Improving tools for monitoring multiple HAB toxins at the land-sea interface in Coastal California (HAB-SICC)

1. Work Accomplishments:

a. Summary of Progress

This is a multi-institution, targeted MERHAB project that is integrated with several other ongoing research and monitoring efforts in coastal California. There is widespread interest from the California management community to utilize monitoring tools such as passive samplers (Solid Phase Adsorption Toxin Tracking, SPATT), as part of routine monitoring programs. The first step towards this goal is to improve the current monitoring tools, field-test, and demonstrate the management application of such tools for routine monitoring programs. This will enhance current HAB monitoring in California, and provide the tools necessary to create a coordinated regional strategy for routine monitoring and event response that can be implemented on a statewide basis and will provide a much needed survey of toxins and toxigenic organisms at the land-sea interface.

The project objectives are the following:

(1) to improve monitoring capabilities of SPATT and demonstrate how it can be used in routine monitoring programs
(2) to conduct a field survey at the land-sea interface in order to determine which HAB species and toxins are predominant
(3) to develop a culture collection and pictoral guide of the major toxin-producing cyanobacterial species in the study area to aid in species identification and management practices.
(4) to determine a HAB monitoring strategy at the land-sea interface that can be provided to management agencies.

We hypothesize that (1) multiple freshwater and marine toxins are routinely present at the land-sea interface, in brackish and marine coastal waters and (2) cyanotoxins produced in lakes, streams, wetlands and reservoirs, accumulate at the bottom of the watershed in estuaries and enter marine waters particularly during storms or times of significant tidal exchange (2A: Intermittent estuaries (created by seasonally, separated from the ocean by berms) accumulate substantial amounts of cyanotoxins when not flowing. These freshwater-brackish sources episodically deliver significant amounts of cyanotoxins to marine waters once flow begins or berms are opened).

The revised workplan includes the following tasks for Year 1:

- SPATT technology development
- Event response
- Project Meeting

i. SPATT technology development

One goal of this project has been to standardize SPATT measurements. It is not possible to *directly* compare SPATT values to the regulatory guidance because (a) SPATT measures dissolved, and not total toxin; (b) SPATT toxins and grab samples for toxins such as domoic acid are not equivalent to toxin levels in mussel tissue; (c) SPATT integrates spatially and temporally. Additionally, SPATT is generally considered to be more sensitive than grab samples (Lane et al. 2010, 2012; Kudela 2011; Gibble and Kudela, 2014). Given these caveats, it is still desirable to relate SPATT concentrations to regulatory limits/guidelines.

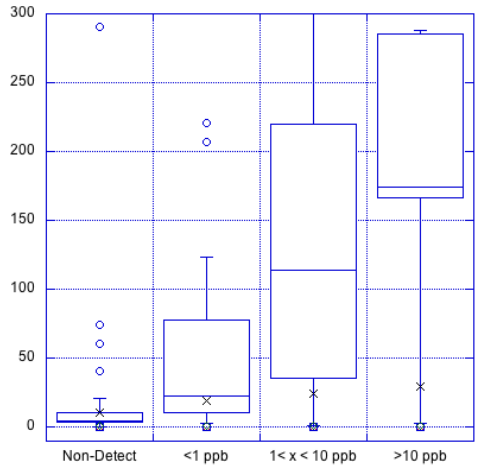

Figure 1. SPATT data from Pinto Lake, showing the correspondence between grab sample bins and SPATT values.

An initial attempt to provide an intercomparison used environmental data from long time-series at the Santa Cruz Municipal Wharf (SCMW) (Lane et al. 2010) and from Pinto Lake, California (Kudela 2011). For both of those programs SPATT, using the HP20 resin, were deployed weekly, with matching samples for dissolved and particulate domoic acid, and mussel tissue (SCMW), and dissolved and total microcystins (Pinto Lake). Using those data, SPATT values were binned into ranges corresponding to grab samples or mussel samples: non-detect, < 1 ppb, 1-10 ppb, and > 10 ppb for microcystins, and 0-5, 5-10, 10-20, and >20 ppm domoic acid in mussel tissue. Ranges were determined by binning the corresponding SPATT data and calculating the median, mean, and standard deviation. These data are depicted graphically for microcystins in Figure 1 and the ranges are provided in Tables 1-2.

As part of laboratory characterization, resin capacity and equilibration times were evaluated when SPATT were developed (Lane et al. 2010; Kudela 2011). Since then, adsorption/desorption of microcystin-LR (MC-LR) was more rigorously evaluated (Zhao et al. 2013) and HP20 was again identified as the optimal resin for environmental use, with linear absorption characteristics over several days. HP20 was also identified as the best resin for use with lipophilic toxins in seawater for prolonged (days) deployment, with reasonably linear uptake and a combination of good adsorption and desorption capabilities; other resins performed better under some circumstance, but were found not to be as universally applicable to a broad range of toxins, deployment times, and recovery methods (Zendong et al. 2014). Thus there is growing acceptance of HP20 resin as a “universal” SPATT resin, with the best overall combination of characteristics.

Table 1. SPATT concentrations of microcystins corresponding to total microcystins from matching grab samples.

Microcystin Grab Sample (ppb)	SPATT (ng/g)
Non-Detect	5-13
< 1 ppb	20-50
1< x < 10 ppb	50-200
> 10 ppb	175-275

Table 2. SPATT concentrations of domoic acid corresponding to domoic acid concentrations from matching mussel samples (SPATT were deployed weekly; mussel samples were collected weekly).

Domoic Acid Mussel (ppm)	SPATT (ng/g)
0-5 ppm	0-30
5-10 ppm	30-50
10-20 ppm	50-75
>20 ppm	>150

In order to translate the general characteristics of HP20 SPATT, a simulation was set up in the laboratory to mimic conditions using underway (shipboard) measurements; deployment in flowing water such as streams would be similar. The following assumptions were made:

1) Transects include fresh, brackish, and marine waters;
2) Individual SPATT deployments are for no longer than 12 hours;
3) SPATT adsorption may differ when using a flow-through system compared to passive (static) water bodies such as Pinto Lake and Santa Cruz Wharf;
4) Temperature and salinity vary over the transects, potentially influencing toxin adsorption;
5) SPATT samplers are stored frozen prior to analysis.

Given these assumptions, the laboratory experiment was designed to mimic typical field conditions. A large volume (~16 L) of low-salinity water was used (Sacramento River water with Monterey Bay water mixed in, final salinity ~10). A recent study (Fan et al. 2014) showed HP20 adsorption varies with salinity, but not significantly so compared to other sources of variability, it was assumed that salinity did not need to be directly tested again. The water was spiked with an initial concentration of ~34 ppb MC-LR, and 82 ppb domoic acid (a trace amount, ~3 ppb, of microcystin-YR (MC-YR) was also present). The water was subsequently diluted to create a series of toxin concentrations for testing SPATT adsorption.

Adsorption kinetics should also be sensitive to temperature, since adsorption is a physical-chemical interaction between the resin and the sorbents (toxins). This was tested as part of the laboratory trial by testing adsorption at 3 temperatures (22°C, 15°C, 4°C) and three time periods (20 minutes; 1 hour; 2 hours). For each time point 2-3 SPATT were soaked in a large (~2 L) volume, with the ambient toxin

concentration tested before and after each SPATT exposure to account for uptake.

For the SPATT adsorption tests (other than temperature), two methods were employed. First, SPATT were exposed for 15 minutes in a glass, 2L container with spiked water at 5 concentrations. This was designed primarily to calibrate SPATT uptake using the method employed by Peggy Lehman (DWR) in a previous field experiment. For that study, Bay and Delta water were collected into a container and SPATT were added for 15 m. Second, the large (~16L) carboy was connected to a peristaltic pump and water was recirculated through a 2L glass container (about 1.5L was in the container), using a flow rate of 2.5 L/min, which is a typical flow rate for underway mapping systems. The SPATT were prepared/deployed following the same methods as for the USGS cruises in San Francisco Bay Estuary. For each time point, the SPATT were removed, allowed to drain, placed in 50 mL plastic centrifuge tubes, and frozen. The SPATT were subsequently thawed and toxin was extracted using the standard UCSC protocol (10 mL 50% MeOH, 20 mL 50% MeOH, 20 mL 50% MeOH with 1M ammonium acetate). An additional step, collection of the Milli-Q rinse water, was added to test for loss of toxin during processing. As per UCSC protocol, each eluate fraction was run separately on an Agilent 6130 LC/MS, and the total toxin per SPATT sampler was calculated based on volumes and concentrations of extract (see also Lane et al. 2010; Kudela 2011; Gibble and Kudela 2014). Pictures of the flow-through setup are provided in Figure 2.

For the flow-through experiment, replicates (2-3) SPATT were placed in the flow-through container and allowed to absorb for 20 minutes to 24 hours. The spiked water was then diluted to adjust the toxin concentration, and additional SPATT were tested. This was repeated for 4 concentrations. Additional SPATT were tested during the transitions, to determine how quickly SPATT exposed to high toxin concentrations would equilibrate to a lower concentration.

Figure 2. Laboratory setup for the flow-through testing of SPATT. Left photo shows the carboy, receiving container, and pump. Right photo shows SPATT (in colored embroidery hoops) within the receiving container.

Calibration of SPATT--Results

Temperature: There was no significant difference (ANOVA, $p>0.05$) for SPATT toxin concentrations of both microcystin and domoic acid as a function of temperature. This is consistent with previous laboratory experiments conducted when SPATT methodology was first developed.

Milli-Q Rinse: For standard processing of SPATT, the Milli-Q (deionized water) rinse is not tested for toxin. As part of these experiments Milli-Q volumes and toxin concentrations were measured. While toxins were detected in the rinse water, it was a few percent of the total extracted toxin (for both microcystin and domoic acid), as previously reported (Lane et al. 2010, Kudela 2011). While this lost toxin could be important for cases where very low toxin levels are of interest, SPATT is already more sensitive than grab samples so this is considered to be an acceptable loss.

Microcystins, 15-minute exposure: Adsorption of MCY-LR and MCY-YR was linear as a function of concentration. Toxins were easily detected after 15 minutes of exposure. Previous comparison of SPATT to grab samples exhibited a calibration factor of about 10-50x (SPATT is 10-50x more sensitive than grab samples), as exhibited in Table 1. Shorter exposure resulted in a calibration factor of about 5x, as seen in Figure 3. Given typical underway mapping speeds, 15 minutes would roughly correspond to spatial scales of about a kilometer, and assuming exposure to toxin occurred for 0-15 minutes at a given concentration, the SPATT factor would be 1-5x.

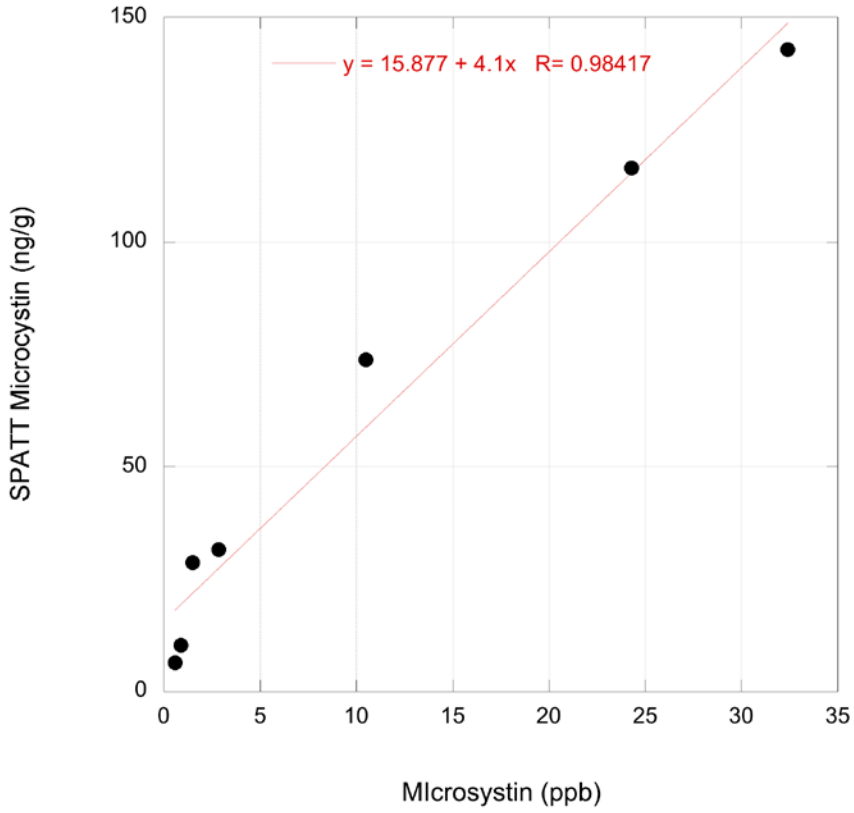

Figure 3. SPATT versus ambient water concentration for microcystins for SPATT exposed to constant concentration of toxin for 15 minutes. When forced to a zero-intercept, the calibration factor is 4.97x.

Microcystins, > 1 hour exposure: Testing of SPATT showed that microcystins equilibrate in approximately 1 hour. The calibration of toxin versus SPATT was therefore recalculated using SPATT exposed for 1-24 hours to estimate the upper-limit calibration factor. Results are presented in Figure 4. Linearity is excellent, and the calibration factor increases considerably compared to the 15-minute exposure, with a calibration factor of 271x.

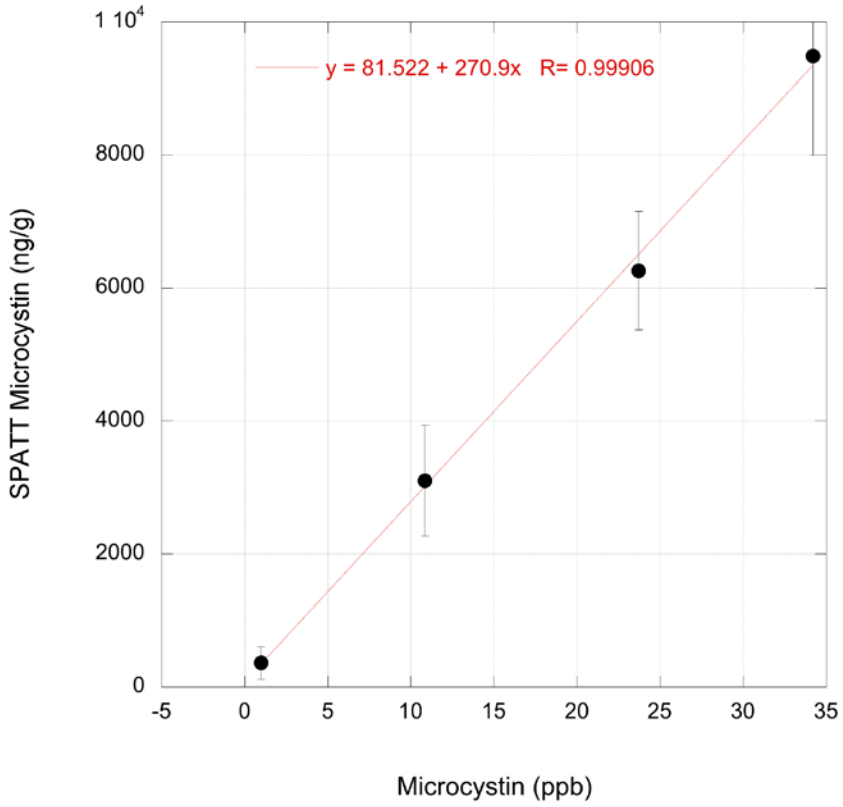

Figure 4. SPATT versus ambient water concentration for microcystins with SPATT exposed to a constant concentration for >1 hour.

Microcystins, transferred to lower concentration: When SPATT were allowed to equilibrate at a higher toxin concentration and were then exposed to water of lower concentration, similar kinetics were observed (not shown) with equilibrium occurring in ~1 hour, and a linear decrease over the first 60 minutes observed.

Field Calibration of SPATT Microcystins: The laboratory data for adsorption kinetics (time) and toxin levels (concentration) were used to develop a matrix showing the relationship between field SPATT observations and potential ambient toxin concentrations. The matrix is shown in Figure 5, together with statistics showing the total microcystin concentrations observed from October 2011-November 2014 for San Francisco Bay. The suggested “alert level” of 1 ng/g microcystins is indicated, along with the estimated non-detect limit.

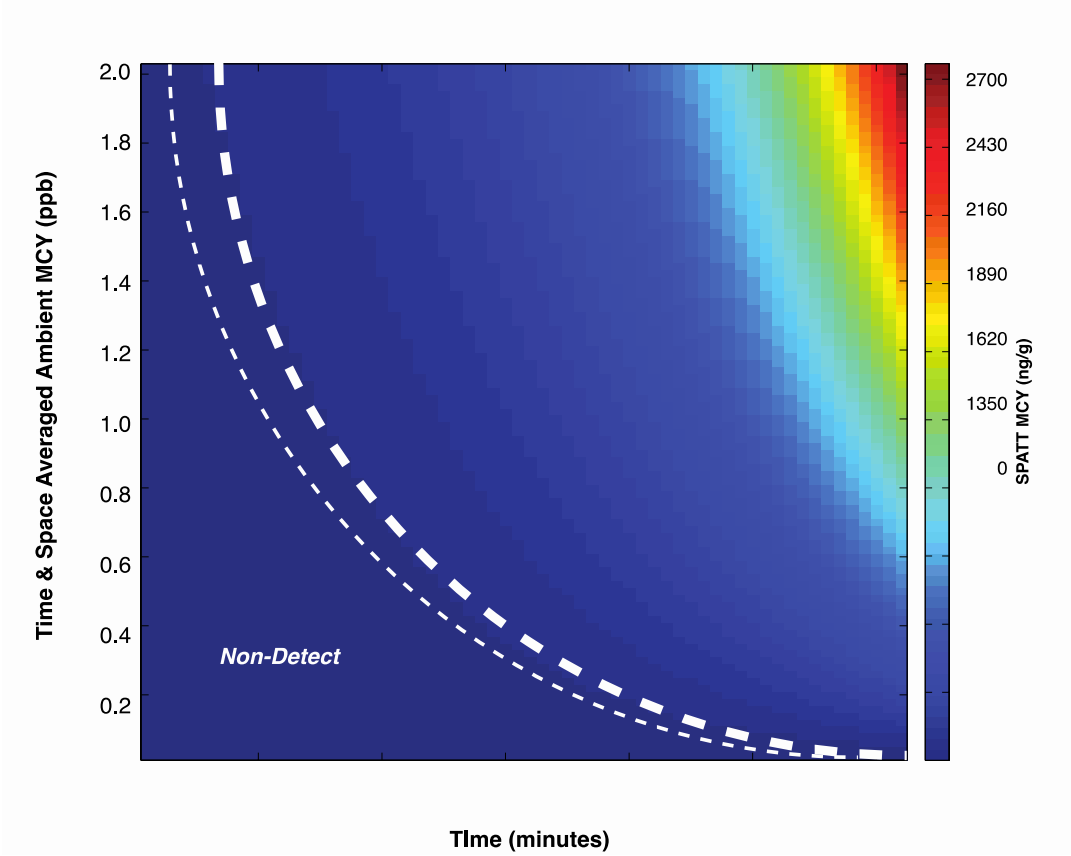

Figure 5. The top graph shows the SPATT concentrations that would be measured as a function of exposure time versus concentration. Note that toxin levels would increase/decrease in response to exposure (x-axis) to water with higher/lower concentration, with an equilibrium time of ~1 hour. The lower panels show the histogram of toxin concentrations observed in SFB (left) and cumulative percent (lower right).

Domoic Acid, 15-minute exposure: Adsorption of DA was exponential rather than linear (as seen for microcystins). Toxins were easily detected after 15 minutes of exposure. This makes calibration of SPATT more difficult, since it strongly depends on how long the SPATT are exposed. Data are presented in Figure 6.

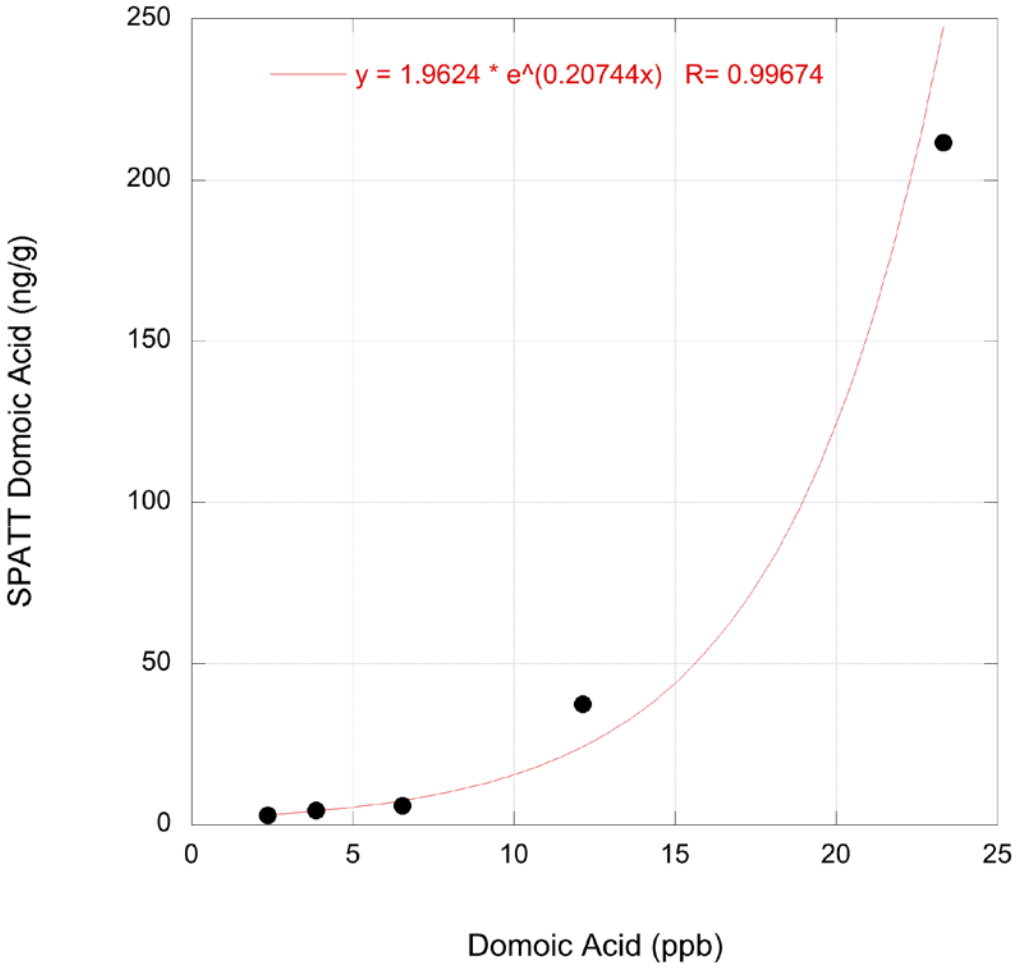

Figure 6. SPATT versus ambient water concentration for domoic acid for SPATT exposed to constant concentration of toxin for 15 minutes.

Domoic Acid, > 20-hour exposure: Testing of SPATT showed that domoic acid continues to be adsorbed for up to 24-hour, while other studies (Lane et al. 2010, Zendong et al. 2014) shows that SPATT continues to adsorb toxins for multiple days, but is quasi-linear when multiple days are included. Results for up to 24-hour exposure for varying concentrations of domoic acid are presented in Figure 7. As with 15-minute exposure, the data fit an exponential curve, suggesting that SPATT concentrations of domoic acid may underestimate low values and overestimate high values, compared to what would be assumed using a linear relationship.

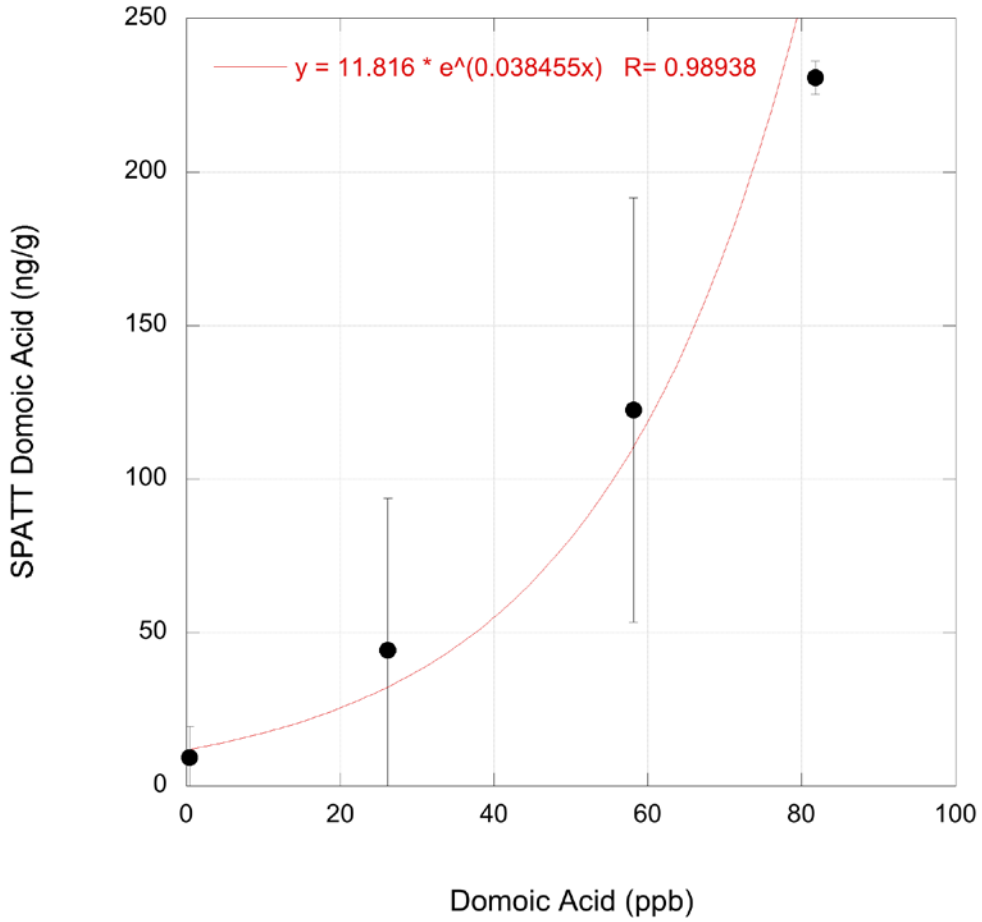

Figure 7. SPATT versus ambient water concentration for domoic acid for SPATT exposed to constant concentration of toxin for >20 hours.

Domoic Acid, transferred to lower concentration: When SPATT were allowed to equilibrate at a higher toxin concentration and were then exposed to water of lower concentration, similar kinetics were observed (not shown) with equilibrium initially fast, and then slowing down. The net result would be to (again) overestimate concentrations when exposed to high levels of domoic acid, compared to a linear response for time-averaged concentrations.

Field Calibration of SPATT Domoic Acid: The laboratory data for adsorption kinetics (time) and toxin levels (concentration) were used to develop a matrix showing the relationship between field SPATT observations and potential ambient toxin concentrations. The matrix is shown in Figure 8, together with statistics showing the total domoic acid concentrations observed from October 2011-November 2014 for San Francisco Bay. The suggested “alert level” of 75 ng/g domoic acid is indicated, along with the estimated non-detect limit.

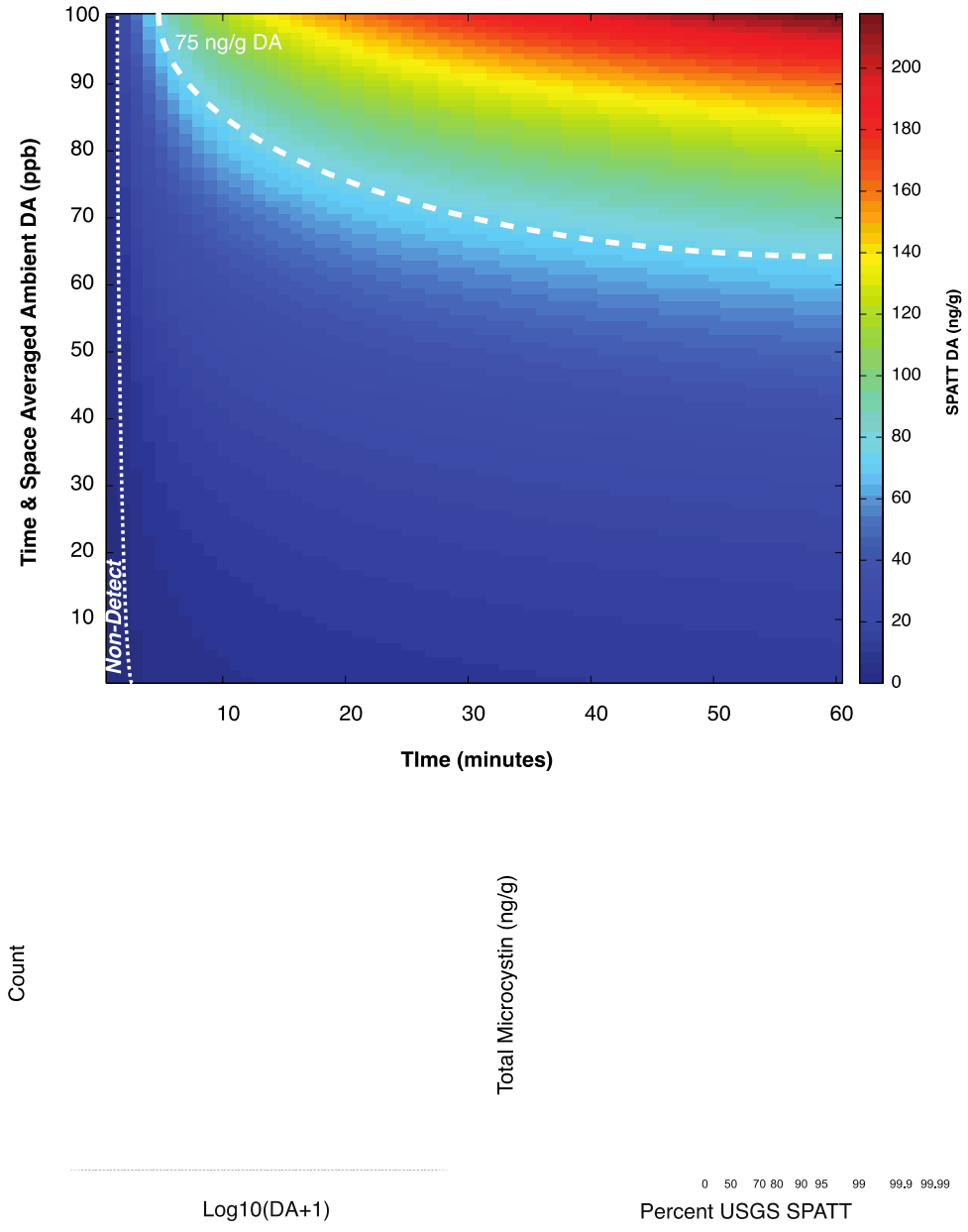

Figure 8. The top graph shows the SPATT concentrations that would be measured as a function of exposure time versus concentration. Note that toxin levels would increase/decrease in response to exposure (x-axis) to water with higher/lower concentration, with an equilibrium time of ~several days. The lower panels show the histogram of toxin concentrations observed in SFB (left) and cumulative percent (lower right).

ii. Event Response

There have not been any bloom events reported as of this writing. However, we expect with the ongoing "exceptional" level drought in California, there will be a large number of estuarine blooms over the summer and into early fall seasons.

The project PIs developed "Event Response Sample Criteria" as a result of the discussions during the PI project planning meeting. While in some areas of California it is clear when an event is occurring (or not), microcystins are present most of the year in the Klamath estuary, therefore the conditions that constitute a 'bloom event' were unclear. The developed criteria was communicated and discussed amongst all of the PIs as well as in a meeting with the Yurok Tribe collaborators (Suzanne Fluharty) on February 18, 2016.

iii. Project Planning Meeting

The PIs, collaborators and program managers all met on November 20, 2016 in Costa Mesa, CA, from 9:30am to noon. Introductions amongst all of the participants were made and Meredith Howard provided a summary of the project goals and objectives. Betty Fetscher (no cost collaborator) from the San Diego Regional Water Quality Control Board presented the study to be conducted in San Diego county in the spring 2016. Raphael Kudela (UC, Santa Cruz) presented background information on SPATT development and plans for Year 1. Neil Chernoff (EPA) presented a summary of the toxicity assessment planned for Year 2.

b. Summary of Work to be Performed and Problems

The tasks for Year 2 of this project are the following:

- Continuation of SPATT technology development
- Field Survey
- Establish Culture Collection
- Event Response
- Project Meeting

The SPATT technology development will continue in order to validate and field-test the improved SPATT tools to determine the best practices for deployment/recovery and analysis of SPATT.

We will begin the field survey in January 2017 (Year 2) to determine the marine and freshwater toxins and HAB species present at the land-sea interface and to demonstrate SPATT technology. A culture collection of regional HAB taxa will be created with samples from the field survey in order to (1) develop a pictoral and

genetic guide to California Cyanobacteria HAB species (this already exists for commonly-occurring marine HAB species), (2) determine the range of toxins produced by cultured HAB species and thus identify potentially important sources of toxins in natural samples, and (3) to employ these cultured HAB taxa to complete and vet the SPATT technology. The field survey will also identify the spatial/temporal patterns of HAB toxins and associated environmental variables at the land-sea interface, focusing on known, logistically feasible HAB "hotspots" along the California coast and will determine which toxins should be included in routine monitoring programs. The field survey will begin in January 2017 and continue monthly through December 2017 (Year 3). The table below lists the sampling locations and the timeframe for sample collection. Several of the sites are leveraged from other monitoring and research programs, and due to the change in duration of this project (3 years initially proposed to a 4 year project) the timeframes differ slightly for some sites. We do not consider this a problem for meeting our objectives and goals of the project.

Table 3. Sampling locations for the field survey.

Sampling Locations	**Waterbody type**	**Timeframe**
Klamath estuary and ocean sites	Brackish	2017
San Francisco Bay; Estuary and ocean sites	Brackish and marine	Ongoing for estuary: 2017 for marine
Santa Cruz Wharf	Marine	2017
Los Angeles River mouth	Brackish	2017
San Gabriel River mouth	Brackish	2017
Santa Ana River mouth	Brackish	2017
Newport Beach Pier	Marine	2017
San Onofre Estuary and Beach	Brackish and Marine	Collected in 2015
San Diego Estuaries (3 sites)	Brackish and fresh	2016

Sites Leveraged from Other Programs

San Francisco Bay Estuarine Sites- in collaboration with USGS and the San Francisco Estuary Institute, SPATT are deployed monthly on cruises transecting the Bay. For 2016-2017, we anticipate also deploying SPATT in parallel with field collection of naturally occurring mussels. MERHAB will be responsible for expanding SPATT analysis to include novel toxins (nodularins, anatoxin-a), and we will use support from MERHAB to evaluate environmental drivers of SPATT toxins.

San Lorenzo River – an emerging issue in the San Lorenzo River (Santa Cruz) resulted in deployment of SPATT in September 2015, to document the simultaneous occurrence of *Nodularia* and *Alexandrium.* Grab samples identified both saxitoxin

(STX) and nodularins at high (>100 ppb) concentrations. SPATT were deployed, and were archived for testing of STXs and nodularins from field samples.

San Onofre Estuary and Beach – The San Onofre sampling location is leveraged from the Estuary Reference Study conducted by SCCWRP and the California State Water Resources Control Board (SWRCB) as part of the State's development of nutrient water quality objectives. The goals are to characterize the natural background concentrations of dissolved oxygen, macroalgal and phytoplankton biomass in reference or minimally developed estuaries. The field sampling at this site was completed at the end of 2015 and the data analysis is underway. The constituents measured as part of this study include the following: in situ continuous measurements (water level, temperature, conductivity/salinity, dissolved oxygen, chlorophyll fluorescence) and discrete samples (nitrate, nitrite, ammonium, phosphate, total phosphate, total nitrogen, chlorophyll *a*, and macroalgal biomass). Cyanotoxin grab samples and SPATT samples were collected and will be analyzed in year 2 using MERHAB funds.

San Diego Estuaries - The San Diego Regional Water Quality Control Board (SDRWQCB) will be conducting a study to determine the transfer of cyanotoxins from upstream into estuarine coastal waters. The study will sample 3 sites (see Figure 9 below), each site having 3 monitoring station locations, (1) in the tributary, just upstream from the coastal discharge point (freshwater), (2) mid-slough (brackish) and (3) coastal receiving water (bay/lagoon). The study sites are the Otay River mouth (flowing out to San Diego Bay), Sweetwater River mouth (flowing out to San Diego Bay) and Peñasquitos Creek mouth flowing out to Peñasquitos Lagoon. The field sampling and toxin analysis is provided by SDRWQCB funding, and MERHAB will be responsible for (1) discrete chlorophyll *a* analysis and (2) identification and relative abundance determination of HAB species. The sampling period will be July through September 2016, and sample collection is every 10 days (toxin grab samples, SPATT, chlorophyll *a*, alkalinity and HAB species identification).

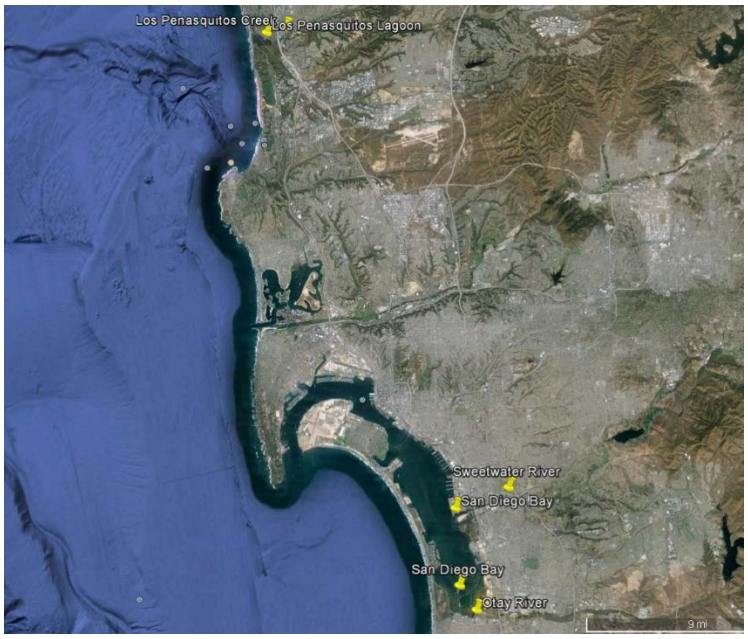

Figure 9. Map of the 3 sample sites and multiple sampling locations within each in San Diego county.

Year 2 will also continue the event response and there will be an annual PI meeting before the start of the field survey (fall 2016).