California Sea Grant College Program
Final Report

R/CONT-101
9/1/2011–8/31/2011
Extending the Use of Solid Phase Adsorption Toxin Tracking
to the Land-Sea Interface

Raphael Kudela
University of California, Santa Cruz
831.459.3290
kudela@ucsc.edu

Melissa Miller
California Department of Fish and Game
831-469-1746
mmiller@ospr.cdfg.ca.gov

Project Hypothesis

Harmful algal blooms (HABs) are an escalating problem in coastal ecosystems, inflicting massive economic losses on commercial fisheries and causing death and illness to both humans and marine fauna. Paralleling the rise of marine HAB problems, cyanobacteria (previously identified as "blue-green algae") have a worldwide distribution and can form extensive blooms in freshwater and estuarine habitat. Microcystins are fast becoming a global health concern, with severe and recurrent blooms with toxin elaboration reported globally, including central California. Until recently, microcystin intoxication was considered a public health issue mainly of freshwater habitat, reflected by the vast body of published literature on potential human health risks due to microcystin exposure in rivers, lakes, reservoirs and freshwater aquaculture. In contrast, monitoring of marine water and seafood for similar risks has been limited, despite confirmation of outflows of microcystin-contaminated freshwater to the ocean. A passive sampling method for determination of dissolved toxins in seawater, Solid Phase Adsorption Toxin Tracking (SPATT), allows for field-deployment of adsorbent resin sealed within a polyester mesh bag and have been used for a suite of lipophilic toxins, but until recently have never been evaluated for use with hydrophilic (water-soluble) toxins such as domoic acid (amnesic shellfish poisoning) and saxitoxin (the primary toxin associated with paralytic shellfish poisoning).

We hypothesized that SPATT could be used in both fresh and salt water, and that routine deployment of SPATT would identify land-sea transfer of toxins as a common occurrence.

Project Goals and Objectives

This proposal represents a "Rapid Response" request for a one-year pilot study focusing on the viability of incorporating the SPATT methodology at the land-sea interface to characterize the land-sea connection for cyanotoxins. Specific objectives include:

1) Add to our existing characterization of SPATT in the laboratory for a broad suite of microcystins (previous Sea Grant funding supported testing of a single form, microcystin-LR), cylindrospermopsin, anatoxin-a, and saxitoxins;
2) Build on our previous field-testing of SPATT by including more sample sites to document the potential pathways leading to cyanotoxin contamination of the coastal ocean;
3) Characterize the effects of "First Flush 2010" on the nearshore enviroment; we suspect that the First Flush event may provide acute (rather than chronic) exposure to cyanotoxins in much of the nearshore environment of Monterey Bay.

Briefly describe project methodology

We propose to conduct basic laboratory evaluation of several microcystin other congeners, including RR, YR, and LA, compounds that are known to be present in central California water bodies (Mekebri et al. 2009), as well as some other common cyantoxins include anatoxin-a and cylindrospermopsin. All analyses will utilize an Agilent 6130 LC/MS system maintained in Kudela's lab, using standard methods (Mekebri et al. 2009). Second, we propose to field-deploy SPATT during the rainy season in the Monterey Bay region to document the environmental conditions,

We propose to conduct basic laboratory evaluation of several microcystin other congeners, including RR, YR, and LA, compounds that are known to be present in central California water bodies (Mekebri et al. 2009), as well as some other common cyantoxins include anatoxin-a and cylindrospermopsin. All analyses will utilize an Agilent 6130 LC/MS system maintained in Kudela's lab, using standard methods (Mekebri et al. 2009). Second, we propose to field-deploy SPATT during the rainy season in the Monterey Bay region to document the environmental conditions, frequency, and intensity of cyanotoxin transfer from land to sea. Our preliminary data clearly indicates that this is occurring, but we did not have sufficient resources in 2009 to conduct a proper field monitoring effort. Specifically, we want to deploy SPATT at more land/sea sites (the major rivers and harbors around the Monterey Bay), and upstream in major watersheds such as the Pajaro River system and Elkhorn Slough, to characterize the gradient in toxin transfer, focusing on times when toxin transfer are likely to occur (late fall when warm weather and rainfall coincide).

Laboratory Evaluation: SPATT has been used in several locations, with consistent results. However, it is still necessary for us to determine that it quantitatively absorbs the toxins of interest, and to determine our percent recovery from the resin as a function of deployment time, environmental conditions, and toxin analysis method. We have so far focused on microcystin-LR. We will conduct a series of simple tests, optimizing the volume of resin and the extraction methods, by loading the resin in the lab with toxin standards and with "natural" toxins obtained from cell cultures (we maintain phytoplankton that produce microcystins). Our group has extensive experience developing analytical methods for toxin analyses (Litaker et al. 2008; Lane et al. submitted). We have budgeted for supplies necessary to set up the toxin analyses on the LC-MS system, primarily for certified standards. As a secondary goal, we also propose to test the applicability of SPATT extracted in 50% methanol (our standard procedure) with enzyme-based competitive ELISA toxin kits, commercially available from Abraxis, Biosense, and Envirologix. When LC/MS is available it is the preferred method, but many regulatory agencies and potential users of SPATT do not have access to that equipment, and are likely to be using commercial ELISA kits. Preliminary results in our lab suggest that the methanol does not interfere with the kits, but the kit manufacturers do not recommend it. By directly testing the kits, we can inform potential users of the pros and cons in using these commercially available kits, potentially greatly extending the group using SPATT. Funds from California Water Boards will be used for this comparison, with the Sea Grant Trainee carrying out the experiments.

Describe progress and accomplishments toward meeting goals and objectives.
We accomplished nearly all of our proposed objectives during this period. We briefly summarize these by topic:
1) Laboratory characterization of more toxins. We fully characterized SPATT for MCY-LR, -RR, -YR, and -LA using both fresh and saltwater. We also characterized the use of SPATT for saxitoxin (STX, the primary compound causing paralytic shellfish poisoning) in the laboratory using both deionized and fresh (Pinto Lake) water. We did not characterize cylindrospermopsin (due to time constraints) and eliminated the characterization of anatoxin-a because a peer-reviewed paper was published at the beginning of our study that outlined the same research.

2) Field sampling. Jenny Lane (Sea Grant Trainee) and Cori Gibble (NOAA Fellow) deployed SPATT throughout the Monterey Bay region during the 12 month period. From these data we identified several "hotspots" for toxin, developed a time-series for Pinto Lake, and tracked the implications of the 2010 First Flush. With support from Sea Grant, we have also extended the use of SPATT to Clear Lake, the San Francisco Bay Delta, and to the Coastal Confluences program.

3) Data Analysis. One manuscript (Kudela 2011) was published from this work, demonstrating a clear linkage between toxins and environmental conditions. Cori Gibble is currently working on data synthesis from the larger SPATT sampling for her thesis.

PROJECT MODIFICATIONS:
We made two primary modifications to the proposal. First, we dropped the analysis of anatoxin-a because a comparable study was published at the beginning of our funding period. We did, however, develop an anatoxin-a protocol for sample measurement, which is now routinely used for other research projects. Second, we did not complete the trials for cylindrospermopsin, because we ran out of time on this project in part due to the difficulty of assessing saxitoxin. We anticipate continuing these aspects of the project beyond the completion of this Sea Grant funding.

PROJECT OUTCOMES:
Our primary outcomes are three-fold.
1) We completed the work proposed, successfully demonstrating the applicability of SPATT for toxin tracking at the land/sea interface, and extending

the list of toxins to include multiple freshwater compounds. These data are now being used by Cori Gibble for her PhD thesis.

2) A manuscript is being published in the journal Harmful Algae fully describing our development of SPATT for microcystins.

3) We participated in several workshops and meetings where results were presented to the scientific and management communities, and to the public.

IMPACTS OF PROJECT:
Impacts resulting from this project include:
• SPATT has been incorporated into monitoring programs funded by the Coastal Confluences program, City of San Jose,USGS Menlo Park, CalFed, and Clear Lake.
• Pinto Lake is being studied under 303(d) funding for ways to mitigate the chronic toxin load present (identified by SPATT).

BENEFITS, COMMERCIALIZATION, AND APPLICATION OF PROJECT RESULTS:
SPATT is now routinely used by several monitoring agencies to augment traditional water quality sampling. Results from this project were also presented at a workshop on passive sampling methods (see below for details). From that meeting, Sigma-Aldrich approached the PI about potential commercialization of the technology. We are currently in discussion with Sigma-Aldrich to determine if this should be pursued.

Kudela, RM. Expanding Applications of SPATT for Algal Toxin Monitoring. Alliance for Coastal Technologies Workshop on Sampling the Aquatic Environment, Monterey, CA, 30 March 2011.

ECONOMIC BENEFITS generated by discovery
No direct economic benefits are documented at this time.

Issue-based forecast capabilities
A statistical model was developed to relate toxin concentrations with environmental conditions in Pinto Lake, CA. This could be used to forecast future toxin events; this is currently being explored with separate EPA funding.

Tools, technologies and information services developed
The SPATT technology was demonstrated for use with multiple toxins in fresh and salt water. It was recently summarized in Harmful Algae (Kudela, RM. Characterization and Deployment of Solid Phase Adsorption Toxin Tracking (SPATT) resin for monitoring of microcystins in fresh and salt water. Harmful Algae, doi: 10.1016/j.hal.2011.08.006)

Publications
Conference Papers, Proceedings, Symposia
California and the World Ocean San Francisco, CA 7-10 September 20, 2011
The Role of Anthropogenic Influences on Harmful Algae in California: What Do We Know? Kudela, RM

Peer-reviewed journal articles or book chapters
RM Kudela. 2011. Characterization and Deployment of Solid Phase Adsorption Toxin Tracking (SPATT) resin for **monitoring of microcystins in fresh and salt water. Harmful Algae**
doi: **doi: 10.1016/j.hal.2011.08.006**

Electronic publications:
RM Kudela. 7.10: video (http://www.cal-span.org/events/CWO/2010/session.php_code=09-b)

MEDIA COVERAGE:
Interviewed for "First Heavy Rains Nourish Dangerous Algae in Monterey Bay" story, 4 May 2011 (Science Communication program, story was published by CeNCOOS)

Interviewed for "Toxic Algae on the Loose", KQED QUEST (public radio and internet) program, 25 April 2011.
http://science.kqed.org/quest/slideshow/toxic-algae-on-the-loose/

Interviewed for "New Efforts to Track and Predict Harmful Algae", http://baynature.org/articles/oct-dec-2010/ear-to-the-ground/new-efforts-to-track-and-predict-harmful-marine-algae

Guest on KUSP (public radio) discussing sea otters and microcystins, 15 September 2010.

DISSEMINATION OF RESULTS:
Our primary dissemination has been through workshops/presentations (listed elsewhere), publication of a peer-reviewed manuscript, and this report.

COOPERATING ORGANIZATIONS:
Federal
NOAA
EPA

Regional
Central and Northern California Ocean Observing System

State
California Water Boards
County of Clear Lake
City of Watsonville

International
Global Ecology and Oceanography of Harmful Algal Blooms (GEOHAB) Programme

Industry
Sigma-Aldrich

Academic
University of California Santa Cruz

INTERNATIONAL IMPLICATIONS:
The technology developed (SPATT) has been of increasing interest globally. Results were presented at the International Program for Harmful Algal Blooms (IOC), and at GEOHAB. Both groups indicated interest in conducting an international workshop to implement and standardize the use of SPATT.

FOR ALL STUDENTS SUPPORTED BY THIS GRANT, PLEASE LIST:
Volunteer Count: None listed

Graduate Student Info
Jenny Q. Lane
University of California Santa Cruz
Department of Ocean Sciences
Degree Program: PhD
Thesis Title: New tools and insight for recognition of Pseudo-nitzschia bloom and toxin incidence
Supported by Sea Grant: Yes
Start Date: 9/1/2010 End Date: 8/31/2011
jennyequay@gmail.com
510-684-8193

Corinne Gibble
University of California Santa Cruz
Department of Ocean Sciences
Degree Program: PhD

Thesis Title: TBD
Supported by Sea Grant: No
Start Date: 9/30/2011 End Date: 9/30/2011
corinnegibble@gmail.com
831-419-8169

AWARDS: None listed.
PATENTS: None listed.