DEVELOPING A COLLABORATIVE VOLUNTEER NETWORK FOR EXPANDING BIOTOXIN MONITORING IN CALIFORNIA: IMPROVING SEAFOOD SAFETY

FINAL REPORT
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Carolynn Culver
California Sea Grant Extension Program
UCSB Marine Science Institute

Gregg Langlois
California Department of Public Health
Environmental Management Branch

Kristine Barsky
California Department of Fish and Wildlife
Marine Region

Bahman 'Bob' Moezzi
California Department of Public Health
Food and Drug Laboratory Branch

EXECUTIVE SUMMARY

Over the past decade, harmful algal blooms (HABs), particularly those that produce domoic acid (DA), have increased in California. Limited data also suggests that these DA blooms may last longer and result in higher concentrations of DA in certain species that support recreational and commercial fisheries in offshore/island areas as compared to the coast. Further, the California Department of Public Health's mandate is to monitor bivalve shellfish for these toxins. However, DA has the ability to readily impact non-bivalve species. Based on this information, we identified a need to expand the State Biotoxin Monitoring Program that collects water and bivalve samples primarily from piers and shore-based areas to also collect crustacean samples at those sites, in addition to collecting all three types of samples from offshore and island areas.

With funding from Collaborative Fisheries Research West, we -- the California Sea Grant Extension Program, the California Department of Public Health (CDPH) Environmental Management Branch, the CDPH Food and Drug Laboratory Branch and the California Department of Fish and Wildlife (CDFW) -- teamed up to address this need. Together we developed and tested a pilot program seeking to improve seafood safety of commercial and recreational fisheries in California by increasing monitoring and evaluation of biotoxins in offshore/island areas of Southern California (Point Conception, Santa Barbara County to the Mexican Border). We did this by:

- Identifying and training volunteers in biotoxin monitoring
- Modifying, as needed, sampling, communication and reporting protocols through pilot testing of the expanded volunteer network (HABNet)
- Identifying needs for HAB outreach materials
- Exploring correlations between nearshore and offshore/island HABs

We contacted and worked with individuals from diverse groups:

- Sportfishing and dive charter operations
- Sportfishing and dive organizations
- Commercial fishermen (divers, trappers) and seafood distributors
- Offshore oil and gas groups
- Community-based marine education groups
- University dive safety officers and research divers
- University research programs
- Local, state and federal agencies

We identified advantages and encountered challenges when engaging the various groups in the different sampling tiers. While we engaged at least one volunteer from most of these groups in one or more tiers of sampling, we were able to most readily involve community-based education groups in Tier 1 (plankton) sampling and university research divers and commercial fishermen and seafood distributors in Tier 2 and 3 (bivalve and crustacean) sampling. Permitting issues, frequency of offshore trips, time and staff availability while offshore, and ability to easily ship

samples were factors that affected the participation of some groups. We addressed two of these issues by identifying and organizing an 'Entity Collecting Permit' for the state biotoxin program, and facilitated the use of a third party to work with some groups to ship their samples for testing.

Sampling, communication and reporting protocols were tested through the newly established HABNet (the network of HAB monitoring volunteers). These protocols were essentially identical to existing CDPH protocols, with the exception of the crustacean protocol that had to be developed by our team to help guide volunteer monitoring. Slight modifications were made to the protocols based on volunteer feedback, with most changes providing clarification of protocol details rather than significantly adjusting the protocols. On a few occasions, there was a bottleneck with getting the sample shipping materials back to volunteers (shipping materials are re-used), but this problem was readily corrected by providing additional supplies to the volunteers so they wouldn't have to wait to receive the initial set of shipping materials before sampling again. Volunteers also indicated that occasionally there were delays in receiving their results, but this was often due to unavoidable backlogs at the laboratory. A review of other similar biotoxin monitoring programs in the United States revealed that some programs use web-based portals to communicate and report findings to their volunteers as well as the public. CDPH may benefit from development of a similar electronic system where sample information can be entered and results posted as soon as they are available. This could be similar to its current phytoplankton maps, but with near real-time data. Importantly, such maps will need to clearly state that the information should not be used to assess whether an area is 'safe', and that consumers should rely on the hotline and posting of advisories.

While working with HABNet participants we identified the need for four types of outreach materials, three of which we developed:

- An information sheet about HABs in California, including levels of concern and symptoms of exposure.
- An information sheet about which California seafood is most affected and details about safe consumption of seafood during blooms.
- A short (few sentences) message in several languages about the potential danger of biotoxins and which parts of the animal to avoid.

These materials are being finalized and will be posted on the California Sea Grant website, with links also provided to CDPH, CDFW and other interested agencies and groups. The fourth outreach item, an information sheet describing the history of the toxins and associated testing in California requires additional data analysis and, as such, was deemed outside the scope of this project.

Volunteers participating in our HABNet program provided 175 samples to the state during the one-year project period, and they have continued to provide samples. PSP-producing HABs were virtually non-existent during the project period, with only 2 HABNet samples detecting the PSP toxin producing dinoflagellate, *Alexandrium* sp. The HABNet samples, however, proved to be quite useful for evaluating various aspects of DA-producing blooms, including the relationship

between coastal and offshore/island DA blooms, intra- and interspecific variation of DA toxins in three important fishery resources (spiny lobster, red rock crab and brown rock crab) and spatial variation in offshore/island DA blooms on a finer scale. Our key findings were:

- Coastal and offshore/island blooms were partially decoupled.
- Offshore/island blooms persisted longer and resulted in higher levels of DA in some wild-caught organisms.
- DA levels varied tremendously, ranging from non-detectable (< 2.5 ppm), to below the critical level (< 20 ppm) to above the critical level (≥ 20 ppm), in individuals of the same species (spiny lobster, red and brown rock crabs) collected from the same site at the same time.
- Rock crabs (red and brown) had higher levels of DA than spiny lobsters collected at the same site and time, but some samples of all species were above the critical level. (This was unexpected, as the highest DA level recorded to date was from a spiny lobster collected at the Northern Channel Islands.)
- Certain sites contained rock crabs (red and brown) with higher DA levels than crabs at other sites.

These results have been useful not only for enhancing the understanding of HABs in California, but also for helping to inform seafood health advisories. In particular, sampling efforts helped to determine the timing and duration of advisories, the species to include in the advisories, and the spatial extent of advisories. Information provided during this project also provided an early warning system for a shellfish grower located down the coast from a newly established sampling site, and it helped identify fishing sites that may be at higher risk to DA exposure.

Based on the results of the samples provided through this project, we also identified a need for additional research. Specifically, it remains unknown whether the differences in DA levels of these species – including the recurring high levels of DA in these organisms after toxin-producing phytoplankton blooms had ceased -- may be due to differences in uptake, retention and/or depuration rates, or continual uptake of DA from benthic sources that are not detected through the shallow water (typically ≤ 15 m (50 ft)) sampling of phytoplankton. Knowing this information will help to identify potential risks associated with consumption of these types of seafood during DA events, and to predict the necessary duration of advisories for each species. In addition, while data collected during this project suggest there may be 'hot spots' - fishing locations that may be at higher risk to DA and other biotoxins - at certain locations or sides of the islands, additional sampling is needed to evaluate whether this is always the case. Moreover, integrating these data with oceanographic patterns also may help identify such potential 'hot spots'.

Overall, additional biotoxin monitoring is clearly needed as the risks of exposure to DA - and potentially other biotoxins - differ among offshore/island sites and the coast, and current coast-based monitoring efforts are not adequate to track and advise on offshore/island HAB events. Through this project and ongoing efforts of the CDPH Biotoxin Monitoring Program, we have illustrated how a collaborative network of volunteers from fishing and coastal communities can provide data useful for more robust evaluations of HABs in California. Resulting data have helped

to identify potential factors important to evaluating the risks associated with HABs (domoic acid blooms specifically), and the process itself has illuminated several key elements required for the program to continue to expand and succeed, including:

- Collaborations with diverse community members
- Balancing the capacity of the state laboratory with sampling needs and effort
- Arrangement of appropriate permits
- Efficient communication and reporting systems
- A primary program coordinator

Due to the limited funding provided for biotoxin monitoring in California, voluntary collaborations with diverse community members will continue to be essential for expanding monitoring and, thus, improving seafood safety in the state. Sampling efforts will need to be balanced with the capacity of the state laboratory, with a need to expand that capacity due to the increase in HAB events and associated sampling. While current communication and reporting protocols have been adequate, the continued expansion of a volunteer network may make these duties more difficult to handle in a timely manner. Thus, appointment of a dedicated position to coordinate the volunteer effort on a statewide basis, maintaining the Entity Collecting Permit, and facilitate enhanced communication of results with volunteers and the public would be beneficial. Further, development of a web-based portal may help improve communication with volunteers and the dissemination of sampling results. Together, these items and the additional resources they require would undoubtedly help to improve seafood safety of California's marine recreational and commercial fisheries while building collaborations among coastal communities.

INTRODUCTION

Harmful algal blooms (HABs) are natural events that pose risks to seafood consumers of both commercially and recreationally harvested seafood. In particular, paralytic shellfish poisoning (PSP) and domoic acid poisoning (DAP, also referred to as amnesic shellfish poisoning, or ASP) result from biotoxins produced by phytoplankton (dinoflagellates and diatoms, respectively), with the toxins passed through the food web. These toxins cause acute problems in humans (and seabirds and marine mammals), including serious illness and even death over a short period of time. Problems occur following consumption of all or part of marine organisms containing PSP toxins and domoic acid (DA); the viscera and roe of lobster and crabs, or whole consumption of filter feeding organisms such as mussels and anchovy. Public health safety advisories are issued when needed to warn the public about hazards related to consumption of commercially and recreationally harvested species in California, including lobster, Dungeness and rock crabs, scallop, mussels, anchovy and sardine.

In California, the PSP toxins have been monitored along the coast since 1929 by the California Department of Public Health (CDPH), the oldest such program in the US. As bivalve aquaculture increased in the 1980's, the California Department of Fish and Wildlife (CDFW) provided funds to partially support expansion of the existing PSP monitoring program to accommodate the growing shellfish aquaculture industry. Following the identification of DA as the link with the deaths of hundreds of seabirds in 1991, CDPH increased its analytical capacity for this new toxin despite the absence of increased funding. A volunteer-based phytoplankton monitoring program was begun in 1991 for early detection of toxic blooms and to focus sampling resources. DA monitoring has relied upon the existing network of: County, State, and Federal program participants; the required weekly sampling by commercial shellfish growers; and intermittent sampling by volunteers via a three-tiered sampling scheme. Phytoplankton is sampled weekly throughout the year at many sites and sent to CDPH for identification of the presence of the toxin-producing species (Tier 1). Other independent programs, such as the California Coastal Ocean Observing System, provide observational data on HABs through phytoplankton sampling as part of their research effort. This Tier 1 sampling, however, does not indicate whether the toxins are present, as they are not continually produced by the phytoplankton. Thus, bivalve (filter-feeding) shellfish (typically mussels) also are sampled throughout the year, but at fewer sites and less frequently (1-2x per month) at non-commercial shellfish aquaculture sites, to determine whether toxins are actually present (Tier 2). If toxins are detected, then recreationally and commercially important animals from higher trophic levels (lobster, crab), as well as filter feeding fishes (anchovy, sardine) are sampled at appropriate locations as possible (Tier 3). This last sampling tier is a relatively new component to the state program, as the program was created specifically for bivalve shellfish monitoring.

While this program has been adequate in the past, it has recently been found to have some shortcomings due to notable changes in the pattern of HABs in California over the past decade. First, the prevalence, intensity and duration of biotoxin blooms, particularly DA, have increased. As a result, there is a need to monitor commercially and recreationally important species (Tier 3) more frequently as they are exposed to higher levels of biotoxins more often and for longer periods of time. Second, these blooms have developed and/or continued offshore, especially in

the Santa Barbara Channel (SBC) region, and appear to often be decoupled from coastal blooms – something not commonly seen in the past. This new pattern in the distribution of HABs now requires that monitoring occur in offshore areas, not just along the coast as is presently done and that Tier 3 monitoring be expanded. The project team has been working together to address gaps in monitoring as they can. However, a more focused and organized effort is now critically needed given 1) the realization that these patterns are persisting, and 2) when HABs occurred unexpectedly throughout the previous lobster season both near- and offshore samples for biotoxin analysis were obtained haphazardly, rendering useful, but incomplete data.

Goals/Objectives

The goal of this project was to improve seafood safety of commercial and recreational fisheries in California by improving monitoring and evaluation of biotoxins. Our objectives included:

- Identifying, training and engaging volunteers in biotoxin monitoring
- Modifying, as needed, sampling, communication and reporting protocols by pilot testing the expanded volunteer network
- Identifying needs for HAB outreach materials
- Exploring correlations between nearshore and offshore HABs

The overarching research question was: Can a collaborative network of volunteers from fishing and coastal communities provide data useful for more robust evaluation of HABs in California?

With funds provided by the Collaborative Fisheries Research West, we built collaborations with and among fishing and other coastal community members (herein referred to as "HABNet" or the "HAB Network") to achieve our objectives and evaluate our research question. Results from this work also enabled evaluation of public health risks for wild-caught fish and invertebrates, thereby informing seafood safety advisories for recreational and commercial fisheries.

METHODS

We conducted this project along the California coast from Santa Barbara south to the Mexican Border. This area includes the Santa Barbara Channel (SBC) region where DA events have been more frequent and severe. It also is where the spiny lobster and the majority of the rock crab fisheries occur, commercially and recreationally important species that are impacted by DA events. We define 'coast' samples as those taken from piers and the shore, 'offshore' samples as those taken nearshore (typically about 1-10 miles from the coastline) and 'island' samples as those taken at or near offshore islands (typically 15-35 miles from the mainland coast). The federal alert level for PSP toxicity is ≥ 0.8 ppm (80 µg/100 g of shellfish tissue), and the detection limit for the PSP bioassay is approximately 0.4 ppm (40 µg/100 g). The alert level for DA is ≥ 20 ppm (20 µg/gram of tissue) with a CDPH FDLB reporting limit of 2.5 ppm (values less than 2.5 ppm are herein referred to as 'non-detect').

Engaging Volunteers

To address our first objective, we contacted many organizations within Southern California coastal communities including:

- Sportfishing and dive charter operations
- Sportfishing and dive organizations (e.g., United Pier and Shore Anglers of California, Council of Divers, Sportfishing Association of California, University dive clubs, Lifeguard Association)
- Commercial fishermen (divers, trappers) and seafood distributors
- Offshore oil and gas groups
- Community-based marine educational groups (e.g., Sea Camp San Diego, Ocean Institute, Voyager Excursions, Island Packers, Ty Warner Sea Center)
- University dive safety officers and research divers
- University research programs (e.g., CalCOFI)

We had to balance the need for obtaining samples with the capacity of the CDPH lab to receive and process the samples. Thus, we did not broadcast or widely advertise the need for volunteers. Instead we contacted individual persons and organizations based on recommendations from coastal community members. We also identified a few groups and individuals through web-based research.

Initial contacts were made by telephone and/or email. A flyer about the project (Appendix A) was provided as background material. For those we were not able to reach this way, we traveled (in most cases) to the place of business and attempted face-to-face contact. We then scheduled a training session with those who were interested in participating in the program.

Volunteers were trained in one or more sampling protocol, depending on their situation and ability to assist with sampling of various organisms. In general, education groups and sportfishing operators were trained in Tier 1 (phytoplankton) sampling, and dive organizations, commercial fishermen and university divers were trained in Tier 2 (bivalve) and/or Tier 3 (crustacean) sampling.

Following training, participants were asked to collect a sample. Once the sample was submitted to and processed by CDPH, we followed-up with participants to discuss how the sampling went, if they received the results from their sample, and if they had any questions or suggestions regarding the overall process.

Also, in collaboration with the Southern California Coastal Ocean Observing System (SCCOOS) and the Scripps Institution of Oceanography, we explored the potential for obtaining phytoplankton samples during quarterly offshore research cruises associated with the CalCOFI program. A presentation was made at the CalCOFI annual conference, with follow-up discussions about sampling sites and methods.

Sampling, Communication and Reporting Procedures & HABNet System Test

For our second objective, we reviewed and discussed with CDPH the current sampling, communication and reporting procedures used in the California Biotoxin Monitoring Program.

We also gathered information about four programs that engage volunteers in monitoring of harmful algal blooms elsewhere (Appendix B):

- Phytoplankton Monitoring Network (PMN)
- Red Tide Offshore Monitoring Program (RTOMP)
- Massachusetts Division of Marine Fisheries (DMF) PSP Monitoring Program
- Olympic Region Harmful Algal Blooms Partnership (ORHAB)

Based on this information, we, in consultation with CDPH, modified the current state procedures as needed. The resulting procedures were then tested and further modified based on input from participants.

Outreach Materials

To address Objective 4, we queried HABNet volunteers about the need for outreach materials on harmful algal blooms. Based on their input, we looked for existing materials that might include the information they had prioritized through both literature, web searches and materials referred by contacts from other HAB programs. In cases where the information was not readily available, we developed new materials. Drafts of the new materials were provided to interested HABNet volunteers and colleagues. The materials were modified based on their feedback.

Data Analysis

HABNet volunteers collected phytoplankton, bivalve and crustacean samples at various locations, and CDPH analyzed the samples for PSP and/or DA. To address Objective 5, the resulting data were examined separately, as well as with data from samples collected by other volunteers already assisting CDPH thereby expanding the data set. The combined data were synthesized and analyzed to explore the relationship between nearshore and offshore/island blooms.

RESULTS

Engaging Volunteers

We contacted 40 groups throughout the region and trained 31 individuals from these groups in one or more tiers of sampling (Tables 1, 2). Although some individuals that were trained submitted only a couple of samples, if any, 24 volunteers have continued to provide samples to CDPH. We encountered different advantages and challenges with engaging each group in biotoxin monitoring, as described in the following section.

Sportfishing and Dive Charter Operations: We initially concentrated our efforts on contacting and working with charter boat operators. We believed these groups would provide several advantages for sampling, in particular having staff and clientele with the necessary sportfishing permits to collect samples of mussels, scallops, crab and lobster (Tier 2 and 3) and having staff available to take water samples (Tier 1) while their clientele were fishing or diving. They also had the added benefit of being able to educate their clientele while out on the water about HABs, sampling and seafood safety. Unfortunately, we had little success in engaging these groups in biotoxin monitoring. Many groups did not respond and those that did lacked the time and staff to commit to monitoring. Still others that were interested and trained did not follow through. We

tried providing weekly reminders, but this did not seem to help. We also encountered regulatory constraints with the volunteers not being able to process the samples onboard to provide the desired parts for testing, while keeping the rest for consumption (see section "Tier 3 Crustacean Sampling" below).

Sportfishing and Dive Organizations: We reached out to several organizations with members that fish and/or dive. While some groups did not respond, some were interested in the monitoring and tried to connect us with their members. Unfortunately, few volunteers were identified. The key representatives indicated the following challenges for their members: 1) insufficient time for monitoring, 2) rarely traveled offshore, with trips often sporadic so sampling would not be consistent, and/or 3) unable to easily consume the 'catch' while also providing material for biotoxin analysis. Our best success with this group of organizations was identifying retired members that were able to conduct land-based monitoring. One volunteer in particular has filled a critical gap in the ongoing coastal pier-based monitoring program.

Commercial Fishermen & Seafood Distributors: Commercial fishermen, both divers and trappers, also were identified early on as potential candidates for biotoxin monitoring. However, permitting issues proved to be challenging, making it difficult to integrate commercial fishermen into our HAB Network. Notably, commercial divers could not obtain bivalves or crustaceans without having additional permits, and they could not combine the take of their targeted commercial species with biotoxin sampling (a non-commercial activity) (see sections "Tier 2 Bivalve Sampling" and "Tier 3 Crustacean Sampling" below). While trappers had the necessary permits for obtaining samples of crabs and lobsters, the shipment of these samples proved challenging and we encountered some additional permitting issues. These problems were resolved by working with a seafood distributor (see section "Tier 3 Crustacean Sampling"), and samples continue to be obtained through this manner.

Offshore Oil and Gas Groups: There are 27 offshore oil and gas platforms in California, with the majority (23) of the platforms south of Point Conception; 16 in the Santa Barbara Channel and 7 south off Orange County. These platforms are two to ten miles offshore in water depths of about 30-365 meters (95-1198 feet). Given their locations offshore, we thought these would be ideal biotoxin monitoring sites especially because some of the platforms are located between coastal and island sampling sites. In the past (and still today), we were able to obtain occasional samples from the platforms through research divers. While these samples have been extremely useful – in fact they were some of the samples that revealed the presence of high toxin levels offshore – sampling is sporadic as research projects at the platforms come and go. To explore ways to obtain more consistent samples from the platforms, we talked to three community and agency representatives that work with oil companies and agencies about the possibility of engaging supply boat operators, platform inspectors and/or platform operators in biotoxin monitoring. Initial discussions concluded that assistance with Tier 1 (phytoplankton) sampling would likely be the easiest because they would not need additional permits and the samples could be easily mailed once back on the mainland. While we have yet to work out continuous monitoring with any oil and gas related groups, discussions are ongoing.

Community-Based Marine Education Programs: We contacted 10 education programs about participating in biotoxin monitoring. These groups were the most responsive and provided the greatest number of phytoplankton (Tier 1) samples. Our success with this group is likely due to two key factors: 1) these programs often have a crew and volunteers available for monitoring, with a coordinator that can schedule and oversee monitoring activities, and 2) monitoring activities coincide with their interests and they typically can be readily integrated with their ongoing educational activities. These groups primarily provided water samples, but some also were interested in assisting with the sampling of mussels. Unfortunately, monitoring of mussels requires additional permits that are not typically held by these groups, resulting in delays that are still being addressed (see section "Tier 2 Bivalve Sampling" below).

University Dive Safety Officers and Research Divers: We contacted dive safety officers at two Cal State and two UC campuses. We also talked to research groups known to frequent the offshore oil platforms. We provided training to the DSO of one Cal State and one UC, and one research diver. Others did not respond to us or were no longer diving in the areas we were interested in. While we initially thought permitting would not be a problem, we later learned that many of these divers did not have collecting permits because they are typically engaged in 'monitoring' of populations and do not remove organisms. Still others that did, had permits that did not include the species we were interested in (mussels, scallops and/or crabs). While some of these people also dive for sport and have a sportfishing license that allows the take of mussels, scallops, lobster and crabs, they could not legally obtain samples for the state program with a sportfishing permit if they were conducting research at the time of the collection. Nonetheless, one group had the required permit and we were able to obtain offshore samples from them.

University Research Groups: We worked with the former Director of the CalCOFI program to explore the possibility of collecting phytoplankton samples for biotoxin monitoring during the quarterly CalCOFI cruises. We contributed to a presentation and associated extended abstract entitled "A Southern California perspective on harmful algal blooms" for the annual CalCOFI conference and proceedings (Appendix C). Following the conference, we continued to work together to prioritize CalCOFI sampling stations for biotoxin monitoring. We prioritized a total of 32 CalCOFI sites, with seven sites being highest priority, another ten sites of secondary priority, and the remaining 15 sites being desirable. We proposed a pilot test where samples would be taken from the high priority sites (n=7) using the established protocols of the state program. Unfortunately, the Director of the program stepped down as we were trying to get things implemented. We continue to work with this program, and other research programs, to obtain offshore samples.

Sampling, Communication and Reporting Procedures & System Test

We found the procedures used by the California Biotoxin Monitoring Program to be fairly similar to those used in other biotoxin monitoring programs. Volunteers from various groups within the community are trained in sampling and shipping methods, with most supplies provided by the coordinating agency. In three of the four programs (PMN, ORHAB, DMF), the volunteer sampling program acts as an early detection system for the state and federal agencies charged with public

health advisories related to biotoxins. That is, the volunteer-associated program notifies the regulatory agencies of a detected bloom, and then the regulatory agencies conduct their own 'official' sampling. This is in contrast to the ongoing program in California and our HABNet efforts, and one program (RTOMP) we reviewed, where the regulators are directly involved, processing the samples obtained by the volunteers and using the resulting data to directly inform seafood health advisories. The coordinating agency usually provides a coordinator who oversees the activities of the volunteers, but these coordinators typically have many other responsibilities. In some cases trainings are conducted by other staff to help spread out the tasks within an organization. Two programs (PMN, RTOMP) also have integrated web-based media into their programs, but in different ways. One program (PMN) uses it for training volunteers, data entry and as a data portal. Volunteers enter data directly, with PMN staff confirming and maintaining the data. These data are informational and not used for regulatory purposes, but they are accessible to viewers, with various options for selecting specific subsets of data that are output as maps and other formats. The other program (RTOMP) uses web-based media for communicating with volunteers. Volunteers use the "Volunteer Info Center" website to submit questions to the coordinator, track their volunteer hours, and post logistical sampling information. There also is a "New Volunteer Inquiry" page that contains a list of areas where monitoring is still needed, along with frequency and sampling specifications which helps with recruiting new volunteers. All programs, including the one in California, maintain general program websites that contain outreach materials and reports on HAB blooms. These systems reduce at least some of the workload of the coordinator and increase the ability of volunteers to access their data and/or see how their efforts are contributing to the program.

Protocols

Based on our review of the other programs, few changes were made to the protocols already in use for the California Biotoxin Monitoring Program. Here we describe the few modifications we made before or after testing the protocols for each tier of sampling with our HABNet volunteers. We also highlight challenges we encountered and solutions we applied based on feedback from volunteers and CDPH.

Sampling Procedures

We developed three sampling protocols for HABNet participants (Appendix D). Two of these protocols – phytoplankton and bivalve shellfish -- were almost identical to existing CDPH protocols. A third protocol was developed to facilitate sampling and shipping of crustaceans (crabs and lobster) for biotoxin analysis. This third protocol was based upon the CDPH bivalve shellfish protocol, with handling information modified for the appropriate species.

Tier 1 Phytoplankton Sampling Protocol (Appendix D.1): The existing CDPH phytoplankton protocol was slightly modified to account for differences in sampling offshore from a boat in deeper water as compared to sampling off a pier. In particular, the number of vertical tows was reduced. This change was necessary because each vertical tow was now longer due to the increased water depth.

Sampling protocols from other programs are similar to the California protocols. However, volunteers of some programs also process the phytoplankton samples and there are protocols and associated data sheets for this activity. Such activities vary from being the normal procedures of a program, to being used only for certain individuals. We facilitated and assisted CDPH with two phytoplankton ID trainings for two groups of volunteers that expressed interest in processing the samples. At these training workshops volunteers learned about 1) HABs in California, 2) ongoing monitoring and sampling efforts, and 3) identification of phytoplankton species, and HAB-species in particular. The workshops also contained a hands-on session where volunteers looked at water samples and identified the various species that were present. One of these groups has continued to provide their analyses to CDPH. The other group has had some recent turn over, but they may start up again.

Volunteers participating in Tier 1 (phytoplankton) sampling found the plankton tows to be easily integrated into their activities. The only bottleneck encountered was a delay in the return of the shipping canisters. This was only a problem once or twice, and it was corrected by providing additional canisters.

Tier 2 Bivalve Shellfish Sampling Protocol (Appendix D.2): The bivalve protocol did not require any major modifications, just some minor changes of wording to clarify sampling procedures. However, the permitting issues regarding this type of sampling proved to be challenging. Unlike taking water (phytoplankton) samples, a permit is required to collect mussels. For commercial divers, they must have a tidal invertebrate permit which not everyone has and, in many cases, the take of bivalves was restricted from shallow intertidal reefs where the mussels occur. Further, there were some regulatory constraints regarding the take of mussels for biotoxin sampling while also conducting commercial fishing activities. Because of these problems, we did not pursue engaging the commercial divers in this tier of sampling.

For sport divers and other volunteers, mussels can be taken with a sportfishing license or a scientific collecting permit. However, not all volunteers have these types of licenses. In order to address this issue, we talked with CDFW and CDPH about obtaining an Entity Scientific Collecting Permit that would cover volunteers. This permit was determined to be a good fit for the program, as it covers numerous volunteers under one permit thereby negating the need for individual permits. It currently (2014) costs $500, which covers a three-year period. CDPH was identified as the ‘entity’ that would hold the permit. We collected the necessary information from volunteers and worked with CDPH and CDFW to develop the text required for the permit application. This permit, which was still being processed when this project ended, allows listed volunteers to provide bivalve (and crab) samples for the state biotoxin monitoring program. However, the permit only covers those listed and there is an additional $100 fee each time you need to update the list (i.e., add/remove volunteers). Regardless, once the permit is approved, current volunteers will be engaged in the collection of mussel samples at additional sites.

Tier 3 Crustacean (Crab/Lobster) Sampling Protocol (Appendix D.3): While we were able to use much of the information from the bivalve sampling protocol for the crustacean protocol, quite a bit of time was spent testing different ways to handle/prepare crab and lobster samples. Two

methods were ultimately included in the final protocol: 1) live or frozen animal samples and 2) cooked animal samples. These different methods were necessary to accommodate both CDPH and the volunteers, some of whom like to consume the lobster tail and/or crab claws but are willing to contribute the rest of the animal for biotoxin analysis. We also developed a submission slip to accompany the shipments of crab/lobster samples to CDPH. The form used for bivalve sampling was not sufficient because it did not allow for samples from more than one site. Working with commercial fishermen, we were able to obtain samples from at least two sites at a time and, thus, we needed to develop a new form.

As with bivalve sampling, permitting issues also were problematic for Tier 3 sampling. Commercial trap fishermen were willing to provide samples but they did not want to have to take the time to ship the samples once back at port. Thus, we explored the possibility of having another person act as courier for the sample, but a different permitting issue arose; fishermen must have a receiver's license in order to provide seafood (the samples) to someone else -- something they don't all have. To remedy this problem, we worked with a crab distributor who obtained the samples from the fishermen as part of his business and then he shipped them to CDPH (with assistance from DFW) for analysis. This set-up has been valuable because the seafood distributor not only has contact with many fishermen, but he also has the permits required for receiving and shipping samples.

For sport fishermen and women, we also encountered regulatory constraints with the handling and processing of samples by volunteers before returning home. For example, lobster tails and crab claws are prized parts for many consumers, while the lab only requires the internal organs. While these parts can be readily separated from each other, such cannot be done legally on a boat or in port because law enforcement officers cannot determine if the catch meets the required size limit if the animal is not intact. That is, the size of the animal cannot be determined from the tail or claws alone; the body is needed. As such, volunteers must transport the intact animal to the place where they will consume it, thereby eliminating the ability of charter businesses and harbors to lend a hand in obtaining and shipping samples.

Despite these hurdles, data were obtained from samples taken by commercial and recreational fishing volunteers that had the appropriate permits to obtain crab samples. To expand the potential for obtaining crab samples from more volunteers, four species of crab were included on the Entity Collecting Permit and we expect more crustacean sampling will be obtained upon approval of the permit.

Communication Procedures

CDPH communicates with volunteers via email predominately, but telephone when needed. When they see a need for Tier 2 or 3 sampling in an area they will send out an email to volunteers in that area asking them to collect and ship samples. This system is similar to that of other programs, although one program (RTOMP) also uses a web-based system to communicate with their volunteers.

We tested the effectiveness of these procedures with new HABNet volunteers. This system worked well in most cases. Some volunteers were best reached via email, while others via telephone. The combination of methods often was used to insure a person was reached.

Reporting Procedures

CDPH typically sends an email with the results of the sample analysis directly to the volunteer who provided the sample(s). These results are later incorporated into a monthly biotoxin report that shows general toxin levels throughout the state. Volunteers also receive this report via email, and it is available to them and the public on the CDPH website. These protocols are similar to those used elsewhere. However, some programs use a password protected web-based reporting system that allows volunteers to access the results from their sample analyses whenever they want. They, along with the public, also can extract data in various ways.

Our volunteers reported a few issues with the reporting procedures. First, some volunteers were not getting the results from their samples analyses. This typically happened when there were two or more volunteers being coordinated by a third person who was listed as the contact person for the group, or if a third party shipped samples for a volunteer. This problem was readily corrected by adding contact information for all persons involved in the sampling/shipping effort. Second, many volunteers found it difficult to understand the state monthly biotoxin reports and how their samples contributed to the findings. Most understood the report once we walked them through it, but they found the coding system too time consuming to decipher. They offered several suggestions for modifying the report and web-based map, and these have been passed along to CDPH for their consideration.

Outreach Materials

Our HABNet volunteers identified a need for four types of outreach materials. First, they requested a short one page information flyer about HABs in California. Volunteers were particularly interested in knowing the levels of concern and the symptoms associated with exposure to the two primary toxins in California (domoic acid and PSP). Second, they were interested in a one page flyer that indicated which California seafood was affected and what parts of the animal to avoid. Third, they wanted to better understand the history of the toxins and associated testing in California. Last, was a request for a short (few sentences) message to let consumers know about the potential danger of biotoxins and to avoid certain parts of the animal. This information was intended to be included with product that was commercially harvested from an area where toxins were detected above the critical level. The volunteer also requested that it be provided in multiple languages so consumers of many ethnicities could read it.

We were able to develop three of the four outreach materials, in collaboration with CDPH (Appendix E). For the first two (Appendix E.1, E.2), we developed a short and long version of each flyer, and gathered feedback from our diverse group of reviewers. While most people appreciated the visual aesthetic of the shorter version because it was less crowded, they liked having the additional information that was included in the longer version. These materials are still under review, but will be posted on the California Sea Grant website, with links provided to other interested groups (e.g., CDPH, CDFW, CFRW). We began development of the third information

flyer -- history of the toxins and testing in California – but realized it would require a more thorough analysis of sampling data. Such analyses were deemed outside of the scope of the project and, thus, we did not finish that information sheet. We did develop the last outreach material; the short message for consumers (Appendix E.3). We worked with CDPH and certain volunteers on the text. Then we had the text translated into seven different languages, including Chinese, Indonesian, Japanese, Korean, Spanish, Tagalog and Vietnamese.

Data Analysis

HABNet project volunteers collected a total of 175 samples, including 81 water samples (Tier 1), 7 bivalve samples (4 mussel and 3 scallop samples) (Tier 2) and 87 crustacean samples (81 crab and 6 lobster samples) during the one year project period (Tier 3) (Table 3). The majority (90.9%) of samples were collected in the Santa Barbara Channel region where we targeted our effort due to the continued presence of domoic acid, with a few samples collected in Los Angeles (6.8%) and San Diego (2.3%) counties.

Plankton Samples

Toxin-producing species were found in 90% of the water samples collected by HABNet volunteers. *Alexandrium* sp., the dinoflagellate that produces the toxin responsible for PSP, was detected in only two (2.5%) of these samples. Both samples were taken in March, but were from different locations; one offshore Los Angeles County and the other off Santa Cruz Island, Santa Barbara County. The other HABNet phytoplankton samples containing toxin-producing species had *Pseudo-nitzschia* spp., the diatoms that produce DA, the toxin responsible for amnesic shellfish poison (ASP). These findings were similar to samples obtained by volunteers with the ongoing CDPH program, with only one sample containing the PSP-producer (*Alexandrium* sp.) during the study period, but the majority of others containing DA (ASP)-producing species.

When considering relative abundance – a standardized estimate that takes into account cell mass and the size of the sample (e.g., from a single 30-ft tow versus four 30-ft tows) – of toxin-producers in water samples collected by both HABNet and CDPH biotoxin monitoring participants, three blooms of differing levels were evident in the Santa Barbara Channel (Fig. 1). The largest bloom occurred in April and May 2013, along the coast, as well as offshore and at the islands. A low level, short duration bloom also was detected at the islands and offshore in early November 2012, with another small bloom evident along the coast in July 2013. The spring (April/May 2013) bloom that occurred in the Santa Barbara Channel also was detected in Los Angeles and San Diego Counties (Figs. 2, 3). Neither small bloom (November 2012, July 2013), however, was evident based on water samples from these two other regions.

Bivalve Shellfish Samples

The seven bivalve shellfish samples provided by HABNet volunteers during this project were collected in the Santa Barbara Channel region, with six samples from offshore sites and one sample from a coastal site that represented a gap in the existing state monitoring program. Domoic acid was not detected in the 6 offshore samples that included mussels and scallops. The seventh sample (mussels) from the coastal site had a detectable, but very low level of DA; 3 ppm. Slightly raised levels of domoic acid were detected in offshore samples of mussels (8 ppm) and

oysters (4.6 ppm) from a nearby shellfish farm a little over a week after this coastal mussel sample was taken. This finding illustrates how some sites also may be beneficial to shellfish growers by detecting toxins before they impact aquaculture leases.

DA levels in bivalves obtained from samples collected by both HABNet and CDPH biotoxin monitoring participants revealed two domoic acid blooms in the Santa Barbara Channel region (Fig. 1). These blooms included two of the three blooms revealed by the phytoplankton data; a large bloom in April/May 2013, and a small short-lived bloom in July 2013. No bivalve samples were taken in early November 2012 when phytoplankton samples revealed a small short lived bloom. Coastal bivalve samples obtained in Los Angeles and San Diego counties also detected a bloom in April, but the bloom appeared to be short lived and did not extend into May as it did in the Santa Barbara Channel (Figs. 2, 3). Bivalve samples from offshore/island areas were lacking at this time, thus it is unknown if the bloom also occurred offshore. However, a single crustacean sample from an offshore reef in Los Angeles County was non-detect for DA, suggesting the bloom may have been restricted to just the coast.

Crustacean Shellfish Samples

Of the 87 crustaceans collected by HABNet volunteers (Tier 3), 43% contained domoic acid above the critical level. The majority (94%) of the samples were collected at the islands, with 5% from the coast and 1% from offshore areas.

Based on DA levels in crustaceans, several blooms apparently occurred in the Santa Barbara Channel region, including the one major bloom in April/May 2013 that also was identified through analysis of phytoplankton and bivalve samples (Fig. 1). Three additional blooms were apparent in December 2012, March 2013 and September 2013. However, phytoplankton samples taken in December 2012 and March 2013 did not indicate a bloom was present then. This also was the case in September 2013 when neither phytoplankton nor bivalve samples supported the occurrence of a bloom. No blooms were evident based on the few crustacean samples collected at offshore/island sites of Los Angeles County (Fig. 2). The lack of crustacean samples from San Diego County (Fig. 3), as well as coastal Los Angeles County inhibited identification of blooms through Tier 3 sampling.

Toxin levels varied within and among crustacean species sampled from the Santa Barbara Channel (Figs. 1, 4, 5). DA levels in individuals sampled on the same day and area often varied widely regardless of species, with some individuals with DA levels above and below the critical level and others non-detect (< 2.5 ppm) for DA (Fig. 1). Overall, red rock crabs had a higher prevalence and average amount of domoic acid than brown rock crab and lobster, with brown rock crab a close second (Figs. 4, 5). Just over half (52%) of the red rock crabs sampled had DA levels above the critical level (≥ 20 ppm), with one third of those crabs with DA levels ≥ 120 ppm (Fig. 4). Red rock crabs had the highest average DA concentration (90.4) (Fig. 5), with a maximum of 360 ppm. Another 34% of the red rock crabs had DA levels that were below the critical level (< 20 ppm), with toxins not detectable (< 2.5 ppm) in 14% of this species of crab. For brown rock crabs, fairly similar percentages of crabs had DA levels that were above and below the critical level; 37% and 43% respectively (Fig. 4). DA concentrations averaged 69.1 ppm (Fig. 5), with 23%

of these crabs having DA levels ≥ 110 ppm, reaching a maximum of 216 ppm. Another 20% of the brown rock crabs had DA levels that were non-detectable. In contrast, only 22% of the lobsters sampled had DA levels that were above the critical level, averaging 24 ppm with a maximum of 26 ppm – just over the critical level of 20 ppm (Figs. 1, 4, 5). While the majority (56%) of lobsters were below the critical level, about one quarter of the lobsters in this category were approaching the critical level. Similar to red rock crab, DA levels of 22% of the lobster were non-detectable (< 2.5 ppm).

Sampling of crustaceans at the coast and offshore were extremely limited during the project. However, one Santa Barbara County volunteer obtained a few rock crabs – three brown rock crabs and one red rock crab - from a coastal site when Tier 1 and Tier 2 sampling had detected toxins in the area. Two of the three brown rock crabs had accumulated toxins just above the critical level (22 and 24 ppm), whereas toxins were not detected in the third brown rock crab. The red rock crab from the same site had a lower level of DA, below the critical level (2.9 ppm).

The prevalence and DA concentrations of crustaceans varied among island sites (Figs. 6, 7, 8). Of the three sites sampled two or more times, a higher percentage of crabs (55%) had DA levels above the critical level at the most northwestern site as compared to crabs from two nearby sites just to the east (Northcentral, 42%; Northeast, 44%), although sample sizes varied among sites (Fig. 6). However, the average concentration of DA was substantially higher at the most eastern site, as compared to the other two sites (Fig. 7), with rock crabs from this area having consistently higher DA concentrations than crabs from the other sites (Fig. 8).

Relationship between coastal and offshore/island blooms

DA blooms were evident both along the coast and at offshore and island sites during this project. The one major DA bloom that occurred in April and May 2013 was evident throughout the Santa Barbara Channel, extending from the coast to offshore and island areas (Fig. 1). The bloom was first evident in coastal phytoplankton samples and island crustaceans. Within several weeks samples from all three tiers along the coast as well as offshore and at the islands had detected the bloom. Another bloom was evident in November 2012, but this one was detected in only offshore and island phytoplankton samples despite water samples being taken along the coast at the same time. No bivalve or crustacean samples were collected then, as the program was just getting started. This bloom was short-lived, with no other phytoplankton samples detecting elevated levels of DA-producing phytoplankton for quite a while after. Elevated levels of DA were detected in crustacean samples from the islands about six weeks after the November bloom, but not in water samples from the coast or offshore/islands. A similar phenomenon also occurred in March and September 2013; crustaceans sampled at the islands had elevated DA concentrations, but DA levels in bivalves and relative abundances of DA-producing phytoplankton from the coast and offshore/island areas were non-detect or virtually zero.

DISCUSSION

Through this pilot project we illustrated the feasibility and usefulness of expanding the State Biotoxin Monitoring Program in offshore areas of Southern California to help inform state seafood safety advisories through a collaborative network of volunteers. With funding a major limiting factor, collaborations remain essential for the success of the current program and its continued expansion. However, recruitment of volunteers must be balanced with the capacity of the state laboratory, as only so many samples can be handled and processed at a time. With this in mind, we have evaluated the potential for engaging various groups and individuals that frequent offshore areas in biotoxin monitoring and have learned that some groups are more readily able to assist. In particular, we were most successful at engaging community-based marine education groups in Tier 1 (plankton) sampling, and commercial fishermen and seafood distributors and research divers in Tier 2 (bivalve) and 3 (crustacean) sampling. However, at least one individual from most of the groups we targeted participated in the pilot program. Further, we are still working with a few groups (e.g., offshore oil and gas groups; oceanographic research cruise programs) to work out details of how they may assist with monitoring efforts.

Permitting issues were, and continue to be, a major hurdle to engaging additional volunteers in bivalve (Tier 2) and crustacean (Tier 3) sampling. While sportfishing licenses, and in some cases scientific collecting permits, allow the take of these species, many of the folks interested in helping did not own such licenses. To address this issue we identified and helped facilitate the set-up of an Entity Collecting Permit to cover volunteers. Nonetheless, this system remains cumbersome and costly. Specific sites and volunteers must be named on the permit, and an amendment to the permit must be filed every time a new volunteer is added with an additional filing fee assessed per amendment. Streamlining this process would help tremendously with the expansion of the California Biotoxin Monitoring Program.

Regulations requiring the transport of intact animals also hindered participation of some individuals and groups in Tier 3 (crustacean) sampling. In particular, dive charter operations cannot be a focal point for collecting biotoxin samples due to these restrictions, unless volunteers provide the entire animal to the dive charter operators who then ship the samples whole to CDPH. Some volunteers were willing to ship the samples themselves once they were home, but this required additional materials when multiple people wanted to participate. Such will be required if one is to engage charter boat customers in monitoring activities. Commercial fishermen also were faced with needing permits to provide the samples to a third party that would ship the samples. In this case, we were able to address this issue by engaging seafood distributors who had the necessary permits and coordinate with multiple fishermen.

The time required to collect samples also deterred some volunteers from participating. While some were eager and able to obtain the samples, the shipping of samples for Tier 2 and 3 became a bottleneck. This was likely due to two factors: 1) samples could only be shipped on certain days based on when the laboratory could receive and process shipments and 2) FedEx locations were not always conveniently located. Neither of these two issues can be easily resolved, but separating the collection and shipment duties was one way we addressed this problem.

One other factor that may have impeded participation by some -- albeit it was never specifically stated --was the sensitivity and seriousness of the issue. In particular, charter boat operators and others may not have been comfortable explaining to their customers and others what they were doing as it would require explaining the potential risks associated with biotoxins and recreational and commercial fisheries. We worked with one group to develop some basic information that could be shared with customers, but there was still a lack of participation in the program. Clearly this is a difficult issue to explain, as the biotoxins can cause serious health issues but it also can be avoided when consuming some species (crabs, lobster, rock scallops) by discarding the parts (internal organs) of the animals where the toxins accumulate. Further, not all sites, species or even individuals within a species, are affected similarly, making it even more difficult to explain the risks. We also believe some commercial fishermen may not have participated for fear of advisories being posted if the samples they provided indicated high levels of domoic acid. Such state advisories have already impacted the ability of some fishermen in certain areas to sell their catch. Nonetheless, some fishermen and seafood distributors participated in the program and provided samples because they wanted to be informed about the status of biotoxin blooms so they could provide information to their customers. Given the sensitivities and complexities of this issue, there remains a need to develop clear, concise and realistic messages about the risks of biotoxins and seafood to help such groups explain the issues to their clientele. We are continuing to gather input from these groups that are willing to review outreach materials.

While the current state biotoxin program has been highly effective at maintaining a volunteer base for monitoring, the program would likely benefit from an updated web-based portal as used in other biotoxin monitoring programs. These systems allow volunteers access to their sample results and provide near real-time maps of where blooms are occurring in the state. HABNet volunteers indicated while they appreciated receiving the results via email, it was somewhat sporadic and they were not sure how their results compared to samples from other sites or informed the seafood advisories. The monthly reports distributed by the state include some distribution maps for the month and they have a web-based map where you can see results from phytoplankton sampling for the past several weeks (http://www.cdph.ca.gov/ HealthInfo/environhealth/ water/Pages/Toxmap.aspx), but they often are a bit delayed (not near real-time). Of course, some delay should be expected as samples have to be processed, but currently a report also has to be compiled and distributed before the state results are known. Development of an integrated sampling database and GIS mapping system, with support to develop and maintain it, may be advantageous not only for the volunteers, but also may improve timely dissemination of the information throughout the state. Other biotoxin monitoring programs have indicated that they and their volunteers have benefitted from these web-based systems. Notably, the HABMap portal (http://www.habmap.info/ data.html) provides near real-time data on phytoplankton monitoring from a small number of coastal sites that are part of the ocean observing system in California. Integration of this web-based mapping system with the ongoing State Biotoxin Monitoring Program that generates the regulatory HAB data may be a cost-effective way to address this need. However, because the state data are used for regulatory purposes, there would need to be very close coordination between CDPH and any outside group helping disseminate this information. Also, one would need to consider how such maps and web portals will interface with the biotoxin hotline and the state advisories. Consumers will continue

to need to rely on the hotline and advisories for specific information regarding seafood safety, as maps can be miss-interpreted.

A dedicated coordinator, or potentially regional coordinators, also would be beneficial to the California Biotoxin Monitoring Program. Currently the CDPH is doing a fine job at maintaining a volunteer program for biotoxin monitoring, especially given that this is just one of many responsibilities of the department and it requires coordination between two different laboratories that handle the three different tiers of sampling. However, some of the time delays experienced with reporting and dissemination of the results may be reduced if a person could be dedicated to overseeing the expansion of the program, recruiting and communicating with volunteers, making sure samples are received and processed as quickly as possible, and updating and disseminating the sample results in a timely manner. These duties will become more demanding as the program expands and more samples are received and communication with more volunteers is required. Given the importance of recreational and commercial fisheries to California, funding for such a position seems warranted.

Contributions of HABNet Data

Expansion of the California Biotoxin Monitoring Program to offshore/island areas through this collaborative volunteer network (HABNet) has resulted in data useful for improving seafood safety of recreational and commercial fishery resources. First, the data have illustrated how coastal and offshore/island blooms may at times be linked with a brief period of overlap, but that blooms may be more intense and persistent at offshore/island areas requiring additional monitoring as compared to coastal sites. This finding is supported by data from the one large, high level bloom (April/May 2013) documented during this project that occurred throughout the channel region with higher DA levels that remain elevated for longer periods in offshore/island bivalve and crustacean samples as compared to those from the coast. Potentially contradicting this finding are the data from the earlier (November 2012) low level bloom. These data suggest that the bloom was completely decoupled between coastal and offshore/island areas, as the bloom was only detected at the islands. However, because we had just started our sampling effort we realized the bloom may have occurred along the coast before we began sampling. This indeed was the case, according to data provided in the Biotoxin Monthly Report for October 2012. A high level domoic acid bloom had started in September 2012, and it was subsiding along the coast but persisting at the islands in October into early November. Thus, this pattern is similar to the large bloom that occurred during this project (April/May 2013), with some overlap in the timing of the blooms between coastal and offshore/island sites. Our documentation of high levels of domoic acid in crabs collected at the islands (December 2012; March 2013; September 2013) also suggest that coast and offshore/island blooms may sometimes be decoupled. However, we are not sure this is the case, as few to no toxin-producers or elevated DA levels were detected in phytoplankton and mussel samples taken around the same time and place. These results suggest that blooms were not present at these times, but instead the crabs and lobsters retained toxins produced by earlier blooms or they continued to update toxins through benthic means that were undetected through plankton samples.

The DA retention/depuration rates for rock crabs and the California spiny lobster are presently unknown. Our data indicate that DA toxins may be retained for many months, especially in rock crab. For example, it could be that the high levels of DA in crab samples from December 2012 and March 2013 were a result of the large, persistent bloom occurring in October/November 2012. Similarly, the high levels of DA in crab samples from September 2013 may have been retained from the bloom that occurred 5-6 months earlier (April/May). Recent data obtained after the end date of this project has recorded concentrations of DA that were lower than those in September for rock crabs from the islands. These low, but still critical levels (20-100 ppm) remain despite the lack of new blooms, further suggesting a slow, continuous depuration process for rock crabs. In contrast, lobster may depurate more quickly than rock crabs, as DA concentrations in lobster were just above the critical level when first sampled in December 2012, following the October/November island bloom. These levels dropped below the critical level by March 2013 when elevated DA levels were still being detected in rock crabs from the same site.

Determining DA uptake/depuration rates would be useful for improving our understanding of the risks associated with these commercially and recreationally important fisheries. Based on data collected during this project, it appears that there may be less risk of exposure to DA through consumption of lobster than red and brown rock crabs. However, the highest DA concentration recorded (1170 ppm) in California came from a lobster collected at the Northern Channel Islands, indicating lobster also can accumulate very extreme levels of DA. Clearly the risks of exposure to DA are very high with all three species, supporting the need to determine how fast toxins are taken up and how long toxins are retained for predicting potential risks associated with consumption of these species during and after DA blooms, and the necessary time frames for seafood advisories.

The variation in DA concentration documented among sites during this project also may be quite useful for developing advisories that are more accurate. While crabs sampled during this project came from the same general areas, we later received some samples from multiple areas, including just offshore from the coast. A comparison of these data indicated that while crabs from our primary island sites still contained DA concentrations above the critical level, crabs from areas on the other sides of the islands, as well as just offshore the mainland coast, were below the critical level or even non-detect. These data illustrate how area-specific the blooms may be, with crabs from some areas exposed more often, for longer periods or to more dense blooms than others. Identifying these areas may be useful for fine-tuning advisories and guiding recreational and commercial fishermen to fishing locations that pose less risk of DA exposure.

The existing CDPH coastal monitoring program for marine toxins has always used the coastal intertidal sampling locations for two purposes: 1) to protect the public that recreationally harvests shellfish; and 2) as an early warning system for toxic blooms that could move into the bays and estuaries where the majority of commercial aquaculture has traditionally occurred. This pilot program illustrated how expansion of the monitoring program similarly may be useful for nearshore and offshore shellfish growers. In particular, strategically located monitoring sites could improve early detection for shellfish growers with leases in the vicinity, beyond what their batch harvest testing can achieve. Further, these collected data may be useful as NOAA moves

forward with expansion of offshore aquaculture. While the responsibility and costs associated with managing and monitoring offshore aquaculture falls under federal jurisdiction, data collected now by HABNet may provide insight for identifying potential offshore/island sites that may be less desirable for raising shellfish due to reoccurring blooms, as well as those areas that may be protected from these events.

SUMMARY

The overarching research question of this project was 'Can a collaborative network of volunteers from fishing and coastal communities provide data useful for more robust evaluation of HABs in California?' Based on the results of this pilot project the answer is 'absolutely yes.' Not only were volunteers from diverse backgrounds engaged in sampling, but the samples they provided resulted in data that furthered the understanding of patterns of HABs in Southern California, particularly the Santa Barbara Channel Region, and helped to inform seafood safety advisories for state recreational and commercial fisheries. Continuation and expansion of offshore sampling is clearly needed, as coastal monitoring is not adequate for determining the prevalence, intensity and duration of offshore/island blooms and the risks associated with consumption of other wild-caught non-bivalve species (particularly crustaceans). Such is supported by the combined data collected by HABNet volunteers and those involved with ongoing CDPH coastal biotoxin monitoring. Funding remains a major hurdle for expanding the state program, and additional funds are critically needed for increased laboratory analyses and for a dedicated coordinator to oversee volunteer efforts that are essential for biotoxin monitoring in the state. With these additional resources and continued collaborations with various groups and volunteers the state will be better able to evaluate the potential risks of HABs to seafood consumers, thereby improving seafood safety of California's valued marine recreational and commercial fisheries.

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Table 1. Effort and participation in HAB Network.

County	Number Contacted	Number Trained	Number Continuing	Number of Groups Monitoring Per Tier		
				Plankton	Bivalves	Crustaceans
Santa Barbara	16	15	10	8	5	6
Ventura	11	14	13	14		
Los Angeles	5	1	1	1		1
Orange	1	0	0			
San Diego	7	1	1	1		
Totals	40	31	24	24	5	5

Table 2. Groups participating in HAB Network.

Group Type	Number Contacted	Number Trained	Number Continuing	Number of Groups Monitoring Per Tier		
				Plankton	Bivalves	Crustaceans
Fish/Dive Charter Operations	12	4	0	3	0	0
Fish/Dive Organizations	4	1	1	1	1	0
Commercial Fishermen/ Seafood Distributors	3	3	3	0	0	3
Offshore Oil	3	0	n/a	n/a	n/a	n/a
Community-Based Educational	10	21	18	19	3	3
University DSOs/Divers	5	2	1	0	1	1
University Research Programs	1	0	n/a	n/a	n/a	n/a
Agencies	2	1	1	1	0	0
Total	40	31	24	24	5	7

Table 3. Sampling effort of HABNet volunteers.

Region	Number of Plankton Samples Taken	Number of Bivalve Samples Taken	Number of Crustacean Samples Taken	Total Number of Samples	Number of Animals Sampled with DA ≥ 20 ppm
Santa Barbara Channel	66	7	86	159	36
Los Angeles	11	0	1	12	0
San Diego	4	0	0	4	n/a
Total	81	7	87	175	36

Figure 1. Domoic acid concentrations in bivalve and crustacean samples and relative abundance index of domoic acid-producers in phytoplankton samples from the Santa Barbara Channel region. A. Coastal samples. B. Offshore and Island samples. Dotted line represents the regulatory critical level for domoic acid. Scales differ.

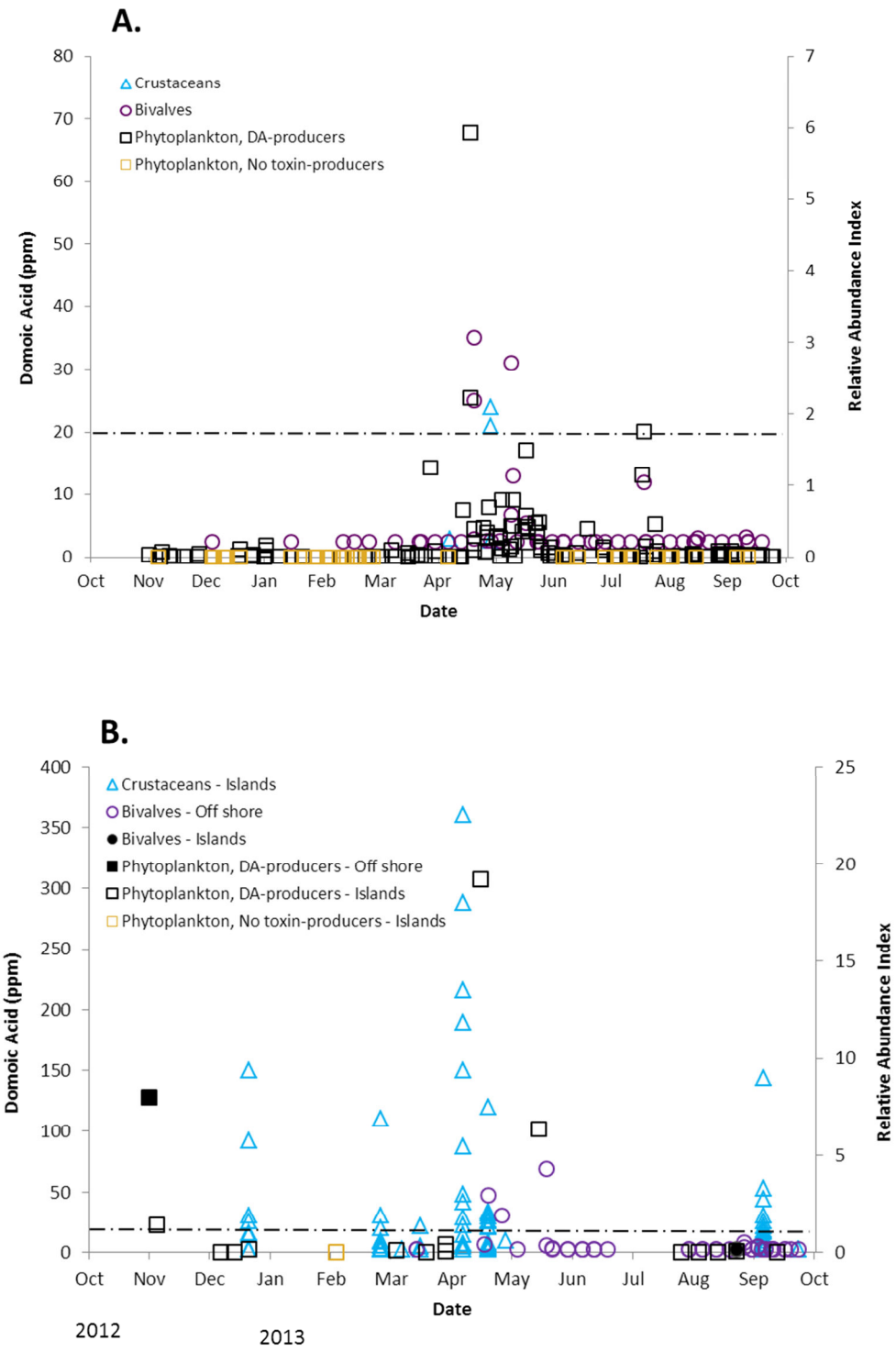
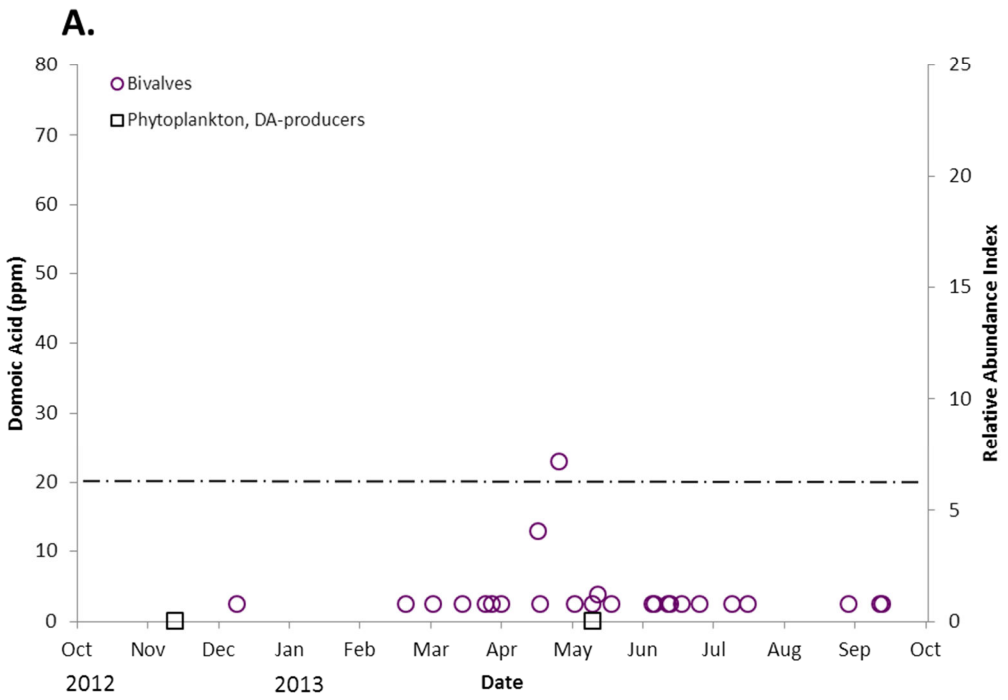


Figure 2. Domoic acid concentrations in bivalve and crustacean samples and relative abundance index of domoic acid-producers in phytoplankton samples from the Los Angeles region. A. Coastal samples (no crustacean samples taken). B. Offshore and Island samples.



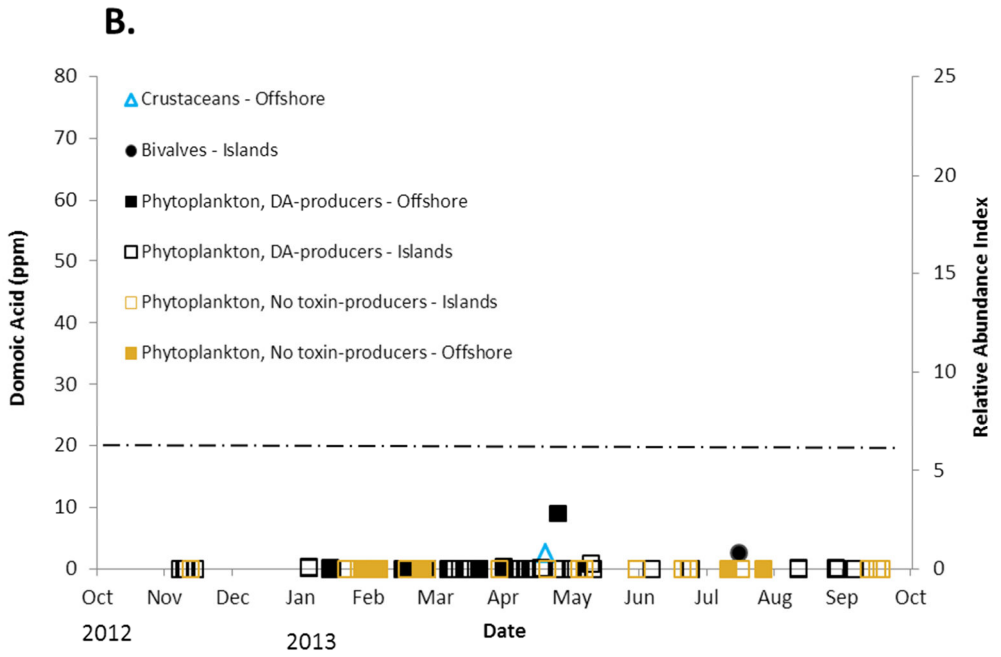
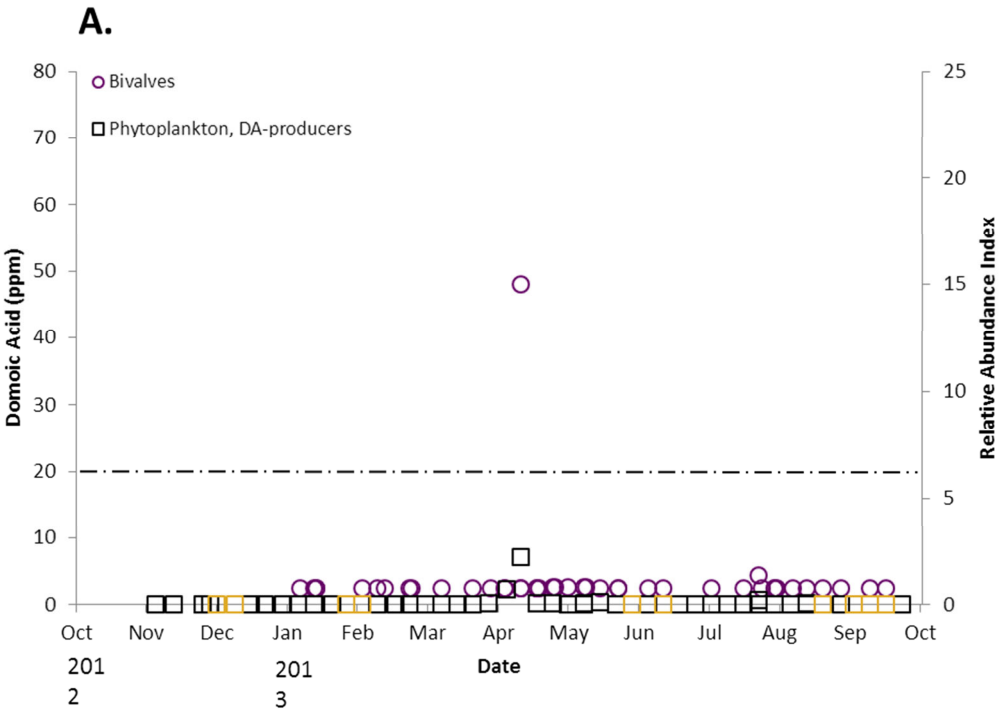


Figure 3. Domoic acid concentrations in bivalve and crustacean samples and relative abundance index of domoic acid-producers in phytoplankton samples from the San Diego region. A. Coastal samples (no crustacean samples taken). B. Offshore and Island samples (no bivalve or crustacean samples taken). Scales differ.



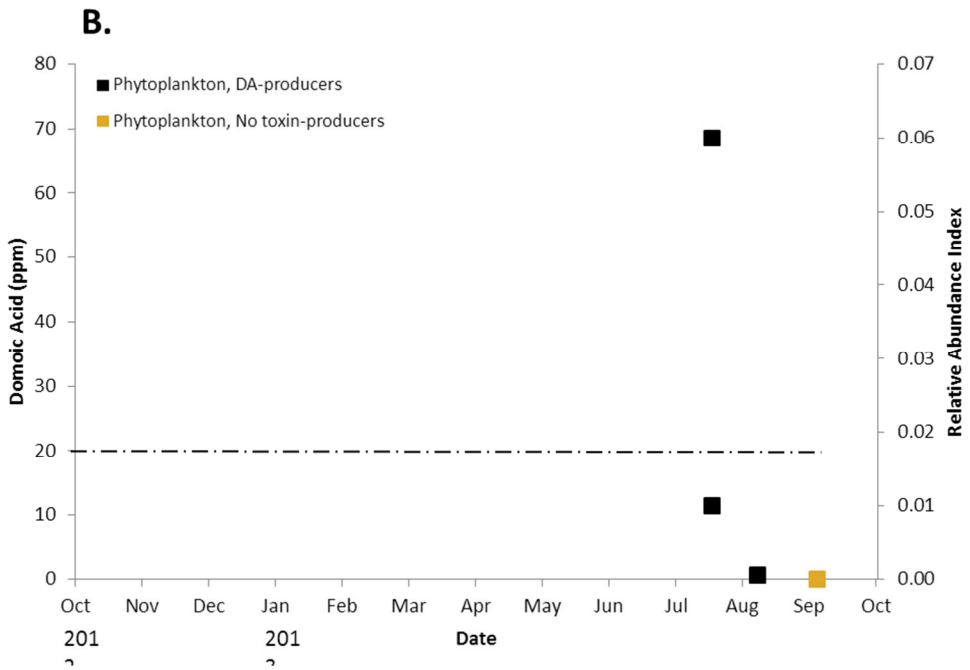


Figure 4. Percentage of samples above and below the critical level (≥20 ppm) and below the reporting limit (< 2.5 ppm) of domoic acid for three crustacean species; red rock crabs (*Cancer productus*), brown rock crab (*C. antennarius*) and California spiny lobster (*Panulirus interruptus*). Sample size indicated above bars.

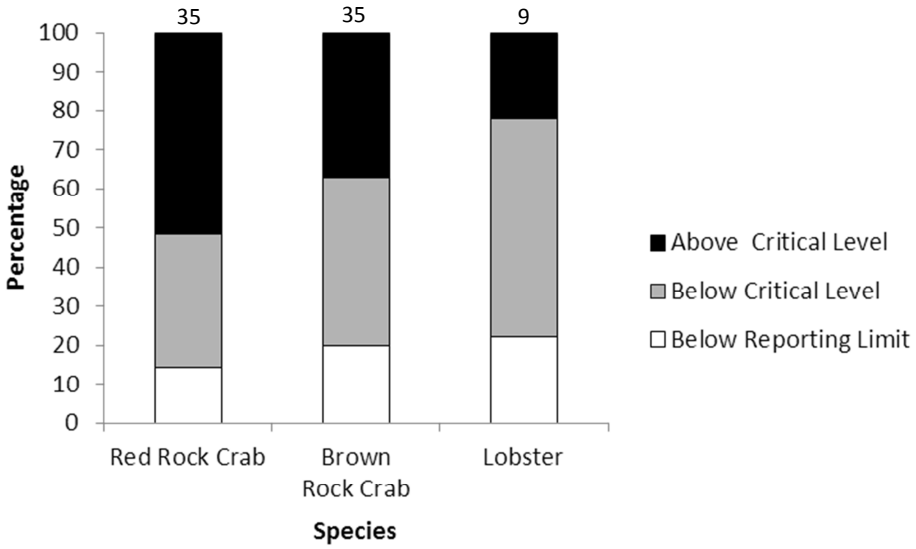


Figure 5. Average concentration of domoic acid for samples above and below the critical level (≥ 20 ppm) for three crustacean species; red rock crabs (*Cancer productus*), brown rock crab (*C. antennarius*) and California spiny lobster (*Panulirus interruptus*).

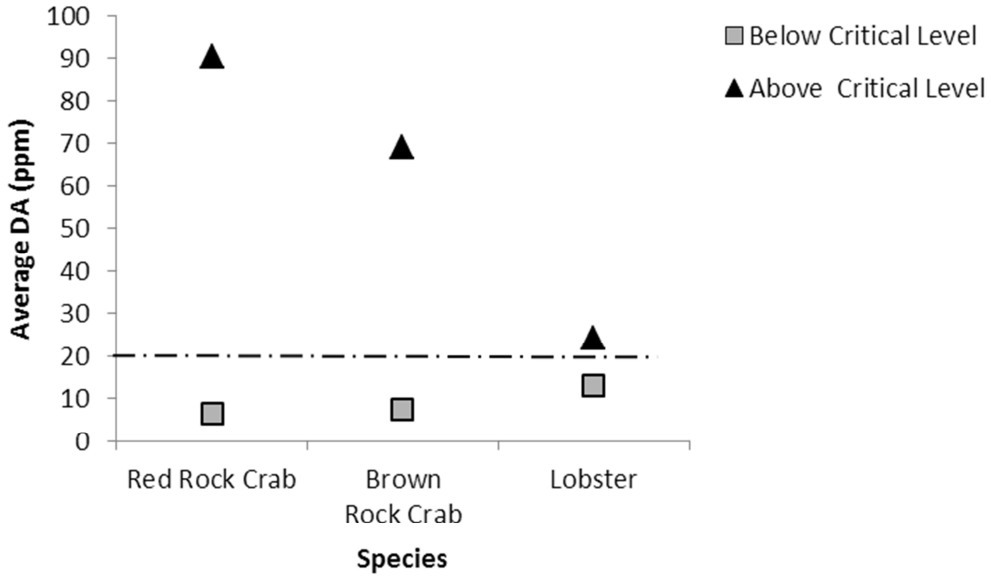


Figure 6. Percentage of samples above and below the critical level (≥ 20 ppm) and below the reporting level (< 2.5 ppm) of domoic acid for three locations at offshore islands, Santa Barbara County; Northwest, Northcentral and Northeast locations. Sample sizes indicated above bars.

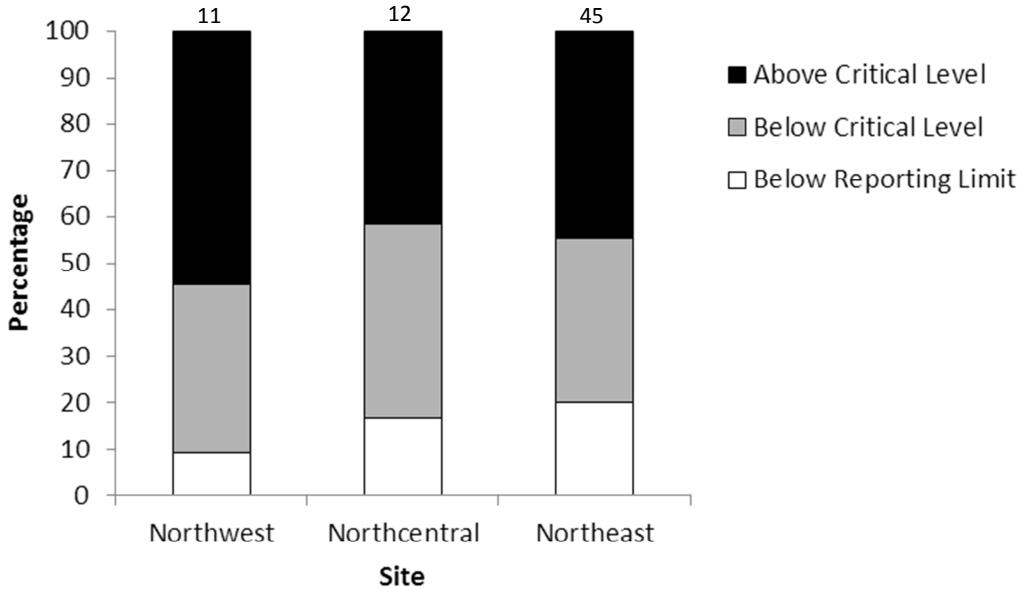


Figure 7. Average concentration of domoic acid for samples above and below the critical level (≥ 20 ppm) for both red (*Cancer productus*) and brown (*C. antennarius*) rock crabs from three offshore/island sites.

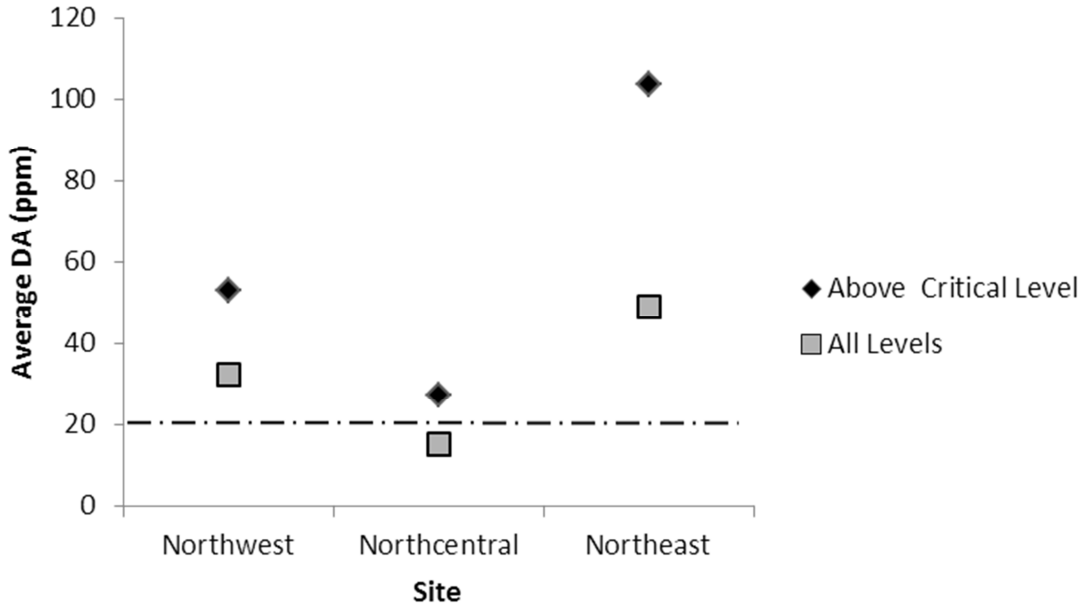
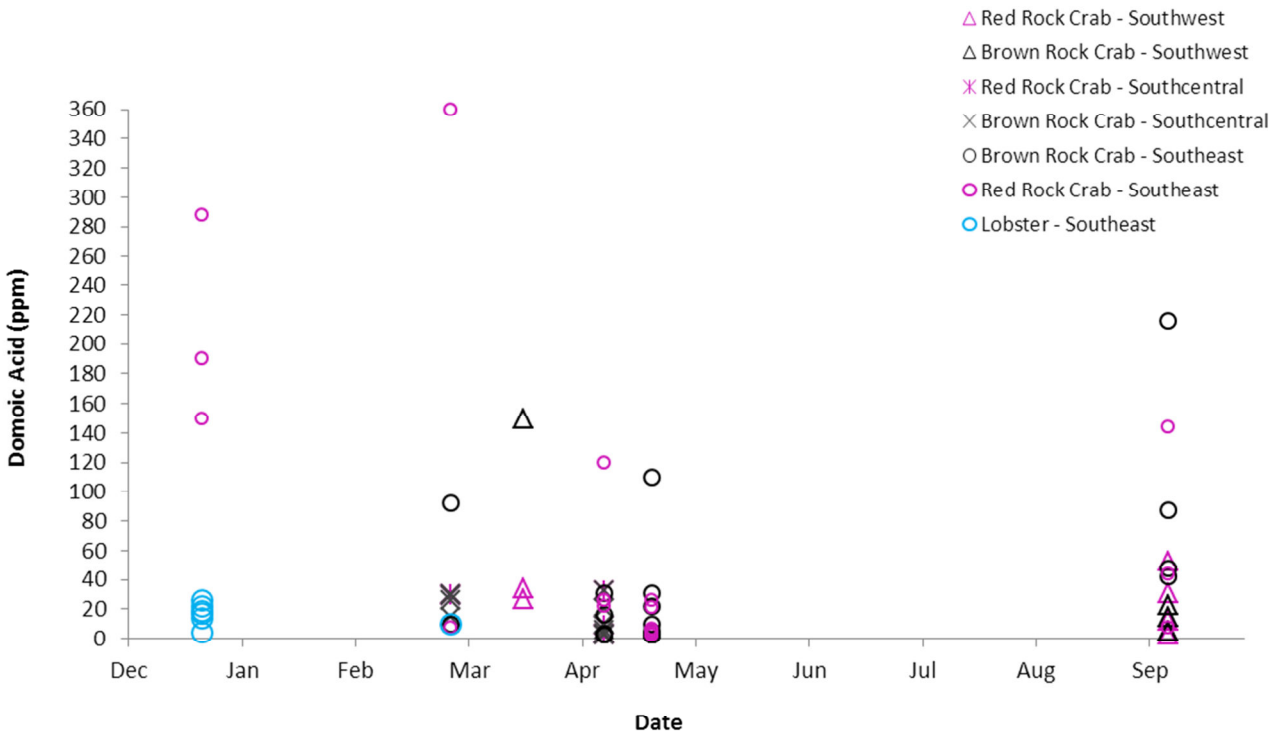


Figure 8. Domoic acid concentrations in various species of crustaceans from three offshore/island sites in the Santa Barbara Channel region. Symbol shape denotes location; Southwest, Southcentral and Southeast. Symbol color denotes type of crustacean; red rock crab, brown rock crab and lobster.

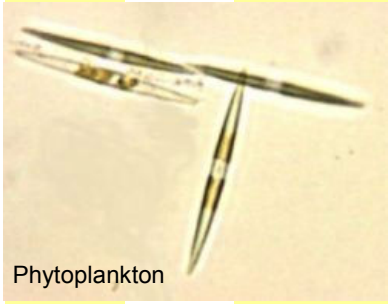


Carolynn Culver

HAB Connection

October 2012

Harmful Algal Bloom Volunteer Monitoring Network



Phytoplankton

Become a Citizen Scientist

Help monitor offshore waters

Come aboard and join our volunteer network! We need help tracking the occurrence of natural toxic events called Harmful Algal Blooms (HABs). These HABs pose a health concern when microscopic phytoplankton produce a toxin that may negatively impact select seafood species.

This is where you come in: we need ***your*** help in expanding HAB monitoring in Southern California. By increasing monitoring efforts to offshore areas, we can better detect and track these events, thereby providing more accurate and timely information for seafood advisories.

Mussels

Volunteers Wanted

The current California volunteer network thrives from the help of dedicated volunteers just like you! We're looking for ocean enthusiasts who can collect samples in **offshore** areas from Point Conception to the Mexican border in one or more of the following tiers, each of which takes about 15-20 minutes to complete:

- Tier 1: Sample *plankton* once a week using a provided net
- Tier 2: Collect *mussels* once or twice a month
- Tier 3: Provide head and stomach of *crabs* and/or *lobster* when notified

Rock Crab © C.Culver

As a member of our volunteer team, you will be provided with:

- A free one hour training
- All of the necessary equipment for sample collection, storage, and shipping
- Data results from your samples

By joining the HAB Connection you will gain more knowledge about your coastal waters while contributing to the science needed to better manage California's ocean resources.

To learn more about or volunteer for the California HAB monitoring program, contact Carrie Culver at c_culver@lifesci.ucsb.edu.

*California Sea Grant Program, UC San Diego ** Marine Science Institute, UC Santa Barbara*
*California Dept. of Public Health ** California Dept. of Fish and Game*

APPENDIX B

A Review of Select
Harmful Algal Bloom Monitoring Programs

To help inform the expansion of the biotoxin monitoring program in California we gathered information about four programs that engage volunteers in monitoring of harmful algal blooms:

- Phytoplankton Monitoring Network (PMN)
- Red Tide Offshore Monitoring Program (RTOMP)
- Massachusetts Division of Marine Fisheries, PSP Monitoring Program (DMF-PSP)
- Olympic Region Harmful Algal Blooms Partnership (ORHAB)

Information for each program is organized into five sections:

- *Program Background*
- *Sampling Design*
- *Volunteer Base & Training*
- *Data Management & Use*: The handling and sharing of data
- *Outreach*: Available newsletters, handouts, fliers etc.

In most cases we gathered the information through telephone discussions and website information (denoted by an asterisks *), but in some cases we used online information exclusively because we were unable to connect with the program coordinator.

A comparative summary table of these programs is included at the end of this document. We also incorporated some aspects of these programs into the main report, especially components that we thought were worth exploring for use in the expansion of the California Biotoxin Monitoring Program.

1. PHYTOPLANKTON MONITORING NETWORK (PMN)*
http://products.coastalscience.noaa.gov/pmn/

Program Background

The Phytoplankton Monitoring Network (PMN), based in Charleston, South Carolina, is a harmful algal bloom (HAB) volunteer network that was established in 2001 by NOAA in an effort to standardize phytoplankton data being collected in the Gulf of Mexico and throughout the Atlantic, including the Caribbean, Puerto Rico, Virgin Islands, and multiple states (Alabama, Connecticut, Delaware, Florida, Georgia, Hawaii, Louisiana, Maryland, Massachusetts, Mississippi, North Carolina, New Jersey, New Hampshire, New York, Rhode Island, South Carolina, Texas, and Virginia). It also includes two west coast states, Alaska and Washington. The main goal is to survey a large span of coastal marine waters and identify the species (both HAB and non-HAB species) and relative (not quantified) amounts of phytoplankton throughout the year. PMN does not have any regulatory authority and serves as a research, education and

outreach entity. They provide assistance to the various states and FDA who perform their own official testing and respond according to their own regulatory procedures and mandates.

Sampling Design

Volunteers are engaged in both the collection and analysis of samples. They collect water samples at least twice a month via horizontal net tows with a 20 µm net for three minutes. They then analyze the samples qualitatively—precise counts are not taken—recording the data on the regional "HAB Screening Data Sheet" provided by NOAA[1]. When a volunteer detects a bloom (via elevated counts as outlined by the program), they send their collected sample(s) to the Charleston lab for confirmation that biotoxins may be present. If the Charleston lab confirms HAB-producing species are present in the sample, they may coordinate the collection of a bivalve sample with the volunteer or others capable of such collections.

Volunteer Base & Training

PMN has about 200 volunteers. When the program first started, PMN engaged middle and high school students in the program. As the network grew, the types of groups participating also expanded. For example, University students conduct sampling in the New York Sound, with government agencies helping out in Chesapeake Bay. PMN continues to expand the program to fill gaps in sampling locations, working with schools, Sea Grant, Centers for Ocean Sciences and Education Excellence (COSEE) and other local groups to identify and engage volunteers.

The volunteers themselves process the collected phytoplankton (water) samples to minimize the work load at the NOAA lab. Prior to sampling, volunteers are trained online through a WebEx seminar that covers background information about the program, identification of a suite of phytoplankton species, and the ecological implications of HABs[2]. They also complete a hands-on training session where they examine a plankton sample and identify and record phytoplankton to the genus level. After practicing their identification skills, they complete a second training session where they process another plankton sample. Upon successful completion of the second training session, volunteers begin collecting and processing samples for the program. Continuing volunteers also are expected to complete a practice identification session each year to illustrate their continued ability to identify HAB toxin-producing species.

Data Management & Use

Volunteers report their data to PMN using an on-line data entry tool developed by the National Coastal Data Development Center (NCDDC)[3]. A volunteer's data entry form is identifiable by its sampling region/site and login ID. The data entry form collects general sampling information (e.g., date, time, conditions), as well as a qualitative evaluation of phytoplankton abundance.

ArcGIS extracts all of the volunteer reported data from the database and marks it as "to be reviewed." PMN staff validate the report before it becomes available on the published ArcGIS

[1] http://products.coastalscience.noaa.gov/pmn

[2] http://products.coastalscience.noaa.gov/pmn/volunteering.aspx

[3] http://products.coastalscience.noaa.gov/pmn/form_screenAtlantic1.aspx

map. This map is easily accessible to the public on PMN's webpage[4]. Website users can locate a site and enter in specific conditions to view or download a data set of their choosing.

When biotoxins are confirmed by NOAA, the lab notifies the appropriate volunteers and state regulators. The state(s) and FDA then perform their own official testing and take regulatory actions as needed.

Outreach
PMN maintains a website for volunteers[2] that contains helpful tips for identifications, spotlights on current volunteers, recent blooms, an in-depth discussion on certain plankton species, and other HAB related information. Their main website also contains general information about HABs, as well as links to other sources of HAB-related information.

2. RED TIDE OFFSHORE MONITORING PROGRAM (RTOMP)*
http://myfwc.com/research/redtide/

Program Background
RTOMP was founded in 2000 by the Florida Fish and Wildlife Research Institute (FWRI) to improve study of *Karenia brevis*, the dinoflaggelate responsible for Florida's frequent red tides in the Gulf of Mexico.

Sampling Design
Volunteers sample water at least one mile offshore once or twice a month, from either one of 15 established sites, or a location they want to sample. The lab receives about 75 samples per month with samples coming from different sites each week. FWRI staff examine the water samples under a microscope, identifying and quantifying HAB-producing species.

Volunteer Base
RTOMP consists of over 150 volunteers, with many sampling along the gulf coast of Florida. These volunteers are fishermen, charter captains, and biologists who are consistently fishing; citizens, biologists, and teachers who are curious about the ocean; and students who may receive volunteer hours. Their website contains a link where new volunteers can sign up[5]. The "New Volunteer Inquiry" contains a list of areas that need monitoring as well as frequency and sampling specifications. RTOMP provides volunteers with all of the appropriate sampling and shipping supplies.

RTOMP mostly actively recruits their volunteers by visiting marinas with charter fishermen, going to environmental organizations' meetings, or by advertising in newsletters or neighborhood associations.

[4] http://www.ncddc.noaa.gov/website/PMN/viewer.htm
[5] http://myfwc.com/research/redtide/monitoring/current/offshore-monitoring/

There is a designated "Volunteer Info Center" hosted by Florida Fish and Wildlife Conservation Commission with a special log-in to identify volunteers. This webpage provides access to service records and news and allows for volunteers to email the RTOMP coordinator, post logistics, hours, and forms.

Data Management & Use
All volunteers receive an e-mail notification of ongoing blooms and are provided with a map, satellite images, and coordinates.

FWC also posts reports under the "Red Tide Current Status" section of their website[6]. These reports also are available for e-mail subscription. For each region (East, Northwest, and Southwest) there is a status and map report. There also is a statewide map. These reports contain a key denoting the concentration of *K. brevis* present and its possible effects on fish and humans.

Outreach
Quarterly newsletters are posted to the RTOMP website that feature articles on HAB species and star volunteers for those months.

3. **MASSACHUSETTS DIVISION OF MARINE FISHERIES PSP MONITORING PROGRAM (DMF-PSP)**
   http://www.mass.gov/eea/agencies/dfg/dmf/programs-and-projects/psp-red-tide-monitoring.html

Program Background
The Massachusetts Division of Marine Fisheries (DMF) strives to protect the safety of seafood by partnering with local management authorities in coastal cities and towns. DMF samples bivalve shellfish to monitor for *Alexandrium*, a species of dinoflagellate that can cause paralytic shellfish poisoning. When samples contain toxins above the critical level, DMF works with local and state authorities to close areas.

DMF is also working with the FDA to engage community and college volunteers to initiate a pilot statewide plankton sampling program. The goal of this volunteer program is to assess the relationship between shellfish toxin levels detected by the traditional DMF program using primarily mussels and *Alexandrium* densities detected in water samples.

Sampling Design
For the traditional biotoxin monitoring program, blue mussels are sampled weekly from April to November at 16 stations along the coastline including Cape Cod[7]. These primary samples are analyzed for biotoxins at the agency's main lab in Gloucester. If the lab detects toxins above the

[6] http://myfwc.com/research/redtide/events/status/statewide
[7] Due to water flowing from the north to south, monitoring in Massachusetts may start earlier than April or go longer into November if elevated toxin levels are detected in Maine.

critical level for PSP (80 ppm) in mussels sampled from the primary sites, secondary shellfish sampling is conducted at the site and at additional nearby sites. Secondary sampling involves analysis of more mussels, plus other bivalves and gastropods such as sea scallops, surf clams and conch. Species that comprise secondary sampling retain toxins for a longer period of time and thus are not good indicators of when the bloom started but they are important for informing the seafood consumption health advisories.

For the new pilot statewide plankton sampling program, volunteers will collect weekly plankton (water) samples from established shellfish sampling sites using equipment (Swift field microscope, sampling nets, containers) provided by the Program. They will be trained to identify *Alexandrium*, the PSP-producing species.

Volunteer base/Recruitment

Shellfish samples are collected by shellfish constables and associated personnel of coastal cities and towns. The pilot phytoplankton monitoring program will engage community and university members in sampling.

Data Management & Use

DMF calls affected shellfish constables and town officials when PSP toxins are detected above the critical level (80 ppm), and state personnel are then notified via an automated e-mail. In addition to the phone calls and e-mails, the Division of Marine Fisheries follows-up with these groups by sending a written notice that specifies the affected area and species. For as long as the bloom persists, a weekly e-mail is sent updating select state agencies on the bloom's status. The affected area remains closed until three consecutive samples are below the critical toxin level. When levels fall below the toxic threshold, written notices are mailed and phone calls are made to town officials to proclaim the re-opening of the previously affected areas. However, due to the variant levels of toxin retention periods among different species, harvesting of some species may be allowed while it remains restricted for other species that continue to test above the critical toxin level. These details are provided in the notices.

Whenever the Division detects high levels of toxins, Woods Hole Oceanographic Institute (WHOI) is notified. WHOI then collects and analyses phytoplankton (water) samples from the affected area, recording counts of *Alexandrium*. These data are being compiled as a time-series, with the intent to elucidate the relationship between dinoflagellate densities and the levels of shellfish toxin accumulation over time.

Outreach

Information on biotoxin blooms is available from local shellfish constables, the local shellfish department and through the Red Tide Hot Line at 978.282.0308, option 6. An informational pamphlet[8] on red tides is available through the MDF paralytic shellfish poison general website[9].

[8] http://www.mass.gov/eea/docs/dfg/dmf/publications/dmf-shellfish-brochure.pdf

[9] http://www.mass.gov/eea/agencies/dfg/dmf/programs-and-projects/psp-red-tide-monitoring.html

4. OLYMPIC REGION HARMFUL ALGAL BLOOMS PARTNERSHIP (ORHAB)

http://www.orhab.org/

Program Background

The Olympic Region Harmful Algal Blooms Partnership (ORHAB) is a collaboration among federal, state and local regulators, coastal tribes, private and academic researchers and universities, marine resource-based businesses, and public interest groups. It came into existence in the summer of 2000 based on the need to investigate the seemingly random commercial product closures due to paralytic shellfish poison (PSP) and domoic acid (DA). The goal of the program is to assess the environmental conditions that are most conducive to HAB events and how to best manage the associated problems, such as health and environmental impacts. A primary focus has been on domoic acid and the associated toxin-producing phytoplankton species. They do not engage in regulatory actions, but instead serve as a research, education and outreach entity.

Sampling Design

The program includes seven sampling sites spanning over 300 miles of coastline; Neah Bay, Makah Bay, Kalaloch, Copalis, Twin Harbors, Willapa Bay, and Long Beach. All sampling sites are in areas where there is considerable harvesting of razor clams, oysters, and mussels. Weekly water samples are collected and analyzed for DA and PSP, chlorophyll, nutrients, salinity, and temperature. Razor clams also are collected twice a month and tested for DA.

Volunteer Base

Samples are collected by people from the Makah and Quinault Tribes, Washington Department of Ecology, Pacific Shellfish Institute, Washington Department of Fish and Wildlife, Olympic Coast National Marine Sanctuary, and Northwest Fisheries Science Center. Training is provided by university scientists. There also is some basic information about sampling on their website. The program partners participate in regular meetings to discuss potential improvements to ORHAB's work procedures and to report new findings.

Data Management & Use

ORHAB technicians regularly post "HAB Alerts" to notify managers of *Pseudo-nitzschia* levels. The information reported to the managers is then provided to regulators.

Outreach

ORHAB maintains a website that contains several outreach materials, including a brochure and various newsletters.

Table 1. Comparison of features of four harmful algal bloom (HAB) monitoring programs in the United States.

Program	**Phytoplankton Monitoring Network (PMN)**	**Redtide Offshore Monitoring Program (RTOMP)**	**Massachusetts Division of Marine Fisheries, PSP Monitoring Program (DMF-PSP)**	**Olympic Region Harmful Algal Bloom Partnership (ORHAB)**
Program Authority	Non-regulatory	Linked w/regulatory	Regulatory	Non-regulatory
Sampling Locations	Numerous states+	Florida	Massachusetts	Washington
Number of Sites	Numerous, varies by state	15 set sites, plus many random	63 16 primary, 47 secondary	7
Number of Volunteers	200	>150	unknown	unknown
Volunteer Base	Students, government agencies, Sea Grant, Center for Ocean Sciences and Education Excellence (COSEE)	fishermen, charter captains, biologists; teachers, students, citizens	Shellfish constables, agencies Pilot Programs: community and university persons knowledgeable in biology	federal, state and local regulators, coastal tribes, private and academic researchers and universities, marine resource-based businesses, public interest groups
Target Species/Toxin	Varies by state	*Karenia brevis*	*Alexandrium* sp. (PSP)	*Pseudo-nitzschia* spp. (DA) *Alexandrium* sp. (PSP)
Sampling Tiers **Tier 1: Water** **Tier 2: Bivalves**	 X X (mussels+, as needed)	 X	 X (pilot program) X (blue mussels consistently; surf clam, conch, sea scallop, etc as needed)	 X X (Razor clam consistently)
Phytoplankton ID	Volunteers	Program staff	Volunteers	Universities (training)
Sampling Frequency	≥2x/month	≥1/month	Weekly (mussels)	Weekly phytoplankton 2x/month razor clam
Sampling Period	Year-round	Year-round	April to mid-November (earlier/later as needed)	Year-round

APPENDIX C

CalCOFI Publication

A SOUTHERN CALIFORNIA PERSPECTIVE ON HARMFUL ALGAL BLOOMS

MELISSA CARTER, MARY HILBERN
Scripps Institution of Oceanography
University of California, San Diego

FERNANDA MAZZILLO
Department of Ocean Sciences
University of California, Santa Cruz

CAROLYNN CULVER
California Sea Grant Extension Program
University of California, San Diego

GREGG LANGLOIS
California Department of Public Health

EXTENDED ABSTRACT

Understanding the complexity of harmful algal blooms (HABs) and their impacts on marine resources requires collaborations that overlaps a variety of disciplines, agencies, and regions. Ongoing monitoring efforts by California Department of Public Health (CDPH), the Southern California Coastal Ocean Observing System (SCCOOS) and the Central and Northern Coastal Ocean Observing System (CeNCOOS) provide the basis for evaluating and assessing the potential of marine biotoxins within commercially and recreationally important fisheries along the California coastline. These programs focus efforts on a particular marine resource (CDPH, farmed and recreationally harvested bivalves) or on a specific toxin (domoic acid only for SCCOOS) to meet regulatory requirements or funding shortfalls that constrain sample collection and processing. Since 2001, prevalence and persistence of offshore toxic blooms, particularly of domoic acid, has compounded this problem and additional monitoring efforts are needed to assess potential risks to consumers and inform seafood advisories within the state. Finding opportunities to collaborate with the California Cooperative Oceanic Fisheries Investigations Group (CalCOFI) and the Southwest Fisheries Science Center (SWFSC) can help assess the potential risks to our marine resources and seafood consumers, and provide novel opportunities for data collection and sharing. This presentation is focused on three main points: 1) providing an overview on the HAB monitoring efforts in southern California, 2) discussing the potential impact on California fisheries, and 3) providing input on how CalCOFI and SWFSC can be engaged in HAB monitoring.

HAB Monitoring in California

One of the oldest HAB programs in the U.S. started in 1929 along the California coast to monitor for saxitoxins that can cause illness or death in extreme cases from paralytic shellfish poisoning (PSP). In the 1940s, such monitoring was mandated for the sale of commercial shellfish by the National Shellfish Sanitation Program. By the 1960s, routine coastal monitoring for PSP toxins in shellfish began as a means to protect those recreationally harvesting shellfish. The regulatory alert level for saxitoxins in shellfish is ≥ 80 μg 100 g^{-1} (0.8 ppm). Several dinoflagellate species within the genus *Alexandrium* spp. (formerly *Gonyaulax*) produce PSP toxins.

The CDPH monitoring program was expanded in 1991 to include phytoplankton monitoring (net tow samples) along the coast as a means to provide an early warning of toxic blooms and prioritize shellfish samples for toxin analysis. At this time the program also began routine monitoring for a second biotoxin: domoic acid (DA), a naturally occurring and toxic amino acid that can cause amnesic shellfish poisoning (ASP; Bates et al. 1989). Toxin production has been confirmed in 12 of 30 species of the diatom genus *Pseudo-nitzschia* (Horner et al. 1997; Bates and Trainer 2006). ASP was first recognized in 1987 when three people died and 105 cases of acute poisoning were reported after consuming DA-contaminated blue mussels (*Mytilus edulis*) from Prince Edward Island, Canada (Bates et al. 1989). Along the West Coast of the U.S., human illness or death from ASP has not been reported though numerous cases of large-scale deaths and illnesses of marine mammals and wildlife have occurred since 1991 (Fritz et al. 1992; Work et al. 1993; Lefebvre et al. 1999; Scholin et al. 2000; Bejarano et al. 2008; Fire et al. 2010; Bargu et al. 2012). The regulatory alert level for DA in shellfish is ≥ 20 μg g^{-1} (20 ppm).

The CDPH program standards to protect consumers includes weekly monitoring of marine biotoxins in shellfish and the relative abundance of toxigenic phytoplankton along the coast, posting of annual quarantines from May 1 to October 31 each year, issuing special health advisories as needed for recreationally harvested bivalves, and public education and outreach. The program relies on commercial growers (7 sites) to provide weekly shellfish and plankton samples, and a volunteer network to provide weekly to monthly shellfish samples (70 sites) and plankton samples (115) from coastal stations (1–4 per county). The resulting data are used to regulate shellfish growers, as well as to inform state health advisories about safe consumption of recreationally harvested shellfish when HABs are present. These data, maps and advisories are available at

http://www.cdph.ca.gov/healthinfo/environhealth/water/Pages/Shellfish.aspx.

Academic and ocean observing research communities interested in understanding the temporal and spatial scales of HABs, factors which promote HABs, as well as improving the detection and prediction of these events began regular, weekly pier-based HAB monitoring efforts in southern California at one site in 2005 (SIO, La Jolla) and an additional four sites in 2008 (SCCOOS, http://www.sccoos.org/data/habs/index.php). These efforts are focused on all HAB species in California that may pose significant impacts to human health, marine life, marine resources, and the economy including both toxin producing (*Pseudo-nitzschia* spp., *Alexandrium* spp., and *Dinophysis* spp.) and bloom forming species (*Lingulodinium polyedrum*, *Akashiwo sanguinea*, *Prorocentrum* spp. *Cochlodinium* spp., *Phaeocystis* spp., and others). Weekly measurements include HAB species abundance estimates, chlorophyll *a* concentration, temperature, salinity, nutrient concentrations (nitrate, nitrite, phosphate, silicate, and ammonia) and particulate DA concentration. These data are posted weekly to the SCCOOS HAB Web site and shared through the California Harmful Algal Bloom Monitoring and Alert Program (CalHABMAP, http://habmap.info) e-mail list serve, which brings together researchers, marine mammal and wildlife rescue groups, managers, and the general public throughout the state of California.

While coastal monitoring efforts and the CDPH program have been effective at protecting and informing consumers of toxic HABs in coastal areas, these efforts have historically focused on nearshore shellfish resources and not on detection of HABs in offshore waters including areas near the Channel Islands. Additional monitoring is now needed for two primary reasons. First, the prevalence, intensity, and duration of these offshore toxic blooms of *Pseudo-nitzschia* have increased in California over the past decade (CDPH data; Lewitus et al. 2012). As a result, there is a need to monitor commercially and recreationally important species more frequently as they are exposed to higher levels of biotoxins more often and for longer periods of time. Second, these blooms have developed and/or continued offshore, especially in the Santa Barbara Channel (SBC) region, often decoupled from coastal blooms—something not commonly seen in the past. This new pattern in the distribution of toxic blooms now requires that monitoring occur in offshore areas, not just along the coast as is presently done.

The California Sea Grant Extension Program, in collaboration with the CDPH and the California Department of Fish and Wildlife (CDFW) recognized that a more focused and organized offshore monitoring program was critically needed given that 1) toxic offshore blooms are persisting, 2) higher levels of biotoxins may pose more risk to consumers, 3) offshore shellfish and fish samples for biotoxin analysis are obtained haphazardly from recreational and commercial fishermen, rendering useful but incomplete data sets, and 4) the value of a more consistent and reliable offshore monitoring program to better manage offshore fisheries and ensure areas not impacted by HABs are not included in health advisories when another offshore area is impacted by a HAB event. This collaborative effort is looking to expand the CDPH biotoxin monitoring program into offshore areas of southern California (Santa Barbara County to the Mexican border) with funding from the Collaborative Fisheries Research West program. They are seeking volunteers to help with one or more of the sampling tiers; Tier 1, phytoplankton; Tier 2, bivalve shellfish (mussels, oysters, scallops, clams) or filter-feeding finfish (anchovies, sardines); Tier 3, crustacean shellfish (crabs, lobster). Several other organizations (e.g., whale watching, dive and island charters, commercial fishermen, National Park Service) and individuals are joining this effort, but coordinating with additional groups that frequent offshore areas, such as CalCOFI and SWFSC, is of great interest.

Impacts on Fisheries

Biotoxins have been detected in a wide variety of species other than bivalve shellfish including but not limited to pelagic filter-feeding species (Pacific sardines and Northern anchovies), California spiny lobster, crab (Dungeness, rock and pelagic red), Humboldt squid, Market squid, and benthic-feeding groundfish including several commercial and recreationally important species (Pacific halibut, Dover sole, and sanddab); (Wekell et al. 1994; Busse et al. 2006; Vigilant and Silver 2007; Mazzillo et al. 2010). Of particular concern has been the high levels of DA found in samples from California over the last 10 years: 1) mussels from an offshore oil platform that contained 610 ppm of DA; 2) anchovies with 2,300 ppm of DA in viscera; 3) lobster viscera with 1,170 ppm of DA, and several samples with 200–400 ppm of DA; and 4) rock crab containing 300–400 ppm (CDPH data). Toxins are typically concentrated in the viscera (internal organs, digestive glands) and not the body tissue (meat), so thorough cleaning and removal of the viscera in larger species (e.g., crab, scallops) can minimize the risk. However many species (e.g., mussels, oysters, sardines, anchovies) are eaten whole and pose the greatest risk to consumers (Mazzillo et al. 2010). Some individuals and ethnicities may also consume the entire rock scallop, rock crab (crab butter) and lobster (lobster tomalley, pâté, bisque) increasing the risk of exposure to biotoxins and other contaminants.

Importantly, even at high DA concentrations, the preliminary data indicates the meat of the larger crustaceans

and game fish remains relatively toxin free even though low concentrations of toxins have been detected in the body tissue (meat) of anchovies (*Engraulis mordax*; Work et al. 1993; Altwein et al. 1995; Lefebvre et al. 2002; Mazzillo et al. 2010), coho salmon (*Oncorhynchus kisutch*; Lefebvre et al. 2007), Dungeness crab (*Cancer magister*; Altwein et al. 1995), mantle of Humboldt squid (*Dosidicus gigas*; Mazzillo et al. 2011) and mantle of octopus (*Octopus vulgaris*; Costa et al. 2004). Overall, these findings are based on a relatively limited number of samples and require more comparative data during HAB events to improve our understanding of the risk exposure to biotoxins for all seafood species.

In general, HABs directly impact California fisheries through the closure of shellfish beds, aquaculture facilities, and even the closure of markets and recreational sport fisheries due to toxin accumulation above regulatory limits and die-offs of natural and farmed fish and shellfish. Almost every year since 2001 CDPH has had to extend the time period of the annual shellfish advisory or issue additional warnings to protect consumers about eating seafood (other than bivalves) such as sardines, anchovies, lobster, and crab that have been found to have biotoxins above the regulatory alert level (20 ppm for DA). The health advisories that have resulted from these findings have impacted commercial fishermen, as some distributors will not buy products coming from the areas under advisory. In most cases, the advisories cover a large area due to a lack of data to pinpoint the location of the bloom and associated affected animals.

Currently, shellfish growers are the most highly regulated in terms of biotoxins, providing the best protection for the consumer, but an equivalent level of monitoring and regulatory oversight for commercial and recreationally important wild-caught fisheries in California does not exist. Ultimately, there are several unanswered questions related to human health impacts of HABs on fisheries. How often are toxins found in offshore populations of shellfish, squid, and finfish? Can one indicator species provide adequate protection to consumers if modes of toxin uptake differ and depuration rates vary for impacted species (bivalves, lobster, crab, squid, and finfish)? Do increased amounts of toxin found in seafood pose a greater risk of acute toxicity to the consumer? Are there human health concerns with chronic exposure to algal biotoxins? These are just a few of the complex questions that need greater attention to protect both the consumer and the seafood industry.

Potential Assistance from CalCOFI

The last goal of this presentation is to provide input on how the CalCOFI and SWFSC groups can be engaged in research and monitoring of HABs. One immediate and cost-saving approach for the offshore monitoring of HABs is to have consistent samples collected during the quarterly CalCOFI cruises and SWFSC fish survey cruises. Sample types consist of water samples (30–100 ml), filtered water samples (400 ml on GF/F filters), net tow samples (20 μm mesh vertical tow), or samples of fish or shellfish (whole or viscera only) and would be analyzed by CDPH and SCCOOS HAB researchers. These samples would be quite beneficial to ongoing research and state monitoring efforts by helping determine HAB species abundance and toxin production in the water and food web at offshore locations on regular intervals. This, in turn, would improve early detection of blooms and increase spatial and temporal data needed to inform health advisories.

Additionally, plankton and hydrographic data sets already being collected by CalCOFI could be reanalyzed to help address HAB related questions. For example, phytoplankton abundance estimates (collected by E. Venrick) and nano- and microplankton biomass and abundance estimates (collected by M. Landry) are currently conducted for some stations and lines throughout the CalCOFI sampling grid. These measurements could also be analyzed to look specifically at HAB species such as *Pseudo-nitzschia* spp., thereby providing information on abundance relative to offshore hydrographic conditions and coastal conditions. More broadly, this increased sampling and analysis of data when combined together would ultimately provide a better understanding of the mechanisms and factors associated with offshore HAB blooms, improve understanding of links with coastal blooms, and potentially improve predictions of HAB events.

Conclusions

Adequate offshore HAB-focused sampling is lacking, hindering the states' ability to provide well-informed seafood health advisories and improve our understanding of the factors related to offshore toxic blooms. Engaging CalCOFI and SWFSC in ongoing HAB monitoring efforts could improve the availability of samples both in space and time thereby helping to identify high-risk areas and improving the resolution of information available to researchers, resource managers, and health regulatory agencies. While some coordination is required, the additional sampling appears to be easily integrated with ongoing activities of CalCOFI and SWFSC. The authors encourage such collaboration, as it would not only increase the knowledge about HABs in California, but it would also enhance the states' ability to provide appropriate seafood health advisories.

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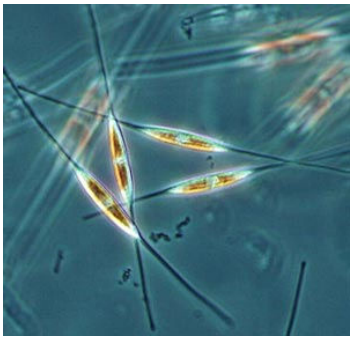


APPENDIX D.1

HAB NETWORK FIELD SAMPLING PROTOCOL
TIER 1: PHYTOPLANKTON

INTRODUCTION

The following protocol is for the field collection and shipping of phytoplankton (Tier 1) samples for detection of toxin-producing phytoplankton associated with amnesic shellfish poison (ASP) and paralytic shellfish poison (PSP). It is important that field collectors follow the sampling guidelines as closely as possible to ensure that comparable samples are collected.

1. SAMPLE SITES

Sampling of the same sites, as well as different sites, is of interest to our program. However, it is critical that we know where the sample was taken and that the same location name be used for sites that are routinely sampled.

a. *Permanent Locations.* Routine monitoring should be conducted at the same site(s) whenever possible. This allows us to make comparisons about the types and numbers of species present between sampling periods.

b. *Special Locations*. The use of fixed locations is valuable for looking at trends in the phytoplankton data. However, your observations in the field are one of our program's most valuable assets. Field samplers that have the ability to sample various locations other than the prearranged permanent sites are encouraged to do so, particularly if you observe signs of possible bloom (e.g., turbidity, discolored water, typically red to brown in color).

2. SAMPLING FREQUENCY

A sampling schedule of once per week per station is ideal, however any effort is valuable.

3. SAMPLE COLLECTION

Field samplers are provided with a 20µm mesh plankton net fitted with a 300mL collection bottle at the cod end. Each net is supplied with about 50 feet of line. Please follow the below guidelines as closely as possible to ensure the comparability of all samples. Collect as dense (thick) a sample as reasonably possible.

a. *Vertical Tows:* Perform vertical tows whenever possible. Many species of phytoplankton can migrate a surprising distance up and down the water column, thus vertical tows are more likely to adequately sample the phytoplankton. Be sure to secure the end of the line to something stable so as not to drop and lose the net. Standard tow depths are 30-50 feet; in shallower water you should sample from approximately 1 foot above the bottom.

In extremely shallow water (< 5 feet) you can conduct horizontal tows (see 3b.). A good rule of thumb is to conduct a total tow length no less than 60-100 feet, meaning a minimum of two tows per sample (2 X 30' = 60' total; 2 x 50' = 100' total).

Keep in mind that the objective is to obtain a ***dense*** sample: the color of water in the net and the rate at which the plankton net drains will provide you with an indication of the sample density. If the water is still clear and the net is readily draining take another tow or two to get a denser sample **(be sure to keep track of the number of tows)**. Retrieve the net in a 'hand over hand' process: make sure the lead hand is close to the back hand to prevent retrieving the net too quickly. A slow, continuous retrieval will help to concentrate the sample as you conduct the tow.

b. *Horizontal Tows:* Whenever it is impractical to collect a phytoplankton sample with vertical tow (e.g., in extremely shallow water) you may use this method. Slowly pull the net horizontally, just below the water surface, either along a pier or behind a boat. **Never** pull the net behind a boat while under way! The fine mesh will easily tear under such stress. A drifting boat will provide enough movement to keep the net moving at the surface. When sampling from a boat keep track of the distance covered or the elapsed time of the tow so that you can be consistent each time you sample.

4. SAMPLE PRESERVATION

Remove the sample collection bottle from the net, gently swirl the contents to re-suspend any settled material, and pour into the 125mL sample bottle provided. Each sample bottle contains 1mL of buffered formalin solution for preserving the sample. There is no need to refrigerate the sample prior to or during shipment.

Note: the cod end may have more water than you need in it. To drain excess water, grab the net above the cod end and rotate it upside down several times. Only the presence of organisms, not the amount of each, is recorded. Thus, it is unnecessary to keep the entire volume of water.

5. FIELD NOTES

Please keep complete records for all samples. The laboratory submission form inside the sample shipping canister should be filled out as completely as possible, with the following information in bold required:

a. ***Date collected***: the date you collected the sample
b. ***Collector***: Your name and/or affiliation (as set up with the program coordinator)
c. *County*: The county where the sample was collected
d. ***Bottle #***: A five digit number located on the top of the plastic sample bottle
e. Time: The time he sample was collected (an estimate is fine)
f. ***Location***: Where the sample was collected. GPS coordinates are most helpful, but fish block number or site name also are acceptable.
g. ***Tow Type***: Vertical or horizontal, depending on the type of tow you conducted.
h. ***Tow Depth***: The maximum *depth* sampled with a vertical tow (see item 3a).

i. ***Number of Tows/Distance***: The number of times you retrieved the net up from the depth indicated above (for vertical tows). This number is used to calculate the tow length which is equivalent to the tow depth multiplied by the number of net retrievals. For horizontal tows estimate the distance you covered as best you can and record that distance here instead of number of tows (which is 1 for a horizontal tow).

j. *Other Information*: Water temperature and salinity information is helpful, but not required.

k. *Comments*: Record your observations of water color, atmospheric conditions, etc. These observations can be very useful for interpreting the data.

6. MAINTENANCE OF NET

Please rinse the plankton net, collection bucket, and all connectors thoroughly with freshwater after each use. It is also advisable to hang up the net to thoroughly air-dry after washing. This is particularly critical if using the net at more than one location. A freshwater rinse is typically sufficient to clean the net. However, if the net begins to accumulate too much debris or becomes coated, please let us know and we can provide additional instructions for cleaning the net.

7. SHIPPING

a. Complete the sample submission form provided in each canister.

b. Place the sample bottle in the sealable plastic bag and the submission form in the mailing canister. Please **do not over tighten the lid**. Include the absorbent material provided in the canister to soak up any leakage.

c. Send the canister to the CDPH laboratory via U.S. Mail (postage prepaid) or next day courier if sending more than one canister (upon arrangement with us). If you are routinely sampling several locations, you may package all sample bottles in one shipping box. Contact us to receive appropriate sample boxes with postage prepaid labels.

d. Ensure that all canisters and packages contain the following address:

California Department of Public Health
Environmental Management Branch
Attn: Specimen Receiving
850 Marina Bay Parkway
Richmond, CA 94804-6403

8. CONTACTS

For questions regarding this protocol and offshore sampling program please contact the Marine Biotoxin Monitoring Program office in Richmond at (510) 412-4635, redtide@cdph.ca.gov or the UCSB California Sea Grant HABNet Coordinator at csg.habnet@gmail.com.

This protocol was adapted from the California Marine Biotoxin Monitoring Program, Phytoplankton Field Sampling Protocol prepared by the California Department of Public Health, Environmental Management Branch. The HAB Network is a collaboration with the California Sea Grant Program UC San Diego, Marine Science Institute UC Santa Barbara, California Department of Public Health and California Department of Fish and Wildlife.


Mussels

APPENDIX D.2

HAB NETWORK FIELD SAMPLING PROTOCOL
TIER 2: BIVALVE SHELLFISH

INTRODUCTION

The following protocol is for the field collection and shipping of bivalve (Tier 2) (e.g., mussels, clams, oysters, scallops) samples. Samples are analyzed for toxins responsible for paralytic shellfish poisoning (PSP) and amnesic shellfish poison (ASP). Because a preservative cannot be used, it is imperative that collectors take care to ensure the integrity of each sample.

1. SAMPLING FREQUENCY

A sampling schedule of every two to four weeks, with additional sampling when notified, is ideal. However, any effort is valuable. Volunteers will be contacted and samples will be requested when toxin-producing phytoplankton are detected near their sampling area.

2. SAMPLE COLLECTION

A sample should consist of a single species of bivalve shellfish (mussels, oysters, clams, scallops etc.) collected randomly from a sampling site. Each sample should include **a minimum of 20 individuals and at least 250 grams of drained shellfish meat**; this provides adequate material for both types of biotoxin analyses and a reserve sample as back-up for additional analyses as needed. The sample amount (250 grams) is equivalent to a volume of about one cup of shucked meats. It takes up to *40 small* sea mussels (about 2 inches shell length) to produce 250 grams of meat. Do not collect only large mussels as only a few individuals would be needed to meet the 250 gram requirement and more individuals are required for proper analysis.

Samples should be collected in accordance with the rules and regulations of the California Department of Fish and Wildlife. A sportfishing license or scientific collecting permit is required. If you do not possess a permit, please ask us to add you to the CDPH volunteer permit.

3. SAMPLE PREPARATION

a. Thoroughly clean the outside of shellfish with water.
b. Using the shucking tool provided, open shell by cutting the muscle(s) that are attached to the inside of the shell. Do not use heat or anesthetic before opening shell. **Cut carefully to avoid damage to body of animal.**
c. Drain off excess liquid from opened shell.
d. Rinse the opened shellfish to remove sand or other foreign materials as needed, and drain off any remaining liquid.
e. For mussels, cut off byssal threads (attachment hairs) and discard, saving only meat.
f. Remove meat from shell by carefully scraping off all tissue attached to the shell (take care to minimize damage to tissue).

g. Place drained meat into a wide-mouthed 16-oz sample bottle. About 1/2 to 2/3 of a sample bottle of shellfish meat provides the desired amount. NOTE: Do not overfill; be sure to **leave an air space** to accommodate expansion upon freezing.
h. Tighten cap securely. Immediately place in cooler with blue ice for transport.
i. **Freeze sample** as soon as possible; ensure that sample is frozen prior to shipment.
j. Fill out sample submission slip; be sure to record sample **bottle number** (five-digit number on bottle's cap) and **type of shellfish** (e.g. sea mussel, bay mussel, Pacific oyster, gaper clam, etc.). In addition, please record the **sample location and sampling date**, and **include your name and telephone number** so that we may contact you immediately. The presence of high toxin concentrations may necessitate immediate resampling.

4. SHIPPING

Rapid testing of samples for PSP and DA is extremely important. Samples should be shipped as soon as possible on an appropriate day of the week (see 'f' below) by standard overnight (next afternoon) service to ensure they arrive in an unspoiled condition.

a. Place **frozen** sample(s) in an insulated shipping container with an adequate quantity of frozen ice packs and sandwiched in absorbent materials (e.g., newspaper) to soak up any leakage or condensation.
b. Place sample submission slip(s) on top of the Styrofoam lid; close and seal the shipping container.
c. Containers returned to you from the laboratory will have mailing labels inside a plastic mailing envelope taped onto the lid or side. Remove the label addressed to you and reverse labels so that the box is addressed to the laboratory. Remove or cover any old UPS or other shipping label(s) that could cause confusion.
d. Package should be addressed to:

California Department of Public Health
Environmental Management Branch
ATTN: Specimen Receiving
850 Marina Bay Parkway
Richmond, CA 94804-6403

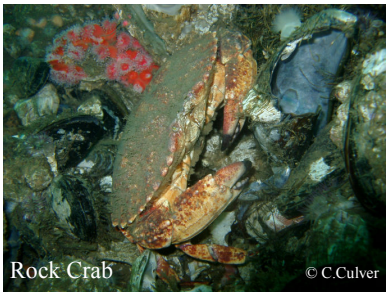
e. Send Package by Courier Service. In accordance with prior arrangements
   1. Next-day **afternoon** courier service may be provided in some locations by EMB: call (510) 412-4635 for information; or
   2. You may use your own courier at your own expense.

f. DO NOT send samples at the end of the week or just before holidays. Prolonged transit time causes increased risk of spoilage.

5. CONTACTS

For questions regarding this protocol and offshore sampling program please contact the Marine Biotoxin Monitoring Program office in Richmond at (510) 412-4635, redtide@cdph.ca.gov or the UCSB California Sea Grant HABNet Coordinator at csg.habnet@gmail.com.

This protocol was adapted from the California Marine Biotoxin Monitoring Program, Shellfish Field Sampling Protocol prepared by the California Department of Public Health, Environmental Management Branch and Environmental Microbial Diseases Laboratory. The HAB Network is a collaboration with the California Sea Grant Program, Marine Science Institute UC Santa Barbara, California Department of Public Health and California Department of Fish and Wildlife.



APPENDIX D.3

HAB NETWORK FIELD SAMPLING PROTOCOL
TIER 3: CRAB AND LOBSTER

INTRODUCTION

The following sampling protocol is for field collection and shipping of crab and lobster (Tier 3) samples for domoic acid (DA) analysis. Because a preservative cannot be used at the time of sample collection, it is imperative that field collectors take care to ensure the integrity of the samples.

1. SAMPLING

A sample consists of an individual crab or lobster collected randomly from a sampling site. Desired species include: 1) red rock crab (*Cancer productus*), 2) brown rock crab (*Cancer antennarius*), 3) yellow rock crab (*Cancer anthonyi*), 4) sheep (spider) crab (*Loxorhynchus grandis)* and 4) California spiny lobster (*Panulirus interruptus)*.

Multiple samples (up to 4 per species) can be provided from the same site, but the samples should be numbered consecutively with appropriate sample ID numbers (see below). Likewise, samples can be collected from multiple sites, but each site should receive its own sample ID number (see below). Crabs and lobster should be collected in accordance with the rules and regulations of the California Department of Fish and Wildlife. A sportfishing license or scientific collecting permit is required. If you do not possess a permit, please ask us to add you to the CDPH volunteer permit.

Sample ID Numbers: Each sample should have a sample ID number that includes your initials, site number and animal number. For example, if John Smith collected 1 red rock crab, 1 brown rock crab and 2 lobster from one site and 1 red rock crab and 1 yellow rock crab from a second site, the samples should be numbered as follows: JS1-1, JS1-2, JS1-3, JS1-4, JS2-1 and JS2-2.

2. SAMPLING FREQUENCY

Sampling of crabs and lobster is required when toxin producing phytoplankton species are present in water samples (Tier 1) and/or when critical levels of biotoxins have been recently detected in bivalve (Tier 2) samples in an area. Volunteer collectors for Tier 3 will be notified when samples are needed.

3. SAMPLE COLLECTION

a. Collect 1-4 individuals per species of crab and lobster from a sampling location. Any effort is valuable so even a single crab or lobster from a single site is useful.

b. If animals are collected from more than one site, be sure to keep them separated by site. You can do this by keeping them in separate coolers or bags, by marking one group using cable (zip) ties (attach it around the body of each animal from the same location), or using other methods to separate or mark the animals.
c. Keep records of at least **the location, species and date collected** (see submission form for other useful information to record). For location, GPS coordinates are most useful, but CDFG block numbers or at least reef name and closest offshore island or coastal county are needed.

4. SAMPLE PREPARATION

The toxins accumulate in the internal organs of crabs and lobster. The meat of the animal – claws, legs, tails and body meat -- are safe to eat. Thus, if desired, collectors can enjoy their catch while also providing useful samples for biotoxin testing.

Because crab and lobster are typically cooked (steamed/boiled) prior to being eaten, such is also done to the collected samples prior to analysis. Thus, it is most helpful if the crab and lobster have been cooked and frozen by the collector prior to them being shipped to the laboratory. However, the samples may be shipped live or frozen without cooking if you do not want to consume any of the catch or if you do not have time to cook and freeze the animals. The catch may also be partially consumed if desired (see Appendix 1). ***The key is to send the body containing the internal organs, using separate bags for separate animals, with all bags labeled as described above.*** See Appendix 1 for additional details on preparing the samples.

5. **SHIPPING**

Minimizing the time between collection and analysis is extremely important when testing samples for biotoxins. Samples should be shipped as soon as possible by the most rapid means available, while taking care that they arrive at the laboratory in an unspoiled condition and on a day that someone is there to receive them.

With this in mind, regardless of how the samples are prepared before shipping, samples should only be shipped to the state laboratory on ***Mondays, Tuesdays or Wednesdays*** so there is adequate time for the samples to be received and processed before the weekend. If you collect samples on a Thursday, Friday, Saturday or Sunday, be sure to freeze them (whether cooked or not) as soon as possible and then ship them on Monday.

a. Complete the sample submission form provided in the sample box. This form requires information you recorded while in the field (see Step 3c).
b. Place absorbent material (newspaper) on the bottom of the insulated container to soak up any leakage or condensation.
c. Place frozen sample(s) in the insulated container with an adequate quantity of frozen ice packs. Place lid over container.
d. Place the completed sample submission slip on top of the lid of the insulated container and then close and seal shipping box.

e. Containers returned to you from the laboratory will have mailing labels inside a plastic mailing envelope taped to the top or side of the box. Remove the label addressed to you and reverse it so that the box is addressed to the laboratory. Remove or cover any old UPS or other shipping labels that could cause confusion.
f. Packages should be addressed to:
   Specimen Receiving
   California Dept of Public Health
   ATTN: EMB Shellfish
   850 Marina Bay Parkway
   Richmond, CA 94804
g. Send package by Courier Service, overnight, next ***afternoon*** (not morning).
   i. Use provided next-day labels provided by CDPH (call 510.412.4635 to make arrangements as needed)
   ii. You may use your own courier service at your own expense if you desire
h. Avoid sending samples at the end of the week or just before holidays when the laboratory is closed. Prolonged transit and holding time increases the risk of spoilage.

6. CONTACTS

For questions regarding this protocol and offshore sampling program please contact the Marine Biotoxin Monitoring Program office in Richmond at (510) 412-4635, redtide@cdph.ca.gov or the UCSB California Sea Grant HABNet Coordinator at csg.habnet@gmail.com.

APPENDIX 1

VARIOUS PREPARATION METHODS FOR CRAB AND LOBSTER SAMPLES

Note: Toxins have not been found in the meat of crabs and lobsters, just the internal organs. The meat is tested when intact (whole) samples are provided.

LIVE OR FROZEN ANIMAL SAMPLES

a. Place the whole intact animal into a sealable plastic bag, remove the air from it and seal it closed. Label the bag with the appropriate sample ID number (see step 1). Note, crab claws and lobster tails may be retained if desired.

b. Ship the sample(s) overnight, next day afternoon if it is an appropriate shipping day (see "Shipping" above). If it is not possible to ship it right away or it is an inappropriate shipping day, freeze the sample overnight or until an appropriate shipping date occurs.

COOKED ANIMAL SAMPLES

a. Steam, boil or grill the crab/lobster. If animals were collected from more than one site it is best to cook them in separate pots to minimize cross contamination and to keep them separated by location. Or if grilling, place them on different parts of the grill. This will minimize cross contamination.

b. Let cooked crab/lobster cool. Place intact (whole) individual animals into a sealable plastic bag, remove the air from it and seal it closed. Label the bag with the appropriate sample ID number. Note, crab claws and lobster tails may be retained if desired.

c. Place all sample bags into the freezer overnight or until the appropriate shipping day (see "Shipping" above).

APPENDIX E

Outreach Materials

APPENDIX E.1

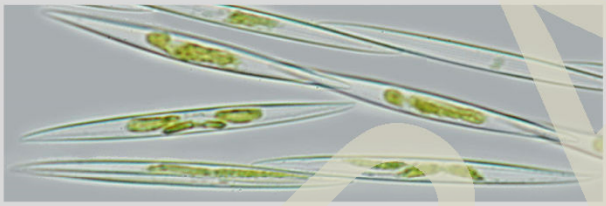
California HABs

A **H**armful **A**lgal **B**loom (HAB) is caused by naturally occurring toxins produced by microscopic algae (phytoplankton). It is currently unclear what stimulates the production of the toxins, but when present, they move through the food web from one animal to the next. Humans can become exposed to the toxins if they eat certain parts of some wild-caught seafood during or after a bloom.

On the West Coast, two primary HAB species are monitored:

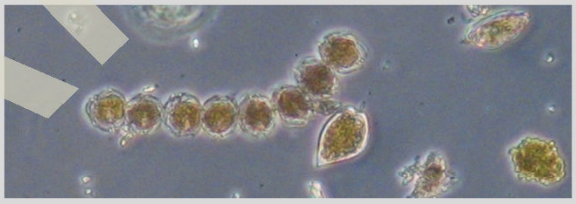
Domoic Acid (DA)/ Amnesic Shellfish Poisoning (ASP)

Pseudo-nitzschia spp. (diatom)



Paralytic Shellfish Poisoning (PSP)

Alexandrium sp. (dinoflagellate)



Range of Potential Physiological Effects on Humans

Potential Impact	DA Levels	DA Symptoms	PSP Levels	PSP Symptoms
Alert Level	20 ppm	None	0.8 ppm	None
Mild	27-75 ppm	GI discomfort	2-4 ppm	Numbness in extremities
Moderate	40-700 ppm	Headaches and disorientation	5-200 ppm	Loss of coordination, difficulty breathing
Severe	> 450 ppm	Short term memory loss, coma, death	> 200ppm	Paralysis, respiratory failure, death

Current Monitoring Efforts

Phytoplankton

Commercial shellfish growers: Weekly
Recreation sites: 1-4 times monthly
Offshore sites: 1-4 time monthly

Shellfish

Commercial shellfish growers: Weekly
Recreation sites: 1-2 times monthly
Offshore sites: Infrequent, opportunistic

Recreational and offshore sites are monitored by volunteers

DRAFT

APPENDIX E.2

Consuming Wild-Caught Seafood During a Harmful Algal Bloom (HAB): What to Know

A **H**armful **A**lgal **B**loom occurs when certain algae produce toxins that move through the food web and accumulate in certain seafood species. Consuming seafood affected by these toxins can pose serious health risks.

During a HAB, it is possible to safely consume most wild-caught seafood by avoiding the internal organs. Here are a few tips for safely consuming commonly affected seafood.

For mussels, clams, oysters, scallops, anchovies and sardines (filter-feeders):

Because the whole animal is typically eaten when enjoying these seafood items, avoid catching and consuming them during a HAB. *Scallops* are an exception; you can usually safely consume them if you remove, clean and eat just the meat. ***Note: commercial seafood farmers only sell toxin-free shellfish because of strict standards and testing.***

For crabs and lobster (animals that eat filter-feeders):

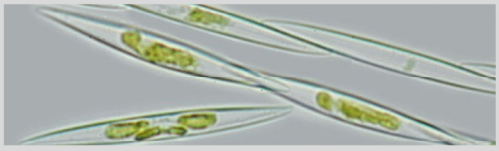
It is safe to eat the meat from the claws, legs, tail and body because the toxins do not accumulate in those parts. Avoid cooking with and consuming internal organs.

GENERAL ADVICE: Avoid eating and even cooking with the internal organs, such as stomach, intestines, and liver (tomalley), of **certain** seafood during a HAB. That is where the toxins typically accumulate. Cooking does not destroy the toxins.

The CA Department of Public Health regularly tests seafood samples and issues advisories when toxin levels are above critical levels. Call **800-553-4133 for current advisories.**

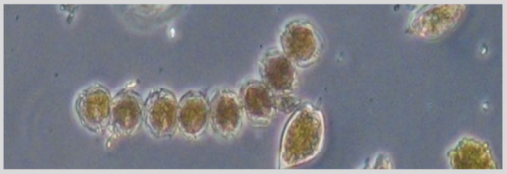
Common HAB Producers of California

Domoic Acid (DA)/Amnesic Shellfish Poisoning (ASP)
Pseudo-nitzschia spp.



Critical Level: 20 ppm
Range of Symptoms : Numbness in extremities to loss of coordination and difficulty breathing to paralysis, respiratory failure , and death.

Paralytic Shellfish Poisoning (PSP)
Alexandrium sp.



Critical Level: 0.8 ppm
Range of Symptoms : GI discomfort to headaches and disorientation to short term memory loss, coma and death.

For more information about HABNet contact the UCSB Sea Grant HABNet Coordinator at csg.habnet@gmail.com and visit www.cdph.ca.gov/healthinfo/environhealth/water/Pages/Shellfish.aspx for information about the state biotoxin monitoring program.

DRAFT

APPENDIX E.3

*** SEAFOOD BIOTOXIN NOTICE ***

ENGLISH

Domoic acid, a naturally occurring biotoxin, has been detected in the Santa Barbara Channel area where these crabs were caught. The meat of the crab is unaffected and safe to enjoy, but please do not consume any part of the stomach and intestines (guts) or even use these parts when cooking soups or chowders. For more info:

KOREAN

살아있는 생물체로부터 유래된 독소 물질인 Domoic Acid (신경 독소 물질:기억 상실증을 일으킴) 가 Santa Barbara 해엽에서 잡힌 게에서 발견되었읍니다.이 게의 살(meat)은 식용으로 섭취하기에는 안전하나 위장이나 내장은 부적합하니 직접 드시거나 수프나 차우더 요리에는 사용하지 마십시요:

SPANISH

Domoic acido, una biotoxina producida naturalmente, ha sido detectada en el area de Santa Barbara donde estos cangrejos fueron encontrados. La carne de los cangrejos no ha sido afectada y su consumo es seguro, pero por favor no consumir o utilizar los partes del estomago e intestinos cuando cocine sopas. Para mas informacion:

CHINESE

Domoic acid, 是一种天然发生的生物毒素，已在圣巴巴拉海峡区域(Santa Barbara Channel)扑抓的螃蟹检测到了。这种天然的生物毒素对螃蟹的肉没有影响，但请不要食用螃蟹的肚或肠，也不要用肚或肠来作汤或稀饭。需要更多信息，请看:

VIETNAMESE

Chất axit Domoic một độc tố thiên nhiên đã được phát hiện trong khu vực eo biển Santa Barbara nơi cua bị bắt. Thịt cua thì không bị ảnh hưởng và an toàn để thưởng thức, nhưng xin đừng ăn hay tiêu thụ bất kỳ phần nào của dạ dày hoặc ruột, hoặc sử dụng các phần của dạ dày hoặc ruột khi nấu canh và cháo.Muốn biết thêm chi tiết, xin coi trang web:

TAGALOG

Domoic acid, isang produktong natural na biotoxin, ay natuklasan sa Santa Barbara Channel kung saan nahuli ang mga alimango na ito. Hindi apektado ang karne ng alimango at ligtas na kainin ng tao, pero huwag kainin, o gamiting panghalo sa sabaw o sopas, ang anumang bahagi ng tiyan o bituka. Para sa karagdagang impormasyon:

JAPANESE

カニをとってるサンタバブラチャネル で生物毒素がみつかりました。カニのみはあんぜんで生物毒素にはえいきょがありません、しかしカニのないぞはス-プやシチューにはしようしないでください。

INDONESIAN

Kepiting ini di tangkap di daerah Santa Barbara, California dimana asam domoic (domoic acid) telah terdeteksi. Asam domoic adalah biotoksin yang berada alami di laut. Daging kepiting ini tidak teracuni oleh asam domoic dan aman untuk dimakan, tetapi awas, jangan memakan atau memasak perut, usus, atau jeroannya. Untung keterangan lebih lanjut, silahkan cek:

CDPH California Department of PublicHealth

www.cdph.ca.gov/healthinfo/environhealth/water/Pages/Shellfish.aspx

Sea Grant California