 Total**
$562,235	$556,803	$586,894	$576,943	$616,497	$2,899,400
				FY22-31 Total	**$5,766,299**

Non-EVOSTC Funds to be used, (round to the nearest hundred) please include source and amount per source:

FY22	FY23	FY24	FY25	FY26	FY22-26 Total
$180,690					$180,690
FY27	**FY28**	**FY29**	**FY30**	**FY31**	**FY27-31 Total**
				FY22-31 Total	**$180,690**

Two existing awards will be provided as match for the proposed project. These are currently funding the establishment of the CROM program, and will provide for materials and limited sample analysis through FY22.

Administration for Native Americans: "Community based Harmful Algal Bloom monitoring to provide an early warning of paralytic shellfish poisoning in Southcentral Alaska"
Award period: September 30, 2020-September 29, 2022
Award amount: FY21: $154,560; FY22: $151,118
This award provides funding for community-based phytoplankton tow sampling and basic microscopic algal speciation and data collection at each of CRRC's Tribal sampling locations. Training for proper microscopic phytoplankton identification and data collection from collaborators at NOAA is included in this workplan. In FY22, this award also provides capacity for shellfish biotoxin testing at APMI using Blue Mussels obtained through community sampling.

USGS Climate Adaptation Science Center: "Building Capacity for Managing Climate Change Strategies Through Tribal Monitoring of Harmful Algal Toxins in Subsistence Harvested Shellfish"
Award period: January 1, 2021-December 31, 2022 (delayed)
Award amount: FY21: $102,853, FY22: $29,572
This award also provides funding for shellfish biotoxin testing capacity at APMI, largely through the purchase of additional laboratory equipment and supplies. Expected award date was January 1, 2021, however, due to the current changes in federal administration, final processing for this funding has not been completed and documentation is unavailable.

1. EXECUTIVE SUMMARY

Please provide a summary of the program including overall goals and program history if this is a continuing program.

Harmful Algal Blooms (HABs) are becoming a topic of increasing concern in coastal Alaskan ecosystems. These blooms may consist of a variety of algal species, which generate toxic products known to cause significant health risks to both humans and wildlife. In the Gulf of Alaska, commonly recorded genera include a suite of potentially harmful phytoplankton. Of these, *Alexandrium* spp. and *Pseudo-nitzschia* spp. are among the most frequently observed (Anderson et al. 2021). These organisms are known to produce harmful saxitoxins (STX) and domoic acid (DA), the causative agents of paralytic shellfish poisoning (PSP) and amnesic shellfish poisoning (ASP), respectively (Anderson et al. 2021). These biotoxins are most commonly observed in lower aquatic tropic levels, and are regularly found in shellfish, mollusks, and other filter feeding organisms (Deeds 2008). Additionally, limited bioaccumulation of these toxins has been recorded in higher trophic levels of both aquatic and terrestrial organisms, indicating the potential for significant risk to coastal and marine fauna (Lefebvre et al. 2016, Turner et al. 2018, Van Hemert et al. 2020). On a global scale, STX and DA are responsible for significant health concerns, in addition to widespread ecological and economic damage (Anderson et al. 2021). Across Alaska, these elevated levels have caused several known cases of illness and even death in shellfish harvesters (Anderson et al. 2021). Blooms of both genera have been documented as far north as the Being Sea, and are regularly observed throughout coastal habitats (Deeds 2008, Natsuike et al. 2013, Trainer et al. 2014, Harley et al. 2020, Anderson et al. 2021). In southeastern portion of the state, collaborative HAB monitoring programs frequently record biotoxin levels well above the regulatory limits in shellfish tissues (Trainer et al. 2014, Harley et al. 2020).

Historical incidences of HABs have been documented across Alaska, however, recent data demonstrate both an increase in frequency and expansion of range for many species (Natsuike et al. 2013, Gobler 2020, Anderson et al. 2021). While current data suggests several components as primary drivers for HABs, a comprehensive profile of the precipitating factors for these blooms is still unclear. Several environmental conditions have been identified as common occurrences prior to HAB events. Significant changes in air temperature, sea surface temperature, winds, photoperiod, freshwater runoff, and both nutrient and mineral availability have all been identified as preceding blooms, and are thought to contribute to the host of potentially necessary factors required for these events to occur (Gobler 2020, Anderson et al. 2021). Increasingly, evidence is emerging to suggest that climate change has contributed to the worldwide increase in the duration, frequency, and geographical distribution of HABs through dramatic variations in some of these precipitating factors (Gobler 2020). Warmer temperatures from climate change are a dominant driver of many of the recorded ecological perturbations contributing to HABs. Thus, the observed global increase in HABs is hypothesized to worsen as climate change continues to rapidly alter ecosystem balance (Gobler 2020). This holds particularly true for Alaskan waters, which

saw some of the warmest temperatures on record in recent years (Stabeno et al. 2017, Walsh et al. 2017).

Subsistence foods from the marine ecosystem make up a significant portion of the rural Alaska Native diet (Naves 2018). Due to these resource utilization patterns, coastal Alaskan Native populations are twelve times more likely to be affected by PSP than the caucasian community (Gessner and Schloss 1966). There is currently no state operated monitoring program for HABs or shellfish safety outside of the department of environmental conservation (DEC) commercial shellfish program in Alaska. This program only monitors commercial harvests, and does not represent many regions in which recreational and subsistence harvest is common. As a result, several organizations have begun to undertake these regular monitoring efforts collaboratively. Many of these programs are Tribally led, and include the involvement of community samplers from rural coastal villages. The Chugach Regional Resources Commission (CRRC) is a Tribal consortium representing seven federally recognized Tribes in the prince William Sound (PWS) and Lower Cook Inlet (LCI) regions of Alaska. These consortium members include the Tatitlek Village IRA Council, Chenega IRA Council, Port Graham Village Council, Nanwalek IRA Council, Native Village of Eyak (Cordova), Qutekcak Native Tribe (Seward), and Valdez Native Tribe. The Tribal members of CRRC have expressed an increasing concern for the safety of subsistence shellfish in the region. In a 2016 Environmental Protection Agency (EPA) workshop hosted by CRRC, all seven consortium Tribes listed HABs and shellfish safety as a priority in their individual EPA Tribal Environmental Plans (ETEP). The CRRC Board of Directors agreed the region would benefit from relevant real-time information about the presence of harmful algae and biotoxins in subsistence shellfish resources. As a result, CRRC has recently begun a Tribal monitoring program for HABs at our Alutiiq Pride Marine Institute (APMI), located in Seward. This effort presently conducts ocean monitoring for harmful algae, biotoxins in shellfish, and seawater carbonate chemistry across the spill-affected region through our newly established Chugach Regional Ocean Monitoring (CROM) program. The current CROM program works with Tribal members in each of the seven communities to conduct regular ecological and biochemical sampling in the field. These samples are then sent to APMI for further analysis to determine presence of HABs, shellfish biotoxins, and seawater carbonate chemistry profiles. These data are disseminated to Tribes, mariculture operations, and the general public through our website (www.alutiiqprideak.org), to provide information on the safe harvest of shellfish for subsistence, commercial, and recreational use. Tribal members may log on to this website to view weekly phytoplankton abundance estimates and shellfish biotoxin levels. Tribe members may use these data, along with information presented on the APMI website to make harvest decisions at their own risk. With “eyes on the water” each week, CRRC member Tribes can advise their communities of the potential dangers of local shellfish. Each CRRC/CROM community can use the data collected to assess both their local and regional vulnerability to HAB events. Finally, data will be shared with the scientific community through the Axiom Research Workspace data archival and sharing platform and Alaska Harmful Algal Bloom (AHAB) Network portal (https://ahab.portal.aoos.org).

While the current CROM program provides basic information to the public, HAB data collection is limited to visual identification and speciation techniques by Tribal field samplers. Additionally, current funding for this project is short-term, and only supports these monitoring efforts through FY22. CRRC is seeking to 1) continue existing HAB speciation, shellfish biotoxin detection, and analytical seawater carbonate chemistry monitoring efforts, 2) expand this project to include more quantitative methodology for algal speciation and detection through Polymerase Chain Reaction (PCR) assays, and 3) add a single major parameter to the current program, in the form of nutrient sampling. While some of these monitoring efforts are occurring at other locations, no single entity is conducting a comprehensive analysis of HABs, biotoxins, and their known precipitating factors in the spill affected region. This assessment will provide

a comprehensive view of these conditions at several locations dispersed across the PWS and LCI area. Furthermore, consistent monitoring of phytoplankton species affecting safe shellfish harvest using quantitative PCR (qPCR) has never been employed in the state of Alaska. CRRC and APMI are currently working with several academic and agency partners on protocol development, and are seeking to establish the CROM Laboratory in Seward as a central flagship location for these analyses.

Overall, the goal of this proposal is to **build upon the CRRC/APMI CROM program in southcentral Alaska by establishing long-term analytical capacity in Seward to provide accurate, informative data to community subsistence, commercial, and recreational harvesters with the goal of reducing the risk of illnesses associated with HABs and shellfish consumption**. Data from this program will unify southcentral Alaska Tribes in monitoring HAB events that pose a human health risk to all shellfish harvesters*. CRRC/APMI and partners have a vested interest in protecting traditional natural resources as well as the health of their local communities. CRRC seeks to represent our Tribes in maintaining stewardship over the natural resources of the region to restore valuable injured subsistence resources to the Native Peoples of the spill affected region.

*CRRC/APMI acknowledges the liability of providing harvest recommendations. CRRC/APMI will disseminate data, but specific declarations of safe shellfish harvest WILL NOT be made. Members of the general public may access the APMI website to view current phytoplankton species concentrations, as well as HAB biotoxin levels across Prince William Sound and Lower Cook Inlet. CRRC/APMI does not assume responsibility for interpretation of these data by the public, and all harvest must be conducted at the risk of the individual.

2. RELEVANCE TO THE INVITATION

Discuss how the project addresses the overall Program goals and objectives. Describe the results you expect to achieve, the benefits of success as they relate to the LTRM Focus Area, and the potential recipients of these benefits.

This program seeks to address the need for long term monitoring of coastal ocean conditions in the spill-affected region through the lens of safe shellfish harvest. Through this program, we hope to accomplish two major goals. First, we would like to create a centrally located comprehensive monitoring laboratory for all aspects of HABs and shellfish safety in southcentral Alaska (located at APMI in Seward). Through weekly data dissemination on our website, we would like to share these monitoring efforts with all members of the public to inform safe shellfish harvest*. With specific focus on our Tribal members, this goal will assist in restoring self-sufficiency in monitoring and access to traditional subsistence resources for Native Peoples across the Spill Area. Second, this project will compile baseline monitoring data on seawater chemistry parameters, phytoplankton species abundance, and the prevalence of algal biotoxins in native shellfish species. The collection of these data will be used to create a comprehensive longitudinal picture of costal conditions in the oceanic Gulf of Alaska region. This dataset will also be shared with collaborators, and disseminated to both scientific and community stakeholders. Finally, through this initiative, the development of a rapid and accurate qPCR assay for harmful phytoplankton monitoring purposes will be the first in use for regular monitoring in the State of Alaska. CRRC/APMI are currently working with several academic and agency partners to develop accurate qPCR monitoring protocols, and has volunteered to serve as the flagship institution for this technique in Alaska.

*CRRC/APMI acknowledges the liability of providing harvest recommendations. CRRC/APMI will disseminate data, but specific declarations of safe shellfish harvest WILL NOT be made. Members of the

general public may access the APMI website to view current phytoplankton species concentrations, as well as HAB biotoxin levels across Prince William Sound and Lower Cook Inlet. CRRC/APMI does not assume responsibility for interpretation of these data by the public, and all harvest must be conducted at the risk of the individual.

3. PROJECT HISTORY (maximum 400 words)

Is this a new or continuing project? If continuing, please describe the history of the project and what has been accomplished to date (i.e., numbers of publications, presentations, podcasts etc.). Please include detailed references to products (i.e., publications, reports, and websites) in the literature cited section.

The proposed project will incorporate several of CRRC/APMI's previous and ongoing projects into a single consolidated monitoring effort. CRRC began both continuous and discrete monitoring of seawater carbonate chemistry at APMI in 2013. Continuous monitoring of conditions in Resurrection Bay is done in house at APMI, and discrete monitoring is currently managed through our seven Tribal sampling partners, as well as presently funded EVOSTC Gulf Watch partners at Kachemak Bay Estuarine Research Reserve (KBNERR) and Prince William Sound Science Center (PWSSC). Together, these Tribal and academic partners supply water samples on a weekly basis from 8 PWS locations (5 Tribal, 3 with PWSSC) and 23 LCI locations (2 Tribal, 21 with KBNERR). The results of these initial efforts were published in 2015 by APMI and monitoring partners (Evans et al. 2015), and we expect to continue future publication and data dissemination for the current projects. CRRC has also recently received short-term funding to expand existing monitoring efforts to include phytoplankton tows and shellfish sampling. These funding sources allow for limited analyses; phytoplankton identification is currently being conducted via basic visual identification and rudimentary speciation onsite by all seven Tribal sampling partners, and analysis of biotoxins in shellfish is being conducted using enzyme linked-immunosorbent assays (ELISAs) in house at APMI. Full implementation of these programs will begin in May 2021 and run through, and APMI staff and tribal field samplers are presently receiving training from several academic and agency partners in these techniques. The outcome of these projects is also expected to be a peer-reviewed publication, in addition to the regular presentations at scientific conferences. APMI has created a new website (www.alutiiqprideak.org) through which regular data dissemination to all stakeholders is readily accessible. We plan to use this as a means to inform both southcentral stakeholders and scientists of the observed phytoplankton and biotoxin abundance levels on a real-time basis. The funding requested in this proposal will build on the current analytical methods at APMI to create a more comprehensive and quantitative monitoring program.

CRRC would also like to note that as an organization, we have recently undergone a significant re-branding and staffing change. Under the leadership of new Executive Director Willow Hetrick-Price (at CRRC) and new Science Director Maile Branson (at APMI), CRRC/APMI are redesigning its operational and program structure to reflect an expansion into a broader scope of natural resource projects within the region. As part of this effort, the Alutiiq Pride Shellfish Hatchery has been re-named and is now the Alutiiq Pride Marine Institute. Furthermore, APMI has expanded its facility to include two complete laboratory spaces, and has built an entirely new biology laboratory to house many of the capacities detailed in this proposal.

4. PROJECT DESIGN

A. Objectives and Hypotheses

List the objectives of the proposed research and concisely state why the intended research is important. If your proposed project builds on recent work, provide justification that the data are valuable and will remain valuable and if any changes are proposed. If the proposed project is for new work, provide

justification of how the project will provide data useful to addressing management objects, Program goals, and further the Council's mission of recovering injured natural resources and their services. Clearly state the hypotheses, describe how these hypotheses contribute to natural resources and services in the Spill Area, and explain how the hypotheses support the monitoring efforts.

This program seeks to define the baseline dynamics of potentially harmful phytoplankton in PWS and LCI. We plan to accomplish this through investigation of known harmful phytoplankton species, coastal oceanic conditions, and HAB biotoxin levels in shellfish. Through this initiative, we seek to establish a Tribally-led program to inform safe subsistence, recreational, and mariculture shellfish harvest efforts. While CRRC currently conducts much of this work in the short term, consistent recording of these conditions is extremely valuable from a continuous long term monitoring perspective. Continuation of these efforts will provide crucial data to both inform safe shellfish harvest in the region, and to create a comprehensive longitudinal picture of coastal ecosystem dynamics contributing to HABs in the spill affected region. This will be done on a weekly basis by sampling:

1. Relevant environmental and meteorological parameters.
2. Relevant ocean chemistry parameters.
3. Prevalence of known harmful phytoplankton genera.
4. Levels of DA and STX in shellfish.

It is our hope that these data will be used to restore safe access to coastal subsistence resources for both our Tribes, residents, and the general public.

B. Procedural and Scientific Methods

For each objective listed in A. above, identify the specific methods that will be used to meet the objective. Project proposals that seek to continue to contribute new data to the data sets collected in previous years using the same protocols and project design must provide justification that the past methods applied are still appropriate. If changes are needed based on current information a justification for the changes must be provided.

In describing the methods for collection and analysis, identify measurements to be made and the anticipated precision and accuracy of each measurement and describe the sampling equipment in a manner that permits an assessment of the anticipated raw-data quality.

If applicable, discuss alternative methods considered, and explain why the proposed methods were chosen. In addition, projects that will involve the lethal collection of birds or mammals must comply with the EVOSTC's policy on collections, available on our website.

Tribal Community Sampling

All of the following samples will be conducted on a weekly basis throughout the year, for a total of 52 sampling sessions annually (Figure 1). At each of the seven community sites, samplers will take:

1. **Environmental and metadata:** In addition to physical sampling, Tribal community samplers will each take associated metadata for every sampling event, including; date, time, location. Samplers will also take temperature, pH, salinity, and dissolved oxygen (DO) using and a YSI meter [Yellow Springs Instruments] currently in use at all seven communities. Samplers will enter these data through APMIs existing online data entry portal and submit them directly to APMI during the data collection session. All metadata collected will be recorded in a format compliant with federal geographic data committee standards.
2. **Seawater carbonate chemistry sample:** A single 350mL water sample will be collected in a glass bottle. This sample will be fixed with 80ug mercuric chloride preservative (Kattner 1999), capped onsite and sent to APMI for analyses.
3. **Seawater nutrient chemistry sample (beginning FY26):** A single 500 mL water sample will be collected in a polycarbonate bottle. This sample will be frozen at -20C (Gardolinski et al. 2001) and shipped to AMPI for analyses.

4. **Seawater phytoplankton sample:** A single 1L sample polycarbonate sampling bottle of seawater will be collected for molecular analyses. This sample will be fixed with 35mL neutral Lugol's preservative, capped onsite and sent to APMI for analyses.

5. **Phytoplankton tow:** A three-minute surface (horizontal) phytoplankton tow sample will be conducted according to protocols established by researchers at the National Oceanic and Atmospheric Administration (NOAA), and communicated to Tribal field samplers during annual trainings. These tows will be evaluated onsite by Tribal field samplers and both visual genus/species identification and rudimentary counts of cells per mL will be made and entered into the APMI data entry portal.

For a 6 month period, on a weekly basis from April 1 to September 30 of each year (for a total of 26 annual samples), samplers at each site will also take:

6. **Shellfish biotoxin sample:** A blue mussel (*Mylitus trossulus*) sample (*n*=20 organisms) will be collected from previously established collection baskets hanging from local docks at each sampling location. These organisms will be frozen at -20C onsite by field samplers and sent to APMI on gel ice for biotoxin analyses.

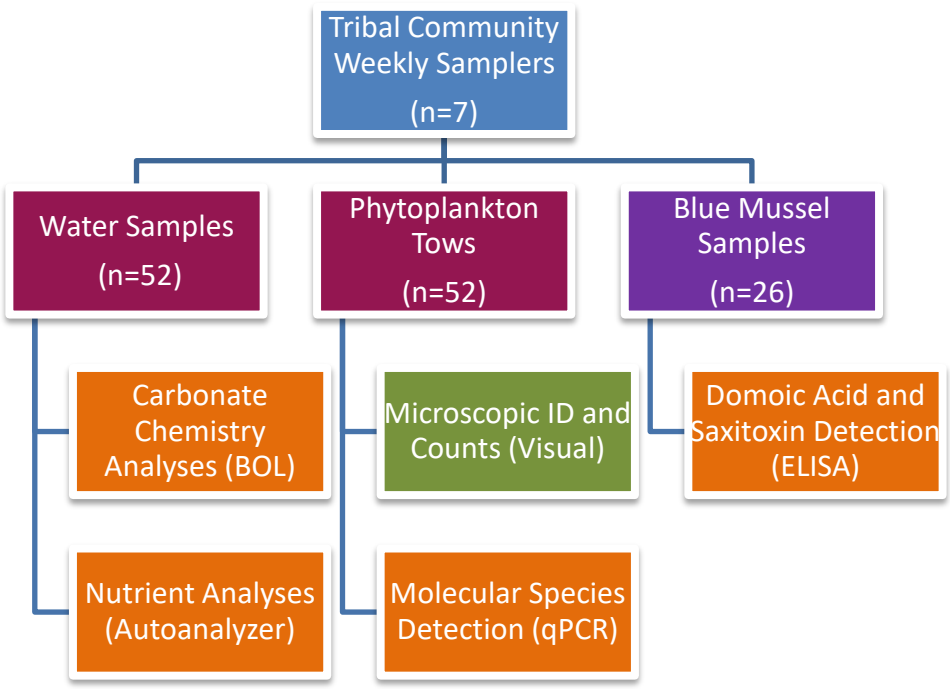

Figure 1. Sample collection flow chart. Blue=activities completed by community samplers, Red=samples collected on a weekly basis throughout the year (n=52), Purple=samples collected on a weekly basis from April 1-September 30 (n=26), Orange=analyses conducted from samples at APMI, Green=analyses conducted by samplers in the field.

Sample Analysis at APMI

All samples shipped to APMI will be sent via same day or next day air. The following methodology will be implemented once samples and data arrive at APMI:

7. **Environmental and metadata (ongoing):** QA/QC of field measurements will be conducted by Laboratory Technicians at APMI, and up to date discrete time series figures will be generated for each parameter on a weekly basis. Additional environmental data such as; air temperature, water

temperature, windspeed, rainfall, and tidal phase will also be added to the master datasheet using agency established metrological datasets. All metadata collected will be recorded in a format compliant with federal geographic data committee standards.

1. **Seawater carbonate chemistry (ongoing):** These water samples will be analyzed by the Chemistry Lab Technician and Manager for dissolved inorganic carbon (DIC) as a measure of ocean acidification, as well as several biochemical parameters essential for shellfish shell formation using a Burke-O-Lator (BOL) and standard protocols (Bandstra et al. 2006, Dickson et al. 2007, Bockmon and Dickson 2015). These measurements include DIC in the forms of pCO_2 and TCO_2, aragonite saturation, and carbonate saturation. In addition to discrete samples from communities, APMI also conducts continuous monitoring of these parameters onsite, and will continue to do so for the proposed project. Full analytical protocols are included in Appendix 1.
2. **Seawater nutrient chemistry (beginning FY26):** These water samples will be analyzed by the Chemistry Lab Technician and Manager immediately upon arrival for phosphate (PO_4) using methods from (Murphy and Riley 1962), as well as nitrate (NO_3), nitrite (NO_2), and ammonium (NH_4) using methods adapted from (Armstrong et al. 1967, Kérouel and Aminot 1997, Grasshoff et al. 2009). All analyses will be conducted using an Autoanalyzer 3 [SEAL] by the Technician, with supervision from the Manager. In addition to discrete samples from communities, APMI will also conduct continuous monitoring of these parameters onsite. Protocol development is underway, and in early phases for this capacity.
3. **Phytoplankton qPCR (beginning FY23):** Water samples will be filtered with an existing filtration system currently in use for phytoplankton sampling at APMI [Millipore]. Filtrate from samples collected prior to analysis start date will be frozen at -80C. From the filtrate, total DNA extraction and qPCR detection will be conducted for *Alexandrium* spp. using protocols and primers adapted from (Vandersea et al. 2017, Zhang & Li 2012). Similarly, protocols and primers used for the detection of *Pseudo-nitzschia* spp. will be adapted from (Fitzpatrick et al. 2010). Assays will be conducted in-house on a CFX Opus thermocycler [Bio-Rad] by the Biology Lab Technician, with supervision form the Biology Lab Manager. Protocol development is underway, and proposed analytical protocols are included in Appendix 2.
4. **Biotoxin detection (ongoing):** On arrival at APMI, mussels will be thawed and shucked by the Biology Lab Technician. A 10g sample of shellfish tissue will be utilized to conduct extraction and analysis using the Saxitoxin (PSP) ELISA Kit and protocols [Abraxis]. Similarly, a 50g sample of shellfish tissue will also be used for the ASP ELISA Kit for Quantitative Determination of Domoic Acid [Biosense], and will follow these methods for extraction and analysis (Appendices 3 & 4). Quantification of these assays will be conducted using a Tecan Nano M+ Plate reader in house at APMI, and will be evaluated by both the Lab Technician and Manager. Final quantification and QA/QC will be conducted by the Full analytical protocols are included in Appendices 3 & 4.

Analytical Support and Interlaboratory Calibrations

Environmental and metadata: All data collected by field samplers will undergo a weekly QA/QC by APMI Science Director to ensure accurate data collection protocols and recording techniques are being met. In the event that field samplers did not enter correct data, substitutes for environmental data may be retrieved from established federal monitoring databases. These QA/QC activities will be recorded in their own log on the APMI google drive. The majority of data collected in the field with a YSI [Yellow Springs Instruments] meter are validated using the Burke-O-Lator (BOL) [Dakunalytics] upon arrival at APMI. Meter calibration occurs on a monthly basis. Field samplers are provided with calibration materials and instructions for conducting these tasks. A calibration log will also be included for field samplers to verify calibration activities on the APMI google drive.

Seawater chemistry: All seawater chemistry data will be collected on regularly calibrated equipment. Calibration logs will be kept on the APMI google drive. Standard curves are also generated for each discrete sample analysis using standards either ordered from established providers or made in-house. Logs for each sample processing run will be maintained in the APMI google drive and will contain both sample data and standard curves for reference. **Support:** APMI regularly works with Dr. Burke Hales (Oregon State University, Dakunalytics LLC), marine chemist and creator of the BOL. Dr. Hales provides regular trainings, and consistent support in protocol development for all seawater chemistry analyses. Interlaboratory comparisons are conducted annually between several institutions, including the laboratories of Dr. Burke Hales (Oregon State University), Dr. Wiley Evans (Hakai Institute), and Dr. Andrew Dickson (Scripps Institute of Oceanography).

ELISAs: All biotoxin data will be collected on regularly calibrated equipment. Calibration logs will be kept on the APMI google drive. A standard curve of serial dilutions is included with each ELISA to ensure precision and accuracy of resulting data. Logs for each sample processing run will be maintained in the APMI google drive and will contain both sample data and standard curves for reference. **Support:** As part of a separate award, Dr. Shannon Atkinson (University of Alaska Fairbanks) conducts interlaboratory calibration and validations with APMI. Dr. Atkinson has validated 20 positive and negative samples from each kit to ensure proper ELISA techniques are transferred to CRRC staff and the data are of a caliber that can be published in peer-reviewed scientific journals. In May of 2021, UAF set up this equipment at APMI and train staff in the proper techniques for ELISA analyses.

qPCR: All DNA or RNA quantification will be conducted using standards provided with the Qubit Fluorimeter [Invitrogen]. Positive and negative controls will be ordered from known suppliers for each target organism. Logs for each sample processing run will be maintained in the APMI google drive and will contain both sample data and controls for reference. **Support:** APMI science director has specific training in development of molecular detection and PCR methodology, and plans to adapt and establish protocols onsite in the CROM Laboratory. APMI science director is currently working with a specialized working group led by Dr. Thomas Farrugia (Alaska Ocean Observing System) to conduct protocol development with the support of several academic and agency collaborators nationwide.

Existing infrastructure available for the implementation of the project

APMI is in the process of an extensive remodel of the current laboratory spaces, and has received funding to provide adequate equipment and supplies for many of the proposed activities. APMI currently has an M Nano + Plate Reader [Tecan], BOL [Dakunalytics], RiOs water purification system (for distilled/deionized water) [Milipore], fume hood, adequate cold storage (4C, -20C, and -80C), water sample (phytoplankton) filtration system [Milipore], microcentrifuge, and laboratory computers.

Infrastructure needed for implementation of this project

APMI is requesting several pieces of equipment for this program:

- A new Burke-O-Lator [Dakunalytics] ($50,000).
- An Autoanalyzer 3 [SEAL] and associated hardware for nutrient analyses ($40,000)
- A CFX Opus quantitative thermocycler [Bio-Rad] ($30,000)
- A Tapestation 2200 [Agilent] electrophoresis system for DNA/RNA PCR product visualization ($25,000)
- A Qubit Fluorometer [Invitrogen] for quantification of extracted genetic material ($5,000)

The following supplies are requested:

- DA and STX ELISA Kits and associated reagents

- qPCR primers and accompanying reagents
- A set of micropipettes
- Field sampling supplies/consumables (bottles, nets, microscope slides, preservatives, etc.)
- Laboratory supplies/consumables (gloves, pipette tips, kimwipes, etc.)

This proposal takes a tiered approach to capacity development. APMI is currently conducting ELISA assays using an existing plate reader. This capacity began in FY21. APMI has also been conducting DIC analyses using the BOL since 2012, however, our current BOL is outdated and cannot handle the significant increase in throughput associated with planned projects. The BOL also requires frequent maintenance and troubleshooting to operate, as it is the second BOL ever produced. Dr. Hales has refined this system since project initiation in 2012, and the new BOL models have the ability to process with greater efficiency/accuracy. APMI is planning on bringing on PCR technology in FY22, and will finalize its analytical capacity with the addition of the Autoanalyzer in FY26. We believe this scaled approach is the most reasonable format with which to add these capacities.

C. Data Analysis and Statistical Methods

Describe the process for analyzing data. Discuss the means by which the measurements to be taken could be compared with historical observations or with regions that are thought to have similar ecosystems. Describe the statistical power of the proposed sampling program for detecting a significant change in numbers based on statistical analyses such as power or sensitivity analysis. To the extent that the variation to be expected in the response variable(s) is known or can be approximated, proposals should demonstrate that the sample sizes and sampling times (for dynamic processes) are of sufficient power or robustness to adequately test the hypotheses. For environmental measurements, what is the measurement error associated with the devices and approaches to be used?

Analyses and methods proposed must be justified. Project proposals that seek to continue to contribute new data to the data sets collected in previous years using the same protocols and project design must provide justification that the past methods applied are still appropriate. If changes are needed based on current information a justification for the changes must be provided.

Evaluation of each monitoring method will occur as follows:

1. **Environmental and metadata:** Up to date discrete time series figures will be generated for each parameter on a monthly basis. These figures will be updated annually to visualize seasonal trends over the course of each year.
2. **Seawater chemistry:** Up to date discrete time series figures will be generated for each parameter on a monthly basis. On an annual basis, these data will be compiled into a yearlong time series for each parameter to visualize oscillations in values, and linear relationships between total alkalinity and salinity may be assessed using basic linear regression models.
3. **Algal detection:** These data will be recorded as cell counts and cycle threshold (Ct) values. Ct values <35 will be considered “probe positives”, and will therefore indicate the presence of the target genera. These Ct values will be translated into binomial data as a “positive” or “negative” detection results for each of the target genera. These data will be presented in two figures; the first as a scatterplot demonstrating cells/mL vs. time, and the second as demonstrating frequency of molecular detection vs. time.
4. **Biotoxin levels:** DA and STX concentration values will be reflected in ug/g of shellfish tissue vs. time, and depicted with a reference at the regulatory limit for each toxin.

Annual data analysis will utilize a number of statistical tests and will occur in in the software package *R*. Analyses may compare any number of the factors recorded in this study, including (but not limited to):

- Correlation between phytoplankton abundance and shellfish toxicity.

- Correlation between macronutrient concentrations/chemical parameters and phytoplankton abundance/biotoxin levels.
- Correlation between environmental factors such as temperature, tide, windspeed, etc. and phytoplankton abundance/biotoxin levels.
- Data may also be complied and analyzed to determine most likely time period in which shellfish toxicity may occur.
-More complex models, such as principal component analysis or random forest modeling may be used to determine factors contributing to bloom events.
All models may be constructed both for each site, and for the region. Variability between regions and interannually may also be assessed. The overall longitudinal compilation of these parameters can be used to compare with observations in other systems to gain a clearer understanding of HAB dynamics in the region.

All metadata collected will be recorded in a format compliant with federal geographic data committee standards. Data will be archived in-house in both the APMI Google Drive, and in CRRC's cloud-based organization drive. Finalized data will be shared with the scientific community through the Research Workspace data archival and sharing platform. These data will be uploaded by the Science Director to the Research Workspace platform on a monthly basis, and will also be shared through the Axiom driven AHAB Network website (https://ahab.portal.aoos.org).

D. Description of Study Area

Is the study area within the Spill Area? Describe the study area, including maps and figures, if applicable, decimally-coded latitude and longitude readings of sampling locations or the bounding coordinates of the sampling region (e.g., 60.8233, -147.1029, 60.4739, -147.7309 for the north, east, south and west bounding coordinates).

The study area is within the Spill Area and includes sample sites at each of CRRC's seven Tribal consortium locations. These include Nanwalek, Port Graham, Seward, Chenega, Tatitlek, Valdez, and Cordova (Table 1). These locations span the entire spill-affected region and provide thorough sampling coverage across PWS and LCI (Figure 2).

Table 1. Sampling coordinates for participating community sampling locations.

Sampling Location	Coordinates
Nanwalek	59.3564° N, 151.9208° W
Port Graham	59.3514° N, 151.8297° W
Seward	60.1042° N, 149.4422° W
Chenega	60.1003° N, 147.9448° W
Valdez	61.1308° N, 146.3483° W
Tatitlek	60.8671° N, 146.6774° W
Cordova	60.5424° N, 145.7525° W

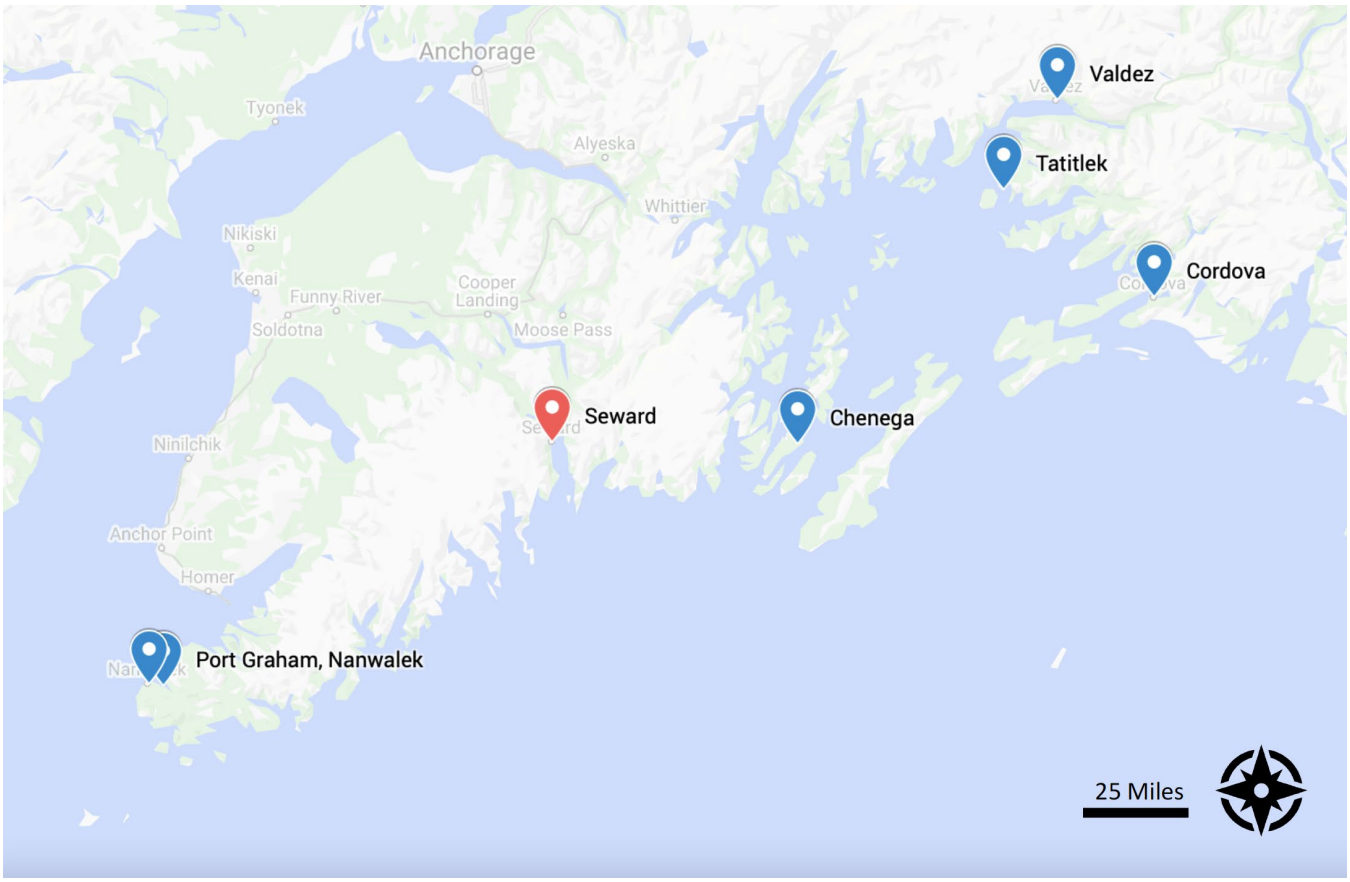

Figure 2. Chugach Regional Resources Commission (CRRC) Tribal consortium members. Each of the seven villages will participate in this study as a sample site for phytoplankton tows, blue mussel (*M. trossulus*) collection, and seawater chemistry sampling.

5. DELIVERABLES

List and describe expected products that will come from this project. Deliverables include but are not limited to papers, reports, recordings, films, websites, presentations, data, and metadata. Project PI(s) will be responsible for all deliverables unless otherwise noted below.

Community Data Dissemination

Deliverables for this project will include weekly dissemination of analyzed data in graphical form to community members and stakeholders in the spill-affected area (and beyond!) via the APMI website (www.alutiiqprideak.org),CRRC/APMI Facebook page (https://www.facebook.com/crrcalaska), and emails to the CRRC Board of Directors and each Tribes' Tribal Administrator for distribution throughout their networks. Data summaries will also be presented to representatives from each of our seven consortium Tribes during quarterly board meetings. These data will include several major sections:

1. **Environmental data:** Discrete environmental data from both community samplers and agency weather monitoring entities in the region at sample collection times. These will include basic parameters such as air temperature, water temperature, windspeed, rainfall, and tidal phase.
2. **Chemical seawater analyses:** Discrete community collected temperature, pH, salinity, and DO measurements; BOL generated pCO_2 and TCO_2 levels, as well as aragonite and carbonate saturation; and profiles of phosphate (PO_4), nitrate (NO_3), nitrite (NO_2), and ammonium (NH_4).

3. **Phytoplankton ID and species prevalence:** Phytoplankton speciation and counts from community samplers, as well as binomial molecular detection results indicating presence or absence of *Pseudo-nitzschia* spp. and *Alexandrium* spp. throughout the year.
4. **Biotoxin levels:** Levels of DA and STX in sampled blue mussels from April-September of each year.

Conference Proceedings and Peer Reviewed Publications

The secondary product of this data collection effort will include regular presentation of ongoing detection methods at three regional conferences annually. A final peer-reviewed publication is expected to be produced, and will detail baseline coastal oceanic conditions, phytoplankton abundance, and shellfish biotoxin levels. The final goal of this project is to evaluate a comprehensive profile of these parameters as it relates to mariculture, recreational, and subsistence shellfish harvests in PWS and LCI.

6. PROGRAM PERSONNEL

*Provide the names of key personnel involved and their role(s) in the program including their roles as they relate to the program and the percentage of their time that will be dedicated to the program. Attach CVs to the end of the proposal. Each CV is limited to **two** consecutively numbered pages and must include the following information:*

- *A list of professional and academic credentials, mailing address, and other contact information (including e-mail address)*
- *A list of up to 10 of your most recent publications most closely related to the proposed project and up to five other significant publications. Do not include additional lists of publications, lectures, etc.*
- *A list of all persons (including their organizational affiliations) in alphabetical order with whom you have collaborated on a project or publication within the last four years. If there have been no collaborators, this should be indicated.*

CRRC/APMI Project Personnel

Willow Hetrick, Executive Director: Hetrick will conduct general project oversight and financial management for the duration of the proposed study.

Maile Branson, Science Director: Branson will oversee operational management of the program, including project deliverables, and management of activities conducted by both staff and Tribal field samplers. Upload final QA/QC into AOOS Axiom Research Workspace and AHAB portal, as well as on APMI website.

Jacqueline Ramsay, Chemistry Laboratory Manger: Ramsay will both conduct and oversee all sample collection, analysis, and data QA/QC/management for seawater chemistry analyses.

Hanna Hellen, Chemistry Laboratory Technician: Hellen will conduct analyses at APMI and coordinate in sample procurement with Tribal samplers. Hellen will also travel to sampling locations to work with samplers in both field methods and general project outreach.

Biology Laboratory Manager (Unfilled Position): The Biology Laboratory Manager will both conduct and oversee all sample collection, analysis, and data QA/QC/management and dissemination for harmful algae qPCR and ELISA analyses.

Annette Jarosz, Biology Laboratory Technician: Jarosz will conduct analyses at APMI and coordinate in sample procurement with Tribal samplers. Jarosz will also travel to sampling locations to work with samplers in both field methods and general project outreach.

Additional Personnel

Indian General Assistance Program (IGAP) Environmental Coordinator (Unfilled Position): This position acts as a liaison between CRRC and Tribal community environmental staff and field samplers. Appropriate and timely sample collection and shipment by tribal samplers will be overseen by the IGAP Environmental Coordinator.

Tanja Davis, Accounting: Davis will oversee implementation of payroll, fringe benefits, purchasing, and award-based financial management tasks as needed.

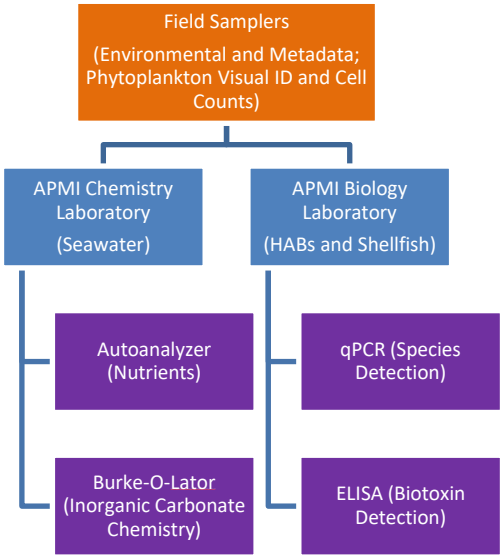

Figure 3. Sampling and data analysis flow chart. Orange=Data collected by field samplers, managed by the IGAP Coordinator; Blue=APMI laboratories responsible for each analyses, managed by the Biology Lab Manager and Chemistry Lab Mangers; Purple=Assays to be conducted by each laboratory at APMI, undertaken by the Biology Lab Technician and Chemistry Lab Technician.

1840 Bragaw Street, Suite 150
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willow@crrcalaska.org

WILLOW HETRICK-PRICE

Dynamic biologist and Executive Director with over eleven years specialized experience providing project management, regulatory compliance, financial management and program development to sea and land-based environmental projects throughout Alaska.

- ➔Possess unique combination of marine ecology expertise, outstanding community outreach record, strong program development background, and wide network of business and professional contacts throughout Alaska's commercial, government, and non-profit sectors.
- ➔Comprehensive knowledge of non-profit management, marketing, client development, financial management, community relations and customer service. Strong desire for success, embracing organizational goals as personal challenges.
- ➔Extensive experience in grants management and accounting concepts, principles, practices, techniques, and procedures, as well as experience in reviewing and analyzing grant applications and summaries, ensuring effective management and accountability of funds.

AREAS OF EXPERISE

- ➔ **Scientific Analysis**
- ➔ **Biological Studies**
- ➔ **Regulatory**
- ➔ **Aerial Wildlife Survey Methods**
- ➔ **Wildlife Management**
- ➔ **Stakeholder Relations**
- ➔ **Complex Presentations**
- ➔ **Training/Supervision**

EDUCATION

UNIVERSITY OF ALASKA SOUTHEAST, Juneau, AK
Master of Public Administration, GPA: 4.0, 05/2018

UNIVERSITY OF ALASKA, Anchorage, AK
Graduate Certificate in Environmental Regulations & Permitting, 05/2018

UNIVERSITY OF HAWAII AT MANOA, Honolulu, HI
Master of Science in Natural Resources and Environmental Management, 05/2009
Bachelor of Science in Marine Resource Management, 12/2006

RECENT PROFESSIONAL EXPERIENCE

CHUGACH REGIONAL RESOURCES COMMISSION, Anchorage, AK
09/2018 - Present
Executive Director
Responsible for a non-profit Inter-Tribal fish and wildlife commission involved in projects and programs related to the natural resources, subsistence, climate change, environmental management and research, as well as community economy development related to natural resources and the environment. Responsible for subsistence advocacy, development of traditional natural resource management programs in the Chugach Region villages, as well as addressing other natural resources and environmental issues, including food security and food sovereignty, development of natural resource education and training programs and conducting training and special issues workshops in the communities in areas related to CRRC's mission. Work directly with Tribal

leaders and their respective Councils to plan and implement community and economic development projects and other areas germane to CRRC's mission. Responsibilities include:

- General oversight of the financial management system of the organization, as well as developing annual budgets and monitoring the budgets for each program.
- Perform financial statement preparation, analysis of accounting reports, establishing, or reviewing of internal control systems, and management of financial accounting systems.
- Built solid relationships and developed network throughout Alaska with businesses and external stakeholders, greatly enhancing company ability to maintain positive customer satisfaction and maximize revenue-enhancing opportunities.
- Interpret grant application guidelines, performing comprehensive research on all necessary data, and successfully securing grants due to strict adherence to writing and qualifications requirements.
- Foster and maintain professional relationships with funders and community leaders, serving as agency's representative on community committees and work groups.
- Serve as communications liaison, facilitating more organized flow of information, and allowing for greater cooperative community relations strategy implementation.
- Coordinate with organizational leaders to monitor current organizational goals developments, recommend priorities, and assist in revising positions.

SELECTED PUBLICATIONS

Peer-Reviewed Publications

Branson, M.A., **Hetrick-Price, W.,** Wisdom, S. Polar Bear (Ursus maritimus) behavioral response to vessel presence in the Chukchi and Beaufort Seas. In review.

Hetrick, W., Cox, L.J, Atkinson, S.K., Malecha, S.R. (2010) Survival of Red King Crab (Paralithodes camtschaticus) Juveniles on Natural and Artificial Substrates. *Journal of Life Sciences* 4(3) pages 1-8

Conference Abstracts and Proceedings

Branson, M.A., Hetrick J.J., & **Hetrick-Price, W**. (2021). Tribal Monitoring and Recovery of Native Clams in the Chugach Tribe's Subsistence Shellfish Use Areas. Alaska Marine Science Symposium. Anchorage, AK, USA.

Branson, M.A., Hetrick, J.J., Ramsay, J., Atkinson, S., & **Hetrick-Price, W**. (2021) The Chugach Regional Ocean Monitoring program: comprehensive biotoxin, phytoplankton, and water chemistry monitoring throughout southcentral Alaska. Kachemak Bay Science Conference and Kenai Peninsula Fish Habitat Science Symposium. Homer, AK, USA.

Branson, M.A., Hetrick, J.J., Ramsay, J., Atkinson, S., & **Hetrick-Price, W**. (2021) Building capacity for safe and sustainable harvest of traditional shellfish resources in Southcentral Alaska. University of Alaska Fairbanks OneHealth Conference. Fairbanks, AK, USA.

Kovalcsik, C., **Hetrick-Price, W.** & Schwalenberg, P. Preserving Traditional Food Resources in a Changing Environment. Alaska Food Policy Council Festival and Conference. Homer, AK, USA.

COLLABORATORS

- Boyd Selanoff, Member of the CRRC Board, Member of the Chenega IRA Council
- Jackie Keating (project), Subsistence Resource Specialist III, Division of Subsistence, Southern Region, Alaska Department of Fish & Game
- Jenn Mintz (project), Education & Outreach Coordinator, NOAA Ocean Acidification Program
- Jim Ujioka (project), Vice Chairman of the CRRC Board, Vice President of The Eyak Corporation and President of the Valdez Native Tribe
- Melody Wallace (project), Member of the CRRC Board, Council member of the Qutekcak Native Tribal, Board member of North Pacific Rim Housing Authority
- Nanci Lee Robart (project), Member of the CRRC Board, The Tatitlek Corporation Board, Chief of the Tatitlek IRA Council
- Patrick Norman (project), Chairman of the CRRC Board and Chief of the Port Graham Village Council
- Priscilla Evans, Secretary/Treasurer of the CRRC Board, Second Chief of the Nanwalek IRA Council
- Roberts Henrichs (project), Member of the CRRC Board, ANTHC Board (bylaws and Policy Committee, Executive Committee, Finance and Audit Committee, Leadership Planning Committee, Maintenance and Improvement Resource Allocation Committee), Healthy Alaska Natives Foundation Board of Directors, the Chair of the ANMC Joint Operating Board and Chairman of the Board at Alaska Village Initiatives
- Sydney Thielke (project), Regional Wetlands Coordinator, U.S. Fish and Wildlife Service

Maile Branson
PO Box 3404, Seward, AK 99664□(907) 360-6790□mailebranson@gmail.com

Education

- 2018-Present: Doctor of Philosophy: Biological Sciences (candidate-anticipated graduation: 2021)
  University of Alaska, 505 S. Chandalar Dr. Fairbanks, Alaska 99775
- 2016: Master of Fisheries and Aquatic Sciences: Aquatic Animal Health Concentration (summa cum laude)
  University of Florida, 876 Newell Dr. Gainesville, Florida 32611
- 2016: Graduate Certificate: Aquaculture and Fish Health (summa cum laude)
  University of Florida, 876 Newell Dr. Gainesville, Florida 32611
- 2012: Bachelor of Arts: Biological Sciences, Minor: Psychology
  University of Alaska, 3211 Providence Dr. Anchorage, Alaska 99508

Current Certifications

- 24 hour HAZWOPER
- Transportation Worker Identification Card (TWIC)
- Basic Safety and Cold Water Survival Emergency training
- Hazard Analysis and Critical Control Point (HACCP) for seafood processors
- PADI SCUBA diver - Endorsements: dry suit, open water
- Family Educational Rights and Privacy Act (FERPA) training
- Title IX training
- CITI Training: Blood Borne Pathogens, Biomedical Responsible Conduct of Research, Wildlife Research, Field Animal Researchers, and Lab Animal Researchers
- State of Alaska Certified Contact Tracer

Current Employment

Employer: Chugach Regional Resources Commission | Alutiiq Pride Marine Institute, 101 Railway Ave., Seward AK 99664
Position: Science Director
Dates worked: October 2020-present
Duties: Responsible for overseeing the science programs at the Chugach Regional Resources Commission (CRRC) and Alutiiq Pride Marine Institute (APMI). I develop and implement research alongside project partners and other staff members. I report on and publish research in scientific journals, oversee data integrity and quality control measures, and conduct project permitting and grant writing. I participate in natural resource projects and issues with the Tribal communities served in the Chugach region, and serve as a spokesperson representing both APMI and CRRC to the public. I serve on several forums and groups that promote issues important to the organization, and attend all major regional natural resource and policy meetings including: Seward Board of Fish/Board of Game Advisory Committee meetings, State Board of Fish/Board of Game meetings, North Pacific Fisheries Management Council meetings, and any Federal Subsistence Board meetings on topics pertaining to Tribal natural resource management.

Publications

1. **Branson, M. A.**, Atkinson, S., & Ramos, M. F. (2016). Hormonal profiles of captive Galapagos tortoises (Chelonoidis nigra). Zoo Biology, 35(3). https://doi.org/10.1002/zoo.21281
2. **Branson, M. A.**, Larkin, I., Parkyn, D. C., & Francis-Floyd, R. (2018). Disease, injury, and sea louse parasitism rates of Copper River and Prince William Sound Sockeye (*Oncorhynchus nerka*), Pink (*Oncorhynchus gorbuscha),* and Chum (*Oncorhynchus keta*) Salmon. Florida Scientist, 81(1), 25–32.
3. Atkinson, S., **Branson, M.A.**, Burdin, A., Boyd, D., & Ylitalo, G. M. (2019). Persistent organic pollutants in killer whales (*Orcinus orca*) of the Russian Far East. Marine Pollution Bulletin, 149. https://doi.orgp/10.1016/j.marpolbul.2019.110593
4. Atkinson, S., Rogan, A., Baker, S., Dagdag, R., Redlinger, M., Polinski, J., Urban, J., Sremba, A., **Branson, M.A.**, Mashburn, K., Pallin, L., Klink, A., Steel, D., Bortz, E., Kerr, I. Application of Unmanned Aerial Systems for Assessment of Cetacean Health. Wildlife Society Bulletin. In press
5. **Branson, M.A.,** Hetrick-Price, W., Wisdom, S. Polar Bear (*Ursus maritimus*) behavioral response to vessel presence in the Chukchi and Beaufort Seas. In review

6. Atkinson, S., **Branson, M.A.**, Hoover-Miller, A. Organochlorine Contaminants in Alaskan Harbor Seals (*Phoca vitulina*). In prep

7. **Branson, M.A.,** Dagdag, R., Redlinger, M., Klink, A., Kosten, T., Soloview, V., Maniaci, B., George, W.J., Causey, D., Chen, J., Murphy, M.D., Winker, K., Bortz, E. Profiling the respiratory microbiome of Northern Fulmars (*Fulmarus glacialis*) in the Bering Sea using metagenomic analysis. In prep

8. **Branson, M.A.,** Kovalenko, G., Dagdag, R., Redlinger, M., Klink, A., Kosten, T., Soloview, V., Maniaci, B., George, W.J., Causey, D., Chen, J., Murphy, M.D., Winker, K., Bortz, E. Identification of respiratory Influenza A Viruses within the breeding seabird colonies of the Alaska Maritime Wildlife Refuge (2018-2019). In prep

9. **Branson, M.A.,** Causey, D., Bortz, E. Paralytic shellfish toxin profiles in the digestive tracts of Northern Fulmars (*Fulmarus glacialis*) in the Bering Sea 2018-2019. In prep

Current and Past Publication Collaborators

- Alexander Burdin, Russian Academy of Sciences
- Amy Klink, University of Las Vegas Nevada: School of Life Sciences
- Andrew Rogan, Ocean Alliance
- Angela Sremba, Oregon State University: Hatfield Marine Science Center
- Anne Hoover-Miller, Pacific Rim Research LLC
- Brandon Maniaci, University of Alaska Anchorage: Department of Biological Sciences
- Daryl Parkyn, University of Florida: School of Forest Resources and Conservation
- Daryle Boyd, NOAA-NMFS
- Debbie Steel, Oregon State University: Hatfield Marine Science Center
- Douglas Causey, University of Alaska Anchorage: Department of Biological Sciences, University of Alaska Fairbanks: College of Fisheries and Ocean Sciences, Harvard University: Belfer Center for Science and International Affairs
- Eric Bortz, University of Alaska Anchorage: Department of Biological Sciences
- Ganna Kovalenko, Institute of Veterinary Medicine (IVM): National Academy of Agrarian Sciences of Ukraine
- Gina Ylitalo, NOAA-NMFS
- Iain Kerr, Ocean Alliance
- Iskande Larkin, University of Florida: College of Veterinary Medicine
- Jack Chen, Alaska State Virology Laboratory, University of Alaska Fairbanks: Department of Biology and Wildlife
- Jennifer Polinski, Gloucester Marine Genomics Institute
- Jorge Urban, Universidad Autónoma de Baja California Sur: Departamento Académico de Ciencias Marinas y Costeras
- Kendall Mashburn, University of Alaska Fairbanks: College of Fisheries and Ocean Sciences
- Kevin Winker, University of Alaska Fairbanks: Department of Biology and Wildlife, Museum of the North
- Logan Pallin, Oregon State University: College of Agricultural Sciences
- Matthew Redlinger, University of Alaska Anchorage: Department of Biological Sciences
- Molly Murphy, University of Alaska Fairbanks: School of Veterinary Medicine
- Ralf Dagdag, University of Alaska Anchorage: Department of Biological Sciences
- Ruth Francis-Floyd, University of Florida: College of Veterinary Medicine
- Scott Baker, Oregon State University: Hatfield Marine Science Center
- Shannon Atkinson, University of Alaska Fairbanks: College of Fisheries and Ocean Sciences
- Sheyna Wisdom, Alaska Ocean Observing System
- Thomas Kosten, University of Alaska Anchorage: Department of Biological Sciences
- Vera Soloview, University of Chicago: Department of Biological Sciences
- William George, University of Alaska Anchorage: Department of Biological Sciences

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Jacqueline Ramsay

25 years of scientific field/laboratory experience with marine invertebrates on the West Coast and Alaska

- ➔ Managed and directed scientific laboratories at UAF and APMI
- ➔ Collaborated in the creation of the water quality monitoring program at APMI and in villages around Alaska
- ➔ Trains students, interns, scientific technicians and community samplers
- ➔ Possesses a diverse skillset for scientific research in the marine environment

EDUCATION

UNIVERSITY OF ALASKA FAIRBANKS, Fairbanks, Alaska
Master of Science in Fisheries, 2007
UNIVERSITY OF CALIFORNIA DAVIS, Davis, CA
Baccalaureate of Biological Science in Evolution and Ecology, 1980

PUBLICATIONS

• Evans, W., Mathis, J.T., Ramsay, J., Hetrick, J., 2015. On the Frontline: Tracking $CaCO_3$ Corrosivity in an Alaskan Shellfish Hatchery. PLOS ONE.
• Weekes, C., Evans, W., Hales, B., Ramsay, J., Hetrick, J., Mathis, J. "Enhancing ocean acidification observing in Alaska through citizen science initiatives and land- based measurement strategies" Frontiers in Earth Science (submitted)

PROFESSIONAL EXPERIENCE

Alutiiq Pride Marine Institute, Seward, AK 9/2011-Present
(A Division of the Chugach Regional Resources Commission)

Shellfish Biologist - Ocean Acidification Research Lab Director
• Directs the Ocean Acidification Research laboratory functions; supervises OAR laboratory technician
• Manages the CRRC coastal seawater monitoring program for baseline carbonate chemistry
• Shellfish brood stock and larval animal husbandry including abalone, king crab, oyster and clam species
• Monitor water quality, microbiology and pathogen monitoring
• Writing: Reports, grants, permit requests and acquisition.

University of Alaska Fairbanks, Seward Marine Center 10/2006-11/2009
Research Professional II, Endocrinology Laboratory Manager
• Daily management of the UAF Endocrinology Laboratory, a radioisotope lab under NRC jurisdiction
• Oversaw graduate students and their projects, complied with nuclear regulatory commission guidelines for acquisition and disposal of radioisotopes. Produced reports and wrote grants. Responsible for training lab users in chemical hygiene and safety as well as laboratory protocols. Helped design and implement experimental parameters for the field and laboratory. Participated in field and laboratory monitoring of Stellar Sea Lions and sample acquisition. Coordinated decommissioning of the lab and commissioning of a new laboratory twice under NRC guidelines.

National Oceanic and Atmospheric Administration, Auke Bay Laboratory, Juneau, AK 2006
Scientific Technician Contractor
• Identification and analysis of zooplankton samples in the lab and on field research cruises for the Southeast Coastal Monitoring Program.
Associate Science Editor of the Fishery Bulletin Contractor 2005.
• Established new office, characterized manuscript status, identified and assigning priority to editorial tasks and assigned reviewers and screened and edited manuscripts.
Analytical Chemist Contractor 2005.
• Produced esterified proteins for fatty acid analysis of sea lion prey using mass spec.

University of Alaska Southeast, Juneau, AK 2003-2004
Teaching Assistant
• Taught laboratory component of upper division Marine Biology and Physiology science course, corrected assignments, assigned grades.

California Department of Fish and Game, Shellfish Health Laboratory 6/1996 – 11/2000
University of California Davis, Bodega Marine Laboratory, Bodega Bay, California.
Post Graduate Researcher
• Conducted research and creel surveys on red and black abalone as well as pacific oysters for pathogens in wild and hatchery animals and the management of their health. Conducted field studies on oyster health in Tomales Bay.

LABORATORY/FIELD EXPERIENCE

• HPLC – High Pressure Liquid Chromatography • CO2 /DIC analysis of seawater samples
• Microscopy • Microbiology • Stress protein analysis
• Isotopic hormone assays • Spectrometry • Cellular immune assays
• Invertebrate spawning and larval husbandry • Chemical hygiene

COLLABORATORS

Alexis Valauri-Orton, Ocean Foundation
Burke Hales, Oregon State University
Jeremy Mathis, NOAA
Kaitlyn Lowder, Ocean Foundation
Wiley Evans, Hakai Institute

HannaMHellen@gmail.com | 907.717.9964 | www.linkedin.com/in/hanna-hellen

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University of Alaska Fairbanks Graduated May 2021 BS Fisheries and Ocean Science, minor in Biological Sciences
 Undergraduate Research and Scholarly Activity Award recipient
  Differences in stable isotope signatures between Chukchi Sea and Southern Beaufort Sea polar bears

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ALUTIIQ PRIDE SHELLFISH HATCHERY May 2021-Present
Biological Technician

SEALASKA CORPORATION
Research Analyst Internship-Summer 2020 August 2020-April 2021
 Started as intern for Sealaska, completed a research project on potential kelp mariculture opportunities in Alaska and was hired as a part-time employee at the end of my internship.
 Research and provide findings on Sealaska's scientific goals, as well as plans to accomplish them, primarily within the field of fisheries and ocean science.

UNIVERSITY OF ALASKA FAIRBANKS
College of Fisheries and Ocean Science, Student Assistant February 2019-Febuary 2020
 Provided administrative support and front desk assistance to the Human Resources, Procurement and Financial departments.

ALASKA SEALIFE CENTER
Interpretation and Steller Sea Lion Remote Monitoring Intern May-August 2019
 Assisted with animal photo identification, behavioral observations, data entry and camera operations on a Steller Sea Lion remote observation research project.
 Conducted education and awareness activities in addition to providing guided tours and scientific talks.

Annette Jarosz
PO Box 1515, Seward, AK 99664 | (443) 878-0159 | annettejarosz1@gmail.com

Education

Appalachian State University, Boone, NC **Spring 2021**
B.S. in Biology with a concentration in Ecology, Evolution, and Environmental Biology (Summa cum laude)
Certificate in Geographic Information Systems

Current Employment

Employer: Chugach Regional Resources Commission | Alutiiq Pride Marine Institute, 101 Railway Ave., Seward AK 99664
Position: Harmful Algae Bloom Lab Technician
Dates worked: May 2021-present
Duties: I oversee and conduct the day-to-day operations in the HAB lab at Alutiiq Pride Marine Institute (APMI). I collect and identify algae from resurrection bay. Additionally, I collect different species of bivalves and test for saxitoxin and domoic acid by conducting enzyme-linked immunosorbent assays.

Current and Past Collaborators

- Freddy Ortega, Appalachian State University: Department of Biology
- Kendall Mashburn, University of Alaska Fairbanks: College of Fisheries and Ocean Sciences
- Locke Revels, Appalachian State University: Department of Biology
- Michael Gangloff, Appalachian State University: Department of Biology
- Shannon Atkinson, University of Alaska Fairbanks: College of Fisheries and Ocean Sciences

7. PROGRAM ADMINISTRATION

Provide an administrative plan for overall program management including an organizational chart. At a minimum the plan should include a list of what services are covered by your indirect rate (clearly report what this rate is); a schedule for the production and implementation of data and reporting policies which must include a plan for addressing non-compliant PIs and programs; and a listing of any costs and staff time associated with meetings.

The indirect cost rate at CRRC is 14.92%. The following indirect costs incurred for the operation of the administration of CRRC have been classified as indirect costs in accordance with the standards for indirect costs as described in 2 CFR Part 200 issued by the Office of Management and Budget. For reasons itemized below, these costs have been determined to comply with the above stated criteria and are therefore to be consistently charged as indirect costs within the funding formulas determined by the cognizant agencies. All items listed below have been determined to benefit more than one cost objective and are not readily allocable to benefiting contracts, grants, and programs:

1. Salaries paid to the administrative staff of the Commission.
2. Fringe benefits including payroll taxes, worker's compensation, health insurance (medical/dental), life insurance and retirement benefits for the administrative staff.
3. Travel, including meals, lodging, and transportation costs for indirect personnel conducting Commission business that is of an indirect nature (i.e., the benefit is not directly related to one program, but instead benefits many programs). Per diem follows the guidelines established for the Federal Government by the General Services Administration.
4. Equipment purchases costing $5,000 or less used for administrative purposes are included as indirect costs.
5. Building/equipment maintenance, janitorial, and utilities expenses are for those buildings and equipment used by administrative staff and programs.
6. Office space costs are for rental of buildings used for administrative and program activities.
7. Telephone, electricity, and fuel costs related to administrative activities and programs where the costs cannot be directly identified to those programs.
8. Office supplies, printing/postage, and other supplies used that cannot be specifically identified with a particular contract, grant, or program.
9. Insurance costs for the general umbrella policy and the fidelity bond of Commission.
10. Consultants, including attorney fees, accounting fees, computer, and contract services associated with obtaining audits, technical assistance, and legal guidance necessary to meet the needs of programs, and the various requirements of federal and state laws and regulations.
11. Training costs include training for the administrative staff in performance of their daily activities, including computer classes, and training which benefits all contracts and grants.

Other indirect costs may be included in the administration of the indirect cost pool. Costs included in the indirect cost pool may not be limited to the above list; however, they are the only such costs foreseen at this time. Each cost is examined for compliance with prescribed criteria before being classified as an indirect cost.

The North Pacific Rim Housing Authority has a Business Services Department that provides financial accounting and management expertise to CRRC. They have assigned three people to work with our organization to provide accounts payable, payroll, and financial management services. The Business Services Department also assists with conducting our annual audits and developing our indirect cost proposals. We use the AccuFund software system to manage our accounts, using fund accounting. The CRRC Board has adopted a financial management policy as well as a system of checks and balances for requesting checks or purchase orders, travel requests, and credit card purchases. CRRC has a double

entry, accrual system of accounting and uses fund accounting concepts. Each grant, contract, or other funding source in the direct cost base is accounted for in a separate fund. A separate fund is also maintained for the indirect cost pool. Each fund is an independent fiscal and accounting entity with a self-balancing set of accounts, recording revenues, expense, assets, liabilities, and fund balances. These funds are segregated for the purpose of carrying out specific programs or attaining certain objectives in accordance with the stated purpose of each fund. CRRC also has an internal Cost Controller to provide support and real-time management financial management, grant management, and cost saving opportunities related to purchasing and procurement.

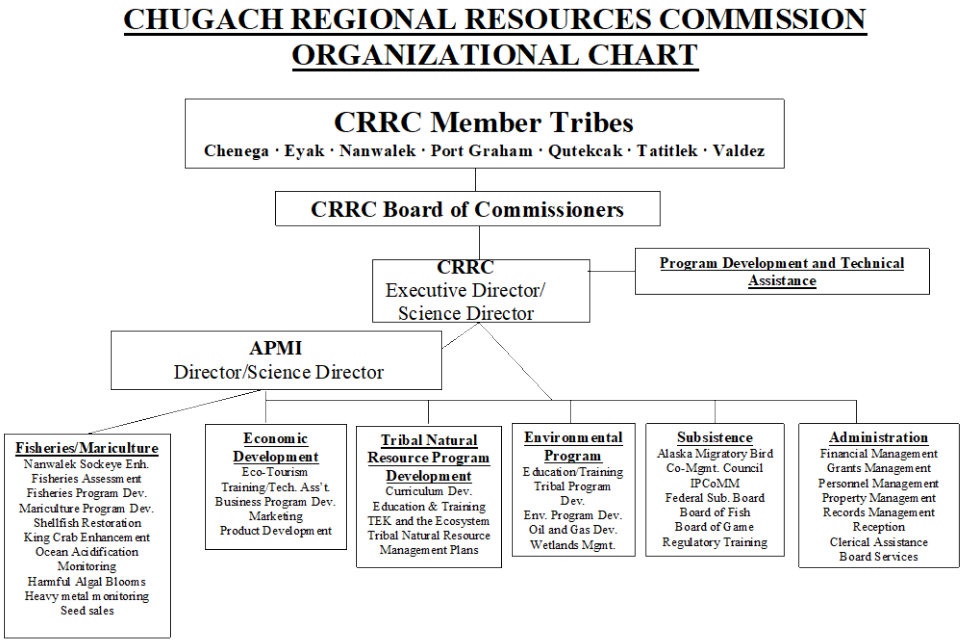

Figure 4. Chugach Regional Resources Commission organizational structure.

Proposed Program Management

This program will be overseen by both the science director and the executive director (Figure 3, Figure 4). Data will undergo weekly entry/QA/QC and annual quantitative analysis for reporting purposes. All of these activities will be undertaken by staff members specifically dedicated to this project (Figure 3). Data dissemination to the public will occur on a weekly basis, and quantitative analyses and scientific dissemination will occur both with annual reporting to EVOSTC, and at annual scientific conferences (three annually). The final data management product of this program is expected to be a peer reviewed publication in a scientific journal. Compliance in reporting and program management is integral to the positions of the employees listed above. In the event a program is non-compliant, these employees may be terminated and replaced to maintain adequate program progress and reporting.

8. PROGRAM SCIENCE MANAGEMENT: DESIGN AND IMPLEMENTATION

Describe the plan for the management of scientific projects. At a minimum, the plan should include:

E. Selection and implementation of an internal science panel

How will members be selected and to what degree will they be involved with the program?
While CRRC does not have a formal internal science panel, this role is fulfilled at CRRC by both of the PIs listed for this project. PIs Branson and Hetrick-Price are the most experienced and educated members of the CRRC staff, and will review all finalized data analyses before submission to publicly available databases.

F. Distribution of and addressing program science panel and EVOSTC work plan comments to the PIs
How will the science panel and EVOSTC work plan comments be circulated to the PIs? How will you ensure that internal science panel comments and recommendations will be addressed?
PIs Branson and Hetrick-Price met weekly throughout the duration of the review period to address all reviewer comments provided for this proposal.

G. Evaluation of progress
Describe a plan for the evaluation of program projects to ensure that they are meeting their milestones and are still relevant to the goals of the program.
To ensure deliverables are being met in a timely fashion, CRRC/APMI staff will meet on a bi-weekly basis throughout the duration of the project. These meetings will be led by the Science Director (Branson) and/or Executive Director (Hetrick-Price), and will focus on task management to meet the goals as proposed. Both laboratory managers will be required to submit analyzed QA/QC data to the Science Director on a weekly basis. Science Director will then review and upload these data to the APMI website on a weekly basis, and the Research Workspace portal on a monthly basis. As much of the work currently being conducted under this project is already underway, CRRC/APMI anticipates that undertaking this project will require minimal adjustments to current operations.

Data and data products generated by this project will be posted on the Research Workspace together with standards-compliant metadata for access by the EVOSTC.

9. PROGRAM DATA MANAGEMENT

Describe how the program will ensure that data and associated metadata collected by the projects are accurate and provided to the data managers based on the data policy. The data policy must provide a clear timeline for the submission of data and metadata by individual researchers and when the data will be made available to the public (see Section 9). Data collected by researchers employed by any federal agency must comply with Federal Open Data Policy Requirements.
The *Exxon Valdez* Oil Spill Trustee Council's data policy encourages full and open access to, and confident use of, the data and information used in and produced by programs and projects of the *Exxon Valdez* Oil Spill Trustee Council (EVOSTC). These data need to be easily understandable, electronically accessible and well organized to allow policy makers, researchers, managers, and the general public to make well-informed decisions. As such, Axiom Data Science, through its partnership with the Alaska Ocean Observing System (AOOS) have considerable experience developing scientific data management infrastructure, and they provide experienced personnel to manage both data and metadata documentation according to federal quality control standards. This project will use the AOOS data management infrastructure (developed and maintained by Axiom Data Science) to manage and share the data generated through this effort, in accordance with the EVOSTC Data Management Procedures. This system uses the standards and best practices defined by the NOAA U.S. IOOS Data Management and Communications committee (IOOS, 2010). Among this infrastructure is an operational stack of open source software components developed by Axiom Data Science, with support from the NOAA Integrated Ocean Observing System (IOOS), EVOSTC, the National Science Foundation and more, which manages

large numbers of continuous data feeds and a data catalog framework to integrate and disseminate a variety of data products. Data and data products generated by this project will be posted on the Research Workspace together with standards-compliant metadata for access by the EVOSTC. At the end of the project term, final QA/QC'd data and metadata will be made publicly available through the Gulf of Alaska data portal and made publicly accessible through the AOOS Gulf of Alaska data portal and distributed to DataONE for long-term preservation.

Data Types, Formats, and Metadata

This project will generate the following data:

- Time series environmental data: Continuous environmental data from both community samplers and agency weather monitoring entities in the region. These will include basic parameters such as air temperature, water temperature, windspeed, rainfall, and tidal phase.
- Time series chemical seawater analyses: Discrete community collected temperature, pH, salinity, and DO measurements; BOL generated pCO2 and TCO2 levels, as well as aragonite and carbonate saturation; and profiles of phosphate (PO4), nitrate (NO3), nitrite (NO2), and ammonium (NH4).
- Phytoplankton ID and species prevalence: Phytoplankton speciation and counts from community samplers, as well as binomial molecular detection results indicating presence or absence of Pseudo-nitzschia spp. and Alexandrium spp. throughout the year.
- Biotoxin levels: Levels of DA and STX in sampled blue mussels from April-September of each year.

Data will be stored in non-proprietary formats to ensure re-use and long-term preservation. Project data may initially exist in proprietary or binary formats as primary-level data, depending on the source provider. Though the data may be in a state which can be easily utilized by the research team, in many cases the primary-level data is not in a form ready to be shared with the broader science community or integrated with other datasets. As such, the final format for project data will be in open standard suitable for long-term archiving, such as:

- Containers: TAR, GZIP, ZIP
- Databases: CSV, XML
- Tabular data: CSV
- Geospatial vector data: SHP, GeoJSON, KML, DBF, NetCDF
- Geospatial raster data: GeoTIFF/TIFF, NetCDF, HDF-EOS
- Moving images: MOV, MPEG, AVI, MXF
- Sounds: WAVE, AIFF, MP3, MXF
- Statistics: ASCII, DTA, POR, SAS, SAV
- Still images: TIFF, JPEG 2000, PDF, PNG, GIF, BMP
- Text: XML, PDF/A, HTML, ASCII, UTF-8
- Web archive: WARC.

Comprehensive metadata using the latest national and international technology and community standards will be written for each data collection generated. The Research Workspace includes an integrated metadata editor, allowing researchers to generate metadata conforming to the FGDC-endorsed ISO 19110 and 19115-2 suite of standards. Axiom will provide technical assistance to project researchers to ensure robust and standards-compliant metadata are generated for final project datasets prior to data publication and archive.

Data Access and Timeframes

Among the Axiom data system infrastructure is the Research Workspace, a web-based scientific collaboration and data management tool used by researchers to secure and centralize project data, generate standards-compliant metadata, and ultimately elect data files and derived data products to be published openly on public data portals and in long-term data archives. Following the EVOSTC data sharing policies, all monitoring data from this project will be transfer as they become available to the Research Workspace. These data shall be replaced in the Research Workspace with QA/QC'd and metadata when available and no later than 1 year after collection, after which they will be made publicly available through the GOA data portal. The Research Workspace is the gateway for PIs to elect and publish data and metadata to the GOA data portal. The exception is for process studies which are research-oriented in nature and do not have annual timeseries data. Process studies require data and metadata to be made publicly available through the GOA data portal by the end of the project term.

Data Storage, Preservation, and Archiving

The Axiom data center and services are housed on highly redundant storage and compute resources at a data center in Portland, OR, and are geo-replicated using Amazon Glacier Cloud Archive Services. All databases and code repositories are routinely backed-up, and servers undergo routine maintenance to swiftly address security vulnerabilities. Servers containing source code and databases are located behind an enterprise-level firewall and are physically secure with environmental regulation systems, redundant power, and fire suppression. Axiom's HPC resources are composed of approximately 2500 processing cores staged in a series of interconnected blade arrays as well as 1.8 petabytes of storage. Dedicated disc-space in the amount of 30 TBs will be allocated for long-term storage of all preliminary and finalized data resources produced by this effort.

For long-term preservation, all final data and metadata will be transferred to a national data center. The data developed in this project will be open source and licensed in the public domain. The planned archive for the data collected by this effort is the Research Workspace's DataONE Member Node. The Research Workspace hosts an integrated system for automating dataset submission to the NSF-sponsored DataONE federation of data repository. The Research Workplace supports and issues Digital Object Identifiers (DOIs), so datasets can be confidentially referenced in the published literature. Upon final permission from the project PI at the end of the project term, final data or data products will be submitted for archive with technical support by Axiom data management staff to ensure appropriate use and compliance with the data center archive requirements.

10. PROGRAM OUTREACH

Provide a public outreach plan focused on providing information to the Trust Agencies for use in their respective outreach and education materials. Outreach efforts by the program should focus on (continued) development and maintaining accurate and timely content for the program's website as a primary source of information on the program. How often will website(s) be updated with information for the general public and other researchers?

This project incorporates the involvement of community samplers from all seven of our Tribal consortium communities. Within the CRRC network, regular board meetings are held on a quarterly basis, through which we disseminate data to attending Tribal members, as well as each regional Tribal representative. CRRC is working in collaboration with The Alaska SeaLife Center, Alaska Sea Grant, Alutiiq Museum and Archaeological Repository, Center for Alaskan Coastal Studies, and Prince William Sound Science Center on a proposal to the EVOS TC titled: Community Organized Restoration and

Learning [CORaL] Network. This project aims to create and maintain an ongoing framework that builds the capacity of existing resources within the *Exxon Valdez* Oil Spill impacted region to ensure that current scientific information, skills, and activities are publicly accessible and serve ongoing needs as identified by local communities. the CORaL Network will ensure that: science outreach is relevant, co-created, and culturally responsive to our regional communities, leading to increased public utilization of available knowledge related to the EVOS; the participation of regional youth in community-based science projects increases diversity in future science projects; EVOSTC-funded Long-Term Research & Monitoring, Mariculture, and Restoration projects are integrated with community-identified needs; and increased understanding of Alaska Native knowledge and relations, cultural competency, and collaborative community research principles lead to active, community-informed restoration projects.

The Alaska SeaLife Center's Education Department will produce the following outreach products annually: one distance learning program, four Virtual Visit programs, one Virtual Field Trip, two in-person programs, and content for one rotating mini-exhibit installation. CRRC will work closely with the Alaska SeaLife Center to develop content for one rotating mini-exhibit. Additionally, to create a regional system of demonstrating current EVOSTC-funded work, the Alaska SeaLife Center, Alaska Sea Grant, Alutiiq Museum and Archaeological Repository, Center for Alaskan Coastal Studies, CRRC, and Prince William Sound Science Center will each install at least one video kiosk at their site. Most of these kiosks will be assembled using tablet technology and may be mobile stations that can be used in different locations or in outreach programs. The Alaska SeaLife Center and the Prince William Sound Science Center will construct more robust kiosk stations appropriate to their exhibit halls with heavy visitation.

These kiosks will be linked to a video library, also available on the CORaL Network website. Videos will be created by these core partners and may also be created through mini-grant proposals with EVOSTC-funded scientists. The Valdez Museum has also expressed an interest in creating videos that integrate current scientific data with historical community contexts. These videos are expected to be 2-5 minutes long and may be played on a loop or be available for visitors to select from a touchscreen.
The ability to install a system of networked video kiosks, linked to a shared video library, is made possible by the fact that the core CORaL Network partners include visitor centers and outreach sites. These kiosks will provide current data to community members, tourists, and visitors across the region. Thus, the potential for collaborative outreach through a number of partner institutions is being addressed via separate proposals. If accepted, the proposed program will be incorporated in this educational program.

11. COORDINATION AND COLLABORATION

A. With the Alaska SeaLife Center or Prince William Sound Science Center

A preferred requirement for all proposals is to partner with the ASLC, PWSSC, or both Centers. If not collaborating with either of these Centers, please provide information as to the inquiries and efforts extended to ASLC and PWSSC researchers and/or administrators.

CRRC and APMI staff have reached out to several potential collaborators to establish partnerships, however, formation of these partnerships is still pending. Furthermore, CRRC believes that in representation of the indigenous Peoples of the region, our projects represent those most affected by the spill, and should not be justified by mandated collaboration with specific entities.

Chugach Regional Resources Commission acknowledges the Council's efforts to create Centers of Excellence to reduce the administrative burden on the Council's staff in administering grants. Just as the Alaska Sea Life Center and the Prince William Sound Science Center have been deemed 'centers of

excellence' by the EVOS Trustee Council for their history in successful fiscal management of EVOS-funded projects, CRRC has been deemed a 'center of excellence' by federal grantors. Since its inception, CRRC has grown over the years to become not only important regional facilities, but valuable statewide resources with reputations of excellence. In FY21 the organization's grant portfolio exceeded $5 million.

CRRC has an ISDEAA of 1975 (Public Law 93-638), a law that recognizes member Tribes inherent status as sovereign nations, which is distinguished by their relationship with the federal government. Chugach Regional Resources Commission serves as the ISDEAA-sponsored natural resource management agency on behalf of the following sovereign Tribal governments– Chenega, Eyak (Cordova), Nanwalek, Port Graham, Qutekcak (Seward), Tatitlek, and Valdez. The signing and passage of the ISDEAA meant Congress understood the inherent right tribes possess to set their destiny through tribally run programs for natural resources operated by the federal government to the benefit of tribal nations. It allows Tribes to manage and control their own assets.

Chugach Regional Resources Commission recognizes that the call for proposals specifically requires consultation with Alaska Natives and to consult with the Centers of Excellence; the Prince William Sound Science Center and/or the Alaska SeaLife Center. This requires an additional and unnecessary % on top of the existing budget that could be better spent on meaningful projects in the Spill Area. What we propose is that the costs associated with including a center of excellence be given to the Chugach Regional Resources Commission. This could bring new revenue to the organization to allow for further development of staff, management capacity, and internal infrastructure instead of these overhead funds going to existing, well-established, and top-heavy organizations such as the centers of excellence. There is no better way to ensure the long-term sustainability of the projects proposed as part of this solicitation than investing in Alaska Native organizations that have worked and will work in the Spill Area for decades benefiting the People who have lived off the land since time immemorial. Should the Trustee Council determine that this project requires administration through a center of excellence, Chugach Regional Resources Commission will expeditiously work with the Alaska Sea Life Center due to its proximity to our organization's operations.

B. Within the EVOSTC LTRM Program

Provide a list and clearly describe the functional and operational relationships within the LTRM Program. This includes any coordination that has taken or will take place and what form the coordination will take (project guidance, shared field sites or researchers, research platforms, sample collection, data management, equipment purchases, etc.).

Environmental Drivers Component

None, however, CRRC feels that valuable partnerships could be made within these research components.

Pelagic Monitoring Component

None, however, CRRC feels that valuable partnerships could be made within these research components.

Nearshore Monitoring Component

This project will compliment APMIs existing Gulf Watch Ocean Acidification Monitoring project (#207101) as a continuation and extension of these monitoring activities. While these proposals are similar with respect to ocean acidification monitoring efforts and an overlap in regional coverage, they do not share the same sampling locations. Instead, these two projects encompass entirely separate monitoring activities, each with distinctly unique sampling locations.

Lingering Oil Monitoring Component
None.

Herring Research and Monitoring component
None.

Synthesis and Modeling Component
None, however, CRRC feels that valuable partnerships could be made within these research components.

Data Management Project
None, however, CRRC feels that valuable partnerships could be made within these research components.

C. With Other EVOSTC-funded Projects (not within the LTRM Focus Area)

Indicate how your proposed project relates to, complements, or includes collaborative efforts with the existing projects funded by the EVOSTC that are not part of an EVOSTC-funded program. Anticipated continuing individual projects for FY22 include project numbers 21210128, 21200127, and 21110853. Use the project search function for project details.

The data obtained in the proposed program may be useful to inform a number of projects, specifically those including mariculture, general marine and coastal terrestrial organism health, human health and subsistence, and oceanographic dynamics. Outreach and education to inform any number of users may also be a potential collaborative portion of this project. CRRC welcomes collaboration and data sharing efforts between these projects and programs. CRRC and APMI staff have reached out to several potential collaborators to establish partnerships, however, formation of these partnerships is still pending. Furthermore, CRRC believes that in representation of the indigenous Peoples of the region, our projects represent those most affected by the spill, and should not be justified by mandated collaboration with specific entities.

D. With Proposed EVOSTC Mariculture Focus Area Projects

Indicate how your proposed project relates to, complements, or includes collaborative efforts with proposed EVOSTC mariculture focus area projects.

APMI was established as the first mariculture research facility in the State of Alaska and is considered a leader in mariculture research initiatives. The proposed monitoring program will contribute to these efforts by establishing baseline oceanic conditions, harmful phytoplankton prevalence, and biotoxin levels across the spill affected region, and may be used a means to inform safe shellfish harvest for all users. This is particularly applicable as an early warning tool to inform risk levels. Using this system, shellfish harvesters may observe an increased risk level, and adjust monitoring within their respective facilities. This project will assist in providing both food safety and economic security to mariculture operations across the spill-affected region.

E. With Proposed EVOSTC Education and Outreach Focus Area Projects

Indicate how your proposed project relates to, complements, or includes collaborative efforts with proposed EVOSTC education and outreach focus area projects.

This project incorporates the involvement of community samplers from all seven of our Tribal consortium communities. Within the CRRC network, regular board meetings are held on a quarterly basis, through which we disseminate data to attending Tribal members, as well as each regional Tribal representative.

CRRC is working in collaboration with The Alaska SeaLife Center, Alaska Sea Grant, Alutiiq Museum and Archaeological Repository, Center for Alaskan Coastal Studies, and Prince William Sound Science Center on a proposal to the EVOS TC titled: Community Organized Restoration and Learning [CORaL] Network. This project aims to create and maintain an ongoing framework that builds the capacity of existing resources within the *Exxon Valdez* Oil Spill impacted region to ensure that current scientific information, skills, and activities are publicly accessible and serve ongoing needs as identified by local communities. the CORaL Network will ensure that: science outreach is relevant, co-created, and culturally responsive to our regional communities, leading to increased public utilization of available knowledge related to the EVOS; the participation of regional youth in community-based science projects increases diversity in future science projects; EVOSTC-funded Long-Term Research & Monitoring, Mariculture, and Restoration projects are integrated with community-identified needs; and increased understanding of Alaska Native knowledge and relations, cultural competency, and collaborative community research principles lead to active, community-informed restoration projects.

The Alaska SeaLife Center's Education Department will produce the following outreach products annually: one distance learning program, four Virtual Visit programs, one Virtual Field Trip, two in-person programs, and content for one rotating mini-exhibit installation. CRRC will work closely with the Alaska SeaLife Center to develop content for one rotating mini-exhibit. Additionally, to create a regional system of demonstrating current EVOSTC-funded work, the Alaska SeaLife Center, Alaska Sea Grant, Alutiiq Museum and Archaeological Repository, Center for Alaskan Coastal Studies, CRRC, and Prince William Sound Science Center will each install at least one video kiosk at their site. Most of these kiosks will be assembled using tablet technology and may be mobile stations that can be used in different locations or in outreach programs. The Alaska SeaLife Center and the Prince William Sound Science Center will construct more robust kiosk stations appropriate to their exhibit halls with heavy visitation.

These kiosks will be linked to a video library, also available on the CORaL Network website. Videos will be created by these core partners and may also be created through mini-grant proposals with EVOSTC-funded scientists. The Valdez Museum has also expressed an interest in creating videos that integrate current scientific data with historical community contexts. These videos are expected to be 2-5 minutes long and may be played on a loop or be available for visitors to select from a touchscreen. The ability to install a system of networked video kiosks, linked to a shared video library, is made possible by the fact that the core CORaL Network partners include visitor centers and outreach sites. These kiosks will provide current data to community members, tourists, and visitors across the region. Thus, the potential for collaborative outreach through a number of partner institutions is being addressed via separate proposals. If accepted, the proposed program will be incorporated in this educational program.

F. With Trustee or Management Agencies

Please discuss if there are any areas which may support EVOSTC trust or other agency work or which have received EVOSTC trust or other agency feedback or direction, including the contact name of the agency staff. Please include specific information as to how the subject area may assist EVOSTC trust or other agency work.

If the proposed project requires or includes collaboration with other agencies, organizations, or scientists to accomplish the work, such arrangements should be fully explained, and the names of agency or organization representatives involved in the project should be provided. If your proposal is in conflict with another project or program, note this and explain why.

No trustee or management agency coordination is involved in this program. To our knowledge, this program is also not in conflict with any other projects or programs.

G. With Native and Local Communities

Provide a detailed plan for local and Alaska Native community involvement in the project. ***This is a mandatory requirement for all proposals.***

CRRC is an intertribal consortium of seven Alaska Native villages located throughout the PWS and LCI regions of Alaska. These consortium members include the Tatitlek Village IRA Council, Chenega IRA Council, Port Graham Village Council, Nanwalek IRA Council, Native Village of Eyak, Qutekcak Native Tribe, and Valdez Native Tribe. CRRC's primary mission is to develop the natural resource capability and capacity for each of our seven member Tribes. CRRC continues to achieve this through improvement of Tribal economies, generation of sustainable jobs, long-term cultivation of native fisheries and mariculture efforts, and engagement in cutting edge biological research efforts undertaken with our academic, agency, and private partners. Since 1994, CRRC has been at the forefront of mariculture research in Alaska, and has led many programs to develop fish and shellfish mariculture, in addition to undertaking important research to better understand oceanic, atmospheric, and climate conditions that affect natural resources and habitats. A major foundation of this project will rely on the community involvement of samplers in each of our consortium locations, providing long term sustainable employment in Tribal communities throughout the region. In addition, CRRC staff members include several Alaska Native Tribe members and stakeholders of the spill-affected region. These staff members are also major participants in this project, and assist with data analysis, information dissemination, and hands-on research. CRRC has engaged with the University of Alaska to begin hosting Alaska Native undergraduate students from the region, some of whom will be regularly working in this program. CRRC acknowledges the disparities in STEM, and actively seeks to close this gap through representation of disadvantaged groups.

12. PROGRAM SCHEDULE

Specify when critical program tasks will be completed including field sampling, data management, meetings, and reporting.
C = completed, X = planned or not completed. For multi-year projects, reviewers will use this information in conjunction with project reports to assess whether the project is meeting its objectives and is suitable for continued funding.

Project milestone and task progress by fiscal year and quarter, beginning February 1, 2022. C = completed, X = planned or not completed. Fiscal Year Quarters: 1= Feb. 1-April 30; 2= May 1-July 31; 3= Aug. 1-Oct. 31; 4= Nov. 1-Jan 31. *Annual review and reporting policy will be discussed at the January 2020 Council meeting. Any changes will be posted on the website.

Milestone/Task	FY22				FY23				FY24				FY25				FY26			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Milestone																				
Water sampling and carbonate chemistry analysis	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Water sampling and nutrient analysis																	X	X	X	X
Phytoplankton sampling and visual analysis	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Phytoplankton sampling and qPCR analysis					X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blue mussel sampling and biotoxin analysis	X	X	X		X	X	X		X	X	X		X	X	X		X	X	X	

Milestone/Task	FY22				FY23				FY24				FY25				FY26			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Annual trainings	X				X				X				X				X			
Data QA/QC	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Reporting																				
*Annual reports					X				X				X				X			
FY work plan				X				X				X				X				X
Science synthesis report									X											
Deliverables																				
Data posted online	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Milestone/Task	FY27				FY28				FY29				FY30				FY31			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Milestone																				
Water sampling and carbonate chemistry analysis	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Water sampling and nutrient analysis	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Phytoplankton sampling and visual analysis	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Phytoplankton sampling and qPCR analysis	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blue mussel sampling and biotoxin analysis	X	X	X		X	X	X		X	X	X		X	X	X		X	X	X	
Annual trainings	X				X				X				X				X			
Data QA/QC	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Reporting																				
*Annual reports					X				X				X				X			
FY work plan				X				X				X				X				
Science synthesis report									X											
Final report																	X	X	X	X
Deliverables																				
Peer reviewed publication																	X	X	X	X
Data posted online	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

13. Budget

A. Budget Forms (Attach)

Please provide completed budget forms (Excel workbook). Please note that costs associated with international travel for meetings, symposia, or presentations will not be considered for funding. Costs associated with outreach or education should be included in the Program budget. Include a screen shot of the "Summary" worksheet (example below).

Budget Category:	Proposed FY 22	Proposed FY 23	Proposed FY 24	Proposed FY 25	Proposed FY 26	5-YR TOTAL PROPOSED	ACTUAL CUMULATIVE
Personnel	$343,695	$352,287	$361,095	$370,122	$379,375	$1,806,574	
Travel	$17,452	$35,860	$17,452	$35,860	$17,452	$124,076	
Contractual	$23,925	$17,725	$17,725	$22,725	$18,925	$101,025	
Commodities	$21,150	$16,800	$17,050	$16,800	$19,900	$91,700	
Equipment	$150,000	$0	$0	$0	$40,000	$190,000	
Indirect Costs (report rate here)	$60,608	$63,063	$61,668	$66,470	$64,999	$316,808	
SUBTOTAL	$616,830	$485,735	$474,989	$511,977	$540,651	$2,630,182	
General Administration (9% of subtotal)	$55,515	$43,716	$42,749	$46,078	$48,659	$236,716	N/A
PROJECT TOTAL	**$672,345**	**$529,451**	**$517,738**	**$558,054**	**$589,310**	**$2,866,899**	
Other Resources (In-Kind Funds)	**$0**	**$0**	**$0**	**$0**	**$0**	**$0**	

INSTRUCTIONS: This summary page provides a five-year overview (FY 22-26) of proposed funding and actual cumulative spending which includes the **non-trustee agency** and **trustee agency workshseets**. **This Summary Page should automatically populate as the formulas reference the cells in the non-trustee agency and trustee agency worksheets. Please make sure the totals given are correct.** The column titled 'Actual Cumulative' will be updated each fiscal year and included in the annual report (include information on the total amount actually spent for all completed years of the project). On the Project Annual Report Form, if any line item exceeds a 10% deviation from the originally-proposed amount; provide detail regarding the reason for the deviation.

Budget Category:	Proposed FY 27	Proposed FY 28	Proposed FY 29	Proposed FY 30	Proposed FY 31	5-YR TOTAL PROPOSED	ACTUAL CUMULATIVE	**TEN YEAR TOTAL**
Personnel	$388,859	$398,581	$408,545	$418,759	$429,228	$2,043,972		$3,850,546
Travel	$35,860	$17,452	$35,860	$17,452	$35,860	$142,484		$266,560
Contractual	$7,325	$7,325	$7,325	$7,325	$9,525	$38,825		$139,850
Commodities	$16,800	$21,150	$16,800	$17,050	$17,550	$89,350		$181,050
Equipment	$0	$0	$0	$0	$0	$0		$190,000
Indirect Costs (report rate here)	$66,968	$66,321	$69,905	$68,719	$73,431	$345,343		$662,151
SUBTOTAL	$515,812	$510,828	$538,435	$529,305	$565,594	$2,659,974		$5,290,157
General Administration (9% of subtotal)	$46,423	$45,975	$48,459	$47,637	$50,903	$239,398	N/A	$476,114
PROJECT TOTAL	**$562,235**	**$556,803**	**$586,894**	**$576,943**	**$616,497**	**$2,899,372**		**$5,766,271**
Other Resources (In-Kind Funds)	**$0**	**$0**	**$0**	**$0**	**$0**	**$0**		**$0**

INSTRUCTIONS: This summary page provides a five-year overview (FY 27-31) of proposed funding and actual cumulative spending which includes the **non-trustee agency** and **trustee agency worksheets**. **The formulas reference the cells in the non-trustee agency and trustee agency worksheets and should automatically populate. Please make sure the totals given are correct.** The column titled 'Actual Cumulative' will be updated each fiscal year and included in the annual report (include information on the total amount actually spent for all completed years of the project). On the Project Annual Report Form, if any line item exceeds a 10% deviation from the originally-proposed amount; provide detail regarding the reason for the deviation.

B. Sources of Additional Funding

Fill out the summary table below (should match the table on page 2). Provide a narrative that Identifies non-EVOSTC funds or in-kind contributions used as cost-share for the work in this proposal. List the amount of funds, the source of funds, and the purpose for which the funds will be used. Do not include funds that are not directly and specifically related to the work being proposed in this proposal. Please attach documentation from additional project funding sources which confirms and describes matching funds, including date(s) the matching funds are/will be authorized.

Non-EVOSTC Funds to be used, (round to the nearest hundred) please include source and amount per source:

FY22	FY23	FY24	FY25	FY26	FY22-26 Total
$180,690					$180,690
FY27	**FY28**	**FY29**	**FY30**	**FY31**	**FY27-31 Total**
				FY22-31 Total	**$180,690**

Two existing awards will be provided as match for the proposed project. These are currently funding the establishment of the CROM program, and will provide for materials and limited sample analysis through FY22.

Administration for Native Americans: "Community based Harmful Algal Bloom monitoring to provide an early warning of paralytic shellfish poisoning in Southcentral Alaska"
Award period: September 30, 2020-September 29, 2022
Award amount: FY21: $154,560; FY22: $151,118
This award provides funding for community-based phytoplankton tow sampling and basic microscopic algal speciation and data collection at each of CRRC's Tribal sampling locations. Training for proper microscopic phytoplankton identification and data collection from collaborators at NOAA is included in this workplan. In FY22, this award also provides capacity for shellfish biotoxin testing at APMI using Blue Mussels obtained through community sampling.

USGS Climate Adaptation Science Center: "Building Capacity for Managing Climate Change Strategies Through Tribal Monitoring of Harmful Algal Toxins in Subsistence Harvested Shellfish"
Award period: January 1, 2021-December 31, 2022 (delayed)
Award amount: FY21: $102,853, FY22: $29,572
This award also provides funding for shellfish biotoxin testing capacity at APMI, largely through the purchase of additional laboratory equipment and supplies. Expected award date was January 1, 2021, however, due to the current changes in federal administration, final processing for this funding has not been completed and documentation is unavailable.

14. LITERATURE CITED

Provide literature cited in the proposal.

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Appendix 1: Dissolved Inorganic Carbon Analytical Protocols

The Ocean Acidification Research (OAR) Laboratory at the Alutiiq Pride Marine Institute (APMI)

A description of the OAR lab practices for analyzing discrete samples

Community Monitoring Program

The OAR lab at APMI analyzes discrete ocean seawater samples from locales around the state in order to obtain baseline data to assess possible community vulnerability to changing ocean chemistry. In order to attain a data set that temporally represents a locale over time, with robust data, a weekly sampling protocol was adopted. The weekly sampling, ideally on the same day and time, performed by a citizen sampler has proven to be an achievable model that can capture statistically meaningful trends over time and provide needed data to inform management and local community resilience planning efforts.

All the laboratory processes at APMI, from liquid bag construction and glassware preparation to data finalization, are adapted from other state of the art laboratories protocols for analyzing like samples with identical equipment. Additionally, system maintenance is performed according to manufacturer's suggestions for specific system components. The analytical equipment used to process the seawater samples is a unique state of the art system developed for analyzing TCO_2 and pCO_2 (developer Burke Hales, Professor of Oceanography at Oregon State University).

Sample Acquisition and Analysis

- **Dissolved inorganic carbon (DIC) sampling technique:**

Our method of collecting samples for DIC analysis was adapted from the protocols posted on NOAA's Pacific Marine Environmental Laboratory website. The document, DIC sampling version 3.0 SOP 1, October 12, 2007, is presented in Reference 1. and can be found online at https://www.ncei.noaa.gov/access/ocean-carbon-data-system/oceans/Handbook_2007/sop01.pdf

The above referenced sample acquisition protocol was modified to a apply to shore side collection (versus typical shipside Niskin bottle collection) through collaboration with The Hakai Inst., and OSU (Wiley Evans, Oceanographer at the Hakai Institute, Burke Hales at Oregon State University, and Jacqueline Ramsay at Alutiiq Pride Marine Institute) to make field sampling at remote Alaskan sites more efficient and streamlined. The resulting sampling method and kit developed for community sampling with protocols is illustrated in Reference 2.

- **Discreet Sampling Analysis**

The methods developed for analyzing discreet bottle samples using the Burke-O-Lator were developed by the Hales laboratory at OSU and written into protocol by the Hakai OA laboratory. Several QA/QC steps are employed before during and after sample analysis that will not be covered here. The complete protocol (Reference 3) details specific instrumentation and analysis parameters to be followed to acquire acceptable data within the standards set forth by the OA research community. The peer reviewed literature containing established analytical methods are listed on page 2 of the Hakai protocol.

In brief - Liquid standards are prepared gravimetrically, from laboratory grade reagents of sodium carbonate (Na_2CO_3), sodium bicarbonate ($NaHCO_3$) and hydrochloric acid. Technicians are guided by a an excel template recipe developed through the Hales laboratory that allows

users to input actual values and acquire real time chemical concentrations targeting specific alkalinity and TCO_2 targets. Three liquid standard concentrations are produced in the lab semi-monthly and stored in airtight Mylar bags. The 3 concentrations are run in series to calculate a liquid standard curve for TCO_2. Certified reference material, also called CRM's which are known liquid standards, produced by the Andrew Dickson lab at U.C. San Diego (Reference 4) are used to ascertain the error of the liquid standards. Gas standards of 3 CO_2 concentrations (150, 750 & 1500 ppm), manufactured by Praxair are used to run standard curves for pCO_2. The gas, liquid standards and CRM's are analyzed at the beginning and end of each set of samples that are analyzed in a day to correct for drift over time (changing temperature or atmospheric pressure) during a sample run. Data acquired during sample analysis through LabView software developed by Dr. Hales, are input into an excel spreadsheet designed to integrate the liquid, gas and CRM standard values and return data that is ready to be processed through CO2SYS (with selected constants, Hakai protocol pg. 14) to obtain finalized TCO_2, pCO_2, pH, saturation state of aragonite (Ω_{Arag}) and total alkalinity.
With the pandemic of COVID-19 the Dickson laboratory has dramatically cut the production of CRM's. The lack of availability of these standards initiated a change in protocol that is being adopted by all laboratories that utilize a Burke-o-lator for their seawater sample analysis. The Hakai laboratory developed targeted Alkalinity and TCO2 standards that are made in batches in the laboratory, fixed and stored back for daily reference during the analysis of seawater samples.

- Data Management & Analysis

The OAR lab director evaluates the data for outliers and trends over time relative to the above-mentioned carbonate data and relies on collaborating oceanographers to interpret data sets. Finalized data are furnished to the respective principal investigator for that set of samples, or in the case of village sample data the data is made available to the local community council. Currently our data is stored and shared on the Google platform using Excel spreadsheets. The new APMI website will feature finalized data as it is available. Future plans for our data repository are to also place data sets on the National Centers for Environmental Information (NCEI) website at www.ncei.noaa.gov, where NOAA hosts and provides public access to one of the most significant archives for environmental data on Earth.

References

Reference #1
NOAA DIC sampling version 3.0 SOP 1, October 12, 2007
https://www.ncei.noaa.gov/access/ocean-carbon-data-system/oceans/Handbook_2007/sop01.pdf
Reference #2

APMI sampling kit developed for community sampling with protocols

Sea Water Sample protocol;

1. Log the date and locale of the water to be sampled on the data sheet as well as any comments.
2. Label the bottle with the date, bottle # and location using the paint pen and allow to dry. *It's best to get the paint pen working before going out to the field and label the bottle so that the*

paint has a chance to dry. Sharpies can be used to # the caps with the bottle # but please do not use them to label the bottle.

3. Grab your sample in a bucket and record the temperature on the data sheet.
4. Triple rinse the bottle and cap; using the same sample water that you will fill the bottle with dunk the bottle and cap in the sample water and toss the rinse water out; do this 3 times and then fill the bottle with the sample water carefully without introducing bubbles. Toss out a bit of water leaving a short 0.5" head space in the bottle.
5. Immediately spike the sample with one drop of fixative (please read the MSDS for Mercuric Chloride included in your kit). Since this is a saturated solution it should always have a precipitate at the bottom that will not go into solution, that is normal. A clean drop of fixative without the sediment at the bottom is ideal.
6. Cap bottle; place bottle in secure spot, place cap on and then place the capper on top of the cap and press down on the capper arms firmly. Invert the sample bottle a few times. The sample is now shelf stable at room temperature.
7. Log data in the data log sheet provided.

The samples can be stored in the action packer until all the bottles are used and then the action packer with the samples and log can be returned to the Ocean Acidification Research lab at APMI. We will provide a prepaid shipping label for you, just give us the weight of the full action packer and the date you wish to ship it to Seward.

Reference #3

Hakai Institute
Burke-o-Lator pCO_2/TCO_2 Analyzer
Discrete Sample Analysis Protocols

2017.3.20 Version 1.0

Katie Pocock[1], Wiley Evans[1], Alex Hare[1], and Burke Hales[2]

[1]Hakai Institute, BC, Canada
[2]Dakunalytics, OR, USA

The following protocol includes:

The Burke-o-Lator (BoL) pCO_2/TCO_2 analyzer measures carbon dioxide partial pressure (pCO_2) and total dissolved inorganic carbon (TCO_2) both continuously from a flow-through seawater stream and from seawater collected in discrete samples. The Hakai Institute operates BoL units at shore-side facilities along the Pacific coast in Alaska and British Columbia, and our discrete sample analyses of seawater marine carbonate system parameters are conducted using this instrumentation. This protocol is intended for BoL users that are familiar with the operation and troubleshooting of the BoL and control software, as well as general lab safety. If the user is unfamiliar with any of the chemical solutions and reagents used in the following procedures, they are required to read the appropriate MSDS forms.
NOTE: These protocols are based on information in the following references:

Hales, B., A. Suhrbier, G. G. Waldbusser, R. A. Feely, and J. A. Newton (2016), The Carbonate Chemistry of the "Fattening Line," Willapa Bay, 2011-2014, *Estuaries and Coasts*, DOI 10.1007/s12237-12016-10136-12237.

Barton, A., B. Hales, G. Waldbusser, C. Langdon, and R. A. Feely (2012), The Pacific oyster, *Crassostrea gigas*, shows negative correlation to naturally elevated carbon dioxide levels: Implications for near-term ocean acidification effects, *Limnology and Oceanography*, *57*(3), 698-710.

Bandstra, L., B. Hales, and T. Takahashi, 2006: High-frequency measurements of total CO_2: Method development and first oceanographic observations. *Marine Chemistry*, **100,** 24-38.

Hales, B., D. Chipman, and T. Takahashi, 2004: High-frequency measurements of partial pressure and total concentration of carbon dioxide in seawater using microporous hydrophobic membrane contactors. *Limnology and Oceanography: Methods*, **2,** 356-364.

Takahashi, T., J. Olafsson, J. G. Goddard, D. W. Chipman, and S. C. Sutherland (1993), Seasonal Variation of CO_2 and Nutrients in the High-Latitude Surface Oceans: a Comparative Study, *Global Biogeochemical Cycles*, *7*(4), 843-878; doi:810.1029/1093GB02263.

Wanninkhof, R., and K. Thoning (1993), Measurement of fugacity of CO_2 in surface water using continuous and discrete sampling methods, *Marine Chemistry*, *44*, 189-204.

1. Liquid Standard Preparation

1.1. Bag Construction

Materials Needed:

- Food grade vacuum sealer bag
- 3/4” Through-wall fitting (1/4 Thread/Pipe Size)
- Straight adapter (1/4” female push-to-connect fitting to male threaded 3/4” pipe)
- 3/4” Plastic washer
- 3/4” Silicone sealing washer
- 3/4” hole punch
- Plastic or wooden cutting board
- Teflon tape
- Hammer
- 100% Silicone adhesive sealant
- Hair straightener
- Ball valve (1/4” female push-to-connect fittings)
- 90° elbow fitting (1/4” female push-to-connect fittings)
- 1/4” polyethylene tubing (<2 ft.)
- DI water

1. **Make the fitting hole.** Place a plastic or wooden cutting board inside one of the bottom corners of the bag, place the hole punch on the bag approximately two inches away from the bottom and side of the bag and tap gently with a hammer.
2. **Glue the washers to both sides of the hole.** Take one silicone sealing washer and one plastic washer and glue them together with silicone sealant, then apply glue to the remaining side of the silicone sealing washer and glue to the inside of the hole. Repeat for the outside of the hole. Spread the glue thinly to provide the best seal, and avoid getting glue anywhere else on the inside of the bag as pieces can come loose and clog the delivery line to the BoL system.
3. **Attach the through-wall fitting.** Insert the fitting into the hole, with the main fitting body on the outside of the bag. Apply a thin layer of silicone glue to the flat side of the through-wall nut and to the screw threads of the through-wall fitting. Screw the nut onto the through-wall fitting, and hand tighten, making sure to not deform the washers.
4. **Attach the straight adapter to the through-wall fitting.** Wrap the threads on the straight adapter with Teflon tape and screw into through-wall fitting, tighten using pliers so that it is fully screwed into the fitting, being careful to not let the through-wall fitting twist as this could compromise the silicone seal.
5. **Assemble the remaining plumbing.** Attach a 1” length of tubing to the straight adapter’s push-to-connect fitting, followed by the elbow fitting, another 1” length of tubing, and ball valve.
6. **Seal all of the washer joints on the outside of the bag.** Apply silicone glue to the washer joints from the bag to the through-wall fitting body, making sure to not leave any gaps.
7. **Allow glue to set for 24 hours**
8. **Test the fitting for leaks.** Fill the bag with water and leave it to sit on the bench for a few hours, if a leak is found, dismantle the fitting parts and reseal with silicone where necessary. Rinse out the inside of the bag with DI water to remove any particles, and store open upside down to air dry.

9. **Seal the bag closed.** Using a hair-straightener (at ~250°C) press the top two inches of the open end together between the heating plates of the straightener, hold for approx. 5 seconds, and repeat across the top of the bag until the entire end of the bag is sealed
10. **Test the bag for leaks.** Allow the bag to cool for an hour or so, then test by filling the bag with DI water and leaving it to sit for a few hours, if no leak is found, bag is ready to be used.

1.1. Primary Standard Preparation

A batch of CO2-stable primary standard solution may be prepared ahead of time, and used for multiple batches of working standards, provided it is stored in a tightly sealed bottle and is used within 2-3 months.

Materials Needed:

- High precision balance (to 0.0001 g)
- Large Capacity balance (>5000 g)
- DI water
- Sodium carbonate reagent (dried)
- Sodium bicarbonate reagent
- Scoopula
- Sealable bottle/container (>2 L)
- 1L Graduated cylinder or jug
- Plastic weigh boats (x2)
- Tin weigh boat
- Squeeze bottle
- Small plastic funnel

1. **Create a worksheet.** Open the Lab_TCO2_Std_Prep template, and "Save As" with the lab and date in the file name (ex.*Lab_TCO2_Std_Prep_Hakai_April_29_2016.xlsx*)
2. **Enter reagent information.** Ensure that the formula weights and corrections for the carbon content of the dry reagents are consistent with those entered in the datasheet, and correct if necessary. Provide any additional information about the dry reagents used (e.g. manufacturer, batch number, catalogue number, etc.).
3. **Enter primary batch information.** Determine the target final primary solution total mass in grams, and enter into the datasheet. Enter preparation date, and name of analyst
4. **Weigh out 75% of DI water.** Place the final solution bottle (must have tight seal if storing) on the large capacity balance and tare (leave this bottle on the balance for the remainder of the preparation). Using a jug or graduated cylinder, fill the solution bottle with DI to within ~75% of the target final mass. Ensure that DI water has been pre-equilibrated with ambient atmospheric CO_2 by venting for ~ 3 days. A primary standard may be made without assessing the DI blank (DI TCO_2 will typically be ~20 umol/kg or less, or 0.0001% of typical primary concentration targets).
5. **Weight out dry reagents.** On a high-precision balance, weigh out the Na_2CO_3 (sodium carbonate) and $NaHCO_3$ (sodium bicarbonate) into plastic weigh boats to within 0.1 g of the target mass indicated in the datasheet. Na_2CO_3 absorbs moisture, therefore it is important to dry it before weighing. To do this, spread a thin layer of the reagent in a tin weigh boat or glass dish and dry in a muffle furnace at 250°C for 2 hours. For continued use, store the dried reagent in a desiccator and re-dry every month.
6. **Add dry reagents to solution.** Using a plastic funnel, add the reagents to the final solution bottle, thoroughly rinsing the weigh boats and funnel into the solution bottle with a squeeze bottle of DI water. Enter the actual masses of weighed reagents in the red-bordered cells of the datasheet.

7. **Top up with DI to final mass.** Top up the solution bottle with DI until target final solution mass is reached, use a pipette or squeeze bottle to get within 1 g of the total target mass. Close the bottle and shake to prevent powder caking on the bottom of the bottle (do this prior to topping up with DI if there is insufficient headspace). Label bottle with solution preparation date, final Alk and TCO_2, and name of preparer.

1.2. Working Standard Preparation

The number and target concentrations of BoL working standards is user-specified, and dependent on the expected TCO_2 range of discrete samples or continuous seawater flow analyses.

Materials Needed:

- High precision balance (to 0.0001 g)
- Large Capacity balance (>5000 g)
- DI water (vented for >3 days)
- 0.1N Hydrochloric acid
- Primary Standard solution
- Saturated mercuric chloride solution (optional)
- Intermediate containers/jugs (>4 L)
- 1L Graduated cylinder or jug
- Small pipette/dropper (x3)
- Squeeze bottle
- 50 mL glass beaker (x2)
- Final containers/bags (>4 L)

1. **Create a worksheet.** Open the Lab_TCO2_Std_Prep template, and "Save As" with the lab and date in the file name (ex. *Lab_TCO2_Std_Prep_Hakai_April_29_2016.xlsx*). If primary standard was prepared on the same day, the same worksheet may be used for both primary and working standard prep.
2. **Enter primary and acid information.** Enter the information regarding the primary standard, and certified hydrochloric acid (HCl) solution used (do not use diluted HCl stock as this will introduce error into the standard concentration calculations).
3. **Enter standard information.** Enter the target TCO_2 concentrations and final masses for each working standard. Enter the DI blank for each standard (see Section 1.3 for DI blank determination).

For each working standard solution:

1. **Weigh out 90% of DI water.** Place the secondary container on a large capacity balance and tare (leave this container on the balance for the remainder of the preparation). Using a jug or graduated cylinder, fill the container with DI to within ~90% of the target final solution mass.
2. **Weigh out acid.** Place a 50 mL beaker on a high-precision balance and tare, weigh out the 0.1 N HCl into the beaker, use a small pipette or dropper to get final weight within 0.1 g of the target mass indicated in the datasheet.
3. **Add acid to solution.** Add the acid to the secondary container, thoroughly rinsing the beaker into the solution container with a squeeze bottle of DI water. HCl is highly volatile at this concentration, add the acid to the container as soon as the weight is recorded. Enter the actual mass of weighed HCl in the red-bordered cells of the datasheet.

4. **Weigh out primary.** Place a second 50 mL beaker on a high-precision balance and tare, weigh out the primary standard into the beaker, use a small pipette or dropper to get final weight within 0.1 g of the target mass indicated in the datasheet.
5. **Add primary to solution.** Add primary standard to secondary container, thoroughly rinsing the beaker into the solution bottle with a squeeze bottle of DI water. Enter the actual mass of weighed primary in the red-bordered cells of the datasheet.
6. **Add mercuric chloride to solution.** using a pipette or dropper, add 5-10 drops (200-400 µL) of saturated mercuric chloride ($HgCl_2$) solution to the secondary container. This step may be omitted if the working standard solution will be used within ~1 month, or the lab does not have the capacity for $HgCl_2$ waste removal.
7. **Top up with DI to final mass.** Top up the container with DI until target final solution mass is reached, use a pipette or squeeze bottle to get within 1 g of the total target mass. Close the container and shake to homogenize the solution.
8. **Rinse and fill standard bag.** As the intermediate containers are not air tight, working standards must be transferred to bags immediately following preparation. To rinse the bag, dispense approx. 200 mL of the new working standard into the bag, shake, and empty. Fill with the remaining working standard solution. Remove the air inside the bag by laying it down flat on the bench with the spigot open, push down on the bag until liquid begins to dribble out, and close the spigot while keeping pressure on the bag.

1.3. Blank TCO_2 Determination

The blank TCO_2 value may be determined either before, or after working standard preparation. If done after, the user must ensure that the working standard actual TCO_2 is updated with the appropriate DI blank data in the preparation worksheet.

1. **Collect a DI sample.** Using a borosilicate or beer bottle, collect a sample of DI water. It is important that the DI used for the blank determination comes from the same batch of DI that was used for the working standard preparation.
2. **Perform gas and liquid calibration.** Run a gas and liquid standard sequence on the system (protocol in Section 3.2). Enter the gas and liquid calibration slopes into the datasheet.
3. **Perform DI TCO_2 analysis.** Analyze the DI sample as a discrete TCO_2 sample on the BoL (protocol in Section 3.3), and record the TCO2_xCO2 average in the "matrix detector response" cell of the datasheet.
4. **Record BoL detector zero reading.** While the system is in wait mode, step the gas selection valve selector to the "Scrubber" position. Wait for the LI-COR reading to stabilize and enter the average value in the "detector zero-gas reading" cell of the datasheet. If the BoL has a nitrogen gas cylinder hooked up to it, then you may use this to determine the detector zero-gas reading instead. The nitrogen gas zero reading can also be compared with the reading of the scrubber gas to assess the scrubber's efficiency.
5. **Enter DI TCO_2 into standard calculations.** Copy the final DI TCO_2 value (in the green shaded cell "matrix TCO_2, µmol/kg"), into the "estimated DI blank" cell of the working standard prep table.

1.4. Working Standard Analysis

A new set of working standards may be analyzed for TCO_2 using the previous liquid standard calibration curve, to determine the consistency of the liquid calibration slope, offset, and r^2 value between the two sets of standards. That is, it allows the user to "test" the new working standards before taking the old set offline.

1. **Plumb standard bag into discrete TCO_2 loop.** Remove the liquid line from the discrete sample bottle cap, and plumb it into the working standard bag and open the valve.
2. **Analyze standard TCO_2.** Follow the discrete TCO_2 sample analysis procedures (outlined in Section 3.3), and repeat for each working standard (gas and liquid standard sequences must be performed prior to and following analysis).
3. **Calculate standard curve.** Graph the analysis final TCO_2 values against the target TCO_2 values to determine the slope, offset and r^2 value of the new working standards (target calibration curve slope=2.0, offset=0, $r^2 = \geq 0.9999$).

1.5. Working Standard Installation

1. **Determine standard sequence.** Decide the order in which each standard will be run within the sequence, with the preference that the system be driven across the largest range in order to better test the system response, and that the sequence end on the standard most comparable to the sample reading, in order to minimize the extent of transition data.
2. **Install standards.** Plumb each working standard bag into the liquid standards selection valve, and open the spigots. The order in which the standards are run is dependent on their position on the selection valve, so ensure that the bags are plumbed into the correct line before running.
3. **Enter standard information.** In the "Standardization parameters" tab of the system software click "Enter new standards" and enter the final calculated TCO_2 values of the new standards according to their valve positions.
4. **Run standard sequence.** Run the new standards as a sequence by selecting the "Liquid Standards" mode in the software. Re-run the new standards until the calibration slope, offset and r^2 value are satisfactory (target calibration curve slope=2.0, offset=0, $r^2 = \geq 0.9999$).

2. Discrete Sample Collection

2.1. Sample Bottle Preparation

1. **Rinse bottle.** Following emptying of bottle, immediately rinse with hot water, filling the bottle completely, and shaking. Repeat for a total of three rinses.
2. **Air dry and store for proper cleaning**

2.2. Sample Bottle Cleaning

1. **Rinse and sterilize bottles.** Fill bottles with DI water to approx. 25% volume, shake vigorously and empty. Repeat for a total of two rinses. Place bottles inside autoclave basket, fill autoclave with tap water, close autoclave, select "instrument" setting, and turn timer dial to 20 minutes. The use of an autoclave may be substituted for submerging the sample bottles in a pot of boiling water for 10 minutes.
2. **Dry and store bottles.** Once bottles are sterilized, allow them to cool, and place upside down on a drying rack or in an oven (at ~50°C) to dry. For long-term storage, cover amber glass bottle opening with Parafilm. Store bottles in a clean dry place.

2.3. Discrete Sample Collection

Materials Needed:

- Cleaned beer bottles
- Bottle caps
- Capping stand
- Sampling noodle (surgical tubing)
- 1 mL pipette

- Pipette tips
- Saturated mercuric chloride solution
- Disposable gloves
- Waste container (ex. Ziploc bag)
- NIST certified thermometer

During sample collection, all sample metadata should be recorded on a paper log sheet.

1. **Prepare sampling equipment.** Pre-label bottles with unique ID, sampling location, collection date, and depth. Put on gloves, and prepare sample bottle and cap to be sampled.
2. **Perform an integrity check of the Niskin bottle to be sampled.**
    a. Visually inspect the Niskin bottle for leaking water.
    b. Open the spout and inspect it for leaking water.
    c. Close the spout, and open the air vent slightly by unscrewing it.
    d. If any water leakage is observed during the above steps, check if adjusting an end cap or tightening the air vent stops the leak, and record any observations in the sampling log sheet.
3. **Rinse bottle with sample water.** Attach the sampling noodle to the Niskin petcock, and open the air vent. Open the spout, as water begins to flow out of the Niskin bottle, massage the noodle to flush out any bubbles (especially nearest the spout). Fill the sample bottle ~20% full with sample water then squeeze off the flow from the sample noodle. Cover the bottle mouth with a cap, and shake vigorously to rinse the bottle, pour sample rinse over the cap, and repeat the full rinse process for a total of three rinses.
4. **Collect sample.** To fill the bottle, insert the sampling noodle to the bottom of the bottle, slowly release pinch on the sampling noodle, allowing seawater to flow into the bottle in a controlled manner (try to prevent any bubbles or turbulence in the bottle). Slowly lift the noodle out as the bottle fills, while keeping the end of the noodle submerged. Fill bottle with sample up to the rib near the top of the bottleneck.
5. **Record sample information.** Once full, insert NIST thermometer into bottle (making sure to not let the probe touch the glass) and record sample temperature on log sheet. Information such as date, time, and GPS coordinates should also be recorded at this time.
6. **Preserve and cap sample.** Using the pipette, carefully dispense 200 µL of saturated mercuric chloride solution ($HgCL_2$) into the sample. Position bottle in stand capper, place cap in magnet of capper, push capper lever down until the cap is securely snapped over the bottle lip. Invert the bottle to allow $HgCL_2$ to mix throughout the sample.
7. **Clean work area.** Place gloves, pipette tips, and any other waste in the waste container and mark as "$HgCL_2$ HAZMAT".

3. Burke-O-Lator Discrete Sample Analysis

When running a batch of discrete samples for pCO_2/TCO_2 analysis, the following sequence of analyses are performed:

1) Gas Standards
2) Liquid Standards
3) CRM TCO_2 analysis (x3)
4) Discrete Sample Processing
    a) TCO_2 analysis
    b) pCO_2 analysis
    c) Salinity measurement (when needed)
5) CRM TCO_2 analysis (x3)
6) CRM pCO_2 analysis (1x)
7) Liquid Standards
8) Gas Standards

During batch sample processing, all analysis data should be recorded on a paper log sheet.

3.1. System Start-Up

1. **Start system.** Turn on power switch on the side of the electronics box.
2. **Open system software.** Turn on the computer, and open "pTCO2_combo" software file with appropriate version (e.g. dot9 is current as of Feb/2017) in LabView. Press the white run arrow in the top left corner. Verify that all sensors/inputs are actively updating in the "real-time raw data" tab.
3. **Start software.** Select the "start run" button, select a location where output files will be stored, and a prefix for filenames (e.g. "Data/FieldData"). Select data file prefix specific to your site (e.g. "quadra"). Enter/verify standard values and valve positions
4. **Allow system to warm up.** Wait until LI-COR cell temp has reached stable operating temperature (~51°C), verify that the mass flow controller is maintaining flow at 500 sccm, and that the LI840 xCO_2 reading is consistent with ambient air pCO_2 (~400 uatm). Check system fluid levels (waste carboy, 1N HCl bottle, and rinse water jug)

3.2. Gas and Liquid Standard Sequences

1. **Generate pCO_2 (gas standard) calibration curve.**
   a. Select "Gas standards" mode.
   b. Check that all gas standards are flowing at proper rate. Mass flow controller (MFC) should be set to 900 sscm, gas cylinder delivery pressure set to 10-20 psi, and gas cylinder internal pressure should be constant at ~1200 psi
   c. Verify that calibration curve information is acceptable and atmospheric signal is legitimate (target calibration curve slope=1.0, offset=0, r^2= ≥0.9999)
2. **Generate a TCO_2 (liquid standard) calibration curve.**
   a. Select "Liquid Standards" mode.
   b. Check that strip-gas is flowing at the correct rate (i.e. MFC set to 900 sccm).
   c. Check liquid-side backpressure is at 5-15 psi, and gas-side pressure is about half of liquid-side pressure (gauges on wet bench).
   d. Check that the FMI pump flow rate is appropriate by (1) checking the mass flow reading in the "real time data" tab of the system software, and (2) checking the percentage output on the pump controller (set to 14.3 % ±0.1). If the controller output is fluctuating over 0.1 units, then the pump controller should be operated manually (turn setting to manual on controller box).
   NOTE: LI-COR xCO_2 response to the TCO_2 analysis is dependent on the ratio of gas to liquid flows. By maintaining the MFC at the set point, and adjusting the FMI output, the user will subsequently adjust the response of the system and alter the slope of the calibration curve. The target slope is 2; to get a higher (lower) slope, decrease (increase) the FMI output.
   e. Check that no bubbles cross the stripper membrane contractor.
   f. Verify that calibration curve information is acceptable (target calibration curve slope=2.0, offset=0, r^2= ≥0.9999).
3. **Validate calibration curves.** If the initial sequence run does not achieve calibration curves within target slope, offset, and r^2 ranges, re-run the sequence. Generated calibration curves should be within the following approximate ranges to continue with sample analyses; slope±0.02, offset±25, r^2±0.001. If standard calibration curves are not consistent between runs, troubleshoot for possible sources of error (such as sample flow control at FMI pump)

3.3. Discrete TCO_2 Sample Analysis

During TCO_2 analysis, sample water is pumped through the stripper by the Fluid Metering Inc. (FMI) pump mounted to the wet bench. The pump flow is regulated by a motor controller that may be operated manually, or through the computer software. The following protocol is specific to manual operation of the FMI pump controller.

1. **Prepare sample for analysis.** Ensure that there is sufficient headspace in the sample bottle, this need only be enough so that insertion of the tubes & bubbler do not cause sample to overflow. If sample is too full, extract the appropriate amount using a pipette.
2. **Set up sample on wet bench.** Uncap bottle, insert thermometer and discrete sample lines into bottle, rest cap on top, attach lanyards to bench hooks, and sinch until cap is securely fastened to the bottle top. Turn on sample thermometer.

Note: If you are using borosilicate bottles with greased lids, insert the thermometer/tubing carefully so that no grease enters the bottle or gets on the probe or discrete sample lines. To help with this, an insert may be made using a 10 mL pipette tip with the tip end cut off and a gap cut down the side of the tip. Insert this into the bottle neck, pass the thermometer, bubbler and tubing through the insert until it passes the greased bottle neck, and remove the insert, using the gap in the side to allow the tubing and thermometer to remain inserted into the bottle.

3. **Enter sample information into system software.** Enter sample ID, salinity, and analysis temperature into the Sample_ID, DscSmpl_S, DscSmpl_analysis_T boxes in the "processed discrete samples" tab of the software (if salinity data is not yet recorded leave box as "NaN").
4. **Begin TCO2 analysis.** Turn on the FMI pump (press "ON" switch on FMI pump controller inside BoL box). Select "Discrete TCO2 Sample" mode. Start timer. As specified above, verify operation of gas flow, liquid and gas pressures. After approximately 1-1.5 minutes, signal should start to plateau.
5. **Take sample reading.** At 2.5 minutes, click "start averaging". Mode will automatically switch to "wait" after averaging is complete. Note that the 2.5 minutes duration time matches that of the liquid standard runs and is implemented for discrete samples to standardize operation between users. Shut off FMI pump once reading is taken (press "OFF" switch on FMI pump controller inside BoL box). If running discrete pCO_2 analysis as well, continue to Section 3.5.
6. **Log sample data.** If sample analysis is complete, select "Log sample average data" mode.
   a. Verify that the information in the upper row in the pop-up window is correct. If it is, select 'Accept Data and Write to File".
   b. If the data is incorrect, but salvageable with some editing, select 'Accept Edited Data'. Enter corrections in the appropriate boxes (update analysis temperature if thermometer reading has changed) and click the 'Edit finished' button.
   c. If the data is bad, select 'Reject data'.

3.4. Discrete pCO_2 Sample Analysis

If the BoL system is setup for continuous seawater pCO_2 analysis, the pCO_2 gas loop will need to be re-plumbed to go to the sample bottle instead of the equilibrator. To do this, re-place the equilibrator supply and return gas lines (entering and exiting the box), with the sample supply and return lines that go to and from the bottle cap (ie. replace equilibrator supply with bottle supply (bubbler), and equilibrator return with bottle return).

1. **Prepare sample for analysis.** Ensure that there is sufficient headspace in the sample bottle, a single TCO_2 analysis is usually sufficient (approx. 60 mL).
2. **Begin pCO_2 analysis.** Select "Discrete pCO2 Sample" mode on system software. Start timer. Check inside bottle that bubbler is functioning correctly, and that liquid is not foaming up into the headspace gas line. Blow around bottle cap to test for air leaks (LI-COR response will spike in the event of contamination). After approximately 3-5 minutes, signal should start to plateau.
3. **Take sample reading.** At 10 minutes, click "start averaging". Mode will automatically switch to "wait" after averaging is complete.
4. **Log sample data.** Once sample analysis is complete, select "Log sample average data" mode
   a. Verify that the information in the upper row in the pop-up window is correct. If it is, select 'Accept Data and Write to File"
   b. If the data is incorrect, but salvageable with some editing, select 'Accept Edited Data'. Enter corrections in the appropriate boxes (update analysis temperature if thermometer reading has changed) and click the 'Edit finished' button.
   c. If the data is bad, select 'Reject data'

3.5. CRM Analysis

Certified reference materials (CRM's) are seawater samples of known carbonate composition prepared by Andrew Dickson's laboratory at the Scripps Institution of Oceanography of the University of California, San Diego. CRM's are analyzed on the BoL system in order to determine the accuracy of the TCO_2 analyses, and to perform a CRM correction in order to maintain consistency with other seawater CO_2 laboratories. One CRM bottle is analyzed for every batch of discrete samples. The CRM bottle is analyzed for TCO_2 three times before beginning the sample batch, and three times at the end of the sample batch. Following the final CRM TCO_2 analysis, the CRM bottle undergoes pCO_2 analysis in order to evaluate consistency with the calculated pCO_2 of the CRM bottle. Triplicate CRM TCO_2 analyses conducted at the beginning and end of a sample batch are each averaged and then compared with the certified TCO_2 value of the CRM bottle in order to produce correction factors. The CRM correction factors are then interpolated between the time intervals of the sample batch analyses, and used to correct sample TCO_2 measurements. Standard deviations are also calculated from the triplicate CRM analyses in order to gauge native analytical precision.

1. **Analyze CRM TCO_2.** Follow the protocol for discrete TCO_2 sample analysis (Section 3.3). Repeat for a total of three runs, logging the TCO2_xCO2 reading for each
2. **Validate analysis readings.** If the runs are highly repeatable (i.e. TCO2_xCO2 standard deviation is <1%), continue to sample analysis. If runs are highly variable (i.e. TCO2_xCO2 standard deviation is >2%), do not continue with analyses but rather troubleshoot for possible sources of error. The CRM correction factor (i.e. native analytical TCO_2 accuracy) is calculated during the data processing steps, therefore only CRM repeatability (i.e. system TCO_2 precision) is evaluated at this stage.

3.6. Discrete Sample Salinity Measurement

If the sample salinity was not recorded in the field, then it may be measured following TCO_2/pCO_2 analysis using a 4310 YSI probe with 4010 MultiLab interface. Prior to use, the YSI probe accuracy must be verified using a CRM bottle, and manually calibrated if necessary. Once all sample analyses are complete, the YSI probe accuracy must be verified again with the same CRM bottle to determine whether the sensor drifted over the analysis period.

1. **Set up probe.** Turn on the MutiLab interface, select the salinity setting by pressing "M" until the appropriate parameter is displayed.
2. **Take CRM salinity reading.** Using the same CRM bottle used for TCO_2 analysis, measure the CRM salinity by inserting the probe into the bottle, submerging as much of it as possible, and gently stir the probe to remove any air bubbles around the sensor. Wait 1-2 minutes for the reading to stabilize, and record in the analysis log sheet. If the CRM salinity reading is not accurate (based on known CRM batch salinity), leave probe inserted in CRM bottle and proceed to step 3. If the reading is accurate, remove probe from bottle and continue to sample processing.
3. **Perform manual sensor calibration.**
    a. While leaving YSI probe submerged in CRM bottle, go to calibration settings by pressing "ENTER" on the MultiLab. Scroll down to "Cell const. man" and press "ENTER" (calibration "Type" must be set to "man" in order to do this).
    b. Adjust the cell constant in the calibration settings until the salinity reading displayed at the bottom of the settings menu is correct. The cell constant should be within 0.46-0.47, if it is not, the probe may need cleaning or servicing.
    c. Exit the settings menu by selecting "Back" and verify that the displayed salinity is now accurate and stable. Record salinity reading in analysis log sheet, and remove probe from bottle.
4. **Measure sample salinity.** Rinse a 100 mL graduated cylinder with ~25% volume of sample water. Fill cylinder to 90% volume with sample water, and insert the probe into the solution, submerging as much of it as possible. Gently stir the probe to remove any air bubbles trapped around the sensor. Wait 1-2 minutes for YSI reading to stabilize and record in analysis log sheet.
5. **Determine sensor drift.** After all sample measurements have been taken, take a second CRM salinity reading from the same CRM bottle (i.e. repeat step 2). Record salinity reading in analysis log sheet, and make a note of any sensor drift.

3.7. System Shut-Down

1. **Shut-down software.** Select 'Stop All' once. Do not click the stop button repeatedly. Allow ~60 seconds for system to rinse, air-out
2. **Turn off system.** When system has finished shutting down, as indicated by illumination of the green 'is shutdown?' button, and return of the run arrow to white, the system box can be powered down by the main power switch.

4. Discrete Sample Data Processing

The following steps should be used to evaluate a completed analysis worksheet and compute CO2 system parameters from analyzed TCO2, pCO2, salinity, and sample analysis temperature.

1. Open completed analysis worksheet and examine the pre- and post- sample batch gas and liquid standard analyses, including calibration gas and working standard values, shown on the "calcs and cals – START HERE" sheet. Fits should all be linear with low offsets (e.g. < 20; ideally < 10), and gas and liquid slopes near 1 and 2, respectively.

   NOTE: Large offsets for the gas calibration curve may mean the LI-COR needs to be cleaned, re-zeroed and spanned. Large offsets for the liquid calibration curve may mean the TCO_2 analysis loop needs troubleshooting or there is a problem with 1 or more of the working standards.

2. Examine the raw data in “Raw sample average data” sheet, and confirm it is consistent with the green highlighted data below the gas and liquid calibration information on the “calc and cals – START HERE” sheet.
   NOTE: The green area in the “calcs and cals – START HERE” sheet is where all initial calculation takes place. Do not alter this section.
3. Examine the CRM TCO2 data in column H on the “final results and CO2 calcs” sheet. Make sure columns I, J, and K are all aligned properly such that the average, standard deviation, and correction factor is calculated for each triplicate.
4. Confirm that the CRM Batch number (cell H,1) is correct, and is the TCO_2 concentration for that batch number (cell H,2).
5. Confirm that the CRM Time Points are correct; these should be the start and end times for the sample batch shown on the “Raw sample average data” sheet.
6. Confirm column L is properly applied to each cell (i.e. the formula to interpolate the correction factors is dragged to the last sample cell).
7. Confirm column M is properly applied such that the TCO2 data in column H have the correction factors in column L applied for each sample. The CRM corrected TCO2 is the final TCO2 of the sample.
8. In order to obtain the correct total alkalinity (TA) for the seawater sample, TCO2 (CRM corrected TCO2) needs to be adjusted by a headspace gas correction to account for the bubbling of headspace through the sample volume. Accounting for the change in TCO2 during pCO2 analysis is done by mass balance of CO2 in both sample and headspace, and is detailed in Equation 8 in Wanninkhof and Thoning (1993). Column N performs this calculation.
9. Compute the correct TA using CO2SYS with the Lueker et al 2000 constaints (to be consistent with following CO2SYS calculations and ensuring output pH is on the total hydrogen scale) with inputs as: pCO2@analysisT (column G), headspace gas corrected TCO2 (column N), analysis T (column F), and salinity (column E). Paste output TA from CO2SYS to column O.
10. Compute output pCO2@SST, pH_T, and Omega-arag with CO2SYS using Lueker et al. 2000 constants with input terms: CRM corrected TCO2 (column M), TA (column O), salinity (column E), and in situ temperature (column C). Paste output pCO2@SST, pH_T, and Omega-arag from CO2SYS to columns P, Q, and R, respectively.

4.1. Sample Collection Log sheet

HAKAI CO2 SAMPLING LOG

REGION:

SITE ID:

DATE:

NISKIN SAMPLER(S):

FIXER:

Drop #	Niskin Bottle #	Niskin Integrity	Sample Depth (m)	Sample ID	NIST T (°C)	Time at Fixing	Notes

4.2. Sample Analysis Log sheet

pCO$_2$/TCO$_2$ Logsheet

Hakai OA Lab
Quadra Isl.
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Date: __________ Lab Temp: __________
User: __________ Lab P: __________
Sample Source: __________

				TCO$_2$	**pCO$_2$**		
Sample Name	$T_{collection}$	S_{YSI}	$T_{analysis}$	xCO_2	P	xCO_2	Comments

Reference #4
Dickson Laboratory Certified Reference Material for CO2 analysis

https://www.usgs.gov/labs/carbonlab/about/laboratory-overview

Appendix 2: DNA Extraction and qPCR Protocol

Extraction: Adapted from Invitrogen Purelink RNA/DNA Mini Kit Protocol

- Before beginning extraction, hydrate lyophilized Carrier RNA at a 1:1 ratio (add 310 µl). This will yield a concentration of (1 µg/µL). This may be prepared ahead of time and aliquots may be stored at -20C for individual use.
- Create Master Mix with: 25 µL Proteinase K, 200 µL Lysis Buffer, and 5.6 µL Carrier RNA (1 µg/µL) per sample.
- If liquid sample, add 200 µL of sample and vortex for 15 s, if tissue sample add tissue and 200 µL PBS and manually homogenize, then vortex (with glass beads is optional for maximum yield) for 15s.
- QIAshredder tubes may be used to further increase tissue homogenization. Pipet liquid homogenate only from the 1.5 ml tube into QIAshredder tube. In some cases, tissue may have absorbed liquid, and may be amplified with PBS. Spin up to 12,000xg(rcf) for 2 min. Eluate contains sample DNA. Place eluate into new 1.5 ml tube, and AVOID pipetting any small tissue chunks from this step. This QIAshredder process generally takes 10-15 minutes and should be done before the incubation step.
- Incubate at 56C for 15 min.
- Briefly centrifuge the tube to remove any drops from the inside of the lid.
- Add 250 µL 96–100% ethanol to the lysate tube, close the lid, and mix by vortexing for 15 seconds.
- Incubate the lysate with ethanol for 5 minutes at room temperature.
- Briefly centrifuge the tube to remove any drops from the inside of the lid.
- Transfer the above lysate with ethanol (~675 µL) onto the Viral Spin Column.
- Close the lid and centrifuge the column at ~6800 × *g* for 1 minute. Discard the flow-through. Precipitated DNA remains in the spin column during and after this step.
- Add 500 µL Wash Buffer (WII) with ethanol to the spin column.
- Close the lid and centrifuge the column at ~6800 × *g* for 1 minute. Discard the flow-through and place the spin column back into the Wash Tube.
- Add 500 µL Wash Buffer (WII) with ethanol into the spin column.
- Close the lid, centrifuge at ~6800 × *g* for 1 minute. Discard the Wash Tube containing the flow-through.
- Place the spin column in another clean, Wash Tube (2 mL) included with the kit.
- Centrifuge the column at maximum speed in a microcentrifuge for 1 minute to dry the membrane completely. Discard the Wash Tube with the flow-through.
- Place the Viral Spin Column in a clean 1.5-mL Recovery Tube supplied with the kit.
- Add 60 µL of Sterile, RNase-free water to the center of the column. Close the lid.
- Incubate at room temperature for 1 minute.
- Centrifuge the column at maximum speed for 1 minute. The Recovery Tube contains purified viral nucleic acids. Remove and discard the spin column.
- Store the purified DNA at –80°C or use the DNA for the desired downstream application.

Alexandrium spp. Probe-Based PCR (adapted from Vandersea et al., 2017; Zhang & Li, 2012)

Forward (5'-3') GATGAAGAATGCAGCAAAATG

Reverse/Probe (3'-5') CAAACCTTCAAGAATATCC

Reagent	Quantity
Template DNA	0.2ug
dNTPs	200uM
F Primer	0.3uM
R Primer	0.3uM
DNA Polymerase	2.5U

Thermal Cycles (50ul Reactions)

	Temp (Celsius)	Time	Step
1	95	0:02:00	Denaturing
2	95	0:00:20	Denaturing
3	55	0:00:20	Annealing
4	72	0:00:30	Extension
5	72	0:05:00	Extension
6	4	Forever	Cold Storage
	Repeat 30 times		

***Pseudo-nitzchia* spp. Probe-Based PCR (adapted from Vandersea et al., 2002)**

Forward (5'-3') CTGTGTAGTGCTTCTTAGAGG

Reverse/Probe (3'-5') AGGTAGAACTCGTTGAATGC

Reagent	Quantity
KCl	100mM
Tris-HCl	40nM
dNTPs	0.4mM
$MgCl_2$	6mM
F Primer	400nM
R Primer	400nM
DNA Polymerase	0.4mM

Thermal Cycles (25ul Reactions)

	Temp (Celsius)	Time	Step
1	95	0:00:30	Denaturing
3	61	0:00:30	Annealing
4	72	0:00:30	Extension
5	4	Forever	Cold Storage
	Repeat 45 times		

Appendix 3: Saxitoxin Extraction and ELISA Protocol

Adapted from: https://abraxis.eurofins-technologies.com/media/8997/saxitoxin-plate-insert-r08182020.pdf

Extraction

Position the blue mussel so that the dorsal side is facing up and the ventral side is face down. Insert a scalpel between the two valves and run it along the perimeter until both the anterior and posterior adductor muscles are severed. The mussel can then be opened up with just your fingers and laid flat against the table. Next, take a scalpel and run it between the mantle and one of the valves until all the soft tissue is removed from the valve. Repeat this process for the second valve. Using a pair of tweezers, grasp the tissue and place in a 50 ml test tube. Repeat this process until you get 10g of soft tissue.

Sample Preparation

Using a tissue tearer, homogenize the 10g of soft tissue. Mix 1.0 g of homogenized mussels with 6 mL of methanol/DI water (80/20) using a Polytron or equivalent. Centrifuge the mixture for 10 minutes at 3000 g and then collect the supernatant. Add 2 mL methanol/deionized water (80/20) to the mussel tissue residue. Re-centrifuge the mixture for 10 minutes. Add supernatant to first portion. Bring the volume of the collected supernatant to 10 mL with methanol/deionized water (80/20). Filter extract through a 0.45 µm filter (Millex HV, Millipore, or equivalent). Remove 10 µL and dilute to 1.0 mL with 1X Sample Diluent (1:100 dilution).

Test Preparation

Adjust the microtiter plate and the reagents to room temperature before use. Remove the number of microtiter plate strips required from the foil bag. The remaining strips are stored in the foil bag and zip-locked closed. Store the remaining kit in the refrigerator (2-8°C). The standard solutions, enzyme conjugate, antibody, substrate and stop solutions are ready to use and do not require any further dilutions. Dilute the 5X Wash Buffer Concentrate at a ratio of 1:5. If using the entire bottle (100 mL) add to 400 mL of deionized or distilled water. Dilute the 10X Sample Diluent Concentrate at a ratio of 1:10 with deionized or distilled water as needed for sample dilutions.

Assay Procedure

Add 50 µL of the standards, control, or sample extracts into the wells of the test strips according to the working scheme given (duplicate). Add 50 µL of enzyme conjugate solution to the individual wells successively using a multi-channel pipette. Add 50 µL of antibody solution to the individual wells successively using a multi-channel pipette. Cover the wells with parafilm and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 to 60 seconds, being careful not to spill the contents. Incubate the strips for 30 minutes at room temperature. Decant the contents of the wells into an appropriate waste container. Wash the strips four times using the diluted wash buffer. Please use a volume of at least 250 µL of 1X wash buffer for each well and each washing step. Remaining buffer in the wells should be removed by patting the inverted plate dry on a stack of paper towels. Add 100 µL of substrate (color) solution to the wells successively using a multi-channel pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 to 60 seconds. Be careful not to spill the contents. Incubate the strips for 30 minutes at room temperature, protected from direct sunlight. Add 100 µL of stop solution to the wells in the same sequence as for the substrate (color) solution using a multi-channel pipette. Read the absorbance at 450 nm using a microplate ELISA photometer within 15 minutes after the addition of the stopping solution.

Appendix 4: Domoic Acid Extraction and ELISA Protocol

Adapted from: https://abraxis.eurofins-technologies.com/media/6472/domoic-acid-biosense-users-guide.pdf

Extraction

Position the blue mussel so that the dorsal side is facing up and the ventral side is face down. Insert a scalpel between the two valves and run it along the perimeter until both the anterior and posterior adductor muscles are severed. The mussel can then be opened up with just your fingers and laid flat against the table. Next, take a scalpel and run it between the mantle and one of the valves until all the soft tissue is removed from the valve. Repeat this process for the second valve. Using a pair of tweezers, grasp the tissue and place in a 50 ml test tube. Repeat this process until you get 50g of soft tissue.

Preparation of Buffers and Reagents

Washing buffer (PBS-T; 0.05% Tween 20 in PBS): dissolve one tablet (C) in distilled water and dilute to 500 mL. May be stored at 4ºC for one week. Extraction solution (50% methanol in water): prepare sufficient solution for the required number of samples by mixing equal volumes of methanol and distilled water. Prepare fresh each day. Standard/Sample buffer (10% methanol in PBS-T): mix 5 mL of methanol with 45 mL of Washing buffer. May be stored for 2-3 days at room temperature. Antibody-HRP ovalbumin buffer (1% ovalbumin in PBS-T): add 6 mL of washing buffer to 60 mg of ovalbumin (vial F). Prepare fresh for each assay.

Preparation of DA Calibration Solutions

The 10-point calibration curve is freshly prepared using standard dilutions in the range of 10,000 – 0.16 pg DA/mL. Prepare one Eppendorf tube containing 450 µL Standard/Sample buffer (10% methanol in PBS-T) - “tube 1”, and 9 Eppendorf tubes containing 300 µL Standard/Sample buffer - “tubes 2-10”. Add 50 µL of the DA standard (100 ng/mL, vial D) to tube 1 and vortex, to obtain a 10 ng/mL DA solution. Transfer 125 µL of the 10 ng/mL solution (tube 1) to tube 2 and vortex. Complete the 3.4-fold dilution series by transferring 125 µL from tube 2 to tube 3 and vortex. Repeat this step for all tubes 3-10.

Extraction of DA From Shellfish Samples

Shellfish flesh should be prepared as a finely blended homogenate. Preferably analyzed fresh, but it may be stored frozen at -20°C for up to 14 days before use. Prepare shellfish homogenate with a tissue terror from no less than 50 g shellfish flesh. Accurately weigh 4g into a 50 mL centrifuge tube. Add 16 mL of Extraction solution (50% methanol). Mix well by vigorous shaking on vortex for 1 min. Centrifuge at 3000xg for 10 minutes at room temperature. Retain the supernatant for further dilution prior to analysis. The extracts can be stored at -20˚C for up to 14 days, although with a possible reduction in DA content.

Assay Procedure

Incubation of standards and samples with antibody. Equilibrate pre-coated plate strips and all reagents to room temperature before use (1 hour max). Open the packet(s) with pre-coated plate strips gently and place the strips in the strip frame. Label each strip e.g. A, B, C and D etc. Add 300 µL Washing buffer to each well. Pre-soak the wells for 5-10 minutes. Remove the Washing buffer by inverting the strips over a sink and tap against a pile of paper towels to remove all the remaining liquid. Add 50 µL Standard/Sample buffer (10% methanol in PBS-T) to each of the duplicate Amax and Blank wells. Add 50 µL Antibody-HRP ovalbumin buffer (1% ovalbumin) to the Blank wells. Add 50 µL of each DA

standard dilution to each of two wells. Add 50 µL of each sample dilution to each of two wells. Shake vial E briefly, and tap the vial gently on a hard surface to ensure that all the content is in the bottom of the vial. Transfer 0.5 mL (for 4 strip assay) or 1.0 mL (for 8 strip assay) from vial E (concentrated Anti-DA-HRP) to a Falcon type tube containing 2.5 mL (for 4 strip assay) or 5.0 mL (for 8 strip assay) AntibodyHRP ovalbumin buffer (prepared vial F). Vortex briefly. Add 50 µL of the diluted Anti-DA-HRP conjugate to all wells except the Blank wells. Seal the strips with the plate sealer (B) and incubate at room temperature (20- 25°C) for 1 hour. Protect from light (e.g., cover with aluminum foil or place in a drawer).

Developing and Reading the Microplate Strips

Carefully remove the plate sealer. Remove all the contents by inverting the strips over a sink and tap to remove remaining liquid. Wash the wells 4 times with 300 µL Washing buffer per well. Add 100 µL of TMB peroxidase substrate (vial G) to all wells. Incubate at room temperature (20-25°C) for 15 minutes. Protect from light. Stop the reaction by adding 100 µL 0.3 M H_2SO_4 to all wells. After 2-5 minutes, read the absorbance in a microplate spectrophotometer using a 450 nm filter.

AXIOM DATA MANAGEMENT PLAN

The *Exxon Valdez* Oil Spill Trustee Council's data policy encourages full and open access to, and confident use of, the data and information used in and produced by programs and projects of the *Exxon Valdez* Oil Spill Trustee Council (EVOSTC). These data need to be easily understandable, electronically accessible and well organized to allow policy makers, researchers, managers, and the general public to make well-informed decisions. As such, Axiom Data Science, through it's partnership with the Alaska Ocean Observing System (AOOS) have considerable experience developing scientific data management infrastructure, and they provide experienced personnel to manage both data and metadata documentation according to federal quality control standards. This project will use the AOOS data management infrastructure (developed and maintained by Axiom Data Science) to manage and share the data generated through this effort, in accordance with the EVOSTC Data Management Procedures. This system uses the standards and best practices defined by the NOAA U.S. IOOS Data Management and Communications committee (IOOS, 2010). Among this infrastructure is an operational stack of open source software components developed by Axiom Data Science, with support from the NOAA Integrated Ocean Observing System (IOOS), EVOSTC, the National Science Foundation and more, which manages large numbers of continuous data feeds and a data catalog framework to integrate and disseminate a variety of data products. Data and data products generated by this project will be posted on the Research Workspace together with standards-compliant metadata for access by the EVOSTC. At the end of the project term, final QA/QC'd data and metadata will be made publicly available through the Gulf of Alaska data portal and made publicly accessible through the AOOS Gulf of Alaska data portal and distributed to DataONE for long-term preservation.

Data Types, Formats, and Metadata: This project will generate the following data:

- Time series environmental data: Continuous environmental data from both community samplers and agency weather monitoring entities in the region. These will include basic parameters such as air temperature, water temperature, windspeed, rainfall, and tidal phase.
- Time series chemical seawater analyses: Discrete community collected temperature, pH, salinity, and DO measurements; BOL generated pCO2 and TCO2 levels, as well as aragonite and carbonate saturation; and profiles of phosphate (PO4), nitrate (NO3), nitrite (NO2), and ammonium (NH4).
- Phytoplankton ID and species prevalence: Phytoplankton speciation and counts from community samplers, as well as binomial molecular detection results indicating presence or absence of Pseudo-nitzschia spp. and Alexandrium spp. throughout the year.
- Biotoxin levels: Levels of DA and STX in sampled blue mussels from April-September of each year.

Data will be stored in non-proprietary formats to ensure re-use and long-term preservation. Project data may initially exist in proprietary or binary formats as primary-level data, depending on the source provider. Though the data may be in a state which can be easily utilized by the research team, in many cases the primary-level data is not in a form ready to be shared with the broader science community or integrated with other datasets. As such, the final format for project data will be in open standard suitable for long-term archiving, such as:

- Containers: TAR, GZIP, ZIP
- Databases: CSV, XML
- Tabular data: CSV
- Geospatial vector data: SHP, GeoJSON, KML, DBF, NetCDF

- Geospatial raster data: GeoTIFF/TIFF, NetCDF, HDF-EOS
- Moving images: MOV, MPEG, AVI, MXF
- Sounds: WAVE, AIFF, MP3, MXF
- Statistics: ASCII, DTA, POR, SAS, SAV
- Still images: TIFF, JPEG 2000, PDF, PNG, GIF, BMP
- Text: XML, PDF/A, HTML, ASCII, UTF-8
- Web archive: WARC.

Comprehensive metadata using the latest national and international technology and community standards will be written for each data collection generated. The Research Workspace includes an integrated metadata editor, allowing researchers to generate metadata conforming to the FGDC-endorsed ISO 19110 and 19115-2 suite of standards. Axiom will provide technical assistance to project researchers to ensure robust and standards-compliant metadata are generated for final project datasets prior to data publication and archive.

Data Access and Timeframes: Among the Axiom data system infrastructure is the Research Workspace, a web-based scientific collaboration and data management tool used by researchers to secure and centralize project data, generate standards-compliant metadata, and ultimately elect data files and derived data products to be published openly on public data portals and in long-term data archives. Following the EVOSTC data sharing policies, all monitoring data from this project will be transfer as they become available to the Research Workspace. These data shall be replaced in the Research Workspace with QA/QC'd and metadata when available and no later than 1 year after collection, after which they will be made publicly available through the GOA data portal. The Research Workspace is the gateway for PIs to elect and publish data and metadata to the GOA data portal. The exception is for process studies which are research-oriented in nature and do not have annual timeseries data. Process studies require data and metadata to be made publicly available through the GOA data portal by the end of the project term.

Data Storage, Preservation, and Archiving: The Axiom data center and services are housed on highly redundant storage and compute resources at a data center in Portland, OR, and are geo-replicated using Amazon Glacier Cloud Archive Services. All databases and code repositories are routinely backed-up, and servers undergo routine maintenance to swiftly address security vulnerabilities. Servers containing source code and databases are located behind an enterprise-level firewall and are physically secure with environmental regulation systems, redundant power, and fire suppression. Axiom's HPC resources are composed of approximately 2500 processing cores staged in a series of interconnected blade arrays as well as 1.8 petabytes of storage. Dedicated disc-space in the amount of 30 TBs will be allocated for long-term storage of all preliminary and finalized data resources produced by this effort.

For long-term preservation, all final data and metadata will be transferred to a national data center. The data developed in this project will be open source and licensed in the public domain. The planned archive for the data collected by this effort is the Research Workspace's DataONE Member Node. The Research Workspace hosts an integrated system for automating dataset submission to the NSF-sponsored DataONE federation of data repository. The Research Workplace supports and issues Digital Object Identifiers (DOIs), so datasets can be confidentially referenced in the published literature. Upon final permission from the project PI at the end of the project term, final data or data products will be submitted for archive with technical support by Axiom data management staff to ensure appropriate use and compliance with the data center archive requirements.