NCCOS FY17 ECOHAB AND PCMHAB PROGRAM AWARDS

UNCOVERING THE MECHANISMS BEHIND WINTERTIME OCCURENCES OF PARALYTIC SHELLFISH TOXICITY IN GEODUCK CLAM FISHERIES IN SOUTHEAST ALASKA

Institutions: University of Alaska Fairbanks, Sitka Tribe of Alaska, Southeast Alaska Regional Dive Fishery Association

Project Period: October, 2017 - September, 2020

Total Approved Funding: $742,941

Project Summary: The Southeast Alaska geoduck clam fishery, worth $4.9 million annually in past years, is an economically important wintertime fishery. However, the fishery has been plagued by unexplained toxicity from paralytic shellfish toxins (PSTs) resulting in substantial economic losses. In Alaska, PSTs are attributed to the marine dinoflagellate, *Alexandrium sp.*, and typically threaten shellfisheries during summer months when conditions support *Alexandrium* growth. Yet, geoduck clams show elevated and erratic patterns toxicity in fall and winter. It is currently not understood what factors lead to fall and wintertime toxicity in geoduck clams. While it has been proposed that benthic resting cysts contribute to the toxicity of certain shellfish, this alternate mechanism for geoduck clam toxicity through direct cyst ingestion or germination following sediment disturbance has not been tested. Further, nothing is known about cyst distributions in relation to geoduck clam harvest areas in Southeast Alaska. Knowledge about distributions of cyst populations and if cyst resuspension contributes to geoduck clam toxicity would provide critical information to reduce the impacts of PSTs to geoduck clam fisheries and inform management response. Thus, the primary objectives of this proposal are to: 1) examine the relationship between cyst distributions and geoduck toxicity patterns within commercial harvest areas to identify areas of greater or lower exposure risk to paralytic shellfish toxins; 2) identify whether cyst ingestion is a mechanism for geoduck clam toxicity to improve understanding of toxin transfer dynamics for geoduck clams and other shellfish species; 3) determine if current geoduck harvest and/or management approaches contribute to the frequent occurrence of wintertime toxicity in geoduck clams; and 4) involve geoduck clam dive industry and resource managers in research to ensure project outcomes meet stakeholder information needs. The proposed research meets the two overall goals stated in the 2017 ECOHAB RFP.

INVESTIGATING DOMOIC ACID BIOSYNTHESIS AND TOXIC BLOOM FORMATION THROUGH *PSEUDO-NITZSCHIA* – MARINE BACTERIA INTERACTIONS

Institutions: University of California Santa Cruz

Project Period: September 2017 – August 2020

Total Approved Funding: $ 389,946

Project Summary: Domoic acid (DA) poisoning is a pervasive problem in the coastal oceans of the Northeast and Northwest United States. Discovered in 1987 from algal bloom forming diatom *Pseudo-nitzschia*, the trigger for its production and the chemical and molecular mechanism for its biosynthesis is still not completely understood to date. Although several physical and biological factors have been implicated for the production of DA, bacterial metabolites as potential triggers for DA synthesis has not been investigated. The overarching goal of this proposal is to gain an understanding of the biosynthetic machinery of domoic acid in *Pseudo-nitzschia* and the influence of marine bacteria that leads to the synthesis of domoic acid and other kainoid families, which will provide fundamental new insights about chemical, molecular and biotic factors at work during *Pseudo-nitzschia* bloom. The proposed goals and approaches aim to (1) map out the diverse but unknown major and minor biosynthetic DA congeners potentially synthesized via interaction between *Pseudo-nitzschia* and epibiotic bacteria using state of the art isolation and identification techniques, (2) shed light into the mixed terpene-amino acid hybrid biosynthetic machinery and pathways hypothesized to produce the domoic acid framework by using labeled substrates that can elucidate the chemical pathways at work during DA synthesis (3) investigate the modulation of DA production in the ocean and in the laboratory by studying the metabolome of *Pseudo-nitzschia*-bacteria cocultures and natural associations during bloom, (4) employ molecular genetic tools including integrated pathway analysis to define the basis for the *Pseudo-nitzschia* – marine bacteria interaction required to produce DA analogues in culture and natural blooms, (5) Use Bayesian statistical approach to quantify the contribution of marine bacteria to toxic *Pseudo-nitzschia* bloom formation against other co-varying physical and biological factors. Important outcomes include the generation of transformative information about the DA biosynthetic machinery at work that can lead to a better mitigation and management of *Pseudo-nitzschia* toxic bloom. Understanding the influence of bacteria on toxic *Pseudo-nitzschia* bloom formation can lead to the use of this biotic factor as a potential determinant of *Pseudo-nitzschia* bloom toxicity and can be integrated with other environmental factors in making better predictive models for forecasting *Pseudo-nitzschia* bloom. Our findings will be disseminated to managers of ocean monitoring systems to provide information about the potential adoption of bacteria and metabolites as additional important markers for monitoring and predicting *Pseudo-nitzschia* bloom toxicity.

INTEGRATION OF ALTERNATIVE METHODS OF ANALYSIS INTO THE NEUROTOXIC SHELLFISH POISONING MONITORING AND MANAGEMENT FRAMEWORK

Institutions: Florida Fish and Wildlife Conservation Commission, Fish and Wildlife Research Institute; U.S. Food and Drug Administration, Gulf Coast Seafood Laboratory

Project Period: September 2017 – August 2020

Total Approved Funding: $285,269

Project Summary: Blooms of the dinoflagellate Karenia brevis threaten the productive Gulf of Mexico shellfish industry. Brevetoxins produced by K. brevis are toxic to humans and can result in Neurotoxic Shellfish Poisoning (NSP) if contaminated shellfish are eaten. To prevent NSP, shellfish harvesting areas (SHAs) are closed when K. brevis concentrations exceed 5,000 cells/L and are re-opened once K. brevis levels decrease and testing demonstrates that shellfish are no longer toxic. This biotoxin plan successfully prevents occurrences of NSP from lawfully harvested shellfish, but NSP closures come at a

steep economic cost to the shellfish industry. The APHA mouse bioassay - the only approved method for regulatory NSP testing - has many drawbacks, and the delays caused by the time required to analyze samples (two days) and low sample throughput compound economic losses. To mitigate economic harm to the shellfish industry and ensure the continued protection of public health, rapid alternative methods for NSP testing are needed. Rationale: Among the many chemical and biological methods developed for brevetoxin detection, enzyme-linked immunosorbent assays (ELISAs) have performed well and shown favorable comparability with mouse bioassays. A brevetoxin ELISA kit marketed by MARBIONC Development Group is routinely used to monitor brevetoxins in Florida's marine systems and to diagnose human and animal exposure. This assay has been validated according to Interstate Shellfish Sanitation Conference (ISSC) guidelines, and a proposal will be submitted to the ISSC by January 31, 2017 for acceptance of this ELISA as a limited use method. Scientific objectives: The objectives of this project are to 1) implement the MARBIONC ELISA as the first non-mouse based NSP testing method within the U.S. shellfish sanitation regulatory framework, 2) expand the list of species for which the method can be used, 3) transfer this technology to appropriate end-users, and 4) evaluate other methods of sample preparation and analysis for potential use in harmful algal bloom (HAB) biotoxin management programs. Summary of work to be completed: Additional work required by the ISSC to gain proposal acceptance will be completed. Following ISSC approval, rapid NSP ELISA testing will be integrated into Florida's NSP monitoring and management program. This includes analyzing all regulatory NSP samples by both ELISA and MB while the ISSC proposal is in process and increasing shellfish testing in key SHAs both during K. brevis blooms and in the absence of blooms. In Year 2, NSP testing capabilities in affected states will be expanded through training workshops conducted in Texas, Louisiana, Mississippi, and Alabama. The ELISA will also be validated for use with Southern hard clams (Mercenaria campechiensis), a native species gaining renewed interest among Florida clam farmers. In Year 3, another widely used NSP ELISA kit (manufactured by Abraxis) will be evaluated for comparability with the MARBIONC ELISA and its potential for use in NSP management, and methods to simultaneously extract multiple HAB toxins from shellfish will be developed to enable shellfish management programs to incorporate more proactive screening for HAB toxins of concern.

A STUDY OF *GAMBIERDISCUS* "SUPER BUGS" AND CIGUATOXIN FATE IN CORAL REEF FOOD WEBS

Institutions: Florida Gulf Coast University, Marine University of South Alabama & Dauphin Island Sea Lab, Woods Hole Oceanographic Institution, University of the Virgin Islands

Project Period: September 2017 – August 2020

Total Approved Funding: $749,976

Project Summary: Ciguatera fish poisoning (CFP) is the most prevalent phycotoxin-borne illness worldwide. This debilitating human poisoning syndrome is caused by consumption of tropical marine organisms contaminated with ciguatoxins (CTXs) and related precursors produced by benthic dinoflagellates in the genus *Gambierdiscus*. Ecological understanding and resultant management solutions lag far behind for CFP versus other HAB syndromes, due to insufficient information regarding the identification of the truly toxigenic *Gambierdiscus* species/strains (e.g., the "super bug"), and elucidation of the ciguatoxin precursors produced by these species. In addition, the introduction and

biotransformation of these precursors into the marine food web is poorly understood. These knowledge gaps severely limit the ability of resource managers to monitor for and protect the public against ciguatera fish poisoning. To date, forecasting/predictive capabilities are limited to non-existent, and responses are reactionary and ex post facto when CFP outbreaks occur. This project builds on several important breakthroughs in ciguatera research in the Caribbean including the identification of *Gambierdiscus* "super bugs" in the region, the development of molecular probes to characterize community structure in field samples, the development of toxin detection methods and reference materials for CTX assessment and quantification, and the development of a numerical toxicity model. The overarching goal identified by investigators in this targeted proposal is to characterize ciguatoxin production and flux into Caribbean reef food webs for prediction and prevention of CFP. Specific objectives are to determine: 1) the spatio-temporal population dynamics of newly characterized ciguatoxin-producing *Gambierdiscus* "super bug" species; 2) how *Gambierdiscus* cellular toxicity translates into toxin body burden in reef herbivores; and 3) the environmental conditions and habitats that produce the highest abundances of *Gambierdiscus* "super bugs". The outputs of these objectives will be incorporated into the next-generation toxicity risk model to forecast and predict toxigenic *Gambierdiscus* populations and toxin entry into the food web (Objective 4) to aid in management strategies to minimize human illness. Leveraged in this proposal is an extensive collection of archived algae and fish samples, which will allow exploration of "super bug" population dynamics. The analysis of archived samples will be supplemented by quarterly field sampling to validate findings of cell and toxin thresholds. This approach will provide six full years of data to characterize spatio-temporal community dynamics that would otherwise be impossible or impractical to collect.

EXPANDING THE OPTIONS FOR MONITORING OF DSP BY PROMOTING THE ISSC APPROVAL OF LC-MS/MS AND TWO RAPID SCREENING APPROACHES

Institutions: Bigelow Laboratory for Ocean Sciences, Maine Department of Marine Resources

Project Period: September 2017 – August 2020

Total Approved Funding: $ 246,673

Project Summary: Diarrhetic shellfish poisoning (DSP) toxins constitute a substantial economic threat to shellfish industries globally, including in the USA, and present a major food safety issue for shellfish consumers worldwide. The major production of DSP-toxins around the coast of the United States is by *Dinophysis* species of planktonic dinoflagellate. Filter-feeding bivalves accumulate these DSP-toxins and monitoring programs have been established worldwide to prevent the intoxication of consumers. Although DSP-outbreaks were considered rare in both USA and Canadian waters, there is growing evidence of the increasing occurrence of DSPoutbreaks around the coast of North America. Rationale: Methods of toxin analysis for the purposes of regulating shellfish in the USA are determined by the Interstate Shellfish Sanitation Conference (ISSC) and the Food and Drug Administration (FDA), and are adopted for use in the National Shellfish Sanitation Program (NSSP). Currently, no reference methods for the analysis of DSP toxins have been approved by the ISSC or adopted by the NSSP. This puts the onus on the relevant State departments to develop strategies relying on 'best available science' for the monitoring of shellfish DSP levels and for decision making regarding the closing and opening of shellfish fisheries and aquaculture. Objective: The overarching aim of this project is to increase the

number of ISSC validated options available to State departments and associated laboratories for monitoring DSP-toxins, and to contribute to a framework in which these analytical approaches can be incorporated into the monitoring and management of shellfish safety for the public. Work to be completed: Three analytical tools have been chosen that are currently most commonly used for 'best science' based monitoring for DSP in the United States, a lateral flow immunoassay test, a phosphatase inhibition assay and an LC-MS/MS approach. These methods contrast in their levels of quantitation, ease of use, cost and analysis time; providing a range of options for their use for monitoring purposes. A database comprising comparable DSP data for the three approaches will be developed by analyzing naturally-incurred, DSP-containing shellfish samples from around the North American Coast using all three methods. Additional experimental analyses will be aimed at addressing the key criteria identified in the ISSC guidelines for method validation. Outcomes: The results generated from these experiments will be used to develop reports for each of the methods that address the specific requirements of the ISSC. These reports will be produced through collaborations within this proposal and will take into account previous work on each of the methods, with the intention that they will be used to develop proposals for the ISSC validation process. Information on the performance of the individual analytical tools will also be used to review how monitoring for DSP is carried out in different regions and for different species of shellfish around the coastline of the USA.

DEVELOPMENT OF A MECHANISTIC ROMS-RCA-HAB MODEL FOR PREDICTING *PROROCENTRUM MINIMUM* AND *KARLODINIUM VENEFICUM* BLOOMS IN CHESAPEAKE BAY

Institutions: University of Maryland Center for Environmental Science

Project Period: September 2017 – August 2020

Total Approved Funding: $598,888

Project Summary: The over-enrichment of Chesapeake Bay (CB) by nutrients has been well recognized and documented. Among the effects of this over-enrichment is an increasing proliferation of HABs, ranging from toxic dinoflagellates (e.g. *Karlodinium veneficum*), to ecosystem disruptive high-biomass dinoflagellates (e.g., *Prorocentrum minimum*) as well as toxic and high-biomass cyanobacterial blooms. HABs in CB are now more frequent, and of significantly higher densities, than several decades ago. Although many HABs develop when nutrients are not in Redfield proportion, traditional biomass-based plankton models assume fixed stoichiometry and operate using Monod kinetics. These models are unsuitable, and are considered as dysfunctional for descriptions of algal growth under variable nutrient conditions. This proposal aims to develop a new, spatially explicit, mechanistic HAB model for CB that integrates physics, nutrient cycling, food web interactions, physical factors and nutrient physiology to improve predictions of the long-term changes of two dominant HABs, *P. minimum* and *K. veneficum* seasonally and under various scenarios of change. A coupled hydrodynamic biogeochemical model (ROMS-RCA) will be coupled to a new HAB model for *P. minimum* and *K. veneficum*. RCA includes 3 phytoplankton functional groups (cyanobacteria, diatoms and flagellates), separate cycling of N, P, Si and C, and is coupled to a sediment diagenesis model. The HAB model will consider nutrient preferences, life history including diel regulation, internal cellular nutrient storage and mixotrophy. The models will be applied to 1) spatially explicit seasonal forecasts, i.e. forecast summer HAB based on

winter-spring nutrient loading and spring bloom development, and 2) scenario analysis to examine how HABs respond to nutrient management strategies (higher or lower loading) and climate change (warming, salinity change due to sea-level rise or changes in stream flows etc.) as projected by global and regional climate models. Model comparisons will be made in hind-cast mode with the extensive CB monitoring data. The proposed effort will also include a suite of laboratory experiments to obtain the necessary parameters to quantify cell quota, nutrient acquisition and growth for multiple nutrients and under a range of nutrient, temperature, and light conditions. Seminars and training on the model will be provided to management agencies, such as the Maryland Department of Natural Resources, the Department of the Environment, BayStat working groups, and the Harmful Algal Task Force. Based on their input, specific scenarios for various specific nutrient reduction goals will be run. The proposed analysis of nutrient loading and climate change scenarios will be useful for designing restoration strategies for CB. The end result will be more effective nutrient reductions that will have significant effects on reducing the numbers of HABs, their frequency or their duration, with positive consequences on fisheries and shellfish habitat.

LINKING PROCESS MODELS AND FIELD EXPERIMENTS TO FORECAST ALGAL BLOOM TOXICITY IN LAKE ERIE

Institutions: The Ohio State University, Limnotech, NOAA Great Lakes Environmental Research Laboratory, Michigan Technological University, University of Toledo, Wayne State University

Project Period: September 2017 – August 2020

Total Approved Funding: $ 749,525

Project Summary: Contamination of drinking water by freshwater cyanobacterial toxins may be the greatest threat to human health associated with harmful algal blooms (HABs). This was demonstrated dramatically by the HAB-induced drinking water ban in Toledo, Ohio in 2014 due to elevated microcystin concentrations, which affected almost 500,000 people who depend on Lake Erie as their water supply. Lake Erie has been plagued by cyanobacterial blooms since the late 1990s and it seems that the major driver of the blooms is the spring phosphorus load from the Maumee River. In recent years, a seasonal bloom forecast has been issued in early July based on the spring phosphorus load from the Maumee River. Although HAB size and location can be reasonably well forecasted, fundamental questions remain about the controls and predictability of toxin production in blooms. One complexity in field studies has been the coexistence of toxic and non-toxic strains of the same species of cyanobacteria. The factors influencing the dynamics of toxic and non-toxic strains within blooms are only beginning to be understood, but they may be at the point where reasonable predictions of changes in toxicity could be possible. While phosphorus has a clear role in HAB dynamics, nitrogen may also be important in the occurrence and biomass of cyanobacteria and the toxicity within the blooms. Other factors, such as light intensity and temperature, may also interact with nitrogen to impact HAB toxin production. The study proposed here will consist of three primary components (1) examination of historical data to look for correlations between changes in environmental variables and changes in HAB toxin concentrations, (2) incorporation of results of Component 1 analyses into numerical models of ecological and physical processes to develop hindcasting, nowcasting, and forecasting capabilities, and (3) field and laboratory experiments to better constrain knowledge gaps identified in Components 1 and

2, including the influence of nitrogen, light, temperature, and other factors on the production and decay of HAB toxin. The products of the research study will be a suite of presentations, publications, and tools to inform stakeholder and scientific audiences of the advances made in understanding HAB toxicity and specific technical guidance to NOAACOOPS and drinking water utilities on incorporating new information and improved forecasting capabilities into their operational systems.

CROSS-REGIONAL COMPARISON OF *DINOPHYSIS* BLOOM DYNAMICS, DRIVERS, AND TOXICITY

Institutions: Virginia Institute of Marine Science, Texas A&M University, Stony Brook University, NOAA/NMFS/NWFSC, USFDA

Project Period: September 2017 – August 2020

Total Approved Funding: $ 674,108

Project Summary: Species of *Dinophysis*, known to produce toxins that cause diarrhetic shellfish poisoning (DSP), have threatened the safety of shellfish consumers in Asia and Europe for decades. Recently, harmful algal blooms (HAB) caused by *Dinophysis* spp. have emerged as a human health threat in the US. Since first detected on the coast of Texas in 2008, *D. ovum* has been detected in six of the last eight years and has resulted in the closures of shellfish harvesting to prevent DSP. Since 2011, closures due to DSP from *D. acuminata* and D. fortii have also been enforced annually at multiple sites throughout Puget Sound, WA, and toxin levels in shellfish exceeding FDA regulatory limits have been reported in New York, Maine, and Massachusetts due to blooms of D. cf. acuminata and most recently *D. norvegica* (ME). Chesapeake Bay and the larger DELMARVA region (Delaware, Maryland, and Virginia) harbor toxin-producing species of *Dinophysis*. The region, however, provides contrast as a relatively new area of concern, with evidence of an approaching tipping point. Rationale: Given the rapid increase in frequency of *Dinophysis* blooms on nearly every US coast, with clear regional variability in timing and species, it is essential that the drivers of *Dinophysis* success in coastal ecosystems be identified using a coordinated, nationwide effort. Such a cross-regional comparative study will identify not only potential drivers, but create a baseline for understanding how future climate and eutrophication scenarios will influence intensity, frequency, and toxicity of blooms. Objective: The goal of this project is to identify and quantify factors controlling *Dinophysis* blooms and DSP across the US as a means of developing optimized regional early warning systems and management plans. It is hypothesized that a combination of temperature, stratification, prey, and nutrient input combine to determine the success of *Dinophysis* in US coastal ecosystems. A carefully coordinated and collaborative study including high-resolution phytoplankton time series, field collections, and multi-factorial laboratory experiments using isolates of *Dinophysis* species from important shellfish harvesting sites in the US (Gulf of Mexico, Puget Sound, Long Island Sound and Chesapeake Bay), will be undertaken to address the following objectives: 1) Identify the environmental factors that control *Dinophysis* blooms and toxicity within and across regions; 2) Use isolates to characterize genetic variability and potential toxicity, under a variety of conditions; 3) Develop and optimize an early warning system for *Dinophysis* blooms and DTXs in US coastal regions; and 4) Partner with state and industry groups to closely match management needs, disseminate results, and aid regional management programs. Outcomes: Implementation of optimized DSP early warning systems will be a

critical first step towards ensuring public safety while also minimizing negative economic impacts on local communities. The proposed work will also identify the environmental regulators of DSP toxin production and *Dinophysis* species success, providing the context with which to better understand, predict, and detect potential threats now and under future climate and nutrient management scenarios. As evidenced by the increasing frequency and duration of DSP-related harvesting closures, and unfortunately human illness events, there is a critical and urgent need to develop improved early warning systems for *Dinophysis* and DSP in Washington State, New York, Texas, and DELMARVA, serving as a template to guide ecoforecasting efforts in the rest of the U.S.

TOXICITY AND POTENTIAL FOOD-WEB IMPACTS OF *ALEXANDRIUM MONILATUM* AND ITS TOXINS

Institutions: College of William and Mary, Virginia Institute of Marine Science

Project Period: September 2017 – August 2020

Total Approved Funding: $ 749,826

Project Summary: *Alexandrium monilatum* is a common HAB species that historically has bloomed along the US southern Atlantic and Gulf coasts, but very dense blooms now occur almost annually in lower Chesapeake Bay, VA. In 2007, we identified *A. monilatum* as the dominant species of a late summer bloom persisting for several weeks. Although *A. monilatum* cells have been shown to cause toxicity in a variety of organisms, the pathway and mechanisms of toxicity including the uptake, tissue distribution, metabolism, depuration and the potential for food-web transfer of the toxin goniodomin A (GDA) all remain poorly understood. Rationale: The recent intensification and expansion of *A. monilatum* activity in the Chesapeake Bay region is cause for concern due to its potentially important ecological and economic impacts. With the shellfish aquaculture industry rapidly expanding and now worth over $48 million to Virginia alone, it is critical that we understand the risk posed to the health of oyster stocks, as well as to humans through consumption of shellfish exposed to *A. monilatum*. This team of scientists is poised to rapidly assess impacts of this species and its toxins on multiple trophic levels including determination of effects on aquaculture stocks and possible human health risks. We have purified toxin in hand and twelve *A. monilatum* isolates from Chesapeake Bay are in culture. Preliminary studies have demonstrated toxicity of *A. monilatum* and its toxin GDA to marine vertebrates and invertebrates. Scientific Objectives: The overarching goals of this project are to characterize 1) the impacts of *A. monilatum* and it goniodomin (GD) toxins, and 2) the association between bloom dynamics and toxigenicity as they relate to the accumulation and transfer of toxin through trophic levels. The specific objectives are to 1) Investigate the toxicity of *A. monilatum* and GDA in vertebrate and invertebrate organisms both in the laboratory and field; 2) Determine the toxicokinetics of GDs in a vertebrate and invertebrate model and the relevance to resource management; 3) Determine the food-web transfer of GDs to higher trophic levels; and 4) Disseminate key information to end-users to communicate results, receive feedback and improve research directives. Summary of Work to be Completed: The impacts of *A. monilatum*, and specifically GDA, on shellfish and finfish will be determined through laboratory dose response bioassays on sheepshead minnows and field assays on juvenile oysters. Reliable methods will be developed

for quantifying toxin in crab and vertebrate tissues. Quantification of toxin in environmental waters and animal tissues from laboratory feeding studies and oyster samples collected during blooms will be coupled with molecular and microscopic methods to follow transport of *A. monilatum* and toxin through animal tissues providing insight into mechanisms of toxicity and metabolism of toxin, as well as elucidating pathways of trophic transfer. Two workshops will be held to communicate results to end-users including regional regulators, aquaculture industry members, NGOs and citizen groups involved in oyster restoration activities, and other scientists involved in HAB research in the Chesapeake Bay region.